MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF MORINGA (*Moringa stenopetala* L.) ACCESSIONS SEEDLING TO WATER STRESS UNDER GREENHOUSE CONDITION AT HAWASSA, SNNPR, ETHIOPIA

MSc THESIS

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Morphological and Physiological Responses of Moringa (*Moringa stenopetala L.*) Accessions Seedling to Water Stress under Greenhouse Condition at Hawassa, SNNPR, Ethiopia

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SCHOOL OF GRATUATE STUDIES

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ADIVISORS' APPROVAL SHEET

This is to certify that the entitled "**Morphological and Physiological Responses of Moringa (*Moringa stenopetala L.*) Accessions Seedling to Water Stress under Greenhouse Condition at Hawassa, SNNPR, Ethiopia"** submitted in partial fulfillment of the requirement for the degree of Master's with Specialization in Horticulture, the Graduate program of the school of plant and Horticultural science, and has been carried out by Mr. Gebre Garmame, Id. No SGS/HORT/006/10, under my supervision. Therefore I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

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We, the undersigned, members of the Boards of examiners of the final open defense by **Mr. Gebre Garmame** have read and evaluated his thesis entitled "**Morphological and Physiological Responses of Moringa (*Moringa stenopetala L.*) Accessions Seedling to Water Stress under Greenhouse Condition at Hawassa, SNNPR, Ethiopia",** and examined the candidate**.** This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of masters of Science in plant and horticultural sciences (Horticulture program).

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DEDICATION

I dedicate this work to my beloved parents, GARMAME GALGAYE BARISSO and KAMIYA GEBINO GEMECHU for their love and soft hearted.

STATEMENT OF THE AUTHOR

I, the undersigned candidate, declare that this MSc thesis is my original work and all sources of materials used for this thesis have been duly acknowledged. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Name	Signature	Date
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LIST OF ABBREVIATIONS AND ACRONYMS

AARC	Arbaminch Agricultural Research Center
AAU	Addis Ababa University
ABA	Abscicic Acid
ANOVA	Analysis of Variance
CRD	Completely Randomized Design
DPSMCHSAAU	Department of Pharmacology, School of Medicine, Collage of Health Science, Addis Ababa University
EBI	Ethiopian Biodiversity Institute
EEDO	Ethiopian Environment Development Organization
EFMHCAC	Ethiopian Food, Medicine, and Health Care Administration and Control
EIAR	Ethiopia Institute of Agricultural Research
EPHI	Ethiopia Public Health Institution
GLM	General Linear Model
Ho-AREC &N	Horn of Africa Regional Environmental Center and Network
MEF	Ministry of Environment and Forestry
NGO	Non-Government Organization
NMASNNPSMCHB	National Metrology Agency, South Nation Nationality people states, Metrology Center, Hawassa Branch
SAS	Stastical Analysis Software
SNNP	South Nation National People
WHO	World Health Organization

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Morphological and Physiological Responses of Moringa (*Moringa stenopetala* L.) Accessions Seedlings to Water Stress Under Greenhouse Condition, Hawassa, SNNPR, Ethiopia

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ABSTRACT

Moringa stenopetala L. is a plant sometimes called miracle plant due to its adaptability and versatility in use. Indeed, it is considered as a super food, store house of nutrients, remedy for more than 300 diseases, water purification material, oil and biogas source, post-harvest preservative, and climate change mitigate plant. However, in spite of its marvelous importance, the plant has not got research attention for morphological and physiological responses of accession to water stress. Therefore, a pot experiment was conducted to investigate the morphological and physiological responses of Moringa stenopetala L. accessions seedling to water stress under greenhouse condition from October to January, 2018 at the college of Agriculture, Hawassa University, Ethiopia. The experiment was consisted of three accessions (Konso, Arbaminch Zuria and Humbo) and four water stress levels imposed as irrigation interval (daily, 5 days interval, 10 days interval and 15 days interval watering) assigned in completely randomized design with three replications. The results revealed that accessions significantly differed for most of the morphological and physiological parameters studied in this experiment. The accession "Arbaminch Zuria" was higher in seedling height, stem collar girth, leaf area, root length, fresh and dry weights, total seedling fresh and dry weights, chlorophyll a, b and total and instantaneous water use efficiency than other accessions. Oppositely, the accession "Humbo" was lower in all parameters except root length, stomata number and instantaneous water use efficiency. The accession "Konso" and "Humbo" was stastically similar in root length and instantaneous water use efficiency. The accession "Konso" and "Arbaminch Zuria" was stastically similar in leaf number, root diameter, stomata number, length and width. Regarding to water stress, the experiment shown that, increased water stress significantly reduced stem collar girth, leaf area, root length, diameter, fresh and dry weights, total seedling fresh and dry weights, chlorophyll a, b and total. Additionally, fresh and dry leaf weight, photosynthetic rate, transpiration rate, stomatal conductance, and leaf relative water content were significantly influenced due to the interaction effect of accession and water stress. Accession "Arbaminch Zuria" grown under daily watering was higher in all parameters except leaf relative water content those indicated significant due to interaction. The present results therefore, indicated that accession "Arbaminch Zuria" with daily watering performs better in raising Moringa stenopetala seedling. As the experiment was a single trail with limited resources, study is still needed on more indigenous and exotic accession under different water stress including under field conditions.

Key words: *Accessions, Physiological responses, Seedling stage, Moringa stenopetala, Morphological responses and Water stress*

1. INTRODUCTION

The interest of food, nutrition and medicinal plant is putting intense pressure in the world (Jane*,* 2015; FAO, 2018). Therefore, focusing on nutrient full, medicinal, multi-purpose and drought tolerant plants is necessary. Surprisingly, Moringa *(Moringa stenopetala L.)* is one of the world's most valuable, multipurpose, nutritious and medicinal plants with fast growing under wide range of agro ecological adaptation (Daba, 2016).

The annual production rate of Moringa could succeed 1.1 to 1.3 million tons from 38000ha of land (Rajangam *et al*., 2001). In India, small scale farmers get a net income of about USD 1500 per hectare annually under adverse agro ecology (Rajangam *et al*., 2001). While Moringa production and productivity has been improved and gained with good agricultural management (Alatar, 2011; Muhl *et al.,* 2011). Under poor environmental and management conditions, Moringa could be affected and reduce its potential growth, development and functions by a number of biotic and abiotic factors.

While among numerous factors, water does an essential component for the plants life owes to the crucial role that water plays in all physiological processes (Majken *et al.,* 2005). Water comprises 80 to 95% of the fresh weight of most herbaceous plants and 50% of woody plants. The properties of water make it essential for many functions in plants such as: a constituent of most parts of plants, a solvent providing a good medium for biochemical reactions and transport, a reactant in different processes such as photosynthesis, respiration and other enzyme-mediated processes; and in the maintenance of turgidity in plants (Majken *et al.* (2005). However, during increased water stress, there is a reduction in water content, diminished leaf water potential and turgor loss, closure of stomata, inhibit photosynthesis, lead to dehydration, impair metabolism processes, decrease/and inhibit cell expansion, enlargement, growth and development of plants especially during early stage (Kamara *et al.,* 2003; Shao *et al*., 2008; Jaleel *et al.,* 2009).

To meet sustainable development goals (SDGs) like ending hunger, achieving food security, improving nutrition and promoting sustainable agriculture (FAO, 2018), massive important and wide range agro ecology adaptive plants such as Moringa can be focused and established on water shortage nurseries (Mall and Tripathi, 2017). But, before establishing seedlings for commercial production it is necessary to investigate if the selected accessions will positively respond, survive and grow under the water stressful environment so that investors can make the justification. Thus, giving attention to studies focusing on the selection of accessions is dispensable, particularly, taking the painful trend of global warming and shortage of water.

Previously, it has been stated that Moringa is drought tolerant plant (Daba and Adisu, 2017; Mall and Tripathi, 2017). But, do favor hot and humid environments and are thus found throughout a range of eco-zones from dry savanna to rainforests (Foidl *et al*., 2001). Despite displaying drought tolerant mechanisms, the plant has a capacity to save water in their stem and root to cope against water deficit. Nevertheless, different accessions collected from different area could probably have variations in genetic potential to water stress (Baiyeri *et al.,* 2015*;* Sale *et al.,* 2015; Jacob, 2016). As a result, proposing research comparing accessions under water deficit has advocated, being familiar with the extent of the accessions to water deficit and evaluating accessions through their morphological and physiological traits.

Moringa is highly heterozygous because it is highly cross pollinated in nature and there is a wide variability in morphological and physiological traits (Rajangam *et al*., 2001; Amoatey *et al*., 2012). Based on limited traits, several studies revealed that morphological and physiological variations of Moringa accessions are controlled by epigenetics (Kenneth, 2012; Rivas *et al.,* 2013; Jacob, 2016).

But not much is known about morphological and physiological traits of *Moringa stenopetala* to water stress (Sale *et al.,* 2015; Badran, 2016). Rather, the previous researches carried out so far have focused mainly on survey works conducted on the field to assess the traditional uses of *M. stenopetala* based on information from Moringa growers. Furthermore, research has been focused on its potential values for human and plant nutrition, water purification, livestock feed, dyes, herbal medicine and oil production (Mall and Tripathi, 2017).

These constraints directed us to evaluate different Moringa accessions under water stress conditions, regarding to each organ of the seedling. Since Moringa is medicinal plant containing different medical chemicals in each part of the plant (in leaf, stem, and root), focusing on those traits are very important to exploit its use (Daba, 2016). Therefore, advance information on different accessions of Moringa morphology and physiology is required to select superior and adaptive accession, and contribute to a better understanding of the plant's capacity standing to water deficit and provide breeding objectives.

In different countries, morphological (seedling height, stem collar girth, leaf number and area, fresh and dry matters) and physiological responses (stomata morphology, chlorophylls concentration, gas exchange, and leaf water status) to water deficit vary with accessions (Wafa, 2006; Kenneth, 2012; Rivas *et al.,* 2013; Baiyeri *et al.,* 2015; Jane *et al.,* 2015; Sale *et al.,* 2015; Badran, 2016). In Ethiopia, despite the versatility use of Moringa, research on morphological and physiological response of *M. stenepetala* accessions seedling under different water stress has not been conducted and documented (Ashenafi, 2014; Eyassu, 2014;

Sreepada and Vijayalaxmi, 2013). This caused lack of information and knowledge based on adaptability and superiority magnitude of accessions to water deficit for breeding program and commercial production of Moringa. Thus, to maximize the potential value of Moringa under the changing climate, there is a need to understand the physiological and morphological responses to water deficit. The main objective of this study was therefore, to investigate morphological and physiological responses of *Moringa stenopetala* accessions seedlings to water stress.

Specific objectives were:

- ✓ To study morphological responses of *Moringa stenopetala* accessions seedling to different water stress,
- ✓ To study physiological responses of *Moringa stenopetala* accessions seedling to different water stress,
- ✓ To determine watering interval for *Moringa stenopetala* accessions seedling.

2. LITERATURE REVIEW

2.1. Origin, Distribution, and Botanical Facts of Moringa

Moringa (Moringa stenopetala Lam.) is native to southern Ethiopia. It is widely distributed in different India states, Egypt, Philippines, Sri Lanka, Thailand, Malaysia, Burma, Pakistan, Singapore, West Indies, Cuba, Jamaica and Nigeria (Ponnuswami, 2002).

As indicated by Eyassu (2014) that, *M. stenopetala* is a tree 6-12 m tall with a diameter of 60 cm and a smooth bark with corky bark and soft white wood. The leaves are alternate, opposite, compound, and usually thrice pinnate. There are 2- 9 leaflets on the ultimate pinnules. These leaflets are thin, ovate to elliptic and 1 -2 cm long. The flowers are white and 1.5-2 cm long on spreading panicles. The pod is 15-30 cm long, pendulous, three-angled and has nine ribs. The seeds are three-angled and winged on the angles. Its pods are elongated, reddish with grayish blooms and twisted when the fruit is fresh.

2.2. Importance of Moringa

As stated by Mall and Tripathi (2017), Moringa is one of the marvelous plants with an enormous amount of benefits in the world. Moringa is a plant which is sometimes called a Miracle plant and Super food because of all its parts are used for “multi-purposes” and “nutritional and pharmacological” properties respectively. Numerous studies also reveal that, Moringa has a direct effect on agriculture (fertilizer, feed, vegetable and so on), nutrition, health, water, environment, sanitation and others (Daba, 2016). Due to its’ massive importance, wide range adaptability and resilience characteristics, Moringa has been named like “Miracle plant”, “Magic plant”. “Marvelous plant”, “Never die plant”, “Mothers of best friend “, and “The tree of paradise” (Mall and Tripathi, 2017).

2.2.1. Moringa as a source of food and nutrition

Moringa is a storehouse of the nutrients includes vitamins, minerals (iron, zinc), beta carotene, ascorbic acid, calcium, proteins and carbohydrates, amino acids, anti-oxidants and anti-inflammatory nutrients (Anwar *et al.,* 2007; Frison *et al.,* 2011; Mudzviti *et al.,* 2012). These nutrients serve for an essential function in medicinal, nutritional and therapeutic properties which meet the standards of the WHO (Daba, 2016*;* Olson *et al.,* 2016*;* Alli and Arumugam, 2017*;* Sharma *et al.,* 2017). In addition, Rockwood (2013) indicated that Moringa is 10 times the vitamin A of carrot, 7 times the vitamin C of orange, 17 times the calcium of milk, 15 times the potassium of banana, 25 times the iron of spinach, and 9 times the protein of yogurt. As a result, Moringa said to be super food over other agricultural products (Carlsen, 2010).

