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Research Article
Interactive Effect of Solar UV-B Radiation and Planting Density on Sucker Development and Physiology of Enset (*Ensete ventricosum*) Variety-Entada

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Abstract
Background and Objective: Entada, which is one of the enset landraces cultivated in Ari zone southern part of Ethiopia. It produces natural suckers like banana, however, sucker development is not preferred in enset production . The purpose of the study was to evaluate the effect of UV-B radiation and planting density on sucker development and physiology of Entada plants. **Materials and Methods:** The experiment was carried out at field condition using a randomized complete block design with 3 replications. For this study three level of planting density (40,000 plants/ha), (17,777plants/ha) and (10,000 plants/ha) and two level of UV-B radiation (with and without Solar UV-B radiation) were used. Data were collected on light quality, morphological and physiological parameters. **Results:** It was observed that, total number of sucker and suckering ratio were significantly ($p<0.05$) affected by UV-B radiation, planting density and their interaction. An increase in planting density significantly reduced R:FR ratio and leading to significant increase in plant height by 18%. Plant grown under higher planting density significantly reduced sucker number by 45% compared to the effect of lower planting density. Maximum number of suckers were recorded (47.3) from treatment combination of lower planting density and exposed to solar UV-B radiation. Removing UV-B radiation using plastic film significantly increased photo system II efficiency (Fv/Fm) of leaves by 3.8% than leaves treated with solar UV-B radiation under lower panting density. Moreover, Entada plant exposed to solar UV-B radiation significantly reduced stomata aperture than the effect of planting density. **Conclusion:** Generally, change in the composition of light quality using planting density and screening material resulted in significant modification on sucker development and physiology of Entada variety.

Key words: Entada, sucker, red light, far red light, UV-B, planting density, physiological growth, morphology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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[1]. 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[2]. 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*[3]. Cheesman[4] 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.)[5]. 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 these factors, light quality and agronomic practices including planting density have been playing a paramount importance in governing plant morphology, physiology and genetic responses.

To sense and respond, plants are naturally given with an array of photo receptors, these photo receptors control diverse responses of the plant to light parameters, such as spectrum, intensity, direction and duration. These photo receptors 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 (315-400 nm), UV-B (280-315 nm) and UV-C (100-280 nm). Among these, UV-C is the radiation with the lower wavelength or rather with the higher associated energy[6,7].

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 and altitude etc[8]. Novel technologies to manipulate light quality including UV levels are at large in use in protected plant cultivation. Either some of these technologies are by using different selective plastic films, UV blocking or UV-transparent, specific parts of the UV spectrum can be manipulated. This provides new opportunities in protected crop cultivation[9].

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[10,11].

Morphologically UV-B exposure decreases shoot length, shoot dry mass, foliar area and delay flowering[12-14]. In some other species, exposure to supplementary UV-B light significantly increases leaf area as well as fresh and dry biomass[15]. 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[12,16].

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[17,18]. 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[19]. 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[20-22].

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[23]. 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[23,24]. Many plant species significantly accelerate elongation within internode 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[25]. Light that passes through densely populated canopy of leaves has a reduced red to far-red ratio (R:FR)[26]. 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[27]. 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[28,29].

Light can affect the growth morphologies like plant height, branching, leaf area and internode extension of different plant species[30]. Entada is the only Enset landraces that produces natural suckers like banana[5]. 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 pseudo-stem, the economically important part of the crop by diverting assimilates towards the suckers[31]. 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. The objectives of the present study were to evaluate the morphological and physiological responses of Entada to light quality and different planting densities and to assess the physiological responses of Entada to UV- B radiation and planting density.

MATERIALS AND METHODS

Experimental location: A field experiment was conducted at Hawassa University, Research field during the2016/2017 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-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 Valleyzone[32].

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[5]. It has the habit of growing fast and suckering.

