



**Morpho-Agronomical, Physiological, and Molecular Genetic
Diversity of Amaranths (*Amaranthus species*) in Ethiopia**



PHD DISSERTATION

MEKONNEN YESHITILA DEGEFU

HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

AUGUST, 2024

**Morpho-Agronomical, Physiological, and Molecular Genetic
Diversity of Amaranths (*Amaranthus Species*) in Ethiopia**

MEKONNEN YESHITILA DEGEFU

A THESIS SUBMITTED TO THE
SCHOOL OF PLANT AND HORTICULTURE SCIENCES,
HAWASSA COLLEGE OF AGRICULTURE,
SCHOOL OF GRADUATE STUDIES,
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(SPECIALIZATION: PLANT BIOTECHNOLOGY)

MAJOR SUPERVISOR: ANDARGACHEWU GEDEBO (PhD, Asso. Prof)

CO- SUPERVISORS: BIZUAYEHU TESFAYE (PhD, Asso. Prof)

HEWAN DEMMISIE (PhD, Asso. Prof)

AUGUST, 2024

HAWASSA UNIVERSITY
COLLEGE OF AGRICULTURE
SCHOOL OF PLANT AND HORTICULTURAL SCIENCES
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(Submission Sheet – 1)

This is to certify that the thesis entitled “Morpho-Agronomical, Physiological, and Molecular Genetic Diversity of Amaranths (Amaranthus species) in Ethiopia” submitted in partial fulfillment of the requirements for the Doctor of Philosophy (PhD) degree with specialization in **Plant Biotechnology** Graduate program of the School of **Plant and Horticultural Sciences**, College of Agriculture, and is a record of original research carried out by **MEKONNIN YESHITLA** under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged. Therefore I recommend that it be accepted as fulfilling the thesis requirements.

A. Gedebu
Name of major advisor


Signature

5/12/2024
Date

OR

Name of co-advisor

Signature

Date

EXAMINERS' APPROVAL SHEET
SCHOOL OF GRADUATE STUDIES
HAWASSA UNIVERSITY EXAMINERS' APPROVAL SHEET
(Submission Sheet-2)

We, the undersigned, members of the Board of Examiners of the final open defense by **MEKONNIN YESHITLA** have read and evaluated his/her thesis entitled "**Morpho-Agronomical, Physiological, and Molecular Genetic Diversity of Amaranths (Amaranthus species) in Ethiopia**" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the Doctor of Philosophy degree (PhD)

A. Gedebo
Name of Major Advisor

[Signature]
Signature

11/12/2024
Date

HUSSEIN M.B
Name of Internal Examiner I

[Signature]
Signature

13/11/2024
Date

Zerehena Dearewo
Name of Internal Examiner II

[Signature]
Signature

08/12/24
Date

Tilaye Feyissa
Name of External Examiner

[Signature]
Signature

13/11/2024
Date

Hewan Demissie(PhD)
SGS Approval
Research and Technology
Transfer Associate Dean

[Signature]
Signature

11/12/2024
Date

Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the school of Graduate Studies (SGS) through the School Graduate Committee (SGC) of the candidate's School.

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DEDICATION

This piece of work is dedicated to my wife Nardos Seifu and my son Leul Mekonnen, and Kirubel Mekonnen, and my daughter Nuhamine Mekonnen and to late mother in law W/ro Mulunesh Zewude.

STATEMENT OF THE AUTHOR

I declare that this dissertation is my own work and that all sources of materials used for this dissertation have been given recognition through proper citation. This dissertation has been submitted in the partial fulfillment of the requirements for a PhD degree at Hawassa University and is reserved at the University library to be made available to users under rules and regulations of the library. I solemnly declare that this dissertation has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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Name: Mekonnen Yeshitila Degefu

Signature: 

Place of submission: Hawassa University, Hawassa

Date of Submission: 26 August 2024

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ABBREVIATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
AVRDC	World Vegetable Center
EBI	Ethiopian Biodiversity Institute
FAO	United Nation Food and Agriculture Organization
GA	Genetic Advance
GAM	Genetic Advance as Percent of the Mean
GBS	Genotype by Sequencing
GCV	Genotypic Coefficient of Variation
GenAlEx	Genetic Analysis in Excel
GLM	General Linear Model
IBPGR	International Board for Plant Genetic Resources
ISSR	Inter Simple Sequence Repeat
MAS	Marker assisted selection
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCV	Phenotypic Coefficient of Variation
RAPD	Random Amplified Polymorphism DNA
RFLP	Restriction Fragment Length Polymorphism
SAS	Statistical Analysis System
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
UPGMA	Un-weighted pair group method with arithmetic averages

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IV	Agro-morphological genetic diversity assessment of amaranth genotypes from Ethiopia based on qualitative traits	Published	Springer Nature
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Morpho-Agronomical, Physiological, and Molecular Genetic Diversity of Amaranths (*Amaranthus* species) in Ethiopia

The genus *Amaranthus* L. is one of the world's underutilized cosmo-politant and genetically potential orphan crops, with a diverse range of morphological characteristics and geographic distribution. It is one of the few dicotyledonous, non-grass mesophytes that uses specialized C4 annuals or short-lived perennials to produce significant amounts of edible small-seeded pseudo cereals. Amaranths are versatile plants with the potential for high yields. They have great photosynthetic performance as a result of eliminating the rival photorespiration mechanism. A diversified family of food crops known as amaranths is impressively adaptable to new environments despite a variety of biotic and abiotic constraints. Moreover, to maintain a healthy lifestyle and prevent disease, people are becoming more and more concerned with their diet and selective about the foods they eat. People are therefore moving away from the typical cereals and staple meals they have been eating for a long time and toward more nutrient-dense options. Regular cereals lack essential minerals, amino acids, a greater nutritional value, and contain gluten. As a result, pseudo-céréals, particularly amaranth, are excellent alternatives. The potential of *Amaranthus* spp. is still underexploited in Ethiopia. This is also evident from the limited genetic research and selection for desirable traits in amaranths. Consequently, the number of genotypes being cultivated is small, thus reducing the genetic variability of the elite germplasm and ultimately increasing potential vulnerability to biotic and abiotic stresses. In addition, the amaranth genotypes are poorly represented in gene banks. Moreover, the plant is being neglected due to a lack of extensive research, discrimination, ignorance, and, among other things, the long-term genetic growth of the plant in Ethiopia. As a result, there is a lack of data on its genetic diversity, necessitating this research. Therefore, it is important to broaden the germplasm collection of this crop and assess the genetic diversity existing within the germplasm to support future collection, conservation, and crop improvement programs. The objectives of the current study were to explore the potential of the genetic resources of amaranth genotypes for further conservation and exploitation and make it easier to incorporate them into breeding programs in order to increase productivity. In this study, one hundred twenty amaranth genotypes were evaluated over two years using an alpha lattice design with two replications using agro-morphological markers. The analysis of variance indicated that the mean square due to year and genotype-by-year interaction varied significantly for most measured traits. The estimates of variability, heritability, and genetic advance found in this study indicate high genetic diversity in amaranth genotypes and the strength of selection response for these traits in the population. Furthermore, the potential for amaranth improvement through appropriate selection is revealed by the existence of significant differences between the number of superior and inferior genotypes for the majority of examined traits. This suggests that these traits are governed more by additive gene action and that selection based on these traits might be successful in achieving the desired genetic gains for improvement. Aside from this, selection based on yield alone may not be effective for yield improvement in plant breeding initiatives. So yield should be considered along with potential yield contributing traits to progress the genetic gains during selection. On the other hand, the results revealed significant positive phenotypic and genotypic associations on leaf yield, with leaf area, leaf breadth, branch number, leaf number, plant height at flowering, and grain yield all having positive direct effects. Similar strong positive phenotypic and genotypic relationships were found for grain yield and grain sinking and filling rates. This study consequently shows the need for traits with significant positive indirect impacts via leaf area to be considered indirect selection criteria for improving leaf yield in amaranth genotypes. The grain sink filling rate also significantly improved grain yield indirectly at both the

phenotypic and genotypic levels, mainly via days to flowering and leaf yield. This demonstrated that selection that mainly targeted days to flowering, leaf yield, and grain sink filling rate would ultimately boost the grain yield in amaranth genotypes. Principal component analysis showed that the first six principal components with eigenvalues greater than one contributed 80.41% of the variability. However, the first two principal components explained 52.42% of the total variation. The highest contributing traits in the first component were days to flowering, basal stem diameter, plant height at flowering, plant height at maturity, auxiliary inflorescence length, number of branches, terminal inflorescence lateral length, days to maturity, terminal inflorescence stalk length, leaf number, leaf length, top lateral branch length.. The traits with the greatest weight on the second component were leaf area, basal lateral branch length, leaf length, and leaf width, grain filling period, grain sinking filling rate, and grain yield. Therefore, selection based on these traits would be effective for yield improvement in amaranth genotypes. Additionally, the hierarchical clustering grouped all the genotypes into five clusters. The findings suggest that amaranth genotypes in Ethiopia have a lot of genetic variation, which might be used for future breeding and ought to be conserved. Twenty qualitative descriptors were utilized for a morphological diversity study for 120 amaranth genotypes. The overall mean of the Shannon diversity indices (H') was 0.61. The estimated diversity indices showed more intra-regional diversity (0.66) than inter-regional diversity (0.34), demonstrating the existence of gene flow between growing regions. Shannon-Weaver Diversity Index, ranged from 0.00 for auxiliary inflorescence to 1.94 for leaf coloration, with an overall mean of 19 characters (95%) that were found to have high diversity (>0.76) while auxiliary inflorescence was invariant. The hierarchical clustering grouped all the genotypes into three clusters. The first cluster included the most genotypes (58), followed by the second (47), and the third cluster contained the fewest (15). The study unequivocally demonstrated that, even when the genotypes were grouped into a small number of clusters, there was still enough divergence within the clusters to demonstrate the genotypes of amaranth to have a high genetic diversity. These results indicate that there is substantial genetic diversity among Ethiopian amaranth genotypes, which should be safeguarded and may be utilized in breeding in the future. The results of the analysis of variance showed that all examined physio-morphological parameters, except the rate of photosynthesis and stomata conductance, had mean squares that varied considerably ($P \leq 0.001$) owing to genotypes. The estimates of genetic variability, heritability, and expected genetic advance indicated high genetic diversity among amaranth genotypes, with a significant selection pressure for these traits in the population to produce better genotypes for improved amaranth. Selection based on desirable features such as leaf-to-air vapor pressure deficit, transpiration rate, chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, leaf area, plant height, leaf number, and root weight can be useful in achieving the intended genetic gains for improvement since these traits appear to be more controlled by additive gene activity. Thus, selection in amaranth genotypes may consider these desired yield-related features. Moreover, the study showed that certain genotypes (ALE-073) exhibited better intercellular CO_2 concentration (C_i), leaf-to-air vapor pressure deficit (VPD), transpiration rate (E), and leaf number (LN), resulting in better grain yield. Understanding the relationship between LA and E can help in selecting crops for high E and may provide an avenue to improve leaf yield. Furthermore, some of the selected genotypes in this study could be used as potential parents for improving the genetic gain in amaranth breeding programs. The study concluded that there was additive gene action present since the Ch a, Ch b, TCh, and Tca markers exhibited 100% heritability. This showed that the use of these characteristics for selection, which indicated a potentially exploitable variation, would be more effective and successful in the long run in breeding programs than the use of other traits for splitting generations. Hierarchical clustering grouped all the genotypes into seven clusters. The sixth cluster had the fewest genotypes (6), while the seventh cluster had the greatest number of genotypes (35) and was followed by Cluster Five (25), Cluster Three (22), Cluster One (14), Cluster Two (11), and Cluster Four (7). High leaf and grain yield-producing genotypes were grouped into the sixth cluster. The second cluster (C2) was characterized by high mean values for LA, LY, and GY. The maximum and significant genetic divergence was found between clusters C4 and C6 ($D^2 = 103.44$) and the least non-significant genetic divergence was found between clusters C5 and C7 ($D^2 = 17.00$). The first six PCs with eigenvalues greater than one accounted for 79.30% of the total variation in amaranth genotypes. The cumulative variance of 39.40%

was explained by 1st two principal components (PC1 and PC2) with eigenvalues greater than 1. Component 1 with a variance of 21.11 had a contribution from root length, root weight, total chlorophyll, and carotenoid while principal component 2 accounting for 18.29% total variability had a contribution from transpiration rate, rate of net photosynthesis, chlorophyll b and stomata conductance. The remaining variability of 13.02%, 11.33%, 9.37%, and, 6.17 % was consolidated in PC3, PC4, PC5, and PC6. These give scope for the selection of parents for breeding programs from these clusters, genetic divergence, and principal components to realize high genetic variation and novel combinations for yield increment. The present study was conducted to determine the genetic diversity and population structure of 40 amaranth genotypes using four inter-simple sequence repeat (ISSR) markers. The polymorphic information content (PIC), marker index (MI), resolving power (RP) and effective multiplex ratio (EMR) showed average values of 0.46, 10.41, 4.74, and 4.22 per primer, indicating high polymorphism values. The degree of polymorphism among the genotypes ranged from 0.00% for the Amhara population to 100% for the Southern Nations, Nationalities, and Peoples' Region population, with a grand mean of $70.83 \pm 11.34\%$. The observed number of alleles (N_a), effective number of alleles (N_e), expected heterozygosity (H_e), and genetic diversity estimated by Shannon's information index (I) were 1.51, 1.50, 0.28 and 0.42, among collection regions, respectively. The total genetic diversity, H_t (0.400 ± 0.006), and the average intrapopulation genetic diversity, H_s (0.285 ± 0.004). A high level of gene flow ($N_m = 2.12$) between populations implies the presence of gene flow ($N_m > 1$) and reflects high genetic differentiation ($G_{st} = 0.281$). The analysis of molecular variance showed that the maximum value of genetic variation was found within populations (99%), whereas a low value of genetic variance was observed among populations (1%). The structure analysis, principal coordinate analysis (PcoA), and unweighted pair-group method with arithmetic averages (UPGMA) analysis clustered the 40 amaranth genotypes into four distinct subpopulations. The majority of genotypes (35%) were clustered in Pop2, mainly obtained from Southern Nations, Nationalities, and Peoples' regions constituted dominantly of breeding lines and varieties, implying target selection contributed to the formation of distinct populations. The findings of this study provide important and relevant information for future breeding and conservation efforts of amaranths. Therefore, the use of molecular markers would be valuable for the effective utilization of amaranth in breeding programs. In conclusion, the characterization of the genotypes using the molecular marker, and phenotypic traits compositions indicated the presence of diversity and variation among Ethiopian amaranth genotypes. The results from these studies suggest possibilities for identification of amaranth genotypes superior for leaf and grain yield, indicating the need for the initiation of a planned breeding program. The outcome of this study provided new insights into the genetic diversity and population structure in Ethiopian amaranth genetic resources for designing an effective collection and conservation strategies for efficient utilization in future breeding.

Key words: agro-morphological, amaranths, agro-physiological, breeding, diversity, genotypes, molecular marker, qualitative traits

CHAPTER I: General Introduction

1.1 Background and justification

1.1.1 General introduction

Globalization and agricultural industrialization have increased yields and steadily increased food supplies globally. However, the green revolution replaced minor crops and reduced agrobiodiversity by favoring a small number of high-performing kinds of crops. Global food security may be threatened by the decline in the variety of crop species that supply the world's food (Khoury *et al.*, 2014; Govindaraj *et al.*, 2020). The agriculture industry is under tremendous pressure to produce 70% of the food needed to feed the people who are expected to rise in number globally by 40% to feed nine billions by the year 2050 (Aswal *et al.*, 2016).

Africa represents a major block of the globe where malnutrition and poor living standard across the spectrum of the population is conspicuous and most intractable (FAO, 2018; Unicef and WHO, 2020). Therefore, research on underutilized edible crops must continue to provide food security for the global population, as reaching such projections is a fear. An unbalanced nutritional profile affects the three main crops—rice, wheat, and maize—which account for the majority of the world's daily nutrition (Pedersen *et al.*, 1990). Although these crops are a good source of calories, they are deficient in important minerals and vitamins that are needed for a balanced diet (Akin-Idowu *et al.*, 2016), which means that over two billion people globally suffer from malnutrition. Even though these crops are a good source of calories, they are deficient in vital minerals and vitamins that are needed for a balanced diet. As a result, nearly two billion people worldwide—who largely depend on these crops—are malnourished (Cheng *et al.*, 2017). Incorporating substitute crops with advantageous nutritional compositions may enhance dietary diversity and play a significant role in enhancing food quality to combat hidden hunger and boost biodiversity.

To ensure future global food security, rational and efficient exploration of sustainable plant resources is crucial. Despite nature contains about 80,000 species of edible plants, and of the 3000 or so species of plants used throughout human existence (Schnetzler, 2018), only 150-200 plant species are currently widely commercialized worldwide (Secretariat and FAO, 2013; Schnetzler, 2018). Of these, 12 account for about 80% of dietary energy from plants, and just four species—rice, wheat, maize, and potatoes—provide more than 60% of the world's protein and

calorie needs ([Economic and Department, 2005](#)); 30 crops provide 95% of the world food supplies ([Joshi *et al.*, 2018](#)). The shrinking portfolio of world food baskets raises serious concerns about how major crops alone can ascertain the food and nutritional security of the ever-increasing population. Further, the major crops (rice, wheat, maize, and potato) are the reservoirs of carbohydrates providing high amounts of calories but lacking the essential amino acids and minerals for a balanced nutrition ([Kumar *et al.*, 2021](#)). Therefore, diversifying the food basket by incorporation of long forgotten ancient pseudo cereals with unparalleled nutritional profiles is eventually a leading strategy to address global nutritional security and combat the health infirmities and prevalent hidden hunger ([Joshi *et al.*, 2020](#)). Furthermore, in a developing country with a large population like Ethiopia, agricultural diversification has become crucial to meeting the needs of an expanding population. Thus, during the past few decades, many academics worldwide have turned their attention to the assessment of important, if occasionally neglected, crops. Amaranth is one such crop, which has a high potential to act as alternative food grain in most parts of the world.

Amaranth is one of the few multi-purpose crops that serves as a fuel source in addition to being used as a vegetable, cereal, oil, dye, fodder, fuel and decorative plant. It has potential industrial uses in cosmetology and biodegradable plastics ([Lozoya-Gloria, 2018](#)). Thus nowadays it could be classified as a new, forgotten, neglected, and alternative crop of great nutritional value. Researchers and consumers are showing a revived interest in this species as a nutritious cereal, particularly those who are health-conscious and suffering from diseases related to modern global lifestyles like as hypertension and diabetes ([Ruth *et al.*, 2021](#)). The poor nutritional values of the few, but most produced, crop species in the world today and consecutively occurred genetic erosion of cultivated land are some of the reasons for renewed interest in alternative crops. The use of alternative crops would result in product competitiveness, rich nutritional value, tradition, locality, and special quality ([Bavec and Bavec, 2006](#)). There has been an increased interest in amaranth since it appeared in the 1980s, when the US National Academy of Sciences carried out a research project entitled Underexploited Tropical Plants with Promising Economic Value, at that time amaranth was elected from among 36 of the world's most promising crops and identified as a major potential crop; since then, extensive research has been carried out ([Das and Das, 2016](#)).

Amaranths are a pseudo-cereal regarded as a good source of protein and non-saturated fatty acids. Amaranth is one of the few double-duty plants that can supply grains and tasty leafy vegetables of high nutritional quality. The nutritional value of amaranth grain is higher than that of several legumes and traditional cereals. Amaranth was officially approved by NASA as essential to the astronaut's diet for its high nutritional value. In 1985, amaranth grew and flowered in space during the flight of the shuttle (Morrison, 1985; Tucker, 1986). It contains high protein content and a well-balanced quantity of important amino acids, particularly methionine and lysine (Akin-Idowu *et al.*, 2013; Mota *et al.*, 2016). Its lysine level is three times higher than that of maize, twice that of wheat, and even more than milk protein, which is considered the gold standard of nutrition. Contrasting amaranth seed to buckwheat and other common cereals, the lipid content in amaranth seed can be up to three times higher (Alvarez-Jubete *et al.*, 2010). About 77% of amaranth oil is unsaturated fat and contains a significant amount of linoleic acid, which is vital for human nutrition (Martirosyan *et al.*, 2007). Squalene, an unsaponifiable lipid that serves as a biosynthetic precursor to all steroids in plants and animals, is frequently associated with the antioxidant and related characteristics of amaranth oil. Squalene is thought to have potential as a chemopreventive and chemotherapeutic agent, and numerous research has validated its health advantages in nutritional, medical, and pharmacological aspects (Smith, 2000; Güneş, 2013). The largest source of squalene is shark liver and, as far as plants are concerned, amaranth is known (He *et al.*, 2002; Caselato-Sousa and Amaya-Farfán, 2012). Shark liver oil is thought to be the richest source of squalene, including high concentrations of the compound (2300–8400 mg/100 g of oil). As detailed in Lozano-Grande *et al.* (2018), other plants that have been claimed to contain squalene in addition to shark liver oil include rice, wheat, and grapes (<1.0%), followed by palm oil, soybeans, maize, and macadamia. The two most valuable sources of this significant bioactive compound, are amaranth (6000–8000 mg/100 g oil) and olive (150–747 mg/100 g oil) showed the highest yields. In addition to having a high vitamin and mineral content, amaranth is regarded as a healthy source of insoluble fiber and contains riboflavin, niacin, ascorbic acid, calcium, and magnesium (Caselato-Sousa and Amaya-Farfán, 2012).

The African continent is blessed with a rich diversity of food crops, most of which have received little or no attention in terms of research and development of policy frameworks that can promote their effective commercial and industrial utilization. Amaranths (*Amaranthus species*) are one of such neglected and underutilized species. Amaranth is often regarded as an orphan or lost crop

(Tadele, 2019). Orphan crops are generally grown on poor soil and thus are often exposed to various abiotic and biotic stresses (Tadele *et al.*, 2018); they are less affected by major diseases and are more resilient to environmental stresses, including heat and drought (Sarker and Oba, 2018), and salinity (Sarker and Oba, 2018; Sarker and Oba, 2019; Sarker and Oba, 2020) than most major crops. Therefore, it can be successfully cultivated for leaf or grain in many regions and seasons where other crops cannot thrive (Ebert *et al.*, 2011; Grundy *et al.*, 2020). It possesses diverse abiotic stress tolerance traits, is genetically diverse, and is highly phenotypically plastic, making it an ideal crop to thrive in a rapidly changing climate. Despite considerable genetic diversity, there is a lack of detailed characterization of genotypes. Diversifying the food basket by including a variety of underutilized crop species with enhanced abiotic stress tolerance features is one way to reduce agricultural losses caused by climate change (Mayes *et al.*, 2012). Amaranth is a self-pollinating crop, but because of frequent interspecific and inter-varietal hybridization, as well as variable levels of outcrossing, there is significant genetic diversity (Ray and Roy, 2009). Due to their great adaptation to a variety of eco-geographic conditions, amaranths also display amazing diversity (Lee *et al.*, 2008b).

Yet despite this diversity and excellent nutritional quality, the breeding of improved cultivars is widely neglected (Shukla *et al.*, 2006). Many orphan crops are rich in nutrients and have high market potential but yet have been neglected and not targets for modern breeding (Tadele, 2019). Preserving and using orphan crop biodiversity could help to adapt agricultural production to environmental changes and to improve food security (Fahey, 1998). The use of these alternative crops may also provide health benefits for consumers and income for farmers in developing countries (Mohamed *et al.*, 2010; Abdu-Raheem and Worth, 2011). Amaranth is an indigenous leafy vegetable of Africa that has great inherent health-promoting components good for human applications and uses (Kwenin *et al.*, 2011; Zhu, 2020). To enhance their agronomic importance, attention should be given to germplasm conservation, the use of crop diversity for breeding improved cultivars, and the development of sustainable production technologies for this promising crop.

Amaranth should be studied as a viable option to widen agricultural production on marginal land thereby offering support to agricultural systems in demanding environments (Schahbazian *et al.*, 2006). It is an inherent strong market and industrial potential which are yet to be fully tapped

(Olaniyi, 2007; Akin-Idowu *et al.*, 2017). According to Onyango (2010), improvement and wider utilization of amaranths could produce an easy and cost-effective way of eliminating malnutrition and promoting people's health as well as achieving food security. This genus is economically significant, it is regarded as the most important raw resource for food and nutrition, as well as a powerful medicinal plant (Shodiev *et al.*, 2021). These plants exhibit all of the features of a functional food (Ishimoto and Monteiro, 2010), and their nutritional and functional properties, such as lowering blood cholesterol and avoiding gluten, are extremely comparable (Almeida and Sá, 2009). As a result, including these grains in one's diet has the potential to enhance one's diet while also providing health advantages. Amaranth has been hailed as a super grain, and a miracle grain according to Mburu *et al.* (2011). Amaranth is sometimes referred to as "the crop of the future" or "third-millennium grain" because of its high nutritional content and resilience to a broad variety of environmental conditions (Martinez-Lopez *et al.*, 2020). However, knowledge of taxonomic differentiation as well as genetic variations is scanty and sometimes confusing and does not facilitate the recognition of types, cultivars, and species (Das, 2012; Achigan-Dako *et al.*, 2014). *Amaranth species* are often difficult to characterize taxonomically, due to the overall similarity of many of them, small and difficult-to-see diagnostic parts, intermediate forms, and the broad geographical distribution, which are the reasons for many synonyms (Jacobsen and Mujica, 2003).

Ethiopia is one of the Vavilovian of crop diversity. Amaranth has long been a component of traditional African agriculture and is semi-domesticated, mostly as a vegetable, in Ethiopia and other East African countries (Alemayehu *et al.*, 2015). Amaranth is now grown as a grain crop in far-flung areas such as Ethiopia's mountains, South India's hills, Nepal's Himalayas, and Mongolia's plains, with exceptional seed quality and the highest potential for use as a food ingredient (Brink *et al.*, 2006; Sokolova *et al.*, 2021). The widespread usage of local names and the large genetic variation present in Ethiopia have also been suggested as a hub of amaranth diversity. In the Flora region of Ethiopia and Eritrea, eleven species have been identified (Demissew, 2010). The real challenge in biodiversity conservation is the identification of components of biodiversity and their characterization (Husen *et al.*, 2012). Ethiopia has great potential for amaranth crop cultivation and production, however, the actual output and productivity are still very low (Aderibigbe *et al.*, 2022). While amaranth is found all across Ethiopia, its main growers are the Me'enit, Konso, Ari, Ale, and Derashe people of the Southern

Nations, Nationalities, and Peoples Region (SNNPR), where it is grown as an intercrop alongside sorghum and maize. Further, amaranth plants are semi-domesticated in the Ethiopian in Wollega, Konso, Ale, Ari, Derashe, and South Omo. Some farmers have started to cultivate and intercrop the species on their farmlands near their homesteads. These people typically consume ground and popped amaranth grains, which they then mix with other cereal flours like teff, sorghum, barley, and wheat to make bread, porridge, and borda. The Konso people call amaranth passa and they also use its seeds for the preparation of a local beverage known as ‘Chaqa’. The vernacular name of the plant varies from place to place in Ethiopia. It is also called Lishalisho, Aluma, and Ferenjitef in Amharic; Ambertefa, Iyaso, and Jolili in Oromo, and Gagabsa in Wolayita. Unfortunately, due to a lack of thorough research, discrimination, and ignorance, there is very little genetic research on the genus *Amaranth* in Ethiopia, and there has been little progress in the improvement of this plant.

Plant breeders use diversity in genetic resources to develop new and improved crop cultivars to address global challenges that affect food security, sustainability, and adaptation to climate change. Genetic diversity is important in developing superior cultivars for crop improvement. Genetic diversity is the range of genetic characteristics in a crop or species, whereas genetic variation is the genetic differences among individuals for a specific characteristic, where these genetic differences reside in one or more DNA sequences. Genetic diversity can be assessed by examining differences in the DNA sequence in a population of individuals (Haun *et al.*, 2011). Genetic variation gives rise to phenotypic variation, which are differences in observable traits within a population. Plant genomes are dynamic and evolutionarily labile, which results in more frequent genetic and epigenetic changes in plants that serve as the sources of a large amount of genetic and phenotypic diversity, even between cultivars within a species. Greater genetic diversity in plants gives them a remarkable ability to adapt to sudden environmental changes (Raza *et al.*, 2019). Despite considerable genetic diversity, there is a lack of detailed characterization of genotypes. Moreover, the growing world population and rising demand for agricultural commodities have created invincible challenges to achieving this status globally. Amaranth is a good choice for adequate mitigation techniques for the effects of climate change aimed at East Africa. According to Fomsgaard *et al.* (2010), amaranth should therefore be further explored as a potential crop to increase the use of marginal agricultural lands in the area

and enable the improvement of the agricultural systems of arid and semi-arid areas in eastern parts of Africa while utilizing both seed and leaf production for reasons of food security.

The assessment of genetic diversity within and between plant populations is routinely performed using various techniques such as morphological in the pre-genomic era, and DNA (or molecular) marker analysis in the genomic era (Govindaraj *et al.*, 2015). Morphological markers generally represent genetic polymorphisms that are easily identified and manipulated but are highly influenced by the environment (Jiang, 2013). The morphological diversity of amaranths is remarkable, as is their adaptation to a wide range of eco-geographical conditions (Lee *et al.*, 2008a), elevation is not a severe limitation (Council, 2006; D'Amico and Schoenlechner, 2017). Because of these benefits amaranth is cultivated in highly diverse areas, such as tropical lowlands and mountainous regions up to 3500 meters altitude (D'Amico and Schoenlechner, 2017). Despite the enormous morphological variability between and within *Amaranthus species*, there are only a few taxonomic traits that are unique to the genus (Juan *et al.*, 2007; Wolosik and Markowska, 2019; Aderibigbe *et al.*, 2022). Amaranths have a considerable genetic variation, which indicates that they have a lot of room for improvement. The determination of genetic diversity relies heavily on agro morphological characterization. As a result, it is required for the successful conservation of amaranth genotypes and crop enhancement programs (Shah *et al.*, 2018).

Over the last few decades, several DNA marker technologies have been developed for amaranth, starting from the early days of the hybridization-based amplified fragment length polymorphism (AFLP) markers (Vos *et al.*, 1995) to the more recent development and applications of single nucleotide polymorphism (SNP) markers (Jamalluddin *et al.*, 2022; Hoshikawa *et al.*, 2023; Pandey *et al.*, 2023). Molecular markers are becoming essential instruments for accurately and reproducibly characterizing plant genotypes and clarifying genetic variability (Parita *et al.*, 2018). Plant breeders might distinguish between genotypes by assessing the degree of variation within and across species using molecular phylogenetic investigations, which would offer a foundation for suitable breeding techniques. Thus far, various molecular markers have been developed, extensively utilized, and categorized into multiple classes, including, such as repeated amplified polymorphic DNAs (RAPDs) (Anjali *et al.*, 2013), restriction fragment length polymorphisms (RFLPs) (Park YoungJun *et al.*, 2014), simple sequence repeats (SSRs) (Wang and Park, 2013), amplified fragment length polymorphisms (AFLPs) (Chandi *et al.*, 2013), inter-simple sequence

repeats (ISSRs) ([Štefůnová et al., 2014](#); [Gelotar et al., 2019](#); [Hamidzadeh Moghadam et al., 2023](#)). Even though they are all based on various premises and have different appealing characteristics, their purpose is to draw emphasis to the variety across the genome ([Agarwal et al., 2008](#)). The main drawbacks of these techniques include the expensive nature of AFLP analysis, the low reproducibility of RAPD, and the requirement for flanking sequence knowledge to design primers specific to a given species for SSR polymorphism ([Pradeep Reddy et al., 2002](#); [Gupta et al., 2010](#)). SNPs appear to be highly intriguing markers as well, but they demand a significant investment in labor and technology. Therefore, it is extremely improbable that universities and national agricultural institutes in countries that are developing are going to embrace SNPs ([Ramakrishnan et al., 2016](#)).

ISSR markers were developed from the need to explore microsatellite repeats without the use of DNA sequencing ([Lagercrantz et al., 1993](#)). The technique is based on the amplification of DNA segments between two microsatellite repeated regions ([Zietkiewicz et al., 1994](#)) ISSR appears to produce reliable and highly polymorphic band profiles. The key additional feature of ISSR lies in its technical simplicity and speed. ISSR analysis is quick, reproducible, and generates sufficient polymorphisms to have the potential for large-scale DNA fingerprinting purposes ([Prevost and Wilkinson, 1999](#)). Moreover, ISSR are one of the most straightforward and popular marker types among polymerase chain reaction (PCR)-based marker techniques ([Kunjupillai, 2005](#)). According to the Mendelian hypothesis, two phenotypes can be identified at each locus for ISSR markers, which are regarded as dominant markers ([Tsumura et al., 1996](#)). [Ratnaparkhe et al. \(1998\)](#), suggested that ISSR markers could provide important information for genome analysis of plant species as a marker associated with disease-resistance gene clusters. ISSR markers offer great potential for differentiating closely related cultivars and the generated bands are very repeatable on duplicate samples ([Fang and Roose, 1997](#)). Plant breeding, the identification of DNA markers connected to agronomic features ([Ratnaparkhe et al., 1998](#)), the evaluation of genetic diversity and phylogenetic studies of various species ([Ajibade et al., 2000](#)), and the characterization of germplasm have all made use of ISSR markers ([Pradeep Reddy et al., 2002](#)). ISSR markers are evolving into crucial instruments for analyzing plant genetic diversity. In tandem, they offer a thorough explanation of the kind and degree of diversity, as well as its genetic relationships and germplasm management ([Sharma et al., 2011](#); [Kumar et al., 2013](#)).

Studying the genetic diversity of a crop is very important for effective germplasm management, utilization, and genotype selection for crop improvement (Bucheyeki *et al.*, 2009). Moreover, research on population structure, evolution, and plant breeding initiatives all value the assessment of genetic diversity (Fatinah *et al.*, 2013). It is the most important step for conserving and increasing the rate of genetic gain in amaranth breeding programs. The level of genetic diversity within a species is commonly used to measure the level of species adaptability and survival in unpredictable environmental conditions (Ramanatha Rao and Hodgkin, 2002; Govindaraj *et al.*, 2015). Similarly, the level of genetic variation within a population is the basis for germplasm selection in plant breeding and is vital for crop improvement (Yan *et al.*, 2017). Hence, the conservation and utilization of plant genetic variation are crucial to human food security (Ramanatha Rao and Hodgkin, 2002). Breeding efforts are hampered by inadequate understanding of the genetic diversity in these amaranth species and the scarcity of appropriate molecular markers. A thorough understanding of these genetic linkages is crucial for cultivar creation and improvement, as these processes depend on having access to a well-characterized, genetically diverse pool of material. So, assessing the extent of genetic diversity using markers of physio-morphological traits is essential to exploit the existing variations in amaranth genotypes. Additionally, evaluating the genetic diversity in the pool of germplasm is made easier by the physiological characteristics of plants (Council, 1984). Information about the genetic makeup of genotypes contributes to decision-making for conservation activities, identification, characterization, and value addition. Molecular characterization, in conjunction with (phenotypic traits or agro-physio-morphological data), provides reliable information for assessing, among other factors, the amount of genetic diversity (Perera *et al.*, 2000).

Amaranth is a climate-smart, high-yielding plant that is vital to both satiating national demand and lowering reliance on just a few cereal crops. It serves as a fuel source in addition to being used as a vegetable, cereal, oil, dye, fodder, and decorative plant (Mlakar *et al.*, 2009; Sheikh and Singh, 2013). According to Ayalew (2013), amaranth grain yields registered by farmers in Ethiopia are relatively low (1859.4 kg/ha–3495.3 kg/ha) compared to 8,000–10,000 kg/ha in Uganda (Muyonga *et al.*, 2008), 6000kg/ha under conventional agricultural practices have been reported (Svirskis, 2003; Gajdosova *et al.*, 2004). Low crop productivity is a general problem facing most farming systems in sub-Saharan Africa including Ethiopia. In the development of a high-yielding variety, the presence of genetic diversity among available germplasm lines plays a

major role (Shukla *et al.*, 2010). However, information on genotypes or lines with both potential for leaf and grain yield (dual purpose) has not been exhaustively evaluated and documented. This information gap also presents a problem for researchers in advocating the right amaranth genotypes for promotion and adoption by farmers concerning dual-purpose genotypes. The low yields by farmers are partly attributed to lack of diverse germplasm adaptable to varied agro-ecological conditions in the country. As a result, it's critical to carefully consider the available germplasm and select improved genotypes with great potential for leaf and grain yield. Ethiopians may not be aware of the crop's production or use (Yosef Alemu *et al.*, 2018). This could be due to insufficient use, a dearth of better varieties, inadequate agronomic practices, or a lack of understanding of the crop's significance. Nonetheless, it is exclusively popular and consumed in a few parts of Ethiopia. Amaranth, an easy-to-grow nutrient-rich vegetable, has been recommended for enhancing food security and lowering malnutrition in Ethiopian populations that depend on subsistence farming (Alemayehu *et al.*, 2015). Furthermore, researchers are less interested in this "orphan crop" (Jonah *et al.*, 2012). By establishing a value chain for the production of amaranth-based value-added products, which may help with food security and nutritional quality, the research on amaranth has made a significant contribution to boosting amaranth growth. Even though several countries have reported on the distribution of *Amaranth species* used like cereal grains and the production of ancient grain products, Ethiopia has yet to conduct similar research. There is little to no information available regarding the agro-morphological, physiological, and molecular genetic diversity of amaranth because these aspects of the plant have not been researched or recorded throughout this country. The assessment of genetic diversity among populations of diverse phytogeography areas is essential because the existence, perpetuation, and continuance of a species to fulfill the demands of changing environments mainly depend on the level of variability available in the gene pool of particular species (Ray and Roy, 2009). Moreover, the diversity present in particular species is utilized for the development of new varieties using plant breeding. Thus, molecular characterization offers trustworthy information for evaluating the degree of genetic variety, among other things, when combined with agromorphological, or physio-morphological data. Finally, the conceptual framework of this study were indicated in Figure 1.1.

1.1.2 Objective

1.1.2.1 General Objective

The general objective of the study was to explore the potential of the genetic resources of amaranth genotypes for further conservation, and exploitation and make it easier to incorporate them into breeding programs to increase productivity.

1.1.2.2 Specific Objectives

- I. To estimate the genetic diversity of Ethiopian amaranth genotypes based on quantitative agro-morphological traits.
- II. To assess genetic diversity among Ethiopian amaranth genotypes using qualitative morphological traits.
- III. To determine the extent and patterns of genetic diversity in Ethiopian amaranth genotypes using physio-morphological markers.
- IV. To determine the genetic diversity and population structure of 40 amaranth genotypes using inter-simple sequence repeat (ISSR) markers

1.1.3 Research hypothesis

- There are differences in agro-morphological quantitative traits of various genotypes of amaranth.
- There are differences in agro-morphological qualitative traits of various genotypes of amaranth.
- There is a difference in physio- physio-morphological characteristics of different amaranth genotypes.
- There is a genetic diversity difference in the amaranth genotypes by using the ISSR marker

1.1.4 Research questions

- 1) Do the Ethiopian amaranth genotypes differ in terms of quantitative agro-morphological traits?
- 2) Are the agro-morphological qualitative features of the Ethiopian amaranth genotypes different?
- 3) Do the Ethiopian amaranth genotypes differ in terms of physio-morphological features?

4) What is the extent of genetic diversity of Ethiopian amaranth using ISSR markers?

Table 1.1 Outline of the dissertation

The four specific objectives indicated above were addressed in different chapters that comprise part of this thesis. The chapters were written as independent papers and somehow interrelated; therefore, overlaps are most likely to occur in terms of contents and references among the chapters. The thesis is organized into seven chapters as shown below:

Chapter	Title
s	
1.	General introduction
2.	Literature Review
3.	Agro-morphological Characterization, Variability, and Diversity among Amaranth (<i>Amaranthus species</i>) Genotypes from Ethiopia
4.	Agro-Morphological Genetic Diversity Assessment of Amaranth (<i>Amaranthus species</i>) Genotypes from Ethiopia Based On Qualitative Traits
5.	Assessment of Physio-Morphological Traits, Genetic Variability, And Growth Performance among Amaranth (<i>Amaranthus species</i>) Genotypes From Ethiopia
6.	Genetic Diversity and Population Structure in Ethiopian Amaranths (<i>Amaranthus species</i>) Genotypes as Revealed by Inter Simple Sequence Repeat (ISSR) Markers
7.	Summary, Conclusions and Recommendation

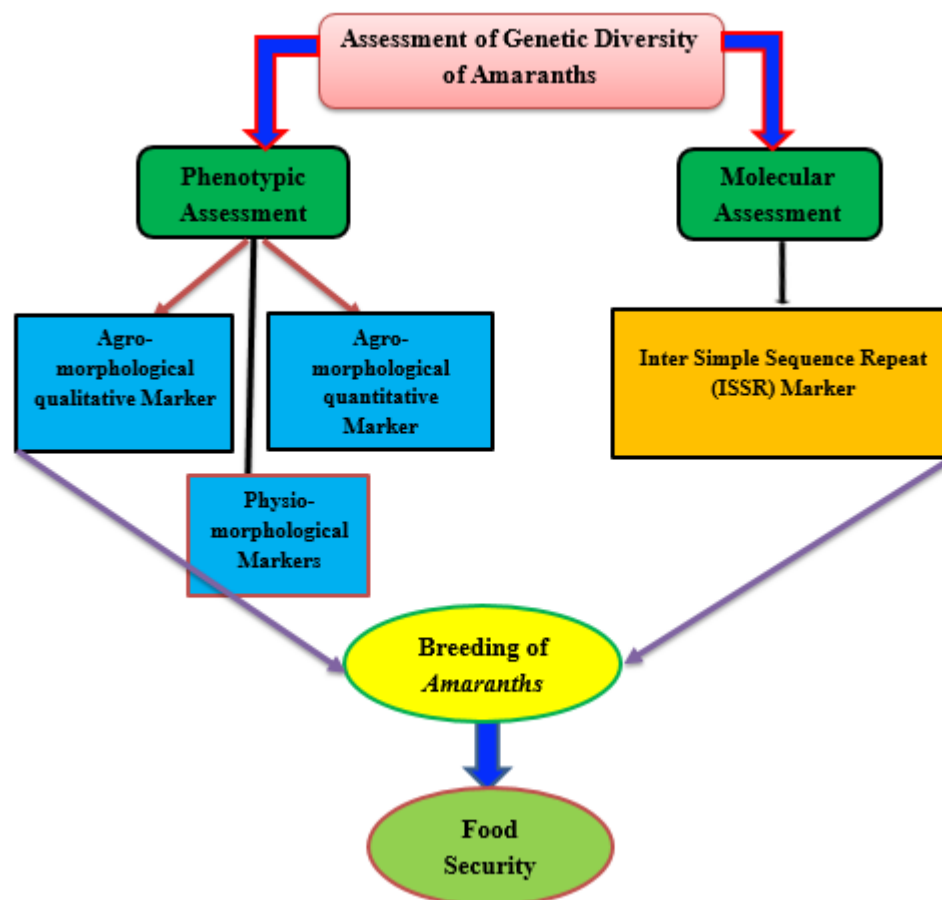


Figure 1.1 Conceptual framework of the study

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CHAPTER II: Literature Review

2.1 Literature Review

2.1.1 Origin and Distribution of Amaranths

Amaranthus L. (Amaranthaceae Juss.) is a cosmopolitan genus of broad-leaved, annual or short-lived perennial plants. It is a paleopolyploid, dicotyledonous plant, which is collectively known as amaranth. It is one of the few non-grasses pseudo-cereals that yield substantial amounts of edible small-seeded grain (Costea and DeMason, 2001; RHS, 2008; Achigan-Dako *et al.*, 2014; Clouse *et al.*, 2016; Das, 2016; Petruzzello, 2016; Peter and Gandhi, 2017; Thapa and Blair, 2018), comprised 65 genera and 850 species (Sreelathaknumary and Peter, 1993). According to reports, there are 87 species in the genus *Amaranthus* (Jacobsen *et al.*, 2000; Jacobsen and Mujica, 2003; Brenner *et al.*, 2010). Furthermore, of the 87 known species of amaranth, 17 are used for vegetable uses, while the other three species *A. caudatus*, *A. cruentus*, and *A. hypochondriacus*—are primarily used for grain (Ebert *et al.*, 2015). The remaining species are weeds that are extensively distributed. The Greek word "amárantos" is where the term "amaranth" comes from which means “unfading flower” (amár for unfading and ánthos for flower) (Liddell and Scott, 1940), “immortal”, “one that does not wither” or “never fading (Anjali *et al.*, 2013), everlasting or un-wilting (Kauffman, 1984; Rastogi and Shukla, 2013). It is one of the oldest known food crops, with evidence of cultivation in Puebla, Mexico, extending back more than 6700 years (Onyango, 2010; Muriuki *et al.*, 2014). There is no concrete evidence of the origin of *Amaranthus* (Rastogi and Shukla, 2013). However, it was reported to have originated both in Central and Latin America (Sauer, 1967), approximately 8000 years B.C. in Central and South America (Arreguez *et al.*, 2013; Rastogi and Shukla, 2013; Thapa and Blair, 2018), and has been domesticated and cultivated for more than 4000 years in Mesoamerica and the Andes mountain region, from where it probably spread to other parts of the world (He and Park, 2013; Oo and Park, 2013). In the Aztec pre-colonial period, *Amaranthus* was a significant plant, as it was central to the worship of gods. The cultivated amaranths are used for food grain, leafy vegetables, forage, ornamental gardening, and other potential uses. It was regarded as a minor grain plant in Russia and central Europe around 1700 AD, and by the early nineteenth century, it had spread to Africa and Asia. Up until the middle of the 1990s, South Asia was the only continent where *Amaranthus* production was rising (Brenner *et al.*, 2000). It is believed that *Amaranthus* is not a true cereal, like wheat, sorghum, millet, maize, or barley, but, somewhat, it is considered a “pseudo-cereal” like the

buckwheat crop (Mlakar *et al.*, 2009; Achigan-Dako *et al.*, 2014). Pseudo-cereal is one of the non-grass plant crops that have been used, like any other true cereals belonging to the grass family. Once a recent study found that grain amaranth protein was of high quality, research on this plant started in the US in the 1970s (Senft, 1980; Quershi *et al.*, 1996).

It is now widespread around the world in various parts of Europe, Asia, and Africa (Myers and Putnam, 1988; Assad *et al.*, 2017). During the 20th century, its production was reported in Asia, Africa, Europe, and North and South America (Sooby, 1998). It was largely ignored in the United States until the 1970s when amaranth grain protein was reported as high quality and could be used as a food ingredient in the American diet (Sooby, 1998). Amaranth is currently grown in both the Old and New Worlds and while its origin and domestication are still up for debate, several lines of evidence support it (Joshi and Rana, 1991). In the highlands of tropical and subtropical America, the three kinds of grain amaranth (*A. hypochondriacus*, *A. caudatus*, and *A. cruentus*) were domesticated in prehistoric times (Sauer, 1967; Joshi and Rana, 1991; Espitia-Rangel, 2018). Thus *Amaranthus* is a historic as well as a contemporary plant (Assad *et al.*, 2017). Legend and ritual were intertwined with amaranth. Aztec women pulverized the seed on different days of the holy calendar, combined it with human blood or honey, and molded it into the shapes of snakes, birds, mountains, deer, and gods that were either eaten at ceremonies, in the large temples or small family gatherings (Council, 1984; Tucker, 1986). These plants are the “holy grain” or “immortal”. They grew it as their basic food, and it was also a sacred plant used in ritual ceremonies (Pavlik, 2012).

Amaranth has long been a component of traditional African agriculture and is semi-domesticated, mostly as a vegetable, in Ethiopia and other East African countries (Alemayehu *et al.*, 2015). Amaranth is now grown as a grain crop in far-flung areas such as Ethiopia's mountains, South India's hills, Nepal's Himalayas, and Mongolia's plains, with exceptional seed quality and the highest potential for use as a food ingredient (Brink *et al.*, 2006; Sokolova *et al.*, 2021). However, only a small number of farmers have started cultivating amaranth grain seriously recently, to the point that they are supplying some millers and shops in Ethiopia, Kenya, Zimbabwe, and Uganda (Achigan-Dako *et al.*, 2014; Cernansky, 2015). The widespread usage of local names and the large genetic variation present in Ethiopia have also been suggested as a hub of amaranth diversity. Amaranthaceae is widely distributed all over the administrative regions of Ethiopia. Twenty

genera and 51 species of Amaranthaceae, several of which are highly variable are recognized in Ethiopia (Eshete *et al.*, 2016). From these species, *Amaranthus graecizans* has three subspecies; *Amaranthus hybridus*, *Amaranthus lividus* and *Digera muricata* have two subspecies each while *Cyathula prostrate* and *Achyranthes aspera* have two varieties each (Hedberg, 1996). In the Flora region of Ethiopia and Eritrea, in the genus *Amaranthus* eleven species have been identified (Demissew, 2010).

2.1.2 Taxonomic Classification

The genus *Amaranthus* L (Amaranthaceae Juss., Caryophyllales Perleb.) was first reported by Linnaeus (1797). The genus *Amaranthus* shows both cultivated and wild species. *Amaranthus* is a taxonomically complex genus with critical morphological similarities, phenotypic variability, and cross-breeding, small, hard-to-see diagnostic parts, a wide geographic distribution, and a significant number of hybrid forms that further confuse taxonomy (Iamónico, 2016a; Wolosik and Markowska, 2019; Aderibigbe *et al.*, 2022). As such, systematists have generally regarded it as a challenging genus, and its taxonomic determinations are frequently incorrect or controversial (Costea and DeMason, 2001; Brenner *et al.*, 2010; Myers, 2010; Iamónico, 2020; Taia *et al.*, 2020). Apart from the taxonomic problems, *Amaranthus*' nomenclature is extremely complex, particularly when it comes to name misinterpretations (Bayón, 2015; Iamónico, 2016b; Iamónico, 2020; Iamónico *et al.*, 2023), which cause, e.g., the use of different names for the same taxon (for example, *A. chlorostachys* Willd. = *A. patulus* Bertol. = *A. hybridus* L. (Iamónico, 2016b), or the use of a name for a wrong taxon (for example, *A. gracilis sensu auct. non desfontaines*, which is supposed to be referred to as *A. viridis* L. (Iamónico, 2016a), or the occurrence of ambiguous names (for example, *A. gangeticus* L (Iamónico, 2014). There is no comprehensive key to the world's *Amaranthus* species and specialists interpret the species' boundaries differently. Some commonly used taxonomy names are synonymous (Chan, 1996). Protein-rich seeds of a few species (*Amaranthus hypochondriacus* L., *Amaranthus caudatus* L., and *Amaranthus cruentus* L.) are consumed as pseudocereals; such species are known as grain amaranths (Das, 2012a).

To solve the taxonomic disputes and systematic ambiguities in amaranths, along with conventional morphological parameters, biochemical and molecular marker techniques have been successfully applied. In some cases, they were supplementing each other, deviating as well in some other instances (Das, 2012a). Phylogenetic relationships within *Amaranthus* have been the

subject of several investigations ([Jain et al., 1984](#); [Kulakow, 2018](#)) and recent studies have utilized molecular techniques. Many studies using biochemical ([Juan et al., 2007](#); [Das, 2012b](#)), and molecular markers ([Costea and DeMason, 2001](#); [Maughan et al., 2009](#)) were conducted to analyze the genetic variability and phylogenetic relationships among *Amaranthus species*.

2.1.3 Genetic Variability

The fundamental source of food, feed, shelter, medicines, and many other goods and methods that make life on Earth feasible and delightful is plant biodiversity ([Biodiversity, 1992](#); [Heywood and Watson, 1995](#)). The crop improvement program largely depends on the extent of variability present. A high genetic variability exists within *Amaranthus* which shows their potential for genetic improvement ([Espitia-Rangel, 2018](#)). Genetic variability is the genetic base for crop improvement ([Bhandari et al., 2017](#)). Analysis of genetic diversity and population structure has direct benefits in research related to evolution and population structure ([Chung et al., 2010](#)). In any breeding program, a wide genetic base is important since a narrow genetic base and low genetic diversity are detrimental to the breeding program ([Phillips and Wolfe, 2005](#)). Knowledge of genetic diversity and trait variations in populations is useful for developing ex-situ conservation strategies for plant genetic resources ([Bartish et al., 2000](#); [Finetto, 2000](#)). Plant phenotype is commonly used for estimating genetic diversity as it provides a simple way of quantifying genotypic variation. However, the efficiency of estimating genetic differences using morphological traits is largely limited by uncontrollable environmental factors and distinctness. Genetic diversity assessment helps in identification of diverse parental combination in breeding program to create segregating progenies with maximum genetic variability and also facilitates in introgression of desirable alleles from diverse germplasm into available genetic base ([Shukla et al., 2010](#)).

To attempt to solve global concerns affecting food security, sustainability, and climate change adaption, plant breeders employ a variety of genetic resources to develop new and improved crop cultivars. Genetic variation refers to the genetic differences between individuals for a particular trait, where these genetic variances are found in one or more DNA sequences, while genetic diversity refers to the range of genetic characteristics in a crop or species. Examining variations in an individual population's DNA sequence can be used to quantify genetic diversity ([Haun et al., 2011](#)). Phenotypic variation, or variations in observable traits within a population, is a result

of genetic diversity. Because plant genomes are dynamic and evolutionarily labile, plants undergo more frequent genetic and epigenetic modifications. These modifications account for a significant portion of genetic and phenotypic variability, even amongst cultivars within a species. Plants with a higher genetic diversity have an amazing capacity to adapt to sudden shifts in their surrounding (Raza *et al.*, 2019).

The main tool used by plant breeders to enhance plant traits is variability. Genetics, environment, and the interplay of genetic and environmental factors were the causes of population diversity. Genetic variables account for the variability exploited in plant breeding (Azrai *et al.*, 2018). A genetic variability is a numerical value that quantifies the difference in appearance caused by hereditary variables. Within a population, individual variance can be explained by genetic variability (Kristantini *et al.*, 2014). Plant breeding programs will be more successful because increasing trait genetic variability increases the possibility of a desired, excellent trait combination (Hapsari *et al.*, 2018). Commercialized crop cultivars now incorporate genetic variation more efficiently and precisely thanks to a variety of genomic tools and breeding techniques, but plant breeding is still a labor- and resource-intensive process (Vaughan *et al.*, 2007). A successful breeding program depends heavily on the type and degree of genetic diversity present in the genotypes since increased genetic variability would make it possible to pick genotypes with a better chance of success (Bhatia *et al.*, 2017). The success of the selection is often determined by heritability and genetic advance (Kumar *et al.*, 2013). Although, genetic diversity is crucial for continuous genetic improvement (Sandhu *et al.*, 2015). Yield and its component is a multi-genic trait, impacted by the contribution of many loci across the genome for various physiological, abiotic, and biotic stress tolerance factors that all interact overtime during the growing season to determine final yield (Das, 2016). Direct selection of yield-related traits, which are easier to precisely quantify than the yield itself, has also been an effective yield improvement technique (Samonte *et al.*, 1998; Kumar *et al.*, 2014). Thus, selection based on yield coupled with yield components can be efficient since yield-contributing traits are less complex in heredity and less impacted by the environment (Gatti *et al.*, 2005; Kumar *et al.*, 2014). The introduction of genetic variability, selection, and usage of variance found in selected genotypes are all components of the contemporary breeding program used to produce new breeding materials. Heritability and variance components are important parameters of interest to disclose the genetic expression

controlling the target trait. A genetic advance is also a potent instrument for looking for the first advance predicted by selection.

Variation arising from genotypic variances between individuals within a group is referred to as genotypic variability (Singh, 2001). Genetic variations of a particular trait are of interest to plant breeders since they have a remarkable impact on the performance of the crop. To develop breeding techniques for crop improvement in the future, studies on genetic variability provide fundamental knowledge about the genetic features of populations (Kristamtini *et al.*, 2014). The most significant components of genetic parameters are the genotypic coefficient of variation and the phenotypic coefficient of variation, which helps identify how much genetic diversity is there in the genotypes. A population benefits from genetic variation when it allows certain individuals to survive the population by adapting to the environment.

The magnitude of variability can be estimated by determining the phenotypic, genotypic, and environmental coefficient of variations (Singh and Singh, 1975). A high level of environmental variation would make it useless to select plants for a particular trait because it could have a significant impact on genetic variation. It is impossible to eliminate environmental variations because they encompass all non-genetic diversity, much of which is outside the scope of the investigation, even though some of them can be minimized by appropriate study (Falconer, 1996). The components of genetic parameters such as the genotypic coefficient of variation and phenotypic coefficient of variation are very important to detect the amount of genetic variation that exists in the genotypes; however, environmental factors are partially controllable through proper implementation of the research. Generally, the achievement of crop improvement through breeding programs largely relies on the extent of genetic and phenotypic variability existing among individuals in the population, and variability can also be created artificially through the hybridization technique by crossing genetically distant parents.

Various researchers have also reported that amaranth genotypes exhibit significant variability (Andini *et al.*, 2013b; Yadav *et al.*, 2014; Thapa and Blair, 2018; Agadi *et al.*, 2021; Oyetunde *et al.*, 2021; Delgado and Martín, 2022; Trivedi *et al.*, 2022; Tomar *et al.*, 2023; BT *et al.*, 2024; Prakash *et al.*, 2024). A high genetic variability exists within *Amaranthus* which shows their potential for genetic improvement (Espitia-Rangel, 2018). The evaluation of both genetic and environmental factors may be made more precisely and efficiently by studying the interaction

between genotype and environment. Stable genotypes are required for sustainable and reliable agriculture production (Kanfany *et al.*, 2021). Different research studies conducted in Ethiopia indicated that there is a considerable variation for most measured traits in amaranth genotypes (Ayalew, 2013; Hailu *et al.*, 2015; Yosef *et al.*, 2018).

2.1.4 Heritability and genetic advance

The value of selection for a specific characteristic depends mainly on its heritability since selection operates on genetic differences (Allard, 1960a). Heritability, a degree of the genetic link between parent and offspring, has been frequently used to determine how much a character may be passed down from one generation to the next. Estimates of heritability reveal the degree to which a trait is under genetic control, as well as the accuracy of phenotypic prediction of its breeding value (Ndukauba *et al.*, 2015). Understanding heritability is crucial because it aids breeders in determining how much improvement is feasible through selection (Robinson *et al.*, 1949; Ene *et al.*, 2016). The most important function of heritability in the genetic study of quantitative characters is its predictive role in indicating the reliability of the phenotypic value as a guide to breeding value (Hill and Mackay, 2004; Dabholkar, 2006). The genotypic coefficient of variation along with heritability estimates provides reliable estimates of the amount of genetic advance to be expected through phenotypic selection (Burton, 1952).

Heritability is the ratio between genetic and all factors (including non-genetic ones) that influence variability (Bernardo, 2002). Allard (1960b), defines heritability in a broad sense as the proportion of the total variability that is due to genetic causes, or the ratio of genetic variance to the phenotypic variance. The extent to which a phenotype is determined by its genetic makeup (genotype) is called heritability in the broad sense. Heritability, in its broadest definition, refers to the degree to which a phenotype is influenced by its genetic composition (genotype). The degree of similarity between relatives is determined by broad-sense heritability, which is the percentage of the overall variance attributable to the average effects of genes at the gene level. Narrow-sense heritability is the heredity represented as a percentage of an additive component of variation. Heritability will be higher in a progeny if there is a large genetic variation about environmental variation, and lower if there is a little genetic variation about environmental variation. Thus, the relative degree of genetic and environmental variation in the population can be determined by estimation of heritability in the broad sense (Marwede *et al.*, 2004; Raffi and

Nath, 2004). Heritability of a trait does not depend only on genetic factors, it also depends on the environmental effects to which an individual is subjected (Falconer *et al.*, 1981). The greater the proportion of the total variability that is due to the environment, the more difficult it would be to select for inherited differences due to the masking effect of the environment on the genotypic effects (Singh and Singh, 1975). On the other hand, if environmental variability is small about genotypic differences, the selection will be efficient because the selected character will be transmitted to its progeny due to a relatively small contribution of the environment to phenotype. However, the yield is greatly influenced by the environment, the heritability of yield itself is low (Hebblethwaite, 1983).

The amount of genetic advancement that would arise from choosing the best individuals is not indicated by heritability alone (Johnson *et al.*, 1955a). However, because high heritability does not always correspond with strong genetic advance, the estimation of heritability in conjunction with genetic advance can indicate the projected genetic gain in the following generation (Johnson *et al.*, 1955b; Amin *et al.*, 1992; Ramanjinappa *et al.*, 2011). The genetic advance would be higher with more genetic variance, yet high heritability values are possible with genotypes having low or high genetic variance (Johnson *et al.*, 1955a; Allard, 1960b). Heritability estimates together with genetic advances are essential to start an efficient breeding program because they give the amount of advance to be expected from selection (Atta *et al.*, 2008). Genetic advancement, which is the product of heritability, genetic variability, and selection intensity, can provide the estimate of the expected gain for a particular character (Allard, 1960b; Hamdi *et al.*, 2003). Genetic advance under selection measures the difference between the mean genotypic values of the selected population over the mean genotypic value of the original population (Allard, 1960b). High estimates of heritability with high genetic advance values can be used as an efficient indicator for phenotype-based selection (Johnson *et al.*, 1955a; Johnson *et al.*, 1955b; Allard, 1960b; Assefa and Labuschagne, 2004), suggested that heritability is not enough in predicting the effectiveness of selection unless otherwise it is considered along with genetic advance.

Heritability in a broad sense is not the true indicator of the inheritance of traits. Since only the additive component of genetic variance is efficiently transferred from generation to generation. Therefore, heritability in a broad sense may mislead in judging the effectiveness of selection for the trait. Considering heritability in a broad sense along with genetic advance may reveal the

prevalence of specific components (additive or non-additive) of genetic variance and thus help in judging the effectiveness of selection for the trait more accurately (Agadi *et al.*, 2021). High heritability accompanied by high genetic advance indicates the prevalence of additive gene effects, and hence, selection would be effective for such traits. High heritability along with high genetic advance in amaranths genotypes were recorded for plant height, days to 80 % maturity and inflorescence length (Yadav *et al.*, 2014); grain yield/plot, 1000 grain weight, petiole length, number of leaves, number of branches, leaf area and leaf width (Showemimo *et al.*, 2021); test weight of seed, plant height, leaf length, number of leaf per plant, leaf width, petiole length, number of branches per plant, dry matter percent, days of 50% flowering, yield in kg per plot (Arti Kujur *et al.*, 2017); leaves/plant (Shukla *et al.*, 2005); plant height at 90 DAS, stem diameter, number of branches per plant, number of inflorescence per plant, number of panicles per inflorescence, panicle length, panicle breadth, thousand seed weight and seed yield per plant (Malaghan *et al.*, 2018); stem girth (Venkatesh *et al.*, 2014), panicle length, panicle width and seed yield per plant (Sravanthi *et al.*, 2012; Selvan *et al.*, 2013; Yadav *et al.*, 2014), BT *et al.* (2024) herbage yield per plant (g); Sharma *et al.* (2024a) a number of branches/plant, leaf yield/plot, gross yield/plot and leaf yield/hectare; leaf length, leaf area, leaf area index, leaf area duration, specific leaf weight, foliage yield per plant, chlorophyll content, ascorbic acid and beta-carotene content (Agadi *et al.*, 2021), recorded high heritability with high genetic advance over mean indicates the predominance of additive gene action and amenable for phenotypic selection in early generations and which revealed that these traits might be under control of additive gene effects and therefore they are more reliable for effective selection. High heritability associated with equally high genetic advance is mainly due to additive gene effect, but if the heritability is due to dominance and epistasis, the genetic advance would be low (Panse, 1957).

Studying the phenotypic expression of the plant characters and yield is controlled by the genetic makeup of the plant and environment, which is composed of additive variance (heritable) and non-additive variance, including dominance and epistasis (non-allelic) interaction (Agadi *et al.*, 2021). Therefore, it becomes necessary to partition the observed phenotypic variability into its heritable and non-heritable components with suitable parameters such as genotypic and phenotypic coefficient of variation, heritability, and genetic advance. Further, genetic advance can be used to predict the efficiency of selection.

2.1.5 Amaranths Morphology

The *Amaranthus* genus is a globally distributed group of species exhibiting great morphological variation and widespread geographic distribution (Das, 2016). *Amaranthus* species show astounding morphological variety and wide-ranging adaptability to various eco-geographical environments (Lee *et al.*, 2008). The morphological evidence provides the basic information for the characterization of plants, identification, classification, and relationships. Radford (1986) and Okwuchukwu *et al.* (2022) showed morphological characteristics as vegetative, phenological floral characters, seeds, and fruit morphology, thus morphological features of plants are those external diagnostic features of the plant (Gasser and Skinner, 2019). The assessment of genetic diversity among populations from various phytogeography regions is crucial since a species' ability to survive, perpetuate, and continue to meet the demands of shifting environments depends largely on the quantity of variability present in its gene pool (Ray and Roy, 2009). Moreover, plant breeding uses the diversity present in a particular species to develop new varieties.

Amaranthus is characterized by the following traits: Annual or (rarely) short-lived perennial life history, herbaceous habit with prostrates to erect stem. Leaves are alternate, ovate to linear, and have an indented or notched apex and smooth margins. Flowers are imperfect, in compound dichasia packed into inflorescences. Amaranth is a beautiful crop with brilliantly colored leaves, stems, and flowers of purple, orange, red, and gold (Tucker, 1986). The plants of *Amaranthus* are monoecious (*A. albus*, *A. blitum*, *A. caudatus*, *A. hybridus*, *A. powellii*, *A. retroflexus* and *A. spinosus*) or dioecious (*A. tuberculatus*, *A. palmeri*, and *A. rudis*). The inflorescence is terminal and/or axillary with three to five tepals and stamens. Monoecious species are generally self-pollinated and wind-pollinated. Seeds are lenticular and tiny (0.9 to 1.7 mm diameter and 1000-seed weights from 0.6 to 1 g) that are typically dispersed by wind, water, or birds, having an extended period of germination with prolific seed production and a base chromosome number of 16 or 17 (Costea and DeMason, 2001; Steckel, 2007; Iamónico, 2020). Morphological terminology in *Amaranthus*, as used in different floristic and taxonomic treatments, is rather confusing, especially regarding the terms applied to flowers and inflorescence. Within each *Amaranthus* species, there are several races defined by their common branching pattern, height, inflorescence size and form, days to maturity, seed size and color, and other morphological characteristics (Brenner *et al.*, 2000; Espitia-Rangel, 2018).

Morphological traits were among the earliest markers used in the characterization of genetic resources, but they have several limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences. On the other hand, DNA marker techniques do not have such limitations. They can be used to detect variation at the DNA level and have been proven to be effective in distinguishing between closely related genotypes. Different types of molecular markers are in use today to assess the genetic diversity in crop species. Morphology contributed a significant part of characters used in taxonomic delimitation in the genus *Amaranthus*. Grain amaranths are characterized by apical, large to moderately large complex inflorescence comprising aggregates of cymes, flowers with 5 tepal lobes, 5 stamens, seeds with variable seed coat color (pale ivory, pinkish, brownish other than black and brownish black), and well-defined flange, utricle circumscissile (Das, 2012a).

Pre-breeding and crop improvement initiatives heavily rely on the morphological characterization of plant genetic resources for qualitative features, which is a fundamental and preliminary prerequisite for producing essential information on beneficial traits (Empilli et al., 2000). In the conservation of plant variety (PPV) systems, morphological characteristics are a crucial group of widely recognized descriptors for the varietal characterization of crop species and aid in determining the uniqueness of crop cultivars (Begum and Kumar, 2011). Qualitative variation is simple to categorize, study, and apply in breeding. It is simply inherited (regulated by one or a few genes) and can be studied using Mendelian genetics. The qualitative traits are the traditional Mendelian traits. A single gene with two or more alleles may be responsible for a particular qualitative characteristic, and there may be little to no environmental interference with the gene's effects. It is believed that the organisms that exhibit qualitative traits belong to distinct (independent) phenotypic classes and show discontinuous variations (Acquaah, 2009). Qualitative traits help characterize the germplasm of various species (Abe and Vegetable, 2015).

As an illustration, the majority of Africans favor eating vegetables with green leaves, while Chinese people prefer vegetables with red leaves. These determine the particular features that can be found in society and have an effect on natural selection, the socioeconomic environment, and consumer preferences (Akaneme and Ani, 2013). Even while qualitative characteristics are frequently independent of environmental factors, they can be used to reliably identify genotypes and possibly facilitate the elimination of duplicates and materials that are strikingly similar

(Esayas *et al.*, 2016; Merrick *et al.*, 2023). Furthermore, when the range of quantitative features is constrained, they help classify varieties (Ghafoor and Ahmad, 2003). The easiest characters, or traits, to deal with are those involving discontinuous, or qualitative, differences that are governed by one or a few major genes (Allard, 1960b). Many such inherited differences exist, and they frequently have profound effects on plant value and utilization. A qualitative trait is expressed qualitatively, which means that the phenotype falls into different categories. These categories do not necessarily have a certain order. The pattern of inheritance for a qualitative trait is typically monogenetic, which means that the trait is only influenced by a single gene. Qualitative plant characteristics such as leaf and stem color, leaf shape, plant habit, flower color, panicle color, and shape were studied in amaranths species by Gerrano *et al.* (2017); leaf color, inflorescence color, seed color, inflorescence spininess, and stem color by Nyasulu *et al.* (2021); revealed a wide range of variation for most of the characteristics studied. The seeds have colors ranging from pale yellow to black (due to their protein content), gold, coffee, and pink (Morales *et al.*, 1988; Paredes-Lopez, 2018). Betalains are present in leaf pigment (Schnetzler, 2018). The different patterns of pigmentation of the amaranth contribute to many practical and aesthetic uses (Paredes-Lopez, 2018), when correctly extracted, allowing it to be used as a natural dye (Schnetzler, 2018).

The genetic variation within and across populations of the amaranth species has been described by evolutionists and geneticists using quantitative morphological features (Shukla *et al.*, 2006; Andini *et al.*, 2013a; Gueco *et al.*, 2016; Molin and Nandula, 2017; Thapa and Blair, 2018). Agronomic and morphological characteristics were used in earlier times by Sammour *et al.* (2012) in a study of 229 genotypes from 20 *Amaranthus* species; Shankar *et al.* (2012) in the 28 amaranths germplasm; Umadevi *et al.* (2020), 125 accession of grain amaranths; Boro *et al.* (2024) 251 accessions of amaranths; Jacques *et al.* (2021) 80 accessions of amaranths; (Bao-Hua *et al.* (2000); Varalakshmi, 2004) different accession of amaranths showed wide variability which was useful in cultivar improvement for agronomic traits, such as plant height among genotypes within the same species and among different *Amaranthus* species. Rashmi Yadav *et al.* (2014), reported that the genotypic variability and character association in grain amaranth genotypes for ergonomically useful and yield contributing traits. Hamidzadeh Moghadam *et al.* (2021) observed variability among morphological traits of 16 *A. retroflexus* L and 17 *C. album* L populations. In general, several genetic diversity studies in amaranths have been carried out based on morphology (Andini *et al.*, 2013a; Hailu *et al.*, 2016; Omondi *et al.*, 2016; Gerrano *et al.*, 2017; Bojórquez-

Velázquez *et al.*, 2018; Thapa and Blair, 2018; Yosef *et al.*, 2018; Oduwaye *et al.*, 2019; Dharajiya *et al.*, 2021; Hamidzadeh Moghadam *et al.*, 2021; Rathod *et al.*, 2021; Showemimo *et al.*, 2021; Adhikari *et al.*, 2022; Delgado and Martín, 2022; Adedibu *et al.*, 2024; Boro *et al.*, 2024; Gomes *et al.*, 2024; Prakash *et al.*, 2024; Sahoo *et al.*, 2024; Sharma *et al.*, 2024a; Sharma *et al.*, 2024b).

2.1.6 Amaranths Physiology

Most terrestrial plants have C3-type photosynthesis, in which there is direct fixation of atmospheric CO₂ via ribulose biphosphate carboxylase oxygenase (Rubisco) in all chloroplast-containing leaf mesophyll (M) tissues (Voznesenskaya *et al.*, 2010). Only ~3% of terrestrial species are C4 plants in which atmospheric CO₂ is fixed initially in the C4 cycle by phosphoenolpyruvate carboxylase (PEPC). This leads to the formation of C4 acids which act as donors of CO₂ to Rubisco in bundle sheath (BS) cells. Through this CO₂-concentrating mechanism which represses photorespiration, C4 plants are recognized as having an increased capacity for carbon assimilation and higher efficiency in nitrogen and water use in warm climates. For this reason, there is interest in genetically modifying major C3 crops with C4 traits to reduce photorespiration, and in developing the use of C4 species for the production of biofuels (Samson *et al.*, 2005; King, 2010).

Amaranthus is a dicotyledonous, herbaceous mesophyte that performs photosynthesis using a specific C4 carbon-fixation pathway, with a four-carbon molecule serving as the first product (Wang *et al.*, 1993; Wang *et al.*, 1999; Meyers *et al.*, 2010). *Amaranthus* are one of the few C4 crop species that are not grasses. *Amaranthus* is a member of the NAD-malic enzyme-type C4 metabolism group and has leaves, cotyledons, and bracts that are characteristic of the Kranz anatomy (C4 anatomy) (Costea and FJ Tardif, 2003). Their photosynthetic pathway is characterized by the use of the mitochondrial NAD-ME to decarboxylate malate in the Kranz bundle sheaths (Long and Berry, 1996). *Amaranthus* tolerate adverse environmental conditions and adapt easily because they synthesize food through the C4 photosynthetic pathway. In the C4 pathway, plants efficiently utilize atmospheric CO₂ such that it is concentrated in the chloroplasts of specialized cells around the leaf epidermis. Unlike C3 plants, which yield 3-phosphoglyceric acid, a three-carbon molecule as the first product of carboxylation, C4 plants produce three C4 acids (oxaloacetate, aspartate, and malate) from the initial fixation of atmospheric carbon dioxide, a reaction facilitated by the enzyme called phosphoenolpyruvate carboxylase (Leegood and

Edwards, 1996; Gardner et al., 2017). The efficient utilization of water by C4 plants is another characteristic. Amaranthus and other C4 plants are promising crops for arid and semi-arid regions because they can maneuver low intercellular concentrations of carbon dioxide at much lower stomatal conductance, which allows them to reduce water loss and still photosynthesize at rates greater than or equivalent to their counterpart C3 plants (Leegood and Edwards, 1996).

The amalgamation of multiple structural attributes of Amaranthus, including C4 metabolism, a strong root system, stomatal conductance, and conservation of leaf area, ability to go dormant under extreme drought conditions (Spreeth et al., 2004; Granato et al., 2018). Moreover, ability to shut down transpiration through wilting and then recovering easily when moisture is available makes (Myers, 1996), it possible to use CO₂ more effectively in situations with higher light levels, moisture stress, and a wide temperature range (from 25 to 40 degrees Celsius) (Williams, 1995). Thus features which enables this plant to adapt under diverse geographic and environmental conditions (Kigel, 2018). Plants that use the C4 carbon-fixation pathway tend to require less water than the more common C3 carbon-fixation pathway plants (Das and Das, 2016). Also, the reduced stomatal opening reduces the water lost by transpiration. This is the reason that Amaranthus performs well under adverse temperature and moisture conditions as compared to many C3 plants such as wheat and soybeans (Spreeth et al., 2004; Nuugulu, 2013), indicating that it is possible to cultivate Amaranthus in places unsuitable for other crops (Rana et al., 2005; Brenner et al., 2010).

2.1.7 Molecular Markers

To gain a deeper comprehension of crop evolution in Amaranthus, more research is required on genetic diversity and phylogenetic links between cultivated and wild species. Furthermore, molecular characterization of germplasm helps plant breeders choose suitable resources for additional cultivar genetic development. Molecular markers comprise macromolecules like proteins and DNA as well as biochemical components like secondary metabolites in plants. However, only plants that produce a suitable spectrum of metabolites that are easily examined and distinguishable by variety are eligible for secondary metabolite analysis (Joshi et al., 2011). In principle, these metabolites—which are being employed as markers—should be insensitive to the impacts of the environment or management techniques. As a result, DNA markers are more widely utilized and appropriate among molecular markers for the majority of living things.

Molecular markers are now essential tools for accurately and reproducibly characterizing plant genotypes and elucidating genetic variability (Parita *et al.*, 2018). Plant breeders would be able to distinguish between genotypes through molecular phylogenetic investigations, which estimate the degree of diversity within and across species and offer a foundation for suitable breeding techniques. Molecular marker-based approaches that are well-established, like random amplified polymorphic DNAs (RAPDs) (Snezana *et al.*, 2012; Anjali *et al.*, 2013; Akin-Idowu *et al.*, 2016; Idehen *et al.*, 2018; Oduwaye *et al.*, 2019), restriction fragment length polymorphisms (RFLPs) (Park and Nishikawa, 2012; Oduwaye *et al.*, 2014; Park *et al.*, 2014), amplified fragment length polymorphisms (AFLPs) (Wassom and Tranel, 2005; Chandi *et al.*, 2013), simple sequence repeats (SSRs) (Nguyen *et al.*, 2019; Vats *et al.*, 2023), and single nucleotide polymorphisms (SNPs) (Wu and Blair, 2017; Hoshikawa *et al.*, 2023), and have been used in genetic diversity studies in different *Amaranthus species*. The degree of polymorphism, genomic abundance, repeatability, locus specificity, dominance or codominance, technological preferences, and financial investment of these genetic markers differ (Omondi *et al.*, 2016).

Inter Simple Sequence Repeat (ISSR) markers have many advantages over other marker systems; the ISSR technique is simple, quick, and less costly than the RAPD technique. ISSR markers have higher reproducibility than RAPD markers due to longer primer lengths, and also do not require prior knowledge of the genome to be analyzed; hence, they can be used universally for plant genome analysis. Based on ISSR molecular markers, various studies on genetic diversity in amaranths are usually conducted on amaranths ((Gelotar *et al.*, 2019; Oduwaye *et al.*, 2019; Rathod *et al.*, 2021; Delgado and Martín, 2022; Hamidzadeh Moghadam *et al.*, 2023).

2.1.8 Uses and Nutritive Value of Amaranths

Amaranths are a very nutrient-dense food source. Exploring the potential for newer plant resources, new crops, and new uses of old ones that hold promise to restore the balance of trade and meet the growing needs of food, clothing, and industrial products for the human population became necessary due to the world's population growth and the rapidly diminishing natural resources. Amaranths, an undervalued crop that is predicted to be a major crop in the twenty-first century, are good possibilities. Amaranth is a pseudo-cereal that has substantially higher nutritional value than cereals (Das, 2016). Amaranth (*Amaranthus species*) has been eaten

throughout history and has been a staple food for the Inca, Maya, and Aztec civilizations since pre-Columbian times for millennia ([Mendonça et al., 2009](#)). Amaranth first attracted attention in the 1980s when the US National Academy of Sciences conducted a study on the grain and noted its excellent nutritional content and agronomic promise ([Monteros et al., 1994](#); [Ulbricht et al., 2009](#)). It is established in several studies that all parts of *Amaranthus species* are edible ([Achigan-Dako et al., 2014](#); [Alemayehu et al., 2015](#)). Holistically, the plant *Amaranthus* is a rich source of nutraceuticals, which are yet to be exploited to their maximum benefits.

Africa has experienced mixed progress in reducing food insecurity and child malnutrition in the past 20 years ([Garcia, 2012](#)). Approximately, one-third of children under 5 years of age in Africa are stunted and more than a quarter are underweight. Amaranth is highly nutritious, with low fat and high protein contents, along with other nutrients such as fiber, vitamins, and minerals ([Schmidt et al., 2023](#)). Proteins are found mainly in amaranth seeds, with a high content of lysine and methionine. The protein content of amaranth seeds (about 14%) is higher than that of other grains with a balanced distribution and high bioavailability of amino acids. Moreover, the proportion of essential amino acids in amaranth protein is similar to the requirements recommended by the World Health Organization (WHO), which is close to the quality of animal protein, making amaranth protein better than ordinary grains ([Rivero Meza et al., 2023](#)). Therefore, it can be determined that amaranth protein has high biological and nutritional value ([Manassero et al., 2020](#)). Differences between the subspecies of amaranth plants, climatic conditions, soil composition, and fertilization methods may result in slight differences in protein profiles ([Baraniak and Kania-Dobrowolska, 2022](#)). Despite this, amaranth protein contains amino acids essential for normal human growth, including lysine, tryptophan, and small amounts of other amino acids such as threonine, leucine, valine, and isoleucine ([Reyad-ul-Ferdous et al., 2015](#); [Schmidt et al., 2023](#)). Additionally, amaranth seeds contain carbohydrates primarily in the form of starch with a content of about 48% to 69% (mainly in the form of amylopectin) ([Coelho et al., 2018](#)), out of a total polysaccharide content of 50 to 60% ([Schmidt et al., 2023](#)). The plant's green sections are high in protein, vitamins A and C, and minerals like potassium, iron, magnesium, and calcium. Its leaves are also eaten raw or cooked; they resemble spinach in terms of texture, color, and nutritional value ([Paredes-Lopez, 2018](#)). Amaranth grains are a rich source of minerals such as copper, sodium, as well as zinc. It also contains vitamins such as, riboflavin, and niacin ([Palombini et al., 2013](#); [Joshi et al., 2018](#)). Regular consumption of amaranth seed or seed oil

provides a significant amount of vitamin E and squalene, which benefit people suffering from hypertension or cardiovascular disease by lowering blood pressure and cholesterol levels and improving antioxidant status (Martirosyan *et al.*, 2007).

Amaranthus has been regarded as a superfood as well. Since they are rich sources of micronutrients—important vitamins and minerals—which are necessary for maximum well-being, this plant is promising and distinctive and deserves more investigation for its great intrinsic, essential elements. Amaranth betalains natural pigments contain antibacterial, anti-inflammatory, anti-carcinogenic, and antioxidant qualities in addition to being utilized as dyes. It has been demonstrated to lower the chance of developing cardiovascular disease as well as other aging-related illnesses (Kanner *et al.*, 2001; Cai YiZhong *et al.*, 2003). Likewise, the grain has attracted attention for having high protein content, complete essential amino acids, and a significant amount of lysine that sets it apart from other grains. It is also well-known for having high-quality vitamins (A, K, B6, C, E, and B), fiber, oil, starch, and important minerals (iron, calcium, etc.) (Peter and Gandhi, 2017). Because it contains a high concentration of high-quality protein and unsaturated fatty acids and is free of gluten, it also offers benefits that might be investigated as a dietary supplement. Table 2.1 gives an overview of the nutritional value of other cereals and grain amaranths in comparison.

Table 2.1 Comparative account of nutritive value of grain amaranths and other cereals

No	Crops	Proteins	Fat	Carbohydrates	Ca	Fe	P	Food Energy (Kca/g)
1	Amaranth	16.00	3.1	60.00	0.49	17.5	0.60	391
2	Rye	12.1	1.7	73.4	0.38	10.5	0.37	334
3	Buckwheat	11.7	2.4	72.9	0.12	15.5	0.28	335
4	Chenopod	12.0	5.0	68.0	0.20	12.6	0.50	342
5	Wheat	13.3	2.0	71.0	0.41	10.5	0.37	333
6	Maize	9.2	3.9	73.7	0.20	3.50	0.25	355
7	Rice	7.00	1.0	78.0	0.20	3.50	0.18	345

Source: (Narwade and Pinto, 2018).