2.2.2. Moringa as an animal feed

According to Peter *et al.* (2016)*;* Elfadil *et al.* (2017*),* Moringa supplement improved feed intake, body weight and hematology of goats. The rich nitrogen source of Moringa leaf meal could further been exploited to boost sustenance and production of West African dwarf goats especially during periods of scarcity when fodder quality and quantity are low (Peter *et al.,* 2016). In addition, Melesse *et al*. (2011) concluded that, dry matter and crude protein intake and average weight gain of Rhode island red chicks increased with increasing levels of moringa stenopetala leaf meal. Thus, the supplementation of chicks’ diet with moringa stenopetala leaf meal could be an alternative feeding strategy in rural and peri-urban chicken production practices in moringa growing tropical regions. According to their result, they indicated also Inclusion of moringa stenopetala leaf meal had not cause any health problems.

2.2.3. Moringa as a medicine and water purification

Worldwide, recent reports claimed that, all parts of Moringa (root, leaf, flower, pod, and seed) are often referred as a remedy for more than 300 diseases (Daba, 2016). Among them, inflammation and infectious diseases has been frequently reported (Anwar *et al.,* 2007; Farooq *et al.,*2007*;* Mudzviti *et al.,* 2012; Ahmad *et al.,* 2014*;* Daba, 2016; Ramesh *et al.,*2016; Kaleab *et al.,* 2017).

As well, it has been reported that, Moringa plays a vital role in water purification (Farooq *et al.,* 2007). Ramesh *et al.* (2016) argued that, Moringa seed powder can be used cleaning dirty water in simple and quick method. It can remove bacteria in water up to 90-99% (Kansal and Kumari, 2014). Work done by Yasabie *et al.* (2014) concluded that, Moringa exhibited the most favorable results in water treatment than *Guar. gum* and *Jatroph curcas*.

2.2.4. Moringa as source of oil and biogas

The gas production from Moringa could be about 580 litters of gas kg-1 of volatile solids (Andinet *et al.,* 2010). The average methane content of the gas is 81 per cent (Andinet *et al.,* 2010; Asante *et al.,* 2014*;* Alli and Arumugam, 2017*).* According to Andinet *et al.,* (2010) report the physicochemical properties of *M. stenopetala* oil is found to comply with both the American and European standard EN 14214. The biofuel derived from Moringa oil is of higher quality compared to those derived from sunflower, soybean, canola and palm oils (Rashid *et al.,* 2008). Moreover, the production of oil by Moringa is higher than that by *Jatropha curcas*, *Aleurites moluccana* and *Pachira aquatic* (Silva *et al.,* 2010).

2.2.5. Moringa as a crop growth hormone

Surprisingly, Moringa leaf juice is rich with growth hormones, especially Zeatin that has been reported to increase the crop yields in the range of 10 to 45% (Muhammad*,* 2014). Rehman *et al.* (2017) reported also that when Moringa leaf extract applied to wheat plants increased plant growth, yield and yield components. Several researchers found similar results with different crops: Mvumi *et al.* (2012) and Emongor (2015) on onion and kidney beans, Muhammad *et al.* (2013) on tomato, Biswas *et al.* (2016) on maize, Mohammed *et al.* (2013) on onions, cereals to oil crops, from fiber to sugar crops and from forages to tuber crops.

2.2.6. Moringa as a post-harvest preservation

The use of plant materials as food preservatives apart from extending the shelf life of foods, are less toxic to humans and animals than synthetic or chemical preservatives (Enzo, 2007*;* Vinoth *et al.,* 2012). According to Irokanulo *et al.* (2015) it has reported that, the leaf and stem bark of the Moringa concentration could able to significantly extend the shelf life of the tomato fruit. They indicated that, tomato fruit treated with the Moringa leaf and stem bark powders remained unspoilt for 21 days.

2.2.7. Moringa for climate change mitigation

According to the study by Villafuerte (2009), the rate of Moringa tree to absorb carbon dioxide (CO_2) is fifty times (50×) higher when compared to the Japanese cedar tree and also twenty times (20×) higher than that of general vegetation. Furthermore, Mall and Tripathi (2017) also indicated that*,* the capacity of the *Moringa* tree is inspiring in mitigating the adverse effects of climate change. Besides, Finding by Assefa *et al.* (2015) revealed that, *M. stenopetala* is efficient to increase soil fertility.

2.3. Moringa Production and Status in Ethiopia

Eyassu (2014) reported that *Moringa stenopetala,* locally called Haleko/shifara; it is grown mainly for its food value (leaves as a staple vegetable) throughout the year even during dry season, when other source of vegetables is unavailable. The agroforestry production system has been adapted in southern Ethiopia, particularly around Konso, Gamo, Goffa, Burji, and South Omo areas for a century (Yohannes and Teshale, 2016). Now a day*,* it is hardly possible to find a household without a "Haleko*"* tree in Konso, Gamo, and Goffa homestead. Hence, *M. stenopetala* is a favorite and main component of the daily meal of the people at the area (Eyassu, 2014*;* Personal observation).

People in the Konso and Gamo Gofa zones have a long tradition of consumption of *M. stenopetala,* the leaf of the "Haleko" tree is eaten like a cooked cabbage (Yalemtsehay, 2002). Thenafter, it has been distributed to Wello, Shoa, Harargie, Sidama, Amhara and Tigria regional state, Fontenina (Wello), Dhera (Arsi) and Ziwai by International Livestock Center for Africa (ILCA) (Dechasa, 1995). Besides using Moringa as a food, the Konso and Hamer people use Moringa as muddy water clearance and traditional medicine to treat different diseases such as malaria, hypertension, stomach pain, asthema, gastrointestinal problems, diabetes, retained placenta in mothers, ear and eye infections, and common cold (Yalemtsehay, 2002; Biniam *et al*., 2016.

The multi-purposeness, adeptness in wide rage agro ecology, and national and international market access has been potential to increase production and productivity of Moringa. Furthermore, the awareness of the plant by the numerous Ethiopian institutions (EPHI, HoAREC &N, EFMHCAC, EBI, EIAR, MEF, DPSMCHSAAU, EEDO and NGOs) in the country (Ethiopia) made the Moringa production potential wide (Sreepada and Vijayalaxmi, 2013; Ashenafi, 2014).

However, several challenges of Moringa production in Ethiopia has been reported by different local and foreign reviewers and pointed out that, despite actual and potential roles of Moringa, *M. stenopetala* has been given a little research attention. Due to the limited improved varieties, research findings, infrastructures, innovation and technologies, there is no any modernized production technology of Moringa in Ethiopia. Hence the commercial production of Moringa in Ethiopia is still very informal and makes it difficult to get reliable information of production volumes and price (Sreepada and Vijayalaxmi, 2013).

2.4. Moringa Seedling Raising, Environmental Conditions and Management

For an improved growth and development of the plant, preferable selection of relevant species, accession, propagation methods, media, environmental condition and management (agronomic practices) are very necessary in the nursery to raise seedlings to enjoy entire use of Moringa (Palada *et al.,* 2007).

Moringa can be raised by seeds and cuttings (Jane, 2015). Comparative studies by Jane (2015); Prevost and Le (1997) had determined that, raising Moringa seedlings through seeds had better results on germination, growth and development than using cuttings. Moringa Seeds can be planted in nurseries, either seedbed or in polythene tubes. However, seedbed seedlings cost less to produce than potted seedlings, but are more susceptible to drought after transplanting (Franzel *et al.,* 2014). Polyethene tubes are therefore important to nursery making to avoid future loses (Sanchez *et al.,* 2006). According to Sanchez *et al.* (2006) seeds should be sown at a maximum depth of 2 cm because planting Moringa above 2cm deep, seeding will greatly reduce the germination rate. According to report by Kumar *et al.* (2011), extremely high and low temperatures may negatively affect the viability of embryos and availability of water. But, the optimum conditions maintained high germination percentage at intermediate rate. As reported by Alatar (2011)*;* Muhl *et al.* (2011), the optimum temperatures of Moringa seedling under greenhouse conditions are ranged from 20 to 35ºC, but can be survive up to 48 ºC. The study conducted by Anber and Abdulrahmn (2017) revealed that, under greenhouse, mid-October was better than mid-February for seed germination and seedling growth of Moringa species.

2.5. Morphological, Fresh and Dry Weights and Physiological Responses of Moringa to Water Stress

2.5.1. Morphological, fresh and dry weights response of different Moringa seedling to water stress

Moringa is highly heterozygous plant because it is highly cross pollinated in nature and there is a wide variability in morphological traits among accessions (Rajangam *et al*., 2001; Amoatey *et al*., 2012). Study by Jacob (2016) revealed that, qualitative and quantitative traits had affected by Moringa accessions. In addition, several scholars had indicated that, different accessions collected from different areas, significantly influenced growth parameters like plant height, stem girth, and leaf number (Baiyeri *et al.,* 2015*;* Sale *et al.,* 2015). These observations could probably be due to variations in the genetic potentials of the accessions and/or the inherent variability across the collection environments (Mridha, 2015; Muhammad *et al.,* 2016).

Seedling height, stem girth, root length and root diameter

Water stress is main factor at initial phase of the plant growth and development (Farooq *et al.,* 2009). It affects both elongation and cell expansion (Shao *et al.,* 2008). Similarly, Even if Moringa is said to be drought tolerant plant, its responded well to different water stress during seedling stage. In young Moringa, seedling height, stem girth decreased as water stress increased (Sale *et al.,* 2015). The same response had observed by Baiyeri *et al.* (2015) who concluded that watering interval significantly influenced stem girth of Moringa. The inhibition of cell expansion and cell growth is mainly due to low turgor pressure under water stress conditions (Shao *et al.,* 2008).

In drought tolerant plants, adaptation to water stress closely associated with root development, which provides plants with a better water extraction ability (Jongrungklang *et al.,* 2011). Increased root length with increased water stress was reported by Alyemeny (1998). However, in Moringa, Daba and Adisu (2017) indicated that root length had not significantly influenced by watering interval, while the highest root length had recorded from well watered seedlings. On the contrary, other report in other species claimed that, the development of roots was inhibited or stopped during water stress (Hamidou *et al.,* 2007b). With regards to root girth, Daba and Adisu (2017) indicated that Moringa root girth had significantly influenced when exposed to different watering intervals. This is due to the presence of different level of water stress in the soil. Thus, the seedling thickness is reduced as water stress (deficit) increased (Jaleel *et al.,* 2009).

Leaf number and area, total seedling fresh and dry weight

Moringa is mostly grown for its leave in most of the countries. Leaf is considered as an important factor in production of photosynthetic by products which serve as an indicator of potential prospective dry matter production. Leaf production and leaf expansion growth were reduced when plants were subjected to water stress (Majken *et al.,* 2005). According to Sale *et al.* (2015); Baiyeri *et al.* (2015) findings conducted on Moringa revealed that, increased water stress was found to reduce leaf number, area and leaf biomass per plant due to decreased production of new leaves, increased leaf shedding and reduced average leaf size.

Study by Farooq *et al.* (2009) indicated that, water stress reduces leaf growth which in turn reduces leaf area of plants. The high number of leaves on plants grown under unstressed condition was interesting result when compared with increased water stressed conditions. Reduced leaf number, leaf area were reported by Blum (2005) as the major mechanism for moderating water use and reducing injury under water stress conditions. In terms of fresh and dry weights, a common adverse effect of water stress on crop species is the reduction of fresh and dry biomass production (Jacob, 2013). In this line, in young Moringa, Badran (2016); Wafa (2006); Majken *et al.* (2005) revealed that, leaf, root and total seedling fresh and dry weight had significantly influenced by water stress. However, Dechasa *et al.* (2006) reported that, biomass had not significantly affected by Moringa accessions, which might be due to the indication of the accessions closeness and interaction of the environmental factor.

2.5.2. Physiological responses to water stress

Stomata traits (number, length and width)

Stomata traits such as number, length and width are considered key determinants of growth rate and water balance in plants (Dillen et al., 2008). Stomata traits are strongly controlled by genetic factors (Gailing *et al.,* 2008). This phenomenon preserves a level of plasticity in response to different water stress conditions. At higher water stress level (water deficit), plants have smaller stomata with less length and width and with a more stomata density to control transpiration and save water (Bosabalidis and Kofidis, 2002; Belhadj *et al.,* 2011*,* Hetherington and Woodward, 2003). On the other hand, as water stress level increased, decreased stomata number has been reported (Xu *et al.* 2003)

Chlorophyll concentration

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Both the chlorophyll a and b are prone to soil dehydration (Farooq *et al.,* 2009). According to Badran (2016) chlorophyll a, chlorophyll b and total chlorophyll has been increased parallel with increasing watering interval in *Moringa oleifera* and *Moringa peregrine*. In other crops, it has been reported that, increasing water stress caused a large declining in the chlorophyll a content, the chlorophyll b content, and the total chlorophyll content (Manivannan *et al.,* 2007b; Farooq *et al*., 2009). Moreover, According to Majken *et al.* (2005), Chlorophyll content had significantly reduced by severe water stress, whereas changes in the Chlorophyll b content were insignificant, indicating greater sensitivity in the former parameter to water stress. The lower amount of Chlorophyll under water stress may be due to increased chlorophyllase activity, although a stimulated activity under water stress might not be purely hydrolytic (Majken *et al.,* 2005). Unlike this, Oliveira *et al*. (2014) reported that, increasing water stress does not affect photosynthetic pigment of *Moringa oleifera*. The unconfirmed idea is may be due to the stage of sampling, different agro ecology and unclear reasons.