Experimental set up and treatments: Using an approach with planting density 0.5×0.5 m (40,000 plants/ha), 0.75×0.75 m (17,777 plants/ha) and 1×1 m (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 0.20 mm thick and 3 m wide Rovero plastic, Raamsdonksveer, The Netherlands) was selectively cut-off of the solar spectrum below 350 nm (UV-Band the shortest wavelengths of UV-A)(Fig. 1). The light transmitted through the plastic film was measured with a spectroradiometer PS-300 (300-1000 nm) apogee instrument connected to cosine-corrected head (Table 1).

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

Uniform age size of (sucker with 3 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 1.5 and 2 m 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.

Planting and crop management: Healthy and well developed suckers were planted on a well-prepared experimental site at

Table 1: Treatment combination

Numbers	Treatment combination
1	1×1 m (10,000 plants/ha)+UV-B radiation
2	0.75×0.75 m (17,777 plants/ha)+UV-B radiation
3	0.5×0.5 m (40,000 plants/ha)+UV-B radiation
4	1×1 m (10,000 plants/ha) with-UV-B radiation
5	0.75×0.75 m (17,777 plants/ha)-UV-B radiation
6	0.5×0.5 m (40,000 plants/ha)-UV-B radiation

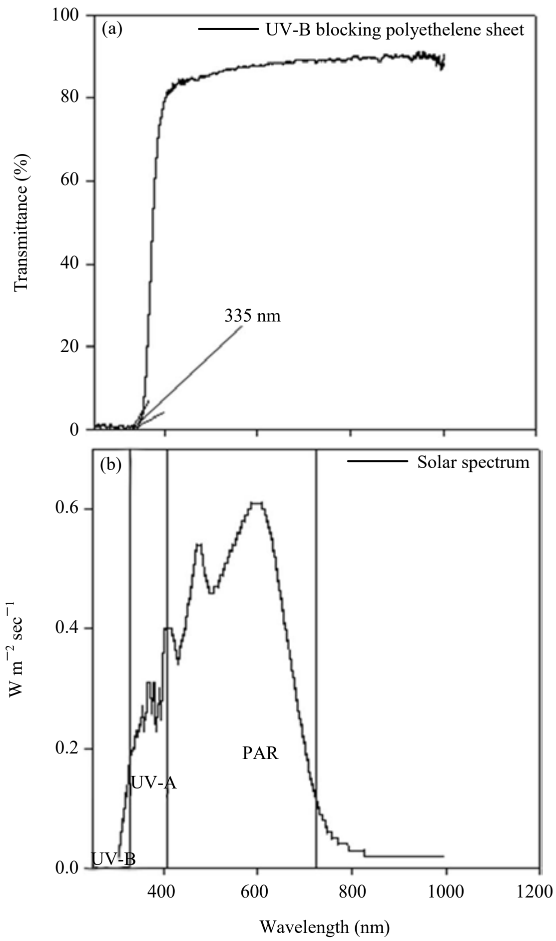

Fig. 1(a-b): (a) Solar spectrum transmission of polyethylene sheets used in the growth experiment with Entada measured during noon time at Hawassa altitude of 1700 m.a.s.l: UV-blocking polyethylene film and (b) Blocks UV-B spectrum (280-315 nm) and the short wavelengths of UV-A ($\leq$350) (Solar EVA-5 0.20 mm thick high diffuse opaque polyethylene film, Rovera plastic, The Netherlands)

Light transmitted through the plastic film was measured with a spectroradiometer PS-300 (300-1000 nm) apogee instrument connected to cosine-corrected head

the spacing of mentioned above. Weeding, cultivation and watering were undertaken whenever it was needed.

Growth parameters measurements: After 6 months of treatment, four plants from each treatment were used for destructive morphological analysis of above ground biomass. At the end of the experiments leaf number, number of suckers (the total number of suckers of the size 5-20 cm 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[33]:

$$\text{TIA} = \Sigma(0.83 \times \text{L} \times \text{W})$$

where, L is the length of lamina, W is 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 2 plants from each plot was measured and dried in oven at 65°C for until a constant weight is obtained. Then, their dry weight was recorded and dry matter (%) was calculated from it.

