GROWTH AND PHYSIOLOGICAL RESPONSE OF ENSET (*Ensete ventricosum*) VARIETY ENTADA TO SOLAR UV-B RADIATION AND DIFFERENT PLANTING DENSITIES

BY

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ABREVIATIONS AND ACRONYM

CPD…….. Cyclobutane Type Primidine

DAP…….. Di Ammonium Phosphate

EOD…….. End of the Day

FR…….. Far Red

HPS…….High Pressure Sodium

LED…….. Light Emitting Diodes

LSD…….. Least Significant Difference

PAR…….. Photosynthetically Active Radiation

R ……Red

UV……..Ultraviolet

GA……..Gibberellic Acid

IAA……..Indole Acetic Acid

DEDICATION

"To my Father Abe Woya, My Mother Dabale Jerjersa and all my family for their inspiration, love and support throughout my life."

STATEMENT OF AUTHOR

I solemnly declare that this thesis is not submitted to any other institution anywhere or any other University and that all the source materials used for this thesis have been duly acknowledged.

Name: ……...…….**Signature:** ……..……

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TABLE OF CONTENT

CONTENTS **PAGES**

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LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURE

Figure **page**

LIST OF APPENDICES

Appendix **Page**

Growth and physiological response of Enset (*Ensete ventricosum*) variety Entada to solar UV- B radiation and different planting density

By Adem Abe

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ABSTRACT

Ensete ventricosum (Welw.) Cheesman, commonly known as enset, is a monocarpic perennial herb originated in Ethiopia. Propagation is commonly practiced through traditional sucker inducing techniques. However, Entada, which is one of the enset landraces mostly cultivated in southern part of Ethiopia around Ari zone, which unlike other enset landraces, produces natural suckers like banana. Information is lacking about the response of Entada to environmental cues for the regulation of natural suckering of the landrace. Therefore, the present study was conducted during the 2016/2017 off season with the aim of evaluating the effect of UV and planting density on morphogenesis and physiology of Entada plants grown at Hawassa at an altitude of 1700masl. The experiment was carried out in a randomized complete block design with three replications at Hawassa University field research station. The experiment had three level of planting density (0.5m x 0.5m (40,000 plants/ha), 0.75m x 0.75m (17,777plants/ha) and 1m x 1m (10,000 plants/ha) and two level of UV radiation (with solar UV-B radiation and without solar UV-radiation). Data were collected on light quality, morphological and physiological parameters. The analysis of variance showed that planting density and UV-B radiation significantly influenced light quality distribution, morphology and physiology of plants. It was observed that, total number of sucker and suckering ratio were significantly ($p<0.05$) affected by UV, planting density and their interaction. The highest planting density induced reduction in R: FR ratio significantly increased plant height by 18% but the number of suckers was reduced by 45% as compared to lowest. However, UV-B had stronger effect on plant height than planting density. Maximum number of suckers were recorded (47.3) from treatment combination of lower planting density (with higher R:FR ratio) and without UV-B radiation. Leaf number significantly responded to UV but not to the changing in planting density. With respect to specific leaf area, dry matter and total biomass no significant effects was observed among planting density, but significantly reduced by solar UV radiation. Photosystem II efficiency (Fv/Fm) significantly responded to UV than planting density. Removing UV-B radiation using plastic film significantly increased photosystem II efficiency (Fv/Fm) of leaves by 2.5%. Although stomata aperture significantly responded to UV than planting density, the stomata number showed a reduction pattern with increasing planting density. Generally, change in the composition of light quality using planting density and screening material approach has a significant effect on the modification of sucker development and morpho-physiological growth condition of Entada.

***Key words**: Entada, Branching (Suckering), Red light, Far Red light, UV-B, Planting Density.*

1. INTRODUCTION

Ensete ventricosum (Welw.) Cheesman, commonly known as enset, is a monocarpic perennial herb originated in Ethiopia. The crop is geographically distributed as a wild species in many parts of Sub-Saharan Africa and Asia. Enset is cultivated only in its native indigenous farming systems of South and South-Western part of Ethiopia. In fact in Ethiopia, *E. ventricosum* is arguably the most important crop contributing to food security and rural livelihoods for about 1/4 (20 million people) of the country's population (Olango *et al*., 2014). It is a multipurpose plant with a range of utilities including food, feed, construction and medicinal uses. Moreover, enset cultivation improves soil by permanent soil tillage due to its high demands to soil fertility and soil structure (Zippel and Karina, 2002).

Enset was considered as member of the genus Musa as it strongly resembles banana morphologically and because of this some of the species names formerly given to enset were *Musa ensete* and *Musa ventricosa* (Lye and Edwards, 1997). Cheesman (1947) was separated enset from banana on the basis of differences in pseudostem morphology and chromosome numbers.

Entada is an enset landraces mostly cultivated in southern part of Ethiopia around Ari zone, which unlike other enset landraces produces natural suckers like banana (Musa spp.), (Bekele and Shigeta , 2011). All other enset except entada initiate suckers by induction through overcoming apical dominance.

The sucker production, changes in morphological, physiological and growth responses in plants are driven by many environmental cues. Amongst such factors are light quality

distribution and agronomic practices including planting density playing a paramount importance in governing these responses.

To sense and respond, plants are naturally given with an array of photoreceptors, these photoreceptors control diverse responses of the plant to light parameters, such as spectrum, intensity, direction and duration. These photoreceptors include the red and far-red absorbing phytochromes, the blue and UV-A light absorbing cryptochromes, phototropins, UVR8 protein that senses the presence of UV-B radiation and other implied photoreceptors absorbing in UV-A and green regions.

Depending on its wavelength; Ultraviolet (UV) can be divided in to three different ranges: UVA (315-400nm), UV-B (280-315nm) and UV-C (100-280nm). Among these, UV-C is the radiation with the lower wavelength, or rather with the higher associated energy (Terfa *et al.*, 2014; Katerova *et al*., 2009).

The spectral radiation of UV- B distribution at the Earth's surface is modified by temporal, geographical and meteorological factors such as time of the day, geographical latitude, season, clouds, surface reflection, altitude and etc. (Frederick *et al.,* 1989). Novel technologies to manipulate light quality including UV levels are at large in use in protected plant cultivation. Some of these technologies are by using different selective plastic films, either UV blocking or UV-transparent, specific parts of the UV spectrum can be manipulated. This provides new opportunities in protected crop cultivation (Jansen et al., 2012)

Different plant responses to supplemental UV-B radiation have been studied. Direct injuries to the photosynthetic apparatus have been studied extensively at molecular level. These effects

include inactivation of photosystem II (PSII), reduced activity of Rubisco, decreased levels of chlorophylls and carotenoids, down-regulation of transcription of photosynthetic genes, and decreased thylakoid integrity and altered chloroplast ultrastructure (Jansen *et al*., 1996; Vass *et al.,* 1999).

Morphologically UV-B exposure decreases shoot length, shoot dry mass, foliar area and delay flowering (Torre *et al*., 2012; Wargent *et al*., 2006; Zhang *et al*., 2014). In some other species, exposure to supplementary UV-B light significantly increases leaf area as well as fresh and dry biomass (Sakalauskaitė *et al*., 2013). Even if the effects of UV-B on branching vary in range according to species, UV-B exposure tends to increase the number of stems / tiller (Barnes *et al*., 1990; Torre *et al*., 2012).

Many physiological processes of plants are also affected by UV-B exposure. It is demonstrated that transpiration is reduced in some UV-B sensitive seedlings (Tevini and Teramura, 1989; Yao and Liu, 2006). The duration time for stomatal closure is rapid at low UV-B levels. Stomatal opening is slowed by higher UV-B levels. Enhanced UV radiation causes a reduction in plant growth and photosynthetic capacity and pigment levels - (Rahimzadeh *et al., 2011*). Photosynthetic activity may be reduced by direct effects on the photosynthetic process or metabolic pathways, or indirectly through effects on photosynthetic pigments or stomatal function. Under high UV-B level chlorophyll fluorescence had been shown to be reduced, which is simple and reliable technique for measuring the performance of photosystem II (Maxwell and Johnson, 2000; Kolb *et al*., 2001; Reddy *et al*., 2004).

Different researchers have demonstrated that, morphological responses to different light spectrum vary based on the plant species and adaptation to previous light conditions. When

plants subjected to shade by other plants many species show the characteristic of 'shade avoidance syndrome' (SAS) response: they start to elongate internodes and leaf petioles in an attempt to reach out of the low shade (Franklin and Whitelam, 2005). SAS is triggered when plants sense a low ratio of Red (R) to Far-red (FR) lights (R: FR) and reduced blue light intensity in their surroundings (Franklin, 2008; Franklin, 2005). Many plant species significantly accelerate elongation within minutes after exposure to FR-rich light. Increased elongation in FR-rich light is often coincides with strengthening of apical dominance, gibberellic acid (GA), indole acetic acid (IAA) that lead to reduced branching. Moreover, FR-rich light also can cause acceleration of flowering, reduced storage of assimilates, reduced seed set, shortened fruit development, and a reduction in seed quality (Whitelam and Halliday, 2007).