Amaranth grains can be malted to produce beer or boiled and consumed as porridge in a family setting (Mugalavai, 2013). Grain amaranth is capable of being popped or roasted to produce flour. This flour can then be combined with other food ingredients or used on its own to create a variety of goods, including morning cereal, bread, biscuits, spaghetti, crackers, pancakes, and muffins (Emire and Arega, 2012). In Ethiopia, pulverized seeds are combined with *Eragrostis tef* flour to

make a pancake-like bread called Injera (in Amharic), or cooked seeds are added to porridge. In the farm fields of Ethiopia districts such as Konso, Ari, and Derashe, the plant is semi-domesticated and quite frequent. On their agricultural areas close to their homestead, a few farmers have begun to cultivate and intercrop the species. The name of amaranth varies throughout Ethiopia; the Konso people refer to it as Passa. It is also known as Iyaso and Jolili in Oromo, Zapina in Arigna, Gegabsa in Wolayita, Lishalisho, Aluma, and Ferenjitef in Amharic ([Getahun, 1976](#)). Amaranth is used by the locals in Guraferda and Jimma to produce alcoholic beverages like "Borde," unleavened bread called "Kita," and a thin porridge known as "Atmit" for newborns and expectant women. Particularly in the African context, simple-to-grow nutrient-rich plants like amaranth can aid in enhancing food security and lowering malnutrition in communities that primarily rely on subsistence agriculture. This is why turning amaranth into value-added food items is a means of rewarding society for its rich nutrients—value-added food products made from underutilized raw materials ([Addis et al., 2013](#)).

The active components of amaranth originate from its leaves and function as phytochemical substances ([Maseko et al., 2017](#); [Qumbisa et al., 2020](#)). Tannins, saponins, phenols, flavonoids, cardiac glycosides, steroids, and triterpenoids are some of these active ingredients ([Reyad-ul-Ferdous et al., 2015](#)). These substances have antiviral, antipyretic, anti-inflammatory, anti-hepatotoxic, anti-ulcer, and anti-allergic properties ([Reyad-ul-Ferdous et al., 2015](#)). In traditional medicines, it is used to reduce labor pain ([Reyad-ul-Ferdous et al., 2015](#)). *Amaranthus* leaves and root paste are applied topically to treat burns, rashes, dermatitis, and psoriasis in Spain ([Khongsai et al., 2011](#)). In addition, it treats intestinal worms, stomach ulcers, and illnesses of the urinary tract. It is also used as a laxative to help with digestive disease. Additionally, it works well for treating respiratory issues including asthma and eye infections ([Reyad-ul-Ferdous et al., 2015](#)). Amaranth leaves are utilized as a preventive meal because of their powerful antioxidant and phytochemical components, which have therapeutic qualities ([Achigan-Dako et al., 2014](#); [Njonje, 2015](#); [Maseko et al., 2017](#)).

Patients with jaundice, rheumatic discomfort, or stomach disease can find remarkable relief if they consume cooked leaves. When applied both inside and externally, root paste has additional health benefits. Amaranth root paste reduces vomiting, making it beneficial for patients with diarrhea; rabies sufferers are administered a paste with black pepper ([Nawaz et al., 2009](#)). You can directly

eat its seeds to stop diarrhea, heavy menstruation, and internal bleeding. It can also be applied externally as a poultice for fractures. Additionally beneficial for treating piles, snake bites, and cholera is the entire amaranth plant (Sebastian and Bhandari, 1984). The roots of *Amaranthus* can be boiled with honey and used as a laxative for infants. In Ghana, the water of macerated *Amaranthus* plants has been used as a wash to treat pains in the limbs. In African countries, amaranth is an important nutritious food in regards to treating those suffering from HIV/AIDS (Alegbejo, 2013). Similarly, in Ethiopia, *A. cruentus* is used as a worm expellent. Moreover, in Sudan, the ash from the stems is used for wound dressing. The heated leaves are used in the treatment of tumors. Furthermore, *A. tricolor* and *A. caudatus* are used externally to treat inflammations and internally as a diuretic diet (Soriano-García and Aguirre-Díaz, 2019). Moreover, the seeds of *A. spinous* are used as a poultice for broken bones. It is used internally to treat internal bleeding, diarrhea, and excessive menstruation (Ganjare and Raut, 2019; Jimoh *et al.*, 2019). In Southeast Asia, a decoction of *Amaranthus* root is used to treat gonorrhea. It is also applied as an emmenagogue and antipyretic (Baral *et al.*, 2011; Achigan-Dako *et al.*, 2014). Among the Nepalese and some tribes in India, *Amaranthus spinous* is used to induce abortion (Peter and Gandhi, 2017). Countries across the world consider the bruised leaves as a good emollient (Soriano-García and Aguirre-Díaz, 2019). *Amaranthus* is a source of various phenolic phytochemicals, including rutin, isoquercitrin, and nicotiflorins, to mention a few. It has been frequently used in industry and people's everyday eating. Furthermore, these phytochemicals can be used in a number of medical specialties, either as illness preventives or treatments (Joshi and Verma, 2020). Fascinatingly, *Amaranthus* is included in the group of medicinal plants that have been shown to have significant promise in the treatment of a number of ailments. Amaranth oil helps lower high blood pressure, cholesterol, and antioxidant levels in the body and immune system, making it advantageous for people with cardiovascular disorders and hypertension (Joshi and Verma, 2020). *Amaranthus* has antiallergic, anticancer, antihypertensive and antioxidant properties (Conforti *et al.*, 2005; Caselato-Sousa and Amaya-Farfán, 2012) and protects against several disorders such as bleeding tendencies, brain stroke, celiac disease, defective vision, diabetes, digestion disorder, functional sterility, haemorrhage, heart diseases, HIV/AIDS, hypertension, kwashiorkor, leucorrhoea, liver disease, marasmus, premature ageing, recurrent colds, respiratory infections, retarded growth, skin diseases, TB and wound healing (Thompson, 2001).

2.1.9 Breeding of Amaranths

The dramatic fall in amaranth farming in the Americas following the arrival of the Spanish is one of the reasons why there hasn't been any amaranth breeding done until lately. To feed the world's population, high-yielding varieties of staple cereals—mostly maize, wheat, and rice—were developed as a result of significant investments made in agricultural genetic modification during the Green Revolution (1960s–1980s). Though these crops are a significant source of calories for human consumption, their protein (especially amino acid content), vitamin, and micronutrient levels are rather low. Furthermore, the global agricultural system is now more vulnerable and agrobiodiversity has been drastically reduced as a result of the widespread production of these staple crops. Typically, orphan crops are traditional crops that are farmed in subsistence farming systems, traded, and eaten. Despite not being traded internationally, they can have a significant impact on the economy and diets of low-income communities throughout the developing globe. Crucially, they are included throughout regional diets. Orphan crops are frequently specially adapted to their local settings with higher nutritional value compared to more regularly farmed grains, vegetables, and fruits, however, research and advancements in orphan crops have lagged significantly behind that of staple crops (Jamnadass *et al.*, 2020). The monoecious plant's inflorescence shape presents a challenge in amaranth breeding. Due to its many intricate blossoms and mostly self-pollinating nature, amaranth makes crosses more challenging than other crops (Sauer, 1967). Crosses that work well are crucial for crop breeding and an important tool for plant research. Large-scale, low-effort crossbreeding is essential to the creation and growth of hybrid agricultural types.

The breeding systems of *Amaranthus species* are complex because they are influenced by both genetic and environmental variation. (Jain *et al.*, 1982; Hauptli and Jain, 1985). The mean outcrossing rates under field conditions have been variously estimated at 3.5 to 34% (Jain *et al.*, 1982; Hauptli and Jain, 1985; Espitia-Rangel, 2018), for the grain species. Substantially lower outcrossing is expected for vegetable species such as *A. lividus* and *A. tricolor* (Pal and Khoshoo, 1973). Some wild *Amaranthus species* are dioecious and, therefore, must outcross (Sauer, 1950; Lemen, 1980). The variation in outcrossing rates can be partly explained by variation in the ratios of staminate to pistillate flowers. Insects may be important pollinators and could also account for some variability in outcrossing rates (Hauptli and Jain, 1985). In amaranth breeding the following

factors are taken into account, high productivity of leaf, large flower head, seed color, stem height, low seed shattering, and satisfactory nutritive and utilization properties (Svirskis, 2003).

To optimize breeding gain from a selection, one must comprehend the relationship between growth and physiological, developmental, and yield-related variables as well as other contributing features. When genotype performance is subject to environmental fluctuations, the additive vigor indices provide a dependable means of evaluation (Reddy *et al.*, 2021). A successful breeding program depends heavily on the type and degree of genetic diversity present in the genotypes since increased genetic variability would make it possible to pick genotypes with a better chance of success (Bhatia *et al.*, 2017). The success of the selection is often determined by heritability and genetic advance (Kumar *et al.*, 2013). Although genetic diversity is crucial for continuous genetic improvement (Sandhu *et al.*, 2015). Yield and its component is a multi-genic trait, impacted by the contribution of many loci across the genome for various physiological, abiotic, and biotic stress tolerance factors that all interact overtime during the growing season to determine final yield (Das, 2016). Direct selection of yield-related traits, which are easier to precisely quantify than the yield itself, has also been an effective yield improvement technique (Samonte *et al.*, 1998; Kumar *et al.*, 2014). Thus, selection based on yield coupled with yield components can be efficient since yield-contributing traits are less complex in heredity and less impacted by the environment (Gatti *et al.*, 2005; Kumar *et al.*, 2014). The methods for generating or restoring variety in the current germplasm are polyploidy breeding, plant hybridization, and mutation.

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CHAPTER III: Agro-Morphological Characterization, Variability, and Diversity among Amaranth Genotypes from Ethiopia

Agro-Morphological Characterization, Variability, and Diversity among Amaranth Genotypes from Ethiopia

Abstract

Amaranth is a versatile plant with the potential for high yields. They have great photosynthetic performance as a result of eliminating the rival photorespiration mechanism. Selection based on yield alone may not be effective for yield improvement in plant breeding programs. So yield should be considered along with potential yield contributing traits to progress the genetic gains during selection. This increases the advantages of expanding the adaptation and cultivation of amaranth in Ethiopia. The objectives of the current study were to estimate the genetic diversity in Ethiopian amaranth genotypes based on quantitative agro-morphological traits. One hundred twenty amaranth genotypes were assessed over two years using an alpha lattice design with two replications. The analysis of variance indicated that the mean square due to year and genotype-by-year interaction varied significantly for most measured traits. The estimates of variability, heritability, and genetic advance found in this study indicate high genetic diversity in amaranth genotypes and the strength of selection response for these traits in the population. The findings showed that very high to moderately high heritability, high to moderate genetic advance, and genetic variability were observed for the traits basal lateral branch length, axillary inflorescence length, leaf area, branch number, plant height at flowering, plant height at maturity, stem diameter, days to flowering, grain filling periods, and leaf width. Furthermore, the potential for amaranth improvement through appropriate selection is revealed by the existence of significant differences between the number of superior and inferior genotypes for the majority of examined traits. This suggests that these traits are governed more by additive gene action and that selection based on these traits might be successful in achieving the desired genetic gains for improvement. The results revealed significant positive phenotypic and genotypic associations on leaf yield, with leaf area, leaf breadth, branch number, leaf number, plant height at flowering, and grain yield all having positive direct effects. Similar strong positive phenotypic and genotypic relationships were found for grain yield and grain sinking and filling rates. Using path coefficient analysis, the direct and indirect effects of yield-related traits on yield were also determined. It was further suggested that leaf yield in amaranth genotypes may increase through the indirect selection of plant height at maturity, leaf length, and terminal inflorescence lateral length, which showed such significant indirect influences, mostly through leaf area, and days to maturity, which displayed such strong indirect effects, primarily through plant height at flowering. This study consequently shows the need for traits with significant positive indirect impacts via leaf area to be considered indirect selection criteria for improving leaf yield in amaranth genotypes. The grain sink filling rate also significantly improved grain yield indirectly at both the phenotypic and genotypic levels, mainly via days to flowering and leaf yield. This demonstrated that selection that mainly targeted days to flowering, leaf yield, and grain sink filling rate would ultimately boost the grain yield in amaranth genotypes. Principal component analysis showed that the first six principal components with eigenvalues greater than one contributed 80.41% of the variability. However, the first two principal components explained 52.42% of the total variation. Additionally, the hierarchical clustering grouped all the genotypes into five clusters. The pairwise generalized squared distance (D^2) among the five clusters based on Mahalanobis's D^2 statistics revealed the maximum and highly significant genetic distance was observed between II and III (277.79), while the minimum inter-cluster distance observed between clusters I and II (39.50). The findings suggest that amaranth genotypes in Ethiopia have high genetic variation, which might be used for future breeding and ought to be conserved.

Keywords: genetic diversity, heritability, multivariate, associations, and path coefficient analysis

3.1 Introduction

The genus *Amaranthus* (L.) belongs to the order Caryophyllales, which includes quinoa, spinach, and beetroot (D'Amico and Schoenlechner, 2017). Amaranth is a mesophyte (Assad *et al.*, 2017; Stetter and Schmid, 2017), a dicotyledonous plant; the majority of the species in the genus *Amaranthus* currently inherit their chromosomes as diploids due to an old allotetraploidization process (Clouse *et al.*, 2016). Some species, on the other hand, are tetraploid due to further genome duplication (Sahoo *et al.*, 2024). There are no systematic studies on the evolution of genome size in the *Amaranthus* genus. Amaranth is a herbaceous plant or shrub that is found all over the world and is either annual or perennial (Stevens, 2012). According to reports, there are 87 species in the genus *Amaranthus* (Jacobsen *et al.*, 2000). It is now referred to be a third-millennium crop plant (Rastogi and Shukla, 2013). The genus encompasses both cultivated and wild species, according to historical records. The Mayan civilization of South and Central America was the first to domesticate and cultivate amaranths some 8000 years ago (Thapa and Blair, 2018; Ruth *et al.*, 2021). They are one of the few non-kinds of grass that produce considerable amounts of small-seeded grain (Santra and Schoenlechner, 2017).

Amaranth has long been a component of traditional African agriculture and is semi-domesticated, mostly as a vegetable, in Ethiopia and other East African countries (Alemayehu *et al.*, 2015). Amaranth is now grown as a grain crop in far-flung areas such as Ethiopia's mountains, South India's hills, Nepal's Himalayas, and Mongolia's plains, with exceptional seed quality and the highest potential for use as a food ingredient (Brink *et al.*, 2006; Sokolova *et al.*, 2021). The widespread usage of local names and the large genetic variation present in Ethiopia have also been suggested as a hub of amaranth diversity. In the Flora region of Ethiopia and Eritrea, eleven species have been identified (Demissew, 2010). Ethiopia is endowed with a wide range of food crops, the majority of which have received little to no attention in terms of research and the creation of regulatory frameworks that can encourage efficient commercial and industrial exploitation. One such underappreciated and neglected but double-duty species is amaranth.

The morphological diversity of amaranths is remarkable, as is their adaptation to a wide range of eco-geographical conditions (Lee *et al.*, 2008), elevation is not a severe limitation (Council, 2006; D'Amico and Schoenlechner, 2017), has a high tolerance to arid conditions, poor soils, withstand heat, drought, and pests, and they easily acclimate to new habitats, including ones that are

unfavorable to conventional cereal crops (Yadav *et al.*, 2014). Because of these benefits amaranth is cultivated in highly diverse areas, such as tropical lowlands and mountainous regions up to 3500 meters altitude (D'Amico and Schoenlechner, 2017). Despite the enormous morphological variability between and within *Amaranthus* species, there are only a few taxonomic traits that are unique to the genus (Juan *et al.*, 2007; Wolosik and Markowska, 2019; Aderibigbe *et al.*, 2022). The determination of genetic diversity relies heavily on agro morphological genotype characterization. As a result, it is required for the successful conservation of amaranth biodiversity and crop enhancement programs (Shah *et al.*, 2018).

Amaranth is a high-yielding, climate-smart, nutrient-rich plant that is essential for meeting rising world demand while also reducing dependency on a few cereal crops. Ethiopians may not be aware of the crop's production or use (Yosef Alemu *et al.*, 2018). This could be due to insufficient use, a dearth of better varieties, inadequate agronomic practices, or a lack of understanding of the crop's significance. Nonetheless, it is exclusively popular and consumed in a few parts of Ethiopia. Amaranth, an easy-to-grow nutrient-rich vegetable, has been recommended for enhancing food security and lowering malnutrition in populations in Ethiopia that depend on subsistence farming (Alemayehu *et al.*, 2015). This genus contains a large number of edible plants, some of which are grown primarily for their leaves. In Ethiopia, two evaluated and released varieties are most frequently utilized (AC-NL and Madiira II).

The amaranth plant not only thrives in a variety of climates (Lakshmi and Vimala, 2000), but it is also one of the few exceptionally nutritionally multi-purpose crops (Ruth *et al.*, 2021). It is used as a vegetable, cereal, medicinal plant, dye plant, forage plant, ornamental plant, as well as a fuel source (Mlakar *et al.*, 2009; Sheikh and Singh, 2013). As a result, it's critical to carefully consider the available germplasm and select improved genotypes with great potential for leaf and grain yield. Moreover, studying the phenotypic expression of the plant characters and yield is controlled by the genetic makeup of the plant and environment, which is composed of additive variance (heritable) and non-additive variance, including dominance and epistasis (non-allelic) interaction (Agadi *et al.*, 2021). Therefore, it becomes necessary to partition the observed phenotypic variability into its heritable and non-heritable components with suitable parameters such as genotypic and phenotypic coefficient of variation, heritability, and genetic advance. Further, genetic advances can be used to predict the efficiency of selection.

The complex variable of yield is influenced by many different variables, notably polygenes, the environment, and genetic variability (Wattoo *et al.*, 2010; Usman *et al.*, 2017). Understanding the relationships between quantitatively inherited crop traits and their direct and indirect effects on yield is crucial for the success of breeding program selections (Binodh *et al.*, 2008). Moreover, studies on the genotypic and phenotypic correlations among crop plant traits are useful for planning, assessing, and creating selection criteria for the targeted characters for selection in the breeding program (Tarekegne, 2001; Agadi *et al.*, 2021). The strength of character association has a significant impact on the effectiveness of any crop development effort.

Path coefficient analysis is an efficient statistical technique specially designed to quantify the interrelationship of different components, and their direct and indirect effects on yield. Partitioning of total correlation into direct and indirect effects by path coefficient analysis helps in making the selection more effective (Verty *et al.*, 2017). Keeping these facts under consideration, a planned effort was undertaken to evaluate different genotypes of amaranth under the agro-climatic conditions of Ethiopia. The selection of better genotypes for breeding programs depends on this information. There hasn't been a remarkable study conducted in Ethiopia to date that evaluates the relationships between yield components and plant characteristics; grain and leaf yield, as well as the direct and indirect effects of these relationships on grain and leaf yield in different amaranth genotypes.

Various crop species, such as the *Arachis* and *Amaranthus* genotypes, have had quantitative variations classified using multivariate statistical methods (Chandran and Pandya, 2000; Ahammed *et al.*, 2013b; Dhakal *et al.*, 2020; Nyasulu *et al.*, 2021). Although studies on the morphological variety of *Amaranthus* (Shah *et al.*, 2018), and quinoa (El-Harty *et al.*, 2021) have been conducted, in-depth agronomic suggestions for yield and yield-related improvement are uncommon. To effectively manage genotypes and make proper use of them in breeding programs, the current study was conducted with the following aims in mind: To optimize yield, it is crucial to assess genetic diversity using multivariate analysis. To enhance leaf and grain yields through selection and subsequent breeding programs, this study set out to evaluate the genetic diversity in amaranth genotypes. The amaranth genus, according to several studies, has high morphological diversity (Srivastava and Mahavidyalaya, 2015; Molin and Nandula, 2017; Oyetunde *et al.*, 2021).

The introduction of genetic variability, selection, and usage of variance found in selected genotypes are all components of the contemporary breeding program used to produce new breeding materials. Heritability and variance components are important parameters of interest to disclose the genetic expression controlling the target trait. A genetic advance is also a potent instrument for looking for the first advance predicted by selection. To take advantage of the existing variations in amaranth genotypes, determining the extent of genetic diversity using markers of agro-morphological traits is crucial. Historic and less expensive morphological markers, which are the most straightforward measures of phenotypes, are frequently used to evaluate the extent of genetic diversity in populations ([Jifar Daba, 2018](#); [Samarina *et al.*, 2022](#)). Therefore, this study was done to i) quantify the extent of genetic variability, heritability, and expected genetic gain in amaranth genotypes using markers of agro-morphological traits ii) to suggest an effective selection scheme and specify promising genotypes for future amaranth breeding programs iii) quantify the magnitude of the correlation coefficient and path coefficients between traits of amaranth genotypes grown in Ethiopia iv) put out the selection criteria for boosting amaranth genotypes' grain and leaf yields v) evaluate the genetic variation using cluster analysis, genetic divergence, and principal component analysis in amaranth genotypes for leaf and grain yields enhancement in Ethiopia.

3.2 Materials and Methods

3.2.1 Experimental site

In the years 2020 and 2021, the experiment was carried out at Hawassa University, which is located at the agricultural experimental site. Geographically, the location is in Ethiopia's Sidama region, around 275 kilometers from the capital, Addis Ababa. The experimental area is located at latitude 7°2' 54.7503"N and longitude 38°30' 17.1608" E, at an elevation of 1709 meters above sea level. The soil texture class in the experimental area was clay loam, and the pH ranged from 6.0 to 6.5. The district's mean monthly minimum and maximum temperatures are 14.10 °C and 27.9 °C, respectively. For two growing seasons, the experimental farm received an average of 1379.16 mm of rain throughout the growing seasons.

3.2.2 Plant materials

A total of 120 amaranth genotypes were used in this study (Table 3.1), 34 from the Ethiopian Biodiversity Institute (EBI), 2 from the Melkasa Agricultural Research Center, 15 from the Werere Agricultural Research Center (Afar Region), and 8 from Sidama. The remaining 61 genotypes were collected in the SNNP, Amhara, Benishangul & Gumuz, Oromia, and Tigray Regions of Ethiopia in 2019 and characterized for various agro-morphological traits. One hundred and eighteen genotypes have passport information, while the other two do not and are considered released varieties. A two-season experiment was conducted at Hawassa University in Ethiopia to achieve the aforementioned objective.

Table 3.1 List and location of plant materials included in the study.

Genotype Name	Easting	Northing	Altitude	Genotype Name	Easting	Northing	Altitude
KEN-065	321813	583339	1756	KEN-012	319703	586470	1760
YRC-048	411783	675273	1995	215567	372960	768376	2100
KAZ-077	327268	599608	1202	ALE-025	300048	597628	1349
KEN-067	321745	585148	1760	SHD-001	434789	761187	1874
211457	326440.36	589244.3	1560	ALE-073	301337	604237	1693
242917	812954	793136	2200	DAL-043	429799	738442	1790
KEN-011	299762	603070	1739	SA-001/07	624955	1028024	736
241764	306077	591230	1520	SRA-002	632177	1031734	742
HA-003	636126	1046188	726	219284	358268	783157	1750
KAZ-076	327267	599336	1202	HA-008	634804	1044976	730
208683	758052	1043629	2270	ALE-071	299973	597729	1372
KEN-016	322071	584756	1745	240814	322866.04	764187.65	1250
MEK-084	552161	1490423	2155	KAZ-060	319338	585751	1734
HAL-039	407697	806621	1834	MEK-083	552491	1490761	2151
212892	315406	622936	2200	BKD-031	234460	634949	1400
211455	328390	645017	1150	WON-049	418632	699405	1743
HAW-041	444076	776871	1750	KAZ-058	327268	599608	1202
KAZ-055	326719	609804	1213	KAZ-006	327308	598524	1188
DRA-053	324631	614748	1290	BKD-029	234471	634953	1409
ALE-023	299964	597729	1370	WA-003	628204	1033787	741
KEN-015	320119	586057	1819	YRB-002	424075	769481	1857
ALE-069	299971	597724	1376	KEN-021	321818	585166	1752
DIL-050	421120	709519	1459	KAZ-079	326433	604711	1204
KAZ-057	326722	609738	1214	KEN-064	321816	585168	1762
KEN-022	321811	585162	1751	SHL-040	433633	805425	1662
SHIA-007	627807	1036846	735	KEN-017	322084	584791	1762
240815	354053.54	754795.3	1950	242532	665945	1167179	1250
209056	312304.71	699421.8	1660	SA-008/07	624844	1026573	740
ABL-004	369796	734542	1367	240813	323825.31	589809.06	2750
ALE-034	295826	597501	1316	208764	698483.15	943746.8	1850
ALE-024	299885	597965	1368	MEK-080	552552	1490147	2162
SAA-004	636126	1046188	726	BA-016	629359	1027955	740
212582	572790	1234490	1840	WA-001	627878	1033651	740
ALE-068	299970	597736	1384	SA-025/07	625039	1026746	735

212581	581861	1245570	2920	DMG-038	380667	777365	1881
KAZ-007	327352	599054	1187	KAZ-078	327269	599337	1200
204645	327492.26	590316.2	1600	242531	195529	1185259	1250
225717	348942	740795	1440	DA-006	630310	1034519	735
KEN-019	321816	585168	1754	211456	314087.91	599272.76	1570
91003	233325	835289	2040	CHU-045	431835	721533	1910
242533	665945	1167179	1250	DUF-003	394497	770836	2113
KEN-013	319707	586409	1772	ADZ-037	389243	797508	1912
BNT-026	247228	615060	1618	212583	570917	1258445	1640
AC-NL	Released variety	Released variety	1550-2400	208025	300580	1354876	2100
Madiira II	Released variety	Released variety	1550-2400	225713	311940	696674	1600
BKD-027	232336	637249	1427	KEN-014	319660	586850	1690
204644	334751.47	653902.5	1200	91001	315299	584231	1890
225718	348942	740795	1440	212893	324653	628440	1380
ALE-070	299981	597743	1329	ALE-033	282131	591864	589.5
OTI-044	431913	721434	1893	KEN-020	321817	585166	1753
KAZ-008	326435	604708	1204	K3A-005	631670	1035541	738
MEK-081	552793	1489902	2143	242530	665945	1167179	1250
KAZ-056	326684	609708	1214	BKD-028	234471	634953	1409
KEN-018	321785	585144	1761	SA-005/07	624847	1026721	737
BA-001	628326	1026376	743	KAZ-009	325782	604450	1213
ALE-074	299762	603000	1655	242534	665945	1167179	1250
209057	341220.34	740810	1580	212890	382230	795996	2180
KAZ-059	327268	599608	1202	DAL-042	431596	753041	1780
KEN-010	319325	585745	1729	MEK-082	552972	1490517	2140
ALE-075	299919	603075	1640	225715	258450	696873	1780

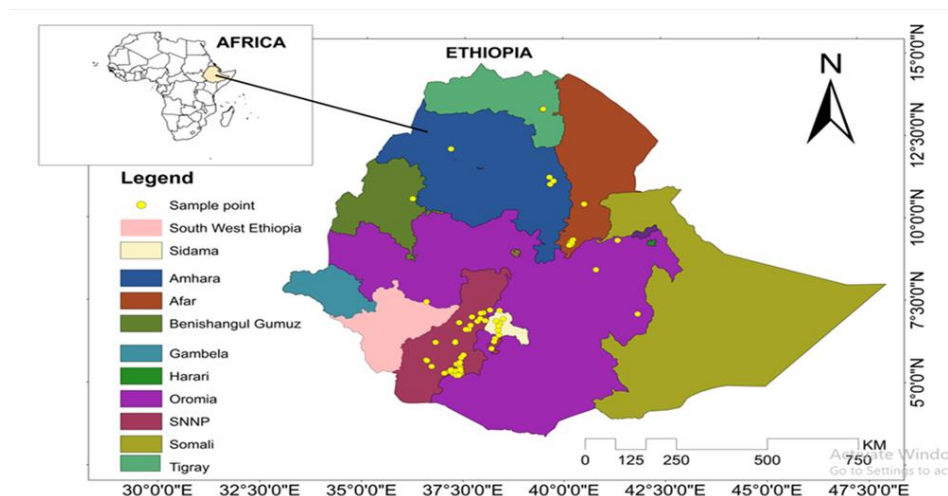


Figure 3.1 Map of Ethiopia showing the collection site for the different genotypes of Amaranths from different agroecological Zones. Open Source Geospatial Foundation; 2020.

3.2.3 Experimental layout and crop management

The experimental field was cleared, properly plowed, and harrowed by a tractor, with ridge preparation done by hand hoe. The experimental design was alpha lattice. A layout with 2 replications, 16 blocks, and 15 plots per block for amaranths was conducted. Each unit plot was separated by a 0.60-meter way between plots, a one-meter between blocks, and a three-meter between replications, with a plot size of 1.80 m in length and 1.50 m width of 2.7 m² area. Each season required a total space of 1,345.2sq.m (38 m × 35.4 m). On April 15th, 2020, and 2021, the seed was sown at one location in each season, which is under ideal growing circumstances and during the agricultural season. One growing season on the experimental site in one year is considered the environment.

Seeds of various genotypes (Table 3.1) were consistently sowed in two rows with a gap of 0.75 m between them. Seeds are quite tiny, with sizes ranging from 0.37 to 1.21 g per 1000 seed weight ([Paredes-Lopez, 2018](#)), and they were planted in seedbeds and covered with powdered, finely diced cow farmyard manure after being combined with sand in a 1:4 ratio ([Figure 3.3](#)). At 14 and 22 days after sowing (DAS), thinning was done twice at a distance of 75 cm between rows and 30 cm between plants. According to [Grubben and Van Sloten \(1981a\)](#) and [Shukla et al. \(2006b\)](#), the experiment followed standard cultural practices. Hand-hoeing was used to control weeds at 2-week intervals following germination and whenever necessary.

A total of 12 plants were maintained in each plot. During the first season of the trial cutworms, were identified as important amaranth pests ([Figure 3.2](#)). Diseases such as root rot, stem rot, and white rot were also observed on some plants ([Figure 3.2](#)). Nevertheless, no pests were identified in the second season. Five percent karate was sprayed twice a day for 5 days at a



rate of 25 ml per 15 liters of water to control cutworm and leaf folder. 250 EC (0.05 percent) was sprayed around the collar region to reduce root rot, and Redomil Gold (0.2 percent) was sprayed twice to control white rus

Figure 3.2 Root rot (A), and cutworm (B) in amaranth plants

3.2.4 Data collection and measurements

To characterize the material under study, observations were made on various morphological traits at distinct phenological stages (Table 3.2). In each plot, the phenotypic characteristics of 10 randomly tagged plants were assessed. Plants were grown to maturity and defined using amaranth descriptors, as recommended by the International Board for Plant Genetic Resources based on taxonomic keys ([Grubben and Van Sloten, 1981b](#)). Characters not on the list that were considered necessary for the characterization were included. In this investigation, a total of 24 quantitative characters were recorded. In each season, data was collected from the field at four phases of growth: germination, vegetative stage, 50% of the plants' developing inflorescences, and 80% plant maturity shortly before and after harvesting. Plant height at flowering and maturity (cm), petiole length (cm), leaf width (cm), leaf thickness (mm), terminal inflorescence stalk length (cm), inflorescence lateral length (cm), number of branches per plant (number), number of leaves (number), number of nodes (number), stem diameter (mm), number of days to emergence (days), number of days to flowering (days), number of days to maturity (days), leaf area (cm²), and 1000-seed weight (g), leaf yield tone ha⁻¹, grain yield tone ha⁻¹, grain filling period (days), grain filling rate (kg ha⁻¹day⁻¹) are among them. Seedling data, vegetative data, inflorescence data, and seed data were among the 24 agro-morphological variables evaluated, which were assessed four times per plot for each replication. Except for days to emergence, days to flowering, and days to maturity, which were recorded at the plot level, all ten planted genotypes for each population (two replications) were used to collect quantitative trait data (Figure 3.3).



Figure 3.3 Some of the measured traits: Stem diameter (A); Terminal inflorescence length (B); Plant height (C); Basal lateral branch length(cm) at maturity (D)

For the traits, the mean value of 10 plants per plot was measured and recorded. Three different leaf lengths, leaf thickness, leaf width, petiole length, and leaf area (small, medium, and large) were randomly picked per plant for measurement. Leaf length, breadth, thickness, petiole length, and area were computed using the averages of 30 measurements (10 plants per plot for each of the 3 different leaf and petiole sizes). The harvest was done manually; panicles were carefully removed to minimize grain spilling, and then the panicles were threshed by utilizing a flail, a sieve, and a basket. After the removal of fine debris, the seeds were cleaned, and they were held in an electric oven at 100 °C for 48 hours to regulate the moisture content to 12 percent, as advised by (Biru, 1978; de Jesus Souza *et al.*, 2016). A digital caliper was used to measure the diameter of the stem and the thickness of the leaves (Digimatic Solar DC-S15 m, Mitutoyo, Japan). The LI-3100 AREA METER, an electronic leaf area meter, was used to measure the leaf area (LI, Cor, Inc, Lincoln, Nebraska , USA).

Table 3.2 List of quantitative agro-morphological traits used along with their phenotype code, description, and phenotype scores.

Traits	Phenotypic code	Descriptions
Plant height at flowering(cm)	PHF	Measured from the ground surface to the tip of the main stem (terminal inflorescence) in in cm at the 50% flowering stage by using a meter tap
Plant height at maturity (cm)	PHM	Measurement of the height of a plant from the soil surface to the top of the inflorescence in cm at the 80% maturity stage by using a meter tap.
Leaf length (cm)	LL	Three different representative leaf sizes (small, middle, and large) per plant were randomly sampled for measurement of leaf length at the flowering stage by using a meter tap.
Leaf width (cm)	LW	Three different representative leaf sizes (small, middle, and large) per plant were randomly sampled for measurement of leaf width at the flowering stage by using a meter tap.
Stem diameter (mm)	SD	Measured thickest part (10 cm) above ground) of the stem at the lower part at the maturity stage by using a digital caliper.
Leaf thickness (mm)	LT	Three different representative leaf sizes (small, middle, and large) per plant were randomly sampled for measurement of leaf thickness in the middle of the leaf at the flowering stage by using a digital caliper.
Petiole length (cm)	PL	Three different representative petiole sizes (small, middle, and large) per plant were measured in centimeters from the base of the stem to the petiole of the leaf with the help of a meter scale at the flowering stage. measured from the point of insertion of the petiole to the beginning of the leaf blade.
Number of the branch (number)	BN	The number of branches was counted along the main stem at the maturity growth stage.
Terminal inflorescence stalk length (cm)	TISL	The mean length of 10 randomly selected terminal inflorescence stalks was measured with a meter rule starting from the base up to the tip of the inflorescence at the maturity stage.
Terminal inflorescence laterals length(cm)	TILL	The mean length of 10 randomly selected terminal inflorescence laterals length (cm) was measured at the maturity stage.
Number of nodes on the main stem (number)	NN	Many nodes were counted along the main stem at the flowering stage.
Grain yield (t ha ⁻¹)	GY	Grain yield was estimated at an adjusted moisture content of 12 % and weighed to determine the dry matter of the net plot and then extrapolated per hectare.
Leaf area(cm ²)	LA	Three different representative leaf sizes (small, middle, and large) per plant were measured by using a digital leaf area meter.
1000 Seed weight (g)	TSW	A Sample of thousand seeds from each genotype was counted and weighed using an analytical balance
Number of Leaf (number)	LN	The main countable leaves were counted along the main stem at the flowering stage.
Days to maturity (days)	DM	The number of days between 90% emergence and the physiological maturity of 80% of plants. (The time of plant maturity is when the seed taken from the central part of the inflorescence does not change shape when pressed between fingers and when the inflorescence changes its color from green to brown).
Days to flowering (days)	DF	From the 90 percent emergence date to the stage where ears had appeared in the terminal inflorescence from 50 percent of the plants in a row, days to 50 percent bloom.
Basal lateral branch length(cm) at maturity	BLBL	Measured from the base of the stem branches to the top of a branch at the maturity stage by using a meter tap.
Top lateral branch length(cm) at maturity	TLBL	Measured from the top of the stem branches to the top of a branch at the maturity stage by using a meter tap
Axillary inflorescence length (cm)	AIL	The mean length of ten randomly selected auxiliary inflorescence was measured at the maturity stage.
Days to Emergence (days)	DE	The number of days from planting to 90% of seedlings germinates in each plot was recorded and expressed in days.
Leaf yield (tone ha ⁻¹)	LY	The separated parts of the leaves were dried for a week (7 days) and weighed to determine the dry matter of the net plot and then extrapolated per hectare.
Grain filling period (days)	GFP	The number of days between days for 50% flowering and days to grain maturity.
Grain sink filling rate (kg ha ⁻¹ day ⁻¹)	GSFR	GSFR is stated as a ratio of grain yield to the number of days from 50% blooming to seed maturity and is represented in kilograms per hectare per day.

3.2.5 Statistical analysis for agro-morphological variability

3.2.5.1 Analysis of variance

The SAS computer program first confirmed Bartlett's test for homogeneity of variance before executing ANOVA for each year's analysis. The F-max technique of [Hartley \(1950\)](#), which is based on the ratio of the larger mean square error (MSE) from the separate analysis of variance to the smaller MSE, was also used to test the homogeneity of error variance for the combined ANOVA. The error variance is considered homogenous if the bigger MSE is not three times greater than the smaller MSE ([Gomez and Gomez, 1984](#)). After determining that the error variance was homogeneous, the combined ANOVA was carried out using the SAS PROC GLM procedure. Descriptive statistics were also done for the mean, range, standard deviation, and standard error of the mean. The genotype and year effects were both accounted for using a linear random model. The degrees of freedom for these combined mean squares were estimated following Satterthwaite's approximation approach, and the mean square of the random effect was compared to the sum of replication and interaction mean squares minus residual mean squares. The interaction effect, however, was assessed against the residual mean square whereas the genotype random effect was tested against the interaction (genotype by year) mean square. Therefore, using the SAS statistical tool, ANOVA was performed on the pooled data from a two-year alpha lattice design. The quantitative data was evaluated using a two-way ANOVA in SAS 9.4 ([Lehman *et al.*, 2013](#)), taking into account all sources of variation as random effect blocks, replications, genotypes, and year of planting as variables. All sources of variation were considered random effects, and the interactions between genotypes and years were evaluated. Following [Gomez and Gomez \(1984\)](#), a mean comparison between years was performed using Duncan's Multiple Range Test (DMRT) at a 5% probability level. The numerator degree of freedom for the year F-test was Y-1 while the denominator (combined mean squares) degree of freedom was estimated using Satterthwaite's ([Satterthwaite, 1946](#)) formula as indicated below.

$$F1 = \frac{MS_y}{(MS_r + MS_{gy-MSe})}$$

$$DF_{cms} = \frac{(MS_r + MS_{gy-MSe})^2}{\frac{(MS_r)^2}{(r-1)} + \frac{(MS_{gr})^2}{(g-1)(y-1)} + \frac{(MSe)^2}{y(g-1)(r-1)}}$$

Where Fl is F-test for the year; MSy is the mean square of the year; MSr is the mean square of replication; MSgy is the mean square of genotype by year interaction; MSe is the mean square of

error; DFCms is the degrees of freedom for combined mean square; r is replication; y is the year, and g is genotype.

The statistical models for each year and combined across the two years are presented below.

The statistical model of the alpha lattice design for individual years is given by:

$$Y_{ijkl} = \mu + Y_i + R_j(i) + B_k(ij) + G_l + (GY)_{il} + \epsilon_{ijk}$$

The statistical model of the alpha lattice design across years is given by:

$$Y_{ijkl} = \mu + Y_i + R_j(i) + B_k(ij) + G_l + (GY)_{il} + \epsilon_{ijkl}$$

where yijk is the measured response of genotypes i at year j and block k; μ is the overall mean; G (k) is the k-th genotype random effect; Y (i) is the i-th year random effect; GY (ki) is the genotype k by year i interaction is random effect; Bj is the j th block random effect, and ϵ (ijkl) is the random error.

Table 3.3 Combined analysis of variance (ANOVA) and expected mean square for the random model of alpha lattice design

Sources of variation	Degree of freedom (Df)	Mean square (MS)	Expected means square (EMS)	F- test
Year (y)	y-1	MS ₁	$\delta^2e + r\delta^2_{gy} + g\delta^2_{r(y)} + gr\sigma^2_y$	MS ₁ /MS ₂ +MS ₅ -MS ₆
Rep(year)	y(r-1)	MS ₂	$\delta^2e + g\delta^2_{ry}$	MS ₂ /MS ₃
Block(rep*year)	yr(b-1)	MS ₃	$\delta^2e + \delta^2_{gy_r}$	MS ₃ /MS ₆
Genotypes (g)	g-1	MS ₄	$\delta^2e + r' \delta^2_{gy} + r'y \delta^2_g$	MS ₄ /MS ₅
Genotypes*year (g*y)	(g-1)(y-1)	MS ₅	$\delta^2e + r' \delta^2_{gy}$	MS ₅ /MS ₆
Error	[y(r-1)(g-1)]-[yr(b-1)]	MS ₆	δ^2e	-

σ^2g = genotypic variance; σ^2e = variance; σ^2gy = genotype by year interaction variance; y is the number of year; r = number of replication; b = block; g = genotype and σ^2e = environmental variance.

3.2.5.2 Comparison of selected genotypes with the original population

To compare with the original population, the means of the top 5% genotypes for each trait were independently computed. The student t-test table was used to determine the significance of the difference between the sample mean and population parameter. When the calculated t-value is higher than the tabulated t-value, the difference is deemed significant (Singh *et al.*, 2001). To compare the performance of the 5% best-selected genotypes with the size of a population, the absolute t-value was obtained using the student's t-test formula as follows:

$$T = (\bar{x} - \mu) / (S / \sqrt{n})$$

Where n is the number of genotypes selected from the size of a population for better performance, $\bar{x}$ is the mean of the genotypes that were selected, S is the sample standard deviation, and μ is the mean of the size of the population.

3.2.5.3 Estimation of variance components

The total variance components are partitioned into different components using ANOVA by assuming the mean square of each source of variation is equal to their expected mean squares (Table 3.3), as suggested by [Singh and Chaudhary \(1977\)](#). The mean, phenotypic, genotypic, and coefficient of variation were all measured to evaluate the population's level of diversity. According to the approach recommended by [Johnson *et al.* \(1955\)](#), the combined over-year mean squares computed using SAS statistical techniques and the causes of variations in alpha lattice design were utilized to estimate variance components as follows:

$$\begin{aligned}\sigma^2_{gy} &= \frac{[(MS_{gy}) - (MSe)]}{r'} & \sigma^2_p &= \sigma^2_g + \frac{\sigma^2_{gy}}{y} + \frac{\sigma^2_e}{r'y} \\ \sigma^2_y &= \frac{(MS_y + MSe) - (MS_r + MS_{gy})}{gr'} & \sigma^2_e &= MSe \\ \sigma^2_r &= \frac{[(MS_r) - (MSe)]}{g} & \sigma^2_g &= \frac{[(MS_g) - (MS_{gy})]}{r'y}\end{aligned}$$

where δ^2_y is the variation over the year; The term "MS_y" refers to the mean square of a year. g represents the number of genotypes, r represents the number of replications, δ^2_r represents the replication variance, MS_r represents the mean square of replication, δ^2_b represents the block variance, MSe represents the mean square of error, and δ^2_g represents the genotypic variance. MS_{gy} stands for the mean square of genotype by year interaction. "y" specifies the number of years, while MS_g refers to the genotypes' mean square. The phenotypic variance is represented by δ^2_p , the environmental variance by δ^2_e , and the genotype by year interaction variance is represented by δ^2_{gy} . On the other hand, the technique suggested by [Singh and Chaudhary \(1977\)](#) was also applied to estimate the genotypic (GCV), phenotypic (PCV), and environmental coefficients of variation (ECV).

These estimates of variance were categorized as low when values were less than 10%, medium when values were between 10% and 20%, and high when values were over 20% ([Johnson *et al.*, 1955](#); [Sivasubramanian and Menon, 1973](#); [Deshmukh *et al.*, 1986](#)).

$$ECV = \frac{\sqrt{\delta^2_e}}{\mu} * 100$$

$$GCV = \frac{\sqrt{\delta^2_g}}{\mu} * 100$$

$$PCV = \frac{\sqrt{\delta^2_p}}{\mu} * 100$$

Where GCV denotes the genotypic coefficient of variation, PCV denotes the phenotypic coefficient of variation, and ECV denotes the environmental coefficient of variation. Whereas the genotypic variation is represented by δ^2_g , the phenotypic variation is represented by δ^2_p , the environmental variation by δ^2_e , μ is the mean of a trait, and the genotype by year interaction variation is represented by δ^2_{gy} .

3.2.5.4 Estimation of heritability

Heritability was defined by (Allard (1960) and Falconer, (1989), as a proportion of the (δ^2_g) the genotypic variance, and phenotypic variance (δ^2_p). Singh *et al.* (2001) and Pandey and Singh (2011) determined that the heritability estimates were classed as low when they were less than 40%, medium when they were between 40 and 59%, fairly high when they were between 60 and 79, and very high when they were higher than 80% estimated these values as follows:

$$h^2_{bs} = \frac{\delta^2_g}{\delta^2_p} = h^2_{bs} = \frac{\frac{[(MSG) - (MSG_y)]}{ry}}{\sigma^2_g + \frac{\sigma^2_{gy}}{y} + \frac{\sigma^2_e}{ry}} * 100$$

Whereas h^2_{bs} = heritability in broad sense; δ^2_g stands for genotypic variance, and δ^2_p for phenotypic variance.

3.2.5.5 Estimation of genetic advance

Using the approach described by Allard, (1960), expected genetic advancement as a component of the mean (GA) for each characteristic at 5% selection intensity ($k = 2.06$) was calculated. Additionally, using the Comstock and Robinson (Comstock and Robinson, 1952) method, the expected genetic advance as a percentage of the mean (GAM) was calculated to examine the magnitude of the predicted advance of various characteristics under selection. Johnson *et al.*, (1955) defined the estimated GAM values as low when values were less than 10%, medium when values were between 10% and 20%, and high when values were greater than 20%.

$$GA = K * (\sqrt{\sigma^2 p} * h^2_{bs}) \qquad GAM = \frac{GA}{\bar{X}} * 100$$

Where GA is the genetic advance; K is the standardized selection differential at 5% selection intensity ($k = 2.06$); $\sqrt{\sigma^2 p}$ is the square root of phenotypic variance; h^2_{bs} is heritability in broad sense; GAM is the genetic advance as percent mean; and $\bar{X}$ is the mean of the population in which selection is employed.

3.2.6 Statistical analysis for correlation and path analysis

3.2.6.1 Diagnosis of multicollinearity

One of the requirements for accurate and trustworthy route coefficient estimation is the analysis of multicollinearity between any explanatory variables ([Sari et al., 2018](#)). The key plant breeding phenomena of collinearity diagnostic boosts the effectiveness of indirect selection for genetic improvement when the target characteristics have low heritability ([Cruz and Souza Carneiro, 2006](#)). When there is a moderate to severe multicollinearity, the computed coefficients may have utterly irrational (higher than unity) values, and the variances linked to estimators of path coefficients may inflate, generating incorrect estimates ([Figueiredo Neto et al., 2005](#)), and the variances associated with estimators of path coefficients may become inflated and leads to unreliable estimates ([Olivoto et al., 2017](#)). By examining the correlation matrix or by using the variance inflation factor (VIF), tolerance limit (TOL), and eigenvalues, multicollinearity can be identified. Two explanatory variables may be multicollinear if there are strong coefficients (near +1) between them in the correlation matrix ([Viotto et al., 2020](#)). This possibility can be verified using the VIF, TOL, and eigenvalues. Using the VIF, TOL, and Collin option methods, the PROC REG was used to perform the VIF, TOL, and eigenvalues (SAS).

A number greater than 10 indicates that the related regression coefficients are underestimated due to multicollinearity. The VIF quantifies how much the variance of an estimated regression coefficient is increased due to the effect of multicollinearity ([Montgomery et al., 2021](#)). The TOL, which is the inverse of the VIF, indicates that there is severe collinearity in the database when it is larger than 0.1 ([Montgomery et al., 2021](#)). According to [Montgomery et al. \(2021\)](#), the correlation matrices' maximum eigenvalue (max) can be divided by the subsequent smallest eigenvalue (min) to determine the condition number (CN) for each trait. [Montgomery et al. \(2021\)](#) determined that the degree of multicollinearity in the matrices was categorized as

weak when the estimates of CN were less than 100, moderate when they were between 100 and 1000, and severe when they were greater than 1000. Either eigenvalues or eigenvectors can be used simultaneously to find the multicollinearity issue. Therefore, removing the traits responsible for boosting the variance of the regression coefficient is essential unless the trait is of importance for improvement. Traits with a low eigenvalue and a high matching eigenvector indicate a striking collinearity problem (Olivoto *et al.*, 2017). Therefore, removing the traits that cause multicollinearity from the study is a quicker and more efficient way to solve this issue and enables obtaining the most precise path coefficients (Viotto *et al.*, 2020). As a result, the correlation matrices, VIF, TOL, CN, and Eigensystem analysis of the correlation matrix were used to perform the multicollinearity test between the researched features. In total 24 traits were measured and based on the multicollinearity diagnosis tests. These multicollinearity diagnosis tests allowed us to exclude variables like TSW, GFP, BLBL, TLBL, SD, NN, PL, and LT from the analysis of correlation since they were so strongly linked to multicollinearity in the matrix. Due to their low multicollinearity, the remaining 16 variables (or around 66%) were taken into consideration for the study.

3.2.6.2 Phenotypic and genotypic correlations

The collected data from the two years were combined and subjected to correlation and path coefficients analysis. Correlation analysis was performed for all possible combinations of traits after diagnosing the multicollinearity test. Phenotypic correlation, the observable correlation between two explanatory traits, which includes both genotypic and environmental effects, and genotypic correlation, the inherent association between two explanatory traits, were computed from the components of variance and co-variances as described by (Singh and Chaudhary, 1977). The coefficient of correlation was tested using 'r' tabulated value at n-2 degrees of freedom, at 5, 1, and 0.1% probability level, where n is the number of genotypes.

$$rp_{XY} = \frac{PCOV_{(x,y)}}{\sqrt{\sigma^2_{p_x} * \sigma^2_{p_y}}}$$

$$rg_{XY} = \frac{GCOV_{(x,y)}}{\sqrt{\sigma^2_{g_x} * \sigma^2_{g_y}}}$$

Where rp_{xy} and rg_{xy} are the phenotypic and genotypic correlation coefficient between traits x and y, $Pcov(x,y)$ and $Gcov(x,y)$ are phenotypic and genotypic covariance between traits x and y; $\sigma^2_{p_x}$ and $\sigma^2_{p_y}$ are phenotypic variances for traits x and y; $\sigma^2_{g_x}$ and $\sigma^2_{g_y}$ are genotypic variances for traits x and y, respectively.

3.2.6.3 Path-coefficient analysis

Using path coefficient analysis, which was proposed by (Dewey and Lu, 1959) and developed by Wright, the correlation coefficient was further divided into direct and indirect effects (Wright, 1921). It is possible to tell whether a cause-and-effect relationship between two explanatory traits is real and independent of other traits by using path analysis. LY and GY were chosen for the path-coefficient analysis as the result (dependent) variables and the other traits as the causal (predictor) traits. The LY was also considered as an independent trait in path coefficient analysis for GY and vice versa. The direct and indirect effects of the independent traits on LY and GY were estimated by the simultaneous solution of the following equations which may be represented by the following general formula as applied by (Dewey and Lu, 1959).

$$r_{ij} = P_{ij} + \sum r_{ik} * P_{kj}$$

Where r_{ij} is the mutual association between the independent trait (i) and dependent trait (j) as measured by the genotypic and phenotypic correlation coefficients; P_{ij} is components of direct effects of the independent trait (i) on the dependent trait (j) as measured by the genotypic and phenotypic path-coefficients; and $\sum r_{ik} * P_{kj}$ is the summation of components of indirect effects of a given independent trait (i) on a given dependent trait (j) via all other independent traits (k). To determine values, square matrices of the correlation coefficients between independent traits in all possible pairs were inverted and then multiplied by the correlation coefficients between the independent and dependent traits.

Based on average data from the two years, path coefficient analysis was conducted using phenotypic and genotypic correlation matrices built up as $X = Y^{-1} * Z$ for LY and GY. The phenotypic and genotypic correlation coefficients of dependent traits (LY and GY) versus independent agro-morphological traits are represented in this matrix by the vector 'X'. Vector Z is the direct effect of the route coefficients, and vector Y' is the inversed value of the phenotypic and genotypic correlations for all conceivable combinations among the examined traits. The matrix inverse (MINVERSE) function of Microsoft Excel 2010 was used to compute the inverse of matrix Y' after inverting the reciprocals and autocorrelations of the correlation coefficient matrix. Using the matrix multiplication (MMULT) function of Microsoft Excel 2010, the direct influence of path coefficients was estimated as the product of vector 'X' and each row of vector Y' inverse. As recommended, the value of the direct effect path coefficient

was multiplied by the correlation coefficient in the matrix to assess the indirect effect of the path coefficient (Dewey and Lu, 1959). The direct effect product in the path analysis and the dependent trait coefficient in the correlation analysis via all predictor traits are added to create the coefficient of determination (R^2). The contribution of the remaining unknown factor was measured as the residual factor (RF), which was calculated with the following formula;

$$RF = \sqrt{1 - R^2} ; \quad \text{Where } R^2 = (\sum P_{ij} * r_{ij})$$

The value of RF reveals how effectively the causative factors explain the dependent factor's variability (Singh, 1985). That is, if the RF value is low (for example, close to zero), the variability in the predictor characteristics fully accounts for the variance in the dependent trait, but a greater RF value suggests that other factors that have not been taken into account must be included in the study.

3.2.7 Statistical analysis for multivariate analysis

The mean values of the genotypes of the two seasons were subjected to the procedures of a multivariate analysis. Because the parameters considered in this study did not share a common measurement scale, the quantitative traits for the principal component, hierarchical agglomerative cluster, and inter-cluster and intra-cluster distance analyses (standardized Mahalanobis's D^2) were all standardized to a mean of zero and a standard deviation of one (Sneath and Sokal, 1973; Manly, 1986; Mohammadi and Prasanna, 2003).

3.2.7.1 Clusters analysis

To group the 120 genotypes and to bring out the patterns of similarity and dissimilarity based on 24 quantitative morphological characters, the R version (4.1.2) has been used with various packages to perform cluster-based analysis based on ward D^2 hierarchical grouping using Euclidian distance and determine the optimum number of clusters in the data set using the average silhouette method for the dendrogram (Maechler, 2019). The silhouette technique and the NbClust software in R Studio were used to determine the optimum number of clusters (Charrad and Ghazzali, 2014).

3.2.7.2 Genetic distance analysis

Using the "biotools" package of the R-studio, the means of each replication and the overall means of each trait were used to calculate the D^2 distance and group the genotypes according to D^2 statistics (Da Silva and Da Silva, 2017). The important proportion and the cumulative proportion of each variable were determined using the "Singh" statistic from the same package based on the squared generalized Mahalanobis distance (Singh, 1981). The significance is determined by the generalized Mahalanobis distance squared. Using the SAS system software package, genetic divergence between clusters was computed based on a correlation matrix and Mahalanobis' D^2 statistic (Mahalanobis, 1936) as suggested by (Rao, 1952). Intercluster distances were calculated based on the standardized Mahalanobis's D^2 statistics as $D^2_{ij} = (x_i - x_j)' \text{cov}^{-1}(x_i - x_j)$ Where, D^2_{ij} = the distance between cases i and j ; x_i and x_j = vectors of the values of the variables for cases i and j ; and cov^{-1} = the pooled within groups variance-covariance matrix.

3.2.7.3 Principal component analysis

Principal component analysis, eigenvalues, eigenvectors, biplot, and clusters were obtained using FactoMineR and factoextra packages using R studio. At each axis of differentiation, principal component analysis (PCA) displays the importance of the largest contributor to the total variance (Menzir, 2012). PCs with an eigenvalue ≥ 1.0 were included in the criteria for determining the number of main components (PCs) (Kaiser, 1960). In addition, the first PCs that accounted for at least 70% to 80% of the cumulative variance were kept and used to explain the variability (MacCallum *et al.*, 1999; Stevens, 2012).

3.3 Results

3.3.1 Analysis of Variance

Table 3.4 illustrates the variance results from a pooled analysis of all traits for 120 genotypes. For all characteristics other than leaf thickness (mm), petiole length (cm), leaf yield (t/ha), top lateral branch length (cm), and thousand seed weight (g), the mean squares differed significantly among genotypes ($P < 0.001$). The majority of the replicates showed no discernible differences. The effect of cropping season was significant for all traits, including plant height at flowering (days), leaf area (cm^2), leaf yield (t/ha), branch number (number), top lateral branch length (cm), auxiliary inflorescence length (cm), terminal inflorescence stalk length (cm), grain filling periods (days), and thousand seed weight (g). Similarly, genotype by

cropping season's interaction effects was significant for all morphological and agronomic traits, except leaf yield (t/ha), and thousand seed weight (g.). Significant genotype by cropping season's interaction effects was mostly a 'cross-over' type; i.e., interactions were associated with rank order changes among the genotypes (Figure 3.6).

Table 3.4 Combined analysis of variance (over the year) for 24 traits of 120 amaranth genotypes grown in the 2020 and 2021 cropping seasons.

Traits	Rep(Year) DF= 2	Block(Rep*year) (DF=28)	Year (DF=1)	Genotype (DF= 119)	G*Y (DF=119)	MSE (DF=210)	CV (%)
Days to emergence (days) [DE]	0.42 ^{ns}	0.319 ^{ns}	156.41**	2.475***	1.328***	0.334	8.67
Days to flowering(days) [DF]	58.16 ^{ns}	24.35 ^{ns}	1836.92**	295.27***	73.17***	22.27	8.92
Plant height at flowering(days) [PHF]	348.176 ^{ns}	235.763 ^{ns}	439.01 ^{ns}	1318.99***	468.187***	179.59	18.48
Leaf number (number) [LN]	475.967***	18.491***	41458***	70.193***	37.976***	9.982	10.75
Leaf area(cm ²) [LA]	33869***	1079.06***	6537.352 ^{ns}	1986.13***	423.752 ^{ns}	395.42	30.01
Leaf length(cm) [LL]	15.236***	1.607 ^{ns}	1122.87**	24.03***	5.45***	1.810	9.30
Leaf width(cm) [LW]	3.633***	0.481 ^{ns}	298.67**	5.911***	1.159***	0.446	9.28
Leaf thickness(mm) [LT]	0.046***	0.0028 ^{ns}	3.015**	0.0025 ^{ns}	0.003***	0.0014	10.09
Petiole length(cm) [PL]	4.079**	0.642 ^{ns}	289.99**	2.86 ^{ns}	2.580***	0.57	12.06
Leaf yield(t/ha) [LY]	369.875***	25.526**	403.59 ^{ns}	22.57 ^{ns}	18.722 ^{ns}	15.567	39.41
Branch number(number) [BN]	75.015**	21.589 ^{ns}	73.907 ^{ns}	203.614***	32.486***	16.583	14.30
Basal lateral branch length (cm) [BLBL]	2797.86***	191.421 ^{ns}	39905.05 ^{ns}	5318.31***	1671.72***	315.72	33.84
Top lateral branch length(cm) [TLBL]	88.290**	42.599**	290.75 ^{ns}	165.63 ^{ns}	173.70***	25.044	24.28
Node number (number) [NN]	245.54***	11.53 ^{ns}	65114**	41.13 ^{ns}	31.89***	8.175	10.84
Days to maturity(days) [DM]	22.10 ^{ns}	67.56**	682.59**	616.34***	133.81***	43.77	6.56
Plant height at maturity(days) [PHM]	59.25 ^{ns}	370.89 ^{ns}	377692***	8801.80***	1557.40***	333.12	8.69
Stem diameter (cm) [SD]	85.37**	10.82 ^{ns}	9515.79**	113.29***	20.29***	12.29	12.45
Auxiliary inflorescence length (cm) [AIL]	91.50***	11.66 ^{ns}	70.58 ^{ns}	112.32***	31.13***	9.52	25.73
Terminal lateral inflorescence length (cm) [TILL]	30.57**	6.02 ^{ns}	23433**	50.37**	33.65***	4.52	11.95
Terminal inflorescence stalk length (cm) [TISL]	175.38**	29.89 ^{ns}	3.06 ^{ns}	210.34**	121.56***	25.51	17.30
Grain filling periods(days) [GFP]	31.95 ^{ns}	79.26 ^{ns}	279.99 ^{ns}	406.91***	149.37***	58.51	15.40
Grain sink filling rate (kg ha ⁻¹ day ⁻¹) [GSFR]	170.23 ^{ns}	516.81**	8900.84**	765.95**	551.09***	313.00	59.26
Thousand seed weight(g) [TSW]	0.0019 ^{ns}	0.160 ^{ns}	0.26 ^{ns}	0.27 ^{ns}	0.22**	0.13	41.90
Grain yield (t ha ⁻¹) [GY]	0.91 ^{ns}	0.89**	17.05**	1.28**	0.92***	0.57	54.00

DF = Degree of freedom; CV = Coefficient of variation; NS = Non-significant; *, **, and *** = significantly different at 5, 1, and 0.1%, respectively; Rep (Y) = Replication nested under year

3.3.2 Descriptive statistics

The mean, range, standard deviation, and standard error of the mean for all 24 quantitative traits showed remarkable variations among amaranth genotypes in the studied phenotypic traits (Table 3.5).

Table 3.5 Mean, range, standard deviation, and standard error of the mean for 24 traits of 120 amaranth genotypes grown in the 2020 and 2021 cropping seasons.

StDev = Standard deviation; SEM ($\pm$) = Standard error of the mean.

Traits	Mean	StDev.	Minimum	Maximum	Range	SEM($\pm$)
Days to emergence (days)	6.65	1.24	5.50	9.50	4.00	0.06
Days to flowering(days)	46.27	10.50	28.50	69.00	40.50	0.48
Plant height at flowering(cm)	73.70	23.94	28.38	144.94	116.5	1.09
Leaf number (number)	29.69	11.13	12.04	38.29	26.25	0.51
Leaf area(cm ²)	69.91	33.07	11.06	120.15	108.5	1.51
Leaf length(cm)	14.39	3.29	4.24	17.70	13.46	0.15
Leaf width(cm)	7.09	1.64	2.53	10.01	7.48	0.08
Leaf thickness(mm)	0.38	0.09	0.30	0.48	0.18	0.00
Petiole length(cm)	6.26	1.53	2.49	8.39	5.90	0.07
Leaf yield(t/ha)	10.31	4.69	5.11	21.69	16.58	0.21
Branch number(number)	28.96	8.48	15.79	42.92	27.13	0.39
Basal lateral branch length(cm)	51.93	45.82	10.88	156.22	145.3	2.09
Top lateral branch length(cm)	21.05	10.13	10.79	45.27	34.47	0.46
Node number (number)	26.51	12.75	12.96	33.96	21.00	0.58
Days to maturity(days)	96.20	15.37	79.75	155.00	75.25	0.70
Plant height at maturity(cm)	209.25	60.71	118.65	343.36	224.7	2.77
Stem diameter (cm)	28.33	7.86	18.33	43.58	25.26	0.36
Auxiliary inflorescence length(cm)	12.04	6.61	2.73	25.33	22.60	0.30
Terminal inflorescence lateral length(cm)	17.91	8.64	13.10	28.77	15.67	0.39
Terminal inflorescence stalk length(cm)	28.96	10.11	13.35	47.98	34.64	0.46
Grain filling periods(days)	49.94	13.52	34.50	100.25	65.75	0.62
Grain sink filling rate (kg ha ⁻¹ day ⁻¹)	30.11	22.99	3.76	94.00	90.24	1.05
Thousand seed weight(g)	0.86	0.44	0.30	1.45	1.15	0.02
Grain yield (t ha ⁻¹)	1.39	0.96	0.23	3.32	3.09	0.04

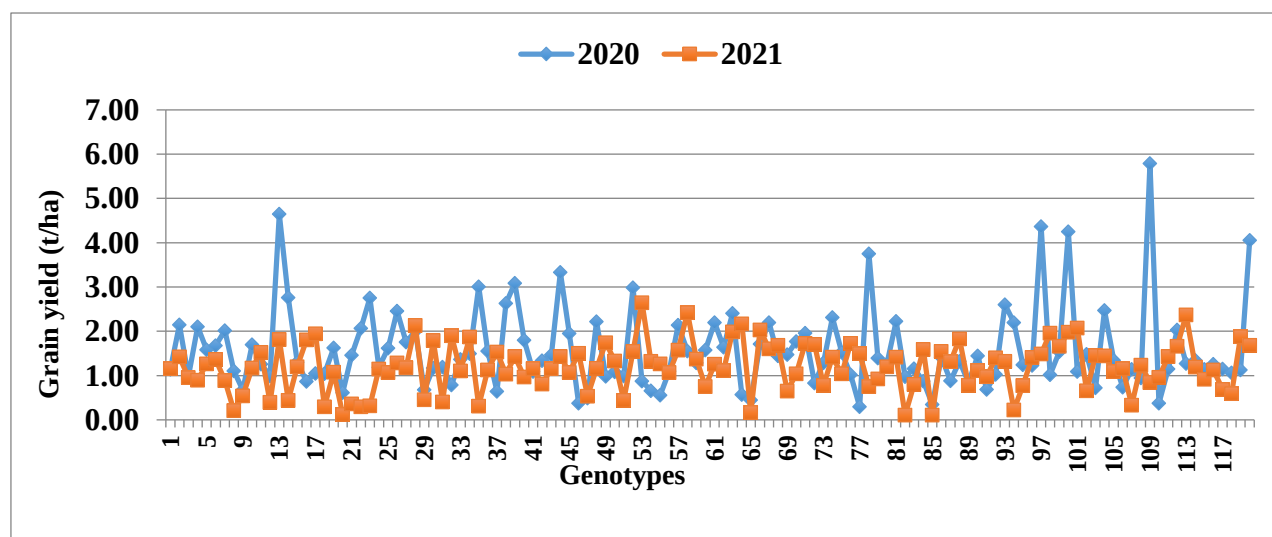


Figure 3 2 Genotype-by-year interaction (crossover) for grain yield of 120 amaranths genotypes grown at two years in 2020 and 2021 cropping seasons.

3.3.3 Effect of test cropping seasons

Table 3.6 indicates how experimental cropping seasons impacted the mean performance of agro-morphological characters in amaranth genotypes. Several agro-morphological parameters of the amaranth genotypes were significantly different between the two cropping seasons. Days to emergence (days), days to flowering (days), leaf length (cm), leaf width (cm), leaf thickness (mm), petiole length (cm), node number (number), days to maturity (days), plant height at maturity (cm), stem diameter (cm), terminal lateral inflorescence length (cm), grain sink filling rate (kg ha⁻¹day⁻¹), thousand seed weight and grain yield (t ha⁻¹) were all significantly higher in 2020 than in 2021. Whereas, plant height at flowering (cm), leaf area (cm²), leaf yield (t/ha), branch number (number), top lateral branch length (cm), auxiliary inflorescence length (cm), terminal inflorescence stalk length (cm), and grain filling periods (days) did not differ significantly between cropping seasons. The mean performance of all traits was greater in the cropping seasons in 2020 than in 2021.

Table 3.6 Mean performance for 24 agro-morphological traits of 120 amaranth genotypes grown in the 2020 and 2021 cropping seasons

Traits	Cropping seasons (Years)		R ²	P- Value
	2020	2021		
Days to emergence (days)	7.20 ^a	6.10 ^b	0.90	0.003
Days to flowering(days)	48.2 ^a	44.3 ^b	0.91	0.030
Plant height at flowering (cm)	74.70	72.70	0.85	0.378
Leaf number (number)	39.00 ^a	20.40 ^b	0.96	0.011
Leaf area (cm ²)	73.60	66.20	0.81	0.703
Leaf length(cm)	15.90 ^a	12.90 ^b	0.92	0.013
Leaf width (cm)	7.90 ^a	6.30 ^b	0.93	0.011
Leaf thickness (mm)	0.50 ^a	0.30 ^b	0.92	0.015
Petiole length (cm)	7.00 ^a	5.50 ^b	0.89	0.010
Leaf yield (t/ha)	11.20	9.40	0.65	0.406
Branch number (number)	29.40	28.60	0.89	0.426
Basal lateral branch length (cm)	61.10 ^a	42.80 ^b	0.93	0.063
Top lateral branch length (cm)	21.80	20.30	0.88	0.211
Node number (number)	38.20 ^a	14.90 ^b	0.98	0.005
Days to maturity (days)	97.39 ^a	95.00 ^b	0.91	0.021
Plant height at maturity (cm)	237.30 ^a	181.02 ^b	0.96	0.000
Stem diameter (cm)	32.80 ^a	23.90 ^b	0.91	0.008
Auxiliary inflorescence length (cm)	12.40	11.70	0.90	0.472
Terminal lateral inflorescence length (cm)	24.90 ^a	10.90 ^b	0.97	0.001
Terminal inflorescence stalk length (cm)	29.00	28.90	0.89	0.907
Grain sink filling rate (kg ha ⁻¹ day ⁻¹)	34.40 ^a	25.80 ^b	0.85	0.026
Grain-filling periods (days)	50.70	49.20	0.71	0.100
Thousand seed weight (g)	0.88	0.83	0.69	0.007
Grain yield (t ha ⁻¹)	1.60 ^a	1.20 ^b	0.70	0.023

R²= coefficient of determination

3.3.4 Mean Performance of Genotypes

Over several traits, genotype differences were substantial (Figure 3.7). It was clear that most genotypes of amaranth outperformed or had superior agro-morphological performances when compared to the overall mean of the population and newly released variety (AC-NL and Madiira II), including the thousand seed weight (g), leaf number (number), leaf area (cm²), leaf length (cm), leaf width (cm), leaf thickness (mm), days to emergence (days), petiole length (cm), and node number (number), top lateral branch length (cm), and days to maturity (days) (Figure 3.7A). In contrast, several newly introduced varieties outperformed the population means when evaluated across all agro-morphological parameters, particularly grain, and leaf yield traits. So, comparisons at both levels (with population and average performances of released varieties) showed that there were genotypes comparable to the released genotypes for all 24 traits (Figure 3.7B). The differences between the numbers of superior and inferior genotypes for most studied traits were higher when the tested genotypes were compared with the mean of released varieties than the mean of the population.

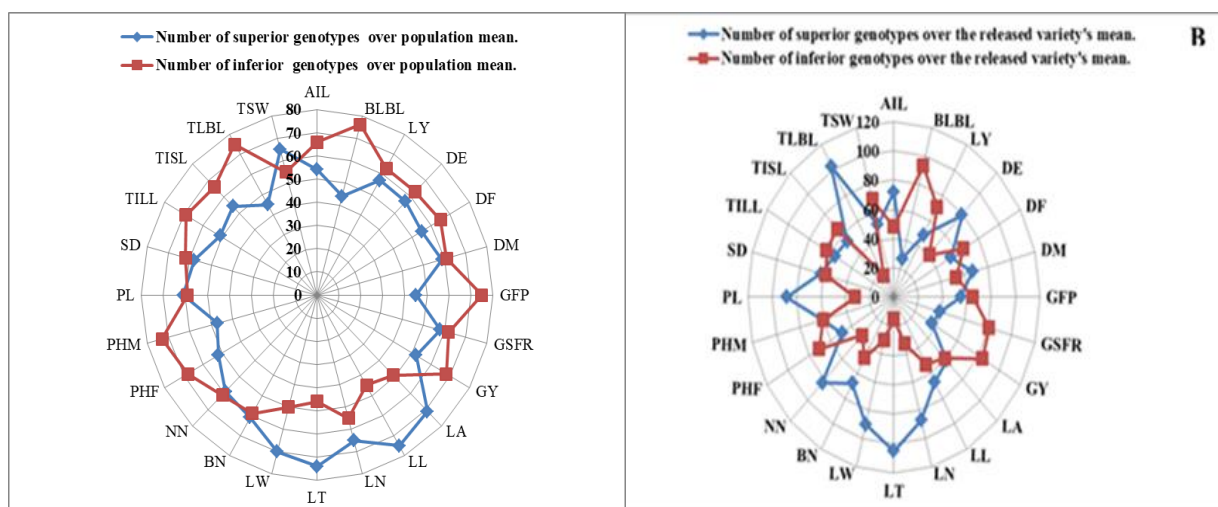


Figure 3.3 The number of superior and inferior amaranth genotypes over (A) the population means and (B) the mean of released varieties grown in 2020 and 2021.

Abbreviated names (codes) of different traits; DE=Days to emergence; AIL= Axillary inflorescence Length; LY = leaf yield; DF= Days to 50% flowering; GY= Grain yield; DM=Days to maturity; LA= Leaf area; LL= Leaf length; LW =Leaf width; BN = Branch Number; LN =Leaf number; NN = Node number; PHF= Plant height at flowering; PHM= Plant height at maturity; SD= Stem diameter; TILL =Terminal inflorescence laterals length; TISL= Terminal inflorescence stalk length; GFP= Grain filling period; GSFR= Grain sink filling rate; BLBL= Basal lateral branch length; LL=Leaf thickness; PL= Petiole length; TLBL=Top lateral branch length; TSW=Thousand seed weight

As shown in Table 3.7, the performance of the top 5% of leaf yielder amaranth genotypes in comparison with the bottom 5% of leaf yielder genotypes, the population means, and the mean of two released varieties. The findings showed that in the majority of evaluated phenotypic traits, the top leaf yielder genotypes were superior to the least leaf yielder genotypes, the

population means, and the mean of released varieties. The top leaf yielder genotypes, however, were inferior to the least leaf yielder genotypes, the population means, and released variety mean in terms of days to emergence (days), basal lateral branch length (cm), top lateral branch length (cm), auxiliary inflorescence length (cm), terminal inflorescence lateral length (cm), terminal inflorescence stalk length (cm), grain filling periods (days), and thousand seed weight. Comparably, Table 3.7 compares the performance of the top 5% grain yielder amaranth genotypes with the lowest 5% grain yielder genotypes, the population means, and the mean of two released varieties. The most grain genotypes outperformed the least grain-yielding genotypes in terms of leaf area (cm²), leaf length (cm), leaf width (cm), leaf yield (t/ha), top lateral branch length (cm), auxiliary inflorescence length (cm), terminal inflorescence lateral length (cm), terminal inflorescence stalk length (cm), grain sink filling rate (kg ha⁻¹day⁻¹), thousand seed weight (g). On the other hand, the top grain yielder genotypes were inferior to the least grain yielder genotypes in terms of the number of leaf days to emergence (days), days to flowering (days), plant height at flowering (cm), leaf number (number), branch number (number), basal lateral branch length (cm), days to maturity (days), plant height at maturity (cm), and grain filling periods (days).

Table 3.8 indicates the results of the top 5% of grain yielder genotypes for the assessed phenotypic variables. The top 5% of grain yielder genotypes were KEN-016, KEN-020, KAZ-060, KEN-010, KEN-018, and 225715. The first top grain yielder genotype (KEN-016) had a higher top lateral branch length (cm), terminal lateral inflorescence length (cm), and grain filling rate (kg ha⁻¹day⁻¹). The second top seed yielder genotype (KEN-020) gave a higher basal lateral branch length (cm) and grain filling rate (kg ha⁻¹day⁻¹). The traits of the third-best grain yielder genotype were longer terminal inflorescence stalk length (cm) and auxiliary inflorescence length (KAZ-060). Higher top lateral branch length (cm), auxiliary inflorescence length (cm), and terminal inflorescence stalk length (cm) were obtained by the fourth-best grain-yielding genotype (KEN-010). The fifth-best grain-yielding genotype, KEN 018, exhibited the most days to emergence (days), days to flowering (days), plant height at flowering (cm), leaf number (number), leaf area (cm²), leaf length (cm), and leaf breadth (cm). The top 5% leaf yielder genotypes were KAZ-059, 225713, KAZ-058, KEN-019, 242530, and 212890. Higher days to flowering (days), leaf area (cm²), leaf thickness (mm), and petiole length (cm) were found in the first top leaf yielder genotype (KAZ-059) (Table 3.8). The genotype with the second-highest leaf yield (225713) had higher plant height at blooming (cm), leaf length (cm), branch number (number), basal lateral branch length (cm), days to

maturity (days), plant height at maturity (cm), stem diameter (cm), and grain filling durations (days). The third top grain yielder genotype (KAZ-058) has larger leaves (cm) and longer terminal inflorescence stalks (cm). The fourth-best leaf-yielding genotype (KEN-019) developed more leaves and nodes (number).

Table 3.7 The mean of 5% high and low leaf and grain yielder amaranth genotypes to the mean of the population and released varieties for 24 amaranth traits grown in the 2020 and 2021 cropping seasons.

Trait	High/low Leaf yielder genotypes							High/low grain yielder genotypes						
					Differences							Differences		
	Top (A)	Bottom (B)	MP (C)	MRV (D)				Top (A)	Bottom (B)	MP (C)	MRV (D)			
					A-B	A-C	A-D					(A-B)	(A-C)	(A-D)
DE	6.50	7.1	6.7	6.1	-0.7	-0.2	0.3	6.6	7.3	6.7	6.1	-0.6	0.0	0.5
DF	49.3	34.7	46.3	53.5	14.7	3.1	-4.2	37.4	47.2	46.3	53.5	-10.4	-8.9	-16.1
PHF	84.3	49.2	73.7	76.0	35.1	10.6	8.3	60.5	72.8	73.7	76.0	-12.3	-13.2	-15.5
LN	29.0	22.9	29.7	27.6	6.2	-0.7	1.5	23.8	29.8	29.7	27.6	-6.0	-5.9	-3.8
LA	83.6	39.5	69.9	72.6	44.1	13.7	11.1	14.3	12.9	69.9	72.6	1.4	-55.7	-58.3
LL	15.9	10.2	14.4	14.6	5.7	1.5	1.3	14.3	12.9	14.4	14.6	1.4	-0.1	-0.4
LW	7.3	5.2	7.1	6.5	2.1	0.2	0.8	7.2	6.1	7.1	6.5	1.1	0.1	0.7
LT	0.4	0.4	0.4	0.4	0.0	0.0	0.0	0.4	0.4	0.4	0.4	0.0	0.0	0.0
PL	6.2	5.2	6.3	5.8	1.0	0.0	0.4	5.7	6.0	6.3	5.8	-0.3	-0.6	-0.2
LY	16.2	6.3	10.3	10.7	9.9	5.8	5.4	9.0	8.5	10.3	10.7	0.5	-1.3	-1.7
BN	28.9	22.3	29.0	26.1	6.7	0.0	2.8	22.2	27.6	29.0	26.1	-5.3	-6.7	-3.9
BLBL	37.8	77.7	51.9	62.6	-39.9	-14.1	-24.8	44.7	59.9	51.9	62.6	-15.2	-7.2	-17.9
TLBL	17.7	28.8	21.1	15.1	-11.1	-3.4	2.6	28.2	20.2	21.1	15.1	8.0	7.2	13.1
NN	27.4	22.2	26.5	25.4	5.2	0.9	2.0	22.1	26.8	26.5	25.4	-4.7	-4.4	-3.3
DM	99.2	90.0	96.2	94.5	9.2	3.0	4.7	87.0	110.6	96.2	94.5	-24.3	43.6	-7.1
PHM	209.	151.0	209.	196.4	58.2	-0.1	12.8	183	191.2	209	196.4	-8.2	-26.2	-13.3
SD	29.7	20.3	28.3	28.0	9.4	1.4	1.7	25.3	26.5	28.3	28.0	-1.2	-3.0	-2.7
AIL	12.8	15.1	12.0	9.6	-2.4	0.7	3.2	18.9	10.0	12.0	9.6	8.9	6.9	9.3
TILL	17.0	18.6	17.9	17.5	-1.7	-1.0	-0.6	24.4	15.9	17.9	17.5	8.5	6.5	6.9
TISL	30.3	32.0	29.0	29.5	-1.8	1.3	0.8	37.6	23.7	29.0	29.5	13.9	8.7	8.1
GFP	49.9	55.3	49.9	47.1	-5.4	-0.1	2.8	49.5	63.5	49.9	47.1	-13.9	-0.4	2.4
GSFR	29.7	25.3	30.1	34.0	4.4	-0.4	-4.3	64.4	7.7	30.1	34.0	56.7	34.3	30.4
TSW	0.8	1.0	0.9	0.9	-0.2	0.0	-0.1	0.9	0.7	0.9	0.9	0.2	0.1	0.0
GY	1.4	1.3	1.4	1.5	0.1	-0.1	-0.2	3.0	0.4	1.4	1.5	2.6	1.6	1.4

Top 5% High leaf yielder amaranth genotypes (1st = KAZ-059; 2nd= 225713; 3rd= KAZ-058; 4th = KEN-019; 5th= 242530; 6th= 212890); and 5% low leaf yielder amaranth genotypes (115th = DAL-042; 116th = KAZ-076; 117th = SRA-002; 118th = 208683; 119th = KEN-010; 120th = HA-008); Top 5% high grain yielder amaranth genotypes (1st= KEN-016; 2nd = KEN-020; 3rd= KAZ-060; 4th= KEN-010; 5th= KEN-018; and 6th=225715); and 5% low leaf yielder amaranth genotypes (115th = DAL-042; 116th = SA-001/07; 117th = 242534; 118th = 225718; 119th = 212583; 120th = 242531). Abbreviated names (codes) of different traits; DE=Days to emergence; AIL= Axillary inflorescence Length; LY = leaf yield; DF= Days to 50% flowering; GY= Grain yield; DM=Days to maturity; LA= Leaf area; LL= Leaf length; LW =Leaf width; BN = Branch Number; LN =Leaf number; NN = Node number; PHF= Plant height at flowering; PHM= Plant height at maturity; SD= Stem diameter; TILL =Terminal inflorescence laterals length; TISL= Terminal inflorescence stalk length; GFP= Grain filling period; GSFR= Grain sink filling rate; BLBL= Basal lateral branch length; LL=Leaf thickness; PL= Petiole length; TLBL=Top lateral branch length; TSW=Thousand seed weight

Table 3.8 Mean traits performance of top 5% leaf and grain yielder genotypes for 24 traits of amaranth grown in the 2020/2021 cropping season.