Leaf Gas exchange parameter's

The gas exchange of the plants (photosynthetic rate, transpiration rate and stomatal conductance) is the principal plant process responsible for plant biomass production and for plant adaptation to adverse environment (Lawlor and Tezara, 2009a). Stomatal opening, which controls gas exchange, is a sensitive indicator for water stress. In young *Moringa oleifera*, Rivas *et al.* (2013) revealed that photosynthetic rate, transpiration rate and stomatal conductance reduced linearly with water stress increased. From other legumes crops, it has reported that, stomatal closure for reducing the water loss is one of the adaptation strategies to water stress (Mabhaudhi *et al.,* 2013). However, stomatal closure also results in reduced CO2 movement for carboxylation within the chloroplast. This can be a major cause of water stress induced decreases in CO2 assimilation capacity (Flexas *et al.,* 2006b; Warren, 2008), and causes reduced leaf expansion and plant biomass production (Chaves *et al.,* 2002; Chaves *et al.,* 2003; Lawlor and Tezara, 2009a).

2.5.2.4. Leaf water related parameter's

Leaf water content is a useful indicator of plant water balance, since it expresses the relative amount of water present on the plant tissues. Different accessions may have different leaf water content due to their genetic contribution under different water stress levels. In young *Moringa olefera*, study by Rivas *et al.* (2013) indicated that, a different water deficit level had remarkably not reduced relative leaf water content (RWC). This may be due to the closeness of watering regime and genetic makeup of the used accessions. On the other hand, as reported by Silva *et al.* (2007), Relative water content of leaves (RWC) decreased drastically in all the genotypes as the crop has subjected to progressive drought stress under receding soil moisture conditions. This indicated that, water tissue content affects physiological processes as stomatal conductance reduced in several plant species under water stress conditions; this is mostly happened due to increased sensitivity to xylem-carried ABA which is induced by low leaf water potentials (Wilkinson and Davies, 2002). Generally, several studies had indicated that accessions maintain high RWC are drought tolerant (Kumar *et al.,* 2008).

Water use efficiency (WUE) is one of the key physiological attribute determining plant productivity under limited water supply (Farooq *et al*., 2009). At the single-leaf level, the relationship between production and water use can be expressed as the transpiration efficiency (carbon fixation/transpiration). In *Moringa oleifera*, water use efficiency is greater at more stressed conditions than unstressed (Rivas *et al.* 2013). The high water use efficiency is a direct consequence of the decrease in stomatal conductance which is a typical response observed in other species when subject to mild drought stress.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The present experiment was carried out under greenhouse condition from October 2018 – January 2018 at Hawassa University, College of Agriculture campus. The area is located in the Sidama zone, Southern Nations, Nationalities, Peoples Region that is 275 km far from Addis Ababa, capital city of Ethiopia. The site lies at 7°05′N latitude, 38°47′E longitude with average altitude of 1750 m above sea level. According to last 11 years (2007-2018) data obtained from the weather station, the average annual rain fall and temperature (maximum and minimum) of the area is 971.9 mm and 27.9°c and 13.8°c, respectively (NMASNNPSMCHB, 2019).

3.2. Experimental Materials

For this experiment, three accessions of *Moringa stenopetala* were provided by Arbaminch Agricultural Research Center (AARC). Brief description of the accessions is stated in the table (1).

Table 1. Description of Moringa stenopetala accessions used for the current experiment.

No	**Accession code**	**Origin of accession**	**Growing altitude**	**Source**
1	Konso	Konso	1320 masl	AARC
2	Arbaminch Zuria	Arbaminch Zuria	1222 masl	AARC
3	Humbo	Humbo	1432masl	AARC

3.3. Soil Sample and Analysis

One gram of the experimental soil (composited soil consisting of the top soil, compost and sand) was sampled and analyzed for most important physical properties (percentage of sand, silt, and clay, and soil textural class) and chemical properties (bulk density, pH, total nitrogen, organic carbon, and organic matter). The sampled media was air dried and grinded using pestle and mortar, passed through a 2 mm sieved. Soil particle size distribution was determined by the Boycouos hydrometric method (Van Reeuwijk, 1992) after destroying OM using hydrogen peroxide (H_2O_2) and dispersing the soils with sodium hexametaphosphate ($NaPO_3$).

Soil bulk density was determined by the undisturbed core sampling method after drying the soil samples in an oven at 105 ^oC to constant weights, while particle density was measured by the pycnometer method (Black, 1965). The pH of the soils was measured in water and potassium chloride (1M KCl) suspension in a 1:2.5 (soil: liquid ratio) potentiometrically using a glass-calomel combination electrode (Van Reeuwijk, 1992). The electrical conductivity (EC) of soils was measured from a soil water ratio of 1:2.5 socked for one hour by electrical conductivity method as described by Sahlemdhin and Taye (2000). The Walkley and Black (1934) wet digestion method was used to determine soil carbon content and percent soil organic matter. Total N was analyzed using the Kjeldahl digestion, distillation and titration method as described by Black (1965) by oxidizing the organic matter in concentrated sulfuric acid solution (0.1N H_2SO_4).

The soil moisture content at field capacity was determined using pressure plate apparatus (FAO, 2006). The gravimetric soil moisture content was calculated using the following relationship (usually termed gravimetric water content):

% water by weight at FC = $\frac{\text{FW at FC} - \text{ODW at FC}}{ODW\ at\ FC}$ x 100, where, FW at FC fresh = weight of soil core at field capacity (-33kpa) and OD at FC = oven dried weight (105 ^{o} C for 24 hours) of soil cores at field capacity (-33kpa), then gravimetric moisture content was converted in to volumetric moisture content by multiplying it by bulk density of the soil as follows;

Θ_{FC} =w (ρb/ ρw) where, ρb = Bulk density of the soil, Pw= density of water

w = gravimetric moisture content at FC

The report of the laboratory analysis for most important physical and chemical properties of the experimental media was presented (Table 2).

Table 2. Physical and chemical properties of the present experimental soil collected from top soil (20cm), compost and sand soil and composited samples were used for analysis

Properties	Determined values
Sand (%)	84.68
Silt (%)	8
Clay (%)	7.32
Soil textural class	sandy loam
PH	7.3
Organic matter	9.076
Organic carbon	5.265
Total nitrogen	0.4538
Bulk density(g/cm^2)	1.02
Electro conductivity(ms cm^{-1})	0.52
Moisture content at FC (v/v %)	31.9

The soil Laboratory report was indicated that, the experimental media is sandy loam in texture with pH value 7.3; the organic carbon content of the media is 9.076%; consisted 5.265% and 0.4538% of organic carbon and total nitrogen respectively (Table 2) which is confirmed with Ramachandran *et al., (*1980); Foidl *et al*. (2001) recommendation.

3.4. Treatments and Experimental Design

The work presented here in was consisted of different water stress imposed as watering interval and *Moringa stenopetala* accessions, in a factorial scheme of 4 x 3, that was, four watering intervals [daily (Control), five day interval, ten days interval and fifteen days interval], in which seedlings were watered keeping the soil at field capacity (FC=31.9) and three *Moringa stenopetala* accessions (Konso, Arbaminch Zuria and Humbo). Totally 12 treatments arranged in completely randomized design (CRD) with three replications (36 experimental units). Each experimental unit was consisted of 15 perforated black polythene tubes with 22 cm x 16 cm size length and width respectively.

Table 3. Treatment combination of the experiment**.**

Watering interval at field capacity	**Accession**
Daily watering	Konso
	Arbaminch Zuria
	Humbo
Five days interval watering	Konso
	Arbaminch Zuria
	Humbo
Ten days interval watering	Konso
	Arbaminch Zuria
	Humbo
Fifteen days interval watering	Konso
	Arbaminch Zuria
	Humbo

3.5. Experimental Procedure

Moringa stenopetala accessions seed was obtained from Arbaminch agriculture research center, the top soil up to 20 cm depth was collected from Hawassa University, College of Agriculture, Plant and Horticultural sciences research site. Then after, Compost and sand was collected and the polyethylene tube with 22*16 cm size were filled with 3:2:1(ratio of top soil, compost, and sand) as suggested by Ede *et al.* (2015) and then arranged in CRD. Then, the amount of water required applying in the perforated black polythene tubes to field capacity was calculated as;

Amount of water to be applied = (θ_{FC}-θAMC)* depth of the Perforated black polythene tubes (cm) x Perforated black polythene tubes area (cm^2).

Where, θ_{FC} = volumetric moisture content at FC and θAMC = actual volumetric moisture content.

Then, two seeds of each *Moringa stenopetala* accessions were sown during October/ 09/2018 in 2cm depth per each number of polyethylene tubes and then thinned after germination. All Experimental units were watered well to field capacity up to October/29/2018 (until the commencements of the water stress treatment made sure that, the seedlings established well. Thereafter, during October/30/2018, the seedlings were subjected to water stress treatment and the plants were maintained in those stress levels for two month while subsequent measurement and observations were made on their morphological and physiological attributes

3.6. Description of the Greenhouse and its condition

The greenhouse used for present experiment was fenced with metal wire to protect the entrance of any undesirable body in to the greenhouse. The top of the greenhouse was covered by transparent corrugated tin.

The daily maximum and minimum air temperatures for the greenhouse were recorded on randomly selected 45 days using mini data logger during the experimental period from October to January, 2018. Data logger was placed inside an open bucket to avoid direct sunlight and hanged close to the seedling canopy. The data logger recorded the climate data every hour for 45 days. The average value of 45 days measurements is represented by Fig 1.

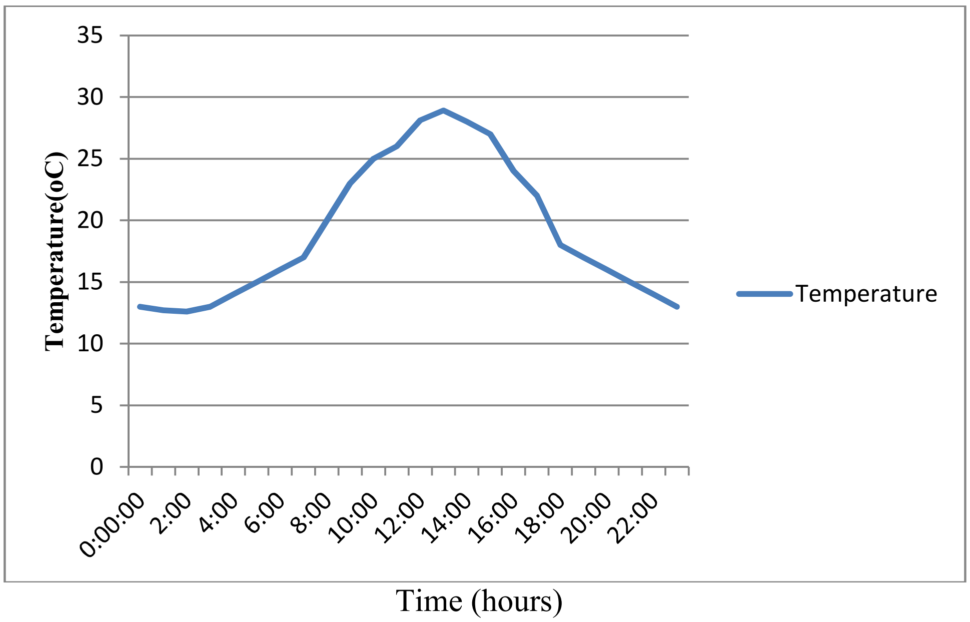

Figure 1. Average daily greenhouse temperature during experimental period (October to January, 2018)

Mean maximum and minimum air temperature in the greenhouse were 28.9^{o}c and 12.6^{o}c respectively (Fig. 1). The greenhouse condition was therefore in the range of optimum temperature (20 to 35ºC) for raising Moringa seedling (Alatar, 2011; Muhl *et al.,* 2011).

3.7. Data Collection and Measurement

3.7.1. Morphological parameter's, Fresh and dry weights

Seedling height and stem collar diameter (cm): they were measured using Ede *et al*. (2015) method. Seedling height was measured from the surface of the seedling above the media up to tip of the seedling by 30cm ruler. Seedling stem collar girth was measured with digital caliper (precision in mm).

Root length and root collar diameter (cm): they were measured by using Jane *et al*. (2015) method that, three black polyethylene tubes with seedlings were randomly selected, and perforated polyethylene tube was removed with sharp scissors, thereafter, in order to protect breaking of roots in to the adhered soil, exactly blow stem- root junction of the seedling was inserted in to the pot containing water until the soil adhered with root had been washed. Then, it was measured using a 30- centimeter ruler placed along-side from the underground surface to the root tip of the seedling. Root collar Diameter was measured using digital caliper (precision in mm).

Leaf area (cm^2): indicates the leafiness of the seedling. Leaf area was measured using LI-3100C Area meter (precision Square cms), LI-COR. Bio science area meter, Australia.

Whole Seedling fresh weight (g) and Leaf fresh weight (g): after destructive sampling, seedling fresh weight was recorded by placing whole seedling on sensitive balance connected with electric power. Thereafter, leaves were disconnected (separated) from selected seedlings and weighed separately as a leaf fresh weight.

Whole seedling dry weight (g) and Leaf dry weight (g): after fresh weights of the samples were recorded, samples were placed in paper bags and then oven dried for 48 hours at 70^{o}C temperature. Whole seedling and leaf dry weight was then recorded by electronic sensitive balance.