Light that passes through densely populated canopy of leaves has a reduced red to far- red ratio (R: FR) (Smith, 1989). In a sparsely populated planting density (e.g. in which leaf area index (ratio of leaf area to ground area) of less than unity) there is almost no shading between plants during most of the day. Under these conditions the effect of reflectance is reduced and the proportion of red light can be higher than the proportion of far red light(Khattak *et al*., 2004). Therefore plant density can affect R: FR ratio. At low plant densities the main effect is an increase in far-red light with no decrease in red, at higher densities red light decreases more than far-red light (Aphalo *et al*., 1999; Ballaré, 1999).

Light can affect the growth morphologies like plant height, branching, leaf area and internode extension of different plant species (Assmann, 1992). Entada is the only enset landraces that produces natural suckers like banana (Bekele and Shigeta , 2011). Planting density and light

quality are inter-dependent in case of R: FR ratio, which have effects on suckering. In Enset, producing suckers naturally may not be important, and regulating sucker production is crucial since, sucker can affect the size of pseudostem, the economically important part of the crop by diverting assimilates towards the suckers (Kefale and Sandford, 1991). Although many plant responses to UV radiation have been reported, complete mechanistic details of most of these responses have not been elucidated in crops like Enset. Therefore, it is important to understand how Enset plant responds to different light quality and plant population.

General objective

The general objective of the present study is to evaluate the morphological and physiological responses of Entada to light quality and different planting densities

Specific objectives

- ✓ To evaluate the impact of UV - B radiation on growth of Entada.
- ✓ To assess the physiological responses of Entada to UV- B radiation and planting density
- ✓ To evaluate the impact of planting density and light quality on sucker development

2. LITERATURE REVIEW

2.1. Taxonomy and Distribution of Enset

Enset (*Ensete ventricosum* (Welw) Cheesman) is a monocot that belongs to the order Schistaminae and family Musaceae. Enset was considered as member of the genus Musa as it strongly resembles banana morphologically and because of this some of the species names formerly given to enset were *Musa ensete* and *Musa ventricosa* (Lye and Edwards, 1997). It was Cheesman (1947) who separated enset from banana on the basis of differences in pseudostem morphology and chromosome numbers. Other than *Ensete ventricosum*, there are species in the genus *Ensete* but there is some ambiguity regarding their number. Cheesman (1947) revised the Musacearum family and transferred about 20 species from the genus Musa to the genus Ensete and listed 24 species which are distributed in Africa and Asia. But other researchers recognized 6 or 7 species under this genus. Taye (1984) has also stated that 7-8 species are known in the genus. This shows that further research is needed on the taxonomy and distribution of enset species (Brandt *et al*., 1997). In any ways all authors agree that *Ensete ventricosum* is the only cultivated species in Ethiopia and has great economic importance.

Currently, enset distribution is restricted to south, southwest and central part of Ethiopia and it is not known as food crop in the northern part of Ethiopia. However, historical evidences suggested that enset may have once played a much more important role in the agricultural practices of central and northern Ethiopia before the mid 19th Century. The possible reasons

for total disappearance of enset culture in the North could be disease, drought and instability in the sociopolitical events between mid 1700 and mid 1800 (Brandt *et al*., 1997).

2.2. Enset Morphology and Ecology

Enset looks like a large, thick, single stemmed banana plant. Usually it is larger than banana and 6-12 meters tall. The leaves are 5-7 meters tall and 1 meter in diameter and are more erect than a banana plant (Kefale and Sandford 1991; Brandt *et al.*, 1997). The stem has three parts: the pseudo stem, which is made of tightly clasping leaf sheaths, is 2-3 meters in height and with an average of 1 meter diameter with an edible pulp and quality fiber. The underground corm is an enlarged lower portion of the stem with an average of 0.7 meter length and diameter. The fibrous rooting system of enset grows out from this part. The true stem is between the pseudo stem and corm near the ground. Usually it grows up during maturity and initiates a single flower head, which forms multiple flower, fruits and seeds. The small banana like fruits produce several irregularly shaped black seeds. Most wild enset plants are produced from seeds unlike the domesticated ones, which are propagated from suckers (Brandt *et al.*, 1997).

Enset cultivation is restricted at altitudes ranging from 1600-3100 masl (Taye and Asrat, 1966). But recent investigations indicated that it grows in areas as low as 1200 m.a.s.l (Endale, 1990). However, the best elevation for enset cultivation is between 2000 and 2750 m.a.s.l with an annual rainfall of 1100 to 1500 mm where the majority falls between March and September (Bermita, 2004). According to Taye and Asrat (1966), enset can with stand relatively long period of drought (about 5 months) and tolerates short period of frost unlike species in genus *Musa*.

The average temperature of enset growing areas is between 16 to 20°C and the relative humidity of 60 to 80 % (Yohannes and Mengel, 1994). Soil types in the enset growing areas of Ethiopia are Utric cambisols, Pellic vertisols and Utric nitosols which are moderately acidic to slightly basic with a pH reaction ranges from 5.6 to 7.3. These soils contain 0.10 to 0.15% total Nitrogen and 2 to 3% organic matters (Taye and Asrat, 1966).

2.3. Economic Importance and Ecological Uses of Enset

Enset is a multipurpose crop of which every part is thoroughly utilized (Shigeta, 1996). It is a good source of starch. The corm and the pseudo stem are the most important sources of food (Kefale and Sandford, 1991). The types of food from these parts are known as 'Kocho', 'Bulla' and 'Amicho' (Spring *et al.,* 1996). Kocho is the bulk of the fermented starch obtained from the decorticated (scraped) leaf sheathes and grated corm. Bulla is obtained by squeezing out the liquid containing starch from scraped leaf sheathes and grated corm and allowing the resultant starch to concentrate into white powder. Amicho is boiled corm of young enset plants known for best quality of corm. It is prepared and consumed in a similar manner to preparation of other root and tuber crops (Brandt *et al.,* 1997). Fiber is the by-product of enset that is left after decorticating the leaf sheathes. Its strength is found to be equivalent to the important fiber crop *Musa textiles* (abaca) (Taye, 1984). Fiber is used for making bags, ropes, twines, cordage, mats, etc where the variety, the age of the plant and the way in which the fiber is extracted and stored determine its length and quality (Kefale and Sandford, 1991; Yohannes and Mengel, 1994).

Enset is one of the major crops that can significantly help to ensure food security in a country like Ethiopia (Brandt *et al.,* 1997). The average yield of refined enset product kocho ranged

from 35 to 51 tons/ha/ year in early maturing enset clones (Atnafua and Endale, 2008). The amount of food attainable from 50-60 enset plants per year could provide enough food for an average family of 5-6 persons (Zeweldu and Ludders, 1998). Enset products are available throughout the year and can be stored in pits for long periods of time without spoiling.

Enset is rich in carbohydrate and mineral substances like calcium and iron (Shigeta, 1990). The energy yield of enset is by far higher than that of several cereals. A mature enset plant could yield 20x106 cal / ha/ year which is 20 times higher than that of barley (Terefe, 1991). Enset energy yield was also reported to be higher than potato, sweet potato and banana (Pijls *et al.,* 1995). This shows that cultivation of enset can significantly improve food security at household and at national level.

Some clones and parts of enset plants are reported to have medicinal value for both human and animals. These clones are claimed to heal bone fractures, for treatment of diarrhea and delivery problems i.e. assisting to discharge the placenta (Spring *et al.,* 1996). Even some farmers believe that eating bulla from clones like Boliae in Wolaita after taking traditional medicines against tapeworm protects the liver from the side effect of the medicine (Zerihun, *et al*, 2013). However, traditional healers in the area confidentially keep ethno-medicinal knowledge of enset landraces., Traditional enset medicines include (i) porridge made of Itima from Agino and Gefetanuwa landraces, for strengthening women after delivery and healing bone fractures in humans respectively; (ii) very highly fermented Uncca from Mazia and Halla landraces, for curing stomach cramps; and (iii) boiled corm of Lochingia, for birth control and abortion in humans and to feed cows to facilitate placental expulsion (Temesgen *et al*, 2014).

2.4. Effect of UV Radiation on Plant Growth and Morphology

2.4.1. The light spectrum controls plant growth and development

The sensitivity range of plants to light is extended from UV through the visible spectrum to far-red radiation (700-800 nm). Regarding ultraviolet radiation, UV-C (100-280 nm) radiation is thoroughly absorbed by the stratospheric ozone layer and the atmosphere; only UV-A (315-400 nm) and UV-B (280-315 nm) radiations reach plants. In the visible light spectrum (400-700 nm), the major wavelengths perceived by plant photoreceptors and pigments are those corresponding to blue (400-500 nm) and red (600-700 nm) and, to a lesser extent, green (500-600 nm). A small fraction of near-infrared radiation, i.e. far-red light perceived by phytochromes with a sensitivity peak at 730 nm, is also essential to plant development (Terfa *et al.*, 2014).

