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INFLUENCE OF LIGHT INTENSITY ON GROWTH AND CROP PRODUCTIVITY OF *VANILLA PLANIFOLIA* ANDR.

Jos Puthur

Post Graduate and Research Department of Botany, St. Thomas College, Pala, Arunapuram – 686 574 Kottayam, Kerala, India

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Summary. To study the influence of light on growth and yield of *Vanilla* plants, 3 plots were marked in a vanillary, receiving sunlight with intensities varying between 300-1500 µE $m^{-2}s^{-1}$. Our results showed that photosynthesis was effective in *Vanilla* plants growing at sunlight of 300-800 µE m^{-2} s^{-1} whereas only plants receiving 600-800 µE $m^{-2}s^{-1}$sunlight were able to effectively partition the accumulated carbon into fruiting structures. Therefore, concerning productivity, light conditions of 600-800 µE $m^{-2}s^{-1}$were most favoured while 300-600 µE m^{-2} s^{-1}sunlight conditions were found to favour vegetative growth. Sunlight above 800 µE m^{-2} s^{-1} affected productivity negatively. It was observed that proline as well as carotenoids accumulated in *Vanilla* plants with increasing light intensities. However, the protective mechanisms against the photodestructive high light were not sufficient to protect *Vanilla* plants from the photoinhibitory damage. This was clearly manifested by the high levels of lipid peroxidation as judged by the malondialdehyde (MDA) levels, low chlorophyll content, low oxygen evolution rate and low productivity in plants exposed to sunlight above 800 µE m^{-2} s^{-1}. These results confirm that shade plants do not have a well-developed mechanism to counteract the after-effects of photoinhibition.

Keywords: crop productivity, growth, light intensity, photosynthesis, *Vanilla planifolia.*

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* Corresponding author, e-mail: jtputhur@rediffmail.com

INTRODUCTION

Orchids, the members of the family Orchidaceae are highly favoured ornamentals from time immemorial. Perhaps the only orchid which is of economic value other than as an ornamental is the Central American taxon *Vanilla planifolia* Andr. (Syn. *Vanilla fragrance*, Ames), the source of commercial vanillin. *Vanilla* is a tropical terrestrial genus of climbing orchids. The principal commercial source of vanillin ($C_8H_8O_3$) is the beans of *Vanilla planifolia* (Purseglove et al., 1981).

The efforts to propagate and popularize *Vanilla* cultivation in India have resulted in a large number of farmers taking it up. *Vanilla* was introduced in India as early as 1835. Recently, the area of *Vanilla* cultivation in India has been reported to be increasing very quickly to 1600 hectares due to the high market price of the beans and the sudden fall in the prices of other spices and plantation crops. During 2001- 2002 the total production of *Vanilla* beans in India was about 60 t^3 (Sudharshan and John, 2003).

Being a new crop, the cultivation of *Vanilla* faces a number of constraints for development. There are some specific bottlenecks such as a narrow germplasm base, inadequate research, a developing package of practices, diseases etc. (Shanmugavelu et al., 2002). Besides the problems mentioned above, the optimal shade conditions for effective productivity in *Vanilla* warrants a thorough investigation. A shade provided less than that required can affect the productivity by causing more light to be incident on the plant and by bringing about photoinhibitory effects that harm the metabolic process of the system (Vyas, 2004). A shade well above that required can also reduce the productivity, since the photosynthetic mechanism of the system does not work to its full efficiency (Shivasankara et al., 2000). Therefore, it is necessary to investigate the optimal light conditions that can maximize productivity. As far as improvement in crop yield is concerned, the aspect of solar energy utilization in photosynthesis is considered to be an area of high potential for further research (Natu and Ghildiyal, 2005)

Variations in light intensity have diverse effects on leaf area development, plant growth and yield (Saini and Nanda, 1986; Singh, 1994; Vyas et al., 1996; Vyas and Nein, 1999). High light is capable of causing stress to the plant and of inducing the production of reactive oxygen species and free radicals which are known to break DNA, destroy the functions of proteins and are also responsible for lipid peroxidation (Alia et al., 2002; Arora et al., 2002). *Vanilla*, which falls into the category of shade-loving plants, shows all characteristic features exhibited by this group of plants. High intensity light falling on shade-loving plants can cause inactivation of reaction centers accompanied by an inhibition of the electron transport through photosystems. Besides, it affects also the activities of the carbon cycle enzymes (Netto et al., 2005).