Root fresh and dry weight (g): three polyethylene tube with seedlings were randomly selected, and perforated polyethylene tube was removed with sharp scissors, thereafter, in order to protect breaking of roots in to the adhered soil, exactly blow stem- root junction of the seedling was inserted in to the pot containing water until the soil adhered with root had been washed. Afterward, above ground part of the seedlings were separated by using sharp scissor, and then sample was rolled by plastic and brought to laboratory. Fresh weight was recorded immediately after harvest using electronic sensitive balance. Finally, roots were placed in paper bag and oven dried with adjusted temperature at 70^{o}C. Then the root dry weight was recorded at 72 hour.

3.7.2. Physiology parameters

At 2 month after treatments applied, the data of physiological parameter's such as stomata number, length, and width, chlorophyll concentration (chl a, chl b, and total chl), relative water content percentage, gas exchange parameter's (Photosynthesis, Transpiration rate, and stomata conductance), and instantaneous water use efficiency from the third leaves of the three seedlings were recorded and computed as stated below:

Stomata number (No mm^{-2}), length and width (μm): stomata number, length and width were counted, and measured following the procedure of Torre *et al*. (2003). Abaxial side of the leaf surface were covered by thin layer of clear nail polish and waited for 10 min until the nail polish dried to capture the epidermal imprint of the leaves, thereafter, a thin layer covering a surface on the leaves were peeled off using a clear tape and attached on the microscope slide. Then, the imprint were mounted on automatic upright Leica microscope DM5000 with 40x magnification lens fixed with digital Leica DFC425/DFC425C image processing camera (Germany) connected with LAS version 4.8 application.

Determination of chlorophyll concentration (µg/ml) (chl a, chl b, and total chl), chlorophylls are pigments which are used to harvest light energy that contribute for the photosynthesis which is very important for carbon assimilation. Thus, in the present experiment, it was determined according to Porra *et al*. (1989).

In the laboratory dark room (to secure disintegration of chlorophylls' by light), circular leaf discs (200 mm^2 surface) were cut out by using 16 mm diameter cork disc cutter. Then after, immediately, the leaf discs were grinded with 2ml of cold methanol (99%) in mortal and pestle, the homogenate, combined with a further three washings of the pestle and mortal (each of 1.5 ml) with cold methanol (99%). Thereafter, the mixture was homogenized and centrifuged at 2500 revolution per minute (r.p.m.) for 10 minutes. After filtering, the supernatant was decanted into 2ml cuvette and absorbance of the centrifuged extracts was recorded at 652.0 and 665.2 nm (as this was a simultaneous extraction of chlorophyll) using spectrophotometer of 6300 (Jenway), made with by varian Techtron pty limited, Australia. Finally, Chlorophyll a, chlorophyll b and total chlorophyll was computed using equation recommended by (Porra et al. 1989).

$$Chl_a = 16.29*A^{665.2}\_8.54*A^{652.0}$$

$$Chl_b = 30.66*A^{652.0}\_13.58*A^{665.2}$$

$$Chl\ a+b=22.12A^{652.0}-2.71\ A^{665.2}$$

Where, chl $_{a =}$ chlorophyll a, Chl $_{b =}$ chlorophyll b, chl a+b= total chlorophylls, and A $_{665.2}$, absorbance at wave length 652.0 nm (nano meter).

Gas exchange parameters (Photosynthesis, Transpiration and Stomata conductance) were measured at 2 months after water stress treatments applied using portable infrared gas exchange analyzer LCA-4 ADC (analytical development company, Hoddeson, England). The measurements was done of the during the time between 2:00pm and 4:00pm hour local time with other adjustments were as follow: leaf surface temperature varied from 32.44 and 40.88°C, average photosynthetic active radiation (PAR) at the leaf surface 1300 µmolm^{-2}s^{-1} atmospheric pressure 834 mbar, and leaf chamber molar gas flow rate was between 250-253.7µ mols^{-1}.

Leaf water related parameters

Leaf relative water content percentage (LRWC %)**:** it was determined by method stated by Chandrasekhar et al. (2000). That was, young fully expanded leaves were collected from each plot and sealed in the tubes, the tubes containing leaf sample were placed most urgently in ice pox to keep the freshness of the sampled leaves, and immediately brought to the laboratory. Leaf discs (200 mm diameter) were cut out by using cork disc cutter, which was immediately weighed as fresh mass (FW) leaves, then the samples were floated on distilled water inside a closed petri dish kept at room temperature for 24 h. After removing superficially adhering water droplets by tissue paper, the turgid weight (TW) was taken. Afterwards, the leaf discs were dried at 70 °C for 72 h until to have a dried weight (DW). Values of fresh, turgid and dry mass were weighed using analytical balance with 0.00001g precision to calculate relative water content percentage as follows:

$$LRWC\% = \frac{FW - DW}{TW - DW} x\ 100$$

Where: RWC = Relative water content,

FW = fresh weight, DM = dry weight, and TM = turgid weight

Instantaneous water use efficiency: It was calculated based on the data [photosynthesis (Ps) and transpiration rate (E)] provided by the portable infrared gas exchange analyzer. It was the ratio of carbon gain in photosynthesis (Ps) and loss of water transpiration (E), mathematically expressed below:

IWUE= Ps / E

Where, IWUE= Instantaneous water use efficiency, photosynthesis = Ps, and Transpiration rate (E)

3.8. Data Analysis

The data collected from growth and physiological parameters were subjected to analysis of variance (ANOVA) using proc GLM procedure of SAS version 9.3 (SAS Institute, 2018). Tukey's HSD test was used to separate means at the 5% level of significance.

4. RESULTS AND DISCUSSIONS

4.1. Morphological, fresh and dry weights Responses of *Moringa stenopetala* Accessions Seedling to Water Stress

In this study, the morphological responses of different *Moringa stenopetala* accessions seedling to varied water stress was determined based on the seedling height, stem diameter, root length, root diameter, leaf number, leaf area. The analysis of variances indicated that accessions and varied water stress imposed as watering interval affected all morphology parameters. In addition, root fresh and dry weights, total seedling fresh and dry weights affected by both accessions and water stress. While Leaf fresh and leaf dry weights were influenced by interaction effect. This is due to the reason that, water stress inhibits cell expansion, cell growth and developmental process of the plant depending on the accession (Majken *et al.,* 2005). Specifically, detail descriptions are presented as follow:

4.1.1. Seedling height (cm), stem collar girth (cm), leaf number and area (cm^2)

Analysis of variance shown that, the seedling height, stem diameter, leaf number and area of *Moringa stenopetala* was significantly ($P< 0.001$) influenced by accession and water stress imposed as different watering interval. However, the interaction of accession and water stress had indicated no significant ($P>0.05$) difference on seedling height, stem collar girth, leaf number and area of *Moringa stenopetala* (Appendix table I).

Result indicated that tallest seedling height, largest collar girth and wider leaves area were found with "Arbaminch Zuria" accession as compared to the other accessions. In opposite direction, accession "Humbo" was the shortest seedling height, smallest stem collar girth, least number of leaves and narrow leaves area than remaining accessions (Table 4). However, Accessions "Arbaminch Zuria" and "Konso" did not show significant ($P>0.05$) difference on the number of leaves produced (Table 4). The result was in agreement with Baiyeri *et al.* (2015) who revealed that, there was a variation among the accessions of *Moringa oleifera* seedlings in seedling height, stem collar girth and area. This might be due to the genetic variability and potential growing area (where the accession adapted the environment) of the accession.

Regarding to water stress, the tallest seedling height, largest stem collar girth, most number of leaves and wider leaves area were produced on seedlings grown under daily watering condition. While seedlings grown under fifteen days interval watering was produced smallest collar girth and narrow leaf area. However, seedling height and leaf number of the seedlings grown under ten days interval and fifteen days interval had not shown statistically significant (P>0.05) difference.

Thus, this study revealed that, the stem collar girth and leaf area of the *Moringa stenopetala* accessions were decreased as watering interval increased and the reverse is also true. This is due to the reduction of water in the soil might leads to initiate decreasing stomatal conductance, one of the first strategies employed by plants to reduce transpiration and to conserve and maintain water. At the sometime, photosynthetic rate is reduced which minimizes the process of further cell division, growth, differentiation and development of leaves. Similar result has been reported by Sale *et al.* (2015) who stated that, stem collar girth, leaf area of Moringa significantly reduced as watering interval increased. It has been argued that, growth is the product of cell division, enlargement and other physiological process of plants. So that, water play a vital role during cell division, enlargement, differentiation and other physiological process of Moringa seedling which tends to initiate and increase height of the seedlings. Likewise, Daba and Adisu (2017) also reported that plant height significantly increased as watering interval decreased. At third month after treatment application, Baiyeri *et al.* (2015) also reported significant difference among watering interval in seedling height and stem collar girth of Moringa. In other plants like citrus seedlings (Wue *et al*., 2008), and *Abelmoschus esculentus* (Sankar *et al.,* 2008) indicated that, increased water stress reduced seedling height and girth of the different cultivars. In general, the inhibition of cell expansion and cell growth is mainly due to low turgor pressure under water stress conditions which leads to reduce seedling girth (Shao *et al.,* 2008). This could be considered as a line of plants defense mechanism against water deficit (Blum, 2005).

Table 4. Responses of seedling height (cm), stem collar girth (cm), leaf number (count) and leaf area (cm^2) of three Moringa stenopetala accessions seedling to water stress.

Treatments	**Parameters**			
Accessions	**Seedling height (cm)**	**Stem girth (cm)**	**Leaf number**	**Leaf area (cm^2)**
Konso	15.41± 0.26^b	0.69±0.01^b	22.88±1.14^a	123.81 ±5.47^b
Arbaminch Zuria	17.07±0.26^a	0.75±0.01^a	25.11±1.14^a	149.50±5.47^a
Humbo	13.87±0.26^c	0.63±0.01^c	18.47±1.14^b	97.56±5.47^c
Tukey/HSD	**0.67**	**0.03**	**2.85**	**13.66**
Water stress				
Daily (control)	20.39±0.30^a	0.98±0.01^a	35.37 ±1.32^a	215.13 ±6.31^a
5 days interval	17.29±0.30^b	0.70±0.01^b	21.96±1.32^b	134.95±6.31^b
10 days interval	12.34± 0.30^c	0.56±0.01^c	17.77± 1.32^c	81.06±6.31^c
15 days interval	11.78±0.30^c	0.51±0.01^d	13.52± 1.32^c	63.35±6.31^d
Tukey/HSD	**0.85**	**0.04**	**3.64**	**17.43**
CV (%)	**4.25**	**5.21**	**12.64**	**10.84**

Where, HSD= honestly significant difference, CV= Coefficient of variation, means with different letter in each column of each factor are statistically significant at P< 0.001.

4.1.2. Leaf fresh and dry weight (gram per seedlings)

The analysis of variance showed that, leaf fresh and dry weights were significantly (P<0.01 and P<0.001) influenced by interaction effect, respectively (Appendix Table II).

The highest leaf fresh weight was observed on accession “Arbaminch Zuria” under daily watering conditions. While, the lowest leaf fresh weight was observed from all accession when grown under ten and fifteen days interval watering (Table 5). The highest leaf fresh weight observed from accession "Arbaminch Zuria" grown under daily watered conditions, is might be due to available of water to express the genetic potential of leaf growth. This indicates that moisture stress limited the growth potential and narrow down the difference in growth of Moringa accessions. The result has shown that evaluations of plants under stressed condition can not reflect the true potential of a particular plant or accession (Chaves and Oliveira, 2004).

The highest leaf dry weight was measured on accession "Arbaminch Zuria" under daily watering condition. The variation in leaf dry weight among accessions narrowed down under higher water stress conditions (Table 5). This might be due to the genetic make-up of the accession to produce more dry matter at that water stress level.

Nevertheless, the present study indicated that increasing water stress reduced leaf fresh and dry weight of *Moringa stenopetala* accessions. In line with our study Majken *et al. (*2005) revealed that as plants subjected to different level of water stress, the stomatal pores progressively close, decreasing the stomatal conductance to water vapour and then slowing photosynthetic rate. Thereby, the rate of carbon fixation is also reduced which tends to reduce the growth of leaf. Furthermore, the reduction of leaf fresh and dry weight might be due to the reason that, leaf production and leaf expansion growth are very sensitive to water availability and were gradually reduced when plants are subjected to different water stress level (Jaleel *et al.,* 2009). The result was also in agreement with works reported by Wafa (2006) and Badran *et al.* (2016) who indicated that, leaf fresh and dry weight of Moringa reduced with respect to prolonged irrigation interval.

Table 5. Response of leaf fresh and dry weight of three Moringa stenpetala accessions seedling to different water stress.

Accessions	Water stress			
	Daily	5 Days interval	10 Days interval	15 Days interval
	Leaf fresh weight (gram per plant)			
Konso	4.88±0.11[b]	2.25±0.11[cd]	1.93±0.11[defg]	1.52±0.11[g]
Arbaminch Zuria	5.33±0.11[a]	2.48±0.11[c]	1.96±0.11[def]	1.63±0.11[efg]
Humbo	4.56±0.11[b]	2.03±0.11[d]	1.70±0.11[efg]	1.58±0.11[fg]
Tukey/HSD = 0.42	**CV (%) = 5.43**			
	Leaf dry weight (gram per plant)			
Konso	2.56±0.04[b]	1.84±0.04[cd]	0.75±0.04[g]	0.50±0.04[h]
Arbaminch Zuria	2.80±0.04[a]	1.70 ±0.04[d]	0.97±0.04[f]	0.43±0.04[hi]
Humbo	1.88±0.04[c]	1.39± 0.04[e]	0.55±0.04[h]	0.32±0.04[i]
Tukey/HSD = 0.15	**CV (%) =3.92**			

Where, HSD= honestly significant difference, CV= coefficient of variation, means with different letters are statistically significant at $P < 0.01$ and 0.001 for leaf fresh and dry weights, respectively.