2.4.2. Impact of UV radiations on plant morphology and vegetative growth

Plant vegetative growth is the result of key processes such as cell division and elongation, directional growth and branching. These processes are dependent on light spectrum, intensity and direction that modulate photosynthetic activity as well as photomorphogenic signals.

In order to optimize photosynthetic activity, plant direct their growth towards incoming light to best position their leaves. In this reaction, direction of blue and UV-A radiations in the incoming light constitutes the signal perceived by plants. In the case of hypocotyl phototropism, the signal will lead to asymmetrical cell growth upon formation of a lateral gradient of auxin distribution, and auxin transfer from the lit side to the shaded side of the

organ where it accumulates (Ding *et al*., 2011; Friml *et al*., 2002). This ultimately causes phototropic growth of the shoot towards the light source through auxin-mediated increased cell growth on the shaded side, as recently reviewed by Christie and Murphy (2013) and by Hohm *et al*. (2013). In this case, phot1 are the main photoreceptors involved in perception of directional light, with CRY and PHYA contributing too. PHOT1 acts as the main phototropin in this response.

UV-B either stimulate stomatal opening or stomatal closing and neither of these responses is readily reversible (Jansen and van den Noort, 2000). These responses depend on the metabolic state of guard cells (Jansen and van den Noort, 2000) but so far, the implicated mechanisms have not been elucidated.

Numerous studies mention morphological effects of UV radiations on plants, which often depend on species and growth conditions (light intensity, relative shares of these wavelengths in the light spectrum).

Exposure to UV also modifies plant growth (Roro *et al.*, 2016). Morphological plant responses to UV-B exposure are also species-dependent (Kakani *et al*., 2003). In numerous species, UV-B exposure decreases shoot length, shoot dry mass and foliar area (Torre *et al*., 2012; Wargent *et al*., 2006; Zhang *et al*., 2014). In some other species, exposure to supplementary UV-B light significantly increases leaf area as well as fresh and dry biomass (Sakalauskaitė *et al*., 2013). The effects of UV-A on plant morphology have been less studied, but Krizek *et al*. (1997) noted that exposure to UV-A without UV-B radiation decreased plant height and leaf area. Even if the effects of UV-B on branching vary in range according to species, UV-B exposure

tends to increase the number of stems / tiller (Barnes *et al*., 1990; Torre *et al*., 2012; Roro *et al.,* 2016).

2.4.3. Plant biomass accumulation and growth

Reduction in growth is one of the most common responses to enhanced and ambient UV-B. Exclusion of UV-B from solar radiation caused a significant increase in the growth (plant height, leaf area, specific leaf weight, leaf weight ratio) and biomass production in many plant species like barley, cotton, mung bean and pea, pumpkin, soybean, Cyamopsis, wheat, sorghum and amaranthus (Guruprasad *et al*., 2007; Kataria, & Guruprasad, 2014). Exclusion of ambient UV-B produced more branches in dicots or tillers in monocots with a larger leaf area in barley, cotton and sorghum (Mazza *et al*., 1999, p. 61; Coleman, & Day, 2004). Root biomass, number of nodules and nodule fresh weight were also enhanced after exclusion of solar UV (Sharma, & Guruprasad, 2012). Rinnan *et al*., (2005, p.11) showed that below ground biomass of *Vaccinium uliginosum* is reduced under ambient UV-B when compared to UV-B exclusion. UV exclusion studies reveals that dicots like mung bean, pea, cotton, soybean and amaranthus are more sensitive to solar UV-B than monocots like maize, sorghum and wheat (Pal *et al*., 2006, p.19; Kataria *et al*., 2013, p.140) while it does not differ between C3 and C4 plants. Thus the UV components present in the solar spectrum seemed to inhibit the growth and biomass accumulation whereas exclusion of solar UV-B from the solar radiation causes significant enhancement in morphological changes in the crop plants.

2.5. Plant Physiological Responses to UV - B Radiation

During the last decade, it has become increasingly clear that UV-B radiation has been considered as damaging environmental factors. However, a low, ecologically relevant UV-B level has a regulatory effect in changing the morphology and the level of accumulation of phenolic compounds, carotenoids and glucosinolates. Fundamental understanding of plant UV-B perception and responses opens up new opportunities for crop manipulation. Thus, targeted low dosage UV-B radiation treatments as emerging technology may be used to generate quality pot plant and fruit, vegetables, and herbs enriched with secondary plant metabolites. The UV-B induced accumulation of secondary plant metabolites is likely to have evolved as a plant defense response against pest, disease and harmful effect of UV-B radiation.

Many different plant responses to supplemental UV-B radiation have been observed (Tevini and Teramura, 1989). The damaging and regulatory effects of UV-B radiation have been demonstrated by different researchers (Tevini *et al*., 1989, Kramer *et al*., 1991; Landry *et al*., 1995). Moreover, previous report indicated UV-B induced growth morphology and change in the level of secondary metabolites in different plant tissue (Schreiner *et al*., 2012; Teramura & Sullivan, 1994).

2.6. Plant Densities and Their Light Environment

Several above-ground environmental factors such as light intensity, spectral distribution, air movement, air humidity, and temperature, influence plant growth and development. Plants interact and compete among themselves for limited resource such as light to survive and to

dominate their territories. Competition for light leads to changes in growth potential and non-structural compounds. It also reduces genetic diversity in mixed stands as many clones are "shaded-out" by their more competitive neighbors (Sin, 2000).

The morphological response to light quality in plants is part of a strategy to adapt to a changing light environment. This is regulated by the interaction between plant photoreceptors sensitive to particular wavelengths and their downstream signaling pathways. The most striking effect of light composition on shoot architecture is the shade avoidance syndrome. This phenomenon describes the elongated growth of plants, growing in high density, to escape canopy shading.

The shade avoidance response includes increased internode elongation, inhibited axil-lary bud outgrowth, petiole elongation and upward bending of leaves (hyponasty). This response to shading is caused by changes in the red (R) to far-red (FR) light ratio, resulting in a FR enhancement that is perceived by the plant photoreceptors(Pierik & de Wit, 2013). In a dense canopy, the surrounding foliage absorbs red light, while much more far-red light reaches the lower canopy. The R: FR ratio is calculated by dividing the photon irradiance between 655 and 665 nm (R) with the photon irradiance between 725 and 735 (FR). This R:FR ratio ranges from 1.2 in daylight to 0.1 under canopy shading(Franklin, 2008).

According to Ballard (1994), reduction in the R:FR ratios initiates an increase in apical dominance and reduces branching. The impact on height growth is greater than the actual shading effect.

Low R :FR ratios reduce nutrient uptake in grasses and reduce the development of root density (Casper *et al*., 1997). Plants grown under high FR light increase shoot dry weight (Kasperbauer *et al*., 1992; Ritchie, 1997). Kozlowski *et al*. (1991) demonstrated that under high competition for light, plant roots are reduced extension, configuration, and density. Therefore plant density can affect R:FR ratio.

Previous research indicated that an increase in the proportion of far red light at lower plant density might be due to reflection from leaves, however, at higher densities the predominance effect of far red light was directly related to a decrease in Red light due to absorption by the canopy (Aphalo *et al.*, 1999).

Therefore, the mechanisms of competition between plants in dense stands should be a major consideration in plant growth improvement. Hall (1994) has provided an outline of plant competition based on spacing management, and suggested that row spacing have their initial effects mediated by the phytochrome shade avoidance response.

The change in the R :FR ratio depends on planting densities, structures, and orientation of leaves. Different *Populus* clone respond differently to spacing, resulting in changing biomass allocations (Panetsos, 1980). Therefore, field spacing experiments comparing different clones and light regimes should provide a good opportunity to test the competitive ability of clones. Good measurements are needed to determine how much of the growth dynamics of plant height and leaf development under plantation can be accounted for on the basis of phytochrome-mediated proximity perception at different plant densities. Ballard *et al*. (1987) have suggested that the shade-avoidance response is a major factor in reducing genetic diversity in stands of mixed genotypes.

When subjected to shade by other plants many species show the characteristic ‘shade avoidance’ response: they start to elongate internodes and leaf petioles in an attempt to reach out the low shade (Franklin and Whitelam, 2005). This plant structure optimizes light interception and photosynthesis by minimizing self-shading, thus outcompeting neighbouring plants, which do not show this response to the same extent. This ability to detect and respond to shade can result in a significant selective advantage to plants growing in natural communities (Van, 2013).

Many plant species direct growth towards light (phototropism). Other than in shade avoidance responses, where the spectral composition plays a central role, in phototropic responses the direction of the light is crucial. In many phototropic responses a phototropin (PHOT1) performs as the essential blue light receptor acting as mediator of phototropic responses (Briggs, 2010).