Traits	Leaf yield-producing genotypes						Grain yield-producing genotypes					
	1 st	2 nd	3 rd	4 th	5 th	6 th	1 st	2 nd	3 rd	4 th	5 th	6 th
Days to emergence (days)	5.8	6.8	5.5	6.8	6.5	7.5	7.0	6.5	6.5	6.5	7.3	6.0
Days to flowering (days)	48.3	59.8	44.5	44.8	46.3	52.5	34.3	43.3	32.3	31.5	50.8	32.5
Plant height at flowering (cm)	71.9	144.9	57.6	62.6	76.1	92.6	44.5	47.4	47.7	52.8	93.0	77.8
Leaf number (number)	28.4	27.8	31.6	34.2	25.8	26.5	15.1	31.3	19.9	17.2	35.3	24.2
Leaf area (cm ²)	92.3	80.7	87.0	62.1	81.0	98.8	63.6	46.0	49.0	49.2	77.7	75.8
Leaf length (cm)	15.6	17.2	14.4	15.6	16.0	16.8	12.5	14.6	12.5	12.6	17.5	16.0
Leaf width (cm)	7.5	6.6	7.9	6.8	7.1	7.8	6.7	6.4	6.4	6.6	10.0	7.0
Leaf thickness (mm)	0.4	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.4	0.5
Petiole length (cm)	7.1	6.1	6.3	5.2	5.9	6.8	4.5	4.6	5.0	4.8	7.5	7.6
Leaf yield (t/ha)	21.7	15.6	15.3	14.9	14.8	14.8	9.3	7.8	8.4	6.8	12.8	8.9
Branch number (number)	25.6	39.9	22.3	25.6	23.9	36.4	18.2	22.8	17.7	17.0	39.2	18.5
Basal lateral branch length (cm)	21.0	71.5	17.9	30.0	58.0	28.4	31.7	57.4	39.7	43.9	48.0	47.7
Top lateral branch length (cm)	15.7	13.2	16.2	17.8	23.2	20.0	38.4	12.3	32.2	34.3	23.9	28.1
Node number (number)	27.2	25.3	27.4	32.3	24.3	27.8	16.6	29.4	19.0	17.9	27.8	22.0
Days to maturity(days)	82.8	128.0	85.5	98.3	93.5	107.3	82.5	80.5	82.5	82.5	106.3	87.5
Plant height at maturity (cm)	181.4	236.3	156.9	190.9	230.0	259.4	153.2	157.7	148.8	137.3	232.5	268.8
Stem diameter (cm)	28.1	36.1	24.9	28.5	32.7	27.8	24.1	29.9	22.0	18.9	34.0	22.9
Auxiliary inflorescence length (cm)	9.0	6.8	16.5	13.1	14.0	17.2	19.7	9.2	23.2	22.4	17.6	21.3
Terminal lateral inflorescence length (cm)	16.6	14.0	19.9	19.4	17.3	14.6	26.8	17.9	28.8	25.6	23.3	24.0
Terminal inflorescence stalk length (cm)	24.7	24.6	32.8	33.4	33.8	32.6	38.2	24.8	42.6	41.8	39.3	39.2
Grain-filling periods (days)	34.5	68.3	41.0	53.5	47.3	54.8	48.3	37.3	50.3	51.0	55.5	55.0
Grain sink filling rate (kg ha ⁻¹ day ⁻¹)	41.1	19.3	32.8	13.1	43.5	28.3	71.6	94.0	64.5	60.0	50.3	46.1
Thousand seed weight (g)	1.1	0.5	1.1	0.6	0.7	0.9	0.7	1.0	0.8	1.2	0.9	0.9
Grain yield (t ha ⁻¹)	1.3	1.3	1.3	0.7	2.0	1.5	3.3	3.2	3.1	2.9	2.9	2.4

Top 5% high leaf yielder amaranth genotypes (1st= KAZ-059; 2nd= 225713; 3rd= KAZ-058; 4th = KEN-019; 5th= 242530; 6th= 212890); Top 5% high grain yielder amaranth genotypes (1st= KEN-016; 2nd = KEN-020; 3rd= KAZ-060; 4th= KEN-010; 5th= KEN-018; and 6th=225715)

Using certain agro-morphological parameters, the performance of the top 5% of genotypes is compared with the population means and the mean of the released variety (Table 3.9). When compared to the released varieties, no single genotype consistently outperformed the top 5% of best performers for various parameters such as grain yield, grain sink filling rate ($\text{kg ha}^{-1}\text{day}^{-1}$), days to maturity (days), and grain filling time (days). Many other superior genotypes for multiple traits of agronomic significance, including grain yield, were also identified. The best genotypes for grain yield (t ha^{-1}) were found to be superior by 71.22%–138.85% to the population mean and 53.55%–114.9% to the mean of grain yield performance of the released varieties; leaf yield (t/ha) 43.06%–110.38% to the population mean and 37.59%–102.33% to the mean leaf yield performance of the released varieties; plant height at maturity (cm) 36.86%–64.09% to the population mean and 45.85%–74.86% to the mean plant height at maturity performance of the released varieties; and grain sink filling rate ($\text{kg ha}^{-1}\text{day}^{-1}$) 67.92% - 212.19% to the population mean and 15.42% - 28.97% to the released varieties' mean grain sink filling rate performance; grain filling periods (days) 38.67%-100.74% to the population mean and 46.93%-112.71% to the released varieties' mean grain filling periods performance; thousand seed weight (g) 53.49%-68.60% to the population mean and 40.43%-54.26% to the released varieties' mean thousand seed weight performance; days to 50% flowering (days) 33.46%-49.12% to population mean and 15.42%-28.97% days to flowering performance of released varieties; days to maturity (days) 30.98%-61.112% to population mean and 33.33%-64.02% days to maturity performance of released varieties; terminal inflorescence stalk length (cm) 46.75%-65.68% to population mean and 43.97%-62.53% terminal inflorescence stalk length performance of released varieties; and leaf area (cm^2) 44.31%-71.86% to population mean, and 39.01%-65.4% leaf area performance of released varieties.

Table 3.9 Mean performance comparison of top 5% genotypes with mean of the population and released varieties for 10 traits of amaranth grown in the 2020 and 2021 cropping seasons.

Relative advantage (%) over				Relative advantage (%) over			
Genotypes	Mean	MP	MRV	Genotypes	Mean	MP	MRV
1. Plant height at maturity (cm)				2. Leaf yield (t/ha)			
215567	343.36	64.09%	74.86%	KAZ-059	21.69	110.38%	102.33%
MEK-084	292.97	40.01%	49.20%	225713	15.56	50.92%	45.15%
KEN-017	292.68	39.87%	49.05%	KAZ-058	15.26	48.01%	42.35%
MEK-083	289.42	38.31%	47.39%	KEN-019	14.88	44.33%	38.81%
BKD-031	287.24	37.27%	46.28%	242530	14.77	43.26%	37.78%
HAL-039	286.39	36.86%	45.85%	212890	14.75	43.06%	37.59%
MP	209.25	-----	6.56	MP	10.31	-----	-3.64

MRV	196.36	-6.16	---	MRV	10.72	3.98	---
3. Grain yield (t ha⁻¹)				4. Thousand seed weight (g)			
KEN-016	3.32	138.85%	114.19%	SAA-004	1.45	68.60%	54.26%
KEN-020	3.23	132.37%	108.39%	ALE-074	1.43	66.28%	52.13%
KAZ-060	3.12	124.46%	101.29%	MEK-081	1.38	60.47%	46.81%
KEN-010	2.93	110.79%	89.03%	212890	1.34	55.81%	42.55%
KEN-018	2.87	106.47%	85.16%	BKD-031	1.32	53.49%	40.43%
225715	2.38	71.22%	53.55%	KAZ-078	1.32	53.49%	40.43%
MP	1.39	---	-10.32	MP	0.86	--	-8.51
MRV	1.55	11.51	--	MRV	0.94	9.30	--
5. Days to 50% flowering(days)				6. Grain filling period (days)			
240815	69.00	49.12%	28.97%	212582	100.25	100.74%	112.71%
211456	63.25	36.70%	18.22%	HA-003	79.50	59.19%	68.68%
242531	62.50	35.08%	16.82%	212583	78.75	57.69%	67.09%
CHU-045	62.25	34.54%	16.36%	242534	73.50	47.18%	55.95%
241764	62.00	34.00%	15.89%	WA-001	70.00	40.17%	48.53%
242534	61.75	33.46%	15.42%	SHIA-007	69.25	38.67%	46.93%
MP	46.27	--	-13.51	MP	49.94	--	5.96
MRV	53.50	15.63	--	MRV	47.13	-5.63	--
7. Grain sink filling rate (kg ha⁻¹day⁻¹)				8. Days to maturity (days)			
KEN-020	94.00	212.19%	176.88%	212582	155.00	61.12%	64.02%
KEN-016	71.57	137.70%	110.81%	242534	135.25	40.59%	43.12%
KAZ-060	64.49	114.18%	89.96%	242531	130.50	35.65%	38.10%
KEN-010	59.99	99.24%	76.70%	212583	130.50	35.65%	38.10%
242533	55.13	83.10%	62.39%	225713	128.00	33.06%	35.45%
KEN-014	50.56	67.92%	48.92%	241764	126.00	30.98%	33.33%
MP	30.11	--	-11.31	MP	96.20	--	1.80
MRV	33.95	12.75	--	MRV	94.5	-1.77	--
9. Leaf area (cm²)				10. Terminal Inflorescence Stalk Length(cm)			
ALE-023	120.15	71.86%	65.54%	215567	47.98	65.68%	62.53%
KAZ-055	119.90	71.51%	65.20%	CHU-045	46.53	60.67%	57.62%
ALE-034	119.66	71.16%	64.87%	240814	45.11	55.77%	52.81%
DRA-053	109.28	56.32%	50.56%	212892	43.83	51.35%	48.48%
ALE-068	108.67	55.44%	49.72%	KAZ-060	42.57	47.00%	44.21%
KAZ-057	100.89	44.31%	39.01%	219284	42.50	46.75%	43.97%
MP	69.91	--	-3.68	MP	28.96	--	-1.90
MRV	72.58	3.82	--	MRV	29.52	1.93	--

MP = Mean of the population; MRV = Mean of released varieties

A student t-test was used to compare the mean of the top 5% genotypes to the mean of the population was varied ($P \leq 0.0001$) for all measured phenotypic traits (Table 3.10). The t-test showed highly significant differences between means of the selected subsets of the top 5% best genotypes ($\bar{x}$) and the population parameters (μ) for days to emergence (days), days to flowering (days), plant height at flowering (cm), leaf area (cm²), leaf length (cm), leaf yield (t/ha), leaf width (cm), petiole length (cm), leaf thickness (mm), top lateral branch length (cm), node number (number), days to maturity (days), plant height at maturity (cm), leaf number (number), stem diameter (cm), auxiliary inflorescence length (cm), terminal lateral inflorescence length (cm), branch number (number), basal lateral branch length (cm), terminal

inflorescence stalk length (cm), grain filling periods (days), grain sink filling rate (kg ha⁻¹day⁻¹), grain yield (t ha⁻¹), and thousand seed weight (g). The findings showed that the top 5% of genotypes had greater relative advantages in all agro-morphological variables, with differences between them and the population means performances ranging from 15.90% for leaf thickness to 184.11% for basal lateral branch length. Each day, the top 5% of genotypes produced 119.05% greater grain, per hectare than the population as a whole. Additionally, the top 5% of genotypes exhibited advantages in leaf area(cm²), leaf yield (t/ha), terminal inflorescence stalk length (cm), thousand seed weight (g), and grain yield (t ha⁻¹) of 61.77, 56.66, 54.53, and 114.03% above the mean performances of the population, respectively, showing the occurrence of various degrees of amaranths enhancements through selection.

Table 3.10 Comparison of mean performances of selected top 5% genotypes with the population mean performances for 24 traits of amaranth grown in the 2020 and 2021 cropping seasons.

Traits	The mean of selected genotypes ($\bar{X}$)	Population parameter (μ)	Change through selection ($\bar{X}-\mu$)	Change as % of a population parameter (μ)	T-test
Days to emergence (days)	8.88	6.65	2.23	33.46	14.37***
Days to flowering (days)	63.46	46.27	17.19	37.15	15.24***
Plant height at flowering (cm)	115.02	73.7	41.32	56.07	6.75***
Leaf number (number)	36.96	29.69	7.27	24.48	25.18***
Leaf area (cm ²)	113.09	69.91	43.18	61.77	13.17***
Leaf length (cm)	17.54	14.39	3.15	21.88	58.16***
Leaf width (cm)	9.06	7.09	1.97	27.79	10.14***
Leaf thickness (mm)	0.44	0.38	0.06	15.90	7.68***
Petiole length (cm)	7.77	6.26	1.51	24.05	11.74***
Leaf yield (t/ha)	16.15	10.31	5.84	56.66	5.24***
Branch number (number)	41.34	28.96	12.38	42.76	19.73***
Basal lateral branch length (cm)	147.54	51.93	95.61	184.11	35.57***
Top lateral branch length (cm)	38.98	21.05	17.93	85.16	11.69***
Node number (number)	32.58	26.51	6.07	22.91	19.04***
Days to maturity (days)	134.21	96.20	38.01	39.51	8.74***
Plant height at maturity (cm)	298.68	209.25	89.43	42.74	9.93***
Stem diameter (cm)	39.07	28.33	10.74	37.92	10.28**
Auxiliary inflorescence length (cm)	23.76	12.04	11.72	97.36	33.19***
Terminal lateral inflorescence length (cm)	27.17	17.91	9.26	51.70	17.13***
Terminal inflorescence stalk length (cm)	44.75	28.96	15.79	54.53	17.51***
Grain-filling periods (days)	105.32	49.94	55.38	110.89	6.11***
Grain sink filling rate (kg ha ⁻¹ day ⁻¹)	65.96	30.11	35.85	119.05	5.64***
Thousand seed weight (g)	1.37	0.86	0.51	59.69	22.27***
Grain yield (t ha ⁻¹)	2.98	1.39	1.59	114.03	11.48***

T = Student t-test;*** = significantly different at 0.1% probability level.; The tabulated t-value for 5 degrees of freedom is 6.869 for two-tailed tests and 5.894 for one-tailed tests at 0.1% probability .

3.3.5 Variance component and coefficient of variation

Genetic parameters such as genotypic variance (δ^2g), phenotypic variance (δ^2p), environmental variances (δ^2e), genotypic variance with year interactions (δ^2gy), genotypic co-efficient of variation (GCV), phenotypic coefficient of variation (PCV), and environmental co-efficient of variation (ECV) were calculated for each of the evaluated quantitative traits (Table 3.11). For all of the traits, the assessment of the variance's components revealed a wide range of variation and substantial disparities. The result reveals that for all examined variables, the phenotypic variance was greater than the comparable genotypic variance. Concerning all examined phenotypic variables; the phenotypic variance was likewise greater than the corresponding genotype-by-year interaction variance and environmental variance. The genotypic variance overshadowed genotype-by-year interactions, except for days to emergence, leaf thickness, petiole length, leaf yield, top lateral branch length, node number, terminal lateral inflorescence length, and terminal inflorescence stalk length, grain sink filling rate, thousand seed weight, and grain yield. Similarly, the genotypic variance was higher than the corresponding environmental variance in the days to flowering, plant height at flowering, leaf area, leaf length, leaf width, branch number, basal lateral branch length, days to maturity, plant height at maturity, stem diameter, auxiliary inflorescence length, and grain filling periods.

More than 33.33 percent of the quantitative variables exhibited high phenotypic and genotypic coefficients of variation (PCV and GCV, respectively). Plant height at flowering, leaf area, branch number, auxiliary inflorescence length, basal lateral branch length, plant height at maturity, grain sink filling rate, and grain yield all had high genotypic and phenotypic coefficients of variation. The traits with medium GCV and PCV comprised days to flowering, leaf length, leaf width, days to maturity, terminal lateral inflorescence length, stem diameter, days to grain filling periods, and thousand seed weight. Leaf yield and top lateral branch length showed high PCV and low GCV values, but days to emergence, the number of leaves, petiole length, and the number of nodes showed medium PCV and low GCV values. Leaf thickness is revealed by low PCV and GCV. Likewise, estimates of the environmental coefficient of variation (ECV) ranged from 6.88 (for days to maturity) to 58.76 for grain sink filling rate. For each trait, the estimates of the genotypic coefficient (GCV) were lower than the corresponding phenotypic coefficient of variation (PCV). The petiole length, leaf yield, basal lateral branch length, node number, top lateral branch length, terminal lateral inflorescence

length, grain sink filling rate, thousand seed weight, and grain yield showed a wide difference between the phenotypic and genotypic coefficient of variations, while the remaining traits all exhibited a slight difference.

Table 3.11 Components of variance and coefficient of variation for 24 traits of amaranth grown in the 2020 and 2021 cropping seasons.

	Variance component						Coefficient of variation		
	δ^2r	δ^2y	δ^2e	δ^2g	δ^2gy	δ^2p	PCV	GCV	ECV
Days to emergence (days)	0.0	0.1	0.3	0.3 ±0.1	0.5±0.09	0.7±0.1	12.2	8.3	8.7
Days to flowering(days)	0.3	0.4	22.3	62.7±10.4	27.1±5.1	82.2±10.7	19.6	17.1	10.2
Plant height at flowering(cm)	1.4	0.0	179.6	226.3±47.8	153.5±33.3	350.8±50.7	25.4	20.4	18.2
Leaf number (number)	3.9	21.6	10.0	8.6±2.7	14.9±2.6	18.7±3.0	14.6	9.9	10.6
Leaf area(cm ²)	278.9	3.2	395.4	415.5±69.4	15.1±6.1.	528.2±71.9	32.9	29.2	28.4
Leaf length(cm)	0.1	0.6	1.8	4.9±0.8	1.9±0.4	6.4±0.9	17.6	15.5	9.4
Leaf width(cm)	0.0	0.2	0.4	1.3±0.2	0.4±0.1	1.6±0.2	17.7	15.9	9.4
Leaf thickness(mm)	0.0	0.0	0.0	0.0±0.0	0.0±0.0	0.0±0.0	6.1	0.0	9.9
Petiole length(cm)	0.0	0.2	0.6	0.1±0.1	1.1±0.2	0.8±0.2	13.9	4.4	12.1
Leaf yield(t/ha)	3.0	0.2	15.6	1.0±0.43	1.7±1.5	6.0±1.3	23.8	9.8	38.3
Branch number(number)	0.5	0.0	16.6	45.5±7.0	8.5±2.4	54.2±7.2	25.4	23.3	14.1
Basal lateral branch length(cm)	20.7	19.9	315.7	969.8±190.4	721.3±115.3	1414.4±199.0	72.4	60.0	34.2
Top lateral branch length(cm)	0.5	0.1	25.0	2.1±1.7	79.1±19.7	44.1±11.3	31.5	0.0	23.8
Node number (number)	2.0	33.9	8.2	2.5±1.8	12.6±2.2	10.9±2.1	12.5	5.9	10.8
Days to maturity(days)	-0.2	0.0	43.8	136.5±21.5	47.9±9.4	172.1±22.1	13.6	12.1	6.9
Plant height at maturity(cm)	-2.3	195.9	333.1	1926.7±305.2	651.2±107.8	2340.9±310.0	23.1	21.0	8.7
Stem diameter (cm)	0.6	4.9	12.3	24.7±3.9	4.3±1.5	30.1±4.0	19.4	17.6	12.4
Auxiliary inflorescence length(cm)	0.7	0.0	9.5	21.6±4.0	11.5±2.2	29.9±4.1	45.4	38.6	25.6
Terminal lateral inflorescence length(cm)	0.2	12.2	4.5	4.4±2.1	15.5±2.3	13.4±2.4	20.4	11.8	11.9
Terminal inflorescence stalk length(cm)	1.2	-0.1	25.5	23.6±8.3	51.1±8.4	55.9±9.3	25.8	16.8	17.4
Grain-filling periods(days)	-0.2	0.1	58.5	68.5±14.8	48.3±10.6	108.2±15.8	20.8	16.6	15.3
Grain sink filling rate (kg ha ⁻¹ day ⁻¹)	-1.2	4.3	313.0	57.1±32.2	126.6±40.9	203.7±38.6	47.4	25.1	58.8
Thousand seed weight(g)	0.0	0.0	0.1	0.0±0.0	0.0±0.0	0.1±0.0	31.2	13.4	41.9
Grain yield (t ha ⁻¹)	0.0	0.0	0.6	0.1±0.1	0.2±0.07	0.3±0.1	42.0	22.3	54.3

σ^2y = year variance; σ^2r = Replication variance; σ^2b = block variance; σ^2g = Genotypic variance; σ^2gy = Genotypic by year interaction variance; σ^2e = Error variance; σ^2p = Phenotypic variance; PCV = Phenotypic coefficient of variation; GCV = Genotypic coefficient of variation; ECV = Environmental coefficient of variation

3.3.6 Heritability and genetic advance

Estimates of heritability in a broad sense (h^2_{bs}) for 24 quantitative traits of amaranth genotypes are presented in (Figure 3.8). The heritability estimates in a broad sense (h^2_{bs} %) in the traits examined revealed large variances, ranging from 4.9% for top lateral branch length to 84.0% for the number of branches. The branch number (84.0%), plant height at maturity (82.3%), stem diameter (82.1%), and leaf width (80.4%) all showed heritability in a broad sense of greater than 80%, revealing very high heritability. Days to maturity (79%), leaf area (78.7%), leaf length (77.3%), days to flowering (76.3%), auxiliary inflorescence length (72.3%), basal lateral branch length (68.6%), plant height at flowering (64.5%), grain filling periods (63.3%), showed moderately high heritability. The estimate of heritability of leaf thickness (50.0%), days to emergence (46.5%), leaf number (45.9%), and terminal inflorescence stalk length (42.2%) was in the medium category, and other traits were low.

As depicted in Figure 3.8 the estimates of genetic advance (GA) for evaluated phenotypic variables in amaranth genotypes vary greatly. The values of GA for the phenotypic traits that were examined ranged from 0.0 to 63.0%. Plant height at maturity (63.0%), followed by basal lateral branch length (44.7%), leaf area (29.26%), plant height at flowering (21.59%), days to maturity (16.8%), grain filling periods (11.88%), days to flowering (11.36%), and grain sink filling rate (10.85%) were all observed relatively as having the highest GA values. The GA (≤ 10) values for the remaining sixteen phenotypic variables varied from 0.0% for top lateral branch length and leaf thickness to 9.68% for branch number.

The genetic advance as a percent of means (GAM) for measured phenotypic traits in amaranths' genotypes is also presented in Figure 3.8. The values of high GAM ($>20\%$) were recorded for basal lateral branch length (86.1%), auxiliary inflorescence length (55.4%), leaf area (41.8%), grain sink filling rate (36%), grain yield (32%), plant height at maturity (30.1%), plant height at flowering (29.3%), stem diameter (25.2%), days to flowering (24.6%), terminal inflorescence stalk length (24.1%), grain filling periods (23.8%), leaf width (22.8%), and leaf length (22.2%). Thousand seed weight (19.2%), days to maturity (17.4%), terminal inflorescence lateral length (16.9%), leaf yield (14.1%), and days to emergence (11.9%) all had medium GAM (10-20%) values. Node number (8.5%), petiole length (6.3%), leaf thickness, and top lateral branch length both had (0.00%) the variables with the lowest GAM value.

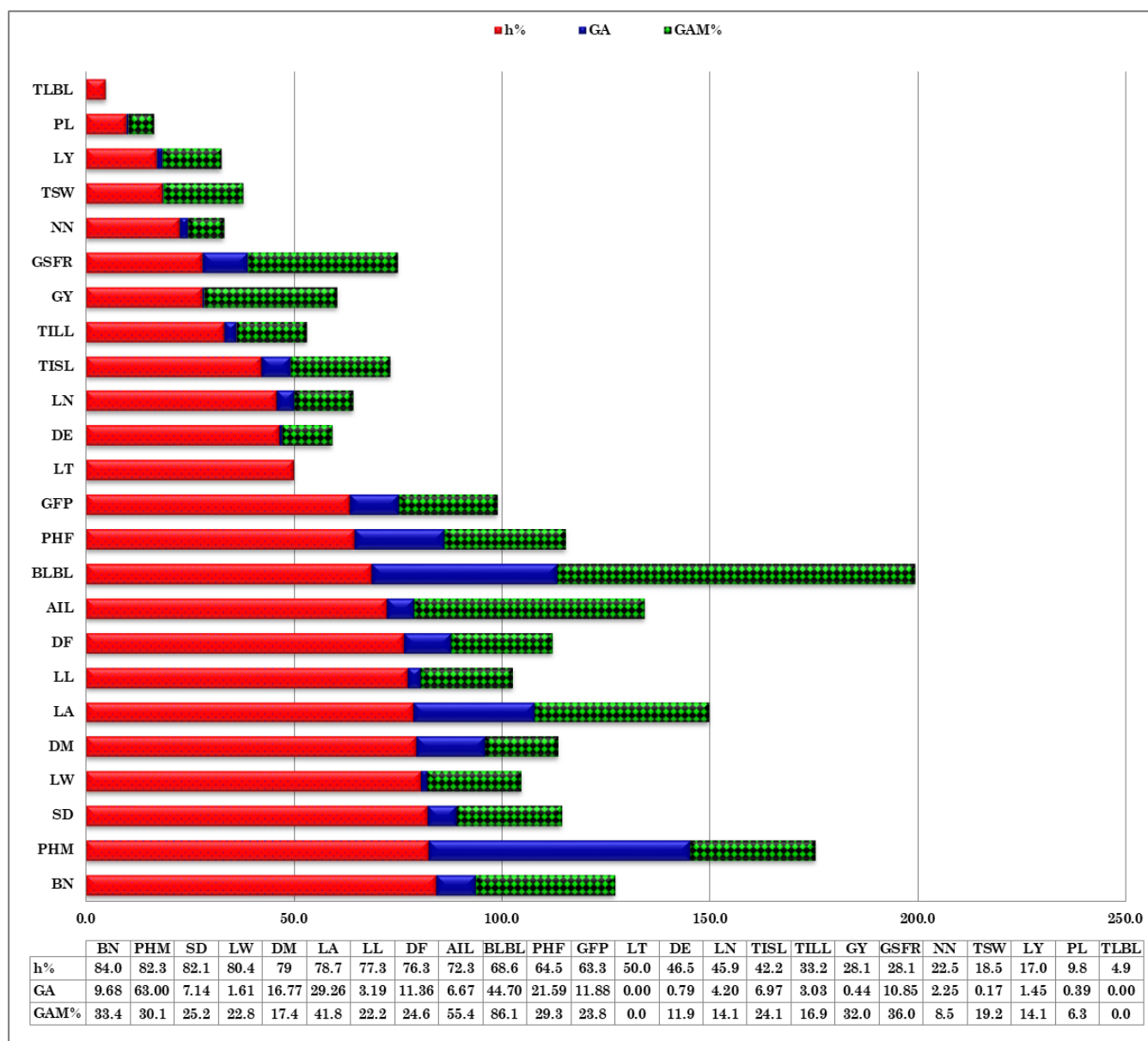


Figure 3.4 Heritability, genetic advance, and genetic advance as percent of the mean for 24 traits of 120 amaranth genotypes grown in the 2020 and 2021 cropping seasons.

Abbreviated names (codes) of different traits; DE=Days to emergence; AIL= Axillary inflorescence Length; LY = leaf yield; DF= Days to 50% flowering; GY= Grain yield; DM=Days to maturity; LA= Leaf area; LL= Leaf length; LW =Leaf width; BN = Branch Number; LN =Leaf number; NN = Node number; PHF= Plant height at flowering; PHM= Plant height at maturity; SD= Stem diameter; TILL =Terminal inflorescence laterals length; TISL= Terminal inflorescence stalk length; GFP= Grain filling period; GSFR= Grain sink filling rate; BLBL= Basal lateral branch length; LL=Leaf thickness; PL= Petiole length; TLBL=Top lateral branch length; TSW=Thousand seed weight

3.3.7 Phenotypic and genotypic correlation of yield-related traits

Out of 91 relations, 71 yield-related trait relationships were significant at the phenotypic level, according to the linear pairwise correlations analysis (Table 3.12). The results revealed a very strong positive correlation between the LW and the LL ($r_p = 0.90$), LA ($r_p = 0.77$), DF ($r_p =$

0.61), PHF ($r_p = 0.48$), and PHM ($r_p = 0.45$). The correlation coefficients of TILL ($r_p = 0.86$), TISL ($r_p = 0.80$), and GSFR ($r_p = 0.30$) were also observed to be highly significant and positive with AIL. On the contrary, it revealed a very highly significant negative phenotypic correlation with PHM ($r_p = -0.56$), BN ($r_p = -0.50$), LN ($r_p = -0.35$), PHF ($r_p = -0.54$), DM ($r_p = -0.42$), and DF ($r_p = -0.64$). BN was significantly and positively correlated with PHM ($r_p = 0.74$), LN ($r_p = 0.39$), PHF ($r_p = 0.66$), DM ($r_p = 0.70$), DF ($r_p = 0.58$), and DE ($r_p = 0.58$), but very negatively correlated with TILL ($r_p = -0.48$) and GSFR ($r_p = -0.36$), as well as highly and negatively correlated with TISL ($r_p = -0.18$). The results also demonstrated a significant negative relationship between LL ($r_p = -0.20$), and DE ($r_p = -0.21$), with the AIL. The LL had a very high positive correlation with LW ($r_p = 0.90$), PHM ($r_p = 0.50$), PHF ($r_p = 0.56$), LA ($r_p = 0.76$), DF ($r_p = 0.62$), and GSFR ($r_p = 0.29$), but a high negative correlation with DE ($r_p = -0.19$). The AIL ($r_p = 0.86$), TISL ($r_p = 0.78$), and GSFR ($r_p = 0.44$) were shown to have a very highly significant positive connection with the TILL. With PHM ($r_p = 0.78$), BN ($r_p = 0.66$), LL ($r_p = 0.56$), DM ($r_p = 0.60$), LA ($r_p = 0.32$), DF ($r_p = 0.82$), and DE ($r_p = 0.32$), it was shown that the PHF had a very highly significant positive association with each of these variables. Strong positive associations with DM were seen for PHM ($r_p = 0.52$), BN ($r_p = 0.70$), LN ($r_p = 0.32$), PHF ($r_p = 0.60$), DF ($r_p = 0.60$), and DE ($r_p = 0.60$). The LA was identified to have a very highly significant negative association with DE ($r_p = -0.34$), but a very highly significant positive connection with LW ($r_p = 0.77$), LL ($r_p = 0.76$), and DF ($r_p = 0.47$). The DE was observed to have a very highly significant negative association with LA ($r_p = -0.34$) but a very highly significant positive relationship with PHM ($r_p = 0.42$), BN ($r_p = 0.579$), PHF ($r_p = 0.32$), and DM ($r_p = 0.36$). DF was significantly positively correlated with BN ($r_p = 0.58$), LL ($r_p = 0.62$), PHF ($r_p = 0.82$), LA ($r_p = 0.47$), and DM ($r_p = 0.82$). AIL ($r_p = 0.30$), LL ($r_p = 0.29$), and TILL ($r_p = 0.44$) were found to have a very highly significant positive correlation with the GSFR, whereas BN ($r_p = 0.36$), LN ($r_p = -0.31$), and DM ($r_p = 0.48$) were found to have a very highly significant negative correlation with the GSFR.

The analysis revealed that among the variables that contribute to yield, 60 associations out of a total of 91 relationships were significant at the genotypic level (Table 3.12). The findings showed that PHM ($r_g = 0.56$), BN ($r_g = 0.17$), LL ($r_g = 0.86$), TILL ($r_g = 0.68$), LN ($r_g = 0.73$), and DF ($r_g = 0.23$) all exhibited a very highly significant positive correlation with the LW. The opposite was true, however, as it showed a very strong negative genotypic relationship ($r_g = -0.35$) with DE. While the AIL displayed a highly significant and negative link with DM ($r_g = -0.14$), it had a very highly significant and positive correlation with TISL ($r_g = 0.38$) and TILL

($r_g = 0.27$) instead. The PHM showed very significant positive relationships with the following variables: BN ($r_g = 0.26$), LL ($r_g = 0.58$), TILL ($r_g = 0.75$), LN ($r_g = 0.70$), DF ($r_g = 0.22$), and GSFR ($r_g = 0.19$); however, the PHM also showed very high negative associations with DE ($r_g = -0.47$). With PHM ($r_g = 0.26$), LL ($r_g = 0.19$), and DM ($r_g = 0.22$), as well as with LN ($r_g = 0.17$), and TISL ($r_g = 0.13$), the BN showed a very strong positive association. The TISL had a very highly significant negative correlation with GSFR ($r_g = -0.25$) but a very highly significant positive correlation with AIL ($r_g = 0.38$) and TILL ($r_g = 0.22$). Concerning LW ($r_g = 0.73$), PHM ($r_g = 0.70$), LL ($r_g = 0.71$), DM ($r_g = 0.21$), and DF ($r_g = 0.28$), the LN exhibited very highly significant positive relationships. Conversely, it showed a very strong negative genotypic relationship with DE ($r_g = -0.55$) and LA ($r_g = -0.24$). The DM noted that BN ($r_g = 0.22$), LL ($r_g = 0.20$), LN ($r_g = 0.21$), and DF ($r_g = 0.28$) all had very highly significant positive correlations. The DF was considerably positively connected with LW ($r_g = 0.23$), PHM ($r_g = 0.22$), LL ($r_g = 0.29$), TILL ($r_g = 0.21$), LN ($r_g = 0.28$), PHF ($r_g = 0.25$), and DM ($r_g = 0.28$), but strongly negatively correlated with DE ($r_g = -0.22$). With LW ($r_g = -0.35$), PHM ($r_g = -0.47$), LL ($r_g = -0.31$), TILL ($r_g = -0.55$), LN ($r_g = -0.55$), DF ($r_g = -0.22$), and GSFR ($r_g = -0.20$), the DE displayed extremely strong negative associations. While TISL ($r_g = -0.25$) showed a very strong and negative association, the GSFR showed extremely substantial and positive correlations with PHM ($r_g = 0.19$).

3.3.7.1 Yields' phenotypic and genotypic associations with other traits

In this study, LY and its yield-contributing characteristics were correlated in 15 ways, with 10 phenotypic associations and 12 genotypic relationships being significant (Table 3.12). The results showed that LY had positive phenotypic associations with LW ($r_p = 0.43$), PHM ($r_p = 0.29$), LL ($r_p = 0.40$), PHF ($r_p = 0.37$), LA ($r_p = 0.45$), DF ($r_p = 0.41$), and BN ($r_p = 0.23$), all of which were highly significant. AIL ($r_p = -0.19$), TISL ($r_p = -0.21$), and TILL ($r_p = -0.19$) all demonstrated a strong negative phenotypic connection with it as well. Additionally, LY had extremely strong positive genotypic correlations with PHM ($r_g = 0.25$), LL ($r_g = 0.21$), LN ($r_g = 0.18$), TILL ($r_g = 0.17$), LA ($r_g = 0.36$), GY ($r_g = 0.20$), and GSFR ($r_g = 0.18$). Additionally, it showed a strong positive genotypic correlation with the BN, PHF, and DF ($r_g = 0.13$, 0.14 , and 0.11 , respectively).

In the current study, GY and its yield-contributing characteristics had 17 correlations, of which 9 genotypic associations and 8 phenotypic relations were significant (Table 3.12). The findings revealed a very strong positive phenotypic correlation between GY and GSFR ($r_p = 0.92$), AIL

($r_p = 0.38$), TISL ($r_p = 0.38$), and TILL ($r_p = 0.51$). Additionally, it showed a substantial positive phenotypic connection with both LW and LL ($r_p = 0.18$ and 0.21 , respectively). Additionally, it had a very strong negative phenotypic correlation ($r_p = -0.24$), ($r_p = -0.30$), and ($r_p = -0.28$) with BN, LN, and DM respectively. GSFR ($r_g = 0.95$), TISL ($r_g = 0.28$), PHM ($r_g = 0.21$), and LY ($r_g = 0.21$) likewise displayed a very highly significant positive genotypic correlation with the GY. Furthermore, it showed a highly significant positive genotypic association with LN ($r_g = 0.16$), a substantial positive genotypic association with DF ($r_g = 0.12$), and a significant positive genotypic association with TILL ($r_g = 0.12$).

3.3.7.2 Phenotypic and genotypic path coefficients on leaf yield

Table 3.13 lists the estimations of the phenotypic effects, both direct and indirect, on LY in Amaranth genotypes. At the phenotypic level, the results showed that LA (0.3849; 47.93%) had the highest positive direct effect on LY, followed by AIL (0.4411; 34.283%), BN (0.3248; 30.17%), LW (0.1465; 17.894%), PHF (0.1420; 12.120%), and LN (0.0417; 7.135%). DM, DF, and GY were additional characteristics that had strong direct effects on LY. TILL (-0.3009; 26.735%), TISL (-0.1246; 12.059%), and PHM (-0.0976; 8.541%) all had detrimental direct effects on LY. Similarly, LL and DE directly have negative effects on LY. Except for AIL, BN, TISL, LN, DM, and DE, the examined characteristics had positive indirect effects on LY via LA. Likewise, GY had positive indirect effects on LY via LW, AIL, PHM, DM, LA, and DE. LW (0.2968; 36.248%), LL (0.2940; 35.758%), DF (0.1826; 14.206%), PHF (0.1230; 10.499%), PHM (0.0796; 6.960%), GY (0.0471; 7.717%), and TILL (0.0027; 0.243%) via LA all had the strongest positive indirect effects on LY. Through LA (0.2968; 36.248%), PHF (0.0681; 8.316%), BN (0.0397; 4.851%), DF (0.0554; 4.118%), TISL (0.0227; 2.777%), DE (0.0080; 0.976%), TILL (0.0044; 0.533%), GY (0.0023; 0.276%), and LN (0.0006; 0.072%), LW had positive indirect effects on LY. Likewise, the positive indirect effects of PHM were observed on LY via BN (0.2396; 20.959%), TILL (0.1317; 11.519%), PHF (0.1110; 9.713%), LW (0.0657; 5.746%), DF (0.0390; 3.415%), TISL (0.0381; 3.329%), and LN (0.0103; 0.903%).

Table 3.14 shows the magnitudes of the direct and indirect genotypic effects of various traits on LY in Amaranth genotypes. LW (0.2225; 38.256%), PHF (0.1378; 73.869%), PHM (0.1277; 23.738%), GY (0.1264; 45.404%), LN (0.0969; 15.269%), TISL (0.0827; 11.643), and BN (0.0168; 8.513%) showed the next largest direct effects on LY after LA (0.3766; 78.608%). TILL (-0.0837; 13.441%), LL (-0.0672; 11.643%), AIL (-0.0760; 30.517%), and

DM (-0.0166; 8.928%) on the other hand, had negative direct effects on LY. DE similarly showed adverse direct effects on LY. Despite having the largest positive direct effect on LY, LN, TISL, TILL, LL, LW, AIL, PHM, and DM had negative indirect effects through LA. BN (0.0351; 17.784%) had strong positive indirect effects on LY via LA, followed by DE (0.0352; 8.591%), GY (0.0218; 7.836%), DF (0.0078; 3.023%), and PHF (0.0066; 3.554%). Similar indirect effects via GY were seen for the following variables: DF (0.0157; 6.104%), PHM (0.0261; 4.858%), LN (0.0202; 3.184%), TILL (0.0110; 2.408%), PHF (0.0041; 2.215%), LW (0.0118; 1.527%), LA (0.0073; 2.408%), and LL (0.0069; 1.198%). But LN (-0.0898; 14.149%) had the most detrimental indirect effect on LY through LA.

3.3.7.3 Phenotypic and genotypic path coefficients on grain yield

Table 3.15 lists the assessed traits' phenotypic direct and indirect effects on GY. According to the findings, the variables that had the largest positive direct effects on GY were GSFR (0.9379; 79.845%), BN (0.1825; 20.246%), PHF (0.141; 18.839%), TILL (0.1620; 17.694%), PHM (0.0678; 9.398%), AIL (0.661; 7.839%), and LA (0.0016; 0.324%). The negative direct effects on GY were also exerted by DF (-0.1182; 16.302%), LW (-0.0789; 13.054%), LY (-0.0306; 10.146%), DE (-0.0452; 8.731%), LL (-0.0349; 5.591%), TISL (-0.0325; 5.053%), and LN (-0.0195; 3.464%). Despite the high positive direct effects of GSFR on GY, its indirect effects via LW, PHM, BN, LL, TISL, PHF, and LY were negative. Along with the strongest positive direct effect on GY, the GSFR also had positive indirect effects via AIL, TILL, LN, LA, DF, and DE. BN positively affected GY both directly and indirectly through PHM, TISL, and PHF. The biggest positive indirect effects via BN were obtained by PHM (0.1347; 18.653%), PHF (0.11; 16.281%), DF (0.1056; 14.567%), LY (0.41; 13.822%), LN (0.07; 12.588%), LW (0.02; 3.694%), and LL (0.210; 3.358%). DE came in second (0.1056; 20.406%). TILL (0.4130; 45.127%), LA (0.2258; 45.042%), LL (0.2679; 42.887%), LW (0.2590; 42.855%), and TISL (0.2446; 37.999%) all contributed high and positive indirect effects via GSFR. However, BN had the largest negative indirect effects through GSFR, followed by LN, DE, PHF, PHM, and DF.

Table 3.16 lists the examined traits' genotypic effects on GY, both directly and indirectly. GSFR (0.9297; 92.706%) had the most positive direct effect on GY, followed by PHF (0.0215; 23.943%), PHM (0.0533; 14.498%), and LN (0.0358; 10.385%). On the contrary, the highest negative direct effect on GY was exerted by DF (-0.0957; 27.051%), followed by BN (-0.0206; 18.309%), DE (-0.0531; 15.039%), TISL (-0.0420; 12.860%), TILL (-0.0354; 11.320%), LL

(-0.0260; 9.961) and LA (-0.0112; 9.125%). GSFR had a highly positive direct effect on GY, whereas it had negative indirect effects via DF, TILL, LL, LW, and LA. PHM's positive direct effects on GY were accompanied by positive indirect effects through LW, AIL, BN, LL, TISL, TILL, and DF. The PHF had a positive direct effect on GY, and through BN, LL, LA, DF, and DE, this trait also had negative indirect effects on GY. For DF (0.0055; 1.542%), LY (0.0030; 1.139%), LL (0.0021; 0.814%), BN (0.0011; 0.983%), PHM (0.0009; 0.233%), GSFR (0.0718; 0.072%), LA (0.0004; 0.309%), DE (0.0002; 0.063%), and LN (0.0001; 0.039%), the highest positive indirect effects via PHF were observed. The highest positive indirect effects via GSFR were recorded for LY (0.1661; 62.387%), DF (0.1889; 53.371%), LA (0.0654; 53.246%), PHM (0.1736; 47.216%), LN (0.1408; 40.816%), LW (0.1047; 36.799%), TILL (0.1081; 34.620%), PHF (0.0310; 34.414%), and LL (0.0742; 28.449%). On the contrary, the highest negative indirect effects via GSFR were observed in TISL, AIL, BN, and DE.

Table 3.12 Phenotypic (above diagonal) and genotypic (below diagonal) correlations of 16 agro-morphological traits in 120 amaranth genotypes grown at Hawassa University agricultural research site in 2020 and 2021 cropping seasons

Traits	LW	AIL	PHM	BN	LL	TISL	TILL	LN	PHF	DM	LA	DF	DE	LY	GY	GSFR
LW		-0.21*	0.45***	0.12NS	0.90***	-0.18*	-0.01 ^{NS}	0.01 ^{NS}	0.48***	0.05 ^{NS}	0.77***	0.61***	-0.14 ^{NS}	0.43***	0.18*	0.28**
AIL	0.08 ^{NS}		-0.56***	-0.50***	-0.20*	0.80***	0.86***	-0.35***	-0.54***	-0.42***	-0.15 ^{NS}	-0.64***	-0.21*	-0.19*	0.38***	0.30***
PHM	0.56***	0.13*		0.74***	0.50***	-0.31***	-0.44***	0.25**	0.78***	0.52***	0.21*	0.70**	0.42***	0.29***	-0.08 ^{NS}	-0.13 ^{NS}
BN	0.17***	-0.04 ^{NS}	0.26***		0.11 ^{NS}	-0.18*	-0.48***	0.39***	0.66***	0.70***	-0.12 ^{NS}	0.58***	0.58***	0.23**	-0.24**	-0.36***
LL	0.86***	0.06 ^{NS}	0.58***	0.19***		-0.15 ^{NS}	0.02 ^{NS}	0.08 ^{NS}	0.56***	0.11 ^{NS}	0.76***	0.62***	-0.19*	0.40***	0.21*	0.29***
TISL	0.17**	0.38***	0.08 ^{NS}	0.13**	0.19**		0.78***	-0.23**	-0.31***	-0.25**	-0.24**	-0.49***	0.002 ^{NS}	-0.21*	0.38***	0.26**
TILL	0.68***	0.27***	0.75***	0.12*	0.66***	0.22***		-0.34***	-0.42***	-0.36***	0.01 ^{NS}	-0.47***	-0.27**	-0.19*	0.51***	0.44***
LN	0.73***	0.11 ^{NS}	0.70***	0.17**	0.71***	0.03 ^{NS}	0.85***		0.24**	0.32***	-0.07*	0.28**	-0.09 ^{NS}	0.13 ^{NS}	-0.30**	-0.31***
PHF	-0.03 ^{NS}	-0.11*	0.04 ^{NS}	0.05 ^{NS}	0.10 ^{NS}	-0.01**	0.004 ^{NS}	0.01 ^{NS}		0.60***	0.32***	0.82***	0.32***	0.37***	-0.09 ^{NS}	-0.14 ^{NS}
DM	0.15**	-0.14**	0.14*	0.22***	0.20***	0.00 ^{NS}	0.15**	0.21***	0.19**		-0.07 ^{NS}	0.60***	0.36***	0.15 ^{NS}	-0.28**	-0.48***
LA	-0.09 ^{NS}	-0.09 ^{NS}	-0.07 ^{NS}	0.09 ^{NS}	-0.13**	-0.01 ^{NS}	-0.18***	-0.24***	0.02 ^{NS}	-0.04 ^{NS}		0.47***	-0.34***	0.45***	0.12 ^{NS}	0.24**
DF	0.23***	-0.06 ^{NS}	0.22***	0.01 ^{NS}	0.29***	-0.17**	0.21***	0.28***	0.25***	0.28***	0.02 ^{NS}		0.22*	0.41***	-0.15 ^{NS}	-0.11 ^{NS}
DE	-0.35***	-0.04 ^{NS}	-0.47***	-0.04 ^{NS}	-0.31***	0.14**	-0.55***	-0.55***	0.01 ^{NS}	-0.13*	0.09 ^{NS}	-0.22***		-0.03 ^{NS}	-0.12 ^{NS}	-0.20*
LY	0.25***	-0.08 ^{NS}	0.25***	0.13**	0.21***	0.03 ^{NS}	0.17***	0.18***	0.14**	0.06 ^{NS}	0.36***	0.11**	-0.16**		-0.04 ^{NS}	0.01 ^{NS}
GY	0.09 ^{NS}	-0.10 ^{NS}	0.21***	-0.06 ^{NS}	0.05 ^{NS}	-0.28***	0.12*	0.16**	0.03 ^{NS}	-0.03 ^{NS}	0.06 ^{NS}	0.12**	-0.24***	0.20***		0.92**
GSFR	0.11*	-0.09 ^{NS}	0.19***	-0.05 ^{NS}	0.08 ^{NS}	-0.25***	0.12*	0.15**	0.03 ^{NS}	-0.16**	0.07 ^{NS}	0.20**	-0.20***	0.18***	0.95***	

Axillary inflorescence Length (cm) =AIL, leaf yield (t/ha)= LY, Grain yield (t/ha)= GY, Days to Maturation = DM, Leaf length (cm)= LL, Leaf width (cm) = LW, Branch Number (number) =BN, Leaf number (number). LN, Node number (number) =NN, Plant height at flowering stage (cm) =PHF, Plant height at maturity (cm) =PHM, Stem diameter (mm)= SD, Terminal inflorescence laterals length (cm)= TILL, Terminal inflorescence stalk length (cm) =TISL, Grain sink filling rate (kg ha⁻¹day⁻¹) =GSFR. Note: The green and yellow colors indicate the strength of correlation; green is positively correlated; green to light green indicates strong to weak positive correlation; yellow is negatively correlated, and yellow to light yellow indicates strong to weak negative correlation. Below the diagonal is the correlation coefficient; while above the diagonal is the significance level. ns=non- significant (p≤0.05), *=Significant (p≤0.05), **=Highly significant (p≤0.05), ***=very highly significant (p<0.0001).

Table 3.13 Estimated phenotypic direct (horizontal) and indirect (off-diagonal) effects of 14 traits on leaf yield of 120 amaranth genotypes in Hawassa University agricultural research site in 2020 and 2021 cropping seasons.

	Traits	LW	AIL	PHM	BN	LL	TISL	TILL	LN	PHF	DM	LA	DF	DE	GY
Path Coefficients	Direct effect	0.1465	0.4411	-0.0976	0.3248	-0.0624	-0.1246	-0.3009	0.0417	0.1420	0.0562	0.3849	0.0554	-0.0569	0.0125
	Indirect effect via														
	LW		-0.0932	-0.0438	0.0397	-0.0562	0.0227	0.0044	0.0006	0.0681	-0.0029	0.2968	0.0337	0.0080	0.0023
	AIL	-0.0309		0.0544	-0.1639	0.0124	-0.1002	-0.2601	-0.0145	-0.0762	0.0237	-0.0572	-0.0356	0.0117	0.0048
	PHM	0.0657	-0.2456		0.2396	-0.0310	0.0381	0.1317	0.0103	0.1110	-0.0292	0.0796	0.0390	-0.0237	-0.0010
	BN	0.0179	-0.2226	-0.0720		-0.0072	0.0227	0.1444	0.0162	0.0942	-0.0391	-0.0474	0.0321	-0.0329	-0.0030
	LL	0.1319	-0.0875	-0.0485	0.0373		0.0183	-0.0063	0.0033	0.0790	-0.0059	0.2940	0.0344	0.0107	0.0027
	TISL	-0.0267	0.3549	0.0298	-0.0591	0.0092		-0.2350	-0.0094	-0.0446	0.0139	-0.0936	-0.0273	0.0001	0.0048
	TILL	-0.0021	0.3812	0.0427	-0.1559	-0.0013	-0.0973		-0.0141	-0.0592	0.0202	0.0027	-0.0261	0.0154	0.0064
	LN	0.0021	-0.1533	-0.0242	0.1258	-0.0049	0.0282	0.1018		0.0347	-0.0181	-0.0254	0.0155	0.0049	-0.0037
	PHF	0.0703	-0.2366	-0.0764	0.2155	-0.0347	0.0391	0.1254	0.0102		-0.0338	0.1230	0.0454	-0.0180	-0.0012
	DM	0.0075	-0.1856	-0.0507	0.2257	-0.0066	0.0308	0.1080	0.0134	0.0853		-0.0282	0.0332	-0.0204	-0.0035
	LA	0.1130	-0.0655	-0.0202	-0.0400	-0.0477	0.0303	-0.0021	-0.0028	0.0454	0.0041		0.0263	0.0192	0.0015
	DF	0.0891	-0.2836	-0.0688	0.1879	-0.0388	0.0613	0.1416	0.0117	0.1164	-0.0337	0.1826		-0.0128	-0.0019
	DE	-0.0206	-0.0905	-0.0407	0.1878	0.0118	0.0003	0.0813	-0.0036	0.0450	-0.0202	-0.1302	0.0125		-0.0015
	GY	0.0265	0.1678	0.0075	-0.0775	-0.0133	-0.0476	-0.1540	-0.0123	-0.0133	0.0158	0.0471	-0.0086	0.0069	
	Direct effect	17.894	34.283	8.541	30.171	7.594	12.059	26.735	7.135	12.120	6.575	47.930	4.311	8.097	2.046
	Indirect effect via														
path Coefficients (%)	LW		11.377	5.346	4.851	6.862	2.777	0.533	0.072	8.316	0.354	36.248	4.118	0.976	0.276
	AIL	2.405		4.227	12.737	0.963	7.791	20.215	1.126	5.921	1.839	4.446	2.770	0.908	0.369
	PHM	5.746	21.487		20.959	2.714	3.329	11.519	0.903	9.713	2.554	6.960	3.415	2.076	0.084
	BN	1.665	20.677	6.693		0.667	2.107	13.419	1.500	8.755	3.631	4.402	2.979	3.058	0.277
	LL	16.035	10.642	5.899	4.538		2.223	0.772	0.398	9.605	0.722	35.758	4.184	1.306	0.323
	TISL	2.588	34.352	2.887	5.721	0.887		22.748	0.914	4.319	1.348	9.061	2.640	0.012	0.462
	TILL	0.189	33.870	3.796	13.849	0.117	8.645		1.253	5.259	1.793	0.243	2.318	1.366	0.568
	LN	0.356	26.243	4.140	21.535	0.840	4.832	17.425		5.936	3.090	4.348	2.657	0.831	0.633
	PHF	5.996	20.193	6.516	18.394	2.964	3.341	10.705	0.869		2.884	10.499	3.878	1.540	0.100
	DM	0.882	21.701	5.929	26.389	0.771	3.607	12.623	1.565	9.978		3.299	3.882	2.389	0.411
	LA	14.069	8.162	2.513	4.978	5.940	3.773	0.266	0.343	5.652	0.513		3.275	2.397	0.190
	DF	6.934	22.057	5.349	14.613	3.015	4.769	11.017	0.908	9.054	2.620	14.206		0.996	0.150
	DE	2.929	12.878	5.794	26.727	1.677	0.039	11.565	0.506	6.405	2.873	18.522	1.774		0.214
	GY	4.334	27.482	1.235	12.686	2.171	7.793	25.215	2.020	2.182	2.592	7.717	1.405	1.122	

$R^2 = 0.9834$, Residual factor = 0.12880, Axillary inflorescence Length = AIL, Leaf yield = LY, Grain yield = GY, Leaf length = LL, Leaf width = LW, Branch Number = BN, Leaf number = LN, Plant height at flowering = PHF, Plant height at maturity = PHM, Terminal inflorescence laterals length = TILL, Terminal inflorescence stalk length = TISL, Grain sink filling rate = GSFR, Leaf area = LA, Days to flowering = DF, Days to emergence = DE

Table 3.14 Estimated genotypic direct (horizontal) and indirect (off-diagonal) effects of 14 traits on leaf yield of 120 amaranth genotypes in Hawassa University agricultural research site in 2020 and 2021 cropping seasons

	Traits	LW	AIL	PHM	BN	LL	TISL	TILL	LN	PHF	DM	LA	DF	DE	GY
Path Coefficients	Direct effect	0.2225	-0.0760	0.1277	0.0168	-0.0672	0.0827	-0.0837	0.0969	0.1378	-0.0166	0.3766	-0.0171	-0.0626	0.1264
	Indirect effect via														
	LW		-0.0064	0.0720	0.0029	-0.0577	0.0138	-0.0571	0.0703	-0.0046	-0.0025	-0.0345	-0.0039	0.0217	0.0118
	AIL	0.0186		0.0171	-0.0006	-0.0039	0.0317	-0.0226	0.0110	-0.0148	0.0024	-0.0338	0.0011	0.0027	-0.0125
	PHM	0.1255	-0.0102		0.0043	-0.0390	0.0068	-0.0624	0.0678	0.0055	-0.0023	-0.0271	-0.0037	0.0295	0.0261
	BN	0.0386	0.0028	0.0329		-0.0131	0.0105	-0.0098	0.0168	0.0071	-0.0037	0.0351	-0.0002	0.0026	-0.0072
	LL	0.1910	-0.0044	0.0742	0.0033		0.0158	-0.0557	0.0684	0.0136	-0.0033	-0.0489	-0.0050	0.0195	0.0069
	TISL	0.0370	-0.0291	0.0105	0.0021	-0.0129		-0.0181	0.0034	-0.0011	0.0000	-0.0043	0.0029	-0.0089	-0.0355
	TILL	0.1518	-0.0205	0.0952	0.0020	-0.0447	0.0179		0.0825	-0.0006	-0.0025	-0.0686	-0.0037	0.0343	0.0150
	LN	0.1615	-0.0086	0.0893	0.0029	-0.0474	0.0029	-0.0713		0.0009	-0.0035	-0.0898	-0.0048	0.0347	0.0202
	PHF	-0.0074	0.0082	0.0051	0.0009	-0.0066	-0.0007	0.0004	0.0006		-0.0031	0.0066	-0.0043	-0.0007	0.0041
	DM	0.0340	0.0109	0.0177	0.0037	-0.0133	0.0000	-0.0125	0.0204	0.0261		-0.0144	-0.0047	0.0078	-0.0032
	LA	-0.0204	0.0068	-0.0092	0.0016	0.0087	-0.0009	0.0152	-0.0231	0.0024	0.0006		-0.0004	-0.0058	0.0073
	DF	0.0513	0.0048	0.0276	0.0002	-0.0196	-0.0139	-0.0179	0.0275	0.0349	-0.0046	0.0078		0.0138	0.0157
	DE	-0.0773	0.0033	-0.0603	-0.0007	0.0210	0.0117	0.0459	-0.0537	0.0014	0.0021	0.0352	0.0038		-0.0307
	GY	0.0207	0.0075	0.0264	-0.0010	-0.0037	-0.0232	-0.0099	0.0155	0.0045	0.0004	0.0218	-0.0021	0.0152	
	Direct effect	38.256	30.517	23.738	8.513	11.643	11.643	13.441	15.269	73.869	8.928	78.608	6.651	15.279	45.404
	Indirect effect via														
Percent Path Coefficients	LW		1.093	12.379	0.500	9.917	2.365	9.820	12.087	0.791	0.434	5.925	0.677	3.735	2.020
	AIL	7.483		6.885	0.253	1.558	12.750	9.082	4.426	5.956	0.956	13.573	0.435	1.096	5.031
	PHM	23.327	1.895		0.804	7.256	1.264	11.604	12.595	1.019	0.427	5.034	0.687	5.492	4.858
	BN	19.569	1.445	16.695		6.631	5.324	4.985	8.502	3.591	1.868	17.784	0.120	1.339	3.633
	LL	33.103	0.759	12.853	0.566		2.744	9.645	11.847	2.351	0.566	8.478	0.862	3.384	1.198
	TISL	14.895	11.728	4.225	0.857	5.176		7.283	1.361	0.460	0.003	1.713	1.157	3.574	14.271
	TILL	24.370	3.292	15.282	0.316	7.171	2.870		13.247	0.104	0.396	11.010	0.587	5.505	2.408
	LN	25.443	1.361	14.069	0.458	7.470	0.455	11.236		0.137	0.549	14.149	0.762	5.460	3.184
	PHF	3.985	4.382	2.725	0.463	3.548	0.368	0.211	0.327		1.683	3.554	2.320	0.349	2.215
	DM	18.318	5.892	9.568	2.015	7.147	0.022	6.734	11.003	14.090		7.782	2.544	4.219	1.738
	LA	4.249	1.422	1.916	0.326	1.822	0.195	3.182	4.821	0.506	0.132		0.073	1.220	1.527
	DF	20.001	1.876	10.761	0.091	7.628	5.428	6.987	10.699	13.601	1.782	3.023		5.370	6.104
	DE	18.867	0.809	14.724	0.173	5.121	2.867	11.207	13.104	0.350	0.505	8.591	0.918		7.486
	GY	7.436	2.705	9.489	0.342	1.321	8.342	3.571	5.568	1.619	0.152	7.836	0.761	5.454	

$R^2 = 0.9851$; Residual factor = 0.1221; Axillary inflorescence Length = AIL, Leaf yield=LY, Grain yield = GY, Leaf length = LL, Leaf width = LW, Branch Number =BN, Leaf number = LN, Days to maturation=DM, Plant height at flowering =PHF, Plant height at maturity = PHM, Terminal inflorescence laterals length = TILL, Terminal inflorescence stalk length =TISL, Grain sink filling rate =GSFR, Leaf area=LA, Days to flowering=DF, Days to emergence=DE.

Table 3.15 Estimated phenotypic direct (horizontal) and indirect (off-diagonal) effects of 14 traits on grain yield of 120 amaranth genotypes in Hawassa University agricultural research site in 2020 and 2021 cropping seasons.

	Traits	LW	AIL	PHM	BN	LL	TISL	TILL	LN	PHF	LA	DF	DE	LY	GSFR
Path Coefficients	Direct effect	-0.0789	0.0661	0.0678	0.1825	-0.0349	-0.0325	0.1620	-0.0195	0.1401	0.0016	-0.1182	-0.0452	-0.0306	0.9379
	Indirect effect via														
	LW		-0.0140	0.0304	0.0223	-0.0314	0.0059	-0.0023	-0.0003	0.0672	0.0013	-0.0719	0.0063	-0.0130	0.2590
	AIL	0.0167		-0.0378	-0.0921	0.0069	-0.0262	0.1400	0.0068	-0.0752	-0.0002	0.0760	0.0093	0.0058	0.2844
	PHM	-0.0354	-0.0368		0.1347	-0.0173	0.0099	-0.0709	-0.0048	0.1096	0.0003	-0.0832	-0.0188	-0.0088	-0.1235
	BN	-0.0096	-0.0334	0.0501		-0.0040	0.0059	-0.0777	-0.0075	0.0930	-0.0002	-0.0684	-0.0261	-0.0070	-0.3360
	LL	-0.0710	-0.0131	0.0337	0.0210		0.0048	0.0034	-0.0015	0.0779	0.0012	-0.0733	0.0085	-0.0122	0.2679
	TISL	0.0144	0.0532	-0.0207	-0.0332	0.0051		0.1265	0.0044	-0.0440	-0.0004	0.0581	0.0001	0.0063	0.2446
	TILL	0.0011	0.0571	-0.0297	-0.0876	-0.0007	-0.0254		0.0066	-0.0584	0.0000	0.0556	0.0122	0.0058	0.4130
	LN	-0.0011	-0.0230	0.0168	0.0707	-0.0027	0.0074	-0.0548		0.0342	-0.0001	-0.0331	0.0039	-0.0038	-0.2907
	PHF	-0.0378	-0.0355	0.0530	0.1211	-0.0194	0.0102	-0.0675	-0.0048		0.0005	-0.0969	-0.0143	-0.0113	-0.1314
	LA	-0.0608	-0.0098	0.0140	-0.0225	-0.0267	0.0079	0.0011	0.0013	0.0448		-0.0561	0.0153	-0.0136	0.2258
	DF	-0.0480	-0.0425	0.0478	0.1056	-0.0217	0.0160	-0.0762	-0.0054	0.1149	0.0008		-0.0102	-0.0124	-0.1052
	DE	0.0111	-0.0136	0.0283	0.1056	0.0066	0.0001	-0.0437	0.0017	0.0444	-0.0006	-0.0266		0.0008	-0.1893
	LY	-0.0337	-0.0126	0.0195	0.0416	-0.0140	0.0068	-0.0305	-0.0024	0.0519	0.0007	-0.0480	0.0012		0.0078
	GSFR	-0.0218	0.0200	-0.0089	-0.0654	-0.0100	-0.0085	0.0713	0.0060	-0.0196	0.0004	0.0133	0.0091	-0.0003	
	Direct effect	13.054	7.839	9.398	20.246	5.591	5.053	17.694	3.464	18.839	0.324	16.302	8.731	10.146	79.845
	Indirect effect via														
Percent Path Coefficients	LW		2.311	5.034	3.694	5.201	0.982	0.389	0.046	11.121	0.207	11.897	1.050	2.159	42.855
	AIL	1.975		4.480	10.921	0.822	3.103	16.598	0.802	8.914	0.029	9.008	1.099	0.691	33.720
	PHM	4.898	5.100		18.653	2.403	1.376	9.817	0.668	15.179	0.047	11.528	2.609	1.214	17.110
	BN	1.070	3.700	5.553		0.445	0.657	8.623	0.836	10.316	0.022	7.583	2.898	0.773	37.277
	LL	11.365	2.100	5.396	3.358		0.764	0.547	0.245	12.480	0.199	11.743	1.365	1.961	42.887
	TISL	2.236	8.263	3.220	5.160	0.796		19.650	0.685	6.841	0.061	9.033	0.015	0.986	37.999
	TILL	0.125	6.242	3.244	9.571	0.081	2.775		0.719	6.382	0.001	6.077	1.333	0.629	45.127
	LN	0.199	4.091	2.992	12.588	0.489	1.312	9.754		6.093	0.019	5.890	0.686	0.681	51.744
	PHF	5.084	4.767	7.131	16.281	2.611	1.374	9.074	0.639		0.070	13.020	1.925	1.521	17.663
	LA	12.131	1.959	2.797	4.481	5.320	1.578	0.229	0.256	8.933		11.182	3.046	2.721	45.042
	DF	6.621	5.863	6.592	14.567	2.990	2.208	10.517	0.752	15.849	0.106		1.401	1.714	14.518
	DE	2.142	2.622	5.469	20.406	1.274	0.014	8.456	0.321	8.588	0.106	5.137		0.151	36.585
	LY	11.177	4.185	6.460	13.822	4.647	2.243	10.125	0.808	17.229	0.241	15.951	0.382		2.584
	GSFR	1.854	1.706	-0.761	5.567	0.849	0.722	6.072	0.513	1.671	0.033	1.129	0.776	0.022	

R²= 0.9899; Residual factor = 0.1005; Axillary inflorescence Length =AIL, Leaf yield=LY, Grain yield = GY, Leaf length = LL, Leaf width = LW, Branch Number =BN, Leaf number = LN, Plant height at flowering =PHF, Plant height at maturity = PHM, Terminal inflorescence laterals length = TILL, Terminal inflorescence stalk length =TISL, Grain sink filling rate =GSFR, Leaf area=LA, Days to flowering=DF, Days to emergence=DE.

Table 3.16 Estimated genotypic direct (horizontal) and indirect (off-diagonal) effects of 14 traits on grain yield of 120 amaranth genotypes in Hawassa University agricultural research site in 2020 and 2021 cropping seasons.

	Traits	LW	AIL	PHM	BN	LL	TISL	TILL	LN	PHF	LA	DF	DE	LY	GSFR
Path Coefficients	Direct effect	-0.0155	-0.0056	0.0533	-0.0206	-0.0260	-0.0420	-0.0354	0.0358	0.0215	-0.0112	-0.0957	-0.0531	0.0344	0.9297
	Indirect effect via														
	LW		-0.0005	0.0301	-0.0036	-0.0223	-0.0070	-0.0241	0.0260	-0.0007	0.0010	-0.0221	0.0184	0.0085	0.1047
	AIL	-0.0013		0.0072	0.0008	-0.0015	-0.0161	-0.0095	0.0041	-0.0023	0.0010	0.0061	0.0023	-0.0027	-0.0813
	PHM	-0.0087	-0.0008		-0.0053	-0.0151	-0.0035	-0.0264	0.0250	0.0009	0.0008	-0.0207	0.0251	0.0086	0.1736
	BN	-0.0027	0.0002	0.0137		-0.0051	-0.0053	-0.0042	0.0062	0.0011	-0.0010	-0.0013	0.0022	0.0044	-0.0444
	LL	-0.0133	-0.0003	0.0310	-0.0040		-0.0080	-0.0235	0.0253	0.0021	0.0015	-0.0279	0.0166	0.0072	0.0742
	TISL	-0.0026	-0.0022	0.0044	-0.0026	-0.0050		-0.0076	0.0012	-0.0002	0.0001	0.0161	-0.0075	0.0010	-0.2338
	TILL	-0.0106	-0.0015	0.0397	-0.0024	-0.0173	-0.0091		0.0305	-0.0001	0.0020	-0.0205	0.0291	0.0060	0.1081
	LN	-0.0112	-0.0006	0.0373	-0.0036	-0.0183	-0.0015	-0.0301		0.0001	0.0027	-0.0271	0.0294	0.0063	0.1408
	PHF	0.0005	0.0006	0.0021	-0.0011	-0.0026	0.0003	0.0002	0.0002		-0.0002	-0.0243	-0.0006	0.0048	0.0310
	LA	0.0014	0.0005	-0.0038	-0.0019	0.0034	0.0005	0.0064	-0.0085	0.0004		-0.0020	-0.0050	0.0124	0.0654
	DF	-0.0036	0.0004	0.0115	-0.0003	-0.0076	0.0071	-0.0076	0.0101	0.0055	-0.0002		0.0117	0.0038	0.1889
	DE	0.0054	0.0002	-0.0252	0.0009	0.0081	-0.0060	0.0194	-0.0198	0.0002	-0.0010	0.0211		-0.0055	-0.1872
	LY	-0.0038	0.0004	0.0132	-0.0027	-0.0054	-0.0012	-0.0062	0.0066	0.0030	-0.0040	-0.0106	0.0085		0.1661
	GSFR	-0.0017	0.0005	0.0100	0.0010	-0.0021	0.0106	-0.0041	0.0054	0.0007	-0.0008	-0.0195	0.0107	0.0062	
	Direct effect	5.4420	3.9795	14.498	18.309	9.961	12.860	11.320	10.385	23.943	9.125	27.051	15.039	12.924	92.706
	Indirect effect via														
Percent Path coefficients	LW		0.166	10.565	1.256	7.840	2.453	8.478	9.135	0.253	0.360	7.765	6.483	3.004	36.799
	AIL	0.913		5.041	0.545	1.057	11.347	6.728	2.870	1.634	0.708	4.279	1.632	1.928	57.337
	PHM	2.375	0.206		1.446	4.105	0.938	7.170	6.811	0.233	0.219	5.634	6.823	2.327	47.216
	BN	2.384	0.188	12.204		4.490	4.731	3.687	5.503	0.983	0.926	1.182	1.991	3.950	39.472
	LL	5.096	0.125	11.872	1.538		3.080	9.013	9.690	0.814	0.558	10.699	6.357	2.749	28.449
	TISL	0.789	0.664	1.342	0.802	1.524		2.341	0.383	0.055	0.039	4.941	2.310	0.304	71.648
	TILL	3.381	0.488	12.722	0.775	5.530	2.904		9.765	0.032	0.653	6.567	9.322	1.922	34.620
	LN	3.257	0.186	10.806	1.034	5.315	0.425	8.731		0.039	0.774	7.868	8.530	1.833	40.816
	PHF	0.575	0.675	2.358	1.178	2.845	0.387	0.185	0.251		0.219	26.970	0.614	5.387	34.414
	LA	1.154	0.413	3.122	1.563	2.749	0.386	5.245	6.956	0.309		1.607	4.042	10.083	53.246
	DF	1.009	0.101	3.257	0.081	2.139	1.997	2.139	2.867	1.542	0.065		3.306	1.074	53.371
	DE	1.522	0.070	7.126	0.246	2.296	1.686	5.487	5.615	0.063	0.296	5.972		1.567	53.015
	LY	1.443	0.169	4.973	1.000	2.032	0.454	2.315	2.470	1.139	1.513	3.973	3.207		62.387
	GSFR	0.174	0.049	0.992	0.098	0.207	1.053	0.410	0.541	0.072	0.078	1.940	1.067	0.613	

$R^2 = 0.987$; Residual factor = 0.1140; Axillary inflorescence Length = AIL, Leaf yield = LY, Grain yield = GY, Leaf length = LL, Leaf width = LW, Branch Number = BN, Leaf number = LN, Plant height at flowering = PHF, Plant height at maturity = PHM, Terminal inflorescence laterals length = TILL, Terminal inflorescence stalk length = TISL, Grain sink filling rate = GSFR, Leaf area = LA, Days to flowering = DF, Days to emergence = DE.

3.3.8 Cluster Analysis

Based on standardized data from two seasons of mean values of 24 quantitative traits, the cluster analysis revealed 120 amaranth genotypes that were typically grouped into five basic clusters based on the level of variation among the genotypes (Figure. 3.9). The average values of the morphological characters from each cluster are shown in Table 3.18

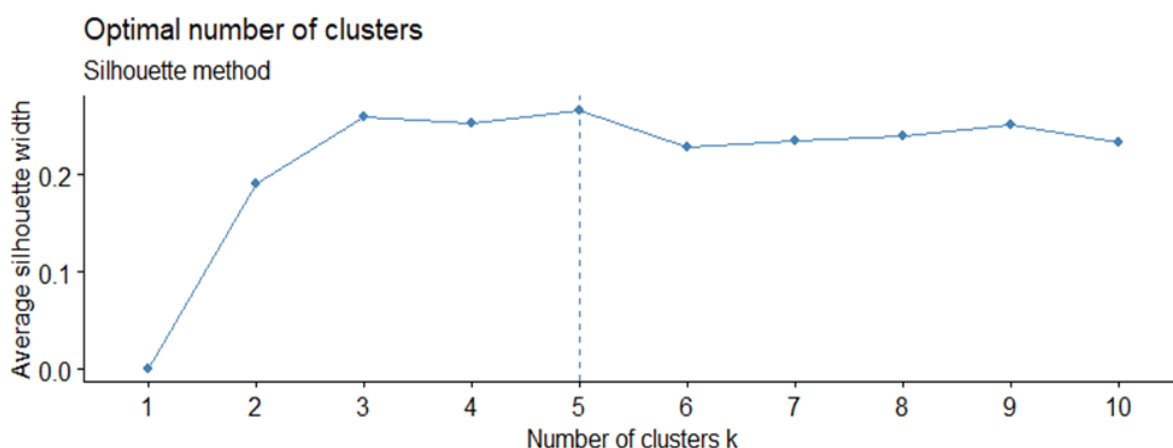


Figure 3.5 Silhouette width to determine the number of clusters (K) for quantitative data

The 120 amaranths genotypes were divided into five groups based on quantitative traits by using the hierarchical agglomerative ward D^2 dendrogram of the Euclidean distance clustering approach (Figure. 3.10).

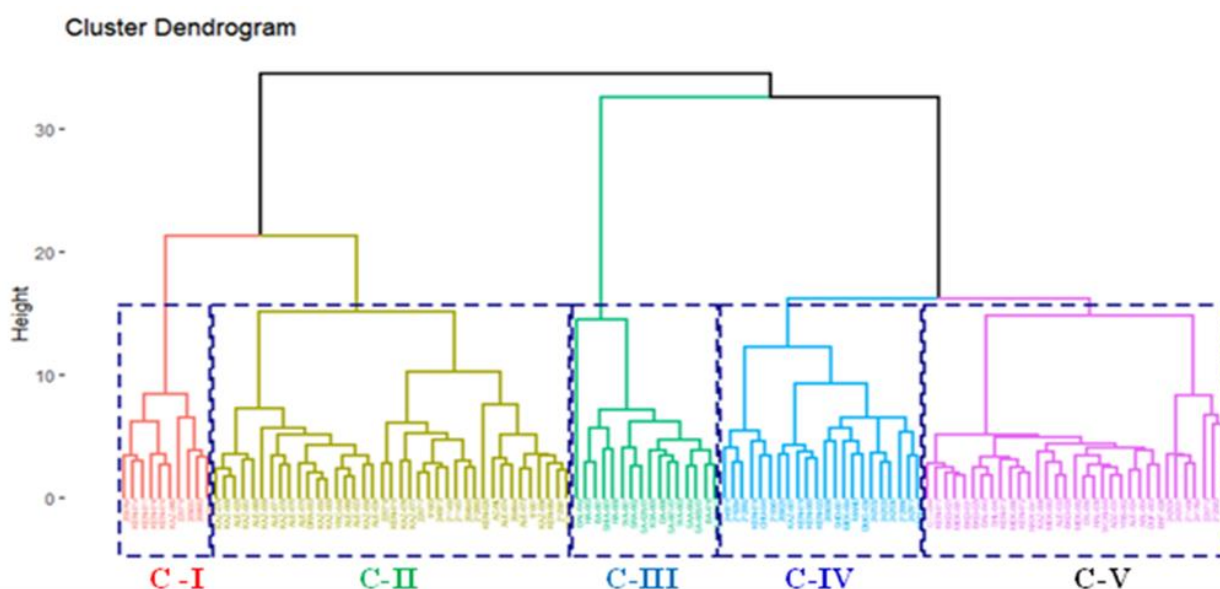


Figure 3.6 Cluster analysis showing the relationship among 120 Amaranths genotypes based on 24 quantitative traits; the abbreviated codes on the dendrogram nodes are described in Table 3.1

Table 3.17 shows the number and names of genotypes found in each cluster, as well as their collecting areas. Clusters (II) included 39 genotypes (32.5%) out of a total of 120, followed by V, IV, III, and I which had 33 (27.5%), 22(18.33%), 16 (13.33%), and 10 (8.3%) genotypes, respectively.

Table 3.17 Clustering of 120 Amaranths species. of genotypes into 5 clusters using the mean of 24 agro-morphological quantitative characters.

Cluster	Number of genotypes	Genotypes comprised	Collection Region
I	10	208683, 240814, 209057 225715, 208764, KEN-014, KEN-010, KAZ-060, KEN-011, KEN-016	South Nation Nationality People's Regional State and Oromia.
II	39	ALE-074, KAZ-078, KAZ-057, KEN-020, KAZ-076,204645,ALE-068, KAZ-059, KAZ-055, 240813, KAZ-008, KAZ-009, ALE-023, 225718, 211457, ALE-073, KAZ-077, KEN-015, 212581, 91001, KAZ-058, KEN-064, KEN-021, 225717, AC-NL, KAZ-056, 211455, KAZ-079, ALE-034, 204644, ALE-025, DRA-053, 91003, 212893, ALE-071, ALE-070, YRC-048, ALE-075, ALE-069	South Nation Nationality, Amhara, Released Varieties, and Oromia.
III	16	SAA-004, HA-003, DA-006, BA-016, SRA-002, K3A-005, SA-025/07, SA-008/07, SA-001/07, SHIA-007, SA-005/07, DAL-042, HA-008, BA-001, WA-001, WA-003	Afar and Sidama
IV	22	212890, CHU-045, 215567, 209056, 242533, KEN-065, 219284, 242917, 242530, 242532, SHD-001, 212892, 208025, MEK-084, KEN-022, MAND, KEN-067, 240815, KAZ-007, KEN-019, DMG-038, KEN-018	South Nation Nationality People Regional State, Sidama, Benishangul & Gumuz, Amhara, Tigray, and Released varieties.
V	33	MEK-081, BKD-031, OTI-044, KEN-012, WON-049,242534, 211456, 241764, MEK-082, 242531, ALE-033, DIL-050, KAZ-006, BKD-027, BKD-029, ALE-024, DAL-043, HAW-041, BKD-028, MEK-083, 212583, KEN-017, HAL-039, MEK-080, SHL-040, ABL-004, KEN-013, DUF-003, 225713, 212582, ADZ-037, YRB-002,BNT-026	Southern Nations Nationalities and Peoples, Released varieties, Tigray, Amhara, Oromia, Sidama, Benishangul & Gumuz.

3.3.8.1 Performance of genotypes under different clusters

The cluster mean values for 24 traits are presented in Table 3.18. Surprisingly, Cluster II had the highest mean values genotypes for LA and TSW. This group of genotypes has the smallest mean value for BLBL, DE, GFP, and TLBL were primarily collected from different regions

of Ethiopia, including South Nation Nationality People Regional State, (n=36, 92.31.13%), Amhara (n=1, 2.56%), Released varieties (n=1, 2.56%), and Oromia (n=1, 2.56%). Clusters (V) had 33 genotypes and accounting for 27.50% of total genotypes, are dominated by genotypes from South Nation Nationality People Regional State (n = 19, 57.58%), Sidama (n = 5, 15.15%), Tigray (n = 4, 12.12%), Benshangul-Gumz (n = 2, 6.06%), Amhara (n = 2, 6.06%), and Oromia (n = 1, 3.03%). In comparison to the other four clusters, genotypes in this cluster had the greatest mean values for DF, DM, LL, LN, LW, BN, PHF, PHM, PL, and SD. Their mean values for AIL, TILL, and TISL were all the lowest. Cluster IV encompassed 22 genotypes, contributing to 18.33% of all genotypes (n=14, 63.64%) from South Nation Nationality People Regional State and (n=3, 13.64%) from Benshangul-Gum, Sidama (n=2, 9.09%), Amhara (n=1, 4.55%), released varieties (n= 14.55%), Tigray region (n=1, 4.55%). In comparison to the other four clusters, genotypes in this cluster had the greatest mean values for LY. AIL, TLBL, TILL, GY, GFP, and TISL all had intermediate mean values (Table 3.18). Interestingly, cluster III is dominated by Afar genotypes accounting for 13.33 % of total genotypes (n = 15, or 93.75 %) and other genotypes from Sidama (n = 1, or 6.25 %). This group of genotypes had the greatest mean values for the longest BLBL, DE, GFP, and NN. GSFP, GY, TSW, LA, LL, LW, LT, and LY had the lowest mean values. Cluster I comprises 10 genotypes, accounting for 8.33 % of total genotypes, collected from South Nation Nationality People Regional State (n = 8, 80.00%), and Oromia (n = 2, 20%). Genotypes in this cluster were characterized by the highest mean value for LT, TLBL, TISL, TILL, AIL, and GSFR. As a result, genotypes from clusters I and IV had the greatest mean values of grain yield-related characteristics and leaf yield-related features, which is desirable for breeding (Table 3.18).

Table 3.18 Mean performance of different quantitative traits in each cluster

Traits	Clusters					Grand Mean
	I	II	III	IV	V	
Days to emergence (days)	6.28	5.92	7.48	7.01	6.98	6.73
Days to flowering (days)	33.88	42.79	36.95	50.70	55.67	44.00
Plant height at flowering (cm)	57.20	60.27	61.15	82.19	95.01	71.16
Leaf number (number)	21.59	30.26	30.41	30.1	30.86	28.64
Leaf area (cm ²)	64.53	79.58	22.04	76.62	78.86	64.33
Leaf length (cm)	14.09	14.48	9.04	15.73	16.06	13.88
Leaf width (cm)	6.80	7.19	4.68	7.71	7.81	6.84
Leaf thickness (mm)	0.40	0.39	0.35	0.39	0.37	0.38
Petiole length (cm)	5.75	6.05	6.44	6.29	6.56	6.22
Leaf yield (t/ha)	8.89	10.04	8.71	11.69	10.92	10.05

Branch number (number)	18.16	22.57	34.18	33.04	34.53	28.50
Basal lateral branch length (cm)	39.55	22.41	134.65	45.71	54.62	59.39
Top lateral branch length (cm)	33.33	18.11	25.56	20.56	18.93	23.30
Node number (number)	20.02	27.05	27.91	26.48	27.16	25.72
Days to maturity(days)	82.88	85.23	101.41	102.02	106.8	95.67
Plant height at maturity (cm)	166.27	169.86	188.00	232.13	263.89	204.03
Stem diameter (cm)	22.79	25.29	22.92	30.29	34.92	27.24
Auxiliary inflorescence length (cm)	22.50	13.58	12.14	13.04	6.32	13.52
Terminal inflorescence lateral length (cm)	25.58	18.78	15.82	19.11	14.76	18.81
Terminal inflorescence stalk length (cm)	38.97	29.00	30.96	34.34	21.31	30.92
Grain-filling periods (days)	49.00	42.44	64.45	51.32	51.12	51.67
Grain sink filling rate (kg ha ⁻¹ day ⁻¹)	48.45	34.59	14.08	31.44	26.15	30.94
Thousand seed weight (gm)	0.96	1.02	0.68	0.91	0.69	0.86
Grain yield (t ha ⁻¹)	2.28	1.42	0.89	1.50	1.24	1.47

3.3.8.2 Intra and Inter-Cluster Distances

The intra and inter-cluster distances of all clusters have been presented in (Table 3.19). The intra-cluster D^2 values ranged from 2.23 (cluster II) to 4.95 (cluster I). The highest intra-cluster distance was observed in cluster I (4.95) followed by cluster III (4.01), cluster IV (3.38), and cluster V (2.63), and was recorded as the lowest in cluster II (2.23). However, the inter-cluster D^2 values varied between clusters II and III (277.79). The pairwise generalized squared distance (D^2) among the five clusters based on Mahalanobis's D^2 statistics revealed the maximum and highly significant genetic distance was observed between II and III (277.79), followed by clusters I and III (275.14), cluster III and V (226.87), clusters III and IV (221.48), cluster I to V (86.07), cluster II and V (52.40), cluster I to IV (49.95), cluster II to IV (27.69), cluster IV and V (21.49), and the minimum inter-cluster distance observed between cluster I and II (39.50). As previously stated, the fundamental reason for variety in a cluster's composition is large inter-cluster distance, which may be leveraged in a hybridization program. The most divergent clusters were observed between clusters II and III. On average, Cluster 3 is the most distant group among the five clusters (Table 3.19).