Plants have evolved diverse strategies for acclimatization and avoidance to cope with adverse environmental conditions. These include the accumulation of compatible solutes like glycine, betaine, proline, mannitol etc. Proline has been shown to protect plants against singlet oxygen and free radical-induced damages (Puthur et al., 1996; Netto et al., 2005). Due to its action as a singlet oxygen quencher and scavenger of free hydoxyl radicals, proline is able to stabilize proteins, DNA and membranes (Alia et al., 2002).

The present study was undertaken with the main objectives to correlate light utility and crop productivity in *Vanilla planifolia* and investigate the effect of high light on its physiological status.

MATERIALS AND METHODS

Plant material

Vanilla planifolia is an orchid species. The plant has long, green, succulent, simple or branched stems producing alternate leaves and nodal aerial roots which cling to tree trunks and other supports.

Two vanillaries at different locations in the Kottayam district of Kerala State, India, were selected for the study. In each vanillary, three plots were selected and the sunlight was regulated by spreading different layers of shade nets 2 feet above the plants. The light intensities between 11.30 am to 12.00 am in the three plots were 300-600, 600-800, and above 800 $\mu E\ m^{-2}\ s^{-1}$, respectively. In each plot, 12 plants of equal and appropriate growth features were selected and labeled. Various parameters such as fruiting, number of inflorescence per plant, number of flowers per inflorescence and number of fruits per plant were recorded for further analyses. Leaves were collected (6th leaf from the apex) for determination of leaf area, fresh and dry weight. The plants selected for the study were 6 years old.

Growth Parameters

Leaf area, leaf fresh weight and leaf dry matter were calculated according to the formulae described by Evans (1972).

Determination of the photosynthetic pigments

For determination of total chlorophyll (chl) and carotenoid pigment content leaf samples were homogenized in 80% chilled acetone, centrifuged at 12000g for 10 min (4^0 C) according to Arnon (1949) and the supernatant was used for quantification of the pigments (McKinney, 1941).

Analyses of oxygen evolution

Healthy leaves were collected from plants acclimatized to all three intensities of sunlight. The collected leaves were in labelled petriplates containing moisture and brought to the laboratory for analyses. Fresh leaf discs of 10 cm^2 were punched under water. The surface of the leaf discs were quickly wiped with a blotting paper and then the discs were immediately transferred to a leaf disc chamber of oxygen electrode (LD/3, Hansatech, UK). The changes in the concentration of gaseous oxygen within the chamber were monitored (Delieu and Walker, 1983). The leaf discs were first acclimatized in the dark for 5 minutes and then exposed to light intensity of 400 µE $m^{-2}s^{-1}$ using LED source (LH36, Hansatech, UK). The photosynthetic oxygen evolution was measured at 25^0 C. To avoid any CO_2 limitation for photosynthesis, 100 µmol of 0.5M bicarbonate buffer was added to the spongy capillary matting of the electrode chamber.

Light Measurements

Light measurements were carried out using a solar radiation meter (EMCON, India).

Quantification of proline

Estimation of proline was carried out according to the method of Bates (1973).

Estimation of malondialdehyde content

MDA content was determined as described by Heath and Packer (1968).

RESULTS AND DISCUSSION

The present investigation was designed in such a way as to find out the influence of light on growth and yield performance of *Vanilla*. Table 1 represents the data collected from the 2 different vanillaries for 3 consecutive years. The leaf dry matter percentage was highest in plots receiving 300-600 µE m^{-2} s^{-1} and 600-800 µE m^{-2} s^{-1} of sunlight. The plants growing at light intensity above 800 µE m^{-2} s^{-1} exhibited lower dry matter percentage.