3. MATERIALS AND METHODS

3.1. Experimental location

A field experiment was conducted at Hawassa University, Research field during the 2016/2017 off season. Hawassa is situated at 7°4’ N, 38°31’ E and it is in a hot to warm sub-moist humid climate zone with warmer temperature especially during the dry season (February to April). It has a longer growing season and a less definitive pattern of rainfall during the growing season. It is a mid-highland area in the Rift Valley zone (Beshir *et al*., 2015)

3.2. Plant materials

Uniform age and size sucker of Entada (sucker with three leaves) were obtained from Hawassa University, College of Agriculture and planted in well prepared experimental site according to their treatments. Entada is an enset accession mostly cultivated in southern people of Ethiopia especially around Ari zone, which unlike other enset landraces and more like banana (Musa spp.), produces natural suckers (Bekele and Shigeta , 2011). It has the habit of growing fast and suckering.

3.3. Experimental set up and Treatments

Using an approach with planting density 0.5m x 0.5m (40,000 plants/ha), 0.75m x 0.75m (17,777plants/ha) and 1m x 1m (10,000 plants/ha) and UV-blocking films, the aim of this study was to evaluate the effect of UV and planting density on morphogenesis and physiology of Entada plants grown at Hawassa altitudes of 1700 in Ethiopia.

UV-B-blocking film (Solar EVA-5 High diffuse opaque film with 0.20mm thick and 3m wide Rovero plastic, Raamsdonksveer, The Netherlands) was selectively cut-off of the solar spectrum below 350 nm(UV-B and the shortest wavelengths of UV-A) (fig: 1). The light transmitted through the plastic film was measured with a spectroradiometer PS-300 (300-1000nm) apogee instrument connected to cosine-corrected head.

The factorial arrangement of three level of spacing with two level of UV – B (3x2) was laid out in randomized complete block design with a three replication. The experimental site had 13m x 18.5m area with plot size 3 x 2.25m. Spacing between plots and blocks were separated by 1m and 2m, respectively.

Uniform age size of (sucker with three leaves) was planted at the spacing distances mentioned above. The plants were placed under plastic covering on the top of 1m high construction for the first time then 1.5m and 2m as plant height increases, and sorghum crop was planted to reduce the entrance of light at the side. The structures were erected in North–South direction over the treatment plots. This orientation ensured that the solar radiation reached the plants only after passing through the filter as the sun moved from East to West.

Table 1: Treatment combination

Treatment No	Treatment combination
1	1m x 1m + UV –B radiation
2	0.75m x 0.75m + UV –B radiation
3	0.5m x 0.5m + UV –B radiation
4	1m x 1m with –UV –B radiation
5	0.75m x 0.75m–UV –B radiation
6	0.5m x 0.5m –UV –B radiation

3.4. Planting and crop management

Healthy and well developed suckers were planted on a well-prepared experimental site at the spacing of mentioned above. Weeding, cultivation and watering were undertaken whenever it was needed.

3.5. Growth parameters measurements

After six months of treatment, four plants from each treatment were used for destructive morphological analyses of above ground biomass. At the end of the experiments Leaf number, Number of suckers (the total number of suckers of the size 5-20cm long that germinated from sample plant) and suckering ratio were calculated as the ratio of total number of sucker to the

number of sample plants. Plant height (cm), the length of the plant from the base to the apex of the plant in each plot was measured.

The total leaf area was estimated by measuring the length and the width of the individual leaves and calculating the area using the formula for banana developed by Turner (1972a):

$$TIA = \sum (0.83 \times L \times W)$$

Where, *L* is the length of lamina, *W* the maximum width of lamina.

Subsequently, plants were dried to a constant weight at 65 ◦C, and the specific leaf area (SLA; ratio of projected leaf area to dry weight) was determined.

Finally, *Total Biomass* (g), the fresh weigh of leaves, pseudo stems, roots and suckers of randomly selected two plants from each plot was measured and dried in oven at 65^{o}C for until a constant weight is obtained. Then, their dry weight was recorded and dry matter percentage was calculated from it.

3.6. Physiological measurements

Number, area and size of stomata: A method that we used for stomata number, area and size was the imprint nail polish technique. Nail varnish was applied on the bottom side of the leaf. After 3-5 min drying the imprint was peeled from the leaf and mounted on glass slide and the stomata density, size and area were measured under leica DM4B stereomicroscope.

Chlorophyll florescence: is light re-emitted by chlorophyll molecules during return from excited to ground states and used as indicator of photosynthetic energy conversion. To evaluate the performance of the plants, maximal photosystem II efficiency (Fv/Fm) of well-developed leaves from randomly selected vegetative plants were measured in the middle of the day with a Handy-PEA fluorimeter following the methodology of Strasser *et al*. (2004). Before measurement, leaves were dark-adapted in the leaf clip for 15min. Light was provided by an array of three high-intensity light-emitting diodes to ensure that the photosynthesis was saturated during the measurements.

Chlorophyll content: Chlorophyll content was determined in samples of fresh leaves according to Arnon, (1949). Chlorophyll extraction was carried out with a mixture of acetone 80%. 2g entada tissue was homogenized with 25ml acetone solution 80%, and the solution was covered with aluminum foil to avoid oxidation of chlorophyll from light. Absorption was measured at 663 and 645nm using spectrophotometer. The chlorophyll content was calculated as mgg^{-1} fresh weight. The concentrations of total chlorophyll, chlorophyll *a*, and chlorophyll *b* were calculated by the following equations (Arnon, 1949). The chlorophyll content in the fresh plant leaves was calculated by equation:

Chlorophyll a (mg/g fresh weight) = [(12.7*A663) - (2.69*A645)]*(V/1000*W)

Chlorophyll b (mg/g fresh weight) = [(22.9* A645) - (4.68* A663)]*(V/1000*W)

Total chlorophylls= (20.08*A645+8.02* A663)*(V/1000*W) = Chlorophyll a+ Chlorophyll b

3.7. Climate data and radiation

Weather data such as temperature, rainfall, relative humidity and sunshine duration of the last ten years (2007 to 2016) were collected from the nearest meteorology station (Ethiopian National Metrology Agency, Hawassa branch).

Table: 2 Climate data for the past ten years (2007 to 2016)

Climate data	Minimum	Maximum	Average
Temperature(^{0}C)	13.7	27.7	20.7
Total monthly rainfall (mm)	670.9	1156.5	913.7
Relative humidity (%)	37.25	70.75	54
Sun shine duration (hrs.)	6.9	7.9	7.4

Average values of minimum and maximum temperature (°C), RH (%), sunshine duration (h) and rain fall (mm) of past ten years (2007 to 2016) recorded by meteorological stations of southern Ethiopia located nearest to the study area (Ethiopian National Meteorological Station, Hawassa branches).

During the study period, temperature and RH at the experimental sites (Table 3) were recorded by Infrared thermometer under leaf canopy as possible without shading to it and hungs close to the plant canopy for 24 hrs.

Table 3 : Climate data during experiment

UV-B	PLSP	T mean(^{o}C)	RH (%)
+UV	1mx1m	21.23	64.84
	0.75mx0.75m	20.47	63.06
	0.5x0.5m	20.09	59.58
	Mean	**20.6**	**62.49**
-UV	1mx1m	19.37	62.13
	0.75mx0.75m	19.25	61.83
	0.5x0.5m	18.7	61
	Mean	**19.12**	**61.65**
Grand mean		**19.86**	**62.07**

Data was sampled under UV-blocking (-UV) films during the dry (January to July) at an elevation of 1700masl altitude in Ethiopia. The mean temperature (T_{mean} ℃) and the relative air humidity (RH) were logged by a mini data logger (Testo 174) at the top of plant canopy. PLSP= plant spacing

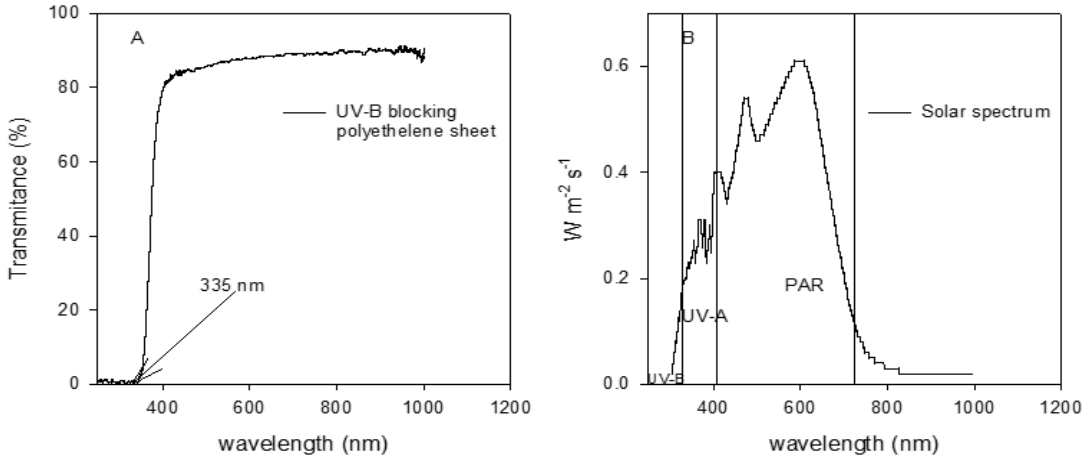


Figure 1: *Solar spectrum transmission of polyethylene sheets used in the growth experiment with Entada measured during noon time at Hawassa altitude of 1700masl*: *UV-blocking polyethylene film (fig: A) blocks UV-B spectrum (280– 315 nm) and the short wavelengths of UV-A (≤350) (Solar EVA-5 0.20 mm thick high diffuse opaque polyethylene film, Rovera plastic, The Netherlands). The light transmitted through the plastic film was measured with a spectroradiometer PS-300 (300-1000nm) apogee instrument connected to cosine-corrected head.*

The UV radiation, red and far red light and PAR (photosynthetic active radiation) was measured with a spectroradiometer PS-300 (300-1000nm) apogee instrument connected to cosine-corrected head. Plants grown under UV-blocking plastic film will hereafter be referred to as minus UV (−UV), those grown without plastic film referred to as plus UV (+UV).