Table 3.19 Pairwise generalized square distance (D^2) between (below diagonal) intercluster and (within diagonal or Boldface) intracluster distance based on the standardized mean values of 120 Amaranths species genotypes

Fro							Mean
m	I	II	III	IV	V		
CLS							
I	4.95						112.67
II	39.50**	2.23					99.35
III	275.14**	277.79**	4.01				158.26
IV	49.95**	27.69 ^{ns}	221.48**	3.38			131.50
V	86.07**	52.40**	226.87**	21.49 ⁿ	2.63		97.46

s

Figure 3.11 shows the biplot loadings for the first two PCs of the 24 agro-morphological parameters in the amaranth genotypes.

* = significant at $P < 0.05$, and ** = highly significant at $P < 0.01$; NS = non-significant at $P > 0.05$. Chi-Square (X^2) = 24, DF = 36.415 at $P < 0.05$ and 42.980 at $P < 0.01$

The findings showed that depending on the length, direction, and proximity of the vector lines, the analyzed traits could be divided into five groups. In the current experiment, principal component biplot loadings analysis and cluster analysis based on Euclidian distances both revealed comparable patterns that appear to support one another. In general, the PCA biplot pattern in the first two PCs reflected the findings of the cluster analysis. Figure 3.11 shows clearly that the features representing the DF, DM, PHF, BN, and SD had a significant impact on the genotypes of cluster I, which correspond to quadrants 1 and 4, as compared to this, eight traits LA, LL, LW, LN, PL, NN, LY, and DE that correspond to quadrants 1 and 2 significantly influenced cluster II genotypes. One of two characters, the BLBL of cluster III, which belongs to the fourth quadrant, was influenced by the genotypes. The AIL, TILL, and TISL of the investigated features had a significant impact on the genotypes of cluster IV, which belongs to the second quadrant. GSFR, GY, and TLBL three of the five traits, all had a significant impact on the genotypes of cluster V, which correspond to the second and third quadrants (Figure 3.11).

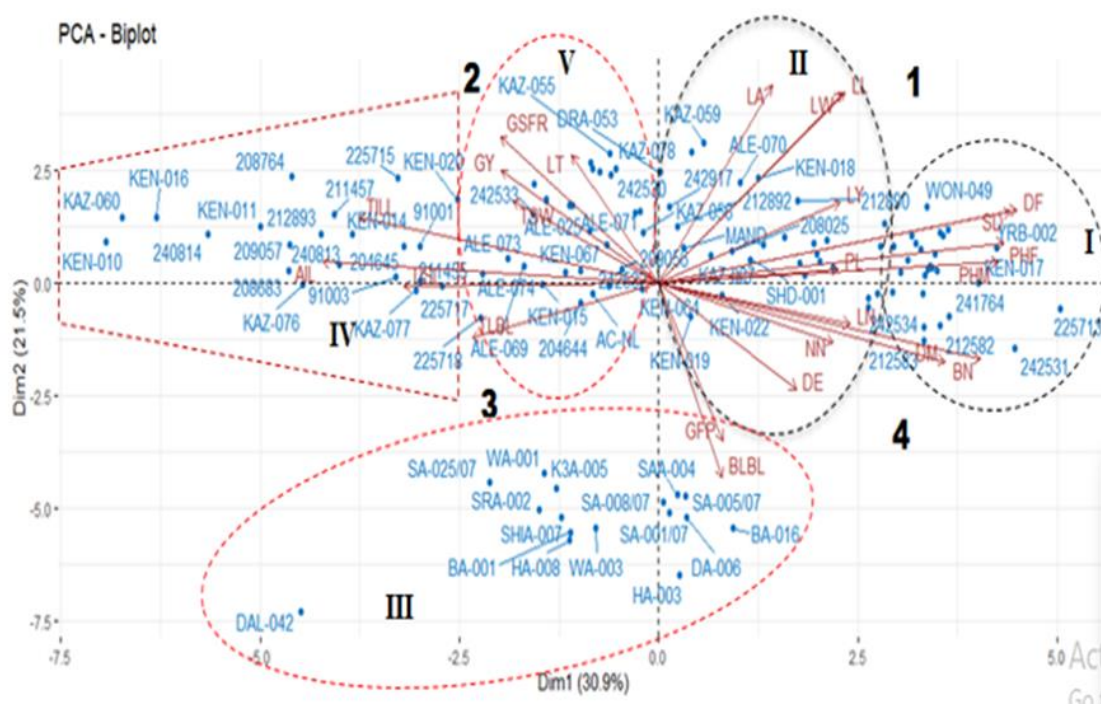


Figure 3.7 Genotype by trait PCA biplots of the first two principal components (PC1 and PC2) combining the score plots of the 120 amaranth genotypes showing their grouping pattern and the corresponding loading plots based on 24 quantitative traits. For abbreviated names (codes) of different traits (see Table 3.4) above.

3.3.9 The relative contribution of different characters to genetic divergence

By estimating the relative contribution of distinct traits to genetic divergence, the D^2 analysis's effectiveness is increased. Figure 3.12 shows the results of the genotypic contribution to diversity. The top five characteristics among all the characters were plant height at maturity (8.5%), leaf width (8.10), leaf length (6.90%), days to maturity (6.0%), and basal lateral branch length (5.60%)., which together accounted for the majority of the overall divergence. These were the main contributors to diversity. The amount of divergence caused by grain sink filling rate (1.5%), leaf yield (2.20%), node number (2.3%), leaf thickness (2.50%), thousand seed weight (2.50%), and other factors are insignificant.

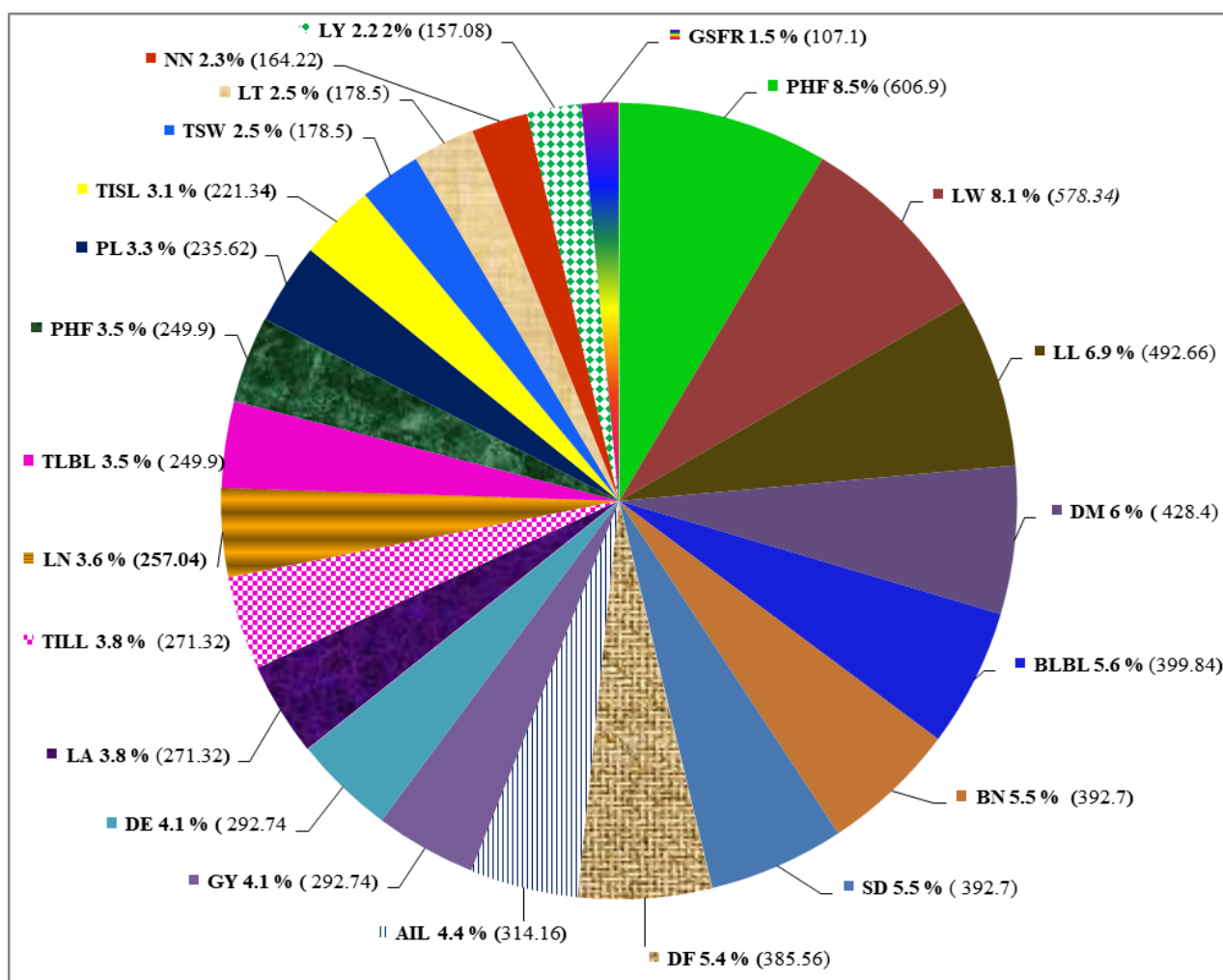


Figure 3.8 Percent contribution and sometimes ranked first of different characters towards diversity in amaranth genotypes. For abbreviated names (codes) of different traits (see Table 3.4 or 3.20) above. Note number under the parenthesis indicates the number of times ranked first.

3.3.10 Principal Component Analysis (PCA)

The six primary components of PCs 1 through 6 were generated from the original data. PC1, PC2, PC3, PC4, PC5, and PC6 were maintained from the overall main components, accounting for 80.41 percent of the total variance, with values of 30.94 percent, 21.49 percent, 10.70, 7.44, 5.16, and 4.69 percent, respectively. In any further data analysis, these main component scores may be used to summarize the original 24 variables, which accounted for the majority of the variance (Table 3.20).

The first principal component (PC1) accounted for 30.94 % of the total variation and was dominated by yield-related traits. The agro- morphological traits that loaded highly for the principal component (PC1) were, DF (0.86), SD (0.84), PHF (0.83), PHM (0.81), AIL (-0.80), BN (0.77), TILL (-0.72), DM (0.69), TISL (-0.61), LN (0.46), LL (0.45), TLBL (-0.45), LW (0.44), LY (0.44), GY (-0.38), GSFR (0.38), and TSW (-0.35). The second principal

component (PC2) demonstrated 21.49 % of the total variation, which related to traits such as LA (0.84), BLBL (-0.83), LL (0.81), LW (0.80), GFP (-0.67), GSFR (0.62), and GY (0.48). The third principle component explained 10.70 % of the total variation and was associated with the TLBL (0.56), DE (0.50), TISL (0.50), GY (0.46), NN (-0.45), the LN (-0.41), PHM (0.38), BN (0.35), and TSW (-0.35). The fourth principal components were selected from 24 components, which accounted for 7.44% of the total variation, for LN (0.58), NN (0.57), TISL (0.42), LT (0.41), GFP (0.38), AIL (0.34), TILL (0.32), DE(-0.31), and DM (0.29). The fifth principal component contributed 5.16 % of the total variation and was mainly related to GY (0.58), GSFR (0.54), and PL. The positive and negative loading indicates the presence of positive and negative correlation trends between the components and the variables. Therefore, the above-mentioned traits that load high either positively or negatively contributed more to the diversity, and they were the ones that most distinguished the clusters. (Table 3.20).

Table 3.20 Eigenvectors and eigenvalues of the first six principal components (PCs) for 24 quantitative characters of 120 genotypes

Traits	Principal Components					
	PC.1	PC.2	PC.3	PC.4	PC.5	PC.6
Eigenvalue	7.42	5.16	2.57	1.79	1.24	1.12
Variance. Percent	30.94	21.49	10.70	7.44	5.16	4.69
Cumulative. variance. Percent	30.94	52.42	63.13	70.57	75.73	80.41
-----Eigenvectors-----						
Days to emergence [DE]	0.33	-0.45	0.52	-0.31	0.07	-0.01
Days to flowering [DF]	0.86	0.31	0.04	-0.03	0.06	-0.22
Plant height at flowering [PHF]	0.83	0.17	0.31	0.04	0.03	-0.09
Leaf number [LN]	0.46	-0.17	-0.41	0.58	0.32	0.20
Leaf area [LA]	0.27	0.84	-0.13	-0.03	-0.22	-0.12
Leaf length [LL]	0.45	0.81	0.13	0.12	-0.07	-0.06
Leaf width [LW]	0.44	0.80	0.13	0.04	-0.17	0.03
Leaf thickness [LT]	-0.21	0.54	0.12	0.41	0.01	0.05
Petiole length [PL]	0.43	0.06	0.3	0.29	-0.43	0.47
Leaf yield [LY]	0.44	0.34	0.03	0.12	-0.21	0.30
Branch number [BN]	0.77	-0.33	0.35	0.14	0.11	0.04
Basal lateral branch length [BLBL]	0.15	-0.83	0.29	-0.05	0.07	0.25
Top lateral branch length [TLBL]	-0.45	-0.23	0.56	0.04	-0.18	0.11
Node number [NN]	0.42	-0.25	-0.45	0.57	0.30	0.25
Days to maturity [DM]	0.69	-0.33	0.21	0.29	0.00	-0.49
Plant height at maturity [PHM]	0.81	0.09	0.38	-0.03	0.10	0.07
Stem diameter [SD]	0.84	0.30	0.18	-0.10	0.10	-0.05
Auxiliary inflorescence length [AIL]	-0.80	0.09	0.31	0.34	-0.15	-0.01
Terminal inflorescence lateral length [TILL]	-0.72	0.28	0.37	0.32	-0.04	-0.15
Terminal inflorescence stalk length [TISL]	-0.61	-0.01	0.50	0.42	-0.03	0.03
Grain-filling periods [GFP]	0.15	-0.67	0.23	0.38	-0.05	-0.43
Grain sink filling rate [GSFR]	-0.38	0.62	0.29	-0.14	0.54	0.14
Thousand seed weight [TSW]	-0.35	0.35	-0.33	0.28	0.00	-0.29

3.3.10.1 Scree plot

The Scree plot depicts the relative distances between eigenvalues, which are used to rank the components in order of preference. The first six numbers are regarded to be important (as their eigenvalues are higher or equal to 1). Then the numbers decrease even more substantially, and we decide that these components aren't as important as they once were, and we'll leave them out of subsequent analyses. As shown in Figure 3.13, PC1 had 30.94 percent variability, followed by PC2 with 21.49 percent, 10.70 percent, 7.44 percent, 5.16 percent, and 4.69 percent, respectively, with eigenvalues of 7.42, 5.16, 2.56, 1.79, 1.24 and 1.12.

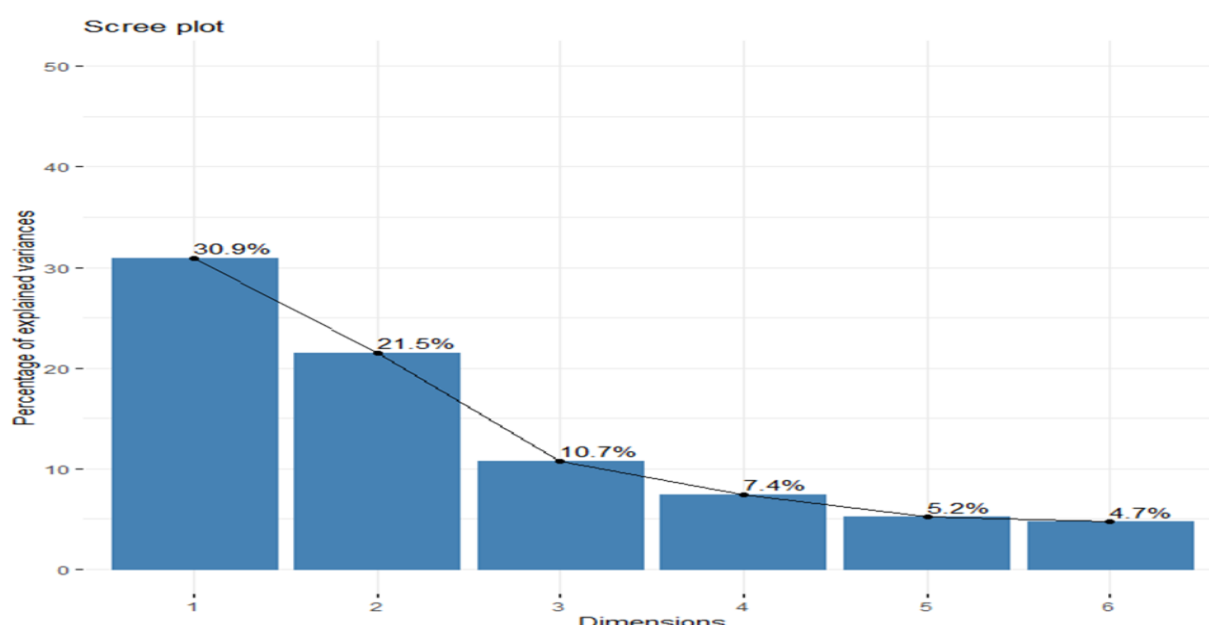


Figure 3.9 Scree plot of principal components (PCs) for 24 quantitative traits in 120 Amaranths genotypes.

3.3.10.2 Clustering Of Genotypes Based On Regions of the Country

A principal component analysis biplot revealed that dim1 distinguishes two groups of amaranth genotypes: one largely comprised of Tigray and Sidama that had the longest PHM, PHF, PL, DF, and high LN, and several NN, thicker SD when compared to genotypes from Oromia, Amhara, South Nation Nationality People Regional State, and Benishangul and Gumuz. The second group, which included genotypes from Amhara, Benishangul & Gumuz, Oromia, and the South Nation Nationality People's Regional State, had longer TISL, AIL, TILL, GSFR, GY when compared to Tigray, Afar, and the Sidama region (Figure 3.14). Dimension 2 also distinguishes between two genotype groups. The longest BLBL, DE, and

NN are observed in Afar and Sidama genotypes, whereas the highest LY, LA, LW, GFP, and LL are observed in Amhara, Benishangul & Gumuz, Oromia, and South Nation Nationality People Regional State genotypes.

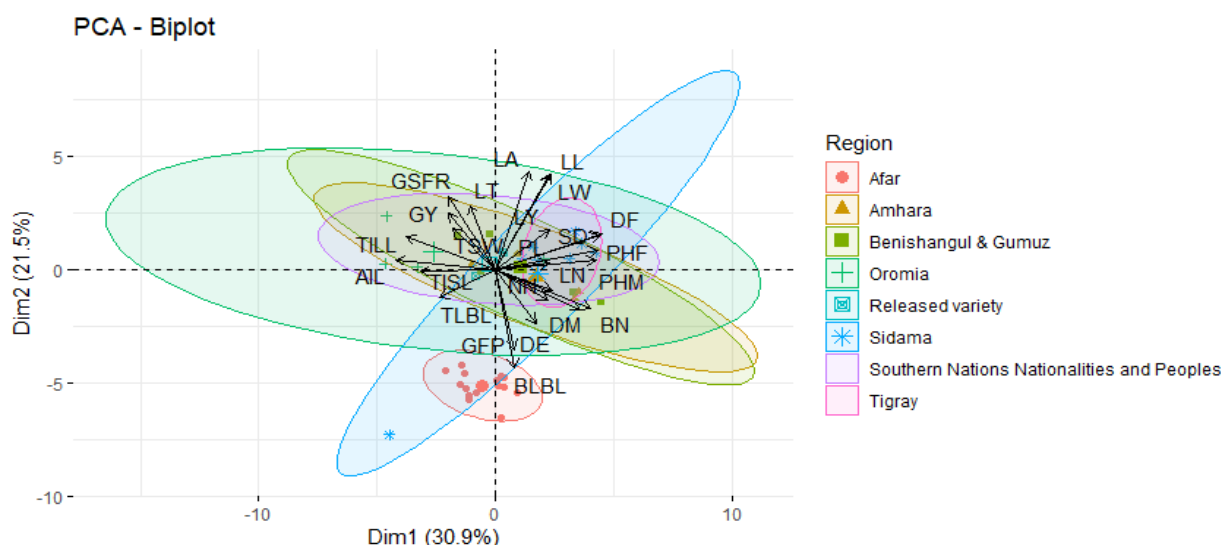


Figure 3 .10 Principal components analysis biplot showing quantitative variables and individuals on Dimensions 1 & 2. For abbreviated names (codes) of different traits. Axillary inflorescence Length (cm) =AIL, leaf yield (t/ha)= LY, Grain yield (t/ha)= GY, Days to Maturation = DM, Leaf length (cm)= LL, Leaf width (cm) = LW, Branch Number (number) =BN, Leaf number (number). LN, Node number (number) =NN, Plant height at flowering stage (cm) =PHF, Plant height at maturity (cm) =PHM, Stem diameter (mm)= SD, Terminal inflorescence laterals length (cm)= TILL, Terminal inflorescence stalk length (cm) =TISL, Grain sink filling rate (kg ha-1day-1) =GSFR. Note: The green and yellow colors indicate the strength of correlation; green is positively correlated; green to light green indicates strong to weak positive correlation; yellow is negatively correlated, and yellow to light yellow indicates strong to weak negative correlation. Below the diagonal is the correlation coefficient; while above the diagonal is the significance level. ns=non- significant ($p \leq 0.05$), *=Significant ($p \leq 0.05$), **=Highly significant ($p \leq 0.05$), ***=very highly significant ($p < 0.0001$).

Variable PCA shows that the first two dimensions accounted for 52.42 percent of the overall variance across amaranth genotypes. Variables that are close to the center of the plot are less important for the first two components. Accordingly, PHM, GSFR, BLBL, LL, DM, DF, LA, AIL, BN, TILL, DE, TLBL, LY, SD, TISL, GSFR, GFP, GY, LW, PHF, and LT were the major variables contributing to the variance of the first two components. NN, LN, PL, and TSW contribute less to principal components 1 and 2. The degree of correlation between the two measured variables is indicated by the angle between the two vectors (Figure 3.15). The strongest correlation was found between LL and LW, followed by SD and DF, all of which had a +1 correlation. There was a highly significant correlation between, GY, GSFR, and TSW; LA, LW, LY, and LT; and DM and BN; DF and DM. There was also a positive correlation between LN, NN, DE, and BN. Positive correlations were found between AIL, TISL, GSFR, GY, and TSW. AIL, TISL, GSFR, and GY, as well as TSW, had a negative connection with PHM and PHF. BLBL and GFP had no or very little association with SD; DF, PHM, PL, and TISL. Furthermore, LT is unrelated to SD, DF, PHF, PL, and TISL. There

was no correlation between TSW, GY, and GSFR with DE, TISLL with DE, and the BN, as indicated by the angle of around 180 degrees ($r = \cos 180 = -1$).

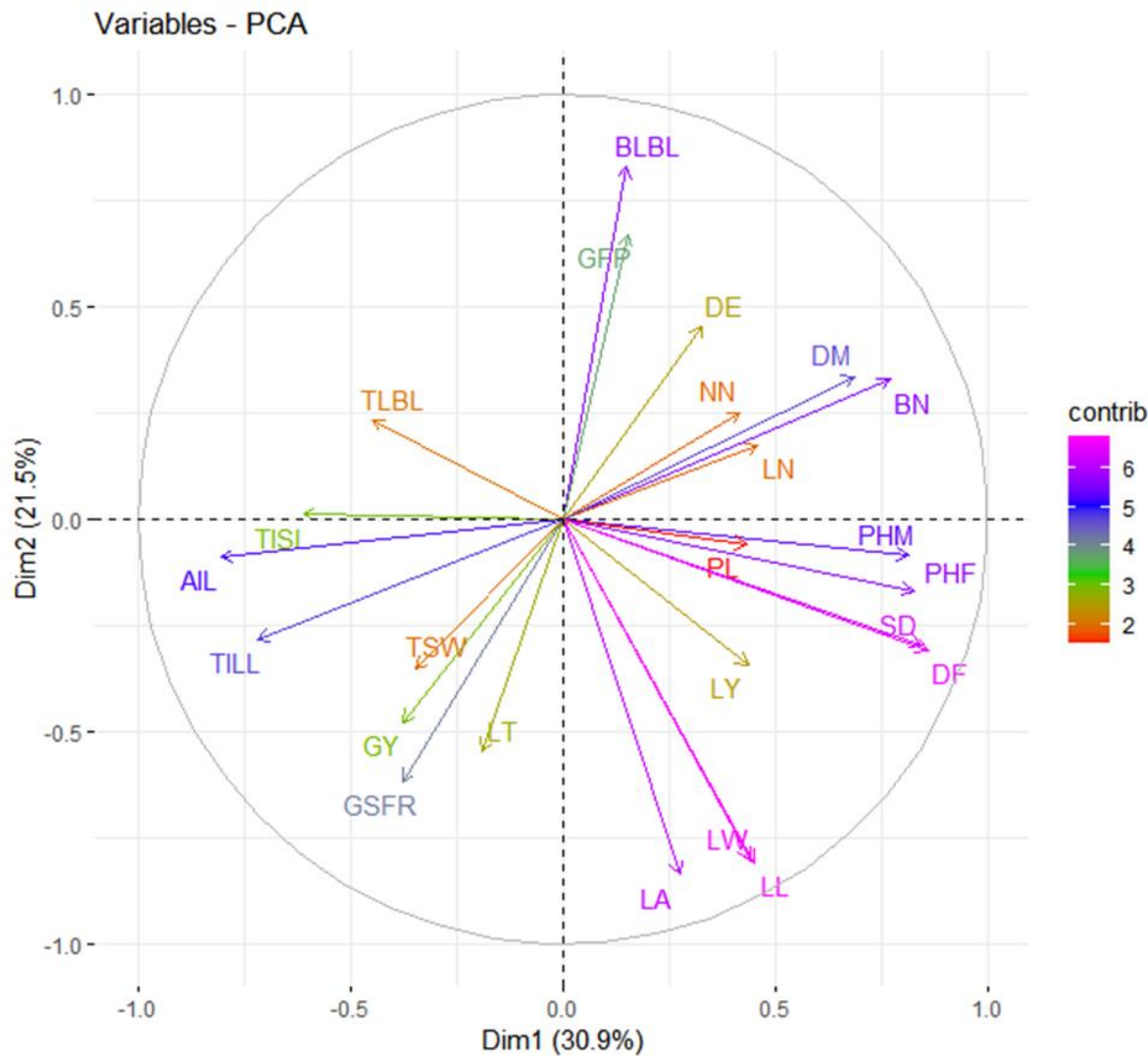


Figure 3.11 Variable correlation plot showing the relationships between 24 quantitative traits in 120 Amaranths genotypes were collected from different regions of Ethiopia. For abbreviated names (codes) of different traits (see Table 3.4 or 3.20).

3.4 Discussion

3.4.1 Agro-morphological variability

3.4.1.1 Effect of Cropping season, genotype, and their interactions

Superior genotypes must be investigated utilizing several traits and multi-environment experiments to make sure that the chosen genotypes perform well in a variety of environments

within the targeted area. Due to the extremely significant changes between the seasons and interactions between genotypes and seasons, the best genotypes for specific traits during the planting season were not always the best genotypes for the subsequent planting season. For the majority of the studied variables, the extent of significant differences was seen among years, genotypes, and the genotype-by-year interaction. In most of the studied traits in amaranth genotypes, the test year had a significant impact. Because weather and farming practices, such as soil characteristics, field management, or weather, affect how genes are expressed, this may help to explain the scenario (Yao *et al.*, 2008; Persaud *et al.*, 2022). Debelo *et al.* (2001), and Mbwambo *et al.* (2013) also observed comparable findings the irregular variations in rainfall from year to year due to the genotypic difference often have an impact on Ethiopia's agriculture and could influence most of the plant variables in a complex way resulting in their plastic responses.

The mean squares of genotypes from the analysis of variance demonstrated that there was significant variation among the genotypes for the studied variables, except for the leaf thickness, node number, petiole length, leaf yield, and top lateral branch length. The considerable observed variances among the genotypes under study suggest that there was a substantial amount of inherent variability among amaranth genotypes for the variables under analysis. Various researchers have also reported that amaranth genotypes exhibit significant variability (Andini *et al.*, 2013; Thapa and Blair, 2018; Trivedi *et al.*, 2022). The evaluation of both genetic and environmental factors may be made more precisely and efficiently by studying the interaction between genotype and environment. Stable genotypes are required for sustainable and reliable agriculture production (Kanfany *et al.*, 2021). In the current studies, the genotypes by year interactions were found significant for DE, DF, LN, LL, LW, DM, PHM, SD, TILL, GSFR, and GY. This is due to both the discrepancy response of genotypes to the test year and the influence of the test year on the genotypes differently. Additionally, the inconsistent performance of the genotypes across years suggested the potential for exploring and cultivating superior genotypes in a variety of environmental conditions (Olaniyi, 2007) and attributed genotype x year to variations in ecological distribution and genetic variations among the genotypes. Contrarily, the observed significant interactions for the PHF, BN, BLBL, AIL, TISL, and GFP traits were only due to the differential response of genotypes to the test years. However, the observed significant interactions for the remaining three (about 12.5%) traits were only due to differential responses of years to the test genotypes. Because growing conditions can vary, it is typical to

expect that a genotype's performance will fluctuate in a variety of environments (Keneni, 2012; Mohammadi, 2017; Fekadu *et al.*, 2022).

The observed crossover interactions in GY were a result of genotypic performance changes brought on by variable environments, which made it more difficult to create genotypes with stable performance. Significant genotype-by-environment interaction effects were mostly of the 'cross-over' type; i.e., interactions were associated with rank order changes among the genotypes. This indicated that the two environments were distinctly different for some of the characters and that better genotypes in one environment may not be better performers in another (Temesgen *et al.*, 2015; Sossou *et al.*, 2021). Moreover, the significant presence of extensive crossover GY interactions in the two cropping seasons suggests that a systematic effort is needed to screen different genotypes across various environments to identify those that perform well there or within a particular target region of environments (environment trials). The inconsistent genotype rankings for the investigated traits would make it difficult to generate genotypes with stability for these traits (Moghaddam and Pourdad, 2009). The results, however, point to significant variations in genotype ranks between the environments; therefore, effort must be used while breeding these characteristics, particularly for grain production.

3.4.1.2 Performance of Genotypes

The estimated 24 quantitative traits showed a massive genetic variation among the genotypes, and a similar variation was reported in the previous studies (Andini *et al.*, 2013; Kumar, 2015; Nyasulu *et al.*, 2021). There are three plausible explanations for the increased variety in morphological appearance that may be in Ethiopian amaranth genotypes: they may have not passed through genetic bottlenecks during the process of speciation; they may not have experienced strong negative natural selection under control or cultivation (Chan and Sun, 1997); and self-pollination is more likely to occur, with mean outcrossing rates ranging from 3 to 32% (Jain *et al.*, 1982). This variation suggests that it would be possible to use it for amaranth varietal enhancement.

Plant height, days to flowering, leaf number, leaf area, leaf length, and individual leaf width are all key contributors to amaranth's leaf yield, as are a variety of other yield elements. Das (2016), reported similar findings. The observed high plant height in the top 5% leaf yielder genotypes might be due to the inherent genetic variation, strong light competition, and

partition of more assimilates for stem elongation. Similar findings were observed by [Yarnia et al. \(2010\)](#) in amaranths and taller plants outcompete weeds more successfully than shorter ones ([Fageria et al., 2004](#)). Similarly, the leaf area is crucial in determining the yield ([Sarker and Oba, 2021](#)). The top 5% of leaf yielder genotypes exhibited the biggest leaf area. This is because each leaf's area is estimated as the sum of its leaf length and leaf width, and when water availability grew, plants were able to photosynthesize more effectively ([Shongwe et al., 2010](#)). The leaf area intercepts sunlight, takes up CO₂ and inorganic nitrogen, and performs photosynthesis and biomass accumulation, among other factors, determining the yield reported in several studies ([Puntel, 2012](#)). Moreover, photosynthesis has been the precondition for a successful breeding program to increase photo-assimilate production in high-yielding genotypes ([Haritha et al., 2017](#)). However, light absorption and the rate of dry matter production increase as leaf number and size increase during crop growth ([Remison and Akinleye, 1979](#)). Besides intercepting most of the solar radiation falling on the crop canopy, high leaf area indices ensure the optimum use of other available environmental factors like moisture, carbon dioxide, and nutrients, to achieve high productivity. Overall, our findings indicate that the genotypes that can be selected to increase biomass have superior photosynthetic efficiency ([Vikram et al., 2016](#)). Therefore, a successful yield improvement strategy has been the direct selection of yield-related traits, which are simpler to quantify precisely than the yield itself ([Kumar et al., 2014](#)). The genotypes of amaranth examined for potential grain and leaf yields generally showed genotypic heterogeneity. These variations in agro-morphological trait performance indicate the amaranth's potential for success in future development efforts for various uses.

Grain yield is an incredibly complex feature that cannot be determined by itself [Voss-Fels et al. \(2019\)](#). It is a resultant effect of actions and interactions of its component traits. Therefore, the identification of plant traits that contribute to high grain yield is essential for breeding efforts. The observed grain yield was markedly higher in the top 5% of grain yielder genotypes, which may be explained by a stronger relationship between GY and GY-related traits LN, LA, LL, LW, LY, BN, AIL, TILL, TISL, GSFR, and TSW. The relative effect of PHM and DM, however, was less significant for grain yield performance in the top 5% of yielder genotypes. This may be tied to the top grain genotypes' considerably shorter plant heights and earlier maturation. Modern high-yielding genotypes have improved grain production mostly through the reduction of plant height, which boosts the harvest index due to lower yields of straw and increased lodging resistance. The photosynthesis and respiration

of the shorter plants are better balanced and thus require less maintenance respiration (Peng *et al.*, 1994).

The study's findings also revealed that genotypes with high grain production typically had shorter plant heights (Shah *et al.*, 2018). According to Sogbohossou and Achigan-Dako (2014), and Brenner *et al.* (2000), grain amaranths were chosen for reduced plant heights. Higher photosynthetic efficiency, lower respiration, and increased grain carbohydrate storage are significant physiological processes that can be used to increase yield potential (Sharma-Natu and Ghildiyal, 2005). Additionally, similar to the photosynthetic rate, leaf area greatly increases grain output (Raza *et al.*, 2019). On the other hand, it seems reasonable that any component that influences a plant's ability to fix carbon and/or transfer available or stored assimilates to the grain will likely also affect physiological maturity. For instance, terminal drought or nitrogen stress, which is known to accelerate leaf senescence, could shorten the filling period and advance physiological maturity (Smith and Hamel, 2012). The top 5% of genotypes for grain-yielding plants began to bloom earlier but took longer for GFP, which resulted in higher GY due to better use of growth resources. The length of the vegetative growth stage may also have an impact on grain yield (Feng *et al.*, 2021). A shorter vegetative growth period makes it possible to reserve more growth resources for the reproductive phase, which raises GY due to the effective use of growth resources for yield production (Feng *et al.*, 2021). The final yield is greatly influenced by the grain filling rate (GFR) and has a positive impact on the final grain weight (Khan *et al.*, 2014). High temperature also increases the rate of grain filling to compensate for the shortened grain growth period. Selecting genotypes with high GFR is therefore probably a wise way to proceed for enhancing grain yield under stress.

3.4.1.3 Phenotypic and genotypic coefficient of variations

Any breeding material must have a high level of genetic diversity since it not only serves as a foundation for selection but also offers important insights into the choice of varied parents for use in hybridization programs (Singh *et al.*, 2016; Upadhyay *et al.*, 2019). To determine the additive or heritable portion of variability, agronomic traits must be divided into genotypic, phenotypic, and environmental influences because they are quantitative and interact with the environment. The extent of phenotypic variances was comparatively higher for all agro-morphological traits in amaranth genotypes in the current study compared to the corresponding genotypic and the interaction of genotypic by year variances, indicating a

relatively high level of environmental influence on the expression of these traits. For 50% of the examined variables, the magnitude of genotypic (heritable) variance was higher than the corresponding environmental (non-heritable) variances. This suggests that the examined traits were mostly influenced by the genotypic component of variance.

In the current study's variability analysis, all of the characteristics exhibited greater phenotypic than genotypic coefficients of variation, which is generally consistent with the findings of different authors ([Sravanthi et al., 2012](#); [Parveen et al., 2013](#); [Yadav et al., 2014](#); [Malaghan et al., 2018](#); [Showemimo et al., 2021](#)). They consequently suggested that the environment had an impact on how they expressed themselves. High genotypic and phenotypic coefficients of variation were revealed in the plant height at flowering, leaf area, branch number, auxiliary inflorescence length, basal lateral branch length, plant height at maturity, grain sink filling rate, and grain yield. Higher PCV values and correspondingly higher GCV values for these traits suggest that they are of economic importance and are under the control of genetics. As a result, these traits can be relied upon, and simple selection can be used to enhance these traits. Results of a comparable ilk have been reported by [Malaghan et al. \(2018\)](#) for the number of branches, and plant height at flowering. A significant difference between PCV and GCV estimates for the traits viz., petiole length, leaf yield, leaf thickness, node number, top lateral branch length, terminal lateral inflorescence length, and thousand seed weight points to a higher level of environmental control or the contribution of non-additive gene effects. The same findings were reported ([Rana et al., 2005](#); [Showemimo et al., 2021](#)). However, it was found that the differences between PCV and GCV were comparatively very small for the traits of days to flowering, leaf length, leaf width, days to maturity, and stem diameter. This suggests that these traits had high of genetic diversity that could be exploited and it is revealed that this estimated phenotypic variability is a reasonable signal of genotypic variability. As a result, environmental influences had a smaller impact on phenotypic performance ([Sawadogo et al., 2014](#)). Six of the examined traits had low ECV estimates (about 25%), suggesting that these traits are less responsive to environmental variables. For the traits of leaf length, leaf breadth, days to maturity, and plant height at maturity, the observed moderate-to-high PCV and moderate GCV along with the accompanying low ECV estimations revealed that improvement in those traits would be possible by direct selection.

3.4.1.4 Heritability and genetic gains

The value of selection for a specific characteristic depends mainly on its heritability since selection operates on genetic differences (Allard, 1960). Heritability, a degree of the genetic link between parent and offspring, has been frequently used to determine how much a character may be passed down from one generation to the next. Estimates of heritability reveal the degree to which a trait is under genetic control, as well as the accuracy of phenotypic prediction of its breeding value (Ndukauba *et al.*, 2015). Understanding heritability is crucial because it aids breeders in determining how much improvement is feasible through selection (Robinson *et al.*, 1949; Ene *et al.*, 2016). The current research revealed that, for assessed phenotypic traits, the estimated heritability generally showed significant variability. This great genetic improvement potential of the traits under study was suggested by the vast range of variability in the studied traits since the degree of wide variability essentially provides a better scope for selection.

The number of branches, plant height at maturity, stem diameter, and leaf width, plant height at flowering, grain filling periods, basal lateral branch length, days to maturity, auxiliary inflorescence length, leaf area, days to flowering, and leaf length all demonstrated substantial heritability in the current investigations. According to Manal (2009) and Yanti (2016), high heritability signifies that environmental influences on the expression of these traits were relatively low and that the dominant genetic influence, due to the presence of high additive gene action on the expression of these traits, was largely responsible for the manifested phenotypic performance. For this reason, special consideration should be given to these particular traits that are passable and may be regulated by additive gene action. Therefore, to make selection effective for improving amaranth, these traits can be enhanced using mass selection or the pedigree technique. Similarly, Showemimo *et al.* (2021) also obtained high heritability estimates for, the number of branches, and leaf width. Similar findings in amaranth for traits including plant height, stem diameter, and the number of branches were reported by Sravanthi *et al.* (2012), Yadav *et al.* (2014), Mobina and Jagatpati (2015), and Selvan *et al.* (2013). Similar results have also been reported by Trivedi *et al.* (2022) for the traits of days to flowering, plant height, and stem diameter. The estimations of heritability, however, were moderate for the length of the terminal inflorescence stalk, the number of leaves, and the number of days to emergence. Selection based on the phenotypic performance of these traits may not be beneficial for improvement due to the medium heritability of these traits, which suggested that the environmental effect was relatively strong on the expression of these traits.

Moreover, moderate heritability, indicating a weak correlation of phenotype with genotypic value and reflecting the high influence of season by genotypes interaction effects. According to [Singh *et al.* \(1993\)](#), the selection is made significantly more challenging by the medium to low heritability estimates since the environment has a considerable obscuring impact on the genotypic effects. So, the genetic potential of such traits would be harnessed by heterosis breeding followed by recurrent selection. The existence of considerable genotype by environment interactions in 22 (or almost 92%) of the examined traits may be the key cause of the low heritability estimates that were observed in the current study.

There is a sign of genetic variation in the genotypes of amaranth that can be selected, according to the estimates of GA and GAM in the present study, which varied significantly across the observed traits. Whenever we select the top 5% of high-yielding genotypes as parents, the mean performance of the offspring is anticipated to improve based on the assessment of genetic gain in the current study. In light of this, it is expected that the genotypic performance of the new population (progeny) will increase, from 1.39 to 1.83 t/ha for grain yield to 10.31 to 11.76 t/ha for leaf yield. Similarly, leaf area (69.91 to 99.17cm²), leaf length (14.39 to 17.58cm), leaf width (7.09 to 8.70 cm), grain sink filling rate (30.11 to 40.96 kg ha⁻¹day⁻¹), plant height at maturity (209.25 to 272.25cm), plant height at flowering (73.7 to 95.29cm), thousand seed weight (0.86 to 1.03 g), auxiliary inflorescence length (12.04 to 18.71cm), terminal lateral inflorescence length (17.91 to 20.94cm), and terminal inflorescence stalk length (28.96 to 35.93 cm) are expected to be improved. The genetic gain (GAM) that could be estimated from selecting the top 5% of the genotypes as a percent of the mean ranged from 0.0% for leaf thickness to 86.1 % for basal lateral branch length.

Only heritability-based trait selection, however, may occasionally be successful since the broad definition of heredity takes into account total genetic variance, which includes additive, dominant, and epistatic variances (i.e. interaction between variations that is not additive). Measuring the heritability of a group of genotypes in conjunction with rapid genetic advance is, therefore, more precise and efficient for the selection of desirable traits for a subset of the population ([Ali *et al.*, 2002](#); [Bhargava *et al.*, 2004](#); [Amegan *et al.*, 2020](#)). High heritability combined with high genetic advance as a percentage of the mean was a more valuable and powerful tool for predicting the effect of selection and producing the resultant effect for selecting the best individuals ([Shukla *et al.*, 2006a](#); [Kuralarasan *et al.*, 2018](#); [Chauhan and Singh, 2019](#)), because heritability is a separate numerical expression of the ratio of the two variances, which may not result in success if the selection is based on heritability estimates

alone. High heritability and GA for a particular characteristic show that it is controlled by additive gene action, which makes it the best candidate for selection (Mohsin *et al.*, 2009). Furthermore, it is thought that traits with high heritability but little genetic advance, which restricts the possibility of improvement through selection, are controlled by non-additive gene action. However, if the trait is governed by additive gene action, it will be highly heritable and have a significant genetic advance, allowing for a significant improvement through selection. High broad-sense heritability (h^2 bs) traits, therefore, may not always result in high genetic gain (Lipi *et al.*, 2020). Estimates of very high to moderately high heritability accompanied with high GAM were identified for basal lateral branch length, auxiliary inflorescence length, leaf area, branch number, plant height at maturity, plant height at flowering, stem diameter, days to flowering, grain filling periods, leaf width, and leaf length. Similar results were recorded by Popa *et al.* (2010); Venkatesh *et al.* (2014), and Mobina and Jagatpati (2015). This implies that additive genetic variables had a significant impact on the development of these traits. The above suggests the existence of additive gene effects and, as a result, a significant genetic gain under phenotypic selection. The remaining traits' moderate to low heritability estimates and moderate to low genetic advance as a percentage mean suggested that non-additive genetic variance played a role in how they manifested themselves. The traits for petiole length, top lateral branch length, and nod number showed low heritability and genetic gain. Through hybridization, these traits with low genetic advancement and heritability can be improved (Samadia, 2005; Rajan, 2012). The superior genotypes of the segregating population obtained from repeated crosses can be accumulated in the lines, whilst the traits that exhibit high heritability with moderate or poor genetic advance can be improved via inter-mating.

3.4.2 Correlation and path analysis

3.4.2.1 Associations between yield attributing traits

Understanding the relationships between characters is crucial for any crop improvement effort since it indirectly influences selection success. A small difference in the genotypic and phenotypic correlation coefficients for different traits was found during the study, which suggests that the environment has a limited impact on how these characteristics are expressed and that there are strong inherent relationships between the traits. To find the component characters that can be used in selection to boost yield, a correlation analysis is conducted to evaluate the underlying correlations between distinct characters. The correlation coefficient

measures the degree to which two variables are positively or negatively associated. The innate association between two variables characterized as genotypic correlation might be caused by linkage, the pleiotropic effects of genes, or both. The importance of correlation study in the selection program is appreciable when highly heritable characters are associated with important characters like yield ([Aruna, 2009](#)). A slight change in the genotypic and phenotypic correlation coefficients for different traits was found during the study, suggesting that the environment has even less effect on how these traits manifest and that there are strong inherent relationships between the traits. Similar findings were also reported by ([Nyasulu *et al.*, 2021](#)).

The phenotypic correlation analysis indicated that the LW was associated significantly and positively with LL which also correlated significantly and positively with PHM, PHF, LA, and DF. Other research findings also showed that the LL had a significant positive association with LW and PHM, and significant positive associations with LA were reported at the phenotypic level ([Jangde *et al.*, 2017](#); [Masum, 2020](#)). The LL had a very high positive correlation with LW, PHM, PHF, LA, DF, and GSFR. Similar to LW and LA, [Jangde *et al.* \(2017\)](#) found a substantial positive association between leaf length and both. In the current finding of PHM, BN, LL, DM, LA, DF, and DE, it was shown that the PHF had a very highly significant positive association with each of these variables. [Singh \(2010\)](#), discovered comparable results in the previous study on amaranth genotypes for plant height exhibited positive significant phenotypic correlations with days to 50% flowering and number of branches. The strong and positive link between leaf width and length also raises the prospect of enhancing both characteristics at the same time. A similar finding was reported by [Olusanya \(2018\)](#). BN was significantly and positively correlated with LN, PHM, and PHF, which indicates that selecting genotypes with taller plant heights would result in a greater number of branches, which is necessary for the production of leaf yields. [Akter *et al.* \(2005\)](#), came to the same results. DF was significantly and positively correlated with BN, PHF, and PHM. Similar findings were reported by [Singh \(2010\)](#). Days to maturity showed a positive and highly significant correlation with plant height. Similar results were reported by ([Shrivastav *et al.*, 2020](#)). The time of flowering and the time of plant maturity are important adaptive traits in grain amaranth ([Paredes-Lopez, 2018](#)). It was observed that leaf number and leaf area correlated negatively, suggesting that the more leaves amaranth produced, the smaller those leaves were. A negative correlation between a number of leaves and leaf area might act as a challenge in the improvement of leaf yield in amaranths. Furthermore, this pattern suggests that the length of the leaf and the area of the leaf decrease as the number of leave increase.

Breeding strategies should focus on increasing the area of each individual leaf or on producing more leaves in each main branch. Similar results were reported ([Gerrano et al., 2013a](#)).

Genotypic correlation between traits is important for identifying the most and least, important characteristics to be considered for the success of selection in breeding ([Tejaswini et al., 2017](#)). At the genotypic level, genes governing two separate traits are correlated positively at the coupling phase of linkage and negatively at the repulsive phase of linkage ([Salini et al., 2010](#)). The relationship between leaf length and leaf width is very strong. Similarly, most of the leaf traits showed high positive correlations with one another, indicating that these traits are crucial for improving and selecting amaranth yields. The results of the trait correlations in this study imply that selection for these features in amaranth genotypes will be efficient and successful. Similar findings on garden peas were reported by [Sharma and Sharma \(2013\)](#). With the following variables BN, LL, TILL, LN, DF, and GSFR, the PHM demonstrated highly significant positive relationships. Similar results were reported by [Shrivastav et al. \(2020\)](#), and inflorescence length and days to 50% flowering were positively and extremely significantly correlated with plant height [Yadav et al. 2014](#). A positive and significant correlation with lateral inflorescence length, leaf length, leaf width, number of branches per plant, and days to maturity was obtained ([Rani et al., 2019](#)). [Varalakshmi and Pratap Reddy \(1997\)](#) reported similar results.

3.4.2.2 Yields' genotypic and phenotypic associations with other traits

For the leaf yield, the phenotypic correlation studies carried out for this investigation revealed a strong and positive association between economically important parameters such as LA, LW, and LL. The results show that any enhancement of these characteristics will increase the potential LY of amaranth. Similar trends were identified by [Kumar, \(2015\)](#), in their analysis. Therefore, consideration for these traits should be given in amaranth breeding programs. LY had significant genotypic and phenotypic relationships with LW, PHM, LL, PHF, LA, DF, and BN. The taller genotypes in the current study produced more leaves, which resulted in higher leaf yields; hence PHM as well as PHF did forecast the genotypes' high leaf-yielding potential due to their significant association. However, the fact that PHM and PHF had a negative association with GY suggested that taller genotypes had poor grain yields. Therefore, selecting a genotype for higher leaf yield by focusing on the taller genotypes and shorter genotypes would produce good grain yields. As a result, the breeding strategy gave higher priority to characters that increased LW, PHM, LL, PHF, LA, DF, and BN, increasing the

chances of obtaining a genotype with a high leaf yield. Similar results were reported in amaranth genotypes by [Varalakshmi and Pratap Reddy \(1997\)](#), who reported that plant height, leaf length, and leaf width were positively and significantly correlated with the leaf yield. Moreover, choosing genotypes with taller plant heights will result in a better number of branches, which is required for the production of leafy vegetables, according to the statistically significant positive association between plant height and the number of branches. A similar finding was reported by [Olaniyi \(2022\)](#) Vegetable amaranths are said to benefit from late flowering and maturation since farmers would have more time to harvest the leaves ([Akaneme and Ani, 2013](#)). A single gene controls early flowering in amaranth, and the dominant allele determines early flowering ([Brenner et al., 2000](#)). For grain production, early flowering and maturity would be preferable. The positive and significant correlations between yield and yield components in amaranth were also reported ([Shukla et al., 2018](#); [Shayanowako et al., 2021](#))

The results showed that at the phenotypic level, GY with GSFR, AIL, TISL, and TILL have extremely high positive phenotypic correlations. Furthermore, it demonstrated a strong positive phenotypic relationship with both LW and LL. The results suggest that these traits might be simultaneously enhanced to raise grain yield either individually or in combination. [Showemimo et al. \(2021\)](#), reported comparable results. As a result, amaranth should be chosen for future breeding programs to ensure the maximum exploitation of grain amaranth, as the photosynthetic capacity of a plant with longer leaves is predicted to be higher than that of a plant with shorter leaves ([Garg, 2017](#)), and traits like a high leaf area, leaf length, and plant height enable optimal crop output when water is accessible as an essential agricultural input in sufficient quantities ([Onasanya et al., 2009](#)). Grain yield exhibited a highly significant and positive phenotypic association with inflorescence length (TISL, TILL, and AIL). [Nyasulu et al. \(2021\)](#); [Kumar \(2018\)](#), and [Yadav et al. \(2014\)](#), obtained consistent findings in amaranth species. As a result, characteristics may be prioritized during selection to produce genotypes with higher grain yield.

The genotypic relationship between GSFR, PHM, and LY with the GY was also very substantial and positive. It also revealed a highly significant positive genotypic association with LN, a substantial positive genotypic association with DF, and a significant positive genotypic link with TILL. These findings demonstrated that selection for any one of these traits that contribute to grain yield will result in increases in the other traits, thereby finally

boosting the grain yield. To select genotypes with higher grain production, primary selection for characteristics like GSFR, PHM, and LY may be prioritized. There were similar trends for the association of GY with other traits (PHM, TILL, and DF) that may be prioritized (Yadav *et al.*, 2014). Grain yield and leaf area, length, and width are strongly correlated with one another. Similar to grain yield, all leaf traits showed high positive correlations with one another, indicating that these features are important for selecting amaranth leaf and grain productivity. The findings of the trait correlations in this study imply that selection for GSFR, PHM, and LY will be efficient and beneficial in improving grain production in grain amaranth genotypes, as also found in garden peas by Sharma and Sharma (2013). In addition, the positive correlation between grain yields and days of flowering revealed that early flowering would be a viable choice for a greater grain yield in this scenario. These results appear to be in line with a general pattern that early blooming is linked to higher grain yields across species (Akin-Idowu *et al.*, 2016; Olusanya, 2018). These findings appear inconsistent with a general trend for late flowering to be associated with high grain yield across species (Olusanya, 2018).

3.4.2.3 Effects of yield contributing traits on yields

The relationship between any two features is merely depicted by their correlation coefficients, which do not reveal any potential underlying causes. The degree of the relationship between the yield and yield components can be determined by path coefficient analysis, which also helps to clarify the cause-and-effect interactions between different characters. Path coefficient analysis was performed to segregate the correlation coefficient into the direct and indirect effects of various features on yield. The yield-contributing features for LY and GY's varied phenotypic and genotypic correlation coefficients were further divided into direct and indirect effects. According to the findings, both phenotypic and genotypic levels of LY were positively affected directly by the LW, BN, LN, PHF, LA, and GY. This suggests that these traits can be used to develop a selection index that is both maximally reliable and effective at improving the leaf yield of amaranth genotypes. These findings confirmed the results of Aycicek and Yildirim (2006), for PHF in amaranths genotypes (Kumar, 2015; Shah *et al.*, 2018), for the number of leaves in amaranths genotypes, Islam (2021) for leaf area, and (Jangde *et al.*, 2017) or leaf width. LL, TILL, and DE, on the other hand, were found to have the greatest negative direct effects on LY, both at the phenotypic and genotypic levels, showing that direct selection of these traits did not improve LY in amaranth genotypes. Following the present findings, the LL had a negative direct influence on LY in amaranth

genotypes ([Kumar, 2015](#); [Masum, 2020](#); [Islam, 2021](#)), However, since PHM, LL, and TILL had such strong indirect effects, mostly through LA, while DM and DE exhibited such strong indirect effects, primarily through PHF, it was anticipated that LY in amaranth genotypes may also rise through an indirect selection of these traits. While TISL had positive direct effects on LY at the genotypic level, AIL had positive direct effects on LY solely at the phenotypic level. Additionally estimated was the residual effect, which establishes the unaccounted variability of the dependent variables (LY and GY). The characteristics that were included in the phenotypic and genotype path coefficient analyses explained 87.12% and 87.79 % of the total variance in LY, respectively, according to the residual effects of 0.1288 and 0.1221. These results suggest that the independent variables taken into account in this study effectively represented the diversity of LY in amaranth genotypes. Other factors (at phenotypic level 12.88% and genotypic level 12.21%) that caused variations in LY, but were not taken into account in the current investigation, did exist.

A path analysis' findings revealed the importance of GSFR, PHM, and PHF traits for direct selection since they had considerable positive direct effects on GY both at the phenotypic and genotypic levels. This suggests that GY improvement in amaranth genotypes might be obtained through selection based on these characteristics. The result was congruity with ([Kumar, \(2015\)](#) and [Masum, \(2020\)](#) for the plant height in amaranth genotypes. On the other hand, LW, TISL, LL, and DF had negative direct effects at both phenotypic and genotypic levels indicating that direct selection based on these traits could not improve the GY in amaranth genotypes. The results were also in agreement with the findings of [Shrivastav *et al.* \(2020\)](#), who reported that DF had a negative direct effect on GY in amaranth genotypes. The direct selection of LW, TISL, LL, and DF at both phenotypic and genotypic levels would decrease the GY; however, their indirect selection through mainly PHM may improve the GY in amaranth genotypes. PHM had beneficial indirect effects on LW, LL, TISL, and DF in addition to its beneficial direct effects on GY. For the indirect selection of high seed-yielding genotypes in amaranths, the DF may be a trustworthy indicator. [Alake and Otusanya \(2020\)](#), obtained consistent findings in amaranths genotypes. The direct effect of an AIL, BN, TILL, LA, and DE on GY was negative only at the genotypic level while LN and LY had a positive direct effect on GY only at the genotypic level. Other research findings also showed that LN had a positive direct effect at the genotypic level on GY in amaranth genotypes ([Tomar *et al.*, 2022](#)). While LY had a negative direct effect on GY only at the phenotypic level, AIL, BN, TILL, and LA had positive direct effects on GY only at the phenotypic level. On grain yield

in amaranth genotypes, the same observation was made for TILL, BN, LA, and AIL ([Kumar and Yassin, 2018](#)). Furthermore, LW, AIL, BN, TISL, TILL, LN, DF, LY, and GSFR might be indirectly selected, mostly through PHM, to improve the GY at the genotypic level. According to the residual values of 0.1005 and 0.1140, the traits included in the phenotypic and genotypic path coefficient analyses, respectively, explained 89.95% and 88.6% of the overall variation in GY. This suggests that additional traits may also be influencing GY.

3.4.2.4 Selection of traits using correlation and path coefficients

When combined with path coefficients, correlation coefficients offer more accurate data that may be successfully predicted in crop development initiatives ([Sahu and Tiwari, 2020](#)). The findings indicated that selection based on the phenotypic expression of LW, BN, LN, PHF, LA, and GY could be successful for LY improvement in amaranth genotypes these traits had positive direct effects on LY along with a significant positive association with LY at both phenotypic and genotypic levels. On the other hand, LL demonstrated negative direct effects on LY both at the phenotypic and genotypic levels, even though LL, DF, and GSFR had significant positive phenotypic and genotypic associations with LY. The occurrence of significant positive phenotypic and genotypic associations along with inverse direct effects between LY and these traits could be due to the high positive counterbalancing potential of the phenotypic and genotypic indirect effects exerted by these traits via mainly LN. The genotypic direct effect of DM on LY was negative despite the non-significant positive genotypic association between the two traits, indicating that the positive genotypic association between these traits was caused by the high and positive neutralizing genotypic indirect effect of this via chiefly by LA. Despite AIL's positive phenotypic direct effect on LY, its negative phenotypic correlation with LY might be because of AIL's significant negative phenotypic indirect effect, which largely acts through LA.

The GSFR had significant positive phenotypic and genotypic relationships with GY as well as positive direct impacts on GY, suggesting that selection for genotypes of amaranth based on the phenotypic expression of these traits may be successful in improving GY. [Wei et al. \(2011\)](#) obtained consistent findings in rice, while [Knott and Gebeyehou \(1987\)](#), reported equivalent results in bread wheat. Likewise, GSFR, PHM, and LY exhibited significant positive genotypic associations with GY as well as positive genotypic direct effects, revealing that genotypic selection based on the phenotypic expression of these traits would be effective for enhancing GY in amaranth genotypes. Although PHM and PHF had positive phenotypic

and genotypic direct effects on GY, their correlations with GY were negative and insignificant on both a phenotypic and genotypic level. This might be because PHM and PHF's indirect actions, which primarily affect BN, LL, and DF, have significant and detrimental neutralizing potential. The phenotypic and genotypic direct effects of LW, LL, and TISL were negative but their associations with GY were positive at genotypic levels. This was due to the high and positive neutralizing potential of the genotypic indirect effects exerted by both traits via mainly PHM, LN, LA, and LY. The phenotypic (DE) and genotypic (DF and DE) direct effects on GY and their respective associations with GY were negative and insignificant indicating that the GY improvement based on the selection of these traits could not be effective in amaranth genotypes.

3.4.3 Multivariate analysis

3.4.3.1 Clustering of genotypes

Cluster analysis is another method for determining the pattern and degree of correlations between or among genotypes. The 120 amaranth genotypes were grouped into five clusters based on the mean performance of quantitative agro-morphological traits. One of the most effective ways to take use of genetic diversity for yield enhancement is to cluster a large number of genotypes according to how well they perform on average. Genotypes obtained from the same region of the country more or less clustered together in some cases, as was observed in Cluster III with genotypes SAA-004, HA-003, DA-006, BA-016, SRA-002, K3A-005, SA-025/07, SA-008/07, SA 001/07, SHIA-007, SA-005/07, HA-008, BA-001, WA-001, and WA-003, which are from the Afar region. This might indicate that some traits are more restricted to specific geographical locations and that genotypes are more suited to the region's agroecological circumstances than others. [Akin-Idowu *et al.* \(2016\)](#), for example, made comparable observations. The clustering pattern of distinct genotypes, on the other hand, did not reflect their geographical distribution and was somewhat fairly random. In this study, the genotypes from South Nation Nationality People's Regional State, Amhara, Sidama, Tigray, Benishangul, & Gumuz region, and released varieties of Ethiopia, for example, showed some regional consistency. Clusters IV and V were created to group them. [Shanmugam and Sree Rangasamy \(1982\)](#), reported that the falling of materials of the same origin into separate clusters was an indication of the broad genetic base of the genotypes belonging to that origin. The current results were consistent with the findings of [Akin-Idowu *et al.* \(2016\)](#). This shows that genotypes from the same origin seen in distinct clusters are indicative of the origin's wide

genetic foundation. This is in agreement with the findings of [Kemal and Hailu \(2019\)](#) in Safflower; and [Gebeyehu et al. \(2021\)](#) in *Guizotia abyssinica*.

3.4.3.2 Performance of genotypes under different clusters

The highest grain yield-producing clusters were typically found in the first clusters, and the highest leaf yield-producing clusters were typically found in the fourth cluster. LT, TLBL, TISL, TILL, AIL, and GSFR all exhibited high values in the first cluster. In terms of LY, PHF, PHM, LL, LW, SD, TISL, TISL, and GY, genotypes classified in the fourth cluster performed better on average. The best-performing genotypes in terms of the aforementioned traits were likewise found in this cluster, and the mean values of these traits were also higher than the overall grand mean of the corresponding trait aggregated across all clusters. The GY produced by the genotypes in the fourth cluster was high despite the fact that grain yield-related parameters, particularly GSFR, TISL, LT, and TILL, were high in this cluster. This could be a result of the smaller seed size (TSW) that the genotypes in this cluster generate, which may have a major impact on the GY per unit area. In terms of DF, LY, LL, LW, LA, PHM, PHF, LT, BN, DM, GFP, SD, TISL, TILL, GSFP, and GY, the genotypes classified in the fourth cluster had higher mean performances, top-performing genotypes, and higher mean values than the grand mean. However, the genotypes included in the fourth cluster had the intermediate mean values of PL, LN, TLBL, BLBL, AIL, and TSW. This result indicated that high GY-producing genotypes had shorter plant height at flowering and plant height at maturity performance. Different scholars reported that modern cultivars (high grain yielders) had short plant heights as compared to landraces which produce low grain yield ([Reynolds et al., 2009](#); [Feng et al., 2021](#)).

Moreover, high cluster means for yield and its components should be given significant consideration when choosing genotypes for inclusion in the hybridization program based on genetic diversity ([Kumar and Singh, 2006](#)). Furthermore, the germplasm from these clusters might be utilized to generate high-yielding varieties or hybridization programs to create materials with desirable traits ([Ajmal et al., 2013](#)). Hybridization of genotypes from distinct clusters with high cluster means for quality attributes will generate *Amaranthus* genotypes that outperform their parents. Furthermore, the greatest inter-cluster variability demonstrated by the genotypes categorized into the various clusters would provide immense potential for *Amaranthus* genotype improvement through hybridization programs involving genotypes from various clusters. A multivariate investigation of bread wheat found similar results

(Ahmad *et al.*, 2014). These results suggest that the genotypes grouped in different clusters may be used as potential parental lines for hybridization programs to develop desirable genotypes, as genetic diversity can be better exploited and the chances of finding better segregants increased. In the future, amaranth tree clusters will be used as parents for hybridization or other population enhancement procedures. The cluster that contributed the most to the divergence was given priority when choosing the kind of cluster for selection and the parents for hybridization.

3.4.3.3 Genetic divergence

The standard Mahalanobis (D^2) statistic is a powerful tool for quantifying the genotypic level variation and may be an effective tool in estimating the genetic diversity in genotypes. Due to their similar performance for the examined variables, the genotypes grouped within a cluster showed restricted genetic variation, as evidenced by the observed intra-cluster genetic distance being smaller than the inter-cluster genetic distance. This finding is also consistent with those of Ahammed *et al.* (2013a), and Kumar (2019), who found that in amaranth genotypes, the intra-cluster genetic distance was typically smaller than the intercluster genetic distance. Although collection regions were far apart, C II and C III, clusters I and III, had the largest distances between groups, but C I and cluster II had the smallest distance between clusters, which should be expected in terms of spatial distribution. In comparison to genotypes in other clusters, the genotypes in these clusters were quite near to each other.

A broad Mahalanobis distance (D^2) between the genotypes of two dissimilar clusters could maximize transgressive segregation (Ayana and Bekele, 1999). Crossing genotypes from those clusters, according to Rahman *et al.* (2012) may not result in a greater heterotic value in F1 and a restricted range of variability in the segregating F2 population. To preserve a somewhat diverse genetic foundation, such analysis was intended to prevent selecting parents from genetically homogenous groups. Spatial dispersion may enhance the accumulation of competing alleles between the two populations, enhancing genetic dissimilarity between them. Clusters II and III, followed by clusters I and III, were the most distant of the five clusters on average. Because crosses between genetically divergent lines will generate heterotic segregates or a broad spectrum of variability in subsequent generations, and thus could be exploited in a hybridization program, the results revealed that a large inter-cluster distance was the major driving force of variation in a cluster's makeup and may be used as parents for hybridization programs to develop desirable varieties (Afroz *et al.*, 2013; Kabir *et*

[al., 2013](#)). This suggests that there are significant genetic differences across these clusters; by choosing genotypes according to the intended use of the crop, yield enhancements may be possible. When parents chosen from these genetically distant clusters are crossed, it is thought that the biggest genetic divergence between them will result in the greatest amount of genetic recombination and differences. However, based on the specific purposes of crossing or hybridizing, the choice of parents from these distant clusters should be made following the peculiar traits of each cluster and the performance of each genotype within each cluster ([Singh, 1990](#)). Crosses involving parents from the clusters with the greatest distances are predicted to have the highest levels of genetic recombination and diversity in the following generation ([Sawarkar et al., 2015](#)). Based on both group performance and the performance of individual genotypes for specific traits, parents with one or more of the desired traits can be chosen from each cluster([Singh, 2016](#)). [Kumar \(2019\)](#) findings are consistent with the present findings. Accordingly, suitable parents for heterosis breeding can be genotypes belonging to clusters C I, C III, C IV, and C V.

3.4.3.4 The relative contribution of different characters to genetic divergence

The contribution of individual characters toward divergence was estimated by [Sneha et al. \(2020\)](#). Out of the 23 traits examined, PHM (8.5%), LW (8.10), LL (6.90%), DM (6.0%), and BLBL (5.6%) contributed the largest contributions to variety. These traits were responsible for expressing maximum variety between the clusters, and so should be given considerable weightage during selection. The remaining characters contributed little to divergence. A similar result was observed in Wheat (*Triticum aestivum* L.) genotypes ([Mecha et al., 2017](#)).

The study of agro-morphological based genetic diversity across genotypes from various phytogeography areas is significant because a species' capacity to adjust to changing conditions is essentially subordinate to the amount of variety accessible in its gene pool. We examined the agro-morphology diversity in *Amaranthus* genotypes from a variety of perspectives. According to [Sarker and Oba \(2021\)](#), genetic variability in amaranths is impacted by both genetic and environmental variability. As a result, the use of molecular markers is essential to supplement morphological data and to increase the precise degree of genetic diversity. As a result, the current characterization information makes strides in the

utilization of amaranth genotypes in breeding programs and guarantees viable genotype collection administration. As a result, further molecular inquiry about the subject is required.

3.4.3.5 Principal component analysis

Without a constant supply of biologically diverse plants, modern plant breeding would not be conceivable, much alone successful (Duvick, 2007). The availability of genetic resources and the evaluation of their diversity are both markers of breeding program success for any crop, including amaranth (Hoisington *et al.*, 1999; Choudhary *et al.*, 2017). The initial stage in plant breeding is to determine genetic diversity in agro morphological variability and characterization in plant breeding. Diversities in morphology result from differences in the crop's genetic makeup (Manimekalai *et al.*, 2018; Soe *et al.*, 2019) as well as the likely effect of planting places and times. Plant breeders may need to gather germplasm from underutilized crops to generate improved cultivars that will feed fast-growing populations (Nelson, 2011). Through trait-assisted selection, trait variation aids in the selection of the best lines for improvement (Fasahat *et al.*, 2016; Moradi *et al.*, 2019; Dewi *et al.*, 2020).

Principal component analysis (PCA) is the re-validation technique of cluster analysis (Khan *et al.*, 2021). PCA was also helpful in detecting the correlations between traits, as well as recognizing the independent principle components that are effective on plant attributes and assessing diversity (Beheshtizadeh *et al.*, 2013). Moreover, PCA is a well-known dimension reduction approach for condensing a big collection of variables into a small set that retains the majority of the information from the large set (Sharma *et al.*, 1998). It reflects the importance of the largest contributor to the total variation at each axis of differentiation (Jolliffe, 2002). According to Chahal and Gosal (2002), characters with greater absolute values close to unity in the first principal component influence clusters more than those with lower absolute values close to zero. As a result, rather than a little contribution from each character, the current study found that the diversification of genotypes into separate clusters was due to the rather substantial contribution of a few traits. Therefore, characteristics with higher coefficients, typically 0.6 or more, on the PC axis are generally considered more important (DeLacy and Cooper, 1990). The positive and negative loading indicates the presence of positive and negative correlation trends between the components and the variables. Therefore, the above-mentioned traits that load high either positively or negatively contributed more to the diversity, and they were the ones that most distinguished the clusters.

The results of multivariate analysis for 24 attributes revealed that the first six PCs accounted for the majority of the variability. All 24 quantitative traits were determined as essential agro-morphological makeup, contributing to total trait variability and hence being appropriate traits for amaranth selection and improvement, according to the PCA. In this study, the first component 30.94% (PC1) accounted for a greater proportion of the variation than the second component 21.49% (PC2). Similar results were reported by [Showemimo et al. \(2021\)](#) of total variations at 39.87% (PC1) and 18.64% (PC2) in *Amaranthus species*. Several studies also support these findings ([Oyetunde et al., 2021](#); [Subaşı, 2022](#)). In addition, PC1 is the most powerful criterion for selection for yield improvement ([Adéoti et al., 2012](#)). Days to flowering, basal stem diameter, plant height at maturity, plant height at flowering, auxiliary inflorescence length, number of branches, leaf length, leaf width, terminal inflorescence lateral length, leaf yield, grain yield, grain sink filling rate, and thousand seed weight were all investigated in this study have a greater impact on PC1. A similar result was identified in pervious report ([Shankar et al., 2012](#); [Akin-Idowu et al., 2016](#)). The substantial relationship between PC1 and grain yield contributing parameters, as well as PC2 and leaf yield contributing traits, suggests a reasonable selection direction. Several researchers ([Showemimo et al., 2021](#); [Subaşı, 2022](#)) supported our findings. Plant height, days to maturity, days to flowering, and length of inflorescence were also recognized as key direct drivers of grain output investigations, This is in agreement with the findings of [Kandel et al. \(2021\)](#) in amaranths.

Another widely used method for displaying graphical groupings of genotypes for examining interrelationships between agro-morphological features in various crops is the biplot ([Mwakha et al., 2020](#); [Oyetunde et al., 2021](#)). According to [Naidoo et al. \(2020\)](#), traits that were exhibited towards the center of the biplot had a smaller impact on the overall variation than those that were placed farther out. In the biplot analysis, the length of the vector lines and their proximity to the axis are often related to the size of the vector loadings. Furthermore, the loading variables in the PCA biplot indicate how strongly each trait affects a PC and how they are connected. For traits with longer and closer vector lines to the X-axis in the first two PCs, as opposed to traits with shorter and longer vector lines with a narrow and wide distance of the vector trait from the axis, respectively, For instance, traits of PHM, PHF, SD, and DF had longer vector lines which are closer to the X-axis as compared to B LBL which also had longer vector lines but the line was far from the X-axis. This indicates the

contribution of PHM, PHF, SD, and DF for the total variation was higher in the first PC as compared to BLBL, and the contribution of these traits to the total variation in the first PC is noticeably higher. However, BLBL had a longer vector line which is very close to the Y-axis indicating that the contribution of this trait for the second PC was higher as compared to other traits.

In general, the cosine of the angle between a vector and an axis indicates the significance of the contribution of the corresponding trait to the PC. As a result, the PHM in the first PC and BLBL in the second PC had a notable contribution to the total variation due to the small cosine of the angle between the traits and the respective PC. The narrower the angle produced by two vectors, the better the relationships. The origin of a PCA biplot shows the average value of all characteristics (DeLacy and Cooper, 1990), whereas the measure of an angle between the vectors of two traits indicates their correlation relative to their variance among genotypes. An angle of zero signifies a complete or perfect positive correlation (+1), an angle of $= 90$ indicates no association, inferring independence or the absence of a relationship between the two traits, an angle > 90 indicates a negative correlation, and an angle of 180 indicates a lack of correlation or perfect negative correlation (-1) (Malik *et al.*, 2014; Feltaous, 2019). Among various quantitative traits, significant and positive relationships were observed. As a result, a positive correlation between the traits suggested that improving one trait would also improve the other required trait. As a result, the biplot loadings revealed that there was a significant positive association between leaf area, leaf breadth, leaf length, and leaf yield. Because leaf area is a function of leaf breadth and length, this was predicted. Across amaranth genotypes, Gerrano *et al.* (2013b), Hailu *et al.* (2015), Akin-Idowu *et al.* (2016), and Mwakha *et al.* (2020) found comparable results.

In addition, TSW GY, and BN showed a strong association. Furthermore, there is a significant link between BN and DM. This is consistent with Yoshihira *et al.* (2020) on soybeans. Furthermore, it was discovered that economically important grain yield and grain yield parameters including TISL, TILL, AIL, GSFR, and TSW are positively correlated. This demonstrated that improving inflorescence-related features leads to an increase in the number of grains and grain yield contributing to production. A similar result was observed in wheat by Sokoto *et al.* (2012); Hailu *et al.* (2015); Guo *et al.* (2018) and Espitia-Rangel (2018) in amaranths. The DM is comprised of the vegetative stage and the GFP. As a result, they should be associated with it. DM was correlated with the vegetative phase and GFP in this study.

The rate of grain filling and the length of the filling period should both influence cereal production (Daynard *et al.*, 1971). In the present study, grain yield and yield-related features were shown to be negatively correlated with grain filling periods but positively correlated with grain filling rates. GY did not correlate with DF, DM, or GFP, as reported in pervious report (Nass and Reiser, 1975). The lack of a correlation between grain yield and vegetative and grain filling periods, in particular, suggests that in our situation, these different growth duration traits would not be yield-limiting factors in *Amaranthus* genotypes and that breeding efforts should be directed toward other grain yield-related characters. In addition, early genotypes are more likely than delayed genotypes to have shorter vegetative and grain-filling periods. Days to maturity are comprised of vegetative and grain-filling stages, thus they should be interconnected, as shown in our research. Others have identified consistency in the correlations between grain filling periods, days to maturity, and grain yield. Others, on the other hand, have observed disparities in the correlations between grain filling time, days to maturity, and grain yield.

The DF, DM, and BN per plant all showed a negative relationship with grain yield-related traits such as TISL, TILL, AIL, GY, and TSW per plant. This is in agreement with the findings of Hailu *et al.* (2015); and Kigel and Paredes-Lopez (1994) in amaranths., meaning that genotypes with late flowering, matured, and a limited number of branches per plant had poor seed yield and vice versa. The grain yield contributing characters were negatively correlated to the leaf yield parameters, indicating that genotypes with high leaf yield also had lower grain production. The result was in line with (Dinssa *et al.*, 2018). Similarly, characteristics associated with vegetable yield contributing characters, such as LN, BN, PH, and LA, are also positively correlated. A similar observation was recorded by Sagar *et al.* (2018) and Dinssa *et al.* (2018). This signifies that the amount of vegetables produced has increased. Tejaswini *et al.* (2017), found a positive correlation between LW, LL, and PH, as well as a negative relationship between vegetable yield traits and the number of branches. In the current study also, DM, DF, and PH were positively correlated. A comparable finding was reported by Yadav *et al.* (2014), and Nyasulu *et al.* (2021). Because the leaf affects the interception of solar radiation, resource allocation, water uptake method, and water stress tolerance, the leaf area is an important characteristic in determining the plant's production (Weraduwege *et al.*, 2015). By enlarging the leaf area, the plant's ability to capture solar radiation can be improved. Valencia *et al.* (2016) reported that a reduced leaf area encourages flowering to begin early, resulting in a positive relationship between leaf area and days to flowering. The current

investigation found a similar positive association between leaf area and flowering time. Flowering occurs later in the season, indicating that more resources are spent to plant development, resulting in more photosynthates available for seed formation. As a result, a plant with delayed blooming and a large leaf will collect more carbon, resulting in a larger seed and a higher yield when harvested (Sharma *et al.*, 2021).

3.5 Conclusions

Overall, the study's findings showed that 120 genotypes of amaranth were analysed using markers of agro-morphological features that were collected over two years in Ethiopia and that there was a significant amount of diversity among them. For the characteristics BLBL, AIL, LA, BN, PHF, PHM, SD, DF, GFP, and LW, estimations of very high to moderately high heritability, high to moderate genetic advance, and genetic variability were found. This indicates that selection based on these traits could be effective in producing the genetic improvements required for advancement. On the other hand, route analysis may be used to enrich the information obtained from correlation to identify traits that greatly boost the grain and leaf yield of amaranth genotypes, hence improving breeding selection efficiency. This suggests that selection based on these features may be successful in generating the needed genetic gains for improvement. Furthermore, information from correlation may be augmented with the use of route analysis to select features that significantly increase the grain and leaf yield of amaranth genotypes and therefore, enhance selection efficiency in breeding. Additionally, the first two major components contributed significantly to the overall diversity in genotypes of amaranth. PHM, BLBL, LL, DM, DF, LA, AIL, BN, TILL, DE, TLBL, SD, TISL, GY, GSFR, GFP, TSW, LW, PHF, LT, and LY were among the variables that contributed significantly to the overall diversity in amaranth genotypes among those that were evaluated. As a result, improving yield in amaranth genotypes may benefit from the selection of these features. Further evidence of the potential of amaranth genotypes in future breeding efforts for enhancing LY, GY, and yield-related variables comes from the presence of remarkable variance across amaranth genotypes for measured traits.

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CHAPTER IV: Agro-morphological genetic diversity assessment of amaranth genotypes from Ethiopia based on qualitative traits

Agro-morphological genetic diversity assessment of amaranth genotypes from Ethiopia based on qualitative traits

Abstract

*Amaranths are a promising plant in the family of Amaranthaceae because of their nutritional and functional properties, such as their high antioxidant content and dietary fiber content. However, it's being disregarded for several reasons, such as ignorance, a lack of in-depth research, and the plant's long-term genetic growth in Ethiopia, among other factors. In the current work, we assess genetic diversity among Ethiopian amaranth genotypes using qualitative morphological traits. The experiment, which was configured with an alpha lattice design and duplicated twice, was run for two seasons in 2020 and 2021. Twenty qualitative descriptors were looked for in the gathered data. Among the 20 qualitative variables that were assessed, the chi-square test result indicated the presence of prevailing phenotypic variation. The results of the agro-morphological characterization also revealed a significant amount of variance. The overall mean of the Shannon diversity indices (H') was 0.61. The indices for germination rate, leaf margin, prominence of leaf veins, and the existence of auxiliary inflorescence varied from 0.12 to 0.99. The estimated diversity indices showed more intra-regional diversity (0.66) than inter-regional diversity (0.34), demonstrating the existence of gene flow between growing regions. Shannon-Weaver Diversity Index, ranged from 0.00 for auxiliary inflorescence to 1.94 for leaf coloration, with an overall mean of 19 characters (95%) that were found to have high diversity (>0.76) while auxiliary inflorescence was invariant. Except for auxiliary inflorescence, all qualitative features showed a lot of variation. Additionally, *Amaranthus hybridus* L. subsp. *cruentus* (L.) Thell recorded the greatest Shannon diversity index (0.47) while *Amaranthus spinosus* L. showed the lowest (0.00). The hierarchical clustering grouped all the genotypes into three clusters. The first cluster included the most genotypes (58), followed by the second (47), and the third cluster contained the fewest (15). Principal component analysis showed that the first six principal components with eigenvalues greater than one contributed 72 % of the variability among genotypes. The study unequivocally demonstrated that, even when the genotypes were grouped into a small number of clusters, there was still enough divergence within the clusters to demonstrate the genotypes of amaranth to have a high genetic diversity. These results indicate that there is substantial genetic diversity among Ethiopian amaranth genotypes, which should be safeguarded and may be utilized in breeding in the future.*

Keywords: agro-morphological, descriptor, diversity, genotype

4.1 Introduction

Plant biodiversity is the primary source of food, feed, shelter, medicine, and a variety of other products and services that make life feasible and enjoyable on Earth (Padulosi *et al.*, 2002). The number of plant species utilized by humans around the world accounts for only one-third of the total number of species used by generations of people from various cultures (Heywood and Watson, 1995). Incorporating alternative crops with a good nutrient profile could promote dietary diversity and be a crucial part of raising food quality to battle unmet hunger and boost

biodiversity. Amaranth is one of the few highly nutritive crops that serve multiple purposes, including those of vegetable, cereal, medicinal plant, dye plant, pasture, fuel, and ornamental (Mlakar *et al.*, 2009; Sheikh and Singh, 2013). Research and development on amaranths could result in a simple and affordable method of eradicating malnutrition, boosting people's health, and ensuring food security.

The name *Amaranthus* comes from the Greek word *amarantos*, which also means "never fading," "immortal," "everlasting," "non-wilting," or "one that never perishes." (Anjali *et al.*, 2013) because its flowers last for a long time (Anjali *et al.*, 2013; Rastogi and Shukla, 2013). High genetic variability is prevalent in *Amaranthus*, which signifies variation in plant form (erect to prostrate), seed color, seed yield, protein content, resistance to pests and diseases, and adaptation to soil type, pH, climate, rainfall, and day length (Assad *et al.*, 2017; Tejaswini *et al.*, 2017). *Amaranthus* is a genus comprising 87 species (Jacobsen *et al.*, 2003). Due to a lack of systematic investigations into amaranth, the number of its species is unclear (Costea and DeMason, 2001). *Amaranthus* is a versatile crop that is grown all over the world for use as a vegetable, cereal, medicinal plant, dye plant, pasture, fuel, ornamental, and weedy plant (Wu *et al.*, 2000; Sheikh and Singh, 2013). Amaranth, a long-standing part of traditional African agriculture, is semi-domesticated in Ethiopia and other East African countries, primarily as a vegetable (Alemayehu *et al.*, 2015). Due to the widespread usage of local names and the large genetic variation present in Ethiopia, the country has been suggested as a hub of amaranth diversity. Eleven species have been identified in the flora regions of Ethiopia and Eritrea (Demissew, 2010).