4.1.3. Root length (cm), collar diameter (cm), fresh and dry weight (gram per seedling)

Root length, diameter, fresh and dry weight of the *Moringa stenopetala* was significantly ($P<0.001$) influenced by accession and water stress (Appendix table I and II). However, interaction effect of the two factors (accessions and water stress) was not significantly ($P>0.05$) influenced in all root traits (Appendix Table I and II).

The longest root length, fresh and dry weights were recorded from “Arbaminch Zuria” Accession. In contrast, the thinnest root diameter and smallest root fresh and dry weights was observed in accession “Humbo” (Table 6). However, there was no significant (P>0.05) difference in root length among accession “Konso” and “Humbo” (Table 6). Regarding root diameter, statistically there was no significant (P>0.05) difference among “Arbaminch Zuria” and “Konso” accession (Table 6). The variability shown in the present study might be due to the genetic variability of the accessions inherited naturally. Moreover, the difference among the accessions observed in root fresh and dry weight could be associated with contribution of root length and girth of the seedlings.

With regard to water stress, the longest root length, highest root girth, fresh and dry weight of *Moringa stenopetala* was recorded from seedlings grown under daily watering. On the other hand, the shortest root length, lowest root girth, fresh and dry weight was recorded on the seedlings watered in fifteen days interval watering. The present study results also indicated that all stress levels were significantly influenced all root traits (Table 6).

The reduction in root length observed in this study was agreed with the findings of Badran *et al.* (2016). However, in *Moringa olifera*, Daba and Adisu (2017) indicated that root length of Moringa was not significantly (P>0.05) influenced by watering interval, Even though non-significant different were observed among watering interval, numerically the longest root length were recorded from daily watered seedlings than seedlings grown under extended watering interval.

The present result was also confirmed with the study conducted by Daba and Adisu (2017), who reported that root collar diameter of *Moringa oleifera* decreased as watering interval increased. Similarly, the study by Sale (2015) concluded that root collar diameter of Persia americana reduced with increased watering interval. A similar work carried by Isah *et al*. (2013) on *Acacia senegal* seedlings indicated also that decreased watering day's interval were increased root diameter.

Similarly, root fresh and dry weight of *Moringa stenopetala* in the present study has been related with patterns reported by Wafa (2006) and Badran *et al.* (2016) who indicated that, prolonging irrigation interval reduced root fresh weight of *Moringa oleifera* and *Moringa* peregriana. Dunford and Vazquez (2005) reported also that, decreased moisture in the soil had a significant reduction in root fresh weight.

In general, in line with our study Wafa (2006); Badran *et al.* (2016) revealed that, root length, collar girth, fresh and dry weight of Moringa has been reduced as water stress increased. This might be due to reason that, water stress is occurred when the available of water in the soil is reduced (Jaleel *et al.*, 2009). It is followed by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and a decrease in cell enlargement and growth which seems in reduction of root length, collar girth , fresh and dry weight (Kamara *et al.,* 2003). Additionally, as described above, the reduction of root dry weight might be associated with root length and fresh weight influenced by water stress. Not only root length and fresh weight, but also associated with all roots and leaf traits, even gas exchange parameters (Kamara *et al.,* 2003). While this phenomenon is mainly due to the decreasing of photosynthesis resulted from a reduction of internal carbon, where the biochemical capacity for carbon assimilation and utilization is reduced during increased water stress (Oliver *et al*., 2009).

Table 6. Response of root length, root diameter, root fresh weight and root dry weight of three Moringa stenopetala accessions seedling to varied water stress.

Treatments	**Parameters**			
Accessions	**Root length (cm)**	**Root diameter (cm)**	**Root fresh weight (g plant^{-1})**	**Root dry weight (gram plant^{-1})**
Konso	14.23±0.47^{b}	1.71±0.05^{a}	21.41±0.72^{b}	10.29±0.56^{b}
Arbaminch Zuria	16.18±0.47^{a}	1.72±0.05^{a}	24.33±0.72^{a}	13.64±0.56^{a}
Humbo	13.08±0.47^{b}	1.50±0.05^{b}	19.23± 0.72^{c}	7.90±0.56^{c}
Tukey/HSD	**1.19**	**0.12**	**1.81**	**1.41**
Water stress				
Daily (control)	18.296±0.55^{a}	2.38±0.05^{a}	51.60±0.83^{a}	19.39±0.65^{a}
5 days interval	14.96±0.55^{b}	1.68±0.05^{b}	17.34±0.83^{b}	12.53±0.65^{b}
10 days interval	13.35±0.55^{c}	1.41±0.05^{c}	10.26±0.83^{c}	6.92±0.65^{c}
15 days interval	11.39±0.55^{d}	1.24±0.05^{d}	7.40±0.83^{d}	3.61±0.65^{d}
Tukey/HSD	**1.52**	**0.16**	**2.31**	**1.80**
CV (%)	**8.06**	**7.45**	**8.2**	**13.07**

Means with different letter in each column of each factor are statistically significant at P-Values < 0.001.

4.1.4. Total seedling fresh and dry weight (gram per seedling)

Total seedling fresh weight of the *Moringa stenopetala* seedling was significantly (P<0.001 and 0.01) influenced by accessions and water stress, respectively. While total seedling dry weight of the *Moringa stenopetala* seedling was significantly (P<0.001) influenced by both accessions and water stress. However, there was no significant (P>0.05) interaction effect between accessions and water stress in both total seedling fresh and dry weight of the *Moringa stenopetala* seedling (Appendix Table III).

Maximum total seedling fresh and dry weights of the *Moringa stenopetala* seedling were recorded from the accession “Arbaminch Zuria”. While the minimum total seedling fresh weight was recorded from accession “Humbo” (Table 7). The Maximum seedling fresh and dry weights recorded from the accession “Arbaminch Zuria” might be due to the highest value recorded in CO_2 assimilation of the accession “Arbaminch Zuria” (Fig. 2). Furthermore, it may associate with the more production of leaf and root fresh and dry weight of the accession “Arbaminch Zuria”. Besides, the maximum seedling dry weight of the accession “Arbaminch Zuria” might be due to the platform of total fresh weight of the accession (Table 7).

With regard to water stress, highest total seedling fresh and dry weight was recorded on the seedlings grown under daily watering. Whereas, the lowest total fresh and dry weights were record from fifteen days interval watering (Table 7). The present experiment revealed that, increased water stress caused in decreased total seedling fresh and dry weights of *Moringa stenopetala* accessions. Similar pattern on *Moringa oleifera* and *Moringa peregrina*, where increased watering interval (increased water stress) resulted in reduced total seedling fresh and dry weights has been reported (Wafa, 2006; Badran *et al.,* 2016). Agreeable results has been also reported by several scholars on most of the other plant (Anyia and Herzog, 2004a; Liu and Stutzel, 2004; Majken *et al.,* 2005; Jacob, 2013). This is due to the reason that, total plant biomass production depends on the amount of water used for growth. This process thus negatively affected by water stress. Additionally, the reduction of the total seedling fresh and dry weight with increment of water stress may be derived from the associated parameters such as seedling height, leaf number, leaf area, root growth and their fresh weight (Table 11). Consequently, the total seedling fresh and dry weight could be seemed declined fresh and dry weight of the seedling.

Table 7 . Response of total seedling fresh weight and total dry weight of three Moringa stenopetala accessions seedling to water stress.

Treatments	**Parameters**	
Accessions	**Total fresh weight (gram plant^{-1})**	**Total dry weight (gram plant^{-1})**
Konso	27.80±0.77^{b}	15.61±0.69^{b}
Arbaminch Zuria	32.09±0.77^{a}	23.08±0.69^{a}
Humbo	24.45±0.77^{c}	13.07±0.69^{c}
Tukey/HSD	**1.93**	**1.74**
Water stress		
Daily (control)	61.80 ±0.89^{a}	31.05±0.80^{a}
5 days interval	25.63±0.89^{b}	20.00± 0.80^{b}
10 days interval	14.92±0.89^{c}	11.62±0.80^{c}
15 days interval	10.11±0.89^{d}	6.34± 0.80^{d}
Tukey/HSD	**2.47**	**2.22**
CV (%)	**6.76**	**9.91**

Means with different letter in each column of each factor are statistically significant at P-values ≤ 0.05.

4.2. Physiological Responses of *Moringa stenopetala* Accessions Seedling to Water Stress

4.2.1. Response of stomata number, length and aperture of Moringa accessions seedling to water stress

Analysis of variance revealed that, number of stomata was significantly (P>0.05 and (P<0.01) influenced by accessions and water stress, respectively. While, Stomata length and aperture of the *Moringa stenopetala* were significantly (P<0.01) influenced by both accession and water stress. But, there was no significant (P>0.05) influence by interaction effect on stomata traits (stomata number, length and aperture) (Appendix Table III).

The significant difference was observed among accession “Arbaminch Zuria” and “Humbo” only. But there was no significant difference among accession “Arbaminch Zuria” and “Konso”, and accession “Konso” and “Humbo” (Table 8). This might be associated with leaf water status and gas exchange parameters like photosynthesis, transpiration, stomata conductance. This parameters determines the size of stomata per given leaf area that, the highest stomata number due to small sized stomata’s and lowest stomata number due to larger size of stomata per leaf area provided by genetic makeup of the accessions (Belhadj *et al.,* 2011).

The longer and wider stomata were observed from the accession “Arbaminch Zuria” and “Konso” (Table 8). Accession with longest and widest stomata seems to the highest photosynthetic, transpiring and metabolic rate resulting high production of dry matter. However, the shortest and narrowest stomata were from accession “H” resulting in least transpiration and photosynthetic rate followed by reduced growth of the whole seedling (Belhadj *et al.,* 2011).

Regarding to water stress, the result indicated that, there was no significant ($p>0.05$) difference among daily watering and five day interval watering in stomata number. As well, there was no significant ($p>0.05$) difference among five, ten and fifteen days interval watering. But significant ($p>0.05$) difference was observed among daily and ten days watering interval (Table 8).

In this regard, the difference observed among daily and ten days interval watering indicated that, stomata number was influenced by increased water stress which is in agreement with study reported by Belhadj *et al.* (2011); Bosabalidis and Kofidis (2002); Hetherington and Woodward (2003). The maximum number of stomata recorded under more stressed condition is might be due to the size of stomata per given leaf area that, the highest stomata number due to small sized stomata's and lowest stomata number due to larger size of stomata per leaf area influenced by water stress (Dillen *et al.,* 2008; Belhadj *et al.,* 2011). Moreover, the higher stomata number is indicator for higher transpiration rate, highest metabolism and absorption of water or not (Munir *et al.,* 2011). However, in our present work, the highest stomata number that produced when seedlings subjected to increased water stress had not increased transpiration rate of the *Moringa stenopetala.* Because, even though more stomata density is produced, the stomata holes was reduced (narrowed) due to increased water stress and then, tends to reduce the photosynthetic and transpiration rate of the seedling.

In terms of stomata length and width as influenced by water stress, the longest and widest stomata were observed from the seedlings grown under daily watering. But, the shortest and narrowest stomata were observed on the seedling exposed to ten and fifteen day's interval watering condition (Table 8). In line with Bosabalidis and Kofidis (2002); Belhadj *et al.* (2011); Hetherington and Woodward (2003), the result revealed that, stomata length and width of *Moringa stenopetla* reduced with increased stress level. Generally, reduced water availability triggers a series of stomata responses in plants (Lawlor, 2009; Chaves and Pinheiro, 2009). As soon as the roots detect a decrease in soil moisture, water potential and cell turgor are reduced. The roots produce abscisic acid (ABA) which is carried to the leaves which tends to promotes reduction of stomata length and width thereby narrowing the stomata. This limit gas exchange and resulted in reduced stomatal conductance, photosynthetic and transpiration rates as well as reduction in leaf traits followed by restriction of entire seedling growth and development (Bedon, 2011; Chaves and Pinheiro, 2009).

Table 8. Response of stomata number, length and width of different Moringa stenopetala accessions seedling to varied water stress.

Treatments	**Parameters**		
Accessions	**Stomata number (count mm^{-1})**	**Stomata length (µm)**	**Stomata width (µm)**
Konso	16.50± 0.85^{ab}	0.39±0.01^{a}	0.22±0.01^{a}
Arbaminch Zuria	15.25±0.85^{b}	0.40±0.01^{a}	0.25±0.01^{a}
Humbo	17.83±0.85^{a}	0.33±0.01^{b}	0.18±0.01^{b}
Tukey/HSD	**2.12**	**0.04**	**0.03**
Water stress			
Daily (control)	13.88±0.98^{b}	0.49±0.01^{a}	0.29±0.01^{a}
5 days interval	16.44±0.98^{ab}	0.40±0.01^{b}	0.23±0.01^{b}
10 days interval	17.11±0.98^{a}	0.32±0.01^{c}	0.19±0.01^{bc}
15 days interval	18.66± 0.98^{a}	0.28±0.01^{c}	0.16±0.01^{c}
Tukey/HSD	**2.71**	**0.05**	**0.04**
CV (%)	**12.63**	**10.96**	**14.67**

Means with different letter in each column of each factor are statistically significant at P-Values< 0.05.