3.8. Statistical analysis

Analysis of variance (ANOVA) was done using a randomized completely block design using proc. mixed model procedure of the SAS statistical software (version 9.0) appropriate for the design. Means were separated using the Least Significant Difference (LSD) test at 5% level of significance. Correlation between plant height, leaf number, sucker number, total fresh weight, total dry weight, dry matter content were evaluated using stepwise regression analysis.

4. RESULT AND DISCUSSION

4.1 Climate and Solar Radiation

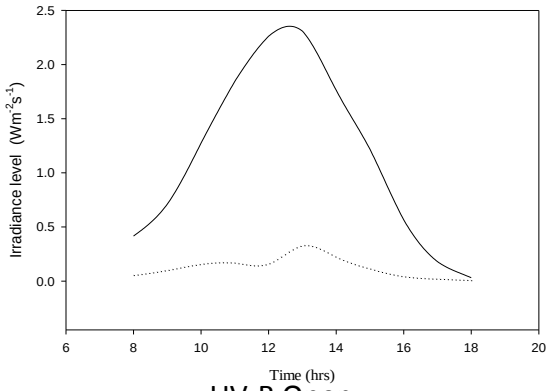
4.1.1. Climate data

Data obtained from the weather stations (Table: 2) indicated that, for the last 10 years (2007–2016) temperature varied in average between 13.7°C and 27.7 °C throughout the year (Ethiopian National Meteorological Station, Hawassa branch, Ethiopia).

During the experimental period the temperature showed similar trends with the previous meteorological data. As planting density increases, the temperature of leaf canopy decreased and slightly lower temperature recorded under plastic film (Table: 3).

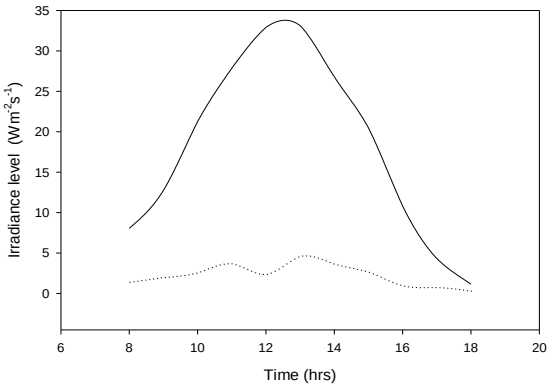
4.1.2. Solar radiation

The open solar UV radiation treated and a UV-blocking filter removing UV-B and the shortest wavelengths of UV-A was used at different planting density. Under both (plastic film and open field), red, far red, PAR, UV-A and UV-B at the experimental sites were measured during clear sky four randomly selected days.



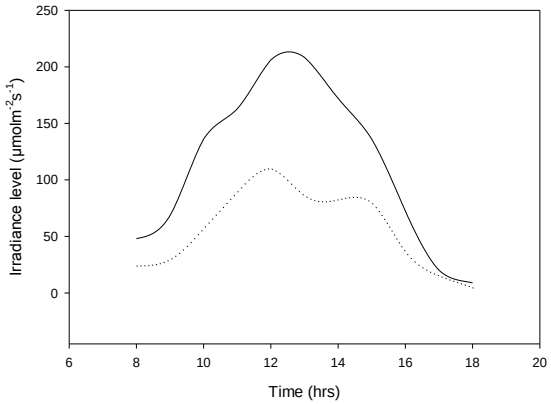
---- UV-B Open

..... UV-B under plastic



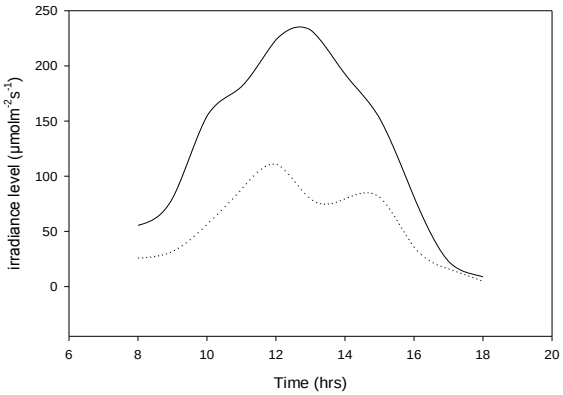
---- UV-A Open

..... UV-A under plastic



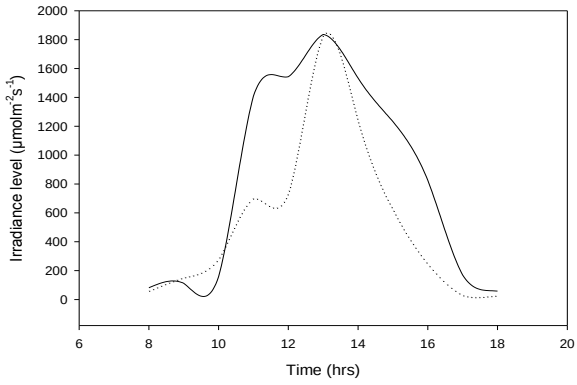
---- Far Red Open

..... Far Red under plastic



---- Red Open

..... Red under plastic



---- PAR Open

..... PAR under plastic

Figure: 2. UV-A, UV-B, PAR, Red and Far Red light distribution under plastic film and open field measured in four randomly selected days.

Table 4: Ambient irradiance level and irradiance level of UVA and UVB as affected by plastic film and planting density (canopy)

Level of Plant spacing	UV-B under canopy ($W\ m^{-2}$)	UV-B below film + canopy ($W\ m^{-2}$)	% UV-B reduction	PAR under canopy ($\mu molm^{-2}s^{-1}$)	PAR below film + canopy ($\mu molm^{-2}s^{-1}$)	%PAR reduction	Aver. Sun shine duration (hrs.)
0.5mx0.5m	0.16	0.08	50	113.6	60.8	46	7.48
0.75mx0.75m	0.39	0.05	87	135.2	54.4	60	
1mx1m	0.98	0.19	80	177.9	89.8	50	
Mean	0.51	0.10	80	142.2	68.3	52	
Ambient (control)			1.76			1835.6	

Ambient irradiance levels and irradiance levels of UV-B ($W\ m^{-2}$) and photosynthetic active radiation (PAR) ($\mu mol\ m^{-2}\ s^{-1}$) below UV blocking films (-UV) and open field were measured in the middle of the day (11:00 to 15:00) at an altitude of 1700 masl in Ethiopia during dry (January to July 2017). Percent reduction in irradiance below films compared with ambient irradiance levels is also shown. The average monthly sun shine duration (January to July 2017) season was calculated based on the secondary data obtained from the nearest meteorological station (Ethiopian national metrology agency, Hawassa Branch).

The mean irradiance level of UV-B measured under UV blocking film was reduced by about 80% compared to ambient solar UV radiation. Higher UV-B levels were measured under open field compared with UV blocking film at clear days. The mean irradiance level of PAR measured under plastic film was reduced by about 52% compared to ambient (Table: 4).

Planting density also affected irradiance level of UV-B. Slightly low UV-B level was recorded under high planting density (Table: 4). This is due to plant canopies effectively filter out UV radiation, including the UV-B (290-315 nm) and changes in UV-B levels can affect plant growth (Flint & Caldwell, 1998).