Amaranthus was named a promising economically valuable underutilized plant by the National Academy of Sciences and it is regarded as the most important raw resource for food and nutrition, as well as a powerful medicinal plant (Shodiev *et al.*, 2021). According to Ishimoto and Monteiro (2010), these plants contain each attribute of a functional food. Moreover, because amaranth flour is gluten-free, it might be a suitable source of nutrition for people who are intolerant to gluten. Amaranth leaves are anti-cancerous; they prevent the erratic division of cancer cells in the breast, colon, and liver, making them suitable for cancer patients as well (Li *et al.*, 2015). As a result, including these grains in one's diet has the potential to enhance one's diet while also providing health advantages. Moreover, amaranth grains have a greater protein content than other typical cereals (Gamel *et al.*, 2004). Pseudo cereals' nutritional value is primarily determined by their protein content. Amaranth grains

are an excellent source of proteins with a well-balanced amino acid profile, with a high concentration of lysine and sulfur-containing amino acids (Haros and Schoenlechner, 2017). Amaranth usually has more protein than quinoa or buckwheat (Haros and Schoenlechner, 2017). The high concentration of lysine, which is required by the human body but cannot be produced by it, is a significant benefit of amaranth. Amaranth is unusually high in this essential amino acid, which is found in low or limited amounts in other grains and plant sources. Amaranth is equivalent to other animal proteins in terms of lysine and tryptophan levels (Correa *et al.*, 1986).

Morphological features are helpful for early evaluation since they are quick, easy, and may be utilized as a generic technique for evaluating genetic diversity across morphologically different genotypes. Regarding the morphological features investigated, the current study showed significant variance among the germplasm. *Amaranthus* has a remarkable morphological diversity and can adapt to a wide range of ecological and geographical environments (Lee *et al.*, 2008). Despite the vast phenotypic diversity across and within amaranthus species, the genus has just a few taxonomic characteristics that are unique to it (Sammour *et al.*, 1993; Juan *et al.*, 2007). Genetic diversity is mostly determined by the agro-morphological characterization of genotypes and it is essential for biodiversity conservation and crop improvement efforts to be successful (Shah *et al.*, 2018). The assessment of a crop species' genetic diversity is an excellent place to start when it comes to crop improvement since it provides a framework and a roadmap for choosing parental lines and designing a breeding program. It's crucial to the creation of new crop varieties with desirable characteristics. As a result, determining the genetic diversity of current genotypes is a prerequisite for crop improvement. Unfortunately, genetic research of the genus *Amaranthus* is very limited in Ethiopia due to lack of extensive research, discrimination, and lack of knowledge, the improvement of this plant is limited. Furthermore, researchers are less interested in this "orphan crop," as it is known (Jonah *et al.*, 2012). The study on amaranth has significantly aided in improving amaranth growth by building a value chain for the manufacturing of value-added products based on amaranth that may aid in food security and nutritional quality. Even though several countries have reported on the distribution of amaranth species used like cereal grains and the production of ancient grain products, Ethiopia has yet to conduct similar research.

The amaranth genus reportedly exhibits high levels of morphological variety (Srivastava and Mahavidyalaya, 2015; Molin and Nandula, 2017; Oyetunde *et al.*, 2021). For describing species genotypes, the qualitative features have been considered advantageous (Gerrano *et al.*, 2015). Amaranth genotypes have not yet been the subject of any specialized research in Ethiopia. The objective of the current study was designed to determine the phenotypic diversity of Ethiopian amaranth genotypes using standard qualitative agro-morphological descriptors. Therefore, the study of genetic varieties is essential for giving information for efforts about domestication, breeding, and propagation, as well as for the preservation of the genetic resources of plant species. Consequently, the results are intended to promote future genetic research aiming at strengthening this unusually nutrient-rich and climate-resilient crop species by employing qualitative agro-morphological features.

4.2 Materials and methods

4.2.1 Experimental site

For the experimental site description please see chapter three and [Figure 4.1](#)

Table 4.1 Description of study site

No	Parameter	Hawassa University Farm and Research Center
1	Altitude (masl)	1709
2	Latitude (N)	7°2' 54.7503"
3	Longitude (E)	38°30' 17.1608"
4	Amount of rain fall (mm)	1379.16
5	Temp.(Oc)	
6	Maximim.temp (°C)	27.9 0C
7	Minimum.temp (°C)	14.10
8	Soil PH	6 - 6.5

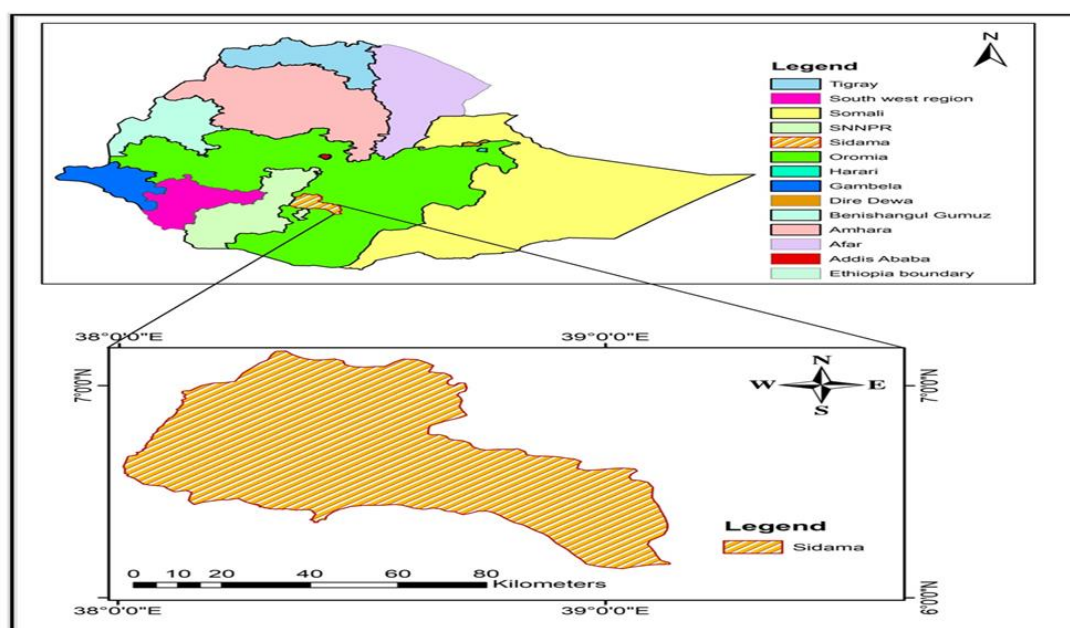


Figure 4.1 Map of the study area.

4.2.2 Plant material and experimental procedures

A total of 120 amaranth genotypes from seven different species— *A. hybridus* L. subsp. *hybridus*, *A. hybridus* L. subsp. *cruentus* (L.) Theil., *A. caudatus* L., *A. viridis* L, *A. spinosus* L., *A. palmeri* S. Wats., and *A. species*—were used for the qualitative genetic diversity study, with 34 coming from the Ethiopian Biodiversity Institute (EBI), 2 from the Melkasa research center, 15 from the Werere research center (Afar region), and the remaining 69 coming from three different regions of Ethiopia in 2019 (Oromia, Tigray, Amhara, and Sidama, as well as the Southern Nations Nationalities and Peoples' Region). While the remaining two genotypes do not and are referred to as released variations, one hundred eighteen genotypes include passport information (for more information on plant materials see under chapter three, Table 3.1).

4.2.3 Experimental layout and crop management

For experimental layout and crop management please see chapter three.

4.2.4 Agro-morphological qualitative traits and data collection

To characterize the genotypes under study, observations were made on various morphological qualitative traits at distinct phenological stages. As suggested by the International Board for Plant Genetic Resources based on taxonomic keys (Grubben and Van Sloten, 1981), data for

amaranth descriptors were recorded. Data also were collected on some characters that were not shown in the list of standard descriptors since these were considered necessary for the characterization. In this investigation, a total of 20 qualitative characters were considered for characterization. Visual observation was used to evaluate all of the qualitative traits. The colors of the different plant parts were determined using the RHS color chart (Voss, 2002). Vegetative, inflorescence, and seed data were among the 20 characteristics evaluated. The list of qualitative agro-morphological traits along with their phenotype code is shown in Table 4.2.

Table 4.2 List of qualitative agro-morphological traits used along with their phenotype code, description and phenotype scores

Qualitative marker	Phenotypic Code	Descriptor and code
Germination rate	GR	1 = Rapid, (< 2 days), 2 = Slow (2-7 days), 3 = Very slow (> 7 days), 4 = Irregular
Growth habit	GH	1 = Erect , 2 = Prostrate
Branching index (score if erect)	BI	1 = No branches, 2 = Few branches (all near the base of the stem), 3 = Many branches (all near the base of the stem), 4 = Branches all along the stem
Stem pubescence	SP	0 = None, 3 = Low, 7 = Conspicuous
Stem pigmentation	SPG	1 = Green, 2 = Purple or pink, 3 = White, X = Mixture
Spines in leaf axils	SLA	1 = Absent, 2 = Present, X = Mixture
Leaf pubescence	LP	0 = None, 3 = Low, 7 = Conspicuous
Leaf pigmentation	LPG	1= Entire lamina purple or pink, 2 = Basal area pigmented, 3 = Central spot, 4 = Two stripes (V-shaped), 5 = One stripe (V-shaped), 6 = Margin and vein pigmented, 7 = One pale green or chlorotic stripe on normal green, 8 = Normal green, 9 = Dark green, 10 = other (specify), X = Mixture
Leaf shape	LS	1 = Lanceolate, 2 = Elliptical, 3 = Cuneate, 4 = Obovate, 5 = Ovatainate, 6 = Rhombic, 7 = Oval, 8 = other (specify), X = Mixture
Leaf margin	LM	1 = Entire, 2 = Crenate, 3 = Undulate, 4 = other (specify), X = Mixture
The prominence of leaf veins	PLV	1 = Smooth, 2 = Rugose (veins prominent)
Petiole pigmentation	PP	1 = Green, 2 = Dark green, 3 = Amaranth, 4 = Dark purple, 5 = White, X = Mixture
Terminal inflorescence shape	TIS	1 = Spike (dense), 2 = Panicle with short branches, 3 = Panicle with long branches, 4 = Club-shaped at tips, 5 = Thyrses, 6 = Raceme, X = Mixture
Terminal inflorescence Attitude	TIA	1 = Erect 2 = Drooping
Inflorescence density index	IDI	3 = Lax 5 = Intermediate 7 = Dense
Inflorescence color	IC	1 = Yellow, 2 = Green, 3 = Pink, 4 = Red, 5 = Amaranth, 6 = Golden, 7 = White, X = Mixture

Seed shattering	SSH	1 = Low (<10%), 2 = Intermediate (10-50%), 3 = High (>50%),
Seed color	SC	1 = Pale yellow, 2 = Pink, 3 = Red, 4 = Brown, 5 = Black
Presence of axillary inflorescence	PAI	1 = Absent 2 = Present
Sex type	SX	1 = Monoecious, 2 = Dioecious, 3 = Polygamous

4.2.5 Data analysis

4.2.5.1 Frequency and Shannon diversity index

The frequency distribution of qualitative traits and basic statistical parameters (number of genotypes, percentage of genotype, Shannon diversity index) based on qualitative morphological traits were analyzed using Minitab 19.1 ([Minitab, 2019](#)). The phenotypic frequency distribution of the phenotypic markers was calculated for each genotype. The Shannon Weaver Diversity Index was constructed using frequency data. The Shannon-Weaver diversity index (H') was used to assess and interpret the phenotypic diversity for each of the markers using phenotypic frequencies. This index, according to ([Perry and McIntosh, 1991](#)), is given as:

$$H' = -\sum_{i=1}^n p_i \ln p_i ; H_{max} = \ln S$$

Where n is the number of phenotypic classes in a given categorical character, and p_i is the relative proportion of the total number of entries (N) in the i^{th} class I (1, 2... n). S is the number of traits category in a qualitative trait of Ethiopia genotypes, H' is the Shannon-Weaver diversity index of a given character, which is lastly standardized to H' as a ratio of H' by $\ln(n)$ to normalize and keep the value between 0 and 1, by pooling various characters across the locations (regions), the additive properties of H' were used to evaluate the diversity of locations and characters within the population. For a monomorphic population, the index has a minimum value of zero. The index value rises as polymorphism grows and reaches its maximum value when all phenotypic classes have equal frequencies ([Yang et al., 1991](#)).

Based on their collecting region and released variety, the genotypes were divided into eight groups for data analysis: Afar, Amhara, Benishangul and Gamuz, Unknown, Oromia, Sidama, Southern Nations Nationalities and Peoples Regions in Ethiopia, and Tigray. The phenotypic diversity of the overall sample and the sample group for each collecting region were both measured using [Shannon and Weaver \(1949a\)](#).

The Wachira *et al.* (1995), techniques were used to divide the phenotypic diversity across regions of collection = $\frac{(H'_{sp} - H'_{cr})}{H'_{sp}}$ and within collections = $\frac{(H'_{cr})}{H'_{sp}}$

Where: H_{cr} and H_{sp} , are Shannon-Weaver diversity index across collection regions and species, respectively.

4.2.5.2 Cluster- analysis

To group the 120 genotypes and to bring out the patterns of similarity and dissimilarity based on 20 qualitative morphological characters, the Past version (4.0.9) and different branches of the trees were differently colored using Fig Tree v1.4.3 (Rambaut, 2014) were used to perform cluster- analysis based on ward hierarchical grouping using Euclidian distance. The optimum number of clusters in a data set was determined by using the average silhouette method for the dendrogram with various packages of R software (Maechler, 2019).

4.2.5.3 Genetic distance analysis

By utilizing Mahalanobis distance (D^2) statistics to calculate the genetic distance between the clusters, the connection between them was evaluated (Mahalanobis, 2018). Using the PROC procedures methods of SAS version 9.4 (Allison, 2012), extended Mahalanobis' D^2 statistics were used to quantify the genetic divergence within and across clusters.

4.2.5.4 Principal component

Principal component analysis (PCA) was used to ascertain which attributes were most responsible for the overall variation. To have a significant genotype dispersion, the PCA components with cumulative variability must account for at least 75% of the overall variability (Govindaraj *et al.*, 2020). To identify the number of principal components (PCs), eigenvalues larger than unity were employed as a criterion (Iezzoni and Pritts, 1991). The eigenvalues frequently decline gradually as the number of PCs rises, and their sum is equal to the number of features employed in the study. The features with the greatest absolute values of eigenvectors for each PC were identified by comparing the eigenvectors of the PCs, and these traits were then taken into consideration for selection because of their significant contributions to yield improvement (Dhakar *et al.*, 2019). Characteristics with higher coefficients, typically 0.6 or more, on the PC axis are generally considered more important (DeLacy and Cooper, 1990; Jolliffe, 2002). The positive and negative loading indicates the

presence of positive and negative correlation trends between the components and the variables. Therefore, the above-mentioned traits that load high either positively or negatively contributed more to the diversity, and they were the ones that most distinguished the clusters.

With the help of the statistical program FactoMineR and factoextra packages using R studio, the eigenvalues, and eigenvectors of the PCs were calculated using FactoMineR and factoextra packages using R studio. Additionally, the first two PCs' scree plots were generated using the FactoMineR and factoextra packages using R studio.

4.3 Results

4.3.1 Qualitative Traits Variation

For various qualitative traits, several morph types were observed in the current study. Nineteen of the twenty qualitative traits were found to be variable (discriminated 120 amaranth genotypes) and one was determined to be invariant (did not discriminate the genotypes). The genotypes could be distinguished by their germination rate, branching index, stem pubescence, stem pigmentation, spines in leaf axils, leaf pubescence, leaf pigmentation, leaf shape, leaf margin, prominence of leaf veins, petiole pigmentation, terminal inflorescence shape, terminal inflorescence attitude, inflorescence density index, seed shattering, seed color, and sex type (**Table 4.3**). Visually collected qualitative data is presented in the form of a table and figure, revealing the frequencies and relative percentages of each phenotype occurring in amaranth genotypes in combined forms, as well as the percentage of genotypes possessing a specific phenotypic trait (**Table 4.3**). In amaranth genotypes' several qualitative traits were observed, examined, and displayed in a histogram **Figure 4.2**.

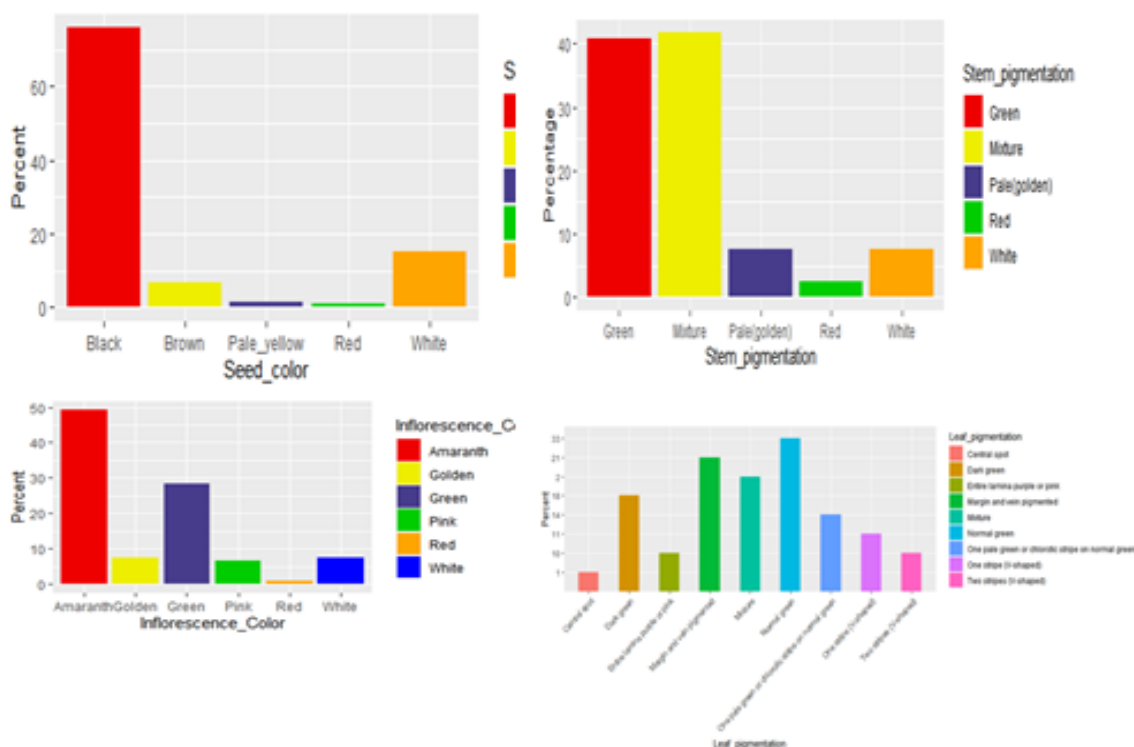


Figure 4.2 Various qualitative characteristics of amaranth genotypes

The genotype's predominantly (75%) germination rate was slow (2–7 days) and the rest of the germination rate was very slow (>7 days). The growth habit of most of the genotypes was erect (86.67%) and 13.33% of the genotypes were prostrate. The branching index of genotypes having an erect type growth habit showed branches all along the stem were 85.83% and many branches all near the base of the stem were 4.17%. Stem pubescence for a majority of the stem was low (79.17%), 19.17% of the genotypes were absent, and the remaining 1.67% of the genotypes had conspicuous stem pubescence. Stem pigmentation showed significant color variation, with approximately 41.67% of genotypes being mixtures, 40.83% being green, white, and pale colors, each having 7.5%, and the remaining 2.53% being red (Figure 4.4a). The terminal inflorescence shape was largely (39.5%) panicle with long branches, followed by a mixture (32.5%), thyrsi (14.17%), club-shaped at tips (8.33%), and raceme (4.17%), and the least common was panicle with short branches and spike (dense) (83%). The majority of genotypes had terminal inflorescence attitudes erect (87.5%), whereas the rest showed drooping (12.5%) Figure 4.3. Inflorescence density indexes of lax, intermediate, and dense types were observed in the present study. Among them, 46 genotypes (38.33%) had a lax inflorescence density index, 42 genotypes (35%) had an intermediate inflorescence density index, and 32 (26.67%) had a dense inflorescence density index. Six types of inflorescence color were observed in the

present study of which 59 genotypes (49.17%) had amaranth color inflorescence, 8 genotypes (6.67%) had pink color inflorescence, 34 genotypes (28.33%) had green color inflorescences, 9 genotypes (7.5%) had golden and white inflorescence colors each, and the remaining 1 genotype (0.83) had red color inflorescence (Figure 4.4b). The majority of genotypes (75.83%) had black seed colors, followed by white (15%), brown (6.67%), pale yellow (1.7%), and red (0.83%) (Figure 4.4c). The leaf pigmentation presented a marked variation, 27.5% were normal green, 15% dark green, 17.5 % margin and vein pigmented, 11.6% one pale green or chlorotic stripe on the normal green, 9.17% one stripe (V-shaped), 8.33% two stripes (V-shaped), 8.33% the entire lamina purple or pink, 1.67 % mixture, and 0.83 % had the central spot (Figure 4.4d). A majority (86.67%) of the genotypes were devoid of spines in the leaf axis, and 13.33% of the genotypes had spines in the leaf axis. The predominant leaf-shape genotypes were lanceolate (57.5%), elliptic (25.83%), and ovate (10.83%), whereas the rest were rhombic (5.83%) in shape. With regards to the distribution frequency of leaf margin, significant variation was observed among the genotypes (98.33%) that had undulated leaf margin, followed by crenate (1.67%). Similarly, it was observed that 98.33% of the genotypes had the prominence of leaf veins that were rugose (veins were prominent), while 1.67% showed smooth leaf veins. Four types of petiole pigmentation were noticed in the present study, among which were green (40.00%), white (32.5%), mixture (25%), and amaranth (2.5%). In general, the distribution frequency of the phenotypes revealed considerable variances, with certain morph types being uncommon while others being frequent and proportionately dispersed over the genotypes (Table 4.3).

Figure 4.3 Terminal inflorescence attitudes: (A) and (B) erect; (C) and (D) drooping





Figure 4.4 a Stem color of amaranth: (a) Red color; (b) Golden (pale) color; (c) Green color; (d) White color; (e) Mixed color



Figure 4 4b Inflorescence color (a) Golden color; (b) Green color; (c) Red color; (d) White color; (e) Amaranth color; (f) Pink color.



Figure 4 4c Seed color of amaranth: (a) Black color; (b) Red color; (c) White color; (d) Brown color; (e) Pale yellow color.

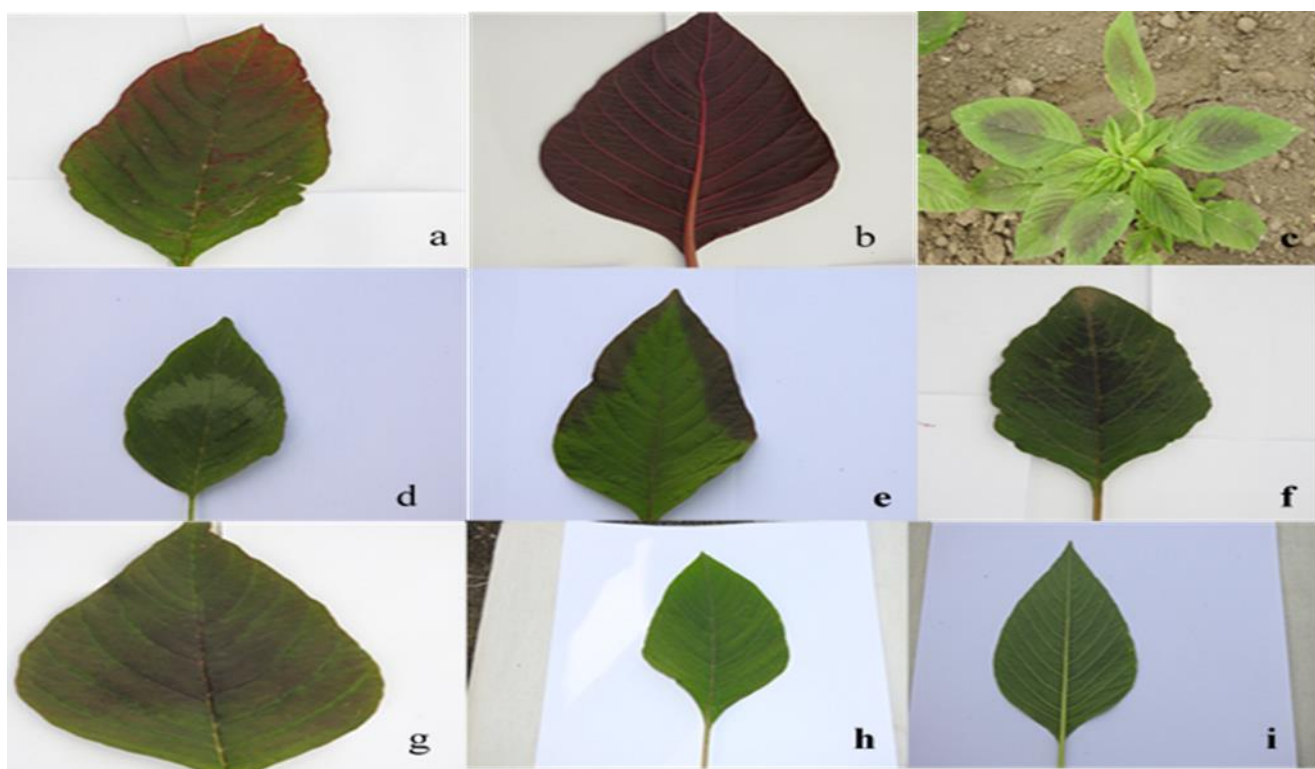


Figure 4 4d Leaf pigmentation: (a) Mixture; (b) Entire lamina purple or pink; (c) Two stripes (V-shaped); (d) One stripe (V-shaped); (e) Margin and vein pigmented; (f) Central spot; (g) One pale green or chlorotic stripe on normal green; (h) Normal green; (i) Dark green.

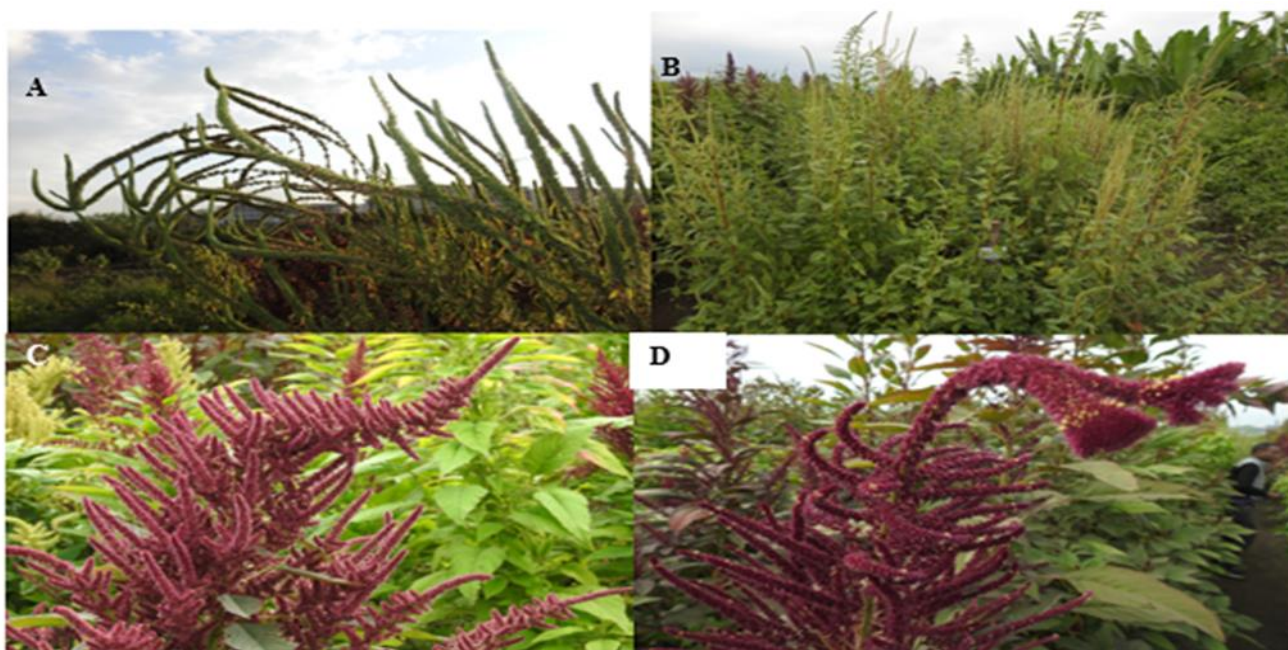


Figure 4.5 Sex type in studied amaranths genotypes:(A) and (B) dioecious genotypes; (C) and (D) monoecious.

4.3.2 Estimation of frequency distribution, and Chi-square

Table 4.3 shows the estimation of frequency distribution, and chi-square. The chi-square test value showed the existence of dominant phenotypic variation among the evaluated 20 qualitative traits. The observed value was higher than the expected phenotypic value. However, all scored qualitative expressive traits showed a very highly significant difference except for the inflorescence density index which is not significant. This indicates the recorded data showed dominant phenotypic classes for seed color (171.5) followed by a terminal inflorescence shape (121.9), stem pubescence (118.95), germination rate (112.1), the prominence of leaf veins (112.1), leaf margin (112), leaf shape (78), sex type (70.5), growth habit (64.5), spines in leaf axils (64.5), branching index (61.63), stem pigmentation (61.70), and leaf pigmentation (58.2) showed dominant than inflorescence density index (2.6), leaf pubescence (13.3), seed shattering (16.1), petiole pigmentation (37.8), and inflorescence color (42.05).

Table 4.3 Specific qualitative morphological traits, their scores, equivalent phenetic characters, (%) contribution to variation, and Chi-square for 20 morphological qualitative traits during the analysis of 120 Genotypes from Ethiopia.

Qualitative marker	Character state	Proportion	Chi-square	% Contribution
Germination rate	Slow (2-7 days)	90.00	112.1***	75.00
	Very slow (> 7 days)	30.00		25.00
Growth habit	Erect	104.00	64.5***	86.67
	Prostrate	16.00		13.33
Branching index	Many branches (all near the base of the stem)	17.00	61.63***	14.17
	Branches all along the stem	103.00		85.83
Stem pubescence	None	23.00	118.95***	19.17
	Low	95.00		79.17
Stem pigmentation	Conspicuous	2.00	61.7***	1.67
	Green	49.00		40.83
	Pale(golden)	9.00		7.50
	Red	3.00		2.53
	White	9.00		7.50
Spines in leaf axils	Mixture	50.00	64.5***	41.67
	Absent	104.00		86.67
	Present	16.00		13.33
Leaf pubescence	None	40.00	13.3***	33.33
	Low	80.00		66.67
Leaf pigmentation	Mixture	2.00	58.2***	1.67
	Entire lamina purple or pink	10.00		8.33
	Central spot	1.00		0.83
	Two stripes (V-shaped)	10.00		8.33
	One stripe (V-shaped)	11.00		9.17
	Margin and vein-pigmented	21.00		17.50
	One pale green or chlorotic stripe on normal green	14.00		11.67
	Normal green	33.00		27.50
	Dark green	18.00		15.00
	Lanceolate	69.00		57.50
Leaf shape	Elliptical	31.00	78****	25.83
	Ovate	13.00		10.83
	Rhombic	7.00		5.83
	Crenate	2.00		1.67
Leaf margin	Undulate	118.00	112***	98.33
	Smooth	2.00		1.67
The prominence of leaf veins	Rugose (veins prominent)	118.00	112.1***	98.33
	Smooth	2.00		1.67
Petiole pigmentation	Green	48.00	37.8**	40.00
	Amaranth	3.00		2.50
	White	39.00		32.50
	Mixture	30.00		25.00
Terminal inflorescence shape	Spike (dense)	1.00	121.9***	0.83
	Panicle with short branches	1.00		0.83
	Panicle with long branches	47.00		39.17
	Club-shaped at tips	10.00		8.33
	Thyrse	17.00		14.17

	Raceme	5.00		4.17
	Mixture	39.00		32.50
Terminal	Erect	105.00	67.5***	87.50
inflorescence	Drooping	15.00		12.50
attitude				
Inflorescence	Lax	46.00	2.6 ^{ns}	38.33
density index	Intermediate	42.00		35.00
	Dense	32.00		26.67
Inflorescence	Green	34.00	42.05***	28.33
Color	Pink	8.00		6.67
	Red	1.00		0.83
	Amaranth	59.00		49.17
	Golden	9.00		7.50
	White	9.00		7.50
Seed shattering	None	20.00	16.1***	16.67
	Low (<10%)	19.00		15.83
	Intermediate (10-50%)	45.00		37.50
	High (>50%)	36.00		30.00
Seed color	White	18.00	171.5***	15.00
	Pale yellow	2.00		1.70
	Red	1.00		0.83
	Brown	8.00		6.67
	Black	91.00		75.83
The presence of	Present	120.00	-	100.00
auxiliary				
inflorescence				
	Monoecious	106.00	70.5***	88.33
Sex type	Dioecious	14.00		11.67

4.3.3 Estimates of phenotypic diversity

Tables 4.4 and 4.5 show the Shannon-Weaver diversity index (H') estimated across all genotypes, across collection regions, and within collection regions, and the maximum Shannon's Weaver diversity index for all 20 qualitative morphological traits examined across all genotypes. Individual morphological qualitative Shannon diversity indices (H') ranged from 0.12 for germination rate, leaf margin, the prominence of leaf veins, and the presence of auxiliary inflorescence to 0.99 for inflorescence density index, with an overall mean of 0.61. Low genetic diversity (0.12) and monomorphic were found in the phenotypic markers auxiliary inflorescence, germination rate, leaf vein prominence, and leaf margin. In contrast, intermediate genetic diversity (0.52-0.60) and polymorphism were found in the phenotypic markers growth habit, sex type, branching index, stem pubescence, spines in leaf axils, and terminal inflorescence attitude. Stem pigmentation, terminal inflorescence shape, seed color, leaf shape, petiole pigmentation, seed shattering, leaf pigmentation, leaf pubescence, inflorescence color, and inflorescence density index all had a high variety index (0.69–0.99).

The average diversity index (H') of individual characters across collection regions ranged from 0.01 (for germination rate) to 0.95 (for leaf pigmentation) with an overall mean value of 0.43 (Table 4.5). Four pigmentation features—leaf pigmentation, petiole pigmentation, inflorescence color, seed color, and one seed trait—seed shattering—were determined to be the most diversified traits across all collecting regions with H' varied from 0.68 to 0.95. However, with $H' < 0.3$, there was less variation across all areas in germination rate, sex type, spines in leaf axils, growth habit, the prominence of leaf veins, inflorescence density index, branching index, leaf margin, presence of auxiliary inflorescence and terminal inflorescence attitude.

Whenever the phenotypic diversity was split within $\frac{(H'_{cr})}{H'_{sp}}$ and between collection regions $\frac{(H'_{sp} - H'_{cr})}{H'_{sp}}$. It was found that there was more intra-regional diversity (0.66) than inter-regional diversity (0.34), demonstrating the existence of gene flow between growing regions. The studied qualitative traits with an H' value greater than 0.83 included stem pubescence, stem pigmentation, leaf pigmentation, leaf shape, leaf margin, petiole pigmentation, terminal inflorescence shape, inflorescence color, and seed shattering, seed color were the main contributors to heterogeneity within the collection region. Even while there was a greater degree of phenotypic variety found within collection regions (0.66), there was also a sizable level of diversity found between regions (0.34) (Table 4.4). The regional diversity was greatly influenced by the branching index (0.97), germination rate (0.92), growth habit (0.88), spines in leaf axils (0.88), and sex type (0.88). Although characters from various regions were pooled together, the mean value of H' varied from 0.20 for Tigray to 0.63 for Sidama (Table 4.5). Except for Unknown, Tigray, and Afar, which had lower diversity indices ($H' = 0.20, 0.25$, and 0.33 , respectively), the majority of the collection regions had H' values above 0.40. In each collecting location, specific characteristics showed various degrees of variability. Overall, Sidama, Oromia, Benishangul & Gumuz, Amhara, and SNNPR genotypes showed higher diversity indices for most of the evaluated characters.

Table 4.4 Qualitative morphological traits, their Shannon-Weaver diversity index, across genotypes, within and among collection regions, and maximum Shannon's Weaver diversity index for 20 morphological qualitative traits during the analysis of 120 Genotypes from Ethiopia

Qualitative marker	H'_{sp}	H'_{cr}	H'_{cr}/H'_{sp}	$(H'_{sp} - H'_{cr})/H'_{sp}$	H_{max}
Germination rate	0.12	0.01	0.08	0.92	0.69
Growth habit	0.57	0.07	0.12	0.88	0.69
Branching index	0.59	0.02	0.03	0.97	0.69
Stem pubescence	0.52	0.44	0.85	0.15	1.1
Stem pigmentation	0.69	0.55	0.80	0.20	1.61
Spines in leaf axils	0.57	0.07	0.12	0.88	0.69
Leaf pubescence	0.92	0.52	0.57	0.43	0.69
Leaf pigmentation	0.88	0.95	1.00	-0.08	2.2
Leaf shape	0.78	0.84	1.00	-0.08	1.39
Leaf margin	0.12	0.14	1.17	-0.17	0.69
The prominence of leaf veins	0.12	0.07	0.58	0.42	0.69
Petiole pigmentation	0.84	0.80	0.95	0.05	1.39
Terminal inflorescence shape	0.70	0.88	1.26	-0.26	1.95
Terminal inflorescence attitude	0.55	0.25	0.45	0.55	0.69
Inflorescence density index	0.99	0.59	0.60	0.40	0.69
Inflorescence color	0.95	0.80	0.84	0.16	1.79
Seed shattering	0.87	0.75	0.86	0.14	1.39
Seed color	0.76	0.68	0.89	0.11	1.61
The presence of auxiliary inflorescence	0.12	0.1	0.83	0.17	0.69
Sex type	0.60	0.07	0.12	0.88	0.69
Mean	0.61	0.43	0.66	0.34	1.10

H'_{sp} , the diversity index for each character calculated from the entire data set; H'_{cr} , the average diversity index of each character pooled over the seven collection regions and one unknown; H'_{cr}/H'_{sp} , the proportion of diversity within collection regions; $(H'_{sp} - H'_{cr})/H'_{sp}$, the proportion of diversity between collection regions, H_{max} =maximum Shannon's weaver diversity indexed.

Table 4.5 Estimates of the Shannon-Weaver diversity index across the seven collection regions and one released variety (Unknown) for each qualitative character

Qualitative Traits	Regions							
	Afar	Amhara	Benishangul & Gumuz	Unknown n	Oromia	Sidama a	SNNPR	Tigray
GR	0.00	0.00	0.00	0.00	0.00	0.00	0.1	0.00
GH	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.00
BI	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00
SP	0.57	1.00	0.00	0.00	0.81	0.81	0.31	0.00
SPG	0.00	1.00	0.96	0.00	0.81	0.89	0.74	0.00
SLA	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.00
LP	0.00	0.81	0.97	0.00	0.81	0.81	0.74	0.00
LPG	0.84	1.00	0.96	1.00	1.00	0.93	0.89	0.97
LS	0.93	0.81	0.67	1.00	0.81	0.95	0.60	0.97
LM	0.57	0.00	0.00	0.00	0.00	0.55	0.00	0.00
PLV	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PPG	0.93	0.81	0.96	0.00	0.95	0.96	0.81	0.96
TIS	0.85	0.99	0.96	1.00	0.81	0.99	0.70	0.73
TIA	0.00	0.00	0.00	0.00	0.81	0.55	0.66	0.00
PAI	0.00	0.00	0.72	0.00	0.00	0.00	0.10	0.00
IDI	0.00	1.00	0.97	0.00	1.00	0.81	0.95	0.00
IC	0.72	0.95	0.95	0.00	1.00	0.93	0.91	0.96
SS	0.00	0.81	0.97	1.00	1.00	0.77	0.72	0.73

SC	0.57	0.00	0.97	1.00	1.00	0.96	0.91	0.00
SX	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.00
Mean	0.33	0.46	0.50	0.25	0.54	0.63	0.43	0.20

SNNPR (Southern Nations, Nationalities, and Peoples' Region).; Refer to abbreviated code chapter four, Table 4.2

Table 4.6 displays the Shannon diversity indices for each of the seven *Amaranth* species that were the subject of the investigation. *A. hybridus* L. subsp. *cruentus* (L.) Theil had a mean value of 0.47 while *A. spinosus* L. had a mean value of 0.00. The greatest Shannon diversity index was recorded by *Amaranthus hybridus* L. subsp. *cruentus* (L.) Theil (0.47) is followed by *A. hybridus* L. subsp. *hybridus* (0.41), *Amaranthus caudatus* L. (0.38), *Amaranthus viridis* L. (0.36), *Amaranthus palmeri* S. Watson (0.35), and *Amaranthus* spp. (0.21), while *Amaranthus spinosus* L. had the lowest (0.00). On the other hand, all of the studied species displayed monomorphism, sex, growth habit, and spines in the leaf axils. *Amaranthus hybridus* L. subsp. *cruentus* (L.) Theil, *A. hybridus* L. subsp. *hybridus*, *A. caudatus* L., and *A. viridis* L., genotypes exhibit monoecious character and upright growth. However, *Amaranthus palmeri* S. Watson is primarily prostrate and dioecious. On the other hand, *A. hybridus* L. subsp. *cruentus* (L.) Theil was found to have the highest values (which are highly polymorphic) in seed color, terminal inflorescence shape, petiole pigmentation, inflorescence density index, stem pigmentation, and stem and leaf pubescence. Whereas *A. hybridus* L. subsp. *hybridus* had the highest values in petiole pigmentation, inflorescence color, seed color, and leaf pigmentation (which is highly polymorphic). In contrast, *Amaranthus palmeri* S. Watson has the highest values for leaf shape, petiole pigmentation, leaf margin, and the prominence of leaf veins. The presence of axillary inflorescence was highest for *A. spp.*, and petiole pigmentation was highest for *Amaranthus caudatus* L.; *Amaranthus viridis* L. showed the highest values in terminal inflorescence shape, inflorescence color, and seed shattering. The lowest values for all the studied features were revealed in *A. spinosus* L, but only for one phenotypic class (monomorphic) (Table 4.6).

Table 4.6 Estimates of the Shannon-Weaver diversity index across the seven *Amaranthus* species for each qualitative character

Traits	<i>A. hybridus</i> L. subsp. <i>Cruentus</i>	<i>A. hybridus</i> L. subsp. <i>Hybridus</i>	<i>A. caudatus</i> L.	<i>A. palmeri</i> S. Wats	<i>A.spp</i>	<i>A. viridis</i> L.	<i>A. spinosus</i> L.
GR	0.00	0.14	0.00	0.00	0.00	0.00	0.00
GH	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BI	0.20	0.14	0.00	0.00	0.00	0.00	0.00
SP	0.60	0.41	0.00	0.55	0.00	0.00	0.00
SPG	0.70	0.52	0.70	0.00	0.00	0.00	0.00
SLA	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LP	0.95	0.69	0.35	0.57	0.81	0.00	0.00
LPG	0.89	0.83	0.77	0.84	0.00	0.81	0.00

LS	0.88	0.76	0.78	0.92	0.81	0.81	0.00
LM	0.00	0.00	0.35	0.57	0.00	0.00	0.00
PLV	0.00	0.00	0.00	0.57	0.00	0.00	0.00
PPG	0.80	0.94	1.00	0.92	0.00	0.81	0.00
TIS	0.83	0.66	0.57	0.84	0.00	0.95	0.00
TIA	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PAI	0.00	0.00	0.00	0.00	1.00	0.00	0.00
IDI	0.92	0.69	0.80	0.00	0.00	1.00	0.00
IC	0.95	0.86	0.72	0.72	0.81	0.95	0.00
SS	0.64	0.67	0.97	0.00	0.81	1.00	0.00
SC	0.94	0.81	0.59	0.57	0.00	0.81	0.00
SX	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	0.47	0.41	0.38	0.35	0.21	0.36	0.00

Refer to the abbreviated code chapter four, Table 4.2

4.3.4 Cluster analysis of 20 qualitative traits

Based on qualitative data of 20 traits, 120 amaranth genotypes were typically grouped into three basic clusters (Figure 4.6). Three distinctive genotype clusters were observed based on the diversity among several qualitative morphological traits (Figure 4.7). Cluster I comprised (58) genotypes collected from Tigray, Amahara, Benishangul & Gumuz, Oromia, Southern Nations Nationalities and Peoples' Region, and Sidama, followed by cluster II, which had 47 genotypes collected from Amhara, Southern Nations Nationalities and Peoples' Region, Benishangul & Gumuz, Tigray, Sidama, Oromia, and released variety. Cluster III had the fewest genotypes (15) collected from the Afar region (Table 4.7).

Cluster I was mainly distinguished by high seed shattering (54.83%), intermediate seed shattering (43.55%), and low seed shattering (1.61%), all of which were monoecious sex types. Out of the total 80.65% had mixture stem pigmentation, 9.68% had white stem pigmentation, and 8.06% had green stem pigmentation. In terms of branching index, 96.77% had all branches along the stem and the remaining had many branches (all at the base of the stem), also distinguished by the absence of spines in the leaf axils. Cluster II was mainly characterized by monoecious sex types with intermediate and low seed shattering (each accounting for (40.47%), no seed shattering (11.90%), and strong seed shattering (7.14%). Likewise, genotypes in cluster II lacked spines in the leaf axils, and inflorescence color varied, with 35.71% having green inflorescence color, (23.80%) having white inflorescence color, 14.29% having pink inflorescence color, 9.52% having purple inflorescence color, and 4.76% having a mixture of inflorescence color. Cluster III genotypes are characterized mainly by dioecious sex types, no seed shattering, having spines in the leaf axis, and a high inflorescence density index.

Table 4.7 Clustering of 120 Amaranths species of genotypes into 3 clusters using the mean of 20 agro-morphological qualitative characters.

Cluster No	Genotypes	Regions	No of Genotypes
I	MEK-081,BKD-031,KAZ-078,OTI-044,208683,KEN 012, 204645,WON-049,215567,209056,240814,242533,ALE-033 ,KAZ-055, 240813, 209057, DIL-050, KAZ-006, BKD-027, BKD-029, KAZ-008, 225715, KAZ-009, 225718, 211457, ALE-024, ALE 073, KEN-015, 212581, 91001, 242530, DAL-043, 225717, HAW-041, 211455, KAZ-079, 242532, SHD-001, BKD-028, ALE-025, DRA-053, MEK-083, 212583, KEN-017, DAL-042, 91003, 212893, MEK-084, SHL-040, ABL-004, KEN-013, YRC-048, DUF-003, ALE-075, 225713, ADZ-037, YRB-002, BNT-026	Tigray, Amahara, Benishangul and Gumuz, Oromia, Southern Nations, Nationalities, and Peoples' Region and Sidama.	58
II	ALE-074, 212890, KAZ-057, KEN-020, CHU-045, KAZ-076, 242534, 211456, 241764, ALE-068, MEK-082, 242531, KEN-065, KAZ-059, 219284, 242917, ALE-023, 208764, KAZ-077, KAZ-058, KEN-064, KEN-021, KEN-014, AC-NL, KAZ-056, ALE-034, 204644, 212892, HAL-039, 208025,MEK-080, KEN-022, MAND, KEN-067, 240815, ALE-071, KEN-010, ALE-070,KAZ-007, KAZ-060, KEN-011, KEN-016, KEN-019, 212582, DMG-038, ALE-069, KEN-018	Amhara,Southern Nations,Nationalities, and Peoples' Region, Benishangul& Gumuz,Tigray, Oromia, and Sidama, Released a variety	47
III	SAA-004, HA-003, DA-006, BA-016, SRA-002, K3A-005, SA025/07, SA-008/07, SA-001/07, SHIA-007, SA-005/07, HA-008, BA-001, WA-001, WA-003	Afar	15

4.3.4.1 Inter-cluster distance (D^2) analysis on qualitative traits

Table 4.8 presents intra- and inter-cluster distances. There were marked variations in intra-cluster distances, which ranged from 1.32 to 4.03. The highest intra-cluster distance was recorded in cluster III (4.03), which contained fifteen genotypes, followed by cluster II (2.10), which contained forty-seven genotypes. The lowest intra-cluster distance was observed in cluster I (1.32) with fifty-eight genotypes, and cluster II showed the second lowest intra-cluster distance (2.10) with the highest (47) number of genotypes. The highest inter-cluster distance was observed between clusters I and III (253.23), followed by clusters II and III (250.20). The lowest inter-cluster distance was observed between clusters I and II (29.63).

Table 4.8 Intra (bold) and inter-cluster distances (D^2) for 20 qualitative traits of Amaranth Genotypes from Ethiopia.

Generalized Squared Distance to clusters				Mean Distance
Cluster	I	II	III	
I	1.32*	29.63 ^{NS}	253.23***	141.43
II		2.10^{NS}	250.20***	139.92
III			4.03^{NS}	251.72
Overall mean				177.69

* = significant at $P < 0.05$, ** = highly significant at $P < 0.01$, and *** = very highly significant at $P < 0.001$; NS = non-significant at $P > 0.05$. Chi-Square (χ^2) 20 DF = 31.41; at 37.57 at $P < 0.01$; 45.32 at $P < 0.001$; Inter-cluster is off-diagonal; and intra-cluster is diagonal bold

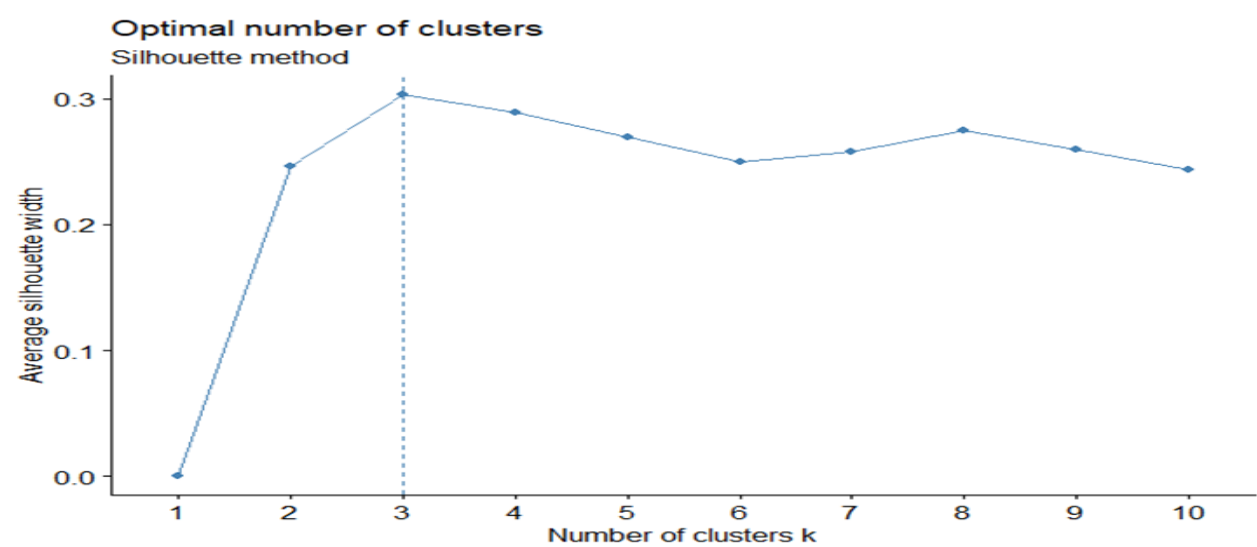


Figure 4.6 Silhouette widths to determine the number of clusters (K) for qualitative data

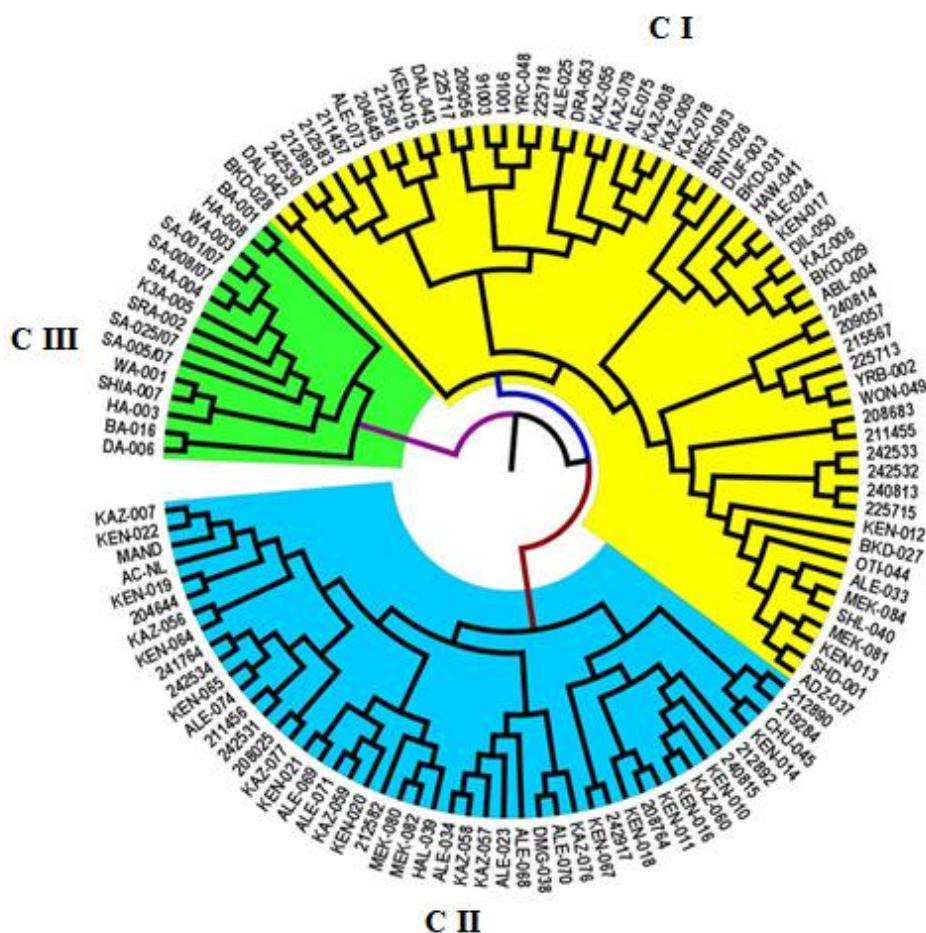


Figure 4.7 Cluster analyses of the 120 amaranths genotypes based on 19 qualitative traits. (Codes represent the genotypes. See Additional Table 3.1, in chapter three.

4.3.5 Diversity based on qualitative traits

Six main components were preserved based on eigenvalues and scree plots in the principal component analysis (PCA) of 19 qualitative traits in 120 amaranth genotypes, accounting for almost 72% of the overall variance (Table 4.9). The scree plot also showed that the first six PCs were important in exploiting the variation in amaranth genotypes (Figure 4.8). According to the qualitative traits, the first six PCs explained 32 %, 13 %, 10%, 6%, 6%, and 5% of the total variation, respectively. For PC1, growth habit, branching index, spines in leaf axils (SLA), and seed shattering (SSH) were the main causes of the relationship between variables and components. PC2 explained 13% of the total variation, with the highest weights assigned to stem pigmentation (SPG), leaf pigmentation (LPG), petiole pigmentation (PP), inflorescence density index (IDI), and seed shattering (SS) (SSH). PC3, which accounted for 10% of the overall variance, was mostly associated with seed color (SC), leaf shape (LS), and

terminal inflorescence shape (TIS). The terminal inflorescence attitude (TIA), prominence of leaf veins (PLV), and leaf pubescence (LP) account for 6% of the variation in the PC4. Similarly, only leaf pubescence (LP), terminal inflorescence shape (TIS), and germination rate (GR) scored heavily for PC5, which accounted for 6% of the overall morphological variance in these genotypes. Leaf margin (LM), prominence of leaf veins (PLV), and terminal inflorescence attitude (TIA) weighted significantly in PC6, which explained 5% of the overall variance.

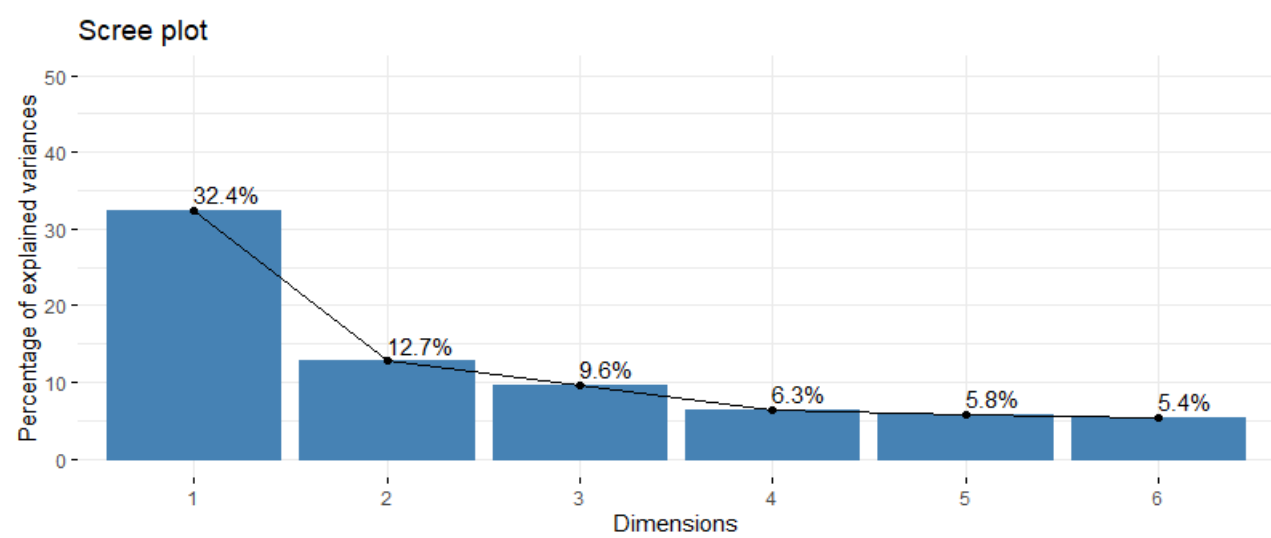


Figure 4.8 Scree plot showing eigenvalues in response to some principal components for 19 agro-morphological traits of 120 amaranth genotypes

Table 4.9 Eigenvectors and eigenvalues of the first six principal components (PCs) for 19 qualitative characters of 120 genotypes.

Traits	Principal Components					
	PC1	PC 2	PC 3	PC 4	PC 5	PC 6
<i>Eigenvalue</i>	6.15	2.42	1.82	1.20	1.11	1.03
<i>Proportion</i>	0.32	0.13	0.10	0.06	0.06	0.05
<i>Cumulative</i>	0.32	0.45	0.55	0.61	0.67	0.72
Eigenvectors						
Growth habit [GH]	0.96	0.08	-0.08	0.08	0.10	0.03
Branching index[BI]	-0.91	-0.09	0.16	-0.11	0.08	-0.03
Stem pubescence[SP]	-0.70	-0.03	-0.10	0.31	-0.08	-0.08
Stem pigmentation [SPG]	0.23	0.77	0.06	0.22	0.03	0.08
Spines in leaf axils [SLA]	0.96	0.08	-0.08	0.08	0.10	0.03
Leaf pubescence [LP]	-0.62	0.16	-0.10	0.45	-0.09	0.00
Leaf pigmentation [LPG]	-0.10	-0.65	0.18	0.23	0.04	0.15
Leaf shape [LS]	-0.10	-0.10	0.67	0.10	0.38	0.20
Leaf margin [LM]	-0.39	-0.10	0.03	0.17	0.29	-0.51
Prominence of leaf veins [PLV]	-0.35	0.09	0.18	-0.60	-0.07	0.45
Petiole pigmentation [PP]	-0.02	0.83	0.03	-0.05	-0.04	0.09

Terminal inflorescence shape [TIS]	0.16	0.15	0.63	-0.09	-0.31	-0.08
Terminal, inflorescence attitude [TIA]	-0.24	-0.12	-0.31	0.36	0.24	0.68
Inflorescence density index [IDI]	0.61	-0.50	0.24	0.07	0.01	0.06
Inflorescence color [IC]	0.59	0.28	0.32	0.27	0.03	-0.03
Seed shattering [SSH]	-0.75	0.46	-0.04	0.15	0.00	0.06
Seed color [SC]	0.14	-0.08	-0.76	-0.22	0.03	-0.04
Germination rate [GR]	0.06	0.23	0.01	-0.24	0.81	-0.11
Sex [SX]	0.93	0.03	-0.11	0.16	-0.08	0.05

Dim. 1 (32.4%) is defined by nine variables, seven of which are strongly positively correlated, including namely: spines in leaf axils (SLA; 98%), growth habit (GH; 96%), sex (SX; 93%), branching index (BI; -91%), inflorescence density index (IDI; 61%), inflorescence color (IC; 59), seed, and two of which are negatively correlated, such as shattering (SSH; -75%), stem pubescence (SP; -70%), and leaf pubescence (LP; -62%). Dimension 2 (12.4%) is defined by five variables, two of which are strongly positively correlated: petiole pigmentation (PP; 83%), stem pigmentation (SPG; 77%), and three of which are negatively correlated: leaf pigmentation (LPG; -65%), inflorescence density index (IDI; -50%), and seed shattering (SSH; -46%) (**Figure 4.9**).

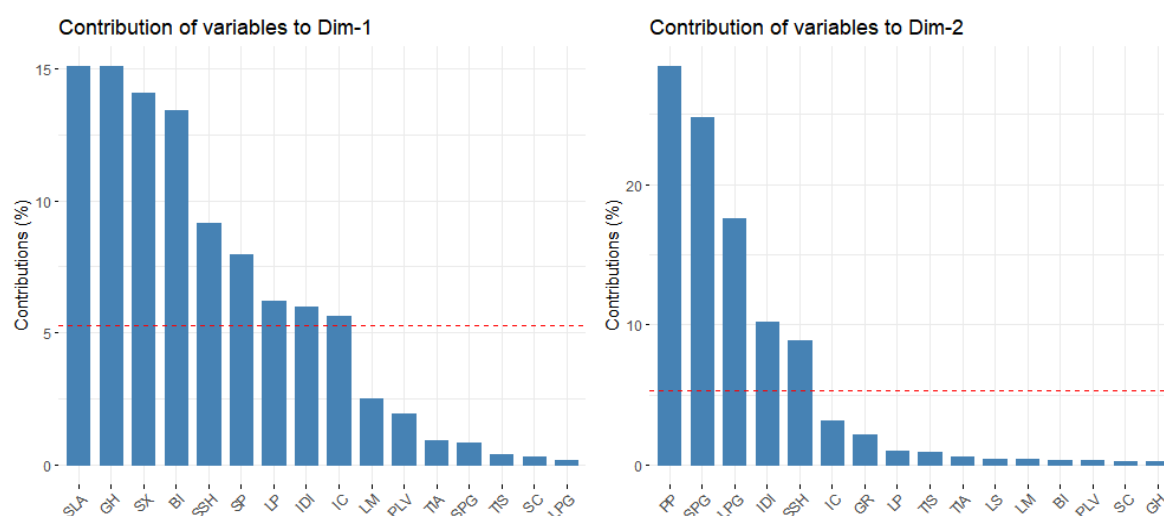


Figure 4.9 Contribution of the variables to the formation of the two principal components.

4.4 Discussion

Plant breeders can only succeed in any crop improvement program if they understand the genetic heterogeneity that exists across a species' genotypes (Roy *et al.*, 2012; Mavengahama *et al.*, 2013). Modern plant breeding would be difficult to imagine, let alone being successful, without a constant supply of genetically varied plants (Duvick, 2007). To offer information for plant breeding initiatives, an agro-morphological assessment of available germplasm is essential (Lin, 1991), and they can use the information to evaluate genotypes to include in a

breeding program. Due to the extensive phenotypic variety, inter-specific relations of the genus *Amaranthus* are poorly recognized. Trait variation assists in the selection of the best lines for improvement in trait-assisted selection (Fasahat *et al.*, 2016; Moradi *et al.*, 2019; Dewi *et al.*, 2020). Moreover, extensive genetic variability among the genotypes offers several opportunities for breeding to select desired features (Laxmi *et al.*, 2020).

It has been thought that qualitative characteristics are useful for describing species germplasm (Abe and Vegetable, 2015). For instance, most Africans prefer to include green-leaved varieties in their diet, whereas the Chinese prefer red-leaved varieties. These have an impact on consumer preferences, the socioeconomic environment, and natural selection as they determine the specific traits that can be found in society (Akaneme and Ani, 2013).

Although qualitative features are often unaffected by environmental variables, they may be used to accurately identify genotypes and perhaps aid in the removal of duplicates and closely similar materials (Esayas *et al.*, 2016; Merrick *et al.*, 2023). Furthermore, when the range of quantitative features is constrained, they help classify varieties (Ghafoor and Ahmad, 2003). In this study, the results showed the existence of a significant morphological variation of genotypes in qualitative characteristics such as germination rate, branching index, stem pubescence, stem pigmentation, spines in leaf axils, leaf pubescence, leaf pigmentation, leaf shape, leaf margins, vein prominence, petiole pigment, inflorescence terminal shape, inflorescence terminal attitude, inflorescence density index, seed burst, and seed color. Similar reports for the existence of large variability have been made by different researchers (Juan *et al.* (2007); Khanam *et al.*, 2012; Gerrano *et al.*, 2013; Gueco *et al.*, 2016; Paredes-Lopez, 2018; Gherase *et al.*, 2020; Nyasulu *et al.*, 2021; Sarker and Oba, 2021; Delgado *et al.*, 2022; Lakshmidhevamma *et al.*, 2022). Nyasulu *et al.* (2021), and Gherase *et al.* (2020) reported a slightly larger range of variances for qualitative features such as leaf color, inflorescence, and seed color. Furthermore, the majority of genotypes had an erect growth habit; erect plant types might be used for breeding genotypes in irrigation-dependent areas, whereas prostrate plant types could be used for creating elite genotypes for rain-fed places that will aid in moisture conservation. Similarly, the consistent result was also reported by Iqbal *et al.* (2003) for cowpea germplasm.

The predominance of genotypes with lanceolate leaf shapes in the current study contributes to the adaptation of the genotypes used for hybridizing the introduced rainfed variety and their capacity to tolerate the drought. A similar result was reported by Iqbal *et al.* (2003).

Genotypes with ovate and rhombic leaf forms are anticipated to be the best for absorbing moisture and producing dietary proteins. These findings coincide with previous reports (Dwivedi *et al.* (2001); Iqbal *et al.*, (2003). As a result, the observed discrepancy in leaf phenotype may be exploited as a key marker for selection in the breeding of better genotypes for different purposes.

High diversity within cultivated crops may be caused by human or natural selection, exchange of seeds, genetic drift, and natural variances (Joshi and Baniya, 2006; Yeshitila *et al.*, 2023). Wider inflorescence trait variabilities seen in the genotypes under study demonstrate the divergence of the genotypes and might be useful in selection and enhancement procedures. Large inflorescence variability, which has been cited as a key characteristic in identifying amaranth genotypes, has also been demonstrated in earlier investigations (Yadav *et al.*, 2014).

The current studies reveal that amaranths exhibit a variety of pigmentation in many phenotypic markers, including the coloring of the seed, stem, leaves, petioles, and inflorescence. This is a result of the variety of betacyanin pigmentation patterns (Das, 2016; Paredes-Lopez, 2018). Amaranth can therefore be used for several utilitarian and aesthetic uses due to the pigmentation's apparent variance. They serve as beneficial genetic markers for managing breeding hybrids, evaluating natural outcrossing, identifying varieties, and mapping the genome. Moreover, according to Wu *et al.* (2000), Khanam *et al.* (2012), and Sarker and Oba (2021) different expressions of natural pigments such as betacyanins, including amaranthine, iso-amaranthine, betanin, iso-betanin, anthocyanin's, carotenoids, and chlorophylls, cause broad-spectrum coloration in *Amaranth species*.

As compared to other leafy vegetables, *Amaranthus* leaves offer a unique source of antioxidant pigments like betalain, β -xanthin, and β -cyanin. They are also a great source of other antioxidant pigments like anthocyanins, carotenoids, and chlorophylls (Sarker *et al.*, 2018b), as well as naturally occurring antioxidant phytochemicals like vitamin C, phenolic acids, and flavonoids (Sarker *et al.*, 2018a). These naturally occurring antioxidant molecules are important for the food business because they act as natural preservatives in food items in addition to their health-promoting properties (Repo-Carrasco-Valencia *et al.*, 2010). Vegetables' inherent antioxidants have drawn the attention of scientists and consumers lately. These organic antioxidants guard against several diseases such as retinopathy, arthritis, emphysema, cancer, heart disease, cataracts, and neurological conditions (Isabelle *et al.*,

2010; Steffensen *et al.*, 2011). It is resistant to abiotic circumstances like salinities (Sarker and Oba, 2018a). More than six key genes have been identified to govern pigment expression patterns (Wu *et al.*, 2000; Paredes-Lopez, 2018). This suggested that the genotypes based on these data had several important genes that expressed a wide range of changes in the color of the stem, inflorescence, leaves, and seeds. The taste, color, and flavor of food are the most important characteristics that influence its popularity. As a result, people all across the world are becoming more and more interested in colorful foods. Because of consumer interest in the aesthetic, nutritional, and safety aspects of food, colored food items have increased the need for natural pigments like betacyanins, along with amaranthine, iso-amaranthine, betanin, iso-betanin, anthocyanins, carotenoids, and chlorophyll (Sarker and Oba, 2021).

The red coloration of the stem/leaves is caused by anthocyanin. Anthocyanin is associated with enhanced resistance to abiotic stressors such as drought (Dabravolski and Isayenkov, 2023), heavy metals (Kgang, 2010) (opposition to herbivores and pathogens (Coley and Aide, 1989). It acts as a sunscreen against damaging UV -B radiation, and as an antioxidant, which protects the plant under stress. *Amaranthus* leafy vegetables are known to have a greater concentration of antioxidant components than beet, cabbage, leafy lettuce, and carrots (Hunter and Fletcher, 2002; Amin *et al.*, 2006; Kgang, 2010).

The results of the chi-square test indicated that among the 20 qualitative markers examined, dominant phenotypic variation existed. The observed value exceeded the phenotypic value that was predicted. However, there was a highly significant difference in all evaluated qualitative expressive traits except the inflorescence density index. It implies that the dominating phenotypic classes were apparent in the data compiled. Abaynesh (2019), reported a similar result in *Coffea arabica* L.

The standardized Shannon diversity index Shannon and Weaver (1949b) was classified as low (0-0.33), intermediate (0.34-0.66), and high (0.67-1). Auxiliary inflorescence, germination rate, prominence of leaf veins, and leaf margin had a low genetic diversity index and were monomorphic, whereas growth habit, branching index, stem pubescence, spines in leaf axils, and terminal inflorescence attitude had an intermediate diversity index. Seed color, stem pigmentation, leaf pigmentation, leaf shape, petiole pigmentation, terminal inflorescence shape, inflorescence density index, inflorescence color, and seed shattering all had a high genetic diversity index and a wide range of polymorphisms. The genetic diversity found using qualitative traits in this study suggests that the tested genotype differs in genetic variants.

Morphological diversity index (H') estimates for individual traits range from 0.12 for auxiliary inflorescence, germination rate, leaf vein prominence, and leaf margin to 0.99 for inflorescence density index, with an overall Shannon Weaver index (H') of 0.61. The high H' in this study was largely due to stem pigmentation, terminal inflorescence shape, seed color, leaf shape, petiole pigmentation, seed shattering, leaf pigmentation, leaf pubescence, inflorescence color, and inflorescence density index all had a high variety index, indicating that these traits contributed to most of the genetic variability among the genotypes. Similar results were also obtained in studies reported by [Adhikari et al. \(2022\)](#) in grain amaranth and [Zavinon et al. \(2019\)](#) in pigeon peas. Therefore, wide genetic variation among genotypes offers a larger genetic pool, providing several possibilities for breeding to select desired features. In amaranths, [Gerrano et al. \(2017\)](#) found comparative results in terminal inflorescence. The Shannon index (HI) increased with both genotype abundance and evenness. [Gueco et al. \(2016\)](#), reported a high diversity index (0.67) in 18 amaranth germplasm collections in the Philippines.

It was additionally pointed out that other crops in Ethiopia and other countries have higher levels of variability for several qualitative traits within collecting locations ([Nsabiyera et al., 2013](#); [Aklilu et al., 2016](#); [Awol, 2018](#); [Zigene et al., 2022](#)). All growing regions might be a source of notable characteristics since there is a higher amount of within-region variability, and all regions should be given a comparable weight for future initiatives including the collection, characterization, and conservation of amaranth crops. For amaranth and other crops, comparable results were also reported ([Gerrano et al., 2013](#); [Nyasulu et al., 2021](#); [Adhikari et al., 2022](#)).

Low Shannon's diversity index signifies a lack of variety and imbalanced frequency classes for a particular trait. The results of H' between 0 and 1 range from the lowest value of 0.12 to the highest value of 0.99 revealing the existence of variability among tested traits of overall amaranths genotypes of Ethiopia. A similar result was also indicated in most studied traits by [Lakshmiddevamma et al. \(2022\)](#).

All of the amaranth species were examined for various qualitative morphological traits, both individually and collectively. Morphological markers were shared by a variety of species because frequent outcrossing and hybridization widened the *Amaranthus* gene pool and produced a large number of morphotypes ([Sauer, 2017](#)). In addition, from a taxonomic standpoint, *Amaranthus* is regarded as a difficult genus because there aren't many

characteristics that set its species apart from one another, the diagnostic features are small and hard to see, the genus has a wide geographic distribution, and there are lots of hybrid forms. These factors make taxonomy difficult and contribute to the genus' general reputation as a challenging genus among systematists (Costea and DeMason, 2001; Das, 2016). As a result, it was difficult to classify genotypes into morphotypes or relate traits to species. According to the study's findings, however, amaranth species classified as grains (such as *Amaranthus hybridus* L. subsp. *cruentus* (L.) Theil, *Amaranthus hybridus* L. subsp. *hybridus*, and *Amaranthus caudatus* L.), exhibit more variation in morphological traits like stem pigmentation and seed color than the weedy types (*A. viridis* L., *A. spinosus* L., and *A. palmeri* S. Wats). *Amaranthus* species differ from one another in several characteristics, including the kind of reproduction (monoecious vs. dioecious). Similar results were reported by Stetter and Schmid (2017).

Samples were classified according to how similar or close together they were (Tahir *et al.*, 2013). There is a lot of genetic variation among the amaranth genotypes, as seen by the distribution pattern of all the genotypes into three groups. Additionally, Ward's method was employed since it is widely acknowledged to be the most effective method for conducting hierarchical cluster analysis (Murty and Arunachalam, 1966; Shankar *et al.*, 2012). Clustering is a valuable tool for examining links between closely linked cultivars or genotypes by grouping units based on their similarity in particular features or response patterns (Hair *et al.*, 1995). Moreover, clustering is a useful tool for studying relationships between closely related genotypes by categorizing units based on their similarity in certain characteristics or response patterns. Crop species' genetic relationships are important because they enable a particular set of breeding populations as well as the collection of data on genetic diversity (Aghaee *et al.*, 2010).

Although genetic diversity is typically linked to geographic variety, it is not always directly connected to geographic distribution (Rahman and Munsur, 2009). When the amaranth genotypes were grouped in this study using the three clustering techniques, it became clear that two of the clusters had no discernible pattern (cluster I and II) whereas the third did (cluster III). While some genotypes from the same collecting site were grouped, others from various sites were clustered. This suggests that there is no relationship between the population and its place of origin. Furthermore, cluster analysis was used to demonstrate the spatial grouping of genotypes and the co-occurrence of species in a single cluster. As a result, cluster

III was established by all of the genotypes from the Afar region. However, despite the genotypes obtained from Afar, genotypes from Southern Nations, Nationalities, and Peoples' Region, Amhara, Tigray, Sidama, Oromia, and Benishangul & Gumuz were grouped into clusters I and II, where the genotypes differed substantially from those in cluster III, demonstrating similarity in adaptability. Nevertheless, the lack of a geographic location correlation in this study shows that the populations of various regions share genetic similarity and may have derived from the same breeding materials (Tahir *et al.*, 2013) or the random grouping of populations from different geographic regions into different clusters suggests that factors other than geographic influence, such as breeding material exchange, genetic drift, and natural and artificial selects, are responsible for diversification (Murty and Arunachalam, 1966). Similar results were found in Indian accessions of amaranth (Shankar *et al.* (2012)). In other words, cluster analysis revealed that the amaranth genotypes obtained from several Ethiopian regions had a high degree of genetic diversity. As a result, crossing these genetically distinct parents could produce desirable recombinants and transgressive segregants, which in turn could produce varieties that perform better than those that have already been released.

Lower heterotic F1 offspring are produced when clusters with short distances between them are crossed. Crossing of best genotypes from distant clusters is expected to produce transgressive segregants that exceed both parents (Mussa *et al.*, 2003). According to Darkwa *et al.* (2016), maximum genetic recombination is expected from the hybridization of the parents selected from divergent cluster groups. However, crossings involving parents chosen from clusters I and III, followed by clusters II and III, are likely to have the highest levels of genetic recombination and variation in the next generation of offspring. The highest inter-cluster distance showed that the genotypes in cluster III have diverged from those of cluster I. Nevertheless, depending on the specific objectives of the hybridization, it is also important to take into account the distinctive advantages of each genotype and cluster (Chahal and Gosal, 2002).

Clusters I and II had the lowest inter-cluster divergence, demonstrating the genetic closeness of these clusters' genotypes. Therefore, based on our findings, it is expected that choosing a parent for hybridization from cluster III may result in the desired heterosis for heterotic amaranth hybrids. In contrast, all of the clusters' inter-cluster distances were greater than their intra-cluster distances, suggesting more genetic diversity among the genotypes of various

groupings. The results were in agreement with previous studies ([Rahman and Munsur, 2009](#); [Kumar, 2019](#)).

The results of PCA revealed that the genotypes of amaranth exhibit significant genetic variety. The first two PCs had a bigger contribution than the other PCs, even though the individual PCs contributed to the overall variance in different ways. In this study, the top two PCs explained 45.1% of the total variation, although the third (9.6%), fourth (6.3%), fifth (5.8%), and sixth (5.4%) PCs made less contribution to the overall variation. Additionally, according to [Chahal and Gosal \(2002\)](#), the first few PCs often contributed more to the overall variance. SLA, GH, SX, BI, SSH, SP, LP, IDI, and IC were the key contributors to the observed variance in the first PC, whereas PP, SPG, LPG, IDI, and SSH had a significant impact on the overall variation in the second PC. Contrarily, it was widely thought that the characteristics inside the first PC with substantially bigger absolute values of the eigenvector that are closer to unity had a greater effect on clustering than those with smaller absolute values that are closer to zero ([Chahal and Gosal, 2002](#)). However, in this study, each of the examined variables alone contributed less (0.02 to 0.96) to the overall variance in the first PC; as a result, the clustering of the genotypes into various groups was more influenced by the traits' additive effects. The value of the eigenvector denotes the relationship between characteristics and PCs. For a particular characteristic, a negative eigenvector denotes a negative correlation between that trait and the given PC, as well as the opposite.

4.5 Conclusions

The diversity patterns among the 120 genotypes that make up our amaranth diversity array are examined in this study. This study lays the groundwork for Ethiopian amaranth genetic advancement since crop improvement and selection may make use of the variety that was identified. The large genetic diversity among genotypes revealed by the morphological qualitative features utilized in the current study will help with the classification, improvement, and preservation of genetic resources. Moreover, the findings also have breeding implications for improved grain or vegetable amaranth variants, particularly for leaf and blossom color uniqueness while selecting for economically relevant seed color features. Therefore, the selection of these traits could be effective for the improvement of amaranth genotypes. This finding indicates that broad collection from a range of altitudes and origin locations is far

more valuable than amaranth collection from particular agro ecology, indicating that it catches significant allele variation from varied growth situations.

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CHAPTER V: Assessment of physio-morphological traits, genetic variability, and growth performance among amaranth (*Amaranthus species*) genotypes from Ethiopia

Assessment of physio-morphological traits, genetic variability, and growth performance among amaranth (*Amaranthus species*) genotypes from Ethiopia

Abstract

Amaranth is a type of plant that belongs to the NAD-malic enzyme-type C4 metabolism category. They have a unique C4 anatomy, which is present in their bracts, cotyledons, and leaves. This allows them to produce food through the C4 photosynthetic pathway and rapidly adapt to unfavorable environmental conditions. The objectives of the current study were to determine the extent and patterns of genetic diversity in Ethiopian amaranth genotypes using physio-morphological markers. Using an alpha lattice design with two replications, 120 amaranth genotypes from Ethiopia were assessed for physio-morphological features, genetic variability, and growth performance assessment during a one-year period. The results of the analysis of variance showed that all examined physio-morphological parameters, except the rate of photosynthesis and stomata conductance, had mean squares that varied considerably ($P < 0.001$) owing to genotypes. The estimates of genetic variability, heritability, and expected genetic advance indicated an incredible extent of genetic diversity among amaranth genotypes, with a significant selection pressure for these traits in the population to produce better genotypes for improved amaranth. Selection based on desirable features such as leaf-to-air vapor pressure deficit, transpiration rate, chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, leaf area, plant height, leaf number, and root weight can be useful in achieving the intended genetic gains for improvement since these traits appear to be more controlled by additive gene activity. Thus, selection in amaranth genotypes may consider these desired yield-related features. Moreover, the study showed that certain genotypes (ALE-073) exhibited better intercellular CO₂ concentration (C_i), leaf-to-air vapor pressure deficit (VPD), transpiration rate (E), and leaf number (LN), resulting in better grain yield. Understanding the relationship between LA and E can help in selecting crops for high E and may provide an avenue to improve leaf yield. Furthermore, some of the selected genotypes in this study could be used as potential parents for improving the genetic gain in amaranth breeding programs. The study concluded that there was additive gene action present since the Ch a, Ch b, TCh, and Tca markers exhibited 100% heritability. This showed that the use of these characteristics for selection, which indicated a potentially exploitable variation, would be more effective and successful in the long run in breeding programs than the use of other traits for splitting generations. The result showed that the hierarchical clustering grouped all the genotypes into seven clusters. The sixth cluster had the fewest genotypes (6), while the seventh cluster had the greatest number of genotypes (35) and was followed by Cluster Five (25), Cluster Three (22), Cluster One (14), Cluster Two (11), and Cluster Four (7). High leaf and grain yield-producing genotypes were grouped into the sixth cluster. The second cluster (C2) was characterized by high mean values for LA, LY, and GY. The maximum and significant genetic divergence was found between clusters C4 and C6 ($D^2 = 103.44$) and the minimum least non-significant genetic divergence was found between clusters C5 and C7 ($D^2 = 17.00$). The first six PCs with eigenvalues greater than one accounted for 79.30% of the total variation in amaranth genotypes. The cumulative variance of 39.40% was explained by 1st two principal components (PC1 and PC2) with eigenvalues greater than 1. Component 1 with a variance of 21.11 had a contribution from root length, root weight, total chlorophyll, and carotenoid while principal component 2 accounting for 18.29% total variability had a contribution from transpiration rate, rate of net photosynthesis, chlorophyll b and, stomata conductance. The remaining variability of 13.02%, 11.33%, 9.37%, and, 6.17 % was consolidated in PC3, PC4, PC5, and PC6. These give scope for the selection of parents for hybridization programs from these clusters, genetic divergence, and principal components to realize high genetic variation and novel combinations for yield increment.

Keywords: physio-morphological, diversity, markers, genetic gain, hierarchical clustering, genetic divergence genotypes.