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 fluorescence: 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.*[34]. Before measurement, leaves were dark-adapted in the leaf clip for 15 min. Light was provided by an array of 3 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[35]. Chlorophyll extraction was carried out with a mixture of acetone 80%. 2 g Entada tissue was homogenized with 25 mL acetone solution 80% and the solution was covered with aluminum foil to avoid oxidation of chlorophyll from light. Absorption was measured at 663 and 645 nm using spectrophotometer. The chlorophyll content was calculated as mg g^{-1} fresh weight. The concentrations of total chlorophyll, chlorophyll a and chlorophyll b were calculated by the following equations[35]. The chlorophyll content in the fresh plant leaves was calculated by equation:

$$\begin{matrix}\text{Chlorophyll a} \\ (\text{mg g}^{-1}\text{ fresh weight})\end{matrix} = \frac{(12.7\times A663)-(2.69\times A645)\times V}{1000\times W}$$

$$\begin{matrix}\text{Chlorophyll b} \\ (\text{mg g}^{-1}\text{ fresh weight})\end{matrix} = \frac{(22.9\times A645)\text{-}(4.68\times A663)\times V}{1000\times W}$$

$$\begin{matrix}\text{Total chlorophylls} \\ (\text{a+b})\end{matrix} = \frac{(20.08\times A645+8.02\times A663)\times V}{1000\times W}$$

Climate data and radiation: Weather data such as temperature, rainfall, relative humidity and sunshine duration of the last 10 years (2007-2016) were collected from the nearest meteorology station (Ethiopian National Metrology Agency, Hawassa branch).

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 h.

The UV radiation, red and far red light and PAR (photosynthetic active radiation) was measured with a spectroradiometer PS-300 (300-1000 nm) 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).

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.

RESULTS AND DISCUSSION

Climate and solar radiation

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 and 27.7°C throughout the year (Ethiopian National Meteorological Station, Hawassa branch, Ethiopia).

Table 2: Climate data for the past 10 years (2007-2016)

Climate data	Minimum	Maximum	Average
Temperature (°C)	13.70	27.70	20.7
Total monthly rainfall (mm)	670.90	1156.50	913.7
Relative humidity (%)	37.25	70.75	54.0
Sunshine duration (h)	6.90	7.90	7.4

Average values of minimum and maximum temperature (°C), RH (%), sunshine duration (h) and rain fall (mm) of past 10 years (2007-2016) recorded by meteorological stations of southern Ethiopia located nearest to the study area (Ethiopian National Meteorological Station, Hawassa branches)

Table 3: Climate data recorded during the experimental period

UV-B radiation	Plant density	T mean (°C)	RH (%)
+UV	1×1 m (10,000 plants/ha)	21.23	64.84
	0.75×0.75 m (17,777plants/ha)	20.47	63.06
	0.5×0.5 m (40,000 plants/ha)	20.09	59.58
-UV	1×1 m (10,000 plants/ha)	19.37	62.13
	0.75×0.75 m (17,777plants/ha)	19.25	61.83
	0.5×0.5 m (40,000 plants/ha)	18.70	61.00
	Grand mean	19.86	62.07

Data was sampled under UV-blocking (-UV) films during the dry (January-July) at an elevation of 1700 m.a.s.l 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

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).

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 4 randomly selected days (Fig. 1).

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[36].

The R:FR ratios were higher under low planting density. Report indicated that R:FR ratio in open conditions, were

Table 4: Ambient irradiance levels and irradiance levels of UV-B (W m^{-2}) and photosynthetic active radiation (PAR) (µmol m^{-2} sec^{-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 m.a.s.l in Ethiopia during dry (January-July, 2017)

Plant density	UV-B under canopy (W m^{-2})	UV-B below film+ canopy (W m^{-2})	UV-B reduction (%)	PAR under canopy (µmol m^{-2} sec^{-1})	PAR below film+canopy (µmol m^{-2} sec^{-1})	PAR reduction (%)
0.5×0.5 m (40,000 plants/ha)	0.16	0.08	50	113.6	60.8	46
0.75×0.75 m (17,777plants/ha)	0.39	0.05	87	135.2	54.4	60
1×1 m (10,000 plants/ha)	0.98	0.19	80	177.9	89.8	50