Table 5: Ambient irradiance level and irradiance level of red light, far red light, R: FR

Level of Plant spacing	Red light ($\mu molm^{-2}s^{-1}$)		Far red light ($\mu molm^{-2}s^{-1}$)		R: FR		Aver. Sun shine duration (hrs.)
	Under canopy	UV-B film + canopy	Under canopy	UV-B film + canopy	Under canopy	UV-B film + canopy	
0.5mx0.5m	8.056	9.112	26.85	21.11	0.298	0.372	7.48
0.75mx0.75m	47.47	40.31	74.73	56.7	0.487	0.522	
1mx1m	72.34	43.65	85.3	62.8	0.83	0.703	
Ambient (control)		200.0		177.4		1.122	

** Ambient irradiance levels and irradiance levels of Red and far red light (µmol m^{-2} s^{-1}) below UV blocking films (-UV) and open field were measured in the middle of the day (11:00 to 15:00) at an altitude of 1700 masl in Ethiopia during dry ((January to July 2017).*

Table 6: Ambient irradiance level and irradiance level of Red Far Red R: FR, PAR, UVA and UVB as affected by plastic film and planting density (canopy)

Treatment	Red	Far Red	R:FR	UVA	UVB	PAR
Control	200.0±5.9[a]	177.7±5.36[a]	1.12±0.01[a]	16.37±2.94[a]	1.77±0.02[a]	1835.6±70[a]
1m +UVB	72.3±31[b]	85.3±34.2[ab]	0.83±0.02[ab]	8.8±5.87[ab]	0.98±0.2[b]	117.9±23.8[b]
0.75m +UVB	47.5±33.8[b]	74.7±29.1[b]	0.48±0.19[ab]	2.95±0.43[b]	0.39±0.1[cb]	54.4±20b
0.5m +UVB	8.05±21[b]	26.9±2.5[b]	0.29±0.02[b]	1.04±0.26[b]	0.14±0.08[c]	113.6±11.4[b]
1m -UVB	43.6±12.5[b]	49.4±0.8[b]	0.89±0.27[ab]	2.69±1.25[b]	0.19±0.1[c]	89.8±6.9[b]
0.75m -UVB	40.3±31.4[b]	56.7±32.7[b]	0.52±0.9[ab]	0.92±0.303[b]	0.05±0.01[c]	135.2±31.8[b]
0.5m -UVB	9.11±4.9[b]	21.11±6.7[b]	0.37±0.1[b]	0.9±0.008[b]	0.07±0.01[c]	60.8±16.6[b]
P-value	0.000	0.002	0.010	0.005	0.0001	0.000

All data are the mean values ±SE and all values sharing the same letter in a column are statistically non-significant at $p \leq 0.05$

The R: FR ratios were higher under low planting density. Report indicated that R: FR ratio in open conditions, were between 1.07 and 1.20. For vertically and horizontally propagated light, average values were 1.22 and 0.75 respectively(Sattin *et al*., 1994). In the present study R:FR ratio increased with decreasing planting density under both open and UV blocking film (Table: 5 & 6). The main reason might be due to absorption of R light by plants for photosynthesis, whereas FR radiation is mostly reflected or transmitted. Elegant studies revealed that internode elongation was strongly accelerated at low R: FR ratios (e.g., Morgan

& Smith, 1978), a response that could be considered adaptive because it would allow the plant to position its leaves in higher, better lit strata of the canopy.

4.2 Impact of UV radiation and planting density on growth of Entada

4.2.1. Total number of sucker and suckering ration

The total number of sucker and suckering ration of Entada were significantly ($p<0.05$) affected by UV, planting density and their interaction (Table: 7).

The highest total number of sucker 47.3 was obtained from the lowest planting density 10000plants/ha (1mx1m) in open field or unscreened solar UV radiation. Under the UV-blocking film, total number of sucker was reduced by about 42% comparing with solar UV-B radiation, unscreened treatments. The study which was confirmed with this work conclude that exclusion of ambient UV-B produced more branches in dicots or tillers in monocots with a larger leaf area in barley, cotton and sorghum (Mazza *et al*., 1999, Coleman, & Day, 2004).

Reduced apical dominance and stimulated branching are a characteristic growth pattern found in plants exposed to UV (Jansen, 2002). Shoot branching is regulated by the complex interactions among hormones, development, and environmental factors. Recent studies into the regulatory mechanisms of shoot branching have focused on strigolactones, which is a new area of investigation in shoot branching regulation (Cunquan YUAN *et al*., 2015).

Roro *et al*, (2016) study on pea confirmed that plant morphological characteristics like plant height and number of branches were affected by UV radiation. Exclusion of UV-B and some UV-A from the solar spectrum enhanced the shoot elongation of pea plants by about 15–19% compared to unfiltered solar spectrum.

Also, previous reports have demonstrated supplementary UV-B radiation for extended periods of time either in controlled environment or field conditions results in significantly reduced shoot length in different plant species including crops like cucumber (*Cucumis sativus*), mung bean (*Vigna radiata*), pot rose (Rosa × hybrida) and spinach (*Spinacia oleracea*) (Krizek *et al*., 1994; Amudha *et al*., 2005; Kumari *et al*., 2009; Jayalakshmi *et al*., 2011; Zlatev *et al*., 2012; Terfa *et al*., 2014).

Planting density also affected the number of sucker significantly. As planting density decreases the ratios of red to far red light was increased and make plants to produce more sucker (Table 5, 6 &7). According to Ballard (1994), the reduction in the R: FR ratio initiates an increase in apical dominance and reduce branching.

4.2.2. Total leaf number

Number of leaves were significantly ($p<0.05$) affected by solar UV radiation (Table: 7). However, planting density had no effects on number of leaves. Under the UV-blocking film, number of leaves was reduced by about 16% comparing with open field getting solar UV radiation.

Production of a larger number of leaves has also been reported for *Triticum aestivum* (Barnes et al. 1990, Teramura, 1980), *Avena sativa, Zea mays, Avena fatua, Amaranthus retroflexus* (Barnes et al. 1990), *Aquilegia caerulea* and *A. canadensis* (Larson *et al*., 1990) under enhanced UV-B radiation.

4.2.3. Plant height

Plant height was significantly ($p<0.05$) affected by UV and plant density. The shortest plant height 63.5cm and 84.7cm were recorded at lowest planting density 10000plants/ha (1mx1m) from the plants grown under solar UV radiation and plants covered by UV blocking film respectively (Table: 7). This could be due to photo-oxidative destruction of the phytohormone IAA followed by reduced cell wall extensibility as demonstrated in sunflower seedlings (Ros and Tevini, 1995). Even though plant height was affected by planting density, it was more affected by UV radiation than planting density (Table: 7).

Planting density had also effects on the height of the plant. The height induction under narrow spacing is corresponds to reduced R: FR signals (Table 5&6). Plant height is increased under conditions in which shade-avoidance reactions were induced (Ballard et al., 1987; Smith, 1984). Shade avoidance responses occur due to radiation reflected from neighboring plants before canopy closure and shading occurs. This could be attributed to the increased competition for light at higher planting density which might have resulted in increased plant height. Similarly, Saiful Islam *et al*. (2002) in pea (*Pisum sativum*) and Sener *et al*. (2004) in maize (*Zea mays*) observed an increase in height with rising density most probably as an adaptation mechanism to increase level of mutual shading.

In our study we observed that the reduction in plant height and vegetative growth which is a typical UV-B response found in many different species, e.g. such as lettuce, mung bean, maize, cucumber, grapevine and Arabidopsis thaliana(Krizek *et al*., 1997; Pal *et al*., 1997; Krizek *et al*., 1998; Jansen, 2002;Wargent *et al*., 2009; Berli *et al*., 2010, 2012).

Table 7: Growth parameters, open field (+UV) and UV-blocking (−UV) plastic films at different planting density.

UV-B	PLSP	Number of sucker	Suckering ratio	Specific leaf area (cm^2/g)	Number of leaves	Plant height (cm)
+UV	1mx1m	47.3±1.8a	15.7±0.6a	180.8±16.9ab	65.6±5.1ab	63.5±3.3c
	0.75mx0.75m	35.0±1.8b	11.6±0.6b	174.5±16.9b	65.6±5.1ab	75.1±3.3b
	0.5x0.5m	16.0±1.8d	5.3±0.6d	193.4±16.9ab	74.0±5.1a	82.8±3.3b
-UV	1mx1m	16.3±1.8d	5.4±0.6d	220.8±16.9ab	58.3±5.1ab	84.7±3.3b
	0.75mx0.75m	22.3±1.8c	7.4±0.6c	230.5±16.9a	55.6±5.1b	105.5±3.3a
	0.5x0.5m	18.6±1.8cd	6.2±0.6cd	231.8±16.9a	58.6±5.1ab	97.6±3.3a
CV (%)		12.3	12.3	14.3	14.1	6.7
p-value						
UV		<0.0001	<0.0001	0.0095	0.0266	<0.0001
PLSP		<0.0001	<0.0001	0.7622	0.5350	0.0008
UV*PLSP		<0.0001	<0.0001	0.8555	0.7368	0.1059

All data are the mean values ±SE of measurements from three plants. All values sharing the same letter in a column are statistically non-significant at $p \leq 0.05$ based on ANOVA followed by LSD.

UV-B radiation is one of the key environmental signals that regulate plant responses including plant morphology (Jansen, 2002; Jenkins, 2009). In the present study, UV radiation affected

most of the growth variables. From this study, it is clear that Entada was responded similar with most crops to UV radiation.

Plants grown under solar UV radiation were in general smaller, with reduced plant height compared to plastic covered plants. However, they developed more sucker compared to the covered plants.

4.2.4. Specific leaf area and total leaf area

Specific leaf area was significantly ($p<0.05$) affected by UV radiation. But total leaf area was unaffected by both UV and planting density (Table: 7&8).