4.2.2. Response of chlorophylls concentration *(µg ml^{-1})* of *Moringa stenopetala* accessions seedling to different water stress

Different accessions and water stress used in the present study had a significant (P<0.001) influence on chlorophylls concentration (a, b and total). In contrast, interaction of the two main factors had not significantly (P>0.05) influenced chlorophylls concentration (a, b and total) of *Moringa stenopetala (*Appendix Table IV*)*.

Maximum chlorophylls concentration (a, b and total) were recorded from accession "Arbaminch Zuria". On the other hand, the minimum chlorophylls concentration (a, b and total) was obtained from accession "Humbo" (Table 9). This might be due to the genetic makeup of the accessions contributed for the variation. The different amount of chlorophylls (a, b and total) in the accession could be associated with leaf water status of the accessions. This tends to influence gas exchange capacity like stomatal conductance, transpiration and photosynthetic rate of particular accessions based on their natural gift which seems to capture solar radiation. This could result in seedling growth and development. As a result, accession "Arbaminch Zuria" has produced the highest values in most of the traits, except stomata number.

The highest chlorophyll concentration (a, b and total) was observed from the seedlings grown under daily watering condition. However, the lowest concentration of chlorophyll a was observed from the seedlings subjected to fifteen days interval watering condition (Table 9). The present study corroborated with study reported by Manivannan *et al.* (2007b); Alireza *et al.* (2011) and Kiani *et al.* (2008) who stated that, the concentration of chlorophyll a was significantly decreased with increased water stress. Similarly, Farooq *et al.* (2009) reported the reduced chlorophylls concentration (a, b and total) of olive cultivars at high water stress. The reduction in total chlorophyll under increased water stress (drought stress) have been also reported previously in fennel and other plant species (Elgamaal and Maswada, 2013; Askari and Ehsanzadeh, 2015). In addition, reduction in chlorophyll content in our study also might be regarded as a drought response mechanism associated with minimization of light absorption by chloroplasts. A decrease of total chlorophyll with increased water stress implies a degradation of chlorophyll due to water deficit (Herbinger *et al.,* 2002). As a whole, the decrease in total chlorophyll concentration as water stress increased led to inactivation of photosynthesis that limits plant growth and productivity (Anjum *et al.,* 2011b).

Oppositely, the present result disagrees with Badran (2016) who reported that chlorophyll a concentration increased parallel with increased water stress in *Moringa oleifera* and *Moringa peregrine.* This might be due to different species and the other reason not clear for the author. In other study by Oliveira *et al*. (2013) chlorophyll a concentration is not influenced by water stress. Study by Correia *et al*. (2014) on *Eucalyptus globulus*, also claimed that, higher chlorophyll a content in stressed plants, linking this result to a reduction in leaf expansion and mass.

However, in our case, the decreased chlorophylls concentration as water stress increased has been considered as a typical symptom of oxidative stress and may be the result of decreased pigment photo-oxidation and chlorophyll degradation (Correia *et al.,* 2014). Furthermore, the decreased chlorophyll concentration under stressed conditions could be due to increased chlorophyllase activity, although a stimulated activity under water stress might not be purely hydrolytic (Majken *et al.,* 2005; Rivas *et al.,* 2013*;* Oliveira *et al.,* 2014).

Table 9. Response of concentration of chlorophyll a, chlorophyll b and total chlorophyll (a+b) of different Moringa stenopetala accessions seedling to different water stress.

Treatments		**Parameters**	
Accessions	**chl a(μgml^{-1})**	**chl b(μgml^{-1})**	**chl a+b(μgml^{-1})**
Konso	5.49±0.08^{b}	2.85±0.07^{b}	8.34±0.15^{b}
Arbaminch Zuria	6.00±0.08^{a}	3.15±0.07^{a}	9.15±0.15^{a}
Humbo	4.48±0.08^{c}	2.45±0.07^{c}	6.93±0.15^{c}
Tukey/HSD	**0.21**	**0.18**	**0.39**
Water stress			
Daily (control)	6.05±0.09^{a}	3.55±0.08^{a}	9.60 ±0.18^{a}
5 days interval	5.60±0.09^{b}	3.10±0.08^{b}	8.70±0.18^{b}
10 days interval	5.06±0.09^{c}	2.47±0.08^{c}	7.53±0.18^{c}
15 days interval	4.53± 0.09^{d}	2.19±0.08^{d}	6.72±0.18^{d}
Tukey/HSD	**0.26**	**0.24**	**0.50**
CV (%)	**3.89**	**6.53**	**4.74**

Where, chl a=concentration of chlorophyll a, chl b= concentration of chlorophyll b, chl (a+b) = concentration of total chlorophyll, and means with different letter in each columns are statistically significant at P-values < 0.001.

4.2.3. Response of gas exchange parameters [Photosynthetic rate (µmol $m^{-2}s^{-1}$), Transpiration rate (mmol $m^{-2}s^{-1}$) and Stomatal conductance (mol$m^{-2}s^{-1}$)] of the *Moringa stenopetala* accessions seedlings to water stress

The present study revealed that photosynthetic rate, transpiration rate and stomatal conductance of *Moringa stenopetala* were significantly affected by accessions, water stress and interaction of the two factors (Appendix table IV and V).

Figure 2 explains that Accession "Arbaminch Zuria" undergone the highest photosynthetic rate when watered daily. The result indicated that variability among accessions for the rate of photosynthesis is narrowed down under much stressed conditions (Fig. 2). Accession "Humbo" undergone similar rate of photosynthesis with accession "Konso" at all water stress. However, under daily watered condition, the great difference was showed among accession "Arbaminch Zuria" than the remained accessions (Konso and Humbo). This indicate that the genetic potential of accessions is determining factor for the rate of photosynthesis but environmental conditions (eg. availability of moisture) is crucial to express full potential of the accessions. As a result accession "Arbaminch Zuria" produced more seedling height, stem girth, leaf traits like leaf number and area, fresh and dry weights of the seedling than the other two accessions (Table 4, 5, 6 and 7).

According to the work done on *Moringa oleifera* and *Moringa peregrine* by Wafa (2000) and Badran (2016), increased water stress has decreased photosynthetic rate which is in line with present work. Seemingly, this indicates that a reduction in photosynthetic rate was caused by both a decrease in stomatal conductance derived from water stress effect on photosynthetic apparatus as suggested in previous studies (Chaves and Oliveira, 2004*;* Flexas *et al.,* 2006b; Warren, 2008; Claudiana *et al*., 2017)

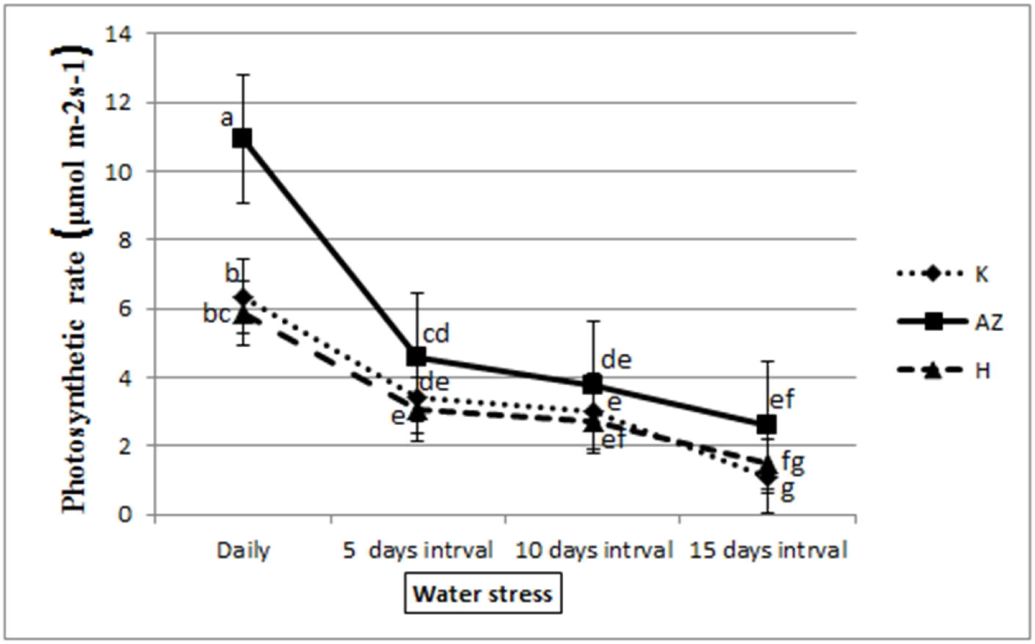

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*Figure 2. Response of photosynthetic rate of the three Moringa stenpetala accessions (K=konso, AZ=*Arbaminch Zuria and H= Humbo) *seedling to different water stress. While, means with different letter on the figure are statistically significant at P-values < 0.001, Tukey/HSD (0.05) = 1.36 and CV (%) = 11.36.*

Similar to photosynthetic rate, the highest transpiration rate was recorded from the accession "Arbaminch Zuria" when grown under daily watering condition (Fig. 3). The rate of transpiration of accessions vary only at daily watering but water stress even at fifteen days interval resulted in similar response of accessions among themselves (Fig. 3). In similar pattern Rivas *et al.* (2013) reported that, transpiration rate has been decreased under more water stressed condition. This could be due to the reduced amount of water in the cell which leads turgor loss and closure of stomata (Kamara *et al.,* 2003).

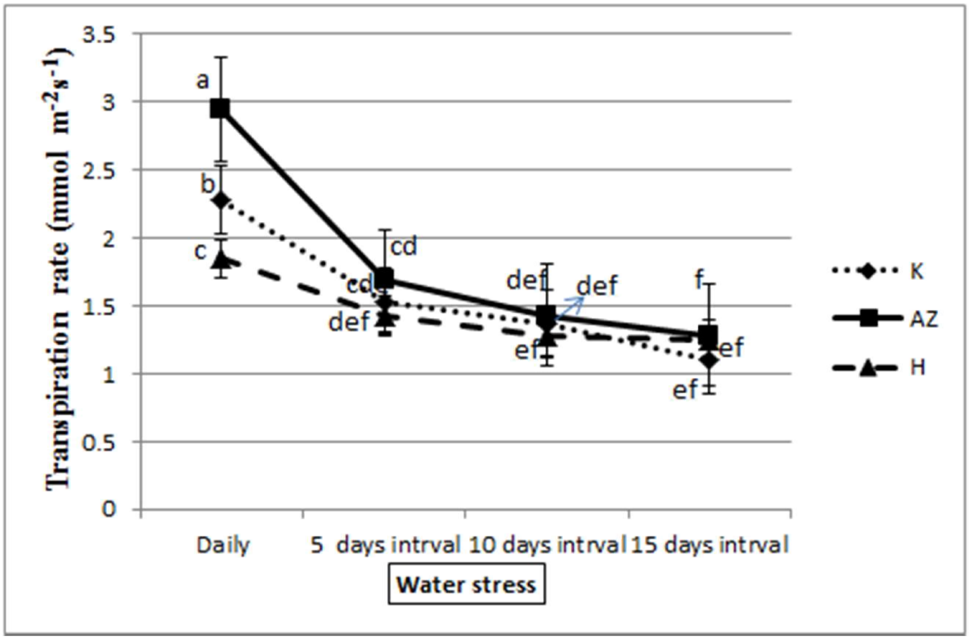

*Figure 3. Response of transpiration rate of the three Moringa stenpetala accessions (K=konso, AZ=*Arbaminch Zuria and H= Humbo) *seedling to different water stress. While, means with different letter on the figure are statistically significant at P-values < 0.001 While, Tukey/HSD (0.05), = 0.38 and CV (%) = 8.18.*

With regards to stomata conductance, the present report revealed that, under daily watering condition, all the accessions studied were statistically different from each other. As it is repeatedly told so from most of the parameters, the highest stomatal conductance was observed from accession "Arbaminch Zuria" on daily watering. Meanwhile, at each of five, ten, and fifteen days interval watering, statistically all accession responded similar (Fig. 4). In line with Wafa (2006) and Rivas *et al.* (2013), the result indicated that, compared with daily watering, under stressed condition the stomatal conductance of Moringa seedlings was decreased. The response of all accessions at each water stress level except daily watering, were not significantly different. This might be due to genetic information contributed for the stomata conductance respond to express its genetic potential under different level of stress.

From other legume plants, it was reported that stomatal conductance reduction for reducing the water loss is one of the adaptation strategies to water stress (Miyashita *et al.,* 2005). And then, stomatal conductance reduction seems to be the main determinant for decreased photosynthesis in this study. This could be a major cause of water stress induced decreases in CO_2 assimilation capacity which results in decline of the growth (Chaves and Oliveira, 2004*;* Flexas *et al.,* 2006b*;* Warren, 2008).

Additionally, it has been hypothesized that the root sense the drying soil (e.g. by reduction in turgor pressure in certain root cells). This then influences the production of hormones (ABA) by the roots and their transportation to the leaves, and then stomatal conductance reduced even partially close in response to hormonal effects on the solute potential of guard cells (Davies *et al*., 1986).