Percent reduction in irradiance below films compared with ambient irradiance levels is also shown, the average monthly sunshine duration (January-July, 2017) season was calculated based on the secondary data obtained from the nearest meteorological station (Ethiopian national metrology agency, Hawassa Branch), ambent UV-B radiation sunshine duration and PAR at 1700 m.a.s.l during January-July, 2017 was about 1.76 W m^{-2}, 7.48 h and 1835.6 mol m^{-2} sec^{-1}, respectively

Table 5: Ambient irradiance levels and irradiance levels of red and far red light (µmol m^{-2} sec^{-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 m.a.s.l in Ethiopia during dry (January-July, 2017)

	Red light (R) (µmol m^{-2} sec^{-1})		Far red light (FR) (µmol m^{-2} sec^{-1})		R:FR	
Plant density	Under canopy	Under UV-B cut off film+canopy	Under canopy	Under UV-B cut off film+canopy	Under canopy	Under UV-B cut off film+canopy
0.5×0.5 m (40,000 plants/ha)	8.056	9.112	26.85	21.11	0.298	0.372
0.75×0.75 m (17,777 plants/ha)	47.47	40.31	74.73	56.70	0.487	0.522
1×1 m (10,000 plants/ha)	72.34	43.65	85.30	62.80	0.830	0.703

At an ambient condition the intensity of red and far red light was recorded as 200 and 177.4 mol m^{-2} sec^{-1} respectively, which gave 1.122 R:FR ratio

Table 6: Ambient irradiance level and irradiance level of red, far red, R:FR ratio, photosynthetic active radiation (PAR), ultraviolet-A (UV-A) and ultraviolet-B (UV-B) as affected by plastic film and planting density

Treatments	Red	Far red	R:FR	UV-A	UV-B	PAR
Control	200.00±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}
1×1 m (10,000 plants/ha)+UV-B	72.30±31^{b}	85.3±34.2^{ab}	0.83±0.02^{ab}	8.80±5.87^{ab}	0.98±0.2^{b}	117.9±23.8^{b}
0.75×0.75 m (17,777 plants/ha)+UV-B	47.50±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
0.5×0.5 m (40,000 plants/ha)+UV-B	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}
1 m (10,000 plants/ha)-UV-B	43.60±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}
0.75 m (17,777 plants/ha)-UV-B	40.30±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}
0.5 m (40,000 plants/ha)-UV-B	9.11±4.9^{b}	21.11±6.7^{b}	0.37±0.1^{b}	0.90±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 $p \leq 0.05$

between 1.07 and 1.20. For vertically and horizontally propagated light, average values were 1.22 and 0.75, respectively[37]. In the present study R:FR ratio increased with decreasing planting density under both open and UV blocking film (Table 5 and 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[38], 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.

Impact of UV radiation and planting density on growth of Entada

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 10000 plants/ha (1×1 m) 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[39,40].

Reduced apical dominance and stimulated branching are a characteristic growth pattern found in plants exposed to UV[41]. 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[41].

Roro *et al.*[42] 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.

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

UV-B	Planting density (PLSP)	Number of sucker	Suckering ratio	Specific leaf area (cm^2 g^{-1})	Number of leaves	Plant height (cm)
+UV	1×1 m (10,000 plants/ha)	47.3±1.8[a]	15.7±0.6[a]	180.8±16.9[ab]	65.6±5.1[ab]	63.5±3.3[c]
	0.75×0.75 m (17,777 plants/ha)	35.0±1.8[b]	11.6±0.6[b]	174.5±16.9[b]	65.6±5.1[ab]	75.1±3.3[b]
	0.5×0.5 m (40,000 plants/ha)	16.0±1.8[d]	5.3±0.6[d]	193.4±16.9[ab]	74.0±5.1[a]	82.8±3.3[b]
-UV	1×1 m (10,000 plants/ha)	16.3±1.8[d]	5.4±0.6[d]	220.8±16.9[ab]	58.3±5.1[ab]	84.7±3.3[b]
	0.75×0.75 m (17,777 plants/ha)	22.3±1.8[c]	7.4±0.6[c]	230.5±16.9[a]	55.6±5.1[b]	105.5±3.3[a]
	0.5×0.5 m (40,000 plants/ha)	18.6±1.8[cd]	6.2±0.6[cd]	231.8±16.9[a]	58.6±5.1[ab]	97.6±3.3[a]
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 3 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

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*)[6, 43-47].