5.1 Introduction

Modern farming systems are very vulnerable to the expected climate change and related weather extremes because they rely on a small number of crops with frequently little genetic diversity. Future generations have serious concerns about food security. These crops are easily obtainable plant sources of micronutrients to mitigate micronutrient deficiency and can be cultivated in most of the country's climates (Smith and Eyzaguirre, 2007). It is currently referred to be a third-millennium crop plant (Rastogi and Shukla, 2013). Improvements to their genetic makeup are imperative, as is research into how these plants reproduce and the potential for population variety. By including so-called orphan crops such as amaranth in our food sources, we can increase the quality of our food security and lessen the impact of environmental change. Though few have been studied during the past century, thousands of traditional crops are recognized, and little has been done in the way of breeding.

Geneticists may deal with some exciting material from the genus *Amaranthus*. Most species are functional diploids with $n = 16$ or 17 (exception: *A. dubius* is a polyploid with $2n = 64$). The detectable genome size of *Amaranthus species* is three to four times larger than that of *Arabidopsis thaliana*, the model plant (Das, 2016). In *A. albus*, *A. palmeri* and *A. retroflexus* chromosome counts of $2n = 34$ (Weaver, 1984). The chromosome number for *A. caudatus*, *A. hypochondriacus*, *A. cruentus*, and *A. hybridus* is normally $2n = 32$, but occasionally it is 34 (Council, 1989). In *A. spinosus*, somatic counts of $2n = 34$ (Al-Turki et al., 2000).

Amaranths are a member of the NAD-malic enzyme-type C4 metabolism group. Its leaves, cotyledons, and bracts all have the characteristic Kranz anatomy (C4 anatomy) (Costea and Tardif, 2003). The C4 photosynthetic pathway allows amaranths to produce food, which allows them to adapt promptly to adverse conditions in the environment (Rastogi and Shukla, 2013). They have developed certain physiological traits that boost their genetic diversity and phenotypic plasticity, enable them to withstand pathogenic organism assaults, and facilitate cultivation (Jimoh et al., 2019; Jimoh et al., 2020). To optimize physiological processes and establish an environment that is most conducive to their functioning, agronomists, soil scientists, and plant breeders should collaborate. For this reason, it seems that research on crop enhancement should heavily rely on plant physiology. To alleviate the constraints on global food security and nutrition, crop genetic enhancement is a crucial component (Ronald, 2011). The physio-morphological characters play an important role in

grain yield. The amalgamation of many structural attributes of amaranths, including C4 metabolism, an enduring root system, stomata conductance, and preservation of leaf area (Spreeth *et al.*, 2004), increases the plant's ability to use CO₂ efficiently in situations with greater light levels, moisture stress, and a wide temperature range (from 25 to 40 degrees Celsius) (Brenner and Williams, 1995). The plant's morphological and physiological characteristics have a significant impact on breeding advance, as distinct morphological and physiological factors determine grain yield. Genetic diversity in pigment traits and gas exchange has been studied and used in various breeding schemes (Kondamudi *et al.*, 2016; Haritha *et al.*, 2017). Although distinct genotypes of amaranth survive a wide variety of climatic circumstances, the plant's genetic and physiological flexibility allows for adaptability to a wide range of habitats, which is why the cultivation of the plant has been successful (Onyango, 2010). In this instance, direct selection for increased yield may not be as effective as indirect selection, which focuses on the underlying physiological features that influence yield (Reynolds and Trethowan, 2007).

Amaranth is a prominent farmed vegetable and grain species, however, despite its popular nutrition function, there have yet to be any efforts to increase its nutritional value and production. Regarding nutrition, grain amaranths yield seeds with balanced amino acid profiles and a high protein content (17–19% of dry weight) (Gamel *et al.*, 2006). Moreover, it has been listed in the group of “superfoods” (Waisundara, 2020), a popular category reserved for natural, nutrient-dense, low-calorie, and health-beneficial food. Unfortunately, it has not entered the mainstream of industrial food processing. Grain amaranths that deserve all of these labels—gluten-free, high in protein, high in fiber, high in nutritional value, high grain yield, resistance to drought, and short production time —cannot be written off as a potential crop in the future. Grain amaranths are a possible model organism for researchers working on the enhancement of other edible dicots because of their desired agronomic features, which include drought resistance, C4 photosynthesis, herbicide resistance, and high dry biomass. Grain amaranths provide an alternative to traditional staple cereals like rice or wheat in the context of rising global population demand for water and other natural resources. Tragically, even though Ethiopia is among the few nations where many landraces of grain amaranths have been noticed, the crop has not received much recognition or succeeded in becoming a staple.

The machinery through which the environment and genotype influence the quantity and quality of growth is comprised of physiological processes, which play a central role in plant growth (**Kramer, 1980**). Indirect selection may be more effective in this situation than direct selection for increased production as it focuses on the underlying physiological features that affect yield (**Reynolds and Trethowan, 2007**). The finding is explained by the fact that traditional yield-based breeding, which measures the impact of a trait of interest on grain production, often uses yield analysis of tens of thousands of plants after each breeding cycle. In contrast, physio-morphological trait-based breeding aims to identify simple surrogate attributes and/or early-season features that contribute to yield (**Nigam et al., 2005**). This indicates that by lowering the dependence on final grain yield, the assessment of yield attributable to physio-morphological features independent of grain yield enhances selection efficiency. By taking advantage of the possibility of additive gene activity, this strategy may raise the likelihood of producing more successful crosses in a breeding effort (**Dolferus et al., 2019**).

The presence of genetic variability in a genotype group can be evaluated using multivariate analysis techniques, which enable the simultaneous use of multiple characters for the best genotype selection as well as the selection of descriptors with greater potential for genotype discrimination in a germplasm bank (**Azevedo et al., 2011**). As a result, it enables breeders to find desirable genetic pairings before conducting crosses (**Nardino et al., 2017**). Group analysis was done to confirm the closeness of *Amaranthus species* genotypes. Since group composition plays a significant role in genotype selection, novel combinations can be based on the degree of genotype dissimilarity.

Assessment and correct genotypic identification of genetic diversity is a key topic in conservation studies and important to maintain ecotypes with desired traits useful for breeding programmes (**Zhao et al., 2007; Jamalluddin et al., 2022**), therefore in to adopt successful conservation methods, it is required to assess the genetic diversity of local amaranths. Breeding efforts are hampered by inadequate understanding of the genetic diversity in these amaranth species and the scarcity of appropriate markers. So, assessing the extent of genetic diversity using markers of physio-morphological traits is essential to exploit the existing variations in amaranth genotypes. Evaluating the genetic diversity in the pool of germplasm is made easier by the physiological characteristics of plants (**Council, 1984**). Therefore, this study was done to (1) estimate the extent of genetic variability, heritability,

and expected genetic gain attributable to physio-morphological traits, and (2) to assess degree of divergence and the relative contributions of each feature to the overall divergence can be ascertained by multivariate studies, such as principal component analysis, clustering, and Mahalanobis's D^2 statistics, (3) recommend a successful selection strategy and identify genotypes promising for further amaranth breeding endeavours. To optimize breeding gain from a selection, one must comprehend the link between growth and physiological, developmental, and yield-related variables as well as other contributing features. Moreover, physio-morphological features of plants are of the utmost importance and are frequently employed in genotype selection for enhanced performance (Sallam *et al.*, 2019). Therefore, quantifying the effects of physio-morphological markers on genetic diversity and yield is a restricted area of extensive research.

5.2 Materials and Methods

5.2.1 Description of the study sites

The field experiment was conducted at the Hawassa University Agricultural Research Site from April to August 2020/2021 to evaluate amaranth growth, with physiological, photosynthetic pigment, and agro morphological parameters evaluated in 2021. The description of the test location for geographical position and physicochemical properties of the soil indicated in the (Table 5.1). The mean annual rainfall of 2020 and 2021 Hawassa district, receives the lowest rainfall (2.9mm) in December and the highest (166.1mm) in April. The average temperature for each month is shown by a line graph (Figure 5.1). In the graph, the lowest temperature is about 11.7°C in November and the highest is about 30.6°C in March.

Table 5.1 Description of the test location for geographical position and physicochemical properties of the soil

Description	Parameters	Hawassa	Soil reaction class/Class and Toxicity level plant nutrients/
Location description	Latitude	7°2' 54.7503 N	Slightly acidic
	Longitude	38°30' 17.1608 E	
	Altitude (masl)	1709asl	
Soil properties and mineral content	Ph	6.22	
	Na	0.07	
	K	1.13	
	Mg	2.81	
	Ca	20.92	
	Mn	47.30	
	Fe	35.00	

Textural class	Cu	1.51
	Zn	1.40
	% sand	45.56
	% silt	25.64
	% clay	28.8
	Sand-clay –loam	

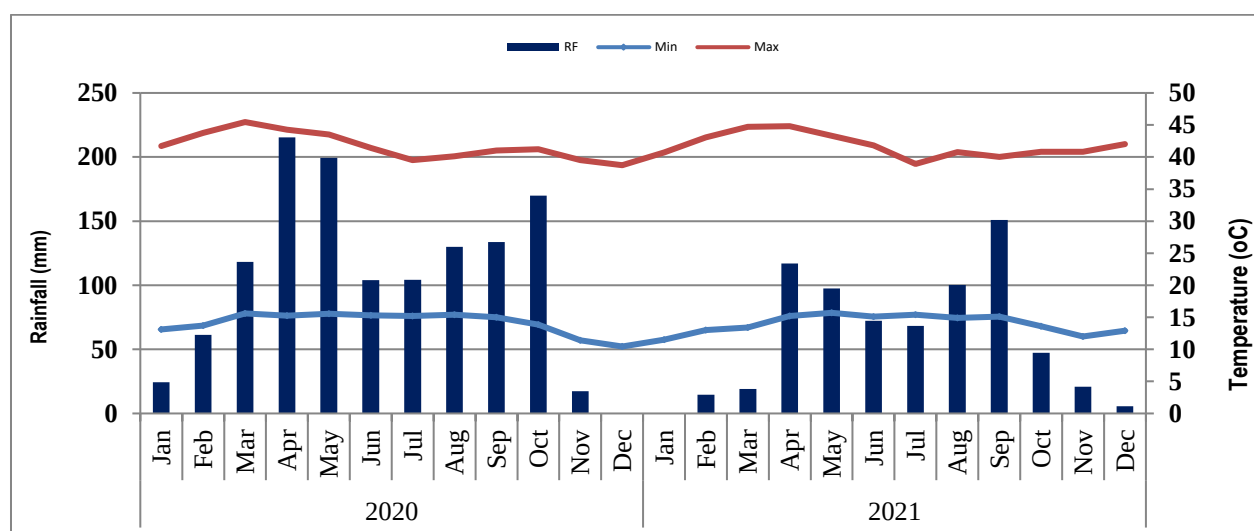


Figure 5.1 The average monthly rainfall (mm) and temperatures (°C) of the Research area.

5.2.2 Experimental treatments and design

For the genetic diversity analysis, seven distinct species—a total of 120 amaranth genotypes—were used, including *A. hybridus* L. subsp. *cruentus* (L.) Theil., *A. hybridus* L. subsp. *hybridus*, *A. viridis* L., *A. caudatus* L., *A. spinosus* L., *A. palmeri* S Wats., and *A. species*. The studied amaranth genotypes were collected from different areas of the country thirty-four genotypes were supplied by the Ethiopian Biodiversity Institute (EBI), two by the Melkasa research center, fifteen by the Werere research center (Afar region), and the remaining sixty-nine by the Southern Nations Nationalities and Peoples' Region, Oromia, Tigray, Amhara, and Sidama of Ethiopia in 2019. Two of the hundred twenty amaranth genotypes were released varieties (AC-NL and Madiira II) while the other 118 one hundred eighteen varieties were not released in Ethiopia. The Ethiopia Biodiversity Institute's field assistants assisted in the collection of voucher specimens. The National Herbarium (ETH) at Addis Abeba University has received specimens that have been dried, numbered, labeled, pressed, identified, and placed. Using taxonomic keys from the Flora of Ethiopia and Eritrea and by comparison with genuine herbarium specimens, specimens were identified both in the field and afterward in Ethiopia. The experiment was designed using an alpha lattice. Using a

layout with two replications, eight blocks per replication, and fifteen plots per block, we conducted our experiment. 3, 1, and 0.6 meters, respectively separated replications, blocks, and plots. 1.80 m in length and 1.50 m in breadth made up each plot's 2.7 m² space. 1,345.2 square meters were needed altogether (38 m x 35.4 m). Before planting, the ground was carefully plowed, and the trial field was cleared and harrowed using a tractor. On April 15, 2021, the first day of the growing season, the seeds were planted at the Hawassa University agricultural experimental site. Each genotype had two rows of identical seeds that were similarly spaced apart by 0.75 m. The tiny seeds were spread on seedbeds in a 1:4 mixture with sand and then covered with finely ground manure from the farmyard. At 14 and 22 days after sowing (DAS), there were only ten seedlings per row, spaced 30 cm apart. After germination, weeding was done by hand-hoeing whenever necessary and at intervals of two weeks.

5.2.3 Measurements of Traits and Data Collection

All 120 amaranth genotypes were assessed using nineteen physio-morphological, agronomic, and photosynthetic pigment attributes. The mean value of ten samples and/or plants per genotype for measuring all physio-morphological, some agronomic, and photosynthetic pigment attributes were randomly selected from rows to reduce environmental impact and repeated two times. Data were collected either on a plot basis or from ten randomly selected plants per plot following the standard descriptors for agro-morphological traits.

5.2.3.1 Growth parameters

At 30, 50, and 70 DAS at intervals of 20 days throughout two years (2020/2021), growth metrics including plant height (cm), the number of branches per plant (number), stem diameter (cm), and the number of total leaves per plant (number) were measured. The sum of the parameters that were measured was averaged for each of the ten plants.

5.2.3.2 Agro-morphological traits measurement

The data on agro-morphological traits were collected from the field at the vegetative stage and plant maturity shortly before harvesting. Root length (cm), root weight (g), number of leaves (number), leaf area (cm²), internode length (cm) plant height at maturity (cm), days to flowering (days), days to maturity (days), grain filling period (days), leaf yield (t/ha), and grain yield (t/ha) are among them. Agro-morphological traits were therefore assessed in each

plot for every replication, for a total of 10 observations in each plot. Data on quantitative traits were gathered using all ten planted genotypes from each population (two replications). Ten plants per plot were used to get the average value for each attribute. For measurement, three distinct leaf areas (small, medium, and big) per plant were chosen at random. Using the averages of 30 measurements (10 plants per plot for each of the three distinct leaf sizes), the area of the leaf was determined. The amount of leaf surface measurement was performed using an electronic leaf area meter from LI, Cor, Inc. in Lincoln, Nebraska, USA.

5.2.3.3 Determination of physiological traits

Seven physiological crop attributes were estimated at the vegetative stage. These are include Relative Humidity inside the leaf chamber (%) [RH], the intercellular CO₂ concentration ($\mu\text{mol mol}^{-1}$)[Ci], stomata conductance ($\text{mmol m}^{-2}\text{s}^{-1}$) [gs], leaf to air vapor pressure deficit (kPa) [VPD], rate of net photosynthesis ($\mu\text{mol m}^{-2}\text{s}^{-1}$) [A], transpiration rate ($\text{mol m}^{-2}\text{s}^{-1}$) [E], photosynthetic water use efficiency) (WUE) ($\text{mmol CO}_2\text{ mol}^{-1}\text{ H}_2\text{O}$) was obtained as the ratio of photosynthetic carbon assimilation to water lost by transpiration (A/E). Physiological parameters (Leaf Gas Exchanges) were measured by choosing a flag leaf with the adaxial surface exposed to sunshine that is clean, dry, completely inflated at the top, and disease-free. At its midpoint, the leaf is inserted into the chamber, making sure that the selected area of the leaf completely encloses the sensor's aperture. While we were taking the measurement, the white filter was facing up and in direct sunshine (we did not allow other plants to shade the filter). On three distinct, arbitrary leaf samples, we recorded three readings. Two replications of each treatment were used to calculate the means. All gas exchange parameters were measured using a CIRAS-3 Portable Photosynthesis System (PP Systems 110 Haverhill Road, Suite 301 Amesbury, MA 01913 U.S.A) infrared gas analyzer (closed system) with a Parkinson's Automatic Universal Leaf Cuvette (PAR Sensor (Internal) range – 0-3000 $\mu\text{mol m}^{-2}\text{s}^{-1}$, air temperature sensor range: -10 o C to 50 °C, equipped with 18 mm diameter and 1.75 cm² area cuvette inserts. Water use efficiency (WUE) was determined as the ratio between *A* and *E* (Figure 5.2).



Figure 5.2 Physiological traits measurement in the experimental field

5.2.3.4 Determination of photosynthetic pigments

Arnon (1949), recommended procedure; the colorimetric method was used to measure the amounts of chlorophyll a, b, total chlorophyll, and carotenoid content. Using a Digital Analytical Balance C.165, the fresh leaves of all the genotypes were weighed at 0.25 g before being transferred to a mortar and pestle and homogenized with the addition of 10 ml of 80% acetone. Whatman's No. 1 filter paper was used to filter the extract before it was collected in a volumetric flask. By continuously adding 2-3 ml of acetone, the green extract is progressively produced. Twenty five milliliter of the extract's final volume was produced with 80% acetone. The extract is maintained out of direct sunshine because it is susceptible to photooxidation. Using wavelengths of 480, 510, 645, 652, and 663 nm, respectively, a spectrophotometer measures the optical density (O.D.) of the extract. Using the following formula, the amounts of chlorophylls a and b, total chlorophyll, and carotenoids were determined: The leaf photosynthetic pigments were expressed as mg g^{-1} on an FW basis using the formula listed below (**Lichtenthaler and Wellburn, 1983**).

Chlorophyll a, b, total chlorophyll, and carotenoid content were calculated using the following formula

$$\text{Chlorophyll "a" (mg/g)} = \frac{12.7 (\text{O. Dx663}) - 2.69 (\text{O. Dx645}) \times V}{W \times 1000}$$

$$\text{Chlorophyll "b" (mg/g)} = \frac{22.9 (\text{O. D x 645}) - 4.68 (\text{O. Dx663}) \times V}{W \times 1000}$$

$$\text{Total Chlorophyll (mg/g)} = \frac{(\text{O. Dx652}) \times 1000 \times V}{34.5 \times W \times 1000}$$

$$\text{Total Carotenoids " (mg/g)} = \frac{7.6 (480) - 1.49 (510) \times V}{W \times 1000} n$$

Where OD = optical density=final volume of 80% acetone (ml); W= weight of sample taken (g).



Figure 5.3 Determination of leaf photosynthetic pigments

5.2.4 Statistical Analysis

5.2.4.1 Analysis of variance

A variety of programs, including Excel 365, Minitab version 19, SAS software version 9.4, and RStudio 4.1.1 were used to analyse the data (<https://www.R-project.org/>; ([Kuhn et al., 2020](#)). Before doing any statistical analysis, we examined for typographical errors and missing data. Descriptive statistics were used to the collected data for all monomorphic physio-morphological and photosynthetic pigment characteristics. The SAS computer software used Bartlett's test for homogeneity of variance before conducting the ANOVA to validate each analysis. To better understand genetic variability in agro-morphology, gas exchange, growth, and photosynthetic pigment, the information generated was employed. The importance of genetic variation for each examined trait was ascertained by doing an analysis of variance (ANOVA) on the 120 amaranth genotypes. At a 5% level of probability, Duncan's multiple range tests (DMRT) were used to statistically compare the significance test for mean differences. The data are then given for all variables as mean plus standard error after the results are averaged over two were submitted to t-test ($P < 0.05$).

The ANOVA followed the model:

$$P_{ijk} = \mu + g/s_i + b_k(j) + r_j + e_{ijk}$$

Where, P_{ijk} = value of phenotypic i th genotype j th replication plus k th incomplete block within j replication; μ = grand mean; g_i = the effect of i th genotype; $b_k(j)$ = the effect of incomplete block k within replication j ; r_j = the effect of replication j ; and e_{ijk} = the residual, s = species.

5.2.4.2 Comparing isolated genotypes to the original population

For each characteristic, the means of the top 5% of genotypes were separately identified to compare them to the original population. The student t-test table was used to determine if the difference between the sample mean ($\bar{x}$) and population parameter (μ) was statistically significant (Singh *et al.*, 2001). When the computed t-value is higher than the tabulated t-value, the difference is deemed significant. The performance of the top 5% genotypes was compared with the base population using the absolute t-value, which was computed using the student's t-test formula as follows:

$$T = (\bar{x} - \mu) / (SD / \sqrt{n})$$

Where $\bar{x}$ represents the mean of the genotypes that were chosen, μ indicates the grand mean, SD represents the standard deviation of the sample, and n is the number of genotypes chosen from the size of a population for better performance.

5.2.4.3 Estimation of variance components

By assuming that the mean square of each source of variation is equal to its predicted mean square, the total variance components can be split into several components using ANOVA (Table 5.2), as suggested by (Singh and Chaudhary, 1977). By using variance components and coefficients of variation, one may determine the level of population variability. The sources of variation in the alpha lattice design were taken into account to estimate the variance components using the procedure recommended by (Johnson *et al.*, 1955)), and the mean squares were computed by SAS's statistical methods as follows:

$$\sigma^2_r = \frac{[(MS_r) - (MSe)]}{g}$$

$$\sigma^2 g = \frac{[(MSg) - (MSe)]}{r'}$$

$$\sigma^2 e = MSe \text{ and}$$

$$\sigma^2 p = \sigma^2 g + \frac{\sigma^2 e}{r'}$$

The term "MSg" refers to the mean square of the genotypes, where g stands for the number of genotypes, r for the number of replications, $\delta^2 r$ for the replication variance, MSr for the mean square of replication, MSe for the mean square of error, and $\delta^2 g$ for the genotypic variance. The environmental variation is denoted by $\delta^2 e$ and the phenotypic variance by $\delta^2 p$.

$$GCV = \frac{\sqrt{\delta^2 g}}{\mu} * 100$$

$$PCV = \frac{\sqrt{\delta^2 p}}{\mu} * 100$$

$$ECV = \frac{\sqrt{\delta^2 e}}{\mu} * 100$$

Nevertheless, it was also used to figure out the genotypic (GCV), phenotypic (PCV), and environmental coefficients of variation (ECV) using the method proposed by (Singh and Chaudhary, 1977). Low values were defined as less than 10%, medium values as between 10% and 20%, and high values as greater than 20% (Deshmukh *et al.*, 1986).

In this scenario, GCV stands for genotypic coefficient of variation, PCV for phenotypic coefficient of variation, and ECV for environmental coefficient of variation. The genotypic variation is denoted by the symbol $\delta^2 g$, whereas the phenotypic variation is denoted by the symbol $\delta^2 p$, the environmental variation by the symbol, and $\delta^2 g$.

5.2.4.4 Estimation of heritability

As outlined by Allard (1960) and Falconer (1989), the estimate of heritability (h^2) is given as a percentage of the ratio of the genotypic variation to the phenotypic variance. According to Singh *et al.* (2001), the heritability estimates were categorized as low when the values were less than 40%; medium when the values were between 40 and 59%; moderately high when they were between 60 and 79; and very high when they were higher than 80%, and it was computed as

$$h^2_b = \frac{\frac{MSg}{r}}{\sigma^2_g + \frac{\sigma^2_e}{r}} * 100 = \frac{\delta^2_g}{\delta^2_p}$$

H^2_{bs} stands for heritability in the broadest sense, r for replication, δ^2_e for environmental variance, δ^2_g for genotypic variation, and δ^2_p for phenotypic variance.

5.2.4.5 Estimation of genetic advance

The technique described by **Allard (1960)** was used to determine the predicted genetic advance (GA) for each characteristic at 5% selection intensity ($k = 2.06$). Similarly, the **Comstock and Robinson (1952)** method is used to determine expected genetic advance as a percentage of the mean (GAM), which compares the magnitude of the projected advance of various characteristics under selection. The predicted GAM values were categorized as low when they were less than 10%, medium when they were between 10 and 20%, and high when they were more than 20%, according to **Johnson et al. (1955)**.

$$GA = K * (\sqrt{\sigma^2_p} * h^2_{bs}) \quad \quad \quad GAM = \frac{GA}{\bar{X}} * 100$$

To clarify the relationships between the studied features, a Pearson correlation analysis was carried out to determine the linear relationship between two variables by using R statistical software version 4.1.1. The correlations between the features under investigation were explained using Pearson correlation analysis.

5.2.5 Statistical analysis for multivariate analysis

5.2.5.1 Data standardization

The mean data were standardized before multivariate analysis (cluster, genetic distance, and principal component analyses) (**Mohammadi and Prasanna, 2003**). Accordingly, the mean data measured for each trait was standardized to means of zero and variances of unity before analysis to avoid bias due to differences in measurement scales (**Martínez-Calvo et al., 2008**). A trait was dropped from the study if it was not relevant for improvement and had a low eigenvalue together with the largest matching eigenvector in absolute value (**Olivoto et al., 2017**). Based on **de Carvalho et al. (2022)** it is critical to exclude these attributes to obtain accurate estimates of advance, as they have minimal effect on individual discrimination. To explain the variability, the first PCs that explained at least 70–80% of the cumulative variance were kept (**Stevens, 2012**), and traits with loadings greater than 0.45 were considered relevant scores for the respective PC (**Tabachnick et al., 2013**).

5.2.5.2 Cluster analysis

The R version (4.1.2) has been used with various packages to perform cluster analysis based on ward D2 hierarchical grouping using Euclidian distance and determine the optimal number of clusters in the data set using the gap-stat method for the dendrogram (Maechler, 2019). The gap stat method and the NbClust software in R Studio were used to determine the optimal number of clusters (Charrad and Ghazzali, 2014). The 120 genotypes were grouped, and the patterns of similarity and dissimilarity based on 19 quantitative physio-morphological traits were brought out. Additionally, using Minitab 19.1 software, discriminant function analysis was carried out to re-evaluate the genotype grouping.

5.2.5.3 Genetic distance analysis

Using Mahalanobis distance (D^2) statistics, the genetic distance between each cluster was calculated to evaluate the link between the clusters (Ghorbani, 2019). Generalized Mahalanobis's D^2 statistics were used to quantify the genetic divergence inside and across clusters using SAS version 9.4's PROC discrim methods (Elliott and Woodward, 2023). The D^2 values for each pair of clusters were taken into consideration when calculating the chi-square (χ^2) values. These values were then compared to the tabulated χ^2 value for the degree of freedom denoted by the letter "P," which stands for the number of traits (Singh and Chaudhary, 1977). Genetic distance between clusters as standardized by Mahalanobis's D^2 statistics was calculated as $D^2_{ij} = (x_i - x_j)' \text{cov}^{-1} (x_i - x_j)$, where, D^2_{ij} = distance between cases i and j; x_i and x_j = vectors of the values of the variables for cases i and j; and cov^{-1} = the pooled within-groups variance-covariance matrix.

5.2.5.4 Principal component analysis

To determine which attributes account for the majority of the variation overall, we employed principal component analysis, or PCA. According to Govindaraj *et al.* (2020), a significant genotype dispersion requires the PCA components with cumulative variability to account for a minimum of 75% of the overall variability. The traits that accounted for the majority of the total variation were identified using the principal component analysis (PCA). A meaningful genotype dispersion requires that the PCA components with cumulative variability account for at least 75% of the total variability (Govindaraj *et al.*, 2020). Principal components (PCs) with eigenvalues greater than unity were used as criteria to determine the number of PCs

(Kaiser, 1960; Iezzoni and Pritts, 1991). The sum of the eigenvalues is equal to the number of traits used for the study and these values often decrease progressively with increasing the number of PCs. The eigenvectors of the PCs were compared among the traits to identify the traits that accounted for the highest variation for each PC, and traits that had higher eigenvectors in the absolute value were considered for selection due to their high contribution to yield improvement (Dhakar *et al.*, 2019). The PCs' eigenvalues and eigenvectors were estimated based on the correlation matrix analysis using the Minitab 18.1 statistical software (Richard, 2014). Moreover, scree plots and biplot loadings and scatter plot diagrams for the first two PCs were constructed using the Minitab 17.1 statistical software (Richard, 2014).

5.3 Results

5.3.1 Analysis of variance

Table 5.2 exhibits the analysis of variance results for 25 physio-morphological markers among 120 amaranth genotypes. Except for stomata conductance and rate of net photosynthesis, the results showed that variance ($P < 0.0001$) was found across all amaranth genotypes. The analysis revealed a broad range of coefficients of variation (CV), ranging from 4.25% to 54.97%. The greatest value was identified in g_s (54.97%), trailed by A (53.08%) and C_i (49.73%). Our result revealed that there was high genetic variability between these traits.

5.3.2 Descriptive statistics

The descriptive statistics like mean, range, standard error, and standard deviation for the examined physio-morphological markers of 120 amaranth genotypes, are provided in Table 5.3. Wide range was observed for all observed characters like CO_2 concentration of sub-stomatal CO_2 concentration, stomata conductance ($mmol\ m^{-2}\ s^{-1}$), plant height (cm), leaf area (cm^2), root weight (g), root length (cm), rate of net photosynthesis ($\mu mol\ m^{-2}\ s^{-1}$), leaf number (number), carotenoids (mg/g), chlorophylls a (mg/g), grain yield (t/ha) and leaf yield (t/ha). The greatest C_i , A , LY , and LN among all genotypes were found in *Amaranthus caudatus* L., whereas its VPD , RH , WUE , TCh , TCa , and RW were the lowest. *Amaranthus hybridus* L. *subsp. hybridus* was identified as having the traits with the highest g_s , TCh , Tca , PH , GY , INL , RW , RL , and the smallest C_i . The smallest g_s , E , INL , and RL , as well as the greatest VPD , $Ch\ b$, have been identified in *Amaranthus palmeri* S. Wats. *Amaranthus spinosus* L. showcased the biggest $Ch\ a$, but the least LA , LN , and GY . *Amaranthus hybridus* L. *subsp. cruentus* Theil. possessed the biggest E , and LA . On top of that, g_s , RH , C_i , PH , and LA

demonstrated significant standard deviations (more than 20) among the examined characteristics. The remaining investigated characteristics also exhibited modest standard deviations, fewer than 10.

Table 5.2 Mean squares from analysis of variance for 25 traits for agro-morphology, physiology, and photosynthetic pigment traits of 120 amaranth genotypes evaluated at Hawassa University Research Site during 2021

Traits	Grand Mean	Rep (Df= 1)	Block(Rep) DF= 14)	Genotype (Df=119)	MSE (DF=105)	CV (%)	The P- value of genotype)	R-Square
Relative Humidity inside Leaf Chamber (%) RH	56.55±0.58	24.41 ^{ns}	105.28**	96.91***	47.45	12.18	0.0001	74.20
Intercellular CO ₂ concentration ($\mu\text{mol mol}^{-1}$) [Ci]	134.53±4.96	341.1 ^{7ns}	6138.47 ^{ns}	6867.94**	4476.11	49.73	0.01	66.71
Stomata conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) [gs]	164.88±6.47	308.80 ^{ns}	5793.34 ^{ns}	9360.57 ^{ns}	8214.48	54.97	0.25	64.03
Leaf to air vapor pressure deficit (kPa) [VPD]	2.40±0.03	0.00 ^{ns}	0.12 ^{ns}	0.36***	0.11	14.00	<.0001	80.44
Rate of net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$) [A]	13.36±0.51	36.27 ^{ns}	84.12 ^{ns}	63.93 ^{ns}	50.27	53.08	0.10	65.29
Transpiration rate ($\text{mol m}^{-2} \text{s}^{-1}$) [E]	4.50±0.15	2.12 ^{ns}	3.83*	5.79***	2.12	32.39	<.0001	82.21
Photosynthetic water use efficiency ($\text{mmol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$) [WUE]	3.24±0.11	0.15 ^{ns}	2.18 ^{ns}	3.37***	2.00	43.67	0.0032	70.03
Intercellular CO ₂ concentration (Ci) to ambient CO ₂ concentrations ratio(Ca) [Ci/Ca]	0.45	0.002 ^{ns}	0.05 ^{ns}	0.06**	0.04	44.25	0.01	68.00
Chlorophyll a to Chlorophyll b ratio [Ch a/Ch b]	1.96	0.47*	0.11 ^{ns}	2.66***	0.08	14.1	<.0001	98.30
Total chlorophyll to total carotenoids ratio [TCh/TCa]	4.8	6.07**	1.5**	5.21***	0.53	15.24	<.0001	93.54
Chlorophylls a (mg/g) [Ch a]	0.80±0.02	0.01*	0.00 ^{ns}	0.16***	0.00	5.07	<.0001	99.17
Chlorophyll b (mg/g) [Ch b]	0.47±0.02	0.01**	0.00 ^{ns}	0.13***	0.00	6.11	<.0001	99.54
Total chlorophyll (Ch a + Ch b) (mg/g) [TCh]	1.28±0.03	0.04**	0.00 ^{ns}	0.31***	0.00	4.25	<.0001	99.23
Total carotenoids(x+c) (mg/g) [Tca]	0.30±0.01	0.01***	0.00***	0.03***	0.00	7.41	<.0001	98.73
Leaf area (cm^2) [LA]	73±1.69	0.03 ^{ns}	482.84***	1169.67***	87.28	12.69	<.0001	94.35
Plant height (cm) [PH]	181.20±2.88	31.79 ^{ns}	504.49*	3524.21***	248.20	8.69	<.0001	94.55
Leaf number (number) [LN]	20.40±0.34	302.95***	15.02*	41.64***	7.61	13.53	<.0001	88.05
Internode length (cm) [INL]	12.53 ±0.21	55.96**	7.03 ^{ns}	15.63***	5.07	17.97	<.0001	80.25
Root weight (g) [RW]	133.91±4.80	35258.51***	575.57 ^{ns}	9062.51***	1364.07	27.58	<.0001	89.14
Root length (cm) [RL]	43.64±0.65	35.81 ^{ns}	60.45 ^{ns}	151.36***	50.85	16.34	<.0001	78.19
Days to flowering [DF]	44.31 ±0.72	114.69*	32.75 ^{ns}	212.04***	29.45	12.25	<.0001	90.00
Days to maturity [DM]	95.01±0.96	12.42 ^{ns}	31.99 ^{ns}	364.36***	32.76	6.02	<.0001	94.00
Grain filling period [GFP]	50.70 ±0.83	51.63 ^{ns}	28.13 ^{ns}	258.13***	50.93	14.08	<.0001	86.70
Leaf Yield (t/ha) [LY]	9.39±0.25	327.88***	20.65**	13.53***	7.92	29.96	0.0027	75.64
Grain Yield(t/ha) [GY]	1.20±0.05	1.85**	0.23 ^{ns}	0.56***	0.23	40.42	<.0001	75.67

Table 5.3 Mean, range, standard deviation, and standard error of the mean for 25 traits of 120 Amaranth genotypes grown at Hawassa in the 2021 cropping season.

Traits	Mean	SEM	SD.	Min	Max.	Diff
Relative Humidity inside Leaf Chamber (%) RH	56.55	0.58	7.34	40.84	84.26	43.42
Intercellular CO ₂ concentration ($\mu\text{mol mol}^{-1}$) [Ci]	134.53	4.96	59.95	28.00	421.50	393.50
Stomata conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) [gs]	164.88	6.47	78.16	50.50	507.00	456.50
Leaf to air vapor pressure deficit (kPa) [VPD]	2.40	0.03	0.44	1.10	3.20	2.10
Rate of net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$) [A]	13.36	0.51	6.05	6.05	27.80	21.75
Transpiration rate ($\text{mol m}^{-2} \text{s}^{-1}$) [E]	4.50	0.11	2.02	1.36	9.99	8.63
Photosynthetic water use efficiency ($\text{mmol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$) [WUE]	3.24	0.02	1.39	2.03	7.78	5.75
Intercellular CO ₂ concentration (Ci) to ambient CO ₂ concentrations ratio(Ca) [Ci/Ca]	0.45	0.01	0.23	0.11	1.25	1.15
Chlorophyll a to Chlorophyll b ratio [Ch a/Ch b]	1.96	0.09	1.41	3.15	6.60	3.45
Total chlorophyll to total carotenoids ratio [TCh/TCa]	4.78	0.12	1.90	1.60	12.60	11.00
Chlorophylls a (mg/g) [Ch a]	0.80	0.02	0.30	0.10	1.49	1.39
Chlorophyll b (mg/g) [Ch b]	0.47	0.03	0.28	0.44	1.19	0.75
Total chlorophyll (Ch a + Ch b) (mg/g) [TCh]	1.28	0.02	0.41	0.45	2.47	2.03
Total carotenoids(x+c) (mg/g) [Tca]	0.30	0.01	0.13	0.02	0.68	0.66
Plant height (cm) [PH]	181	2.89	43.26	93.67	265	171.33
Leaf number (number) [LN]	20.4	0.34	4.75	6.50	32.50	26.00
Leaf area (cm ²) [LA]	73.60	1.68	24.79	12.70	124.08	111.38
Days to flowering [DF]	44.31	0.95	10.45	24.00	76.00	52.00
Days to maturity [DM]	95.01	1.31	14.40	80.50	153.50	73.00
Grain filling period [GFP]	50.71	1.09	11.98	25.00	95.00	70.00
Leaf Yield (t/ha) [LY]	9.39	0.24	2.87	3.56	20.30	16.74
Grain Yield(t/ha) [GY]	1.20	0.04	0.55	0.11	2.65	2.54
Internode length (cm) [INL]	12.54	0.22	2.91	6.89	22.47	15.58
Root weight (g) [RW]	133.91	4.80	68.99	30.25	305.00	274.75
Root length (cm) [RL]	43.64	0.65	8.76	28.25	66.00	37.75

Min. = Minimum; Max. = Maximum; Diff. = Difference between maximum and minimum values; SD = Standard deviation; SEM ($\pm$) = Standard error of the mean

5.3.3 Growth performance of amaranth species

5.3.3.1 Plant height per plant

After 30 DAS, the highest plant height mean was recorded in *A. palmeri* S. Wats (14.02 $\pm$ 0.8. cm), followed by *A. viridis* L. (13.54 $\pm$ 0.4cm), *A. hybridus* L. subsp. *cruentus* (L.) Theil (13.22 $\pm$ 0.4 cm); *A. spp.*(12.71 $\pm$ 0.7); *A. hybridus* L. subsp. *hybridus* (11.74 $\pm$ 0.4); *A. caudatus* L (10.98 $\pm$ 0.5); *A. spinosus* L was found to have the shortest plant height (6.17 $\pm$ 0.8cm). At 50 DAS, the plant height showed significant differences, with an overall average of 61.78 $\pm$ 1.4 cm for *A. hybridus* L., Subsp. *cruentus* (L.) Theil., *A. hybridus* L. subsp. *hybridus* with an average of 61.66 $\pm$ 1.6 cm, and *A. palmeri* S. Wats, with an overall average of 74 $\pm$ 2.5 cm. Similarly, the plant height for *A. viridis* L. is 50 DAS per plant, with a mean of 60.71 $\pm$ 2 for *A. caudatus* L. and an overall average of 57.01 $\pm$ 1.9 cm. The plant height for *A. spinosus* L. at 50 DAS has an overall average of 39.73 $\pm$ 1.48 cm. The plant height per plant for *A. spp.* has an average mean of 61.15 $\pm$ 3.7. After 70 DAS, the highest plant height mean was recorded in *A. palmeri* S. Wats (135.98 $\pm$ 5. cm), followed by *A. hybridus* L. subsp. *hybridus* (103.92 $\pm$ 1.5), *A. viridis* L. (101.63 $\pm$ 4 cm), *A. spinosus* L. (97.45 $\pm$ 2.45cm), *A. caudatus* L. (95.35 $\pm$ 4 cm), and *A.*

hybridus L. subsp. cruentus (L.) Theil (95.02 ± 1.9 cm). *A. species* was found to have the shortest plant height (87.03 ± 3.5 cm) (Figure 5.4A).

5.3.3.2 Stem Diameter per plant

Stem diameter per plant (cm) at 30 DAS had the thickest for *A. hybridus L. Subsp. cruentus* (L.) Theil. (5.01 ± 0.1 cm), followed by *A. spp* (4.90 ± 0.19 cm), *A. viridis* L. (4.78 ± 0.23 cm), *A. hybridus L. subsp. hybridus* (4.61 ± 0.1 cm), *A. caudatus* L. (4.53 ± 0.04 cm), and *A. palmeri* S. Wats (4.50 ± 0.12), respectively. *A. spinosus* L (2.22 ± 0.18 cm) had the thinnest stem diameter per plant. The range of stem diameters per plant at 50 DAS is 9.9 to 20.55 cm, with a mean of 19.18 cm. The *A. hybridus L. subsp. hybridus* was found to have the largest stem diameter per plant (cm), measuring 20.55 ± 0.3 cm, while *A. spinosus* L. had the smallest stem diameter per plant, measuring 9.9 ± 0.71 cm. The stem diameter ranged from 17.18 cm to 34.90 cm, with an average of 26.32 cm, at 70 DAS, showing notable variations. The maximum stem diameter was recorded in *A. hybridus L. subsp. hybridus* (29.29 ± 0.5 cm), however, it was found statistically at par with *Amarantus species viz. A.spp* (28.52 ± 1.14 cm), *A. caudatus* L. (27.75 ± 0.9 cm), *A. hybridus L. Subsp. cruentus* (L.) Theil. (27.01 ± 1.3 cm) and *A. viridis* L. (26.19 ± 1.3 cm), *A. palmeri* S. Wats (21.46 ± 0.9 cm), whereas, the lowest stem diameter was recorded in *A. spinosus* L (19.87 ± 1.4 cm) (Figure 5.4B).

5.3.3.3 Number of branches per plant

The number of branches per plant at 30 DAS ranged from 0.00 to 4.70 with an overall mean of 1.76. The highest number of branches per plant 4.7 ± 0.2 was counted in *A. viridis* L., followed by *A. palmeri* S. Wats (3.6 ± 0.5), *A. spinosus* L. (2.17 ± 0.2), *A. caudatus* L. (1.76 ± 0.2). *A. hybridus L. subsp. cruentus* (L.) Theil., *A. hybridus L. subsp. hybridus* and *A. sp* at 30 days do not bear braches. At 50 DAS the number of branches per plant showed significant differences and it varied from 9.39 ± 0.39 (*A spinosus* L.) to 19.20 ± 0.4 (*A. palmeri* S. Wats) with an overall average of 14.12. After 70 DAS, the recorded number of branches per plant ranged from 19.23 ± 1.7 (*A. spinosus* L) to 31.98 ± 2.7 (*A. palmeri* S. Wats) (Figure 5.4C).

5.3.3.4 Number of leaves per plant

At 30 DAS, there are between 9.74 and 35.43 leaves per plant, with a mean value of 15.68. *A. palmeri* S. Wats had the largest number of leaves per plant (35.43+1.9), while *A. hybridus* L. subsp. *hybridus* had the fewest (9.74+0.2) leaves per plant. At 50 DAS, there were substantial variations in the average number of leaves per plant, which ranged from 39.6- 101.9 (*A. hybridus* L. subsp. *hybridus*) with an overall average of 72.11+7.2, for *A. palmeri* S. Wats ranged from 289.5- 686.8 with an overall average of 392.00+24. After 70 DAS, the reported average leaf counts varied from 314.80+1 for *A. hybridus* L. subsp. *cruentus* (L.) Theil to 888.00+23 for *A. palmeri* S. Wats (Figure 5.4 D).

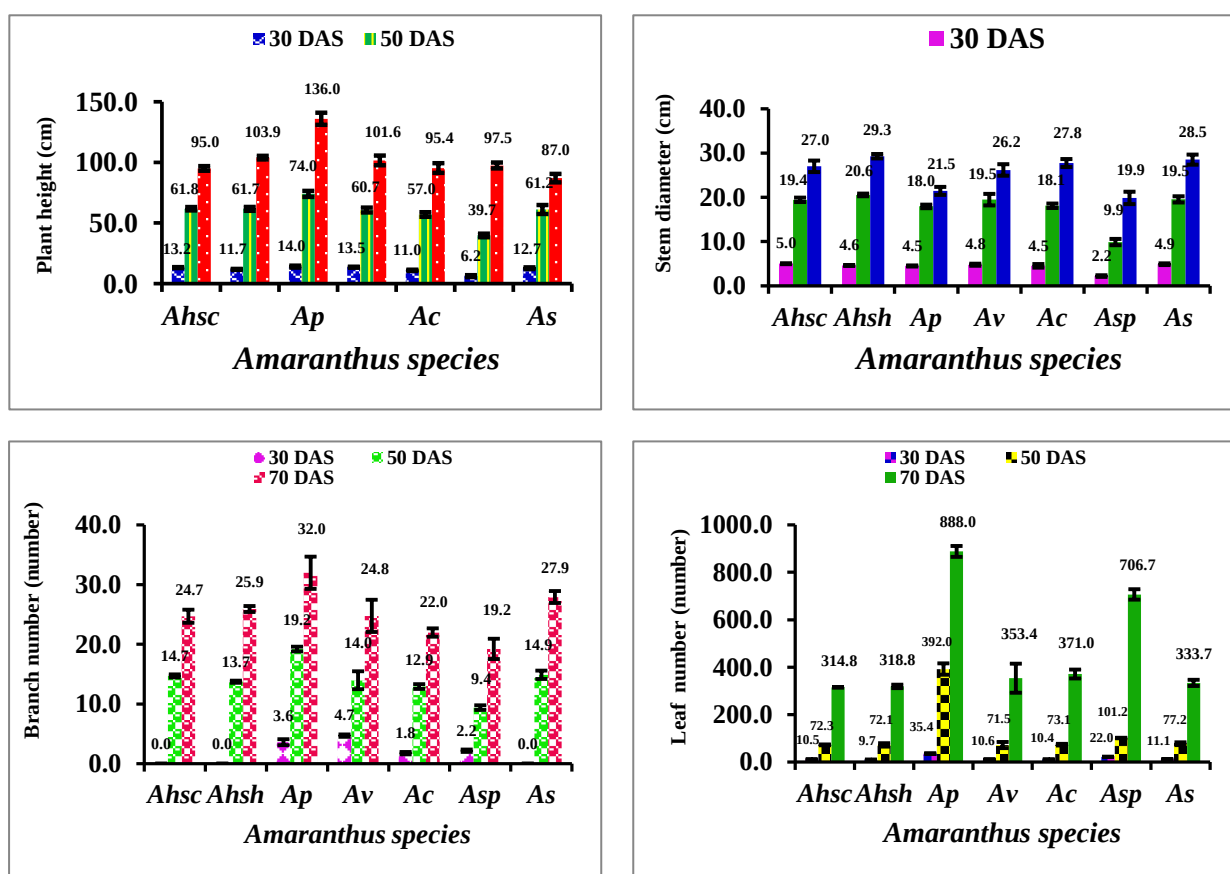


Figure 5.4 Mean performance of plant height (A); stem diameter (B); branch number (C), and leaf number (D) of different species of amaranths under different days after sowing (DAS).

5.3.4 Performance of Genotypes Analysis

5.3.4.1 Performance of top leaf yielder genotypes within species

Table 5.4 presents performance comparisons of the top four genotypes within each six-leaf yielder *Amaranthus species* vis-à-vis each other, the species mean, and the overall mean. Physio-morphological and photosynthetic pigment markers RH, Ci, gs, VPD, A, E, WUE, Cha, Chb, TCh, TCa, LA, PH, LN, LY, GY, INL, RW, and RL were studied on 120 genotypes of amaranth from six different *Amaranthus* species. For the characteristics Ci (mol mol^{-1}), LN (number), and Ch a (mg/g), the top leaf yielder genotype of *A. hybridus L. subsp. cruentus* (L.) Theil (KAZ-007) recorded the greatest value. The top leaf yielder genotypes from *A. hybridus L. subsp. hybridus* (225713) showed the highest value in the recorded features like RH (%), Ci ($\mu\text{mol mol}^{-1}$), and gs ($\text{mol m}^{-1}\text{s}^{-2}$). The top leaf yielder genotype (BA-001) from *A. palmeri* S. Wats showed the largest value for the traits such as WUE ($\text{mmol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$), Ch. a (mg/g), and Tch (mg/g). In the species *A. caudatus L* the top leaf yielder genotype (212890), the features WUE, Ch b, Tca, LA, and RL had the highest values, whilst the characteristics gs, A, E, and LN had the lowest values. The traits RH, and WUE showed the greatest values in the top leaf yielder genotype (KEN-019) from *A. viridis L*. Surprisingly, the top leaf yielder genotype (242534) for the *A. species* showed values for the markers A, E, gs, and WUE, Ch b, TCh, Tca, LA, PH, and LN were the highest.

5.3.4.2 Performance of leaf yielder among species

The results of the first top leaf yielder genotype from six species of amaranth are contrasted with the illustrated species means in Table 5.4. Among the six species of amaranth under study, *A. hybridus L. subsp. cruentus* (L.) Theil, one of the top amaranth species for leaf production, showed the greatest species mean value for the examined markers, such as LA, while its gs, A, and Ch b values were the lowest. The examined amaranth species *A. hybridus L. subsp. hybridus* showed the second greatest species mean values for the leaf yield and had the largest value for the features Ch a, TCh, Tca, GY, RW, and RL, however, the traits gs, A, and Ch b showed the least values. *A. caudatus L*. exhibited the third greatest leaf yielder species mean values had the highest value for the attributes RH, WUE, PH, LN, and INL, as well as the lowest values for VPD, Ch a, TCh, and TCa. The features gs, VPD, E, A, and Ch b showed

the greatest mean values in the unnamed species of amaranth, precisely as a species type *A. spp.*, while the reverse showed the lowest average species values in the traits WUE, LY, GY, and INL. In the *A. palmeri* S. Wats species, the features LA, RL, and RW have the lowest species mean values, whilst the characteristics Ci have the greatest mean values. Surprisingly, the species mean values for the markers RH, PH, and LN were lowest for *A. viridis* L. The other investigated attributes had values that were close to the species mean.

5.3.4.3 Leaf yield comparison of *Amaranthus* species with mean of population and released varieties

The results of each of the top six leaf-yielding *Amaranth* species genotypes are compared to the population mean; the species mean, the overall mean, and the mean of released variants, illustrated in Table 5.4. The results showed that in various measured parameters, such as LA, GY, RW, and INL, the genotypes of the top four leaves yielding species of *A. hybridus* L. *subsp. cruentus* (L.) Theil outperformed the population mean, the overall mean, and the mean of all varieties. The top leaf yielding *A. hybridus* L. *subsp. cruentus* (L.) genotypes, however, performed lower than the population mean and the released variety mean in RH, gs, VPD, A, E, WUE, Cha, Chb, TCh, Tca, PH, LN, and RL. The top four genotypes of the *A. hybridus* L. *subsp. hybridus* species to leaf yield outperformed the population, overall, and released varieties means for the majority of measured markers, including WUE, Ch a, Ch b, TCh, Tca, LA, PH, LY, GY, INL, RW, and RL, whereas CI, VPD, and E values underperformed the population, overall, and released varieties means. Similarly, when the performance of the top leaf yielder *Amaranthus* species, which is not identified as a specific species level genotype (not available), is compared to the overall mean, population mean, and mean of released varieties, the top leaf yielder *Amaranthus* species genotypes were higher in gs, VPD, A, E, Ch a, Ch b, TCh, Tca, PH, and LN than the overall mean, mean of population, and mean of released varieties. Most measured characters, including RH, Ci, gs, A, Cha, TCh, TCa, INL, and RW, were beneath in the top leaf yield *A. viridis* L. genotypes compared to the population mean, overall mean, and mean of released varieties, while the VPD value was lower than the population mean, overall mean, and mean of released varieties. In comparison to the overall mean, the population mean, and the released variety mean, the genotypes of *A. caudatus* L. were higher in RH, gs, A, WUE, PH, LN, and RW. The top leaf yielder genotypes of *A. caudatus* L, on the other hand, perform

lower than the overall, population mean, and mean of released variety in terms of VPD, Ch a, TCh, Tca, and GY.

5.3.4.4 Performance of top grain yielder genotypes within species

Table 5.5 compares the best four genotypes within each six-grain yielding *Amaranthus species* against one another, and the species mean in terms of performance. The physio-morphological markers RH, Ci, gs, VPD, A, E, WUE, Cha, Ch b, TCh, Tca, LN, LY, GY, INL, RW, and RL were evaluated on 120 genotypes of amaranth from six distinct *Amaranthus species*. The superior genotype for grain (242530) had the greatest values for the traits RH (%), gs ($\text{mmol m}^{-2} \text{s}^{-1}$), E ($\text{mol m}^{-2} \text{s}^{-1}$), Ch a (mg/g), Tca (mg/g), Ch b (mg/g), TCh (mg/g), PH (cm), GY (t/ha), INL (cm), and RL (cm) from *A.hybridus L. subsp. cruentus* (L.) Theil., whereas the remainder of the characteristics had intermediate values. The genotypes (ALE-073) for the characteristics Ci, VPD, E, and LN had the highest values in comparison to other genotypes among the top grain yielder, *A. hybridus L. subsp. hybridus*. Furthermore, in terms of Ci, VPD, E, LN, and GY the highest grain yielder genotypes (ALE-073) from the species *A. hybridus L. subsp. hybridus* exhibited high yield advantages of 22.40, 22.97, 42.16, 6.97, and 14.72%, respectively. The highest grain-yielding genotypes of *A. palmeri* S. Wats were SA-025/07 had revealed the highest value for the markers VPD, E, Tca, Ch a, Ch b, and TCh. Among the top grain yielders from *A. caudatus* L, species the genotypes KAZ-060 and KEN-014 had the highest values for the traits RH, Ci, and Tca. Moreover, *A. caudatus* L. genotypes with the highest grain yields (KEN-014) demonstrated substantial benefits above the species mean grain yield in terms of gs, E, Ch b, TCh, and GY of 24.22, 45.78, 153.33, and 36.76%, respectively. With regards to the selected genotypes, the best grain-producing genotype (204644) in *Amaranthus species* showed greater had the highest values for the traits Ci, Ch a, and INL. According to the findings, the genotypes from the species *Amaranthus species* selected for having the maximum grain production the genotypes (20464) had the highest values for Ci and Ch a. The top grain yielder genotypes (KAZ-059) for the traits Ci, gs, E, Tca, and RW performed the highest in comparison to other genotypes within the *A. viridis* L.

5.3.4.5 Performance of top grain yielder among species

Table 5.5 compares the findings of the top six species of grain-producing amaranth with the indicated species means. *A. hybridus L. subsp. hybridus* showed the first highest species mean values for grain yield among the six studied species had the highest value for the traits WUE, Ch a, TCh, Tca, LA, PH, LY, and RW, but the lowest values for the trait E. *A. hybridus L. subsp. cruentus* Theil (L.), one of the second top grain yielder *Amaranthus species*, displayed the highest species mean value for the markers that were investigated, such as RL. The third top grain yielder species (*A. caudatus* L) displayed the highest value for the variables RH, Ci, and INL, on the other hand, for the traits VPD, A, Ch a, Ch b, TCh, and Tca revealed the lowest values. The lowest grain yield was recorded for the species (*Amaranthus species*) had the lowest mean value for the attributes Ch b, LY, and RW, whereas the traits gs, A, E, and LN had the highest value. The species mean values for the marker VPD were notably the greatest for *A. viridis* L. However among the investigated amaranth species, the LN on the main stem was the lowest.

5.3.4.6 Grain yield comparison of *Amaranthus* species with mean of population and released varieties

Comparisons of the genotypes for each of the top six grain-yielding *Amaranth species*, as well as the population mean, species mean, overall mean, and mean of released variants, are shown in Table 5.5. The results showed that the genotype means of the top four grain-yielding genotypes of *A. hybridus L. subsp. cruentus* (L.) Theil was significantly different in a number of the measured parameters, such as E, LA, LN, LY, GY, RW, and RL outperformed the population means, the overall mean, and released varieties means. However, in WUE, Ch a, and TCh, the highest grain-yielding genotypes of *A. hybridus L. subsp. cruentus* (L.) fared inferior to the population mean, overall mean and the released variety mean. For the measured markers VPD, WUE, Ch a, TCh, Tca, LA, PH, LY, GY, and RW, the mean of the top four-grain yielder genotypes of the *A. hybridus L. subsp. hybridus* species outperformed the population, overall, and released varieties means, whereas RH, CI, gs, and E values underperformed these means. The initial genotype of *A. palmeri* S. Wats that produced the top four grain yields had higher values for Ch a, Ch b, TCh, and Tca traits than the population mean, overall mean, and mean of the newly introduced variety. On the other hand, the genotype of *A. palmeri* S. Wats displayed lower values for characteristics than the population mean,

overall mean, and mean of all varieties, notably for the majority of agro-morphological traits including LA, INL, RW, and RL. The highest grain yielder *Amaranthus species* genotypes were higher in gs, VPD, A, E, Ch b, TCh, Tca, PH, and LN than the overall mean, mean of population, and mean of released varieties. This is similar to the performance of the top grain-yielding *Amaranthus species*, which has not been recognized as a specific species-level genotype. As opposed to the average, the mean of the population, and the mean of the released variety, the top grain yielder genotypes fared poorer in Ci, LY, GY, INL, and RL. When compared to the population mean, overall mean, and mean of released varieties, the measured characters—including VPD, WUE, and LY—were below the top grain yield *A. viridis* L. genotypes, while the RH, Ci, E, TCh, Tca, and RW values were lower than these averages. The genotypes of *A. caudatus* L. have greater RH and GY values compared to the genotype mean, population mean, and released variety mean. On the other hand, in terms of Ch a, Ch b, TCh, Tca, PH, LY, RW, and RL, the top grain yielder genotypes of *A. caudatus* L performed lower than the average population mean, and the mean of all varieties. The remaining investigated traits, however, showed intermediate values.

Table 5.4 The mean of selected, population mean, released variety mean, and overall mean for 25 physiological and morphological markers of top leaf performer selected genotypes from each *Amaranth species*

<i>Amaranthus species</i>	genotype s code	Physiological traits													Agro-morphological traits										
		RH	Ci	Gs	VP D	A	E	WU E	Ch a	Ch b	TCh	Ch a/b	Tc h/T ca	TC a	LA	PH	LN	LY	GY	INL	RW	RL	DF	DM	GFP
<i>Ahsc</i>	KAZ-007	53.8	212.0	112.5	2.1	6.1	2.9	2.1	1.0	0.2	1.2	4.5	4.9	0.3	91.6	183.5	23.0	20.3	1.7	12.2	94.8	40.0	43.5	92.5	49.0
	208025	62.5	173.5	146.0	2.1	10.7	3.6	2.6	0.7	0.2	0.9	2.7	7.8	0.1	101.2	186.3	18.0	15.8	1.3	13.3	104.0	38.3	60.5	104.0	43.5
	ALE-070	59.6	84.5	116.0	2.2	13.7	3.1	4.5	0.7	0.2	0.9	3.1	3.7	0.2	106.9	165.5	21.0	14.8	2.0	10.3	205.3	45.3	56.0	81.0	25.0
	ALE-068	48.1	67.5	100.5	3.0	12.2	3.5	3.3	0.5	0.7	1.2	0.8	3.4	0.4	124.1	150.0	13.5	14.6	1.2	12.4	196.5	40.8	43.5	81.5	38.0
	SM	56.0	134.4	118.8	2.4	10.7	3.3	3.1	0.7	0.3	1.0	2.8	5.0	0.2	105.9	171.3	18.9	16.4	1.5	12.0	150.1	41.1	50.9	89.8	38.9
<i>Ahsh</i>	225713	66.2	134.0	177.5	1.5	16.4	3.1	5.3	1.1	0.4	1.5	2.8	4.5	0.3	101.6	230.7	20.0	16.6	1.4	14.9	266.5	44.3	42.0	110.0	68.0
	212582	58.2	48.5	91.0	2.2	14.0	2.5	5.7	1.2	0.3	1.5	3.8	6.2	0.3	106.4	177.2	20.0	15.6	2.4	11.7	305.0	52.0	71.5	147.5	76.0
	ABL-004	58.2	120.5	74.0	2.2	8.1	1.9	4.2	1.3	0.5	1.8	2.9	2.7	0.7	97.2	238.5	21.0	14.9	2.1	15.8	191.8	50.3	55.5	97.0	41.5
	212581	53.3	120.0	172.5	2.5	15.1	4.9	2.9	0.8	0.7	1.5	1.1	3.5	0.4	97.1	173.8	24.0	14.1	1.1	8.2	155.0	42.8	43.0	92.0	49.0
	SM	59.0	105.8	128.8	2.1	13.4	3.1	4.5	1.1	0.5	1.6	2.6	4.2	0.4	100.6	205.1	21.3	15.3	1.7	12.7	229.6	47.3	53.0	111.6	58.6
<i>AP</i>	BA-001	64.0	82.5	98.0	2.1	12.7	2.4	5.1	1.3	0.5	1.8	2.9	6.0	0.3	22.4	153.7	14.0	12.1	0.3	12.7	99.5	33.0	35.0	105.5	70.5
	SA-008/07	57.3	210.0	238.0	2.6	10.7	7.3	1.4	0.9	0.9	1.8	1.0	3.9	0.5	25.4	189.7	21.5	11.3	0.4	12.6	41.3	33.5	38.0	108.0	70.0
	WA-001	58.6	202.0	89.0	2.0	7.5	2.1	2.8	0.9	0.4	1.4	2.5	5.0	0.3	22.6	186.5	19.0	10.4	1.7	10.2	78.0	37.3	26.0	104.0	78.0
	DA-006	56.9	70.0	255.0	2.4	26.8	7.1	3.8	0.6	0.3	0.9	1.7	3.2	0.3	23.2	174.0	24.0	10.2	0.5	11.0	63.8	35.3	36.0	100.5	64.5
	SM	59.2	141.1	170.0	2.3	14.4	4.7	3.3	0.9	0.5	1.5	2.0	4.5	0.3	23.4	176.0	19.6	11.0	0.8	11.6	70.6	34.8	33.8	104.5	70.8
<i>As</i>	242531	51.4	74.5	232.5	2.6	24.3	7.1	3.8	1.0	0.7	1.7	1.5	5.3	0.3	66.6	221.3	30.0	7.9	0.5	8.9	133.4	33.3	45.0	126.5	81.5
	242534	58.5	73.0	267.0	2.7	23.9	7.6	3.5	0.8	0.7	1.5	1.2	4.2	0.4	82.4	189.5	32.5	8.4	0.1	10.6	173.2	46.3	60.0	144.5	84.5
	204644	56.7	182.5	262.0	2.1	12.0	5.0	2.2	1.0	0.3	1.3	3.8	5.7	0.2	76.2	133.2	22.0	6.3	1.4	11.3	70.8	37.5	44.0	87.5	43.5
	241764	53.4	104.5	174.0	2.8	16.2	5.9	2.7	0.5	0.7	1.1	0.7	3.5	0.3	73.4	183.2	23.0	6.8	0.3	12.0	152.0	40.5	55.0	108.5	53.5
	SM	55.0	108.6	233.9	2.6	19.1	6.4	3.0	0.8	0.6	1.4	1.8	4.7	0.3	74.7	181.8	26.9	7.3	0.6	10.7	132.4	39.4	51.0	116.8	65.8
<i>Av</i>	KEN-019	56.1	138.0	98.5	1.8	9.9	2.0	5.0	0.6	0.2	0.8	2.7	5.0	0.2	75.7	156.3	17.0	12.4	1.0	11.2	92.3	40.8	42.5	91.5	49.0
	KEN-021	51.8	51.0	140.5	2.9	18.0	4.9	3.7	0.9	0.3	1.2	3.1	5.4	0.2	72.3	171.5	24.0	10.3	1.1	12.6	80.3	57.5	41.0	81.5	40.5
	KAZ-059	48.0	185.5	224.0	2.6	10.1	6.2	1.6	0.6	0.6	1.2	1.0	3.6	0.3	66.7	164.3	17.5	10.2	1.9	10.5	112.3	43.5	50.0	82.5	32.5
	KAZ-057	50.8	102.5	109.0	3.1	11.9	4.0	2.9	0.7	0.6	1.3	1.2	4.2	0.3	80.4	163.2	15.0	7.3	1.5	12.7	107.3	38.0	44.0	87.5	43.5
	SM	51.7	119.3	143.0	2.6	12.5	4.3	3.3	0.7	0.4	1.1	1.8	4.6	0.3	73.8	163.8	18.4	10.1	1.4	11.7	98.0	44.9	44.4	85.8	41.4
<i>AC</i>	212890	62.0	109.5	69.5	1.7	10.3	1.5	6.8	0.3	0.6	0.9	0.5	3.4	0.3	99.4	246.7	21.0	17.3	0.9	10.1	158.5	51.3	49.5	108.0	58.5
	CHU-045	60.8	75.0	298.0	2.4	27.8	8.1	3.5	0.6	0.3	0.8	2.2	3.3	0.3	54.6	256.2	32.5	10.4	0.5	13.3	200.8	44.8	63.5	120.0	56.5
	KEN-018	81.7	185.5	162.5	1.2	13.4	2.2	6.0	0.3	0.2	0.5	2.2	6.2	0.1	70.4	221.5	29.5	10.1	1.7	14.6	141.0	45.5	50.0	104.0	54.0
	215567	77.9	183.0	159.5	1.3	14.2	2.4	5.9	0.5	0.5	1.0	1.0	9.0	0.0	81.7	236.3	28.0	10.0	0.3	14.8	175.0	35.5	52.5	116.5	64.0
	SM	70.6	138.3	172.4	1.7	16.4	3.6	5.6	0.4	0.4	0.8	1.5	5.5	0.2	76.5	240.2	27.8	11.9	0.8	13.2	168.8	44.3	53.9	112.1	58.3
<i>PM</i>	OAM	58.6	124.6	161.1	2.3	14.4	4.2	3.8	0.8	0.5	1.2	2.2	4.8	0.3	76.2	189.7	22.1	12.0	1.1	12.0	141.6	42.0	47.8	103.4	55.6
	PM	56.6	134.5	164.9	2.4	13.4	4.5	3.2	0.8	0.5	1.3	2.0	4.8	0.3	73.6	181.0	20.4	9.4	1.2	12.5	133.9	43.6	44.3	95.0	50.7
	RVM	54.1	212.3	116.0	2.5	6.0	3.5	1.7	1.0	0.3	1.3	3.0	5.5	0.3	79.9	160.6	17.5	9.9	1.7	11.8	134.3	47.9	41.8	83.5	41.8

RH = Relative Humidity inside leaf chamber; Ci = CO₂ concentration of Sub-stomatal CO₂ concentration; gs = Stomata conductance; VPD = leaf to air vapour pressure deficit; A= Rate of net photosynthesis; E= Transpiration rate; WUE = Photosynthetic water use efficiency; Ch a = Chlorophylls a; Ch b = Chlorophyll b ; TCh= Total chlorophyll; Ca = ambient CO₂ concentrations; Tca= total carotenoids; PH = Plant height; LN= Leaf number; LA= Leaf area; LY= Leaf Yield; GY= Grain Yield; INL = Internode length; RW= Root weight; RL= Root length; GFP= Grain filling period ; DF=, Days to flowering; DM= Days to maturity

Table 5.5 The mean of the selected, population mean, released variety mean an overall mean for 25 physiological and photosynthetic pigment markers of top grain performer selected genotypes from each Amaranth species

Species	Genotypes	Physiological traits														Agro morphological traits										
		RH	Ci	Gs	VPD	A	E	WUE	Cha	Chb	TCh	TCa	Ch a/b	Tch/TCa	Ci/Ca	LA	PH	LN	LY	GY	INL	RW	RL	DF	DM	GFP
Ahsc	225717	52.54	98	145	2.66	14.7	4.41	3.17	0.5	0.15	0.65	0.17	0.65	3.73	0.36	75.73	149	26	8.98	2.17	10.36	48.25	35.75	35	82	47
	242530	61.78	171	237.5	2.54	12	6.88	1.85	0.72	1.1	1.82	0.49	3.4	4.13	0.6	96.74	221.67	17	13.79	2.42	15.15	165	59	49.5	98	48.5
	AC-NL	49.52	223.5	132	2.5	6.2	3.89	1.36	0.7	0.25	0.95	0.15	2.81	6.38	0.74	82.98	155.84	19	9.51	2.04	10.51	157.5	56	49	86	37
	ALE-070	59.59	84.5	116	2.23	13.7	3.12	4.53	0.66	0.22	0.88	0.24	3.07	3.7	0.29	106.88	165.5	21	14.77	1.96	10.31	205.25	45.25	56	81	25
	SM	55.85	144.25	157.63	2.48	11.65	4.57	2.73	0.64	0.43	1.07	0.26	2.48	4.48	0.5	90.58	173	20.75	11.76	2.15	11.58	144	49	47.38	86.75	39.38
Ahsh	ALE-073	46.19	147.5	124.5	3.16	9.6	4.79	1.98	0.6	0.34	0.94	0.28	1.78	3.41	0.51	73.2	167.17	21	9.95	2.65	10.27	87	40.25	34.5	81.5	47
	212582	58.15	48.5	91	2.22	13.95	2.46	5.67	1.18	0.31	1.49	0.25	3.79	6.21	0.16	106.39	177.17	20	15.58	2.37	11.71	305	52	71.5	147.5	46
	242533	51.29	87	144.5	2.75	16.35	4.3	3.35	1.02	0.74	1.76	0.47	1.38	3.77	0.31	86.58	156.67	16.5	9.48	2.14	10.72	123.75	38.75	42.5	87	44.5
	ABL-004	58.24	120.5	74	2.15	8.05	1.94	4.21	1.33	0.47	1.79	0.68	2.85	2.65	0.39	97.24	238.5	21	14.85	2.08	15.77	191.75	50.25	55.5	97	41.5
	SM	53.47	100.88	108.5	2.57	11.99	3.37	3.8	1.03	0.46	1.49	0.42	2.45	4.01	0.34	90.85	184.88	19.63	12.46	2.31	12.12	176.88	45.31	51	103.25	52.25
Ap	SAA-004	63.4	151.5	260	1.96	17.7	4.66	3.95	0.73	0.7	1.43	0.35	0.93	4.14	0.48	16.67	180.67	18	9	1.16	10.63	81	41.25	42	99	57
	SA-005/07	47.89	104	76	2.53	8.6	2.4	3.55	0.54	0.21	0.75	0.11	2.47	5.05	0.36	15.1	187.5	22	9.67	1.5	12.5	95.5	39.8	42	94.5	52.5
	SA-025/07	47.57	127.5	167.5	3.14	14.65	5.95	2.29	1.1	1.19	2.29	0.56	2.67	7.28	0.45	21.27	156.67	16	9.78	1.75	9.87	59.5	29.75	36	104	68
	WA-001	58.62	202	89	2.02	7.5	2.06	2.8	0.94	0.42	1.36	0.27	1.04	4.12	0.63	22.55	186.5	19	10.36	1.67	10.21	78	37.25	26	104	78
	SM	54.37	146.25	148.13	2.41	12.11	3.77	3.14	0.83	0.63	1.46	0.32	1.78	5.15	0.48	18.9	177.83	18.75	9.7	1.52	10.8	78.5	37.01	36.5	100.38	63.88
As	204644	56.66	182.5	262	2.14	12	5.02	2.15	1.04	0.28	1.32	0.24	3.75	5.68	0.65	76.22	133.17	22	6.8	1.42	11.33	70.75	37.5	87.5	44	43.5
	241764	53.35	104.5	174	2.83	16.15	5.86	2.68	0.47	0.65	1.13	0.32	0.73	3.52	0.38	73.42	183.17	23	6.29	0.3	11.95	152	40.5	108.5	55	53.5
	242531	51.42	74.5	232.5	2.64	24.25	7.07	3.78	1	0.67	1.67	0.32	1.5	5.26	0.28	66.6	221.33	30	7.9	0.46	8.85	133.43	33.25	126.5	45	81..50
	242534	58.46	73	267	2.66	23.9	7.57	3.46	0.8	0.67	1.47	0.35	1.2	4.17	0.28	82.4	189.5	32.5	8.38	0.13	10.55	173.23	46.25	144.5	60	84.5
	SM	54.97	108.63	233.88	2.57	19.08	6.38	3.02	0.83	0.57	1.4	0.31	1.79	4.66	0.4	74.66	181.79	26.88	7.34	0.57	10.67	132.35	39.38	116.8	51	65.8
Av	KAZ-057	50.83	102.5	109	3.12	11.9	3.97	2.88	0.7	0.57	1.26	0.3	1.23	4.2	0.35	80.39	163.17	15	7.32	1.53	12.65	107.25	38	44	87.5	43.5
	KAZ-059	47.99	185.5	224	2.59	10.1	6.17	1.64	0.59	0.57	1.16	0.33	1.03	3.6	0.66	66.73	164.34	17.5	10.2	1.9	10.45	112.25	43.5	50	82.5	32.5
	KEN-019	56.07	138	98.5	1.77	9.85	1.98	4.97	0.56	0.21	0.78	0.16	2.68	5.03	0.44	75.66	156.34	17	12.44	0.97	11.22	92.25	40.75	42.5	91.5	49.5
	KEN-021	51.76	51	140.5	2.88	18	4.87	3.65	0.89	0.29	1.18	0.22	3.1	5.4	0.19	72.33	171.5	24	10.26	1.12	12.55	80.25	57.5	41	81.5	40.5
	SM	51.66	119.25	143	2.59	12.46	4.25	3.28	0.68	0.41	1.09	0.25	2.01	4.56	0.41	73.78	163.83	18.38	10.05	1.38	11.71	98	44.94	44.38	85.75	41.38
Ac.	208764	40.84	177	80	2.93	5.15	2.81	1.83	0.67	-0.22	0.45	-0.02	3.05	3.87	0.6	75.25	177.84	18.5	9.08	1.55	12.04	53.75	36.25	33.5	87.5	54
	KAZ-060	59.18	208	156	2.14	6.1	3.67	2.18	0.65	0.22	0.88	0.22	2.95	3.98	0.65	62.01	142.84	14.5	7.11	1.99	11.02	30.25	41.25	26	81	55
	KEN-014	50.14	173	179.5	2.33	13	5	2.29	0.55	0.38	0.93	0.1	1.43	9.98	0.59	72.08	160.84	16.5	8.34	1.99	12.46	56.25	43.25	32.5	81	48.5
	KEN-018	81.71	185.5	162.5	1.19	13.35	2.24	6.04	0.34	0.15	0.49	0.08	2.24	6.22	0.55	70.38	221.5	29.5	10.06	1.69	14.63	141	45.5	50	104	54
	SM	57.97	185.88	144.5	2.15	9.4	3.43	3.08	0.55	0.13	0.68	0.1	2.42	6.01	0.59	69.93	175.75	19.75	8.65	1.8	12.53	70.31	41.56	35.5	88.38	52.38
PM	OAM	54.6	132.41	156.33	2.47	12.9	4.32	3.18	0.77	0.45	1.22	0.28	2.16	4.81	0.45	69.78	176.2	20.72	10.04	1.61	11.54	118.27	42.91	55.25	85.92	50.04
	PM	56.55	134.53	164.88	2.4	13.36	4.5	3.24	0.79	0.47	1.28	0.3	1.96	4.78	0.45	73.6	181	20.4	9.39	1.2	12.54	133.94	43.64	44.31	95.01	50.7
	RVM	54.06	212.25	116	2.5	6.03	3.45	1.65	0.97	0.33	1.3	0.26	2.98	5.48	0.69	79.93	160.58	17.5	9.85	1.72	11.78	134.25	47.88	41.75	83.5	41.75

RH = Relative Humidity inside leaf chamber; Ci = CO₂ concentration of Sub-stomatal CO₂ concentration; gs = Stomata conductance; VPD = leaf to air vapour pressure deficit; A= Rate of net photosynthesis; E= Transpiration rate; WUE = Photosynthetic water use efficiency; Ch a = Chlorophylls a; Ch b = Chlorophyll b ; TCh= Total chlorophyll; Ca = ambient CO₂ concentrations; Tca= total carotenoids; PH = Plant height; LN= Leaf number; LA= Leaf area; LY= Leaf Yield; GY= Grain Yield; INL = Internode length; RW= Root weight; RL= Root length; GFP= Grain filling period ; DF=, Days to flowering; DM= Days to maturity

Table 5.6 Comparison of mean performances of selected top 5% genotypes with the population mean performances for 25 physio-morphological traits of amaranth.

Traits	The mean of selected genotypes ($\bar{X}$)	Population parameter (μ)	Change through selection ($\bar{X}-\mu$)	Change as % of a population parameter (μ)	T-test
RH	77.87	56.55	21.32	37.69	11.43***
Ci	269.00	134.53	134.47	99.96	4.32***
Gs	365.83	164.88	200.95	121.88	6.48***
VPD	3.12	2.4	0.72	29.79	27.00***
A	26.23	13.36	12.87	96.29	23.76***
E	8.83	4.5	4.33	96.02	12.06***
WUE	6.37	3.24	3.13	96.73	9.91***
Ci/Ca	0.83	0.45	0.38	84.44	4.62**
Ch a/Ch b	4.40	1.96	2.44	124.49	5.34**
TCh/Tca	10.22	4.78	5.44	113.81	9.41***
Ch a	1.38	0.8	0.58	72.81	17.00***
Ch b	1.11	0.47	0.64	135.28	21.04***
TCh	2.26	1.28	0.98	76.30	12.98***
Tca	0.60	0.3	0.29	95.56	11.62***
PH	255.00	181	74.00	40.88	27.60***
LN	31.50	20.4	11.10	54.41	19.73***
LA	111.24	73.6	37.64	51.14	13.06***
DF	66.42	44.31	22.11	49.90	8.50***
DM	137.60	95.01	42.59	44.83	8.46***
GFP	83.58	50.71	32.87	64.82	11.80***
LY	16.73	9.39	7.34	78.15	9.28***
GY	2.30	1.2	1.10	91.94	11.81***
INL	18.93	12.54	6.39	50.98	8.50***
RW	280.29	133.91	146.38	109.31	19.08***
RL	62.16	43.64	18.52	42.00	15.90***

T = Student t-test; *** = significantly different at 0.1% probability level; RH = Relative Humidity inside leaf chamber; Ci = CO₂ concentration of Sub-stomatal CO₂ concentration; gs = Stomata conductance; VPD = leaf to air vapour pressure deficit; A= Rate of net photosynthesis; E= Transpiration rate; WUE = Photosynthetic water use efficiency; Ch a = Chlorophylls a; Ch b = Chlorophyll b ; Tch= Total chlorophyll = Tca= Total carotenoids; Ca = ambient CO₂ concentrations ; PH = Plant height; LN= Leaf number; LA= Leaf area; LY= Leaf Yield; GY= Grain Yield; INL = Internode length; RW= Root weight; RL= Root length; GFP= Grain filling period ; DF=, Days to flowering; DM.= Days to maturity

The student t-test performed between the mean of the top 5% genotypes and the mean of the population was varied ($P < 0.0001$) for all measured physio-agro-morphological marker traits (Table 5.6). The result indicated that the top 5% of genotypes had higher relative advantages in all phenotypic traits, which ranged from 29.79% for leaf-to-air vapor pressure deficit to 135.28% for chlorophyll b compared to the mean performances of the population. Furthermore, the CO₂ concentration of sub-stomata CO₂ concentration, stomata conductance, rate of net photosynthesis, transpiration rate, photosynthetic water use efficiency, chlorophyll a, carotenoids, grain yield, and leaf yields of the top 5% genotypes had advantages of 99.96, 121.88, 96.29, 96.3, 72.81, 95.56, and 91.94% over the mean performance of population, respectively, indicating the presence of different level of amaranth improvements through selection.

5.3.5 Variance component and coefficient of variation

The variance components viz., environmental variance, phenotypic, and genotypic variance for measured markers in amaranth genotypes are indicated in Table 5.7. Despite excluding chlorophyll a, b, total chlorophyll, and carotenoids, the results demonstrated that phenotypic variance was larger than the corresponding genotypic variance for all examined attributes. For all examined phenotypic characteristics, except for net photosynthesis rate, photosynthetic water use efficiency, and leaf yield, the phenotypic variance was likewise greater than the corresponding environmental variance. Contrarily, the phenotypic variance was less than the corresponding environmental variance in all measured phenotypic traits except the relative humidity inside the leaf chamber, transpiration rate, leaf area, plant height, internode length, leaf number, root length, root weight, and grain yield. Similarly, the genotypic variance was higher than the corresponding environmental variance in leaf area, plant height, internode length, leaf number, and root weight.

In Table 5.7, the genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and environmental coefficient of variation (ECV) for the examined characteristics in amaranth genotypes are listed. The GCV values ranged from 8.79% for relative humidity inside the leaf chamber to 54.24% for chlorophylls b, and they were higher than the corresponding ECV values for a leaf-to-air vapor pressure deficit, internode length, plant height at grain harvest, leaf number, total chlorophyll, leaf area, chlorophylls a, carotenoids, root weight, and chlorophylls b. Except for chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, which had identical PCV and GCV values, the PCV levels were often greater than the equivalent GCV values for all assessed characteristics. Chlorophylls b had the highest PCV value, followed by root weight, grain yield, sub-stomata CO₂ concentration, net photosynthesis rate, stomata conductance, carotenoids, photosynthetic water use efficiency, transpiration rate, chlorophylls a, leaf area, total chlorophyll, dry leaf yield, plant height at maturity, leaf number, internode length, and root length. All evaluated physio-morphological variables, except for the rate of CO₂ concentration of sub-stomata CO₂ concentration, net photosynthesis, stomata conductance, and photosynthetic water use efficiency, showed PCV values that were greater than the comparable ECV values. Chlorophyll b, carotenoids, chlorophyll a, and total chlorophyll had ECV values as low as 0.00% and as high as 54.97% per plant for stomata conductance.

5.3.6 Heritability and genetic advance

The heritability of measured physio-morphological markers in amaranth genotypes is presented in Table 5.7. The values of heritability ranged from 12.24% (stomata conductance) to 100% for all measured photosynthetic pigment traits (chlorophyll b, carotenoids, chlorophyll a, and total chlorophyll). The result revealed that very high heritability (>80%) values were recorded for leaf number, root weight, leaf area, plant height at maturity, chlorophyll b, total carotenoid, chlorophyll a, and total chlorophyll. The traits that showed moderately high heritability (60–79%) values were transpiration rate (63.39%), root length (66.40%), internode length (67.56%), and leaf-to-air vapor pressure deficit (69.44%). The features of photosynthetic water use efficiency, dry leaf yield, relative humidity inside the leaf chamber, and grain yield had values of medium heritability (40–59%). The remaining eight traits had low heritability values.

The genetic advance (GA) for measured physio-morphological traits in amaranth genotypes is highly variable, as indicated in Table 5.7. The values of GA ranged from 0.25 to 117.80% for measured physio-morphological markers. The highest GA (>20%) value was recorded for sub-stomata CO₂ concentration (42.04%), leaf area (46.10%), plant height at maturity (80.38%), and root weight (117.80%). Likewise, medium GA (10–20%) values were recorded for root length (11.90%) and stomata conductance (17.26%). The remaining thirteen physio-morphological markers had low GA (<10%).

In Table 5.7, the genetic advance expressed as a percentage of means (GAM) for the assessed physio-morphological features in the genotypes of amaranth is also shown. The values of high GAM (>20%) were recorded for dry leaf yield (23.66%), leaf to air vapor pressure deficit (25.29%), root length (27.27%), internode length (31.03%), the CO₂ concentration of sub-stomata CO₂ concentration (31.25%), photosynthetic water use efficiency (33.55%), leaf number (36.66%), plant height at maturity (44.41%), transpiration rate (49.37%), grain yield (53.53%), leaf area (62.64%), total chlorophyll (63.36%), chlorophylls a (2.83%), carotenoids (84.10%), root weight (87.97%), and chlorophylls b (111.4%). Similarly, medium GAM (10–20%) values were recorded for stomata conductance (10.47%), relative humidity inside the leaf chamber (12.94%), and rate of net photosynthesis (18.63%). For the analyzed markers, the lowest GAM value wasn't disclosed.