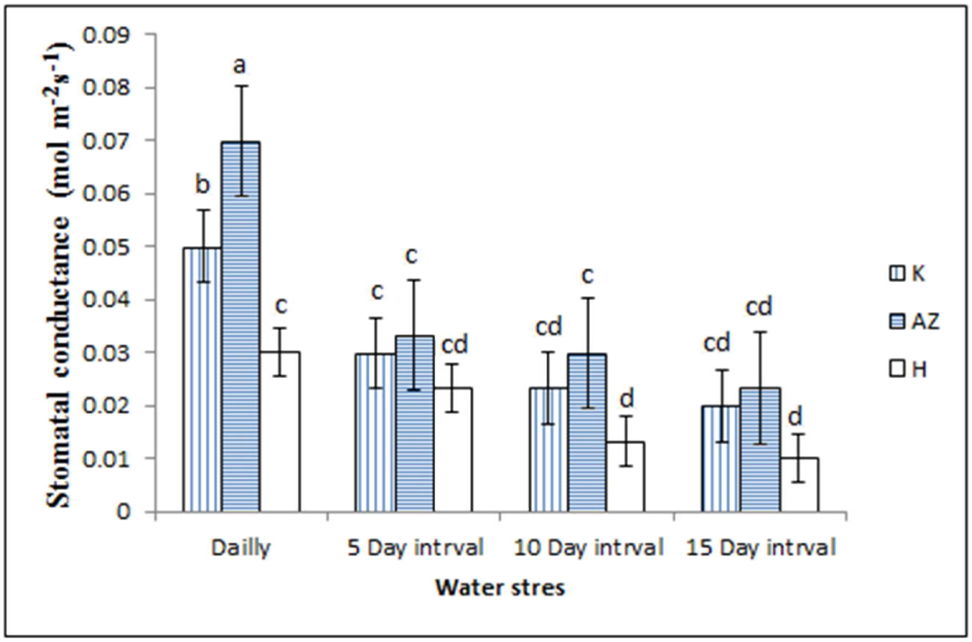

*Figure 4. Response of stomatal conductance of the three Moringa stenpetala accessions (K=konso, AZ=*Arbaminch Zuria and H= Humbo) *seedling to different water stress, while, While, means with different letter on the figure are statistically significant at P-values < 0.05, Tukey (HSD) =0.0163, CV (%) = 18.59, CV= Coefficient of variation, and the error bar indicates the difference among the treatments.*

4.2.4. Response of leaf water related parameters of *Moringa stenopetala* accessions seedling to water stress

Leaf relative water content (%)

Leaf relative water content of *Moringa stenopetala* was significantly ($P<0.001$) affected by accessions and water stress used in the present experiment. It was also significantly ($P<0.05$) affected by the interaction effect of the factors (Appendix Table V).

The result indicated that, the maximum leaf relative water contents of *Moringa stenopetala* were found from accession "Arbaminch Zuria" and "Konso" when exposed to daily watering condition. On the other hand, the minimum leaf relative water content of *Moringa stenopetala* was observed from the seedlings exposed to fifteen days interval watering condition (Fig. 5).

Particularly, the result indicated that, there were no significant ($p>0.05$) difference among all accessions at five days and ten days interval watering. As well, under fifteen day's interval watering there was no significant ($p>0.05$) difference among accession "Konso" and "Arbaminch Zuria" (Fig. 5). The result indicated that, leaf relative water content of Moringa stenopetala accessions was higher under daily watering condition than more stressed condition (ten and fifteen days interval watering).

In line with our result Silva *et al.* (2001), reported that leaf relative water content (LRWC) decreased as the seedlings were subjected to more reduced soil moisture conditions. However, the narrowed different at each stress level is might be due to the ability of different accession with respect to stress level (each accession may have mechanism coping against level of water stress (Kumar *et al.,* 2008). The present result was agreed with study conducted on young *Moringa oleifera* by Rivas *et al.* (2013) indicated that, a different water deficit level had remarkably not reduced relative leaf water content (RWC).

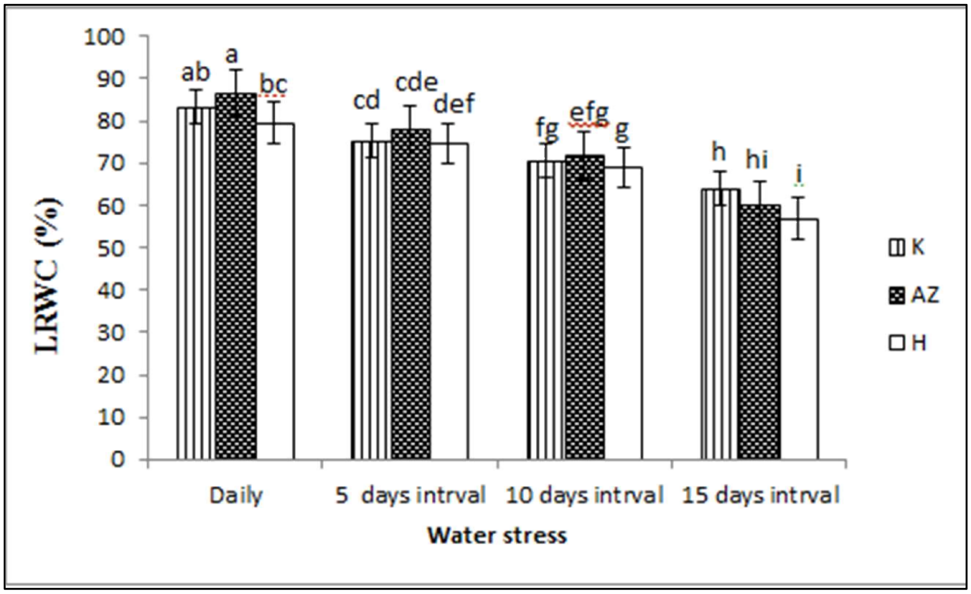

Figure 5. Response Leaf relative water content percentage (LRWC %) of three Moringa Stenopetala accessions (K=konso, AZ= Arbaminch Zuria and H= Humbo) *seedling to different water stress.* Tukey (HSD) for (accessions*water stress) = 4.370 and CV=*2.05*, *means with different letter at each bar of interaction is statistically significant at P-values <0.05.*

Instantaneous water use efficiency

Analysis of variance shown that, accessions and water stresses significantly (P<0.001) influenced water use efficiency of *Moringa stenopetala*. Whilst there was no significant (P>0.05) interaction effects on water use efficiency (Appendix Table V).

The highest water use efficiency was observed from the accession "Arbaminch Zuria" (Fig. 6). This might be associated with leaf area and gas exchange parameters like stomata conductance, photosynthetic and transpiration rate. The higher leaf area and photosynthetic rate character of the accession "Arbaminch Zuria" than other accessions leads accession "Arbaminch Zuria" water use efficient accession. Because, water use efficiency is calculated from photosynthesis and transpiration rate. However, the process of photosynthesis could be affected by stomatal and non-stomatal cases. Restricting CO2 entry into leaves (stomatal case), and variations in leaf biochemistry that results in inhibition or down regulation of photosynthesis (non-stomatal limitation) (Chaves *et al*., 2002). In our study, therefore the observed variations might be stomatal case or non stomatal case that made accession “Arbaminch Zuria” superior than accession “Humbo” and ‘Konso”.

With regard to water stress, the highest water use efficiency was observed from the daily watered condition. Contrarily, the lowest water use efficiency was observed from the fifteen days interval watered condition. Accordingly, Hanafey *et al.* (2018) reported that the highest water use efficiency was reported on unstressed conditions. In addition, Farooq *et al.* (2009) revealed that the decrease in water use efficiency in drought-stressed treatments might be attributed to less leaf area and rate of photosynthesis and transpiration. Furthermore, when plants are subjected to water stress, the stomatal pores progressively close, decreasing the stomatal conductance to water vapour and thus slowing transpiration. Thereby, the rate at which water deficits develop is reduced, but CO_2 entry into leaves is also potentially limited. Hence under more water stressed conditions the rate of CO_2 entry in to the leaves could be less than rate of transpiration when compared to unstressed condition. This concept might be a reason for the decline in water use efficiency parallel to increased water stress.

However, according to Ismail and Hall, 1992*;* Rivas *et al.,* 2013; Andreia, 2014, water use efficiency is greater at more stressed conditions than unstressed in cowpea, *Moringa oleifera,* and *Arundo donax*. The high water use efficiency is a direct consequence of the decrease in stomatal conductance which is a typical response observed in other species when subject to increased water stress.

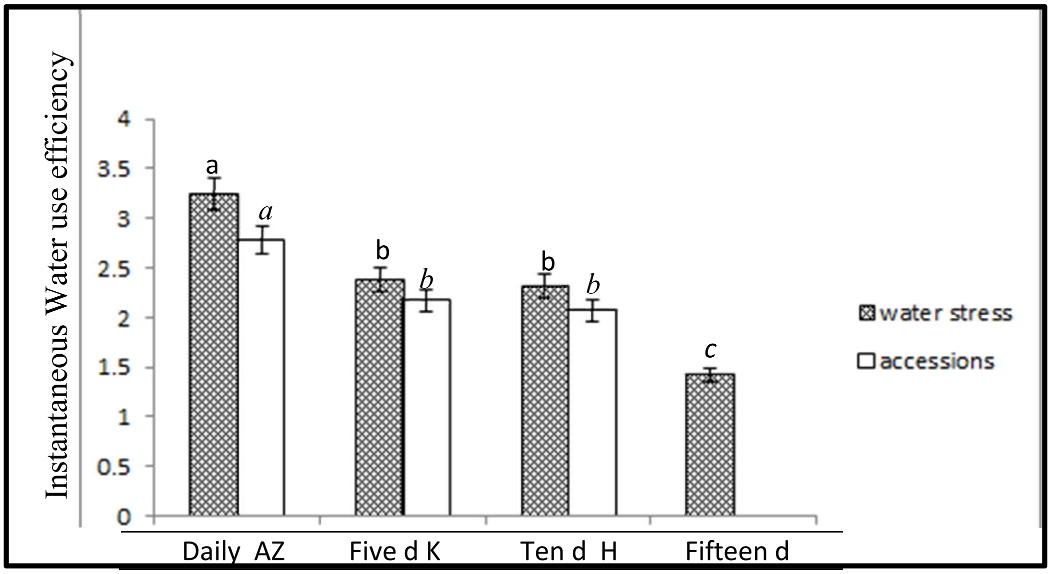

Figure 6. Instantaneous water use efficiency of three Moringa stenpetala accessions (K=konso, AZ=Arbaminch Zuria and H= Humbo) seedling under different water stress. Tukey (HSD) for accessions and water stress are 0.317 and 0.404 respectively and CV=13.26, ***Means with different letter at each bar of each factor is statistically significant at P values ≤ 0.001.***

4.3. Correlation Analysis

4.3.1. Associations between physiological parameters and fresh and dry weights of Moringa *stenopetala* accessions seedling under different water stress.

The associations of different physiological parameter's with fresh and dry weights have been determined (Table 10). Correlation analysis indicated that, the relationship of all physiological parameters except stomata number had significant ($p<0.001$) strong positive correlation with fresh and dry weights (Table 10). Stomata number had significant ($p<0.001$) strong negative correlation with fresh and dry (leaf, root and total seedling) weights (Table 10).

This is due to the reason that, all the physiological parameters except stomata number was higher under daily watering condition than prolonged water stress condition. Consequently, fresh and dry weight of Moringa stenopetala has been experienced strong positive relation with physiology. Since physiological parameters play a crucial role in fresh and dry weights of the plant especially during seedling stage is indispensable (Claudiana *et al*., 2017).

The best example is stomata aperture; stomata serve as both exit and entry points for water vapour and CO2 respectively (Chaves *et al*., 2002). Hence the rate of transpiration and photosynthesis depend on stomatal aperture and closing of the stomata is the first response plants persuade growth and development (fresh and dry weights. Therefore, this is the main mean of regulating carbon assimilation which relates physiological parameter's with fresh and dry weights even growth and development in plants (Hetherington and Woodward, 2003).

Chlorophyll is one of the major chloroplast components which harvest light being input for photosynthesis that lead to produce fresh and dry weights (Farooq *et al*., 2009; Claudiana *et al*., 2017). Therefore, chlorophyll concentration can directly limit photosynthetic potential and hence shown significant positive correlation among fresh and dry weights in the present study.

Water related parameters (leaf relative water content and instantaneous water use efficiency) are an indicator of water status and through its relation to cell volume. They reflect the balance between water supply to the leaf and transpiration affirming carbon assimilation. This will directly influence the seedling growth and development of the seedlings (fresh and dry weights).

Table 10. Pearson's Correlation coefficient among the "physiological traits" and "fresh and dry weights" of Moringa stenopetala accessions seedling grown under different water stress.