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 and 7). According to Ballare *et al.*[48], the reduction in the R:FR ratio initiates an increase in apical dominance and reduce branching.

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*[16,49], *Avena sativa*, *Zea mays*, *Avena fatua*, *Amaranthus retroflexus*[16], *Aquilegia caerulea* and *A. Canadensis*[50] under enhanced UV-B radiation.

Plant height: Plant height was significantly (p<0.05) affected by UV and plant density. The shortest plant height 63.5 cm and 84.7cm were recorded at lowest planting density 10000 plants/ha (1×1 m) 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[51]. 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 and 6). Plant height is increased under conditions in which shade-avoidance reactions were induced[52,53]. 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, Islam *et al.*[54] in pea (*Pisum sativum*) and Sener *et al.*[55] 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*[41,56-60].

UV-B radiation is one of the key environmental signals that regulate plant responses including plant morphology[41,61]. 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.

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 and 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 182.9 cm^2 g^{-1} whereas

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

UV-B	Planting density (PLSP)	Total leaf area (m^2)	FW (kg)	DW (kg)	DMC (%)
+UV	1×1 m (10,000 plants/ha)	5.2 ± 0.71^{ab}	6.60 ± 0.24^{c}	0.38 ± 0.04^{cd}	5.8 ± 0.5^{b}
	0.75×0.75 m (17,777 plants/ha)	6.0 ± 0.71^{a}	5.60 ± 0.24^{d}	0.34 ± 0.04^{d}	6.0 ± 0.5^{b}
	0.5×0.5 m (40,000 plants/ha)	3.5 ± 0.71^{b}	6.20 ± 0.24^{cd}	0.37 ± 0.04^{cd}	5.9 ± 0.5^{b}
-UV	1×1 m (10,000 plants/ha)	4.6 ± 0.71^{ab}	8.80 ± 0.24^{a}	0.69 ± 0.04^{a}	7.8 ± 0.5^{a}
	0.75×0.75 m (17,777 plants/ha)	4.8 ± 0.71^{ab}	8.83 ± 0.24^{a}	0.63 ± 0.04^{ab}	7.0 ± 0.5^{ab}
	0.5×0.5 m (40,000 plants/ha)	5.3 ± 0.71^{ab}	7.90 ± 0.24^{b}	0.49 ± 0.04^{bc}	6.2 ± 0.5^{ab}
CV (%)		25.2	5.7	15.5	14.7
p- value					
UV-B		0.9680	<0.0001	<0.0001	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 3 plants, all values sharing the same letter in a column are statistically non-significant at $p\leq0.05$ based on ANOVA followed by LSD

Table 9: Effect of solar UV radiation and different planting density on chlorophyll content (chl a, chl b and chla b) and chlorophyll inflorescence (Fv/Fm)

UV-B	Planting density (PLSP)	Chl a	Chl b	Chl ab	Fv/Fm
+UV	1×1 m (10,000 plants/ha)	0.80 ± 0.052^{ab}	0.58±0.07	1.38 ± 0.09^{ab}	0.76 ± 0.005^{d}
	0.75×0.75 m (17,777 plants/ha)	0.74 ± 0.052^{ab}	0.55±0.07	1.30 ± 0.09^{ab}	0.77 ± 0.005^{cd}
	0.5×0.5 m (40,000 plants/ha)	0.67 ± 0.052^{b}	0.42±0.07	1.10 ± 0.09^{b}	0.78 ± 0.005^{bc}
-UV	1×1 m (10,000 plants/ha)	0.88 ± 0.052^{a}	0.64±0.07	1.2631	0.79 ± 0.005^{ab}
	0.75×0.75 m (17,777 plants/ha)	0.80 ± 0.052^{ab}	0.57±0.07	1.37 ± 0.09^{a}	0.79 ± 0.005^{ab}
	0.5×0.5 m (40,000 plants/ha)	0.90 ± 0.052^{a}	0.52±0.07	1.43 ± 0.09^{ab}	0.80 ± 0.005^{a}
CV (%)		11.40	22.60	12.19	1.20
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