The productivity in terms of fruits formed on a single plant was highest in plots receiving 600-800 µE m^{-2} s^{-1} of sunlight. The oxygen evolution rate was high in plants receiving 600-800 µE m^{-2} s^{-1} of sunlight at mid noon (1 pm) while it was low in plants receiving 300-600 µE m^{-2} s^{-1} of sunlight. It was still less in plants receiving sunlight above 800 µE $m^{-2}s^{-1}$ (Fig.1). These results clearly showed that effective photosynthesis was taking place in *Vanilla* plants receiving sunlight of 600-800 µE $m^{-2}s^{-1}$. Photosynthesis was at an appreciable level in plants exposed to 300-600 µE m^{-2} s^{-1} of sunlight, but only plants exposed to 600-800 µE m^{-2} s^{-1} sunlight were able to effectively partition the accumulated carbon into fruiting structures. Therefore, as regards productivity, light intensities of 600-800 µE m^{-2} s^{-1} were the most favoured ones. While sunlight of 300-600 µE m^{-2} s^{-1} favoured vegetative growth as judged by the fresh weight, dry weight and leaf area (which is the same as that of the plants receiving 600-800 µE m^{-2} s^{-1} of sunlight), light intensities above 800 µE m^{-2}s^{-1} affected negatively plant productivity (Table 1).

Plants receiving 300-600 µE m^{-2} s^{-1} sunlight have high levels of total chlorophyll content as compared to 600-800 µE m^{-2} s^{-1} and above 800 µE m^{-2} s^{-1} (Table 2). More shade is known to result in the synthesis of more chlorophyll as an adaptation strategy to harvest even the weak light reaching to the leaf (Anderson, 1986). The high chlorophyll content, however, did not favour higher rate of oxygen evolution in *Vanilla* plants. This could be due either to the inactiveness of the existing reaction centers or to a reduced number of reaction centers in the leaves. Although there was appreciable oxygen evolution rate in plants exposed to 300-600 µE m^{-2} s^{-1}sunlight, the carbon accumulated thereon was not partitioned in a productive manner as compared to the plants growing under 600-800 µE m^{-2} s^{-1} sunlight conditions. It is well known that plants which exhibit effective photosynthesis need not effectively partition their assimilated carbon into fruiting structures (Taiz and Zeiger, 1991), instead, they may add up to the vegetative growth of the plant. This partitioning nature of the assimilated carbon is highly influenced by the genetic feature of the plant as well as the physical conditions of the environment. In *Vanilla* plants, it may be assumed that

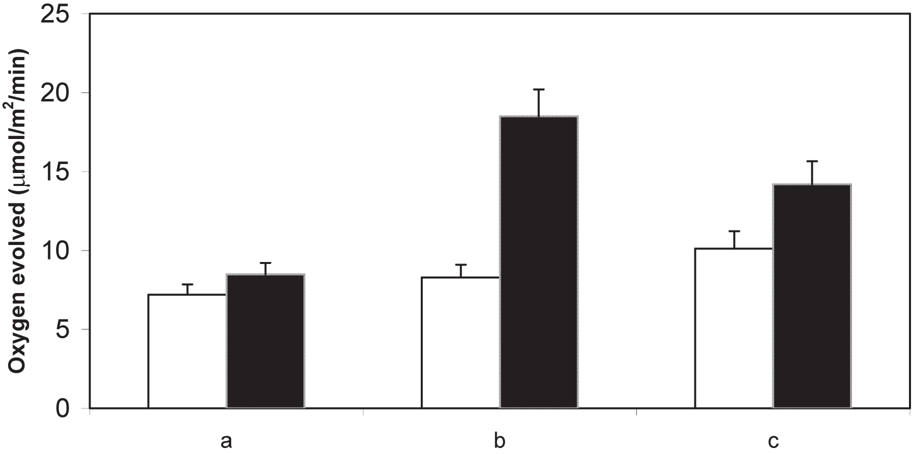

Fig 1. Oxygen evolution in leaves of vanilla plants acclimatized to conditions of varying light intensities. a, above 800 µE m^{-2} s^{-1}; b, 600-700 µE m^{-2} s^{-1}and c, 300-600 µE m^{-2} s^{-1}. (□) and (■) represent measurements done at 8 am and 1 pm, respectively. Vertical bars represent SE of the means from 3 independent experiments with a minimum of 3 replicates each.

Table 1. Growth parameters recorded in *Vanilla* plants growing under conditions of different light intensities. Data are means ± SE of three independent experiments with three replicates each (i.e. n=9).