	SN	SL	SW	chl a	chl b	ch(a +b)	RWC (%)	Ps	E	IWUE	Gs
SH	-0.68	0.87	0.81	0.82	0.92	0.87	0.88	0.83	0.87	0.76	0.84
SCG	-0.72	0.83	0.79	0.76	0.88	0.83	0.89	0.89	0.89	0.81	0.87
RL	-0.68	0.82	0.77	0.82	0.87	0.86	0.85	0.81	0.8	0.82	0.81
RD	-0.74	0.86	0.81	0.78	0.88	0.84	0.87	0.90	0.88	0.82	0.87
LN	-0.72	0.88	0.82	0.78	0.88	0.83	0.87	0.89	0.91	0.78	0.87
LA	-0.68	0.85	0.84	0.81	0.92	0.88	0.88	0.88	0.91	0.78	0.86
TFW	-0.67	0.83	0.77	0.69	0.83	0.76	0.86	0.89	0.91	0.79	0.83
RFW	-0.65	0.80	0.74	0.64	0.80	0.72	0.82	0.88	0.89	0.77	0.81
LFW	-0.65	0.79	0.74	0.65	0.80	0.72	0.82	0.89	0.90	0.77	0.82
TDW	-0.72	0.83	0.82	0.83	0.92	0.88	0.89	0.89	0.89	0.83	0.87
RDW	-0.74	0.87	0.84	0.83	0.93	0.89	0.92	0.89	0.87	0.86	0.88
LDW	-0.67	0.90	0.82	0.79	0.91	0.86	0.93	0.84	0.88	0.76	0.85

Where, SN=stomata number, SL=stomata length, SW= stomata width, Chl a chlorophyll a, Chl b =chlorophyll b, Chl (a+b) =Total chlorophylls, RWC (%) =relative water content percentage, Ps= Photosynthetic rate, E= Transpiration rate. IWUE= instantaneous water use efficiency, gs- stomatal conductance, SH=seedling height, SCG=stem collar girth, RL=root length, RG=root diameter, LN=leaf number, LA=leaf area, TFW=total fresh weight, RFW=root fresh weight, LFW= leaf fresh weight, TDW=total dry weight, RDW=root dry weight, LDW=leaf dry weight, all r values of the traits were significant at $p \leq 0.001$.

4.3.2. Associations between morphological parameters and fresh and dry weights of Moringa *stenopetala* accessions seedling grown under different water stress.

The associations of different morphological parameter's with fresh and dry weights have been determined (Table 11). Correlation analysis indicated that, the relationship of all morphological parameters had significant ($p<0.001$) strong positive correlation with fresh and dry weights (Table 11).

The relationship of morphological parameters such as seedling height and stem collar girth, root length and diameter, leaf number and area with fresh and dry weights is might be due to the sequential processes (Jaleel *et al.,* 2009). Initially, cell division, enlargement, expansion, differentiation leads to different organs of seedling followed by production and expansion leaf number and area, respectively (Majken *et al.,* 2005). Again, the more production and expansion of leaf number and area seems seedling to elongate and expand the seedling. Because leaf number and area has been an important in production of photosynthetic byproduct that initiate further development of fresh and dry weights even whole seedling (Chaves and Oliveira, 2004).

Table 11. Pearson's Correlation coefficient among the "morphological traits" and "fresh and dry weights" of Moringa stenopetala accessions seedling grown under different water stress.

	SH	SG	RL	RD	LN	LA	TFW	RFW	LFW	TDW	RDW	LDW
SH	__											
SG	0.93	__										
RL	0.89	0.89	___									
RD	0.91	0.97	0.91	__								
LN	0.9	0.93	0.88	0.93	__							
LA	0.96	0.95	0.91	0.94	0.94	__						
TFW	0.9	0.97	0.86	0.95	0.93	0.94	___					
RFW	0.86	0.95	0.84	0.94	0.92	0.92	0.99	___				
LFW	0.85	0.95	0.83	0.94	0.92	0.92	0.98	0.99	___			
TDW	0.95	0.94	0.93	0.92	0.91	0.96	0.91	0.88	0.87	___		
RDW	0.95	0.94	0.91	0.94	0.92	0.94	0.92	0.89	0.88	0.96	___	
LDW	0.96	0.94	0.86	0.92	0.92	0.94	0.92	0.89	0.89	0.93	0.95	___

Where, SH=seedling height, SCG=stem collar girth, RL=root length, RG=root girth, LN=leaf number, LA=leaf area, TFW=total fresh weight, RFW=root fresh weight, LFW= leaf fresh weight, TDW=total dry weight, RDW=root dry weight, LDW=leaf dry weight and all r values of the traits were significant at $p \leq 0.001$

5. SUMMARY, CONCLUSION AND RECCOMMENDATION

5.1. Summary

Moringa stenopetala L. is one of the most multi-purpose medicinal plants which adapt wide range of agro ecology. However, even though Moringa is said to be drought tolerant plants, its seedling responses to different accession and water stress level during seedling stage. As a result, this study tested the responses of some morphological, fresh weights, dry weights and physiological parameters to different water stress level and *Moringa stenopetala* accessions seedling under greenhouse condition.

Accordingly, morphological parameters such as seedling height, stem collar girth, root length, root diameter, leaf number and area were influenced by accessions and imposed water stress levels. As well, total seedling fresh and dry weights, root fresh and dry weights were influenced by accessions and imposed water stress levels. While, leaf fresh and dry weights were also influenced by interaction of the accessions and water stress.

It was observed that there were variations among accessions of *Moringa stenopetala*. Accession "Arbaminch Zuria" was superior in all morphological parameters' except leaf number and root diameter. As well, accession "Arbaminch Zuria" was higher in total seedling fresh and dry weights, root fresh and dry weights. Whereas accession "H" was lower in all morphological, fresh and dry weights parameters except root length.

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Regarding to water stress, all morphological parameters, fresh and dry weights except seedling height and leaf number were significantly decreased with increased water stress. However, there were no significant differences among ten and fifteen days interval watering in seedling height and leaf number. Seedlings grown under daily watering recorded higher value in all morphological parameters and fresh and dry weights. However, seedlings grown under fifteen days watering interval recorded lower value in al morphological parameters, fresh and dry weights except seedling height and leaf number.

Physiological parameters like stomata traits, chlorophylls concentration (a, b and total) and instantaneous water use efficiency were significantly influenced by accessions and water stress. Meanwhile, gas exchanges parameters and leaf relative water content were affected by the interaction effect of accession and water stress.

Regarding to accessions, in accession “Humbo” the highest stomata number was observed. Unlike, accession “Arbaminch Zuria” was superior in chlorophylls concentration (a, b and total). However, accession “Humbo” as inferior in chlorophylls concentration (a, b and total).

The longest and widest stomata length and width were observed from daily watering conditions. As well, Chlorophylls concentrations and instantaneous water use efficiency were the highest under daily watering condition. But they were the lowest under fifteen day’s interval watering conditions

It was also shown that, gas exchanges parameters (photosynthesis, transpiration and stomatal conductance) were higher by “Arbaminch Zuria” accession when grown under daily watering condition. While all accessions were relatively similar under five, ten and fifteen day’s interval watering in gas exchanges. But, in terms of leaf relative water content there was no significant difference among all accessions under five and ten days interval watering.

5.2. Conclusion

As a whole, it can be concluded that variations in some morphological, fresh and dry weights and physiological responses of three *Moringa stenoptala* accessions (Konso, Arbaminch Zuria and Humbo) to different water stress levels imposed by watering interval (days) were observed. Accordingly, accession "Arbaminch Zuria" produced the largest values for seedling height, stem collar girth, leaf area, root length, fresh and dry weights, total fresh and dry weights, chlorophylls concentration(a, b and total) and instantaneous water use efficiency. Contrarily, the lowest values were observed from accession "Humbo" in all parameters except, root length, stomata number and for parameters' those indicated interaction effects. Accession "Arbaminch Zuria" grown under daily watering was produced more values in all parameters except leaf relative water content for those interaction effects indicated.

5.2. Recommendation

Based on the present finding, thus, there are some recommendations stated as follow;

1. Accession *"*Arbaminch Zuria*" of Moringa stenopetala* produced more in most of the morphological parameters' and fresh and dry weights (especially root, leaf traits and total seedling biomass).
2. Daily watering should be used during raising *Moringa stenopetal* seedling in the nursery and where Moringa has being produced for vegetable in which water availability is not problem. However, for the place where water scarcity has being challenged ten days interval watering is recommended only for seedling in the nursery but not for vegetable production. Because vegetative parts (leaf traits) of the Moringa plant was reduced under more water stressed conditions.
3. Further research is needed in the responses of morphology, physiology and growth of *Moringa stenopetal to* different accession (indigenous and exotic) and water stress during the life of Moringa tree (due to its perennial character) to follow its field performance and adaptation.

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APPENDICES

Appendix Table I. Mean square values of seedling height (SH), stem girth (SG), leaf number (LN), leaf area (LA) and root fresh weight (RFW) of *Moringa stenopetala* accessions under different water stress.

		Parameters				
Source of variation	Df	SH	SG	LN	LA	RFW
Accession(A)	2	30.749***	0.042***	137.00***	8091.27***	78.55***
Water stress(WS)	3	152.945***	0.407***	805.18***	41837.94***	3743.64***
A* WS	6	0.464ns	0.0018ns	13.46 ns	417.22 ns	1.057ns
Error	24	0.432	0.0013	7.851	179.71	3.614
CV(%)		**4.25**	**5.21**	**12.64**	**10.84**	**8.2**

*Where, ns and *** indicates non-significant difference at P≤ 5% and significant difference at P≤ 0.1% probability levels, respectively. df = degree of freedom, A*WS= interaction among accessions and water stress.*

Appendix Table II . Mean square values of leaf fresh weight (LFW), root length (RL), root diameter (RD), leaf dry weight (LDW) and root dry weight (RDW) of *Moringa stenopetala* accessions under different water stress.

		Parameters				
Source of variation	df	LFW	RL	RD	LDW	RDW
Accession(A)	2	0.454***	29.389***	0.341***	0.6804***	99.781***
Water stress(WS)	3	21.315***	76.769***	2.259***	7.3098***	430.193***
A * WS	6	0.070**	0.601ns	0.010ns	0.1052***	1.775ns
Error	24	0.020	1.367	0.015	0.0026	1.928
CV(%)		**5.43**	**4.25**	**5.21**	**3.92**	**13.07**

*Where, ns, ** and *** indicates non-significant difference at P≤ 5%, significant difference at P≤ 1% and P≤ 0.1% probability levels, respectively. df=degree of freedom, A*WS= interaction among accessions and water stress*

Appendix Table III. Mean square values of total fresh weight (TFW), total dry weight (TDW), stomata number (SN), stomata length (SL) and stomata width (SW) of *Moringa stenopetala* accessions under different water stress.

Source of variation	Df	Parameters				
		TFW	TDW	SN	SL	SW
Accession(A)	2	175.8***	1046.47***	20.0277*	0.0190**	0.0177**
Water stress(WS)	3	4917.46**	324.47***	35.657**	0.0749**	0.0255**
A* WS	6	7.03 ns	6.90ns	1.546ns	0.0033ns	0.00117ns
Error	24	3.614	2.925	4.361	0.0017	0.0010
CV (%)		**6.76**	**9.91**	**12.63**	**10.96**	**14.67**

*Where, ns, *, ** and *** indicates non-significant difference at P≤ 5%, significant difference at P≤ 5%, P≤1% and P≤ 0.1% probability levels, respectively. df = degree of freedom, A*WS= interaction among accessions and water stress*

Appendix Table IV . Mean square values of chlorophyll a (chl a), chlorophyll b (chl b), total Chlorophyll [chl (a+b)] concentration, photosynthetic rate (Ps) and transpiration rate (E) of *Moringa stenopetala* accessions under different water stress.

Source of variation	Df	Parameters				
		chl a	Chl b	Chl (a+b)	Ps	E
Accession(A)	2	6.829***	1.644***	15.059***	17.584***	0.464***
Water stress(WS)	3	3.942***	3.394***	14.564***	58.766***	2.344***
A* WS	6	0.028ns	0.042ns	0.0739ns	3.684***	0.181***
Error	24	0.043	0.034	0.149	0.215	0.016
CV		**3.89**	**6.53**	**4.74**	**11.36**	**8.18**

*Where, ns, and *** indicates non-significant difference at P≤ 5% and significant difference at P≤ 0.1% probability levels, respectively. df=degree of freedom, A*WS= interaction among accessions and water stress.*

Appendix Table V. Mean square values for stomatal conductance (gs), leaf relative water content percentage (LRWC %), and instantaneous water use efficiency (IWUE) of *Moringa stenopetala* accessions under different water stress.

		Parameters		
Source of variation	Df	Gs	LRWC%	IWUE
Accession(A)	2	0.0012***	0.0056***	1.818***
Water stress(WS)	3	0.0018***	0.0834***	4.983***
A * WS	6	0.00014*	0.0011*	0.0889ns
Error	24	0.000030	0.00022	0.0967
CV (%)		**18.59**		

*Where, ns, *, and *** indicates non-significant difference at P≤ 5%, significant difference at P≤ 5%, and P≤ 0.1% probability levels, respectively. df = degree of freedom, A*WS= interaction among accessions and water stress.*

SKETCH OF BIOGRAPHY

The author was born in 1993 at Gesergio kebele (around the "Newwork"), Kena wereda, in the Konso zone of SNNPRS, Ethiopia. He attended primary education (grade 1-4) at Gesergio primary school. He then, joined Fasha elementary school (grade 5-7). He afterward, attended grade 8 at Doha full primary school. After completing full primary school, he joined Fasha secondary school where he attended grade 9 and 10. With scoring well result in Ethiopian national examination, he joined konso secondary and preparatory school where he studied grade 11 and 12. As a result of passing Ethiopian university entrance examination, he joined Wolaita Sodo University, College of Agriculture in 2014 and graduated with the Degree of Bachelor of Science in Horticulture with very great destination in June 28, 2016.

Immediately after his graduation, he employed as a graduate assistant I at Bule Hora University, college of agriculture, department of plant sciences in October 2016. And then, with two consecutive semester (one year) experience, he was sponsored by Bule Hora University in October 2017 to pursue his Degree of Masters in plant sciences (Specialization Horticulture) under school of postgraduates of Hawassa University, Ethiopia.