Table 5.7 Components of variance, coefficient of variation, heritability, genetic advance, and genetic advance as percent for 25 traits of physio-morphological of 120 amaranth genotypes.

Traits	Mean	σ^2_e	σ^2_r	σ^2_g	σ^2_p	GCV	PCV	ECV	h^2_{bs}	GA	GAM
RH	56.55	47.45	-0.19	24.73	48.46	8.79	12.31	12.18	51.04	7.32	12.94
Ci	134.53	4476.11	-34.46	1195.92	3433.97	25.71	43.56	49.73	34.83	42.04	31.25
Gs	164.88	8214.48	-65.88	573.05	4680.29	14.52	41.49	54.97	12.24	17.26	10.47
VPD	2.4	0.11	0.00	0.13	0.18	14.73	17.68	13.82	69.44	0.61	25.29
A	13.36	50.27	-0.12	6.83	31.97	20.00	42.32	53.07	21.37	2.49	18.63
E	4.5	2.12	0.00	1.84	2.90	30.10	37.81	32.36	63.39	2.22	49.37
WUE	3.24	2.00	-0.02	0.69	1.69	25.54	40.06	43.65	40.65	1.09	33.55
Ci/Ca	0.45	0.04	0.002	0.06	0.03	23.43	39.22	44.47	35.70	0.13	28.84
Ch a/Ch b	1.96	0.08	0.47	2.66	1.33	57.93	58.79	14.17	97.10	2.31	117.59
TCh/Tca	4.78	0.53	6.07	5.21	2.60	31.97	33.74	15.24	89.80	2.99	62.42
Ch a	0.8	0.00	0.00	0.08	0.08	35.36	35.36	0.00	100.00	0.58	72.83
Ch b	0.47	0.00	0.00	0.07	0.07	54.24	54.24	0.00	100.00	0.53	111.74
TCh	1.28	0.00	0.00	0.16	0.16	30.76	30.76	0.00	100.00	0.81	63.36
Tca	0.3	0.00	0.00	0.02	0.02	40.82	40.82	0.00	100.00	0.25	84.10
LA	73.6	87.28	-0.73	541.20	584.84	31.61	32.86	12.69	92.54	46.10	62.64
PH	181	248.2	-1.80	1638.01	1762.11	22.36	23.19	8.70	92.96	80.38	44.41
LN	20.4	7.61	2.46	17.02	20.82	20.22	22.37	13.52	81.72	7.68	37.66
INL	12.54	5.07	0.42	5.28	7.82	18.32	22.29	17.96	67.56	3.89	31.03
RW	133.91	1364.07	282.45	3849.22	4531.26	46.33	50.27	27.58	84.95	117.80	87.97
RL	43.64	50.85	-0.13	50.26	75.68	16.24	19.93	16.34	66.40	11.90	27.27
DF	44.31	29.45	114.69	91.30	106.02	21.56	23.24	12.25	86.11	18.27	41.22
DM	95.01	32.76	12.42	165.80	182.018	13.55	14.21	6.02	91.01	25.30	26.63
GFP	50.70	50.93	51.63	103.60	129.07	20.08	22.41	14.08	80.27	18.79	37.05
LY	9.39	7.92	2.67	2.81	6.77	17.84	27.70	29.97	41.46	2.22	23.66
GY	1.2	0.23	0.01	0.17	0.28	33.85	44.10	39.97	58.93	0.64	53.53

RH = Relative Humidity inside leaf chamber; Ci = CO₂ concentration of Sub-stomatal CO₂ concentration; gs = Stomata conductance; VPD = leaf to air vapour pressure deficit; A= Rate of net photosynthesis; E= Transpiration rate; WUE = Photosynthetic water use efficiency; Ch a = Chlorophylls a; Ch b = Chlorophyll b ; TCh= Total chlorophyll; Ca = ambient CO₂ concentrations; Tca= total carotenoids; PH = Plant height; LN= Leaf number; LA= Leaf area; LY= Leaf Yield; GY= Grain Yield; INL = Internode length; RW= Root weight; RL= Root length; GFP= Grain filling period ; DF=, Days to flowering; DM= Days to maturity

5.3.7 Pearson's Correlations of yield with yield-related traits

The result of the association between grain and leaf yield and other components viz., A, g_s , WUE, RL, LA, RW, Ci, Ch a, Ch b, TCh, LN, GY, and LY are presented in Figure 5.5. Correlation among the traits may be the consequence of the genetic association among the characters. From the breeder's viewpoint, the type of association of grain and leaf yield and its component traits is of supreme importance. The results showed that correlation among physio-morphological traits revealed a very highly significant positive association of A with E ($r = 0.50$; $p < 0.001$), g_s ($r = 0.46$; $p < 0.001$), WUE ($r = 0.49$; $p < 0.001$), and LN ($r = 0.23$; $p < 0.001$) were also significantly positively associated with each other. It also showed that WUE had a very highly significant positive correlation with Ch b ($r = 0.23$; $p < 0.001$), whereas LN had a significant association ($r = 0.13$; $p < 0.05$). Chlorophyll b exhibited a very highly significant and positive correlation with TCh ($r = 0.69$; $p < 0.001$) followed by Tca ($r = 0.67$; $p < 0.001$), E ($r = 0.42$; $p < 0.001$), and g_s ($r = 0.29$; $p < 0.001$).

RW had a very highly significant positive association with RL ($r = 0.63$; $p < 0.001$), LA ($r = 0.48$; $p < 0.001$), and LY ($r = 0.32$; $p < 0.001$), TCh ($r = 0.29$; $p < 0.001$), Tca ($r = 0.27$; $p < 0.001$), Ch a ($r = 0.26$; $p < 0.001$), and LN ($r = 0.23$; $p < 0.001$). The correlation studies in the present investigation revealed that the RL per plant recorded a positive and a very highly significant positive correlation with A ($r = 0.23$; $p < 0.001$). It also had highly significant correlations with the GY ($r = 0.19$; $p < 0.01$), whereas WUE had a significant association ($r = 0.13$; $p < 0.05$) with RL. Leaf number also had positive and very highly significant correlations with A ($r = 0.49$; $p < 0.001$) followed by Ch b ($r = 0.29$; $p < 0.001$), Tca ($r = 0.22$; $p < 0.001$), E ($r = 0.19$; $p < 0.05$), and g_s ($r = 0.18$; $p < 0.05$) were highly significant and positively correlated, contrarily WUE ($r = 0.21$; $p < 0.05$) was highly significant and negatively correlated. Stomata conductance had a very highly significant and positive association with E ($r = 0.88$; $p < 0.001$), Ch b ($r = 0.42$; $p < 0.001$), and TCh ($r = 0.21$; $p < 0.001$). However, g_s had a negative and a very highly significant relationship with WUE ($r = -0.31$; $p < 0.001$). Transpiration rate exhibited a very highly significant and negative correlation with WUE ($r = -0.71$; $p < 0.001$) followed by A ($r = -0.66$; $p < 0.001$), but none of the other physio-morphological traits were correlated with each other. Association studies between the CO_2 concentration of sub-stomatal CO_2 concentration and WUE and E indicated a tendency for the CO_2 concentration of sub-stomatal CO_2 concentration to decrease with increasing WUE and A while positively correlated with RL.

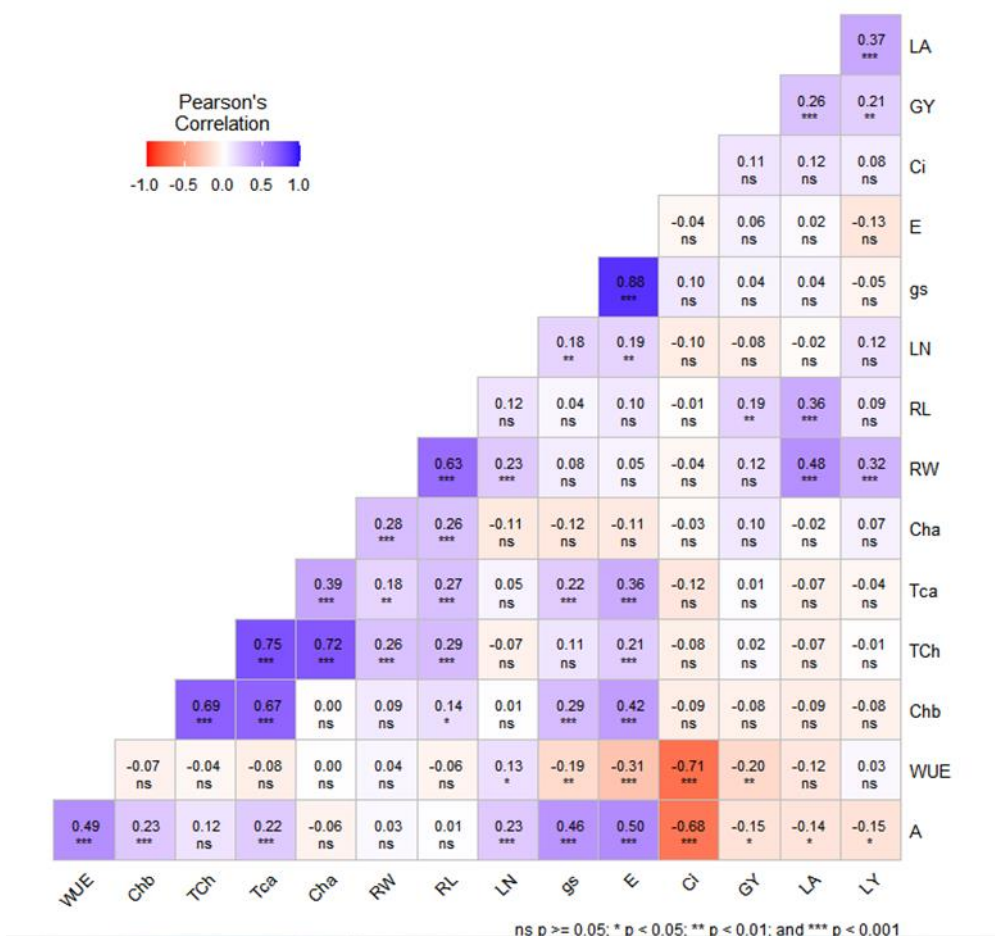


Figure 5.5 Pearson correlations between studied traits

RH = Relative Humidity inside leaf chamber; Ci = CO₂ concentration of Sub-stomatal CO₂ concentration; gs = Stomata conductance; VPD = leaf to air vapour pressure deficit; A= Rate of net photosynthesis; E= Transpiration rate; WUE = Photosynthetic water use efficiency; Ch a = Chlorophylls a; Ch b = Chlorophyll b ; TCh= Total chlorophyll; Ca = ambient CO₂ concentrations; Tca= total carotenoids; PH = Plant height; LN= Leaf number; LA= Leaf area; LY= Leaf Yield; GY= Grain Yield; INL = Internode length; RW= Root weight; RL= Root length; GFP= Grain filling period ; DF=, Days to flowering; DM= Days to maturity

5.3.8 Clustering of genotypes

The 120 genotypes of amaranth were mainly categorized using Ward's approach of classification based on the Euclidean distance into seven hierarchical groups based on the mean of 19 physio-morphological parameters (Figure 5.6). Due to variations in the assessed physio-morphological parameters, there were different numbers of genotypes in each cluster (Table 5.11). The highest number of genotypes was observed in cluster seven (C7), followed by cluster five (C5), cluster three (C3), cluster one (C1), and cluster two (C2), while the lowest was observed in cluster four (C4) and cluster six (C6). The seventh cluster (C7, n = 35)

contributed 29.2% of the total studied genotypes, of which 34 were collected from Oromia, SNNPR, and Afar, while the remaining 1 genotype was a released variety and comprised *Amaranthus hybridus* L. subsp. *cruentus* (L.) Theil, *Amaranthus viridis* L., *Amaranthus caudatus* L., *Amaranthus palmeri* S. Wats, and *Amaranthus hybridus* L. subsp. *hybridus*. The fifth cluster (C5, n = 25) comprised 20.8% of the total genotypes under consideration, of which 9, 6, 5, 4, and 1 genotypes were identified as *Amaranthus hybridus* L. subsp. *cruentus* (L.) Theil, *Amaranthus hybridus* L. subsp. *hybridus*, *Amaranthus palmeri* S. Wats, *Amaranthus* sp., and *Amaranthus caudatus* L, respectively, and collected from SNNPR, Afar, Sidama, and Benishangul & Gumuz. The third cluster (C3, n = 22) consisted of 18.3% of the total genotypes, of which 20 genotypes were identified as *Amaranthus hybridus* L. subsp. *hybridus*, and the remaining were grouped in the *Amaranthus hybridus* L. subsp. *cruentus* (L.) Theil and collected from SNNPR, Sidama, and one genotype was released. The first cluster (C1, n = 14) comprised 11.7% of the total genotypes, of which 11 genotypes were identified as *Amaranthus hybridus* L. subsp. *hybridus*, 1 genotype as *Amaranthus caudatus* L, and 2 genotypes as *Amaranthus hybridus* L. subsp. *cruentus* (L.) Theil, was collected from SNNPR, Tigray and Sidama. The second cluster (C2, n = 11) consisted of 9.2% of the total genotypes, of which 6 genotypes were identified as *Amaranthus hybridus* L. subsp. *cruentus* (L.) Theil, 4 genotypes as *Amaranthus hybridus* L. subsp. *hybridus*, and the remaining were grouped as *Amaranthus caudatus* L, which were collected from SNNPR, Benishangul & Gumuz, and Amhara. The fourth cluster (C4, n = 7) consisted of 5.8% of the total genotypes, of which 4 genotypes were identified as *Amaranthus palmeri* S. Wats, and the remaining were grouped in the *Amaranthus hybridus* L. subsp. *cruentus* (L.) Theil, *Amaranthus hybridus* L. subsp. *hybridus*, *Amaranthus spinosis* and collected from Afar, SNNPR, and Sidama. The six clusters (C1, n = 6) comprised 5.0% of the total genotypes, of which 4 genotypes were identified as from *Amaranthus caudatus* L, while the other 2 genotypes were from *Amaranthus palmeri*, which were collected from SNNPR and Afar.

The accuracy of the identified groups produced was reassessed using discriminant function analysis (Table 5.8). The results indicated that clusters I, II, III, IV, and VI (100%) discriminated correctly followed by cluster VII (97.1%) and cluster V (92.0%) while the fifth cluster had the lowest proportion of correct placement, with only 25 of 23 traits, or 92%, correctly placed into that clusters.

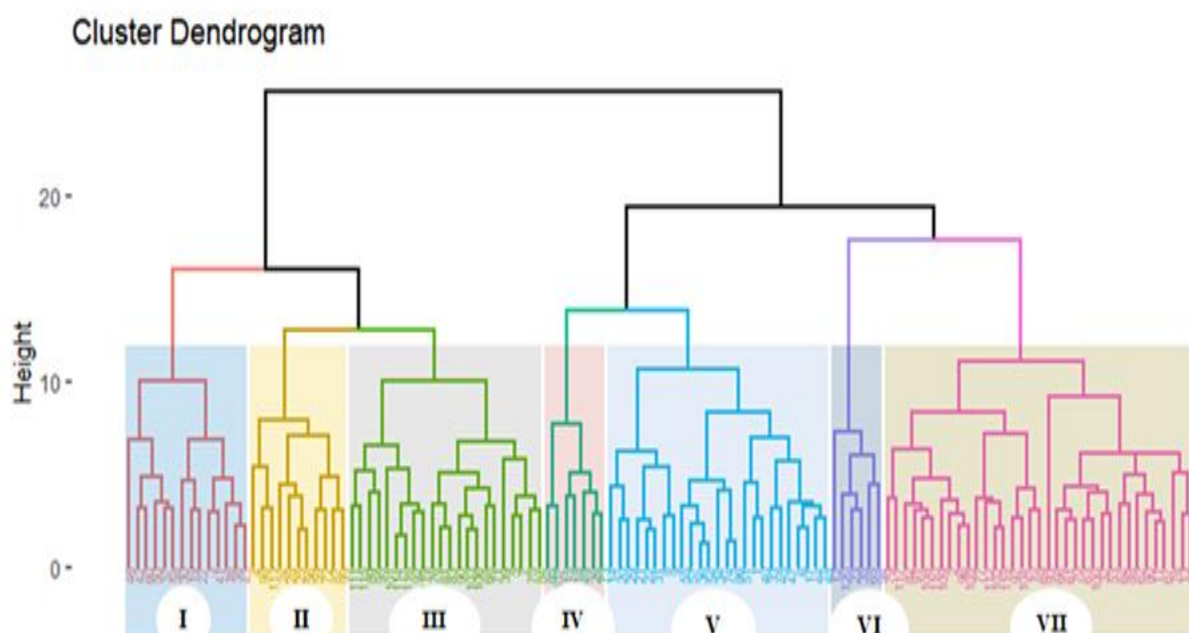


Figure 5.6 Cluster dendrogram developed by hierarchical grouping using Euclidian distance for 19 physio-agro-morphological traits of 120 amaranth genotypes.

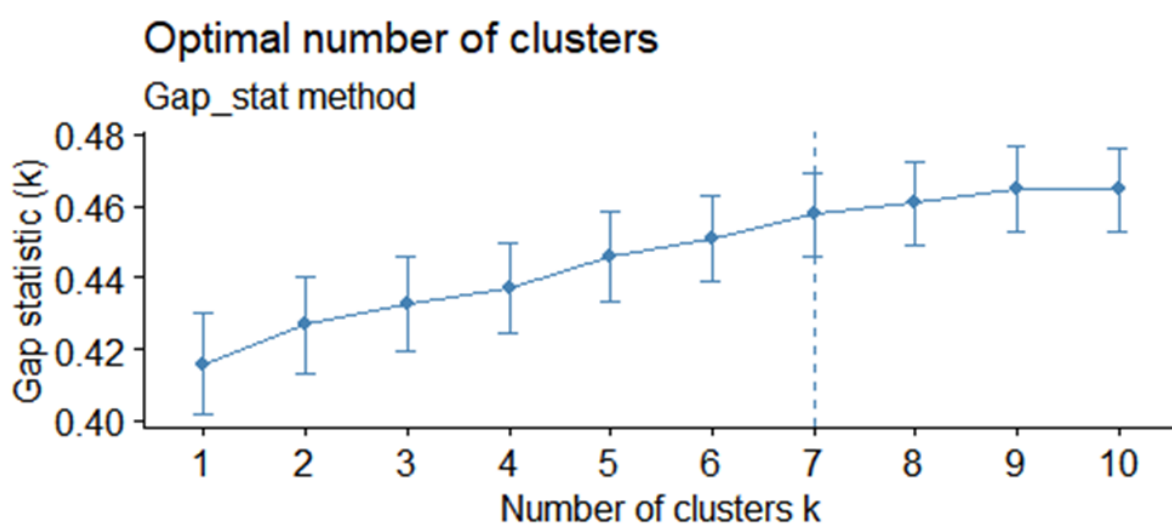


Figure 5.7 Gap statistics to determine the number of clusters (K) for quantitative data

Table 5.8 Determination of clustering accuracy using discriminant function analysis

Cluster Analysis Group	Predicted group membership						
	CLS 1	CLS 2	CLS 3	CLS 4	CLS 5	CLS 6	CLS 7

	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
1	14	100	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	11	100	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	22	100	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	7	100	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	23	9	0	0	1	3
6	0	0	0	0	0	0	0	0	0	0	6	100	0	0
7	0	0	0	0	0	0	0	0	2	8	0	0	34	97
Total N	14		11		22		7		25		6		35	
N correct	14		11		22		7		23		6		34	
Proportion	1		1		1		1		0.92		1		0.97	

5.3.8.1 Performance of genotypes under different clusters

Table 5.10 illustrates the significant differences found in the clusters for the attributes taken into consideration based on inter-cluster mean performances. Each cluster had peculiar characteristics in terms of the traits under consideration. The first cluster (C1) had the highest mean values for TCh, TCa, and RL, while the remaining sixteen traits had intermediate mean values. The second cluster (C2) was characterized by high mean values for LA, LY, and GY. The mean values of Ch a, PH, INL, and RW were the highest in the third cluster (C3); mean values of RH, Ci, gs, VPD, A, E, WUE, Ch a, TCh, Tca, LA, PH, LN, LY, GY, INL, RW, and RL were intermediate; features Ch b exhibited the lowest mean performances. While LA had the lowest mean performances and the remaining features showed moderate mean values, the fourth cluster (C4) showed the highest mean values for VPD, Ch a, and TCh. The fifth cluster (C5) had the highest mean values for gs, A, and E while the remaining traits had recorded intermediate mean values. The six clusters (C6) had the highest mean values for the traits RH, Ci, WUE, and LN while the remaining traits revealed the intermediate mean performances. The seventh cluster (C7) indicated the intermediate mean performance for all the traits except for the traits gs and A which were the lowest values. The seventh cluster (C7, n = 17 traits) had the greatest number of traits with intermediate performance, followed by the second and fifth clusters (C2 and C5, n = 16 traits). The least number of features with maximum performance was found in the second, fourth, and fifth clusters (C 2, 4, and 5, n = 3 traits). In contrast, the maximum number of traits, which were made up of the highest performances, were found in the first, third, and sixth clusters (C 1, 3, and 6, n = 4 traits).

5.3.8.2 Genetic divergence

The genetic distances as measured by the pairwise generalized Mahalanobis's D^2 statistics between each cluster showed high genetic distances among clusters (Table 5.9). The intra-cluster genetic distance was non-significant for all clusters. The highest intra-cluster genetic distance was observed in cluster C6 ($D^2=5.99$) followed by cluster C4 ($D^2=5.68$), cluster C2 ($D^2=4.78$), cluster C1 ($D^2=4.30$), cluster C3 ($D^2=3.39$), cluster C5 ($D^2=3.14$), and cluster C7 ($D^2=2.46$). On the other hand, the inter-cluster genetic distance values ranged from 17.00 to 103.44 and these distances were significant between different clusters except between some clusters. The maximum and significant genetic divergence was found between clusters C4 and C6 ($D^2=103.44$) followed by clusters C2 and C6 ($D^2=95.25$), C1 and C6 ($D^2=89.57$), C3 and C6 ($D^2=85.85$), C5 and C6 ($D^2=71.86$), C6 and C7 ($D^2=70.56$). The inter-cluster genetic distances were significant and intermediate between clusters C1 and C2 ($D^2=37.36$), clusters C3 and C4 ($D^2=36.93$), and clusters C2 and C4 ($D^2=36.13$). The inter-cluster genetic distances were non – significant between clusters C1 and C5 ($D^2=27.58$), C2 and C3 ($D^2=26.23$), C3 and C7 ($D^2=26.14$), C4 and C5 ($D^2=22.73$), C4 and C7 ($D^2=21.51$), C2 and C5 ($D^2=21.32$), C1 and C3 ($D^2=20.08$), C2 and C7 ($D^2=17.59$), while the least non-significant genetic divergence was found between clusters C5 and C7 ($D^2=17.00$). The highest mean inter-cluster genetic distance was observed in cluster C6 ($D^2=86.09$) followed by cluster C4 ($D^2=45.25$), cluster C1 ($D^2=42.96$), C3 ($D^2=39.99$), cluster C2 ($D^2=38.98$), and C5 ($D^2=32.90$) while the least mean genetic distance was recorded from cluster C7 ($D^2=32.16$).

Table 5.9 Intra-cluster means and range for 19 physio-morphological traits of 120 amaranth genotype

From CLS	CLS 1	CLS 2	CLS 3	CLS 4	CLS 5	CLS 6	CLS 7	Mean Distance
CLS 1	4.3 ^{NS}	37.36**	20.08 ^{NS}	42.97***	27.58 ^{NS}	89.57***	40.17***	42.96
CLS 2		4.78 ^{NS}	26.23 ^{NS}	36.13*	21.32 ^{NS}	95.25***	17.59 ^{NS}	38.98
CLS 3			3.39 ^{NS}	44.71***	36.93**	85.85***	26.14 ^{NS}	39.99
CLS 4				5.68 ^{NS}	22.73 ^{NS}	103.44***	21.51 ^{NS}	45.25
CLS 5					3.14 ^{NS}	71.86***	17.00 ^{NS}	32.90
CLS 6						5.99 ^{NS}	70.56***	86.09
CLS 7							2.46 ^{NS}	32.16
Overall mean								45.48

Table 5.10 Mean performance among seven clusters of 120 amaranth genotypes based on 19 physio-agro-morphological traits.

Variable	CLS- 1			CLS-2			CLS-3			CLS- 4			CLS- 5			CLS -6			CLS- 7		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
RH	56.5	48.1	65.4	56.0	48.1	62.5	56.4	44.6	73.1	51.4	43.4	64.0	57.9	51.0	68.1	77.3	69.7	84.3	53.3	40.8	67.7
Ci	126.1	28.0	268.5	132.0	48.5	220.5	133.6	47.0	235.0	96.4	63.0	137.5	129.1	70.0	219.5	189.0	156.5	232.0	141.4	51.0	421.5
Gs	225.5	130.5	372.0	147.7	69.5	276.0	125.5	55.5	235.5	129.9	98.0	167.5	254.4	159.5	507.0	154.4	95.5	254.5	115.6	50.5	224.0
VPD	2.7	2.2	3.0	2.3	1.7	3.0	2.3	1.5	2.9	2.8	2.1	3.2	2.5	1.9	2.9	1.3	1.1	1.5	2.4	1.6	3.2
A	16.2	-6.1	27.6	12.1	6.1	16.4	11.2	3.9	21.4	13.7	11.9	15.2	18.4	7.4	27.8	13.3	9.8	15.1	10.3	-1.5	20.1
E	6.7	4.1	10.0	4.0	1.5	6.5	3.4	1.6	5.7	4.3	2.4	5.9	6.8	4.7	9.9	2.3	1.7	3.2	3.3	1.4	6.2
WUE	2.7	0.2	5.3	3.4	1.5	6.8	3.4	1.3	5.4	3.3	2.3	5.1	2.7	1.2	3.9	5.8	4.7	7.8	3.2	-2.0	5.9
Ch a	0.9	0.5	1.4	0.7	0.3	1.2	1.1	0.8	1.4	1.1	0.5	1.5	0.6	0.2	1.1	0.6	0.3	0.9	0.7	-0.1	0.9
Ch b	0.9	0.7	1.2	0.5	0.2	0.7	0.3	-0.4	0.5	0.7	0.5	1.2	0.6	0.3	0.9	0.5	0.2	1.1	0.3	-0.3	0.9
TCh	1.8	1.2	2.5	1.2	0.8	1.8	1.5	0.8	1.8	1.8	1.3	2.3	1.2	0.6	1.8	1.0	0.5	1.6	1.0	0.4	1.4
Tca	0.5	0.3	0.6	0.3	0.1	0.5	0.3	0.1	0.7	0.4	0.3	0.6	0.3	0.2	0.5	0.2	0.0	0.5	0.2	0.0	0.3
LA	81.7	69.5	111.8	100.5	74.8	124.1	87.2	72.5	104.3	29.4	12.7	56.4	66.8	16.7	104.4	59.1	16.8	83.8	69.6	15.1	105.1
PH	213.0	148.0	243.2	172.5	148.0	246.7	236.1	165.3	265.0	134.1	93.7	160.7	159.4	106.5	256.2	203.1	150.8	236.3	158.0	114.5	230.0
LN	21.3	17.0	27.0	20.1	13.5	24.0	21.1	12.5	32.5	14.8	6.5	20.0	22.6	16.0	32.5	27.3	21.0	32.0	18.0	9.0	27.0
LY	8.7	5.7	13.8	14.5	9.5	20.3	10.6	6.2	16.6	7.3	3.6	12.1	8.5	5.8	11.3	8.6	6.6	10.1	8.5	3.6	13.4
GY	1.3	0.8	2.4	1.5	0.9	2.4	1.3	0.8	2.1	0.8	0.1	1.7	1.0	0.1	1.9	0.5	0.2	1.7	1.3	0.1	2.7
INL	14.9	13.2	18.4	11.1	8.2	13.3	16.4	13.1	22.5	10.3	8.6	12.6	10.5	7.1	13.8	11.6	6.9	14.8	11.6	7.9	16.2
RW	199.9	109.3	278.5	163.1	94.8	305.0	208.1	111.0	300.0	77.2	39.5	127.8	102.7	38.5	214.8	120.8	31.3	175.0	87.6	30.3	240.3
RL	55.6	45.5	66.0	44.1	38.3	52.0	51.2	38.8	61.5	34.8	29.8	38.5	39.7	29.3	48.5	39.4	33.5	50.8	39.3	28.3	57.5

* = significant at $P < 0.05$, ** = highly significant at $P < 0.01$, and *** = very highly significant at $P < 0.001$; NS = non-significant at $P > 0.05$. Chi-Square (χ^2) 16 DF = 30.14; at 36.19 at $P < 0.01$; 43.82 at $P < 0.001$; Inter-cluster is off-diagonal; and intra-cluster is diagonal bold. RH = Relative Humidity inside leaf chamber; Ci = CO₂ concentration of Sub-stomatal CO₂ concentration; gs = Stomata conductance; VPD = leaf to air vapour pressure deficit; A= Rate of net photosynthesis; E= Transpiration rate; WUE = Photosynthetic water use efficiency; Ch a = Chlorophylls a; Ch b = Chlorophyll b ; TCh= Total chlorophyll; Ca = ambient CO₂ concentrations; Tca= total carotenoids; PH = Plant height; LN= Leaf number; LA= Leaf area; LY= Leaf Yield; GY= Grain Yield; IN = Internode length; RL= Root weight; RL= Root length; GFP= Grain filling period ; DF=, Days to flowering; DM= Days to maturity

Table 5.11 Grouping of 120 amaranth genotypes into seven clusters based on the mean performance of 19 physio-morphology-physiological and photosynthetic pigment traits

Cluster No	Number of genotypes	Proportion	Species compositions	Number of species within clusters	List of amaranth genotypes	Regions
1	14	11.7%	<i>Amaranthus hybridus</i> L. subsp. <i>hybridus</i> , <i>Amaranthus caudatus</i> L., <i>Amaranthus hybridus</i> L. subsp. <i>cruentus</i> (L.) Theil.	11 1 2	MEK-081,KEN-012,,WON-049, MEK-082, ALE-033,DIL 050,242917,BKD-027,BKD-029, KAZ-008, ALE-023, ALE-024, 242530, DAL-043	SNNPR Tigray, and Sidama
2	11	9.2%	<i>Amaranthus caudatus</i> L. <i>Amaranthus hybridus</i> L. subsp. <i>cruentus</i> (L.) Theil. <i>Amaranthus hybridus</i> L. subsp. <i>hybridus</i>	1 6 4	212890, ALE-068, 209056, 242533, 212581, KAZ-058, ALE-034, 208025, ALE-070, KAZ-007, 212582	SNNPR, Benishangul & Gumuz, and Amhara
3	22	18.3%	<i>Amaranthus hybridus</i> L. subsp. <i>cruentus</i> (L.) Theil. <i>Amaranthus hybridus</i> L. subsp. <i>Hybridus</i>	2 20	BKD-031,OTI-044, KAZ-006, HAW-041, 242532, SHD-001, BKD-028, MEK-083, KEN-017, HAL-039, MEK-084, MEK-080, MAND, SHL-040, ABL-004, KEN-013, DUF-003, 225713, ADZ-037, DMG-038, YRB-002, BNT-026	SNNPR, Sidama, and released variety
4	7	5.8%	<i>Amaranthus hybridus</i> L. subsp. <i>cruentus</i> (L.) Theil. <i>Amaranthus palmeri</i> S. Wats <i>Amaranthus hybridus</i> L. subsp. <i>hybridus</i> <i>Amaranthus spinosus</i> L.	1 4 1 1	KAZ-076, SRA-002, 240814, SA-025/07, SHIA-007, DAL-042, BA-001	SNNPR, Sidama and Afar
5	25	20.8%	<i>Amaranthus palmeri</i> S. Wats <i>Amaranthus caudatus</i> L. <i>Amaranthus hybridus</i> L. subsp. <i>hybridus</i> <i>Amaranthus hybridus</i> L. subsp. <i>cruentus</i> (L.) Theil.	5 1 6 9	SAA-004, KAZ-078, HA-003, DA-006, CHU-045, 204645, 242534, 211456, 241764, 242531, KEN-065, KAZ-055, 240813, 209057, K3A-005, 225715, KAZ-009, 225718, 211457, SA-008/07,KAZ-077,KEN-015, 91001, 204644, 91003	SNNPR, Afar, Sidama, Benishangul & Gumuz

6	6	5.0%	<i>Amaranthus sp.</i>	4	BA-016, 215567, 219284, SA-001/07, 240815, KEN-018	Afar, and SNNPR
			<i>Amaranthus caudatus L.</i>	4		
			<i>Amaranthus palmeri S. Wats</i>	2		
7	35	29.2%	<i>Amaranthus hybridus L. subsp. cruentus (L.) Theil</i>	12	ALE-074, 208683, KAZ-057, KEN-020, KAZ-059, 208764, ALE-073, KEN-064, KEN-021, KEN-014, 225717, AC-NL, KAZ-056, 211455, KAZ-079, SA-005/07, 212892, ALE-025, DRA-053, 212583, 212893, KEN-022, KEN-067, ALE-071, KEN-010, KAZ-060, HA-008, KEN-011, YRC-048, ALE-075, KEN-016, KEN-019, WA-001, ALE-069, WA-003	Oromiya, SNNPR, Released variety, Afar
			<i>Amaranthus viridis L.</i>	4		
			<i>Amaranthus caudatus L.</i>	8		
			<i>Amaranthus palmeri S. Wats</i>	4		
			<i>Amaranthus hybridus L. subsp. Hybridus</i>	7		

5.3.9 Principal component analysis

Principal component analysis (PCA) indicated that the first nine principal components (PCs) with eigenvalues greater than 0.5 accounted for 90.91% of the total variation among 120 amaranth genotypes studied for the nineteen physio-morphological traits (data not shown). Of these, the first six PCs with eigenvalues greater than unity accounted for 79.30% of the total variation in amaranth genotypes (Table 5.12 and Figure 5.10). The scree plot also showed that the first six PCs were important in exploiting the variation in amaranth genotypes (Figure 5.8). The first PC axis accounted for 21.11% of the total variation and differentiated the population based on RL (0.780), RW (0.752), TCh (0.717), Ca (0.667), PH (0.631), INL (0.567), Ch a (0.504), and Ch b (0.504). Among the studied traits, E, LA, and gs had intermediate while RH and WUE had the lowest contribution for the total variation in the first PC. The second PC axis explained 13.02% of the total variation and had higher magnitudes of positive loadings concerning traits of PH (0.539), INL (0.535), and LY (0.501) and negative loadings for E (-0.719), A (-0.600), and Ch b (-0.576). On the other hand, the contribution of variation observed in the second PC from RW, LA, Ci, and RL were intermediate while the lowest values were recorded from LN, GY, GY, WUE, Ch a, and RH. Eigenvectors of the third PC axis accounted for 13.02% of the total physio-agro-morphological variation and had high positive contributing factor loadings from WUE (0.710), RH (0.701), A (0.522), and LN (0.478) and that of high negative factor loadings from VPD -0.654), and, GY (-0.534). The remaining traits in the third PC contributed the least to the overall variance, whilst gs and PH had moderate contributions. The fourth PC axis explained 11.33% of the total variation due to high positive eigenvector values of Ci (0.692), gs (0.637), and TDW (0.355), and high negative values of WUE (-0.571) while the remaining traits had less contribution for the total variability. The fifth PC axis accounted for 9.37% of the total variation and differentiated the population based on TCh (0.517), Ci (0.487), RH (0.452), and, Ch a (0.452). Among the studied traits, Ca, Ch b, and gs had intermediate while LY and INL had the lowest contribution for the total variation in the first PC. The six PC axes explained 6.17% of the total variation and had higher magnitudes of positive loadings concerning traits of LA (0.430), INL (0.535), LY (0.507), and GY (0.465).

Table 5.12 Eigenvectors and eigenvalues of the first six principal components (PCs) for 19 physio-agro-morphological characters of 120 amaranth genotypes.

Eigenvalue	Principal Components					
Variance per cent	PC1	PC.2	PC3	PC4	PC5	PC6
	4.01	3.48	2.47	2.15	1.78	1.17
	21.11	18.29	13.02	11.33	9.37	6.17
Cumulative variance percent	21.11	39.40	52.42	63.75	73.12	79.29
Traits	PC1	PC2	PC3	PC4	PC5	PC6
Relative Humidity inside Leaf Chamber (%) RH	-0.018	0.137	0.705	0.398	0.452	0.107
CO ₂ concentration of Sub-stomatal CO ₂ concentration ($\mu\text{mol mol}^{-1}$) [Ci]	-0.141	0.318	-0.248	0.692	0.487	-0.221
Stomata conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) [gs]	0.321	-0.569	0.248	0.637	-0.026	0.015
Leaf to air vapor pressure deficit (kPa) [VPD]	0.205	-0.417	-0.654	-0.235	-0.445	-0.116
Rate of net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$) [A]	0.271	-0.600	0.522	-0.167	-0.337	0.074
Transpiration rate ($\text{mol m}^{-2} \text{s}^{-1}$) [E]	0.410	-0.719	-0.009	0.464	-0.201	-0.034
Photosynthetic water use efficiency ($\text{mmol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$)[WUE]	-0.118	0.158	0.710	-0.571	-0.039	0.282
Chlorophylls an (mg/g) [Cha]	0.506	0.186	-0.197	-0.400	0.452	-0.024
Chlorophylls b (mg/g) [Ch b]	0.504	-0.576	0.027	-0.003	0.276	0.138
Total chlorophyll (mg/g) [TCh]	0.717	-0.265	-0.124	-0.292	0.517	0.078
Carotenoids (mg/g) [Ca]	0.667	-0.448	-0.067	-0.150	0.308	0.108
Leaf area (cm^2) [LA]	0.329	0.426	-0.105	0.374	-0.342	0.430
Plant height at maturity (cm) [PH]	0.631	0.539	0.215	-0.080	-0.128	-0.311
Leaf number (number) [LN]	0.255	0.005	0.478	0.145	-0.358	-0.272
Dry leaf yield [LY]	0.202	0.501	0.068	0.137	-0.026	0.507
Grain yield [GY]	0.120	0.156	-0.534	0.140	-0.120	0.465
Internode length [INL]	0.567	0.535	-0.038	-0.029	-0.049	-0.381
Root weight [RW]	0.752	0.431	0.099	0.054	-0.202	0.016
Root length [RL]	0.780	0.317	-0.036	0.090	-0.147	0.002

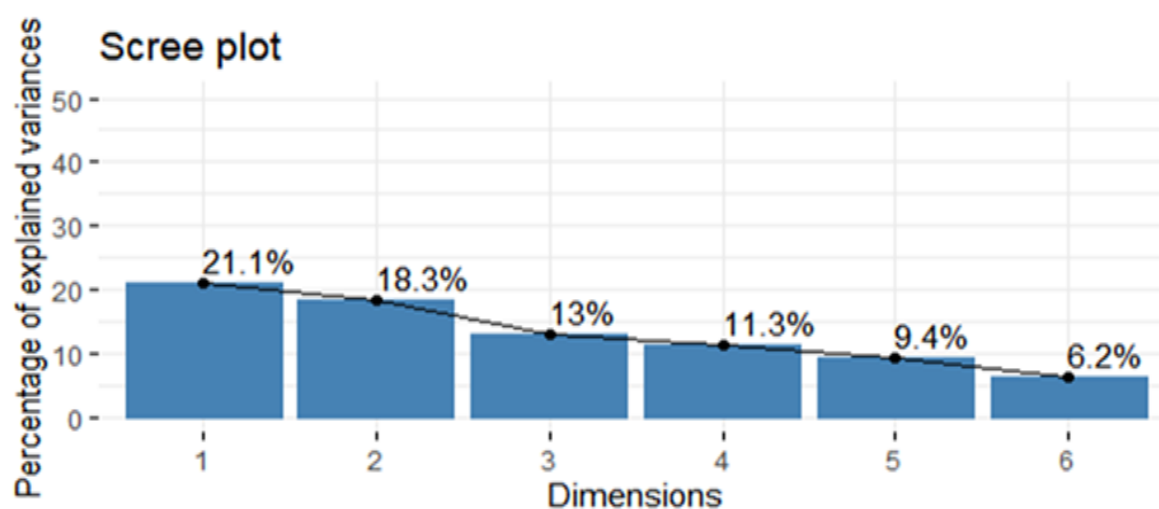


Figure 5.8 Scree plot showing eigenvalues in response to the number of principal components for 19 physio-agro-morphological traits of 120 amaranth genotypes

Figure 5.9 shows the biplot loadings for the first two PCs of the 19 physio-agro morphological attributes in amaranth genotypes. According to the results, based on the vector lines' length, direction, and closeness to one another, the researched features were categorized into six categories. INL, PH, RL, and RW constituted the first group, and their eigenvectors for PCs 1 and 2 were all positive. Three traits—LY, LA, and Ch a—that exhibited positive factor loadings for both PCs comprised the second group. Three traits (WUE, Ci, and RH) with positive PC2 values for WUE, Ci, and RH but a negative PC1 value comprised the third group. The features gs, E, VPD, and A, which exhibited positive eigenvectors for PC1 but negative eigenvectors for PC2, were indicative of the fourth group. For these qualities, Ch b, TCa, and TCh formed the fifth group. They had positive eigenvectors for PC1 but negative eigenvectors for PC2. Two features (GY and LN) in the set of six PCs exhibited positive factor loadings for both PCs (PC 1 and PC 2).

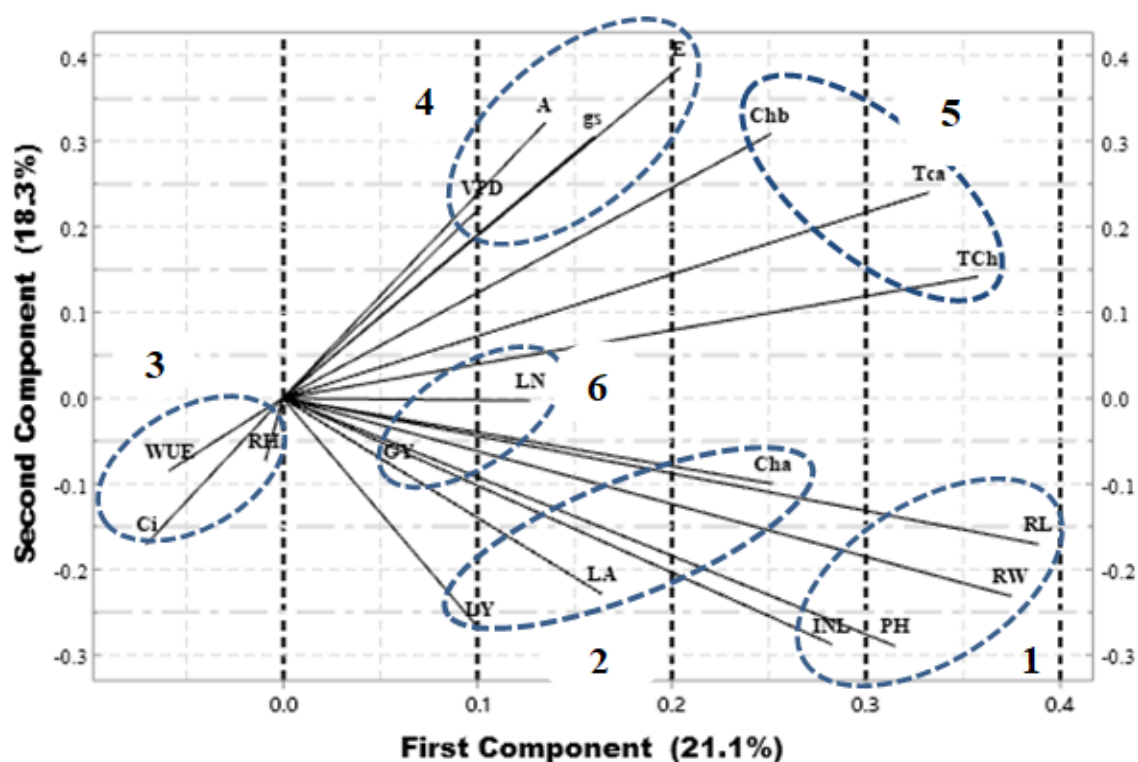


Figure 5.9 Biplot loadings of the first two principal components (PC1 and PC2) showing the association of 19 physio-agro-morphological traits in 120 amaranth genotypes.

RH = Relative Humidity inside leaf chamber; *Ci* = CO₂ concentration of Sub-stomatal CO₂ concentration; *gs* = Stomata conductance; *VPD* = leaf to air vapor pressure deficit; *A*= Rate of net photosynthesis; *E*= Transpiration rate; *WUE* = Photosynthetic water use efficiency; *Ch a* = Chlorophylls a; *Ch b* = Chlorophyll b; *TCh*= Total chlorophyll; *Tca*= total carotenoids; *PH* = Plant height; *LN*= Leaf number; *LA*= Leaf area; *LY*= Leaf Yield; *GY*= Grain Yield; *IN* = Internode length; *RL*= Root weight; *RL*= Root length

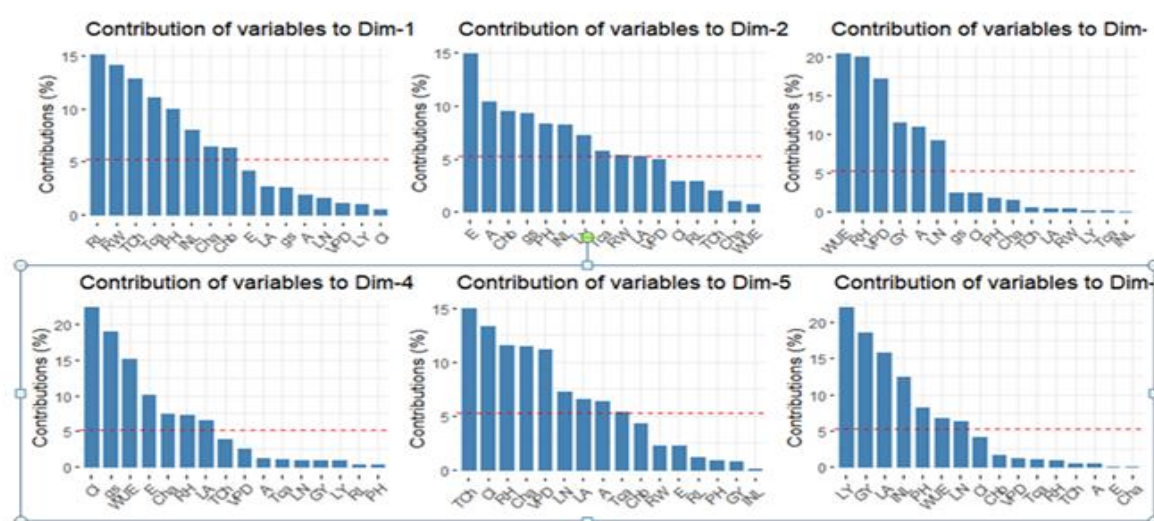


Figure 5.10 Contribution of the variables to the formation of the six principal components

5.4 Discussion

The combination of various anatomical characteristics of Amaranths, such as C₄ metabolism, well-developed root system, stomatal conductance, and maintenance of leaf area (Spreeth *et al.*, 2004), results in increased efficiency of using CO₂ under a wide range of temperatures (from 25 °C to 40 °C), with higher light intensity and moisture stress environments (Brenner and Williams, 1995) which enables this plant to adapt under diverse geographic and environmental conditions (Kigel and Paredes-Lopez, 1994). Plants that use the C₄ carbon fixation pathway tend to require less water than the more common C₃ carbon-fixation pathway plants (Council, 1984). Because of this, contrasted to many other C₃ plants like wheat and soybeans, amaranths thrive in unfavorable temperature and moisture circumstances (Spreeth *et al.*, 2004), indicating that *Amaranthus* can be grown in areas that are not suitable for other crops (Rana *et al.*, 2007). Different breeding efforts have looked at and utilized genetic diversity in traits related to gas exchange and pigment traits (Kondamudi *et al.*, 2016; Haritha *et al.*, 2017). Furthermore, breeding programs should become more effective as a result of developments in precision phenotyping and the integration of genetic and molecular methods (Choudhary *et al.*, 2018). In this instance, targeting the underlying physiological features that affect yield by indirect selection may be more effective than using direct selection to increase yield (Khadka *et al.*, 2020).

5.4.1 Analysis of variance

In this study, a wide range of variations were observed among all measured traits except the rate of net photosynthesis and stomata conductance. High range and variation suggest that most of the traits in amaranth might be successfully improved with straightforward selection. Moreover, the great variations observed among the studied genotypes indicate the presence of remarkable inherent variability among amaranth genotypes for the studied traits. A similar result was reported by Jamalluddin *et al.* (2021) and Hamidzadeh Moghadam *et al.* (2021) in different species of amaranths for most measured traits. *Amaranthus hybridus* L. *subsp. cruentus* Theil possessed the biggest E and LA. On top of that, gs, RH, Ci, PH, and LA demonstrated significant standard deviations (more than 20) among the examined characteristics indicating that these traits had higher genotype-to-genotype variability than other traits. The remaining investigated characteristics also exhibited modest standard deviations, fewer than 10, indicating that the genotypes had limited variability for these traits. Similar to the standard deviation, the standard error of the mean demonstrated that attributes

with low standard deviations also had low standard errors of the mean, and vice versa. The analysis revealed a broad range of coefficients of variation (CV), ranging from 4.25% to 54.97%. According to our findings, there was a significant amount of genetic variation among these characteristics.

5.4.2 Descriptive statistics

Amaranthus hybridus L. *subsp. hybridus* was identified as having the traits with the highest gs, TCh, Tca, GY, and RL. The root is the plant's primary organ for drawing water and minerals from the soil. Water deficiency in the soil results in root system plasticity (Jain *et al.*, 2014), which lowers root proliferation even more, finally preventing upstream water and mineral dissemination and significantly lowering grain yield (Figuroa-Bustos *et al.*, 2019). The increase in root length which helped plants to grow even under extreme water deficit conditions according to Jomo *et al.* (2015), is the second line of defense after leaf number reduction. Besides differential sensitivity, the observed increase in root with an increase in water deficit, in the current study may be attributed to the increased allocation of biomass from shoot to root, which is in agreement with previous results obtained (Jomo, 2013). In addition, improved physiology (gs) gives the genotype the ability to use resources more effectively throughout crucial growth phases, which has the benefit of overcoming unfavourable climatic variability (Naqeebullah *et al.*, 2021). The green portion of plant leaves indicates a plant's capability for photosynthetic activity and offers helpful information on crop yield as well as the physiological and phenological condition of the plant (Kira *et al.*, 2015; Ramya *et al.*, 2016). The high carotenoids' role in the photosynthetic process, either as an antioxidant or as an additional antenna to absorb light, may explain the link between carotenoids and grain yield (Silva-Pérez *et al.*, 2012).

In the current study, *Amaranthus hybridus* L. *subsp. cruentus* Theil possessed the biggest transpiration rate and leaf area. Similarly, Eavis and Taylor (1979), in their experiment with wheat by MA and Tsunoda (1970), concluded that total transpiration increases linearly with the leaf area and also that transpiration rate decreases linearly with the decrease of soil water content. However, transpiration and leaf area were inversely related in soybeans (Bhagsari *et al.*, 1977). The amount of leaf area affects how much light is intercepted, which affects how much dry matter plants produce. In a similar vein, it is crucial to calculate figuring out how much water plants consume (Liu and Stutzel, 2002). Nevertheless, leaf area alone was not a reliable indicator of transpiration rate since large leaves frequently indicated low transpiration

rates and vice versa. The kind or type of stomata feature(s) present in such leaves that truly affect the rate of transpiration in the same plant species might be the decisive variable in such circumstances if documented. [Abdulrahman \(2009\)](#), has observed that stomata density, stomata index, and stomata size had a certain influence on the rate of transpiration in *Canna indica* and *Euphorbia milii*. [Gerrano et al. \(2013\)](#), found that *Amaranthus hybridus* L. subsp. *cruentus* Theil had the maximum leaf area. Transpiration rate and leaf area were directly related in *Amaranthus hybridus* L. subsp. *cruentus* Theil.). Similar results were reported in wheat ([MA and Tsunoda, 1970](#)).

Amaranthus caudatus L. had the highest rate of net photosynthesis; leaf number and leaf yield among all genotypes, although its WUE was the lowest. WUE expresses the relation between root water uptake ability and shoot water use. It is commonly recognized that higher WUE species have better adaptation to drought. The increase in WUE under water deficit was ascribed to the fact that either the biomass production is reduced less than water use ([Bramley et al., 2009](#)) or the net CO₂ assimilation rate is being decreased less than the transpiration rate ([Lawlor and Cornic, 2002](#); [Lessani and Mojtahedi, 2002](#); [Bacelar et al., 2007](#)). *Amarantus caudatus* L had the highest number of leaves, which suggested higher rates of photosynthetic activity and, as a result, a greater distribution of photosynthetic resources to other plant parts ([Jomo, 2013](#)). Moreover, net photosynthesis is a complex process controlled by many genes ([Gu et al., 2014](#)). Many authors report that an increase in the CO₂ concentration favors an increase in the photosynthetic rate ([Taiz and Zeiger, 2009](#)).

In the current studies, *Amaranthus spinosus* L. has the highest Ch a, but the least LA, and GY. A similar result was reported by ([Singh, 2010](#)) in *Amaranthus hypochondriacus* L for chlorophyll content and grain yield. [Morgan et al. \(1990\)](#), found that the reduction in leaf size associated with dwarfing genes concentrated leaf photosynthetic machinery (i.e. Higher: mesophyll cell number, stomata! density, chlorophyll concentration, and ribulose-1,5-bisphosphate carboxylase (RuBPC) per unit LA) and increased photosynthetic capacity. The smallest gs, E, and RL as well as the greatest VPD, Ch b, have been identified in *Amaranthus palmeri* S. Wats. The degree of stomata aperture (and consequently the physical barriers to the passage of gases between the air and the interior of the leaf) determines stomata conductance, which determines the rate of gas exchange (i.e., carbon dioxide uptake) and transpiration (i.e., water loss) through the leaf stomata. Therefore, it depends on the density, size, and degree of stomata opening; larger, more open stomata permit higher conductance, which in turn suggests

that transpiration and photosynthesis rates may be higher. The greatest rate of photosynthesis and high leaf yield were found in *Amaranthus caudatus* L among the 120 studied amaranth species. A similar result was reported by [Haritha et al. \(2017\)](#). To enhance the production of photoassimilates in genotypes with high yields, a breeding program has required photosynthesis as a prerequisite ([Haritha et al., 2017](#)). For instance, genotypes with the stay-green trait or slower Rubisco breakdown led to longer photo assimilation intervals and increased yield ([Shin et al., 2020](#)).

It became known that the populations of various amaranth species varied in terms of biochemical characteristics, namely leaf photosynthetic pigments. By triggering different metabolic and biochemical processes, weed species can withstand stress more readily than domesticated plants ([Pavlovic et al., 2015](#)). Chlorophylls are essential for photosynthesis and their amounts can directly influence plant photosynthetic ability and biomass ([Filella et al., 1995](#)). Besides chlorophylls, carotenoids are also essential for the photosynthesis process ([Ong and Tee, 1992](#)). protecting chlorophylls from photo-oxidative destruction ([Giri et al., 2013](#)). In this study, wide variations of leaf photosynthetic pigments were measured in the *Amaranthus hybridus* L. subsp. *cruentus* Theil., *Amarantus caudatus* L, *Amaranthus hybridus* L. subsp. *hybridus*, *Amaranthus palmeri* S. Wats, *Amarantus spinosus* L., *A. viridis* L. and *A.spp* populations. Photosynthetically efficient populations have been found in this study, which may be useful for cultivated amaranth efforts to grow ([Zhang et al., 2016](#)).

5.4.3 Growth Performance

Plants have different intrinsic rates of growth and can have three times different relative growth rates in the same environment. Certain species have a natural tendency to develop quickly, whereas others grow slowly ([Price and Munns, 2020](#)). Genotype and environment have an impact on plant growth ([Enßlin et al., 2011](#)). Furthermore, different growth rates in a field may result from genetic heterogeneity amongst amaranth species. In terms of the examined features, including plant volumes, branch counts, and total leaf counts, *A. palmeri* S. Wats had the considerably highest mean at 30,50 and 70 days after planting. Perhaps these fast-growing species of amaranth require respiratory energy in addition to increased rates of photosynthesis, more effectively for the absorption of ions, growth, and maintenance. Differences in the percentage of whole-plant respiration attributed to these three processes and the particular costs associated with each process are reflected in the energy user's efficiency variations ([Price and Munns, 2020](#)). The result was consistent with

the findings reported by [Horak and Loughin \(2000\)](#) and [Sellers et al. \(2003\)](#). The high photosynthetic rates enable *A. palmeri* S. Wats to grow rapidly with more than 5cm per day under bright sunlight and ideal conditions ([Horak and Loughin, 2000](#)). After 50, and 70 DAS, the thickest plant stem diameter mean was recorded in *A. hybridus* L. subsp. *hybridus*. Varying mechanical characteristics result from varying stem diameters. The corresponding bend strength increases with the stem's load failure rate. A plant's stem strength is a significant determinant of its lodging and stem bending ([Xia et al., 2018](#)). The diameter of the plant stem positively correlates with its bend strength and load failure, indicating a close relationship between the stem's diameter and mechanical strength. As the stem diameter increases, the load failure and bend strength also increase. The relationship between the plant stem diameter and the content of cellulose in the plant stem was analyzed ([Feng et al., 2022](#)).

5.4.4 Performance of top leaf yielder genotypes within species

The genotype 225713 of *A. hybridus* L. subsp. *hybridus* was highlighted for its high leaf yield and illustrated the greatest levels of the characteristics RH, Ci, gs, A, WUE, LA, and PH these genotypes were 66.2, 134.0, 177.50, 16.35, 5.30, 101.62, and 230.67, respectively. Similarly, the high value of stomata conductance involves slow stomata closure, leading to increased CO₂ intake and high net photosynthesis of cassava ([Shan et al., 2018](#)). Further, a high WUE, indicating that water was more efficiently utilized for assimilate production during the reproductive phase and among the yield groups was related to WUE. Net CO₂ uptake at higher light intensities is two to three times faster per unit leaf area in C₄ than in C₃ leaves largely because C₄ leaves are not subject to oxygen stress and have low rates of photorespiration ([Zelitch, 2012](#)). According to a recent meta-analysis by [Gago et al. \(2014\)](#), the parameters influencing the photosynthetic process at the leaf scale are the primary variable in WUE at the leaf level, which is a complicated feature depending upon physiological responses linking stomata conductance. In addition, [Bindi et al. \(2005\)](#) attested that the rate of leaf area expansion and transpiration, are thought to be proportionate to the plant's capability for gas exchange. In comparison with the less productive species, all higher-yielding leafy species have modest photorespiration fluxes ([Zelitch, 1973](#); [Zelitch, 2012](#)).

Among the top leaf yielder genotypes (242534) of the species *Amaranthus* sp., the characteristics A, Ch b, TCh, Tca, and LA have the highest values. This revealed that one of the key elements affecting photosynthetic capability is leaf chlorophyll concentration since it houses the reaction centers ([Mao et al., 2007](#)). [Zhu et al. \(2012\)](#) showed comparable results in

tomatoes. There have been several reports that, high yield and biomass production are associated with higher leaf photosynthetic rates (Horie *et al.*, 2003). Chlorophylls and carotenoids are significant photosynthetic pigments used in the process of capturing light for photosynthesis (Ramani *et al.*, 2023). Additionally serving as supplementary pigments, carotenoids shield the photosystem from ultraviolet light. Chlorophyll content in plant leaves fluctuates as a result of abiotic stressors (Khadka *et al.*, 2020).

The contribution of intercellular CO₂ concentration (C_i), leaf-to-air vapor pressure deficit (VPD), transpiration rate (E), and leaf number (LN) for grain yield in the top grain yielder genotypes (ALE-073) was the highest among the studied top genotypes from *A. hybridus L. subsp. hybridus* and also from overall selected top grain yielding genotypes. The reason might be related to the reduction in CO₂ assimilation may account for the somewhat elevated internal concentration of CO₂ (C_i) despite the considerable decrease in leaf stomata conductance (g_s) caused by a water deficit (Lu and Zhang, 1998). Although g_s determines the intercellular CO₂ concentration (c_i), which in turn determines A, c_i may act as a partial mediating factor in this link if g_s is somewhat independent of C_i. The superior genotype for grain (242530) had the greatest values for the traits stomata conductance (mmol m² s⁻¹), transpiration rate (mol m² s⁻¹), chlorophyll a (mg/g), total carotenoids (mg/g), chlorophyll b (mg/g), total chlorophyll (mg/g), plant height (cm), INL (cm), and RL (cm) from species *A.hybridus L. subsp. cruentus* (L.) Theil. The recorded grain yield for genotypes (242530) may be due to the highest value for the traits' leaf pigment content. Chlorophyll content has been suggested as the most directly relevant to the prediction of productivity (Dawson *et al.*, 2003). Moreover, higher chlorophyll content in plants' leaves is a desirable characteristic because it indicates a low degree of photoinhibition of photosynthetic apparatus, therefore, reducing carbohydrate losses for grain development (Jones *et al.*, 1989). This result is also in close conformity with the findings of (Kikuchi *et al.*, 2017; Won *et al.* (2020)). The more chlorophyll content in the leaves the faster photosynthesis processes will run, producing more photosynthates (Sitompul and Guritno, 1995).

5.4.5 Performance of top leaf yielder genotypes among species

Amaranthus hybridus L. subsp. cruentus (L.) Theil, one of the top amaranth species for leaf production, showed the greatest species mean value for the examined markers, such as LA and LY, while its g_s, A, and Ch b values were the lowest. On the other hand, other experts contend that excessive leafiness during grain filling might lessen a plant's capacity to tolerate high air

temperatures and decrease its heat resistance. In this regard, drought-resistant genotypes do not have a very high leaf area (Deriglazova and Gavrilova, 2021). As a result, *A. hybridus* L. subsp. *cruentus* (L.) Theil may be less able to withstand droughts because of their extremely large leaf area.

5.4.6 Performance of top grain yielder genotypes among species

Differences among amaranth species were observed in gas exchange parameters and WUE. Leaf photosynthesis, measured, statistically varied with species. The highest WUE and lowest transpiration rate among the studied amaranth species were observed in top grain yielder *Amaranthus hybridus* L. subsp. *hybridus*. Moreover, as field experiments in enriched CO₂ environments have reported a direct relation between increased WUE and reduced evapotranspiration (Drake *et al.*, 1997), the photosynthetic WUE, examined at the leaf level for the top grain yielder, could contribute to screening and evaluation for suitable characteristics for the water-limited environment. Respiration was also used as a selection factor in one case, where selecting for decreased respiration rates led to notable yield gains (Wilson and Jones, 1982).

5.4.7 Phenotypic and genotypic coefficient of variations

For the majority of the physio-agro-morphological variables under investigation, the phenotypic variance magnitudes in the current study were much larger than the corresponding genotypic variances. This suggests that these attributes exhibit a rather high degree of environmental impact. This result is also in line with the findings of (Singh, 2010; Tuhina-Khatun *et al.*, 2015), who reported that the phenotypic variance in all studied traits is higher than the genotypic variances in upland rice genotypes.

The observed wider variability on phenotypic and genotypic coefficients of variation generally indicates the existence of substantial variability in the studied markers. The study illustrated that 10.53 and 36.84% of the studied traits had lower PCV and GCV estimates, respectively; suggesting selection based on these traits may not be effective. However, 89.47 and 63.16% of the studied traits had high values of PCV and GCV, respectively; indicating that the larger proportion of variability was contributed by the genetic component but less by the environment. Thus, selection may be effective based on the physio-morphological performance of these traits. Among the studied traits, Ci, A, E, Ch b, Ch a, LA, LN, A, TCh, Tca, RW,

WUE, GY, and PH had high PCV values and these respective traits gave also high GCV values. Similarly, [Tuhina-Khatun et al. \(2015\)](#) reported similar results in *Oryza sativa* L. For five (about 26.32%) of the examined attributes, the estimates of ECV were comparatively low, indicating that these traits were less susceptible to environmental influences. Conversely, large (>20%) estimates of environmental variation were found in eight (approximately 42.11%) of the characteristics under study. The influence of environment on gas exchange parameter traits (Ci, A, E, and WUE) and agro-morphological and yield traits (RW, GY, and LY) was higher compared to photosynthetic pigment traits. The moderate-to-high PCV and moderate GCV that were found, along with the accompanying low-to-moderate ECV estimations for VPD and RL, suggested that selection may lead to improvements in these characteristics. In populations with significant genetic diversity, PCV and GCV estimations are consistently high. These high values offer a broad range of selection opportunities based on the phenotypic performance of these variables for improvement ([Yadav et al., 2014](#); [Yeshitila et al., 2023a](#)).

Although the estimates of PCV were greater than GCV, the differences between high PCV and the respective high GCV were relatively lower in magnitude for Ch a (0.00%), Ch b (0.00%), TCh (0.00%), Tca (0.00%), LA (1.25%), PH (0.83%), LN (2.15%) and RW (3.94%). This result revealed that the environmental effect on any trait is indicated by the magnitude of the differences between the genotypic and phenotypic coefficients of variation; large differences reflect a large environmental effect, whereas small differences reveal a high genetic influence. The study found that the slight variations in the PCV and GCV for these characteristics indicated a degree of environmental impact on the characters' phenotypic manifestation. Moreover, the lower values showed that phenotypic performance was less affected by environmental influences and that these features had relatively large exploitable intrinsic genetic variability. Thus, selecting for phenotypic performance might be a useful strategy to significantly increase these attributes. The report of [Osman et al. \(2012\)](#); [Tuhina-Khatun et al. \(2015\)](#); and [Acevedo-Siaca et al. \(2021\)](#) are likewise consistent with this result. On the contrary, slightly wider ranges between high PCV and the corresponding moderate GCV were observed for gs (26.97%) and LY (9.86%), suggesting that the influence of the environment on these traits is high and selection based on the phenotypic performance of these traits may not be effective for genetic improvement.

5.4.8 Heritability and genetic gains

The study revealed that the estimated heritability, in a broad sense, generally exhibited considerable variability for measured phenotypic traits. This wide range of variability in the studied traits indicated the possibility of high genetic improvement of traits under consideration because the extent of wide variability virtually offers a better scope for selection. The tested amaranth genotypes had a very high degree of heritability in Ch a, Ch b, TCh, Tca, LA, PH, LN, and RW, while moderately high heritability estimates were recorded for VPD, E, INL, and RL. This indicates the influence of environmental factors on the expression of these traits was relatively small. Consequently, the expressed phenotypic performance was manifested mainly due to the substantial effect of the genetic factor. Therefore, to ensure that selection is successful in improving amaranth, special attention should be paid to these specific transmissible traits. The high heritability estimates observed in this study were also reported by [Acevedo-Siaca et al. \(2021\)](#) and [Tuhina-Khatun et al. \(2015\)](#), who observed high heritability estimates in one or more of their studied traits in rice genotypes. Moreover, the markers Ch a, Ch b, TCh, and Tca revealed 100% heritability, which indicated the presence of additive gene action. This also showed that selection based on these characteristics would be more effective and efficient than the use of other traits for segregating generations in future breeding programs, which indicated an exploitable amount of variation. Similar results were reported by [Tuhina-Khatun et al. \(2015\)](#) for the traits A, E, *gs*, and Ci in rice

The phenotypic-based selection could be effective for yield improvement when the traits have moderate to high heritability ([Shariatipour et al., 2022](#)). The influence of environmental factors on the phenotypes is relatively low for traits having a very high degree of heritability, and selection based on these traits might be effective due to the presence of high additive gene action on the expression of these traits ([Rosmaina et al., 2016](#)). The estimates of heritability were medium for RH, WUE, LY, and GY. This medium heritability of traits indicated that the environmental effect was relatively high on the expression of these traits, and selection based on the phenotypic performance of these traits may not be effective for improvement. [Singh et al. \(1993\)](#), also reported that estimates of medium to low heritability make selection considerably difficult due to the significant masking effect of the environment on the genotypic effects. The heritability estimates in this study were generally higher than those reported by [Acevedo-Siaca et al. \(2021\)](#) and comparable with the study of heritability from photosynthetic traits in *Oryza sativa* by [Tuhina-Khatun et al. \(2015\)](#).

The estimates of GA and GAM in the present study had wide variations among the observed traits, suggesting the existence of considerable expected genetic gain in amaranth genotypes for improvement. Whenever we select the best high-yielding genotypes as parents, the mean performance of the offspring is predicted to increase, according to the assessment of genetic gain in the current study. High to medium GAM estimates were found for every attribute under study. This is in line with that of [Tuhina-Khatun *et al.* \(2015\)](#), who reported high to medium GAM, in one or more of their considered traits in rice. High expected genetic advance confirmed the relatively greater involvement of the additive gene action component indicating that selection based on phenotypic performance might be effective for improvement.

Since total genetic diversity includes additive, dominant, and epistatic variations, selection based just on broad-sense heritability may not be predictive. Thus, estimates of heritability coupled with genetic advancement are more effective in predicting the genetic gain under selection than heritability alone ([Chakraborty *et al.*, 2014](#); [Chauhan and Singh, 2019](#)). High genetic gain may not always result from traits with high heritability ([Johnson *et al.*, 1955](#)). Estimates of very high heritability to moderately high heritability accompanied by high GAM were found in VPD, Ci, E, WUE, Ch a, Ch b, TCh, Tca, LA, PH, LN, INL, RW, RL, LY, and GY. Traits with such high heritability in conjunction with high genetic advance are dominated by additive gene effects thereby selection based on the phenotypic performance of these traits would be promising for improvement. High heritability in combination with high genetic advance resulted in better genetic gain, implying that phenotypic selection can be an effective approach for improving of traits under consideration ([Johnson *et al.*, 1955](#)). On the other hand, very high to moderately high heritability estimates associated with medium GAM were observed in RH. These traits are most likely governed by both comparable additive and no additive gene actions for the manifestation of phenotypic performance. The traits which had very high to moderately high heritability in combination with high to medium GAM estimates in the present study suggest the extent of genetic gain through phenotypic selection would be reliable and effective for genetic improvement ([Burton and Devane, 1953](#)), thus it is evident that the co-efficient of variation alone may not reveal the actual situation of heritable nature of the trait. Hence, to obtain more information on a heritable portion of the variability, it is essential to know the heritability estimates of different characters ([Venkatesh *et al.*, 2014](#)).

The degree of correlation among the traits is crucial, especially for a complex trait such as yield ([Akinwale *et al.*, 2011](#)). The results showed that correlation among leaf gas exchange traits

revealed a very highly significant positive association of A with stomata conductance ($r = 0.46$; $p < 0.001$). Similar results were also reported by [Rajaona *et al.* \(2013\)](#); [Yamori *et al.* \(2020\)](#); [Silva-Pérez *et al.* \(2020\)](#) for *Jatropha*, rice, and spring wheat, respectively. The rate of photosynthesis is frequently controlled by stomata conductance, especially in situations when water is scarce. A positive correlation between A and g_s has been consistently documented in a wide variety of species and environmental circumstances ([Farquhar and Sharkey, 1982](#)). Additionally, the association between photosynthesis and stomata conductance was positive and linear, indicating the role of stomata conductance in photosynthetic CO₂ assimilation ([Cechin *et al.*, 2022](#)). The degree of the stomatal aperture (and consequently the physical barriers to the passage of gases between the air and the interior of the leaf) determines the stomata conductance, which measures the rate of gas exchange (i.e., carbon dioxide uptake) and transpiration (i.e., water loss) through the leaf stomata. Therefore, it depends on the density, size, and degree of stomata opening; larger and more open stomata permit higher conductance, which in turn suggests that transpiration and photosynthesis rates may be higher ([Pietragalla and Pask, 2012](#)).

Our results showed that the grain yield significantly increased with RL, but was not affected by A, suggesting that higher A does not necessarily lead to the higher grain yield. The relationships between root length and grain yield are perhaps due to increased nutrient acquisition from the soil ([Barber and Silberbush, 1984](#)). When the relationships of A with grain yield and agronomic traits have been evaluated, it has been found a negative significant correlation between A and grain yield ($r = -0.15$; $p < 0.01$). It had become increasingly clear that increasing crop yield was not closely associated with a rate of photosynthesis. This result was consistent with the findings of ([Evans, 1996](#); [Chen and Hao, 2015](#); [Sinclair *et al.*, 2019](#)). The increase in the grain yield was limited by the grain number and grain size (sink-limited) rather than by photosynthate production (source-limited), in agreement with the findings ([Richards, 2000](#)). The highest number of leaves in amaranths implied higher photosynthetic rates and subsequently increased photosynthetic allocation to other plant organs. This was in agreement with ([Sah and Zamora, 2005](#)). It has been suggested that enhanced photosynthetic efficiency and capacity are new features necessary for breeding programs to maximize cereal crop output potential ([Furbank *et al.*, 2019](#)). However, negative relationships between A and grain yield have been obtained in high-yielding genotypes ([Murthy and Singh, 1979](#); [Gifford and Evans, 1981](#); [Sadras *et al.*, 2012](#)). Moreover, contrasting results between A and grain yield have been reported by ([Moss, 1976](#); [Reynolds *et al.*, 2000](#); [Tang *et al.* \(2017\)](#)). Increased

photosynthesis cannot be achieved by agronomic or physiological means unless nitrogen intake from the soil, storage in vegetative tissues, or transfer to seed are improved (Barber and Silberbush, 1984).

The result of the correlation analysis showed that there were statistically significant correlations ($P < 0.001$) between chlorophyll a, b, total chlorophyll (a + b), and total carotenoid (x+c) contents of the plant studied. This implies that any increase in one of the components would correspond to an increase in the other components. Several studies showed that there is a correlation between the dominant pigment concentration and one or more chromatic parameters such as carotenoids in amaranth (Singh, 2010; Hamidzadeh Moghadam *et al.*, 2021).

5.4.9 Clustering of genotypes

Based on the average performance of 19 physio-agro morphological parameters, the 120 genotypes of amaranth under study were divided into seven distinct groups. Utilizing genetic diversity to boost yield may be achieved most effectively by grouping a large number of genotypes into distinct clusters according to their average performance. With a 97.5% overall success rate, the discriminant function analysis appears to have been successful in differentiating across groups. It is expected that genotypes inside a cluster will be more closely related to each other in terms of the attributes under examination than genotypes outside of a cluster (Franco *et al.*, 2001). Employing the mean performance of 19 physio-agro morphological parameters, seven distinct clusters were formed from the 120 genotypes of amaranth that were examined.

One of the most effective ways to use genetic diversity for yield enhancement is to partition a large number of genotypes into several groups according to their average performance. The discriminant function analysis's overall success rate was 97.5%, indicating that it was effective in differentiating across groups. In terms of the features under discussion, it is expected that genotypes inside a cluster would be more closely related to one another than genotypes outside of a cluster. This study found that out of the 120 amaranth genotypes, seven clusters developed. Nonetheless, there was a variance in the number of clusters reported by other researchers due to differences in genotype numbers, genetic variation, and analytical methods. Shankar *et al.* (2012) grouped 28 accessions into 12 clusters, Andini (2013) grouped 84 amaranth genotypes into IV clusters, Mbwapbo *et al.* (2013) grouped 14 amaranth genotypes into three clusters, Gerrano *et al.* (2015) grouped 32 amaranth genotypes into VI clusters, Idowu-Agida *et al.*

(2020) grouped 28 genotypes into IV clusters, [Nyasulu et al. \(2021\)](#) grouped 5 accessions into III clusters, [Yeshitila et al. \(2023a\)](#) grouped 120 genotypes into V clusters, ([Kamini and Sandilya, 2023](#)) grouped forty genotypes into VI clusters. The results of the current study indicated that the numbers of amaranth genotypes included in each cluster were generally variable due to their genetic variation and region of collection.

5.4.9.1 Performance of genotypes under different clusters

Clusters I and II had *Amarantus caudatus* L., *Amaranthus hybridus* L. subsp. *cruentus* (L.) *Theil*, and *Amaranthus hybridus* L. subsp. *hybridus* clustered together; such clustering is due to the common parentage of all these three-grain species. According to [Gupta and Gudu \(1991\)](#), *A. cruentus* and *A. hypochondriacus* are homologous in the grain *Amaranthus* because their shared trait of grain production indicates a higher degree of genetic similarity, while cluster IV had *Amaranthus hybridus* L. subsp. *cruentus* (L.) *Theil*, *Amaranthus palmeri* S. Wats, *Amaranthus hybridus* L. subsp. *hybridus*, and *Amaranthus spinosus* L. weedy and grain genotypes. This grouping is also caused by shared phenotypic variations or genetic similarities in terms of physio-agro-morphological traits. The phenotypic differences in many genotypes of *Amaranthus species* tend to vary in plant physio-agro-morphological markers. Weedy types such as *A. spinosus*, *A. viridis*, *Amaranthus palmeri* S. Wats, and *A. species* were the most dispersed in clusters IV, V, VI, and VII. According to clusters IV, V, VI, and V of our findings, weedy amaranths and cultivated grain may have undergone natural introgression in Ethiopia. Our study demonstrated that cluster analysis was a useful technique for classifying the resources of Ethiopia amaranths according to how similar the 19 physio-morphological traits were. Furthermore, the second clusters were generally characterized by high leaf and grain yield-producing genotypes while the first cluster was characterized by a high value for the traits Ch a, PH, INL, and RW. The genotypes grouped in the fourth cluster had higher mean performances in VPD, Ch a, and TCh. This cluster also included the top-performing genotypes in terms of the above-mentioned traits and the mean values of these traits were also higher than the grand mean of the respective trait averaged over all clusters.

Cluster analysis clearly not only showed the geographic grouping of genotypes but also exhibited the co-occurrence of species in single clusters. On the other hand, because planting materials were exchanged across places, there was a lot of overlap among the species in each cluster. The transfer of planting material from one place to another might be the cause of the association that exists between geographical regions and clusters. This might also be explained

by the relationship between the similarity of adaptation and the quantitative clustering that was achieved from each site (Shukla *et al.*, 2010). Additionally, they revealed that a pair of genotypes was formed from plant materials obtained from the same site, suggesting that the 120 genotypes under study had similar adaptations.

5.4.9.2 Genetic divergence

The study's pairwise generalized square distance (D^2) revealed that, except for clusters C1 and C5, C2 and C3, C3 and C7, C4 and C5, C4 and C7, C2 and C5, and C1 and C3, C2 and C7, there was a substantial ($P < 0.05$) inter-cluster genetic distance between the clusters. Because the genotypes within a cluster performed similarly for the variables under study, the observed intra-cluster genetic distance was less than the inter-cluster genetic distance, suggesting that the genotypes inside a cluster exhibited restricted genetic variation. The findings of Pandey and Singh (2011), Ahammed *et al.* (2013), Kumar (2019), and Yeshitila *et al.* (2023a), indicating the intra-cluster genetic distance in amaranth genotypes was often smaller than the inter-cluster genetic distance are also in line with the aforementioned finding. For cluster C6, the intra-cluster genetic distance was comparatively large, suggesting that this cluster's genotypes were more diversified than those of any other cluster. Conversely, cluster C7's intra-cluster genetic distance was quite low, indicating that its genotypes were more homogeneous than those of other clusters and potentially useful for backcross breeding projects. Genetic distances intra and inter clusters as well as cluster means were taken into consideration while choosing a genotype for amaranth that performed better in the chosen cluster.

The genotypes classified in clusters C5 and C7 had a closer association than the genotypes grouped in other clusters, as demonstrated by the lowest and non-significant ($P > 0.05$) inter-cluster genetic distance between them. By choosing the cluster with the greatest inter-cluster distance, the varied and desired genotypes were found. More intimate links between genotypes in distinct clusters with non-significant inter-cluster genetic distance were predicted than between genotypes clustered in considerably distant clusters (Singh, 1985). It is possible that crossing low-distance clusters (clusters 5 and 7) will not result in a reduced range of variability in the segregating F2 population and a greater heterotic rating in F1. Clusters C2 and C6 had the most significant ($P < 0.05$) inter-cluster genetic distances, followed by clusters C1 and C6, C3 and C6, C5 and C6, and C6 and C7. This suggests that there are significant genetic differences across these clusters and that selecting genotypes according to the goal of

production from these clusters may boost output. It is believed that the greatest genetic divergence between these clusters will result in the greatest amount of genetic recombination and a variation in the crossover of parents chosen from these genetically remote groups. Nonetheless, the unique traits of each cluster should be taken into account when choosing parents from these far-off clusters, and the effectiveness of each genotype within each cluster should be taken into account based on the specific purposes of crossing/ hybridization (Singh, 1990; Kabir *et al.*, 2013). The genotypes found in clusters C4 with C6, C2 with C6, C1 with C6, C3 with C6, C5 with C6, and C6 with C7 are predicted to cross and provide relatively better genetic recombination and segregation in their progenies since crosses of parents grouped in divergent clusters are expected to result in maximum genetic recombination and variation in the subsequent generation. Therefore, crossing genotypes from different clusters may produce a high degree of heterosis, which might be used to enhance amaranth (Yeshitila *et al.*, 2023b). From crossings involving parents from clusters characterized by the greatest distance, maximal genetic variation and recombination are predicted in the subsequent generations (Rezene *et al.*, 2011; Sawarkar *et al.*, 2015; Abdella *et al.*, 2022; Kebede *et al.*, 2023a; Tomar *et al.*, 2023). Based on group performance as well as the performance of individual genotypes for particular characteristics, parents from each cluster can be chosen if they possess one or more of the desired features (Singh, 2016). With a wide range of variability in segregating generations and a strong heterotic impact in the first generation, genotypes aggregated in different clusters were heterogeneous and could be exploited for a hybridization program (Ravindra Dangi *et al.*, 2018; Rathinavel, 2019; Chauhan *et al.*, 2020; Yeshitila *et al.*, 2023b). Moreover, In the diallel selective mating system, additive gene variance and increased genetic recombination were employed. For heterosis breeding, genotypes belonging to clusters C2, C3, C4, C5, C6, and C7 might therefore be employed as possible parents. In amaranth, significant heterosis has been seen (Kumari *et al.*, 2023). For the yield per plant, heterosis over superior parents was reported by Pandey (1984) to be 71.36%.

5.4.10 Principal component analysis

Principal component analysis (PCA) preserves data covariance while simplifying datasets. In the majority of population genetic investigations, PCA is utilized as the initial analysis of data exploration and data description (Lazaridis *et al.*, 2016). The PCA analysis demonstrated that amaranth genotypes exhibit significant genetic diversity. The first two PCs contributed more than the other PCs, even though each PC contributed to the overall variance in a different way. In this study, the top two PCs accounted for 39.4% of the overall variance, whereas the third

(13.02%), fourth (11.33%), fifth (9.37%), and 6.17% PCs contributed less. [Elhaik \(2022\)](#) also reported that the first few PCs generally contributed more to the total variation.