Values are Mean±SE of 3 plants in each of the treatment, all values sharing the same letter in a column are statistically non-significant at $p\leq0.05$ based on ANOVA followed by LSD

227.7 cm^2 g^{-1} 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[62] studied on effects of UV radiation and air humidity on morphology, stomatal function and photosynthesis of *Euphorbia pulcherrima* 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[63,64], while specific leaf area in Indian cress (*Tropaeolum majus*) were unaffected by enhanced UV-B radiation[65].

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[66]. Chen[67] investigated the single UV-B effects on sweet potato and found that the projected UV-B exposure (10 kJ m^{-2} d^{-1}) reduced total biomass by 30-62%.

Reduction in biomass accumulation due to UV-B exposure was found in several tree[68,69] and crop[70] species. Negative impact of enhanced UV-B radiation on cotton growth included reduction in height, leaf area, total biomass and fiber quality[71]. As demonstrated by Kakani *et al.*[70] 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.

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

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).

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

UV-B	Planting density (PLSP)	Stomata number	Stomata area (mm^2)	Stomata aperture (mm)
+UV	1×1 m (10,000 plants/ha)	11.1±0.83^{ab}	0.6541±0.07^{ab}	0.50±0.019^{cd}
	0.75×0.75 m (17,777 plants/ha)	11.0±0.83^{b}	0.5657s±0.07^{b}	0.52±0.019^{bcd}
	0.5×0.5 m (40,000 plants/ha)	10.8±0.83^{b}	0.6047±0.07^{ab}	0.48±0.019^{d}
-UV	1×1 m (10,000 plants/ha)	13.6±0.83^{a}	0.8343±0.07^{a}	0.60±0.019^{a}
	0.75×0.75 m (17,777 plants/ha)	12.2±0.83^{ab}	0.6679±0.07^{ab}	0.57±0.019^{ab}
	0.5×0.5 m (40,000 plants/ha)	10.1±0.83^{b}	0.5416±0.07^{b}	0.56±0.019^{abc}
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 3 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

In previous studies Teramura[72] 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[17]. 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[73].

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).

Chlorophyll fluorescence analysis is a powerful technique to conveniently assess the condition of PSII and vitality in intact plants. He *et al.*[74] observed that decrease in the ratios of variable to maximum chlorophyll fluorescence yield and in the quantum yield of photo synthetic 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 10). 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[36].

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[75-77]. 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[78].

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[79], with differing results according to UV-B fluence rate, duration and wavelength[61].

Stomatal opening as a response to low fluence rates of UV-B radiation has been found in *Arabidopsis thaliana*[79], 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.

Correlation between parameters: 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

Table 11: Coefficients of correlations (R2) between growth parameters in Entada plants

Parameters	PLH	LN	SN	TFW	TDW	DMC
PLH	-	-0.327^{ns}	-0.695**	0.626**	0.550*	0.396^{ns}
LN	-	-	0.144^{ns}	-0.472*	-0.287^{ns}	0.0171^{ns}
SN	-	-	-	-0.471*	-0.452^{ns}	-0.333^{ns}
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, *$p \leq 0.05$, **$p \leq 0.01$, ns: Significance differences at $p \geq 0.05$

density and UV-B screening film will be good direction for environmentally friendly approach in the regulation of plant growth and development.

CONCLUSION

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 2.4 kg/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.

SIGNIFICANCE STATEMENT

"This study discovered the interactive effect of solar UV-B radiation and planting density for the regulation of sucker development and physiology of Entada that can be beneficial for farmers for improving Entada yield productivity and physiological adaptability under changing climate. This study will help the researchers to uncover the critical areas of light quality induced sucker regulation that many researchers were not able to explore. Thus a new theory on sucker regulation using light quality may be arrived at".

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