Light intensity	300-600 $\mu E\ m^{-2}\ s^{-1}$	600-800 $\mu E\ m^{-2}\ s^{-1}$	above 800 $\mu E\ m^{-2}\ s^{-1}$
Number of inflorescence	4± 0.22	5± 0.27	3± 0.25
Number of flowers/inflorescence	20± 0.97	20± 0.87	15± 1.12
Number of fruits / plants	39± 2.12	80± 3.12	31± 1.12
Leaf area (cm^2)	89± 6.20	89± 7.20	85± 4.70
Fresh wt. of leaf (g)	14.9± 0.81	15± 1.10	14.6± 0.92
Dry wt. of leaf (g)	1.18± 0.07	1.20± 0.05	0.96± 0.07
Percentage of dry matter	7.92± 0.22	8.00± 0.29	6.58± 0.42

Table 2. Pigment composition in leaves of *Vanilla* plants growing under conditions of different light intensities. Data are means ± SE of three independent experiments with three replicates each (i.e. n=9).

Light intensity $\mu E\ m^{-2}\ s^{-1}$	Chl. a µg/g FW	Chl. b µg/g FW	Chl. a/b ratio	Chl. a+b µg/g FW	Carote-noids µg/g FW	Chl/caro-tenoid-sratio
300-600	784± 59.6	408± 39.0	1.92	1130± 95.8	120± 10.3	9.42
600-700	638± 52.4	378± 35.4	1.69	1022± 92.3	125± 9.7	8.18
above 800	347± 29.0	188± 17.5	1.85	570± 48.6	144± 11.2	3.96

besides the genetic features, the physical conditions in which the plants grow (light, humidity etc.) may influence the partitioning nature of the assimilated carbon between the vegetative and fruiting structures.

Plants adapted to low light and high light conditions are known to have low (~2 and above) and high (~2.8 and above) chl a/b ratios, respectively (Anderson, 1986). But, surprisingly, no significant differences were observed in the chl a/b ratios in *Vanilla* plants acclimatized to all three light conditions studied. A decreasing chl a/b ratio correlates with increased thylakoid membrane stacking in the chloroplast, and chloroplasts with an increased granal cross sectional area are prone to increased photoinhibition (Anderson and Aro, 1994). Our results for the chl a/b ratios helps us to assume that no significant reorganization of thylakoid membranes has taken place in chloroplasts of the plants adapted to higher light intensities so as to decrease the membrane stacking. Thus, the increased rate of photodestruction in the plants exposed to sunlight above 800 $\mu E\ m^{-2}\ s^{-1}$ may be directly influenced by the high degree of membrane stacking as judged by the low chl a/b ratio.

The low chlorophyll content in the leaves of plants receiving sunlight above 800 $\mu E\ m^{-2}\ s^{-1}$ could be a result of increased chlorophyll degradation. Shade adapted plants with large antennae are known to receive high light when exposed to high light conditions but as a lack of effective channelisation of this energy into photochemical reactions, this energy will culminate in the bleaching of chlorophyll (Anderson, 1986) (Table 2). The extent of photo injury was so severe in the leaves of these *Vanilla* plants that chlorosis took place in the major portion of the leaves indicating an irreversible injury.

Plants with shade adaptive features are highly sensitive to high light. The antennae of the photosynthetic machinery of such plants are unable to channel the light energy to the photochemical reaction centers. This unutilized energy goes stray and finally culminates in the production of free radicals (Powles, 1984). These free radicals can cause damage to the metabolism of the plants resulting in a retarded synthesis rate. This is what was precisely observed in the case of the productivity of *Vanilla* plants growing in sunlight above 800 µE m^{-2} s^{-1} which recorded high levels of lipid peroxidation as analyzed from the MDA content (Fig.2). Plants possess several protection mechanisms against free radicals, such as free radical scavenging enzymes, increased accumulation of caroteinoids, proline, etc (Asada et al., 1987; Alia et al., 2002; Arora et al., 2002; Netto et al., 2005). Therefore, it was of interest to see whether any of these mechanisms is operating in *Vanilla* plants to counteract the adverse effects of high light. Our results showed increased proline accumulation with increasing light intensities, the maximum proline being accumulated in the leaves of *Vanilla* plants exposed to light intensities above 800 µE m^{-2} s^{-1} (Fig. 3). Although carotenoids play a major role in light harvesting as accessory pigments (Taiz and Zeiger, 1991), the increase in carotenoid content with increasing light intensities (Table 2) could not justify its role in light harvesting alone. It also acts as a quencher of singlet oxygen species and in avoiding the generation of reactive oxygen species