Specific leaf area (SLA) is lower under solar UV radiation compared to UV blocking film. The average SLA recorded under solar UV radiation was 182.9cm^2/g whereas 227.7cm^2/g was recorded under UV blocking film. It was increased by about 20% under UV blocking film. The lower SLA found under ambient UV radiation may indicate that leaf thickness was increased.

Similarly, (Innes, 2015) Studied on Effects of UV radiation and air humidity on morphology, stomatal function and photosynthesis of *Euphorbia pulcherrima* a reduction in SLA (19 %) was found in plants exposed to UV compared to plants not exposed to UV.

In contradictory, enhanced UV-B reduced leaf thickness (indicated by specific leaf weight) has been reported in maize, *Amaranthus tricolor* and sorghum varieties (Correia et al., 1999; Kataria and Guruprasad, 2012), while specific leaf area in Indian cress (*Tropaeolum majus*) were unaffected by enhanced UV-B radiation (Germ *et al*., 2015).

4.2.5. Plant biomass and dry matter content

Plant biomass and dry matter of Entada were significantly ($p<0.05$) affected by UV. However, they were not statistically affected by planting density (Table: 8).

About 30% and 42% fresh weight and dry weight reduction recorded due to solar UV radiation respectively. Dry matter content was affected by UV radiation. The higher dry matter was recorded under UV blocking film. About 15% reduction of dry matter was recorded due to solar UV radiation.

This may be due to growth reduction is a consequence of the UV-B radiation effects on the rate and duration of both cell division and elongation (Hopkins et al., 2002). Chen (2017) investigated the single UV-B effects on sweet potato and found that the projected UV-B exposure (10kJ m^{-2} d^{-1}) reduced total biomass by 30-62%.

Reduction in biomass accumulation due to UV-B exposure was found in several tree (Searles et al., 1995; Liu et al., 2005) and crop (Kakani et al., 2003b) species. Negative impact of enhanced UV-B radiation on cotton growth included reduction in height, leaf area, total biomass and fiber quality (Gao et al., 2003). As demonstrated by Kakani et al., 2003b increased UV-B radiation exposure reduced the photosynthetic rate of many species and, in general, the reduction was more pronounced under growth chamber or greenhouse conditions than under field conditions.

Table 8: Biomass and dry matter content under +UV and –UV at different planting density

UV-B	PLSP	Total leaf area (m^2)	FW (kg)	DW (kg)	DMC (%)
+UV	1mx1m	5.2±0.71ab	6.6±0.24c	0.38±0.04cd	5.8±0.5b
	0.75mx0.75m	6.0±0.71a	5.6±0.24d	0.34±0.04d	6.0±0.5b
	0.5mx0.5m	3.5±0.71b	6.2±0.24cd	0.37±0.04cd	5.9±0.5b
-UV	1mx1m	4.6±0.71ab	8.8±0.24a	0.69±0.04a	7.8±0.5a
	0.75mx0.75m	4.8±0.71ab	8.83±0.24a	0.63±0.04ab	7.0±0.5ab
	0.5mx0.5m	5.3±0.71ab	7.9±0.24b	0.49±0.04bc	6.2±0.5ab
CV (%)		25.2	5.7	15.5	14.7
p- value	UV-B	0.9680	<.0001	<.0001	0.0355
	PLSP	0.4041	0.0730	0.0925	0.4032
	UV-B*PLSP	0.1532	0.0329	0.1078	0.3276

All data are the mean values ±SE of measurements from three plants. All values sharing the same letter in a column are statistically non-significant at $p \leq 0.05$ based on ANOVA followed by LSD.

4.3. Physiological responses of Entada to UV- B radiation and Red to far red ratio

4.3.1. Chlorophyll contents

The contents of chlorophyll ***a*** and total chlorophyll were significantly ($p<0.05$) affected by UV radiation. The content of chlorophyll ***a*** total chlorophyll was reduced under solar UV radiation. Statistically UV and planting density didn't affect chlorophyll b. However, statistically planting density did not affect chlorophyll in general (Table: 9).

In previous studies Teramura (1983) observed that chlorosis often occurred in leaves of UV-susceptible plants such as soybean, pea and cucumber, and also in less sensitive plant such as barley and cotton after exposure to UV radiation. Several studies have shown that chlorophyll destruction was a function of UV fluence rate (Tevini & Teramura 1989). This effect could be associated with direct changes on PSII and PSI and damages chloroplast membrane and seriously affect net photosynthesis and growth in sensitive plants (Brandle et al. 1977).

2.3.2. Chlorophyll inflorescence

Chlorophyll inflorescence was significantly ($p<0.05$) affected by UV and planting density. Maximal photosystem II efficiency (Fv/Fm) was lower with UV than without UV, and it was increased with increasing planting density (Table: 9).

Table 9: Effect of solar UV radiation and different planting density on chlorophyll content (chla, chlb and chlab) and chlorophyll inflorescence (fm/fv)

UV-B	PLSP	Chla	Chlb	Chlab	Fm/fv
+UV	1mx1m	0.80±0.052ab	0.58±0.07	1.38±0.09ab	0.76±0.005d
	0.75m0.75m	0.74±0.052ab	0.55±0.07	1.30±0.09ab	0.77±0.005cd
	0.5mx0.5m	0.67±0.052b	0.42±0.07	1.10±0.09b	0.78±0.005bc
-UV	1mx1m	0.88±0.052a	0.64±0.07	1.2631	0.79±0.005ab
	0.75m0.75m	0.80±0.052ab	0.57±0.07	1.37±0.09a	0.79±0.005ab
	0.5mx0.5m	0.90±0.052a	0.52±0.07	1.43±0.09ab	0.80±0.005a
CV (%)		11.4	22.6	12.19	1.2
p- value	UV-B	0.0165	0.3385	0.0403	0.0002
	PLSP	0.4080	0.1941	0.1737	0.0152
	UV-B*PLSP	0.2786	0.8422	0.4272	0.3873

The values are mean ± SE of three plants in each of the treatment. All values sharing the same letter in a column are statistically non-significant at $p \leq 0.05$ based on ANOVA followed by LSD.

Chlorophyll fluorescence analysis is a powerful technique to conveniently assess the condition of PSII and vitality in intact plants. He *et al*. (1993) observed that decrease in the ratios of

variable to maximum chlorophyll fluorescence yield and in the quantum yield of photosynthetic oxygen evolution by supplemental UV-B in pea and rice leaves.

Regarding planting density on chlorophyll inflorescence there is no written document. In this study Fv/Fm was increased with increasing planting density (Table 9). This may be due to planting density decreased temperature of plant canopies, and can increase maximal photosystem II efficiency (Table: 1). Additionally, plant canopies effectively filter out UV radiation, including the UV-B (290-315 nm) and changes in UV-B levels to minimum can increase maximal photosystem II efficiency (Flint & Caldwell, 1998).

2.3.3. Stomata measurements

Stomatal number and size did not show any significant differences among all treatments. However, the size of stomata was significantly ($p<0.05$) affected by solar UV radiation. Planting density has no significant effects on the size, number and area of stomata (Table: 9). Size of stomata was lower under solar UV radiation comparing with UV blocking film.

The closure of stomata in response to UV-B radiation is by far the most common response found (He et al. 2005; Negash & Björn 1986; Nogués et al. 1999). Effects of UV radiation on stomatal movements have been reported in several studies. UV-B has been found to induce stomatal closure and thus reduce stomatal conductance (Mirecki and Teramura, 1984).

Table 10: Stomata number, area and size under +UV and –UV at different planting density

UV-B	PLSP	Stomata number	Stomata area (mm^2)	Stomata aperture (mm)
+UV	1mx1m	11.1±0.83ab	0.6541±0.07ab	0.50±0.019cd
	0.75mx0.75m	11.0±0.83b	0.5657s±0.07b	0.52±0.019bcd
	0.5x0.5m	10.8±0.83b	0.6047±0.07ab	0.48±0.019d
-UV	1mx1m	13.6±0.83a	0.8343±0.07a	0.60±0.019a
	0.75mx0.75m	12.2±0.83ab	0.6679±0.07ab	0.57±0.019ab
	0.5x0.5m	10.1±0.83b	0.5416±0.07b	0.56±0.019abc
CV (%)		12.59	20.7	6.29
p- value	UV-B	0.1736	0.2731	0.0007
	PLSP	0.1841	0.1193	0.2568
	UV-B*PLSP	0.1249	0.3163	0.4869

All data are the mean values ±SE of measurements from three plants. All values sharing the same letter in a column are statistically non-significant at $p \leq 0.05$ based on ANOVA followed by LSD.