[Yeshitila et al. \(2023b\)](#), reported that the first six PCs (80.41%), [Yadav et al. \(2022\)](#) from three PCs 91.45% accounted more for the total variation in amaranth genotypes. [Tabachnick et al. \(2013\)](#) criteria state that characteristics with eigenvector absolute values more than 0.45 added more to the overall variance in that specific PC, whilst characteristics with values less than 0.45 were given less weight because of their negligible contribution to the overall variation. The observed variation in the first PC was mainly contributed by PH, INL, RL, RW, Ch a, Ch b, TCh, and Ca while gs, A, E, Ch b, Ca, PH, LY, and INL had a high contribution to the total variation in the second PC, because the eigenvectors of these traits were higher than the cut-off limit as suggested by [Tabachnick et al. \(2013\)](#). Nonetheless, it was generally thought that characteristics with eigenvector absolute values that are closer to unity inside the first PC had a bigger effect on clustering than characteristics with lower absolute values that are closer to zero ([Chahal and Gosal, 2002](#)). In this study, however, all the studied traits individually contributed less (0.018 to 0.780) to the total variation in the first PC, and, therefore, the differentiation of the genotypes into different clusters was rather dictated by the cumulative effects of the traits under consideration. Furthermore, when the characteristics under consideration had lower values of eigenvectors in the first PC, [Wakeyo \(2012\)](#) found that the combined effects of the traits had a large contribution to the classifications of genotypes into various clusters. The eigenvector's sign shows how characteristics are related to PCs. A negative correlation between a characteristic and the given PC is shown by a negative eigenvector for that trait, and vice versa. As a result, the first PC was positively correlated with the remaining attributes, but RH, Ci, and WUE were negatively correlated with it.

The first two PCs were used in a biplot analysis to assess the contribution of the attributes to the overall genetic variance among the genotypes. A trait's significance to the overall variance in a given PC may be determined by measuring the length of the trait vector that emerges from the plot's origin in two dimensions. According to [Naidoo et al. \(2020\)](#), characteristics that were shown nearer the biplot's center contributed less to the overall variance than characteristics that were shown farther from the center. In the biplot analysis, the length of the vector lines and their proximity to the axis are often related to the size of the vector loadings. The contribution of a trait to the total variation in the first PC is significantly higher for traits with longer and longer vector lines in the first two PCs that are closer to the X-axis than for traits with shorter

and longer vector lines that are farther from the axis, respectively. In contrast to E and A, which likewise had longer vector lines but were farther from the X-axis, the features of INL, PH, RW, and RL, for example, had longer vector lines that were closer to the X-axis. This suggests that in the first PC, as opposed to E and A, the contributions of INL, PH, RW, and RL to the overall variance were larger. On the other hand, E and A's vector lines were longer and positioned closer to the Y-axis, suggesting that their feature contributed more to the second PC than the other attributes. Due to the small cosine of the angle between the traits and the corresponding PC, RW in the first PC and E in the second PC had a notable contribution to the overall variation. Typically, the cosine of the angle between a vector and an axis indicates the importance of the contribution of the corresponding trait to the PC.

An additional indicator of the relationship between the respective traits is the cosine of the angle between vector pairs. Smaller angles between vectors in the same direction indicated the presence of a high correlation between the characteristics, which is consistent with the findings of (Asante *et al.*, 2019; Naidoo *et al.* (2020); Kebede *et al.*, 2023b; Yeshitila *et al.*, 2023b), who reported that smaller angles between vectors in the same direction showed the presence of a high correlation between the traits. In contrast to uncorrelated traits, which were almost perpendicular to one another, correlated traits had a high narrow cosine of the angle between vectors pointing in comparable directions. Therefore, gs, E, VPD, and, A were clustered, as demonstrated by the biplot loadings, suggesting that these characteristics have a significant positive correlation. The vector lines Ci from the third group and LY from the second group, as well as E from the fourth group with A and LY from the second group, have an acute coordinate angle (90°) that suggests a non-significant negative connection with these respective traits. Furthermore, there was a substantial negative correlation between the features of the fourth group (Ch b, E, gs, and A) and the vector line of RH, which was found in the third group, that made a high obtuse angle ($>90^\circ$).

5.5 Conclusions

The results of this study indicate that there may be significant genetic diversity in the 120 genotypes of *Amaranthus species* for physio-agro-morphological parameters. This genetic

variety might be a valuable resource for breeding initiatives that target increasing amaranth yield. The study also highlights the importance of selecting traits with high heritability and additive gene effects for successful genetic improvement. Moreover, the study produced information that will help breeders of amaranth choose complimentary parents from various clusters, which will enrich the genetic material available for breeding programs including intra- and inter-species hybridization. The notable heterogeneity seen between amaranth genotypes for defined variables provides more evidence of the potential of amaranth genotypes to improve leaf, grain yield, and yield-related traits in future breeding initiatives.

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**CHAPTER SIX: Genetic Diversity and Population Structure of Ethiopian Amaranths
(*Amaranthus species*) as Revealed by Inter Simple Sequence Repeat (ISSR) Marker**

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Genetic Diversity and Population Structure of Ethiopian Amaranths (*Amaranthus* species) as Revealed by Inter Simple Sequence Repeat Markers

Abstract

The potential of *Amaranthus* spp. is still under-exploited in Ethiopia. This is also evident from the limited genetic research and selection for desirable traits in amaranths. Therefore, the number of genotypes being cultivated is small, thus reducing the genetic variability of the elite germplasm and ultimately increasing potential vulnerability to biotic and abiotic stresses. In addition, the amaranth genotypes are poorly represented in gene banks. Therefore, it is important to broaden the germplasm collection of this crop and assess the genetic diversity existing within the germplasm to support future collection, conservation, and crop improvement programs. The present study was conducted to determine the genetic diversity and population structure of 40 amaranth genotypes using four inter-simple sequence repeat (ISSR) markers. The polymorphic information content (PIC), marker index (MI), resolving power (RP) and effective multiplex ratio (EMR) showed average values of 0.46, 10.41, 4.74, and 4.22 per primer, indicating high polymorphism values. The degree of polymorphism among the genotypes ranged from 0.00% for the Amhara population to 100% for the Southern Nations, Nationalities, and Peoples' Region population, with a grand mean of $70.83 \pm 11.34\%$. The observed number of alleles (N_a), effective number of alleles (N_e), heterozygosity expected (H_e), and genetic diversity estimated by Shannon's information index (I) were 1.51, 1.50, 0.28 and 0.42, among collection regions, respectively. The total genetic diversity, H_t (0.400 ± 0.006), and the average intra-population genetic diversity, H_s (0.285 ± 0.004). A high level of gene flow ($N_m = 2.12$) between populations implies the presence of gene flow ($N_m > 1$) and reflects high genetic differentiation ($G_{st} = 0.281$). The analysis of molecular variance showed that the maximum value of genetic variation was found within populations (99%), whereas a low value of genetic variance was observed among populations (1%). The structure analysis, principal coordinate analysis (PcoA), and unweighted pair-group method with arithmetic averages (UPGMA) analysis clustered the 40 amaranths genotypes into four distinct subpopulations. The majority of genotypes (35%) were clustered in Pop2, mainly obtained from Southern Nations, Nationalities, and Peoples' regions constituted dominantly of breeding lines and varieties, implying target selection contributed to the formation of distinct populations. The findings of this study provide important and relevant information for future breeding and conservation efforts of amaranths. Therefore, the use of molecular markers would be valuable for the effective utilization of amaranth in breeding programs.

Keywords: Diversity, Population, polymorphism, Alleles, ISSR

6.1 Introduction

Variability is the primary capital for plant breeders in improving plant characteristics. Genetic variables are the cause of the variability exploited in plant breeding [Azrai et al. \(2018\)](#). Greater trait genetic variety will enhance the possibility of achieving a desirable good trait combination, which will increase the effectiveness of the program in plant breeding ([Hapsari et al., 2018](#)). Genetic diversity is the base for crop improvement and the existence of crop plant in nature ([Bhandari et al., 2017](#)). The ability to effectively identify and incorporate genetic diversity from a variety of plant genetic sources—such as newly developed cultivars, cultivated cultivars, wild relatives of cultivated cultivars, and germplasm collections containing elite and/or mutant plants—is essential to the success of crop improvement. Plant breeding is still a labor- and resource-intensive process, but it has become more accurate and

efficient with the use of various genomic technologies and breeding techniques in introducing genetic variety into marketable crop cultivars (Vaughan *et al.*, 2007). Diverse plant genetic resource enables plant breeders to develop or improve crop varieties with desirable qualities. While developing new cultivars, due consideration must be given to the farmers' preferences, such as high-yielding varieties, quality, and resistance to diseases and insect pests. Only around 200 of the thousands of plant species that have been domesticated since agriculture started some 12,000 years ago have been widely farmed, which has resulted in reliance on a limited range of genetic diversity in crops (Meyer *et al.*, 2012). Comprehending the molecular underpinnings of fundamental biological events in plants is imperative for the proficient preservation, management, and use of plant genetic resources. In particular, basic science and applied fields like the effective management of agricultural genetic resources benefit greatly from a sufficient understanding of the genetic diversity that currently exists, where it is distributed in plant populations, and how to effectively use it. Crop genetic resources can only be improved via the constant introduction of wild relatives, traditional varieties, and contemporary breeding methods. All of these procedures need some sort of diversity evaluation to choose resilient, extremely fruitful cultivars.

The majority of the food crops that grace the African continent are rich in diversity, but they have gotten little to no attention when it comes to research and the creation of frameworks for policies that might support their efficient commercial and industrial use. Among these underappreciated and neglected species is amaranth (*Amaranthus spp.*). Amaranth (*Amaranthus spp.*) is a climate-smart, nutrient-dense, and ancient crop with a high degree of genetic variety and environmental tolerance. It is an African leafy vegetable that is native to the continent and has several natural health-promoting qualities that are beneficial for human consumption (Zhu, 2020). Due to its ability to grow in a variety of environmental conditions, the amaranth is a possible underutilized food crop. This crop is tolerant to drought and possesses significant market and industrial potential that has not yet reached its full potential (Akin-Idowu *et al.*, 2016). Moreover, in Africa, *Amaranthus species'* potential remains mostly untapped. The scant genetic studies and selective breeding for desired features in amaranths also support this. Moreover, modern genetic improvement initiatives are focused on a variety of phenotypic selection and utilize material with a restricted geographical origin. Because of this, there aren't many genotypes being cultivated, which lowers the elite germplasm's genetic diversity and eventually increases its susceptibility to biotic and abiotic challenges (Olaniyi, 2007). Furthermore, there is a lack of representation for amaranth genotypes in gene banks.

As a result, it's critical to expand the crop's germplasm collection and evaluate the genetic diversity found therein to assist breeding initiatives.

The assessment of genetic diversity across populations in various phytogeography regions is crucial because a species' ability to survive and adapt to changing environmental conditions primarily depends on the degree of variability present in its gene pool (Ray and Roy, 2009). Additionally, the variety found in a given species is used in plant breeding to generate novel varieties. For *Amaranthus species*, morphological features have been utilized for diversity study (Yeshitila *et al.*, 2023). Some characters' adaptability and changes brought about by their surroundings are the reasons behind these characters' limits. In addition, the restricted number of morphological markers to cover the whole genomic regions of a plant makes them less suitable for use as markers (Nadeem *et al.*, 2018).

Molecular markers are becoming essential instruments for accurately and reproducibly characterizing plant genotypes and clarifying genetic variability (Parita *et al.*, 2018). Plant breeders might distinguish between genotypes by assessing the degree of variation within and across species using molecular phylogenetic investigations, which would offer a foundation for suitable breeding techniques. Thus far, various molecular markers have been developed, extensively utilized, and categorized into multiple classes, such as repeated amplified polymorphic DNAs (RAPDs) (Anjali *et al.*, 2013), restriction fragment length polymorphisms (RFLPs) (Park YoungJun *et al.*, 2014), simple sequence repeats (SSRs) (Wang and Park, 2013), amplified fragment length polymorphisms (AFLPs) (Chandi *et al.*, 2013), inter-simple sequence repeats (ISSRs) (Štefůnová *et al.*, 2014). Even though they are all based on various premises and have different appealing characteristics, their purpose is to draw emphasis to the variety across the genome (Agarwal *et al.*, 2008). The main drawbacks of these techniques include the expensive nature of AFLP analysis, the low reproducibility of RAPD, and the requirement for flanking sequence knowledge to design primers specific to a given species for SSR polymorphism (Pradeep Reddy *et al.*, 2002; Gupta *et al.*, 2010). SNPs appear to be highly intriguing markers as well, but they demand a significant investment in labor and technology. Therefore, it is extremely improbable that universities and national agricultural institutes in countries that are developing are going to embrace SNPs (Ramakrishnan *et al.*, 2016).

ISSR markers were developed from the need to explore microsatellite repeats without the use of DNA sequencing (Lagercrantz *et al.*, 1993). The technique is based on the amplification of

DNA segments between two microsatellite repeated regions (Zietkiewicz *et al.*, 1994) ISSR appears to produce reliable and highly polymorphic band profiles. The key additional feature of ISSR lies in its technical simplicity and speed. ISSR analysis is quick, reproducible, and generates sufficient polymorphisms to have the potential for large-scale DNA fingerprinting purposes (Prevost and Wilkinson, 1999).

Moreover, intersimple sequence repeats (ISSR) are one of the most straightforward and popular marker types among polymerase chain reaction (PCR)-based marker techniques (Kunjupillai, 2005). According to the Mendelian hypothesis, two phenotypes can be identified at each locus for ISSR markers, which are regarded as dominant markers (Tsumura *et al.*, 1996). Ratnaparkhe *et al.* (1998), suggested that ISSR markers could provide important information for genome analysis of plant species as a marker associated with disease-resistance gene clusters. This marker involves the amplification of genomic DNA by PCR using a primer typically attached at the 5' or 3' end with 14 arbitrary, often degenerate bases extended into the flanking sequences (Zietkiewicz *et al.*, 1994). ISSR markers offer great potential for differentiating closely related cultivars and the generated bands are very repeatable on duplicate samples (Fang and Roose, 1997). Plant breeding, the identification of DNA markers connected to agronomic features (Ratnaparkhe *et al.*, 1998), the evaluation of genetic diversity and phylogenetic studies of various species (Ajibade *et al.*, 2000), and the characterization of germplasm have all made use of ISSR markers (Pradeep Reddy *et al.*, 2002). ISSR markers are evolving into crucial instruments for analyzing plant genetic diversity. In tandem, they offer a thorough explanation of the kind and degree of diversity, as well as its genetic relationships and germplasm management (Sharma *et al.*, 2011; Kumar *et al.*, 2013).

Studying a crop's genetic diversity is essential for effective genotype selection, management, and utilization of germplasm for agricultural improvement. It is the most important step in crop-breeding programs to accelerate genetic gain and promote conservation. The degree of genetic variability within a species can be used to measure its ability to adapt and thrive in a variety of environmental conditions. In a similar vein, the level of genetic variety within a population serves as the foundation for germplasm selection in plant breeding and is a crucial aspect of crop development. Thus, preserving and employing genetic diversity in plants is crucial to ensuring human food security. Therefore, using ISSR markers, the study intends to ascertain the molecular genetic diversity and population structure of amaranth genotypes

gathered from several administrative regions of Ethiopia. In addition, PIC, MI, RP, and EMR analyses were conducted to assess the potential of ISSR markers to identify polymorphisms in Ethiopian genotypes of amaranth.

6.2 Materials and methods

6.2.1 Plant material

Seeds of 40 genotypes of *Amaranthus species* were collected in 2019 and 2020 from seven different administrative regions of Ethiopia and Melkasa Research Centre (Table 6.1) provided two released varieties. Further information about the methods used to get these particular samples, along with a thorough description and examination of the agro-morphological characteristics, can be devoured by [Yeshitila et al. \(2023\)](#). At the agricultural study site of Hawassa University, the seeds were planted in 2024 in the experimental field. Fresh leaves from two weeks old, seedlings of each genotype were used for DNA extraction.

Table 6.1 Sampling details of *Amaranthus species* obtained from different regions of Ethiopia.

Taxon ID	Region	Woreda/District	Logitude	Latitude	Altitude
SAA-004	Afar	Amibara	636126	1046188	726
K3A-005	Afar	Amibara	631670	1035541	738
SA-025/07	Afar	Amibara	624847	1026721	735
SHIA-007	Afar	Amibara	627807	1036840	735
HA-008	Afar	Amibara	634804	1044976	730
WA-001	Afar	Amibara	627878	1033651	740
212581	Amhara	Werebabu	581861	1245570	2920
242533	Benishangul & Gumuz	Kurmuk	634804	1044976	1250
242530	Benishangul & Gumuz	Kurmuk	665945	1167179	1250
242532	Benishangul & Gumuz	Kurmuk	665945	1167179	1250
SHL-040	Oromia	Shala	433633	805425	1662
208683	Oromia	Deder	758052	1043629	2270
208764	Oromia	Sayo	698483.15	943746.8	1850
91003	Oromia	Seka chekorsa	233325	835289	2040
			Released variety	Released	
AC-NL	Released	Released	Released variety	Released	1550-2400
			Released variety	Released	
MAND	Released	Released		Released	1550-2400
OTI-044	Sidama	Otilicho	431913	721434	1893
CHU-045	Sidama	Chuko	431835	721533	1910
WON-049	Sidama	Wondo	418632	699405	1743
DAL-043	Sidama	Dale	429799	738442	1790
HAW-041	Sidama	Hawassa	444076	776871	1750
SHD-001	Sidama	Shebedino	434789	761187	1874
DAL-042	Sidama	Dale	429799	738442	1780
YRB-002	Sidama	Yirba boricha	424075	769481	1857
KAZ-078	SNNPR	Karat zuria	327269	599337	1200
KAZ-057	SNNPR	Karat zuria	326722	609738	1214
KEN-020	SNNPR	Kenna	321817	585166	1753
KAZ-076	SNNPR	Karat zuria	327267	599336	1202

211457	SNNPR	Konso special	326440.36	589244.33	1560
ALE-024	SNNPR	Ale special	299885	597965	1368
ALE-073	SNNPR	Ale special	301337	604237	1693
KEN-064	SNNPR	Kenna	321816	585168	1762
KEN-021	SNNPR	Kenna	321818	585166	1752
KEN-014	SNNPR	Kenna	321816	585168	1690
BNT-026	SNNPR	Bena tsemaye	247228	615060	1618
KEN-018	SNNPR	Kenna	321785	585144	1761
KEN-016	SNNPR	Kenna	322071	584756	1745
MEK-081	Tigray	Mekele	552793	1489902	2143
MEK-083	Tigray	Mekele	552491	1490761	2151
MEK-080	Tigray	Mekele	552552	1490147	2162

6.2.2 DNA extraction and ISSR analysis

Genomic DNA was isolated from the young leaves of plants of each of the forty genotypes of amaranth was extracted from fresh leaves of each genotype using the cetyl trimethyl ammonium bromide extraction method (CTAB) described by (JJ, 1990) with minor modifications. One gram of leaf tissues was ground with usig mortar and pestle. Eight hudered microliter of 2% CTAB buffer per sample was added to the homogenized leaf material and mixed by gently shaking with hands for 5 minutes. The homogenized material in polypropylene Eppendorf tubes was incubated for 1 h at 65 °C in the water bath while shaking with hands for 3 minutes at 15-minute intervals. After cooling for 5 minutes, 500 µl of Chloroform: isoamyl alcohol (24:1) per each sample, and was shake for 5 minutes gently. This was followed by centrifugation at 12,000 rpm for 10 min at room temperature. Five hundred microliter of the supernatant was transferred to fresh Eppendorf tube to which 500 µl chloroform: iso-amyl alcohol (24:1)were added to each sample was added and then was shake for 5 minutes gently. The procedure for removal of proteins by treatment with chloroform: iso-amyl alcohol (24:1) mixture was repeated twice. The samples were then spun at 15,000 rpm for 10 min at room temperature (25 °C). The aqueous phase was transferred to a new Eppendorf tube. Then centrifuged for 15 minutes at 12000 rpm. Four hundred microlitter cold isopropanol was added to the aqueous phase to precipitate the DNA and then shaken gently with hands. DNA-CTAB complex was precipitated as a fibrous network and keep it for 1hr at -20C for 5 minutes. It was lifted by auto-pipette and transferred to other polypropylene tubes and centrifuged at 12,000 rpm for 10 min. After discarding the supernatant, 400 µl of 70% ethanol was added to the pellet and was mixed for 5 min with gentle agitation. It was then centrifuged at 15,000 rpm for 10 min at room temperature. The supernatant was discarded and the Eppendorf tubes were inverted and drained on a paper towel to completely dry the pellet. The pellet was air-dried for 30 min. Each pellet was re-dissolved in 100 µl of Tris–EDTA (TE)

buffer and all the genomic DNA samples were kept at -20°C overnight (12 hrs). To perform PCR-based analysis, The DNA was quantified using a Thermo™ Scientific NanoDrop™ spectrophotometer and visually verified via 0.8% (w/v) agarose gel electrophoresis.

6.2.3 PCR amplification and electrophoresis

The reaction master mix of 13 µl was placed in a thermocycler (MJ Research Model: PTC 100) and the PCR amplification was performed for 35 cycles with an initial denaturation at 96°C for 3 min followed by denaturation at 96°C for 30 seconds, annealing at 49°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes.

A PCR reaction comprised of 50 ngµl⁻¹ template DNA, 20 pmoleµl⁻¹ concentration of forward and reverse primers, 10X PCR buffer, 10 mM of dNTPs, 2.5 mM MgCl₂, and 1.0 U of Taq DNA polymerase. Eight ISSR primers were screened and four primers produced polymorphic and reproducible bands and used for further studies (Table 6.2).

The integrity of DNA was judged by agarose gel analysis using the following steps: 0.8% agarose gel was prepared in 100ml 1X TBE (Tris-borate EDTA) buffer containing 2.5µl of ethidium bromide. The gel was cast in the tray and allowed to solidify until the wells were properly formed. 20 µl of DNA samples along with 5µl a DNA ladder were individually loaded into each well after mixing them with a drop of gel tracking dye. The gel was run at 80 V for 90 minutes. After the gel was run to about 3/4th of the tray, the gel was removed from the cast and visualized under UV light. The presence of a single compact band at the corresponding band of the DNA ladder indicates that the DNA isolated is of high molecular weight.

Table 6.2 Primers used for differentiation of Amaranth genotypes using ISSR molecular marker

No	Primer name	Primer sequence (5'–3')	Primer size/ Molecular weight range (bp)	(G+C) content (%)	Annealing temperature
1	UBC 835	AGAGAG AGA GAG AGA GYC	350-2500	52.63	49°C
2	UBC 826	ACACACACACACACACC	500–1500	53.00	49°C
3	UBC 842	GAGAGAGAGAGAGAGAYG	355–2400	52.94	49°C
4	UBC 889	ACACACACACACACAC	385–2560	50.00	49°C

6.2.4 Data Analysis

ISSR fragments were scored based on a binary matrix for the presence (1) or absence (0) of bands leading to the construction of a data binary matrix (1, 0). Consequently, before doing a

diversity analysis the number of scored bands (NSB); the number of polymorphic bands (NPB) and the number of monomorphic bands (NMB). To analyze the suitability of ISSR markers to evaluate genetic profiles of *Amaranth* sp., the performance of the markers was measured using five parameters: polymorphic information content (PIC), mean resolving power (MRP), effective multiplex ratio (EMR), marker index (MI) and resolving power (RP).

Calculating the percentage of bands' diversity (polymorphism) in the starter was done according to the following equation:

$$\text{Polymorphism} = \frac{Np}{Nt} * 100$$

Where Np = number of polymorphic loci Nt = total number of loci

Resolving power (Rp) is a parameter used to characterize the ability of the primer combination to detect the differences between a large number of genotypes (Gilbert *et al.*, 1999; Prevost and Wilkinson, 1999).

$$RP = \sum Ib$$

Where I_b (band informativeness) takes the values of. The I_b can be represented on a scale of 0-1 by the following formula:

$$1 - [2 * (0.5 - p)]$$

Where p is the proportion of genotypes containing the band. Further, the mean resolving power (MRP) for each primer was calculated via

$$MRP = \frac{1}{n} \sum Ib$$

Where n is the NPB (number of polymorphic bands for that primer), I_b (Informativeness of a band).

PIC value was calculated according to (Roldan-Ruiz *et al.*, 2000; Soengas *et al.*, 2006), where PIC is the polymorphic information content of the locus i, f_i is the frequency of the amplified fragments (band present) and 1 - f_i is the frequency of non-amplified fragments (band absent). The frequency was calculated as the ratio between the number of amplified bands at each locus and the total number of genotypes (excluding missing data). The PIC of each primer was calculated using the average PIC value from all loci of each primer.

$$PIC = 2f_i(1 - f_i)$$

EMR (Effective multiplex ratio) = n*β

where n is the average number of bands amplified by genotypes to a specific system marker (multiplex ratio) β is estimated from the number of polymorphic loci (n_p) and the number of non-polymorphic loci (n_{np}), $\beta = n_p / (n_p + n_{np})$ (Powell *et al.*, 1996).

The range of PIC must be between 0-0.5 when using a dominant marker (RAPD-ISSR), a high value means a very good primer for genetic diversity between samples.

$$MI = PIC * EMR$$

Where the marker index (MI), a measure of the overall utility of a molecular marker technique, for each primer, was calculated as a product of two functions, the polymorphic information content and effective multiplex ratio (EMR) (Milbourne *et al.*, 1997; Nagaraju *et al.*, 2001). The higher the MI, the better the method is.

The mean effective number of alleles (N_e), expected heterozygosity (H_e), Shannon information index (I), and gene flow (N_m) for each ISSR marker and genotypes were estimated using GenAlEx 6.5 (Peakall and Smouse, 2006). The binary data matrix was analyzed using POPGENE version 32 (Yeh and Boyle, 1997) to examine different genetic diversity parameters including the number of polymorphic loci (PL), percentage of polymorphic loci (PPL), total genetic diversity (H_t), genetic diversity within populations (H_s), and (Nei, 1973) coefficient of genetic differentiation among populations (G_{st}) were calculated via

$$G_{st} = H_t - H_s / H_t$$

Whereas corresponding estimates of gene flow (N_m), i.e. the average per generation number of migrants exchanged among populations, were calculated based on (McDermott and McDonald, 1993).

$$N_m = 0.5 (1 - G_{st}) / G_{st}$$

To evaluate genetic variance, an analysis of molecular variance (AMOVA) was performed to determine the relative amount of variation explained among and within species (Excoffier *et al.*, 1992) was carried out using GenAlEx version 6.5 (Peakall and Smouse, 2006). Principle coordinate analysis (PCoA) to assess genetic diversity was also calculated (Mohammadi and Prasanna, 2003). A dendrogram was constructed based on the genetic distance, UPGMA (unweighted pair-group method with arithmetic averages) with software BIO-R_v3.2 (Pacheco *et al.*, 2016)

To determine genetic composition and account for population diversity, a population structure model evaluation was conducted with the STRUCTURE version 2.3.4 software (Pritchard *et al.*, 2000; Evanno *et al.*, 2005). According to Evanno *et al.* (2005), to assess the exact number of clusters through STRUCTURE HARVESTER, it was compressed into a single “Zip-Rar” file and then uploaded online to “Structure Harvester” (<http://taylor0.biology.ucla.edu/structureHarvester/>).

6.3 Results

6.3.1 ISSR Analysis

To generate DNA fingerprint profiles, eight ISSR primers were utilized. Based on their better amplification and apparent polymorphism across forty genotypes, the four primers, UBC 826, UBC 835, UBC 842, and UBC 889, provided a clearly amplified product and conspicuous banding pattern (Figure 6.1). The remaining primers were considered unsuitable due to poor amplification. The study hired primers with GC contents ranging from 50 to 53%.

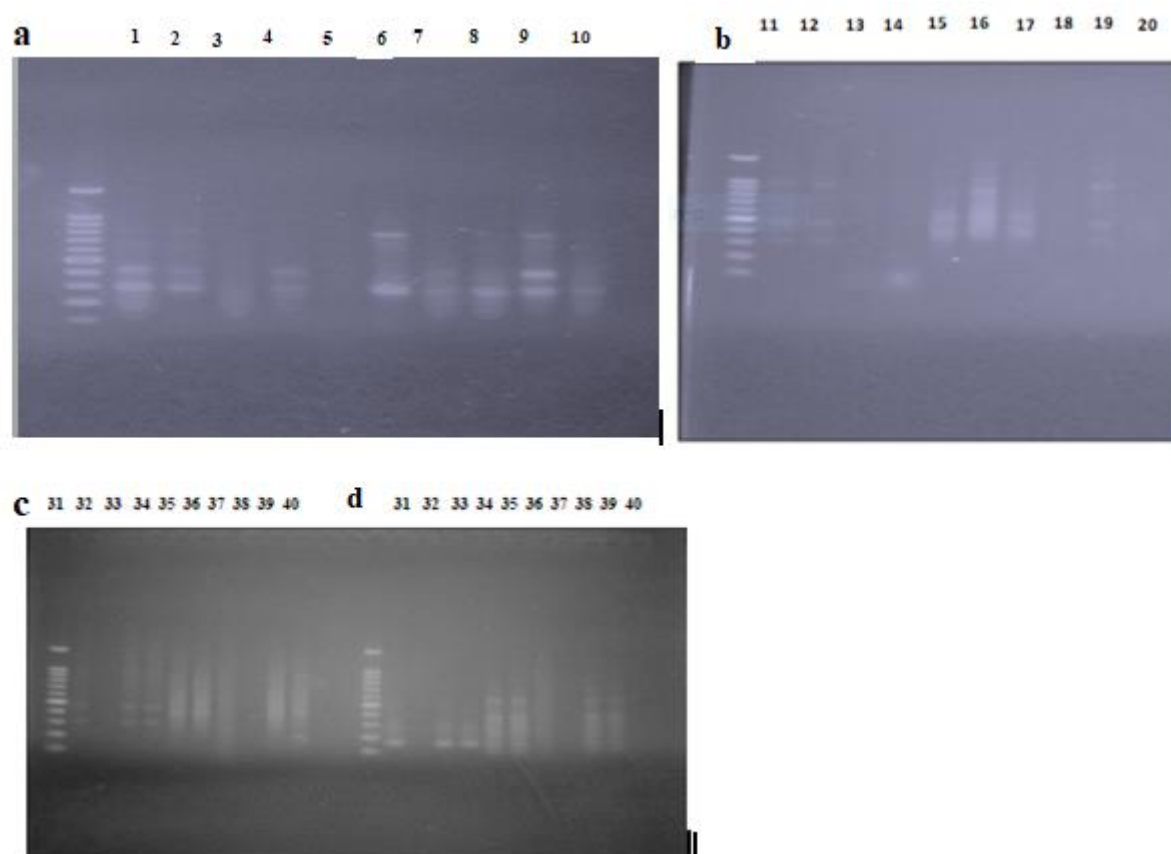


Figure 6.1 Example of banding pattern of primers (a) UBS 889 (1-10), (b) UBS 826 (11-20), (c)) UBS 826 (31-40); (d) UBS 835 (31-40), among amaranth genotypes using the ISSR primer.

6.3.2 Polymorphism and efficiency of ISSR markers

Generally, in our study, the efficiency of markers was determined by effective multiplex ratio (EMR), marker index (MI), and resolving power (Rp) (Table 6.3). Out of the 75 fragments generated by ISSR primers, 54 (72%) were polymorphic. With an average of 18.75 bands per primer, a set of 4 ISSR primers identified polymorphic bands ranging in number from 15 (UBC 835) to 22 (UBC 842 and UBC 889) of different sizes ranging from 350 to 2560 bp. In terms of amplicons, primer UBC 842 produced the highest yield (22) and primer UBC 835, the lowest (15). Between 45.45% and 100.00% for primers UBC 826 and UBC 835, respectively, the proportion of polymorphism varied, with an average of 74.43% for each primer. The results of the present study revealed that the average PIC value per primer was 0.46, with a high PIC value of 0.49 for primer UBC 826 and a low PIC value of 0.43 for primer UBC 889. With an average EMR of 10.41 per primer, the maximum effective multiplex ratio (EMR) of 15.00 was noted with primer UBC 835, and the lowest was 4.55 with primer UBC 826. The maximum resolving power (Rp) was 5.41, which was exhibited by primer UBC 826 while primer UBC 835 displayed a lower Rp value (3.05). MI values varied from 2.23 to 7.05, with a mean of 4.74. UBC 835 exhibited the highest MI value among the four ISSR markers used (Table 6.3).

Table 6.3 Primer sequence, total number of bands (NTB), number of polymorphic bands (NPB), number of monomorphic bands (NMB), percentage of polymorphic bands (PPB%), polymorphic information content (PIC) and marker index (MI), ISSR primer for 40 genotypes of *Amaranthus species*.

Primer No	Annealing temperature	NTB	NPB	NMB	PPB	MRP	PIC	EMR	MI	RP
UBC-826	49	22	10	12	45.45	0.54	0.49	4.55	2.23	5.41
UBC-835	49	15	15	0	100.00	0.20	0.47	15.00	7.05	3.05
UBC-842	49	16	12	4	75.00	0.34	0.45	9.00	4.05	4.05
UBC-889	49	22	17	5	77.27	0.26	0.43	13.09	5.63	4.35
Mean	49	18.75	13.50	5.25	74.43	0.34	0.46	10.41	4.74	4.22

Total number of bands (NTB), number of polymorphic bands (NPB), number of monomorphic bands (NMB), percentage of polymorphic bands (PPB%), PIC (polymorphic information content) = $1 - \sum p_i^2 - \sum q_i^2$, where q is the null allele frequency and p is the visual allele frequency. EMR (effective multiplex ratio) = $np / (np/n)$, where n is the total loci number and np is the polymorphic loci number; Rp (resolving power) = $\sum Ib$, where $Ib = 1 - (2 \times |0.5 - p|)$; MI (marker index) = PIC $\times$ EMR.

6.3.3 Genetic diversity analysis

Table 6.4 illustrates the findings from the genetic polymorphism analysis of various amaranth populations, including the number of effective alleles (Ne), observed alleles (Na), gene diversity (He), Shannon's information index (I), expected heterozygosity unbiased (uHe), number of polymorphic loci (PL), and percentage of polymorphic loci (PPL). The results showed that within the eight studied populations, the number of observed alleles (Na) ranged

from 0.33 in the population from Amhara to 2.00 in the population from the SNNPR region. The effective number of alleles (Ne), ranges from 1.00±0.00 (Amhara) to 1.69±0.06 (SNNPR) with an average of 1.50. The expected heterozygosity (He) lay between 0.00 for the Amhara region and 0.39±0.03 for the SNNPR regions with an average of 0.28. Shannon's diversity Index (I) extended from 0.00 for population Amhara to 0.57±0.03 for SNNPR regions. Based on the (He) and (I) values, Amhara and released varieties showed the lowest genetic diversity, whereas the results for the genetic diversity in Sidama and Afar were convergent. The analysis of the genetic polymorphism obtained from ISSR markers revealed that the SNNPR region's population owned the highest proportion of polymorphic loci (100%), the greatest count of polymorphic loci (18), the highest gene diversity (0.39±0.030), and the highest Shannon's information index and (0.57±0.03). The effective number of alleles (Ne) ranges from 1.00±0.00 (Amhara) to 1.69±0.06 (SNNPR) with an average of 1.50. The expected heterozygosity (He) lay between 0.00 for the Amhara region and 0.39±0.03 for the SNNPR regions with an average of 0.28. Shannon's diversity Index (I) extended from 0.00 for population Amhara to 0.57±0.03 for SNNPR regions.

Table 6.4 Genetic parameter estimates based on ISSR Marker among collection regions.

Region	Pop	Na	Ne	I	He	uHe	NPL	PPL
RV	1	1.44±0.17	1.39±0.99	0.34±0.07	0.23±0.05	0.31±0.7	10	55.56%
Sidama	2	1.67±0.18	1.64±0.08	0.51±0.06	0.36±0.04	0.38±0.04	15	83.33%
Tigray	3	1.39±0.22	1.61±0.11	0.44±0.08	0.31±0.05	0.38±0.07	12	66.67%
Afar	4	1.89±0.11	1.64±0.07	0.54±0.04	0.37±0.03	0.40±0.03	17	94.44%
Amhara	5	0.33±0.11	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.0	0.00%
BG	6	1.56±0.20	1.39±0.06	0.39±0.05	0.25±0.04	0.30±0.04	14	77.78%
Oromia	7	1.78±0.15	1.62±0.06	0.53±0.05	0.36±0.03	0.41±0.04	16	88.89%
SNNPR	8	2.00±0.00	1.69±0.06	0.57±0.03	0.39±0.03	0.41±0.03	18	100.00%
Grand mean		1.51	1.50	0.42	0.28	0.32	13	70.83±11.34

Na (different allele number); Ne (effective allele number) = $1/(p^2 + q^2)$; I (Shannon's information index) = $-1 \times (p \times \ln(p) + q \times \ln(q))$; He (heterozygosity expected) = $2pq$; uHe (expected heterozygosity unbiased) = $(2N/(2N - 1))He$ where, by assuming diploid binary data and Hardy-Weinberg equilibrium, $q = (1 - \text{Band Freq.})^{0.5}$ and $p = 1 - q$; P (percentage of polymorphic loci).

The current study's findings demonstrated that the number of detected alleles (Na) for the four ISSR markers used varied, ranging from 1.313 in the primer UBC 835 to 1.634 in the primer UBC 826, with an average of 1.490. The effective number of alleles (Ne) varies from 1.419 (UBC-835) to 1.550 (UBC-826), with an average of 1,490. The anticipated heterozygosity (He) ranged from 0.247 for the UBS 835 primer to 0.312 for the UBS 826 primer, with a mean value of 0.280. The Shannon's diversity index (I) increased from 0.363 for primer UBS 835 to 0.455 for primer UBS 826 (Table 6.5).

Table 6.5 The genetic diversity of amaranth revealed by ISSR markers

Primers	Na	Ne	I	He	uHe
UBC-826	1.634	1.550	0.455	0.312	0.353
UBC-835	1.313	1.419	0.363	0.247	0.282
UBC-842	1.500	1.503	0.428	0.291	0.335
UBC-889	1.531	1.487	0.393	0.271	0.305
Mean	1.495	1.490	0.410	0.280	0.319

Na (different allele number); Ne (effective allele number) = $1/(p^2 + q^2)$; I (Shannon's information index) = $-1 \times (p \times \ln(p) + q \times \ln(q))$; He (heterozygosity expected) = $2pq$; uHe (expected heterozygosity unbiased) = $(2N/(2N - 1))He$ where, by assuming diploid binary data and Hardy–Weinberg equilibrium, $q = (1 - \text{Band Freq.})^{0.5}$ and $p = 1 - q$; P (percentage of polymorphic loci).

The analysis of the inter- and intra-population genetic structure (Table 6.6) showed that the overall genetic diversity was ($H_t = 0.40 \pm 0.006$) and the intra-population genetic diversity was ($H_s = 0.285 \pm 0.004$). The genetic differentiation among the studied amaranth populations (G_{st}) was 0.281, with effective gene flow observed between populations ($N_m = 2.12$).

Table 6.6 Total number of alleles and Nei's analysis of gene diversity in subdivided populations in 40 amaranth genotypes

Primer	H_t	H_s	G_{st}	N_m
UBC-826	0.44	0.32	0.27	2.13
UBC-835	0.35	0.25	0.28	1.97
UBC-842	0.34	0.29	0.14	3.56
UBC-889	0.45	0.27	0.42	0.80
Mean	0.400±0.006	0.285±0.004	0.281	2.12

Total genetic diversity (H_t), genetic diversity within populations (H_s), coefficient of genetic differentiation among populations (G_{st}), and gene flow (N_m).

6.3.4 Analysis of molecular variance

The partitioning of variations within and among populations was analyzed by AMOVA, which revealed the occurrence of 1% of total genetic variations among the different regions. It implies that the remaining 99% was due to variations within a region (Table 6.7).

Table 6.7 Analysis of molecular variance (AMOVA) based on ISSR data in Amaranth sp. populations

Source	Df	SS	MS	Est. Var.	%
Among Pops	3	13.35	4.45	0.02	1%
Within Pops	36	152.52	4.24	4.24	99%
Total	39	165.88		4.29	100%
<i>PhiPT = 0.005</i>					

Df degree of freedom, *SS* sum of squares, *MS* mean of squares, *Est. Var* estimated variance components, *Var* total variance.

6.3.5 Genetic Distance and Identity

Based on Nei's unbiased genetic distance, and identity pairwise population matrix at the region level, average Nei's unbiased genetic distance was high (0.582) between released variety and Amhara populations and low (0.035) between genotypes of Sidama and SNNPR (Table 6.8

above). Genetic identity values were in the range of 0.559 to 1.000. The highest identity was observed between Southern Nations, Nationalities, and Peoples' Region and Oromia (0.979) populations; however, a lower identity was noticed between Amhara and Released varieties (0.559) populations (Table 6.8 below).

Table 6.8 Unbiased Nei's genetic distance (above diagonal) and identity (below diagonal) among and between populations and collection sites

Regions	Released variety	Sidama	Tigray	Afar	Amhara	Benishangul Gumuz	Oromia	SNNPR
Released variety	***	0.273	0.271	0.226	0.582	0.416	0.284	0.239
Sidama	0.761	***	0.160	0.092	0.325	0.057	0.041	0.035
Tigray	0.762	0.852	***	0.078	0.297	0.135	0.122	0.107
Afar	0.798	0.913	0.925	***	0.314	0.073	0.044	0.053
Amhara	0.559	0.722	0.743	0.731	***	0.322	0.303	0.265
Benishangul Gumuz	0.660	0.944	0.873	0.930	0.725	***	0.027	0.048
Oromia	0.753	0.960	0.885	0.957	0.738	0.973	***	0.021
SNNPR	0.788	0.966	0.899	0.948	0.767	0.953	0.979	***

6.3.6 Principal Coordinate analyses

The association between the various genotypes and the ISSR markers could be visually represented via principal coordinate analysis (PCoA) (Figure 6.2). Using principal coordinate analysis (PCoA), the relationship between the ISSR markers and the different genotypes could be graphically visualized. Based on ISSR marker data, the first three axes represent a 58.10% overall variation. 30.55% was the highest change reflected by the first axis (coord 1), whereas 17.18% and 10.36% were accounted for by the second (coord 2) and third axes (coord 3), respectively. There was no discernible genetic overlap in PCoA. Additionally, a collection of genotypes from several nearby regions was made up of 7 genotypes from Sidama and 2 released varieties; 6 genotypes from Afar, 3 genotypes from Tigray and 1 genotype from Amhara regions; 4 genotypes from Oromia, and 3 genotypes from Benishangul Gumuz regions. Remarkably, genotypes from Southern Nations, Nationalities, and Peoples' Region, only gathered together. This suggests that in both coordinates, genotypes from various administrative regions were grouped. In the present studies, the results of PCoA analysis were consistent with those of the cluster analysis. The genotypes were well distributed in the cluster, which could be due to the relevance of primers used and the wide variety between genotypes. The PCoA demonstrated that there is a robust genetic flow among the Afar, Amhara, Tigray; Oromia; and Benishangul-Gumuz regions, even though this did not follow their origins. Amaranth populations were expanding, according to PCoA data. The PCoA analysis revealed

groups that mirrored those found in the UPGMA clustering analysis as well as in the structure analysis, demonstrating the consistency of the study's findings.

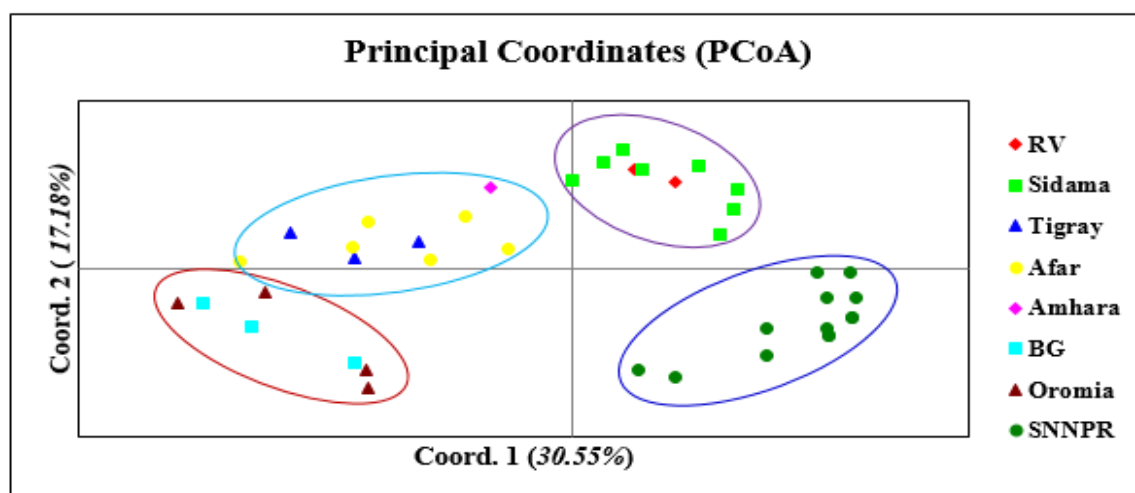


Figure 6.2 PCoA analysis of 40 *Amaranthus* genotypes of eight pre-defined populations using ISSR data based on two-dimensional coordinates. Benishangul Gumuz (BG), Southern Nations, Nationalities, and Peoples' Region (SNNPR)

6.3.7 Clustering analysis

The UPGMA-based dendrogram obtained from the binary data deduced from the DNA profiles of the samples analyzed revealed four distinct clusters out of the pooled ISSR marker data. The dendrogram derived from UPGMA analysis of the whole ISSR data based on regions of collection of *Amaranthus species* revealed four major groups, which mix proximal populations and in some cluster genotypes collected from the same regions clustered together (Figure 6.3 and Table 6.9). The first cluster-contained genotypes collected from Sidama, released varieties; while the second cluster is a representation of the genotypes collected from Southern Nations, Nationalities, and People Regions, and tend to form their grouping. The third cluster contains genotypes collected from the Afar, Tigray, and Amhara Regions. The fourth cluster contained the genotypes collected from Oromia and Benishangul-Gumuz. Like before, the PCoA analysis showed four main clusters confirming the results of the UPGMA clustering. The maximum and the minimum number of genotypes across the four clusters are 14 and 7, respectively. The first cluster consisted of 7 genotypes and two released varieties. Among the four clusters; the second cluster comprised the maximum genotypes (14). While the third cluster contains 10 genotypes. The fourth cluster consisted of the minimum number of genotypes (7). Additionally, there was strong evidence of gene flow between the

populations and grouping based on proximity in the clustering analysis (**Figures 6.2, 6.3, 6.4, and Table 6.9**).

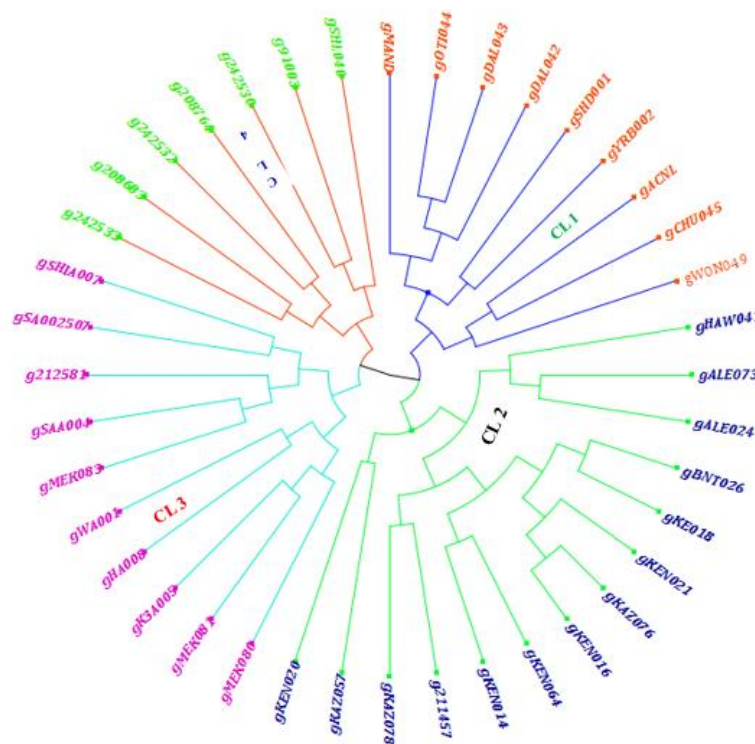


Figure 6.3 UPGMA dendrogram of the Jaccard similarity matrix representing the genetic relationships among the indicated amaranth genotypes.

Table 6.9 Clustering 40 *Amaranthus species* genotypes into four clusters using four ISSR primers.

Cluster	Number of genotypes	Genotypes	Regions
I	9	MAND,CHU045,,OTI044,WON049,SHD001,YRB002, DAL043, DAL042, ACNL	Sidama and released varieties
II	14	BNT026, KE018, KEN064, KEN021 KEN014, ALE073, ALE024, g211457 KAZ078,KAZ057,KEN020, KAZ076, KEN016, HAWA041	SNNPR
III	10	212582,K3A005,HA008,WA001,SAA004,SA0025, SHIA007, MEK083,MEK081, MEK080	Afar,Tigray, and Amhara
IV	7	242530,242533,242532, 208683, SHL040, 208764, 91003	Oromia and BG
Total	40		

6.3.8 Population structure

The program detected the highest value of delta k obtained from the structure harvester, $k = 4$. Based on the best $k = 4$, each assessed genotype is often positioned into four different as the

bar plot reveals (Figure 6.4A). Various subpopulations are represented by various colors. The Y-axis in the figure represents the percentage identity of the cluster to which it belongs. A genotype is considered to belong to a cluster if its membership coefficient is ≥ 0.80 ; if it is less than 0.80, the individual is considered admixed (Figure 6.4B). The 40 genotypes of amaranths were grouped into four subpopulations based on the result of the structural analysis (ΔK value at $K = 4$) according to [Evanno *et al.* \(2005\)](#), hereafter referred to as Pop1, Pop2, Pop3, and Pop4. Pop 4 constituted the lowest number of genotypes 7, with 17.5% of genotypes sourced from two regions (Oromia and Benishangul Gumuz) of Ethiopia. Among 40 genotypes, a larger percentage of genotypes in Pop 2 were from Southern Nations, Nationalities, and Peoples' Regions (35%). Subgroup 3 consisted of all genotypes from Afar, Tigray, and Amhara, whereas all genotypes from Sidama and released varieties were allocated to Subgroup 1 (Table 6.9).

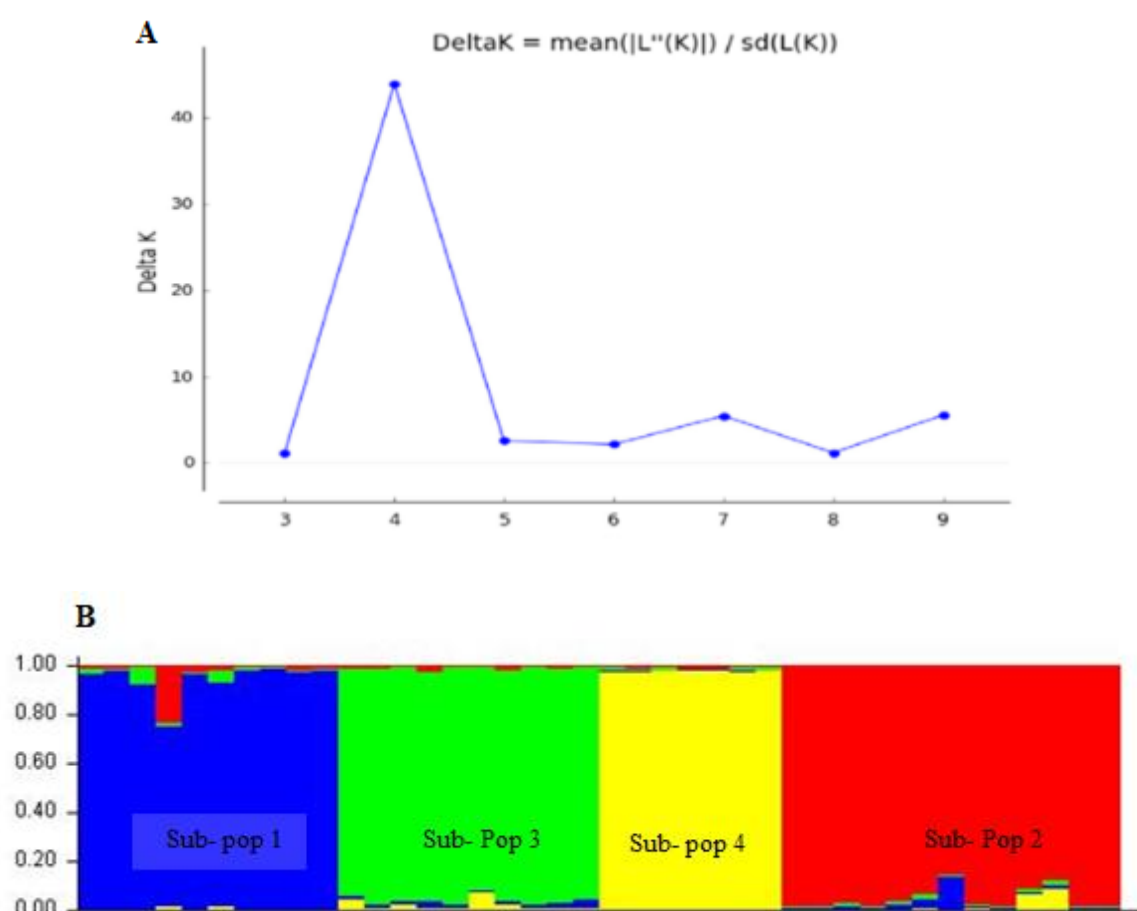


Figure 6.4 STRUCTURE output of 40 genotypes based on ISSR data. (A) Evanno's ad hoc statistic of the K scale. (B) Population structure of 40 amaranths genotypes at $K = 4$. Each color represents a specific population subset.

6.4 Discussion

Since the 1980s, the dynamics of plant breeding have evolved due to the advancement of molecular marker technologies (Hamrick and Godt, 1996). Plant genetics and breeding programs that carry the information for desired genes, alleles, or haplotypes are facilitated by a marker-based genetic map. To properly use and safeguard plant genetic resources and for fundamental research on evolution, it is imperative to evaluate genetic diversity and connections within and between crop species and their wild relatives (Govindaraj *et al.*, 2015; Brozynska *et al.*, 2016). The morphological traits of an organism are the basis of the classical phylogenetic method. *Amaranthus* exhibits a broad spectrum of morphological variants that overlap and are affected by environmental factors. Morphology alone is insufficient for classification (Lewis, 1957). These variables have led to the application of several molecular markers, including AFLP, RAPD, ISSR, internal transcribed spacer (ITS) of rDNA, and SSR in the genetic research of amaranth (Trucco and Tranel, 2011; Das, 2012). Selecting the molecular marker is a crucial step for researchers, particularly those working in genetics and breeding. Using ISSR PCR molecular markers, our study examined the genetic diversity of Ethiopian *Amaranthus* genotypes.

The potential of the markers to reliably assess genetic diversity will determine which type of molecular marker is appropriate for this species' genetic investigation (Roldan-Ruiz *et al.*, 2000). To determine the genetic variety of genotypes of *Amaranthus species* and their profile of ISSR genetic variation, we first evaluated the effectiveness of ISSR markers in the current study.

In the present investigation, each primer generated an average of 18.75 amplicons per primer. However, Saghir *et al.* (2022) found 27.4 bands per primer, which is higher than the findings of the present investigation. Overall, the primers employed in this work generated good amplified results and can be utilized for *Amaranthus species* diversity analyses. The ability of the markers to reliably assess genetic diversity is a determining factor in selecting the right type of molecular marker for the genetic study of the specific species (Roldan-Ruiz *et al.*, 2000). To determine the genetic variety of genotypes of *Amaranthus species* and their profile of ISSR genetic variation, we first evaluated the effectiveness of ISSR markers in the current study. The efficiency of a molecular marker system can be measured by the amount of polymorphism that can be detected among the genotypes under investigation. All four ISSR

primers used in this work revealed extensive polymorphism: in 18.75 bands (average number of bands generated per ISSR primer), 13.50 were polymorphic. [Raut et al. \(2014\)](#), [Oduwaye et al. \(2014\)](#), [Bayer et al. \(2017\)](#), [Ray and Roy \(2007\)](#), and [Labajová \(2013\)](#), reported 4.41, 6.57, 11.36, and 16.00 average bands per ISSR primers in amaranth genotypes, respectively, which are lower than the results of the present study. However, [Nolan et al. \(2010\)](#); [Saghir et al. \(2022\)](#) and [Saghir et al. \(2022\)](#) reported 19.00, and 27.3 27.4 bands per primer, respectively, which is higher than the findings of the present investigation. Overall, primers used in the present study gave good amplification and can be used in the diversity analysis in amaranth.

The average percent polymorphism among *Amaranthus* genotypes detected by the ISSR primers in the present investigation was 74.43%, which is lower than the findings of the previous reports, viz., 93.00% [Oduwaye et al. \(2014\)](#), 87.15% [Gelotar et al. \(2019\)](#), 86.88% [Raut et al. \(2014\)](#) and 80.4% ([Xu and Sun, 2001](#)), whereas it is much higher than the findings of few previous reports, viz., 20% [Mukhlif et al. \(2023\)](#) in maize and 64.77% [Mortada and Ragab \(2023\)](#) in quinoa genotypes using five primers of ISSR. As a result, it can be said that the primers employed in this investigation have good power to distinguish between the genotypes. The findings showed that the ISSR primers employed in this investigation had a high polymorphism percentage, making them suitable for use in evaluating genetic diversity.

It is also possible to assess the effectiveness of ISSR markers using parameters like PIC (polymorphic information content; [Roldan-Ruiz et al. \(2000\)](#) for AFLP), MI (marker index; [Varshney et al. \(2007\)](#) for SNP markers, SSR, and AFLP), and RP (resolution power; [Prevost and Wilkinson \(1999\)](#) for ISSR). The informative value of ISSR markers in various crops has been evaluated more and more through the use of parameters like PIC ([Gomes et al., 2009](#); [Grativol et al., 2011](#); [Saghir et al., 2022](#)). The PIC is determined by the number of detectable alleles, and the gene diversity is reflected in its frequency distribution. The PIC of the equally dispersed markers in the population was greater and stayed up to 0.5 for the dominating markers. Each marker's PIC value indicates its probability of identifying two randomly selected genotypes from the same area. These values can be as low as 0.0 for monomorphic markers and as high as 0.5 for markers that occur in half of the genotypes and not in the other 50%.

The average PIC value of all four ISSR primers in the current studies was 0.46, which indicates a good selection of primers in the present study for the assessment of the genetic diversity of

amaranth. The average PIC value per primer (0.47) is comparable to that found by [Oduwaye et al. \(2014\)](#) using ISSR markers in *Amaranthus species*. The high PIC values for ISSR primers in this study indicate the suitability and efficiency of the markers in the assessment of the genetic diversity of amaranth genotypes in Ethiopia, whereas they are much higher than the findings of a few previous reports, viz., (0.26) [Grativol et al. \(2011\)](#) in maize genotypes and (0.29) [Santhosh et al. \(2009\)](#) in cashew germplasm using ISSR primers.

The EMR was 4.55–15.00 among the 40 amaranths genotypes. [Hamidzadeh Moghadam et al. \(2023\)](#), also found EMR values of 3.00–6.00 using the ISSR primers in *Amaranthus species* from Iran. The higher EMRs illustrate how helpful the marker system is for investigation with an emphasis on diversity ([Powell et al., 1996](#); [Goulão et al., 2001](#)). The marker index (MI), which is a broad indicator of how well a polymorphism is detected in the current investigations, varied between 2.23 and 7.05 (average 4.74), depending on the ISSR primer used. The MI is used to calculate the complete effectiveness of marker systems, and a higher value is always associated with better effectiveness of the procedure ([Goulão et al., 2001](#)). In the current study, the high value of MI was derived from the high average EMR value of 4.74. In contrast to the current study, [Saghir et al. \(2022\)](#) found an average EMR value of 3.055 in thirty-nine rose accessions. The values of resolving power (RP) in this study ranged between 3.05 and 5.41 (average 4.22), suggesting that the set of ISSR primers used was capable of distinguishing among different genotypes. A proficient marker system's potential to discriminate between various genotypes is one of its key characteristics. As stated by [Prevost and Wilkinson \(1999\)](#), the Rp is a metric to assess how well a primer can distinguish between genotypes in ISSR molecular markers. Gene-to-genotype differentiation is typically better with primers having higher resolving power (RP) values.

Maximum values of indices about genetic diversity (N_a , N_e , I , H_e , and PPL) were reported for the Southern Nations, Nationalities, and Peoples' Region. This result indicates that this region could be an important source of diversity for breeding projects which can benefit from new alleles and genes of interest. A large amount of variability was observed in these sampled populations, exhibiting high intraspecific genetic diversity. Our results are in agreement with the findings of [Moghadam et al. \(2021\)](#) in amaranth genotypes in Iran. [Nei \(1978\)](#), stated that G_{st} is classified as low when $G_{st} < 0.05$, medium when $0.05 < G_{st} < 0.15$, and high when $G_{st} > 0.15$. G_{st} is equivalent to the weighted average of F_{st} for all alleles ([Nei, 1973](#)). Generally, G_{st} rates ranging from 0.15 to 0.25 signify a high degree of genetic heterogeneity

or inter-population divergence between groups. Thus, the G_{st} coefficient of amaranths in the current studies ($G_{st} = 0.281$) would be considered high. G_{st} was determined using the Nei approach and is defined as the percentage of genetic diversity that exists among populations (Nei, 1973). The *Amaranthus species* also had a gene flow N_m value greater than 1 ($N_m = 2.12$), indicating that our sampled populations were not subject to genetic drift (JD, 1991). According to Waples (1987), N_m can be classified into three categories: high (defined as $N_m \geq 1$), intermediate (defined as N_m values between 0.25 and 0.99), and low (defined as N_m values between 0.00 and 0.25). Furthermore, Slatkin and Barton (1989) pointed out that it makes sense to prevent significant differentiation from occurring as a result of genetic drift if the gene flow value is higher than 1. According to Wright (1949) if $N_m > 1$, the genetic differentiation is little, and if $N_m < 1$, the genetic differentiation is high. If $N_m > 1$, genetic differentiation does not result from genetic drift only, but if $N_m < 1$ it results from genetic drift only (Slatkin and Barton, 1989). Consequently, in the present study, a significant genetic variation among populations may be caused by the outcrossing pollination phenomena. The high proportion of crossover and high degree of differentiation between populations and species are confirmed by these values, which show that there is, on average, more than one migrant individual every generation between populations and between species. The interactions of several evolutionary processes, such as changes in dispersion, alteration of habitat and population isolation, mutation, genetic drift, mating systems, gene flow, and natural selection, are reflected in the genetic differentiation of a species. Significant population variations may result from breeding systems, genetic drift, geographic isolation, or community fragmentation. In contrast to our findings, low N_m and G_{st} have been detected in amaranth species using ISSR markers by Ruiz Hernández *et al.* (2018) in Mexico and in SSR markers by Suresh *et al.* (2014) in the Republic of Korea.

The AMOVA revealed that most of the genetic diversity occurred within populations (99%), while the genetic diversity among populations was 1%. This indicates that *Amaranthus species* is a relatively outcrossing species. The high value of within-population genetic variation in *Amaranthus* sp. is following other studies in different plant species (de Almeida Pereira *et al.*, 2015). Overall, the obtained results demonstrate that ISSR markers are suitable for use in genome analysis and genetic diversity studies of *Amaranthus species*, as they efficiently generated polymorphism even in closely related genotypes.

The PCoA identified between the species and their variations more precisely as it was based on a genetic distance matrix. The first three axes accounted for 58.10% of the genetic variance in these PCoA data, which shows a strong correlation with the UPGMA clustering. Notably, [Ray and Roy \(2007\)](#) have previously endorsed cluster analysis for ISSR primers using principal coordinate analysis (PCoA). In the recent studies of [Gelotar *et al.* \(2019\)](#), [Oduwaye *et al.* \(2014\)](#), and [Moghadam *et al.* \(2021\)](#), PCoA analysis has been used for the diversity analysis using ISSR markers, which supported the cluster analysis. The variation in floral morphology particularly lays the foundation for the divergence of the species ([Yeshitila *et al.*, 2023](#)). The PCoA analysis results in the current studies coincided with the cluster analysis results. Because of the great variation in genotypes and the significance of the primers employed, the genotypes were well distributed. Based on the ISSR marker, four clusters of genotypes were identified and analyzed. Genotypes that had been collected in the same area were grouped. The UPGMA dendrogram obtained from the ISSR separated the amaranth genotypes relative to their genotypes collection area. This may indicate that certain traits are more likely to be found in specific geographic areas than others. According to [Shukla *et al.* \(2010\)](#), and [Akin-Idowu *et al.* \(2016\)](#) there is a comparable pattern of vegetable amaranth strains from the Tarai and Himalayan regions and grain amaranth in South West Nigeria grouping together into the same clusters. The grouping of amaranth genotypes from Southern Nations, Nationalities, and Peoples' Region, Afar, Oromia, Tigray, and other States suggests that they might be acclimated to the agroecological circumstances unique to the area. The STRUCTURE analysis also generally agrees with the observed clustering pattern although it suggested four genetic populations ($K = 4$) as the best representation of the genotypes studied. The STRUCTURE analysis also agreed with the dendrogram analysis in that four clusters were found in the population. The complementarity of this result suggests the representative nature of the used markers. [Gelotar *et al.* \(2019\)](#) reported similar results with the current study that is the congruency between dendrogram and PCoA results.

Prospects for sustainable breeding and efficient crop genetic material usage are generated by using genome sequencing technology to assess unexplored genetic resources across the genome ([Salgotra *et al.*, 2022](#)). While the entire genome of amaranths has already been sequenced, we recommend additional research on the genetic diversity and population structure of amaranths in Ethiopia that covers a wide geographic area and a larger sample size using single nucleotide polymorphism (SNP) markers to achieve better genome coverage and resolution.

6.5 Conclusions

The current study's molecular markers demonstrated significant genetic diversity within amaranths populations but little genetic diversity between populations, suggesting a high level of gene exchange among populations. These markers can be exploited for molecular breeding for the improvement of grain amaranth. Furthermore, the study generated data that producers and academics can utilize to better the preservation and sensible use of this important amaranths genotypes. Recognizing the genetic variation among and within Ethiopia amaranth populations will be a valuable resource for prospective germplasm conservation, maintenance, and exploitation.

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CHAPTER VII: Summary, Conclusion, Recommendation, and Suggestions for Future Research

Summary, Conclusion, and Recommendation

7.1 Summary and Conclusions

Malnutrition is one of the major public health problems in developing countries including Ethiopia. In Ethiopia, 36% of children under five years are underweight. Biodiversity such as the assessment of genetic diversity of underutilized crops with high nutritional values is promoted as a promising potential to alleviate malnutrition. Even though amaranths are quite popular, Ethiopia has not yet started any breeding programs. Ethiopia has a wide variety of amaranths, which presents a significant opportunity for genetic improvement. However, this enormous variety is either unexplored or has not yet been used to address the issue of malnutrition. Amaranths are considered easy-to-grow crops that may be harvested in as little as four weeks and are accessible year-round. These beneficial characteristics of amaranths have the potential to significantly reduce Ethiopia's malnutrition problem.

A breeding program's potential to succeed largely depends on the type and extent of genetic variation found in the genotypes; higher genetic variability would enable the selection of genotypes with higher chances of success. In order to effectively evaluate and utilize the agro-morphological diversity of 120 amaranth genotypes for selection, conservation, and future breeding efforts in the amaranth improvement program, this study was conducted. Additionally, agro-morphological qualitative characteristics of 120 genotypes of amaranth were assessed. This information is crucial for activities related to domestication, breeding, and propagation, as well as for the ongoing conservation of plant species' genetic resources. In a similar vein, in the 2021 cropping season, 120 genotypes of amaranth from Ethiopia were assessed for their physio-morphological features, genetic diversity, and growth performance. The exploited variability and diversity could be useful for trait-based breeding. The genotypes of amaranth that were collected from different regions varied in yield performances in our earlier investigations on agro-morphological variability. Consequently, 40 genotypes were

selected for molecular characterization out of 120 based on collecting area and yield performance. The following reflects the main findings from these four investigation studies:

Experiment I

The results of this study demonstrated the existence of considerable variation among 120 Amaranth genotypes evaluated based on markers of agro-morphological traits collected over two-year periods in Ethiopia. Furthermore, most of the traits were significantly affected by the test year, suggesting that the test year affects the traits differently. The majority of the assessed traits also showed significant variance in the genotype-by-year interaction. There were estimates of very high to moderately high heritability, high to moderate genetic advance, and genetic variability for the traits BLBL, AIL, LA, BN, PHF, PHM, SD, DF, GFP, and LW. This suggests that selection based on these features may be successful in generating the needed genetic gains for improvement. These traits are thought to be more strongly influenced by additive gene action. By selecting genotypes with high leaf yield together with traits like increased LA, BN, PHM, and PHF, it might be possible to boost leaf yield in amaranth genotypes. Grain yield improvement in amaranth genotypes, on the other hand, might be successful if the selection was carried out with high GY along with greater LA, AIL, TILL, and TISL. Because of this, the selected amaranth genotypes, KAZ-059, 225713, KAZ-058, and KEN-019, 242530, and 212890, had high leaf yields coupled with superior LA, LW, LL, LN, and PH. Conversely, genotypes KEN-016; KEN-020; KAZ-060; KEN-010; KEN-018; and 225715 generated high GY coupled with improved LN, LA, AIL, TILL, TISL, GSFR, and TSW. Therefore, these genotypes were selected to improve the genetic gain in the amaranth breeding program by boosting the genotypes' existing leaf and grain yield productivity. Before making the preferred genotypes available to the farming community, it will be crucial to evaluate the genotypes under various environmental conditions.

Information from correlation may be augmented with the use of route analysis to select features that significantly increase the grain and leaf yield of amaranth genotypes and hence enhance selection efficiency in breeding. The studies of phenotypic and genotypic correlation and path coefficients revealed that LA was the variable that was most crucial for the indirect selection of LY since it exhibited a strong positive direct influence on LY as well as a substantial positive correlation with LY. Furthermore, it was suggested that LY in amaranth genotypes might also rise by an indirect selection of PHM, LL, and TILL, which exhibited such potent indirect influences, mostly through LA, and DM and DE, which showed such

strong indirect effects, primarily through PHF. Therefore, this study pointed out that traits that showed significant positive indirect impacts via LA should also be taken into account as indirect selection criteria for LY improvement in amaranth genotypes. Similarly, the studies of correlation and path coefficients demonstrated strong direct impacts of GSFR on GY, and noteworthy positive correlations of both traits with GY were seen at the phenotypic and genotypic levels. Additionally, PHF strongly influenced GY at the genotypic level and had a substantial positive correlation with GY. At both the phenotypic and genotypic levels, the GSFR also had a significant indirect favorable impact on GY, mostly through DF and LY. This showed that selection mostly focused on DF, LY, and GSFR would ultimately enhance the GY in amaranth genotypes. However, because DE has detrimental direct impacts on LY and GY at the genetic level, breeding progress in these genotypes might be negatively impacted.

The expression of significant genotypic heterogeneity as well as environmental factors may help to explain the current investigation of the phenotypic variability shown in 120 amaranth genotypes in Ethiopia. The first two principal components had a high contribution to the total variation in amaranth genotypes. Among the studied traits, PHM, BLBL, LL, DM, DF, LA, AIL, BN, TILL, DE, TLBL, SD, TISL, GY, GSFR, GFP, TSW, LW, PHF, LT, and LY all had a high contribution to the total variation in amaranth genotypes. Therefore, the selection of these traits could be effective for yield improvement in amaranth genotypes. Moreover, the occurrence of remarkable variation among amaranth genotypes for measured traits indicates the potential of amaranth genotypes in future breeding programs for improving LY, GY, and yield-related traits. Similar to how PCA emphasized the agro-morphological aspects contributing to the components, the biplot further splits the genotypes and associated attributes into two primary principal component axes. Cluster analysis grouped the genotypes into five clusters, based on the performances of measured traits. High-leaf yield-producing genotypes were found in the fourth cluster, but high-grain yield-producing genotypes were identified in the first cluster. The genetic divergence also showed that the clusters' variability existed. The second and third clusters had the greatest inter-cluster genetic distance, followed by the first and third clusters, and the first and second clusters had the smallest inter-cluster genetic distance. The morphological and agronomic distinctions revealed in this study might be used as pre-breeding tools to get Ethiopia's Amaranth genotypes breeding program off to a good start.

Furthermore, the fact that amaranth genotypes exhibit notable variance for the attributes that are examined suggests that amaranth genotypes may be used in future breeding efforts to enhance grain production, forage yield, and yield-related traits.

Experiment II

The diversity patterns among the 120 genotypes that make up our amaranth diversity array are examined using qualitative traits in this study. This study lays the groundwork for Ethiopian amaranth genetic advancement since crop improvement and selection may make use of the variety that was identified. The large genetic diversity among genotypes revealed by the morphological qualitative features utilized in the current study will help with the classification, improvement, and preservation of genetic resources. The majority of the investigated characters showed similarity across collection regions. Still, some phenotypic traits, including germination rate, inflorescence density index, growth habit, spines in leaf axils, sex type, branching index, and inflorescence color, were found to be variable and contributed to regional differentiation. To distinguish genotypes in various growing regions, these traits might be utilized as important markers. Future actions for collection, conservation, and development should be based on real diversity and give equal weight to the growing areas since the genotype variability within the expanding areas is larger, making all regions a potential source of valuable genes. Additionally, growing genotypes with distinct traits, a broader genetic basis, and unique identification may be achieved by selecting for the diversity and distinctive qualities found. Likewise, some qualitative characteristics such as amaranth's leaves provide a special source of antioxidant pigments such as β -cyanin, β -xanthin, and betalain. The development of amaranth varieties can use anthocyanins, carotenoids, and chlorophylls as well as naturally occurring antioxidant phytochemicals like vitamin C, phenolic acids, and flavonoids, among others. This is significant for the food industry because these phytochemicals promote health and act as natural preservatives in food items. Furthermore, the existence of genetic variation was shown by the Shannon-Waver diversity index (H') in many qualitative traits. Therefore, it can be declared that a higher degree of divergence was detected among tested genotypes based on H' . Clusters I and III, then II and III, had the greatest distance between them. Because of this, heterozygous offspring can be produced. The first two main components contributed a significant portion of the variance in amaranth genotypes. A substantial amount of the variance in amaranth genotypes was contributed by the characteristics SLA, GH, SX, BI, SSH, SP, LP, IDI, and IC. Moreover, the findings also have breeding implications for improved grain or vegetable amaranth variants, particularly for

leaf and blossom color uniqueness while selecting for economically relevant seed color features. Therefore, the selection of these traits could be effective for the improvement of amaranth genotypes. This finding indicates that broad collection from a range of altitudes and origin locations is far more valuable than amaranth collection from particular agro ecology, indicating that it catches significant allele variation from varied growth situations. Because of this, this material might be improved by conventional breeding (direct selection) or by using modern methods to investigate the genetic underpinnings of the phenotypic variations of these features. With enough variability to allow for the selection of genotypes for desirable characteristics in Ethiopian breeding programs for amaranth, these genotypes may offer a starting point for the production of amaranth of Ethiopian genotypes.

Experiment III

The current study revealed significant variation in the genotypes of amaranth species in terms of physio-morphological characteristics and growth performance. This variability is essential for selecting amaranth plants for genetic improvement. The study showed that certain genotypes (ALE-073) exhibited better intercellular CO₂ concentration (Ci), leaf-to-air vapour pressure deficit (VPD), transpiration rate (E), and leaf number (LN), resulting in better grain yield. Therefore, these genotypes can be selected for direct production to increase grain yield productivity, as well as for further study in diverse environmental settings in preparation for commercial release. Additionally, selecting genotypes with higher leaf area (LA) could help enhance leaf production. Understanding the relationship between LA and E can help in selecting crops for high E and may provide an avenue to improve leaf yield. Furthermore, some of the selected genotypes in this study could be used as potential parents for improving the genetic gain in amaranth breeding programs. The study found that Ch a, Ch b, TCh, and Tca markers had 100% heritability, indicating the presence of additive gene action. This demonstrated that selection based on these features, which suggested an exploitable amount of variation, would be more successful and efficient than utilizing other traits to separate generations in breeding programs in the future. The VPD, Ci, E, WUE, Ch a, Ch b, TCh, Tca, LA, PH, LN, INL, RW, RL, LY, and GY showed estimates ranging from extremely high to moderately high heritability with high GAM. Selection based on the phenotypic performance of these features would be promising for improvement since traits with such high heritability in conjunction with robust genetic advances are dominated by additive gene effects. Better genetic gain was generated by a combination of resilient genetic advances and high

heritability, suggesting that phenotypic selection may be a useful strategy for enhancing the desired traits. In conclusion, the study suggests that selecting appropriate genotypes of amaranth can lead to better grain yield productivity and enhanced leaf production. The findings also provide useful information for breeding programs aimed at improving the genetic gain in amaranth. The study also highlights the importance of selecting traits with high heritability and additive gene effects for successful genetic improvement. To sum up, no correlations were found between grain yield and leaf photosynthetic rates, which may attest to the infrequency of relationships between grain yield and single-leaf photosynthesis indicating that improved photosynthetic efficiency and capacity may not always lead to increased cereal crop output potential.

The study has generated findings useful for amaranth breeders to select complementary parents from different clusters that could form a better material for genetic improvement through intra-and inter-species hybridization breeding programs. The species groupings were independent of the genotypes' geographical origin. This is likely a result of germplasm origin being registered as where the seeds were donated from, which may not be the actual origin of the genotypes or movement of germplasm in recent historical times. The performance of the evaluated attributes led to the cluster analysis's grouping of the genotypes into seven groups. High leaf and grain yield-producing genotypes were included in the second cluster. Another sign that there was variation among the clusters was the genetic divergence. Genetic distance between clusters was found to be lowest between clusters five and seven and largest between clusters two and six. Furthermore, the characteristics under investigation contributed differently to the overall diversity in amaranth genotypes. The entire variance in amaranth genotypes was mostly accounted for by the first two main components. PH, INL, RL, RW, Ch a, Ch b, TCh, and Ca were the characteristics that were analyzed; on the other hand, gs, A, E, Ch b, Ca, PH, LY, and INL contributed significantly to the overall variance in amaranth genotypes. In a nutshell, selecting these features in amaranth genotypes may be advantageous for boosting productivity. A further indication of the promise of amaranth genotypes for improving leaf yield, grain production, and yield-related features in future breeding endeavors is the noteworthy variation seen across amaranth genotypes for identified variables.

Experiment IV

The understanding of genetic diversity is significantly essential not only for the conservation of many species of plants and animals but also for the maintenance of genetic diversity within

the populations. ISSR marker is one of the powerful tools, which has helped in understanding genetic variations through polymorphism of band patterns among different genotypes. In the present study, genetic variation between genotypes was observed based on the ISSR marker. Moreover, the study showed that clustering and population structure analysis is a powerful method for characterizing genotypes. It was also able to access and evaluate some polymorphic ISSR markers, which can be effectively used in future genetic analysis studies and molecular breeding programs. Further, this study confirmed that genetic diversity exists in the Ethiopian amaranth genotypes. In addition, this study provides information about geographical relationships and genetic structuring of amaranth genotypes and it shows that the amaranth genotypes can have good value as a source of genetic diversity for improvement programs of amaranth in Ethiopia. Overall, the findings point to the possibility of future breeding initiatives taking advantage of the genetic diversity present in Ethiopian amaranth genotypes and developing and putting into practice suitable conservation plans, such as the development of core collections and upcoming national collecting missions. Finally, using ISSR markers, this work is the first to evaluate genetic variation in multiple genotypes of amaranth distributed throughout various regions of Ethiopia. The findings demonstrated a very high degree of genetic diversity, with values for diversity (polymorphic bands) and similarity (monomorphic bands) of 1% and 99%, respectively.

7.2 Recommendations and Suggestions for Future Research

7.2.1 Recommendations

- Top grain yielder genotypes: KEN-016, KEN-020, KAZ-060, KEN-010, KEN-018, and 225715 have the largest LN, LA, AIL, TILL, TISL, GSFR, and TSW. The biggest LA, LW, LL, LN, and PH are found in the top genotypes for leaf yielding, which are KAZ-059, 225713, KAZ-058, and KEN-019, 242530, and 212890. Thus, if selection is done, genotypes could succeed. However, this experiment has to be repeated in several Ethiopian locations to be recommended for similar agroecologies and different amaranth-growing places.
- The phenotypic coefficient of variance (PCV) was higher in magnitude than the genotypic coefficient of variance (GCV) for all the characters of agro morphological as well as physio morphological studied markers except Ch a, Ch b, TCh, and Tca, which suggests

that the variation is not only due to genotypes but also by influence of environment up to some extent.

- The highest estimates of genetic advance as a percentage of the mean were obtained for agro-morphological characters namely PHM, LA, BLBL, PHF, GFP, BN, SD, LL, DF, AIL, TISL, GSFP, LW, and GY. The high value of genetic advance for these traits showed that these characteristics are governed by additive genes and selection will be rewarding for the further improvement of such traits.
- The top five characteristics among all agro-morphological characters were plant height at maturity, leaf width, leaf length, days to maturity, and basal lateral branch length, which together accounted for the majority of the overall divergence. Thus, it can be concluded that involving these traits will be beneficial in improving the yield of *Amaranthus* and used in future improvement programs of *Amaranthus*.
- Most of the agro-morphological features had genotypic correlation coefficients that were greater than phenotypic correlation coefficients, illustrating an inherent relationship between the traits.
- The highest estimates of genetic advance as a percentage of mean were obtained for physio-morphological characters namely for dry leaf yield, leaf-to-air vapor pressure deficit, root length, internode length, the CO₂ concentration of sub-stomata CO₂ concentration, photosynthetic water use efficiency, leaf number, plant height at maturity, transpiration rate, grain yield, leaf area, total chlorophyll, chlorophylls a, carotenoids, root weight, and chlorophylls b. The high value of genetic advance for these traits showed that these characteristics are governed by additive genes and selection will be rewarding for the further improvement of such traits.
- The ISSR molecular markers employed in the current research are thought to be effective in analyzing population genetic diversity, choosing matrices, and maintaining germplasm banks. They may also aid in the genetic enhancement and conservation of populations of *Amaranthus species*.

7.2.2 Suggestions for Future Research

The results of this study allow the suggestions that follow to be made for future *Amaranth*s enhancement initiatives.

- The genotypes under investigation may be assessed in Ethiopia's various agro-climatic zones to determine their stability and actual performance
- Carry out nutritional analysis to find out the nutrient contents of these lines
- To be used in future research, more genotypes may be collected from other unexplored regions of Ethiopia.
- Additional molecular-level genetic diversity studies using high-resolution molecular markers should be conducted to screen more variability, find some desirable genes, and establish a core collection for conservation and breeding programs.
- Genome-wide association study (GWAS) should be implemented to identify markers associated with important chemical traits.

BIOGRAPHICAL SKETCH

The author, Mekonnen Yeshitila was born in Debrebrihan, Ethiopia. He attended his junior school at Koffelle and his secondary school at Hawassa Comprehensive Senior Secondary School. Then he joined Hawassa College in 1997 and graduated with a diploma in biology in 1999. After his graduation, he was employed as a teacher at Hawassa. After serving for five years, he joined Dilla University for BSc study and graduated in biology in 2009. He joined Dilla University in 2013 and graduated with MSc in botanical science in 2015. After his graduation, he served at different institutions, including Dilla College of Teacher Education, and published more than eleven scientific papers. Then, he joined Hawassa University in October 2019 to pursue her PhD degree in plant biotechnology.