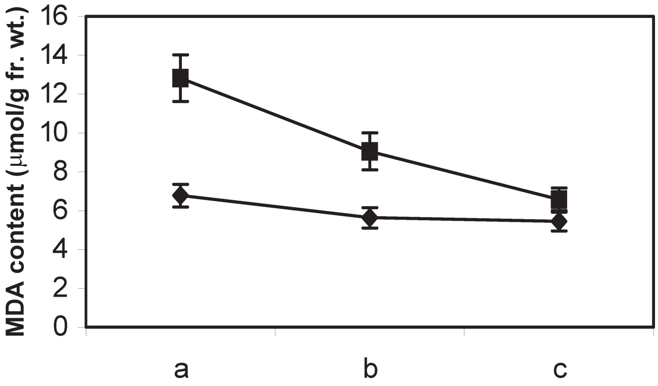

Fig 2. Malondialdehyde (MDA) content in leaves of vanilla plants acclimatized to conditions of varying light intensities. a, above 800 µE m^{-2} s^{-1}; b, 600-700 µE m^{-2} $s^{-1}$and c, 300-600 µE m^{-2} s^{-1}. (■) and (◆) represent measurements done at 8 am and 1 pm, respectively. Vertical bars represent SE of the means from 3 independent experiments with a minimum of 3 replicates each.

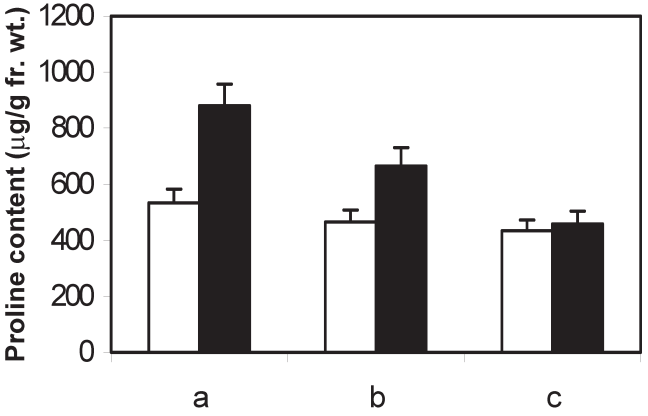

Fig 3. Proline content in leaves of vanilla plants acclimatized to conditions of varying light intensities. a, above 800 μE m^{-2} s^{-1}; b, 600-700 μE m^{-2} s^{-1} and c, 300-600 μE m^{-2} s^{-1}. (☐) and (■) represent measurements done at 8 am and 1 pm, respectively. Vertical bars represent SE of the means from 3 independent experiments with a minimum of 3 replicates each.

by the absorption of excess excitation energy from chlorophyll by direct transfer. Even though both proline and carotenoids were accumulated with increasing light intensities, the percentage of increase was not sufficient to de-energize the free radicals generated. Thus, the after-effects of free radicals generation were exhibited in the productivity of plants exposed to sunlight above 800 μE m^{-2} s^{-1}.

Hendry and Price (1993) have reported that the chlorophyll/carotenoid ratio is a sensitive indicator of photo oxidative damage. Lower values here indicate a higher degree of photo oxidative damage. It was found that *Vanilla* plants exposed to light above 800 μE m^{-2} s^{-1} had the lowest chlorophyll/carotenoid ratio (3.96), whereas plants exposed to light intensities of 300-600 μE m^{-2} s^{-1} and 600-800 μE m^{-2} s^{-1} had higher values for the chlorophyll/carotenoid ratio (9.42 and 8.18, respectively) (Table 2). These results indicate an extensive photo oxidative damage in the leaves of *Vanilla* plants exposed to light above 800 μE m^{-2} s^{-1}.

CONCLUSION

Sunlight varying from 600 to 800 μE m^{-2} s^{-1} favoured maximum productivity in *Vanilla planifolia* plants whereas light intensities of 300-600 μE m^{-2} s^{-1} affected to a greater extent plant vegetative growth. Light intensities above 800 μE m^{-2} s^{-1} led to photo destructive and, in part, photoinhibitory effects on the photosynthetic apparatus. Although the high light conditions are helpful in triggering mechanisms to counteract the photoinhibitory effects, such as accumulation of proline and carotenoids, our results showed that these mechanisms were not fully capable of protecting the plants against photoinhibitory effects. Therefore, sunlight above 800 μE m^{-2} s^{-1} can bring about a drastic reduction in the productivity of *Vanilla*.

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