However, stomatal control by UV-B radiation is slightly more controversial, with studies reporting both stomatal opening and closure in response to UV-B radiation (Eisinger et al. 2000; Eisinger et al. 2003; He et al. 2005; Jansen & van den Noort 2000; Tossi et al. 2014),

with differing results according to UV-B fluence rate, duration and wavelength (Jenkins 2009; Tossi et al. 2014),

Stomatal opening as a response to low fluence rates of UV-B radiation has been found in *Arabidopsis thaliana* (Eisinger et al. 2000; Eisinger et al. 2003), and Jansen & van den Noort (2000) found that higher fluence rates of UV-B induce stomatal closure when given in combination with low PAR fluence rates, yet when given with high PAR fluence rates induced stomatal opening.

4.4. Correlation between parameters

Table: 11 Coefficients of correlations (R^2) between growth parameters in Entada plants.

	PLH	LN	SN	TFW	TDW	DMC
PLH	-	-0.327ns	-0.695**	0.626**	0.550*	0.396ns
LN	-	-	0.144ns	-0.472*	-0.287ns	0.0171ns
SN	-	-	-	-0.471*	-0.452ns	-0.333ns
TFW	-	-	-	-	0.927**	0.650**
TDW	-	-	-	-	-	0.880**
DMC	-	-	-	-	-	-

*PLH = Plant Height, LN = Leaf Number, SN = Sucker Number, TFW = Total Fresh Weight, TDW = Total Dry Weight, DMC = Dry Matter Content. Values followed by * and ** represent significance differences at $P \leq 0.05$ and $P \leq 0.01$. Means followed by ns represent do not significance differences at $P \geq 0.05$.*

Growth and development of a plant is complex character that is controlled by quite a number of factors. Hence, the degree of association of these complex characters formed the basis for yield evaluations and correlation coefficient analysis measures the extent of closeness of the component traits.

Positive and significant correlation was observed between plant height and total fresh weight; total fresh weight and dry matter content; and total dry weight and Dry matter content. Negative and significant correlation also observed between plant height and sucker number; leaf number and total fresh weight and sucker number with total fresh weight (Table 11). This suggested that the strong association exhibited by the growth parameters indicated that modification of microclimate using planting density and UV-B screening film will be good direction for environmentally friendly approach in the regulation of plant growth and development.

5. CONCLUSION AND RECOMMENDATION

The overall result shows that using an approach with planting density and UV-B blocking film, had significant effect on light quality distribution with in the canopy, number of suckers, plant height, leaf number, biomass and chlorophyll fluorescence. Increasing planting density significantly reduced Red, FR, R: FR ratio, PAR and UV-B radiation. The study shows that exclusion of UV radiation and lowering R: FR ratio negatively affected sucker and leaf development, but positively influenced plant height, specific leaf area, stomata number and photosynthetic efficiency II of the leaves. Exposition of Entada plant to solar radiation significantly reduced the biomass by about 2.4kg per plant and this reduction was stronger under densely populated plants than sparsely planted crop. This might be due to reduction in plant height due to UV-B irradiation. From horticultural point of view, sucker producing naturally may not be important for Enset, unless it is used for production of propagules. Sucker may reduce the size of pseudostem of Entada, which is the most important sources of food. The strong association exhibited between morphological and total yield indicated the importance of microclimate modification in terms of ecological importance and the regulation of plant growth and development.

However, growth and development evaluation with few climatic factors is not enough to address all the problems for such Robusta type of plants. This study was carried out on single cultivars at one location for one grown season, hence repeating an experiment with different Entada accession at different location is recommended to get more reliable information.

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APPENDICES

Appendix 1: ANOVA Table for Number of Sucker

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	0.41	0.6724
UV_B	1	10	82.22	<.0001
PLSP	2	10	34.11	<.0001
UV_B*PLSP	2	10	41.69	<.0001

Appendix 2: ANOVA Table for Plant Height

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	0.30	0.7452
UV_B	1	10	67.86	<.0001
PLSP	2	10	16.04	0.0008
UV_B*PLSP	2	10	2.83	0.1059

Appendix 3: ANOVA Table for Suckering Ratio

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	0.41	0.6724
UV_B	1	10	82.22	<.0001
PLSP	2	10	34.11	<.0001
UV_B*PLSP	2	10	41.69	<.0001

Appendix 4: ANOVA Table for Leaf Number

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	1.03	0.3916
UV_B	1	10	6.75	0.0266
PLSP	2	10	0.67	0.5350
UV_B*PLSP	2	10	0.31	0.7368

Appendix 5: ANOVA Table for Leaf Area

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	1.35	0.3018
UV_B	1	10	0.00	0.9680
PLSP	2	10	0.99	0.4041
UV_B*PLSP	2	10	2.28	0.1532

Appendix 6: ANOVA Table for Specific Leaf Area

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	1.85	0.2079
UV_B	1	10	10.24	0.0095
PLSP	2	10	0.28	0.7622
UV_B*PLSP	2	10	0.16	0.8555

Appendix 7: ANOVA Table for Dry Matter Content

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	1.25	0.3272
UV_B	1	10	5.90	0.0355
PLSP	2	10	1.00	0.4032
UV_B*PLSP	2	10	1.25	0.3276

Appendix 8: ANOVA Table for Total Dry Weight

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	2.25	0.1564
UV_B	1	10	43.58	<.0001
PLSP	2	10	3.05	0.0925
UV_B*PLSP	2	10	2.81	0.1078

Appendix 9: ANOVA Table for Total Fresh Weight

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	2.61	0.1221
UV_B	1	10	135.55	<.0001
PLSP	2	10	3.44	0.0730
UV_B*PLSP	2	10	4.90	0.0329

Appendix 10: ANOVA Table for Stomata Number

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	0.11	0.9004
UV_B	1	10	2.15	0.1736
PLSP	2	10	2.01	0.1841
UV_B*PLSP	2	10	2.58	0.1249

Appendix 11: ANOVA Table for Stomata Size

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	31.61	<.0001
UV_B	1	10	23.06	0.0007
PLSP	2	10	1.56	0.2568
UV_B*PLSP	2	10	0.77	0.4869

Appendix 12: ANOVA Table for Stomata Area

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	2.32	0.1491
UV_B	1	10	1.34	0.2731
PLSP	2	10	2.65	0.1193
UV_B*PLSP	2	10	1.29	0.3163

Appendix 13: ANOVA Table for Chlorophyll Inflorescence

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	1.43	0.2844
UV_B	1	10	33.05	0.0002
PLSP	2	10	6.56	0.0152
UV_B*PLSP	2	10	1.04	0.3873

Appendix 14: ANOVA Table for Chlorophyll A

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	0.43	0.6611
UV_B	1	10	8.27	0.0165
PLSP	2	10	0.98	0.4080
UV_B*PLSP	2	10	1.46	0.2786

Appendix 15: ANOVA Table for Chlorophyll B

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	1.38	0.2967
UV_B	1	10	1.01	0.3385
PLSP	2	10	1.94	0.1941
UV_B*PLSP	2	10	0.17	0.8422

Appendix 16: ANOVA Table for Chlorophyll AB

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
REP	2	10	1.11	0.3665
UV_B	1	10	5.55	0.0403
PLSP	2	10	2.10	0.1737
UV_B*PLSP	2	10	0.93	0.4272

Appendix 17: Table of Correlation Coefficient for Growth Parameters

	Plant height	Leaf number	Sucker number	fresh weight	Dry weight	Dry matter content
Plant height	1.00000	-0.32745 0.1847	-0.69506 0.0014	0.62695 0.0054	0.55034 0.0180	0.39629 0.1035
Leaf number	-0.32745 0.1847	1.00000	0.14409 0.5684	-0.47236 0.0478	-0.28748 0.2474	0.01712 0.9462
Sucker number	-0.69506 0.0014	0.14409 0.5684	1.00000	-0.47184 0.0481	-0.45265 0.0593	-0.33303 0.1769
fresh weight	0.62695 0.0054	-0.47236 0.0478	-0.47184 0.0481	1.00000	0.92753 <.0001	0.65080 0.0034
Dry weight	0.55034 0.0180	-0.28748 0.2474	-0.45265 0.0593	0.92753 <.0001	1.00000	0.88021 <.0001
Dry matter content	0.39629 0.1035	0.01712 0.9462	-0.33303 0.1769	0.65080 0.0034	0.88021 <.0001	1.00000

Appendix 18: ANOVA Table for far red light

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	6	50100	8350	6.17	0.002
Error	14	18950	1354		
Total	20	69050			

Appendix 19: ANOVA Table for red light

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	6	77578	12930	9.12	0.000
Error	14	19856	1418		
Total	20	97434			

Appendix 20: ANOVA Table for R: FR

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	6	1.6857	0.28095	4.44	0.010
Error	14	0.8869	0.06335		
Total	20	2.5726			

Appendix 21: ANOVA Table for UVA

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	6	606.0	101.00	5.24	0.005
Error	14	269.9	19.28		
Total	20	875.9			

Appendix 22: ANOVA Table for UVB

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	7.32870216	1.22145036	10.82	0.0001
Error	14	1.58020354	0.11287168		
Corrected Total	20	8.90890570			

Appendix 23: ANOVA Table for PAR

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	6	7804173	1300696	413.91	0.000
Error	14	43994	3142		
Total	20	7848167			