

Growth and Photosynthetic Pigment Accumulation in *Lycopersicum esculentum* in Response to Light and Nutrient Stress

Gideon Olarewaju OKUNLOLA^{1*}, Adekunle Ajayi ADELUSI²

Department of Botany, Obafemi Awolowo University Ile-Ife, Osun State, Nigeria;
lanrog01@yahoo.com (*corresponding author)

Abstract

This study is aimed at determining the responses of some of the growth parameters of *Lycopersicum esculentum* to light and nutrient stress. It is equally aimed at determining the effect of light and nutrient stress on the photosynthetic pigment accumulation in the plant. Tomato seeds were grown in pretreated sand and were watered with distilled water until they were fully established. After this, the plants were transplanted into 60 plastic pots with holes bored at the bottom of the pots to allow for proper drainage of the excess water during the course of the experiment. The plants were divided into four groups of fifteen pots each. A group of plants was stressed of nutrient only by administering 100 ml of complete nutrient solution once in every four days. The nutrient solution if applied daily was considered to be adequate for the plants. Another group of plants were light stressed by placing them under shade while adequate light was gotten in the direct sunlight. Sampling was carried out at weekly intervals starting from seven days after treatment. Plants were randomly picked from each of the four treatments. Three replicates were used for each parameter. The result gotten from the study showed that there was a reduction in photosynthetic pigment accumulation in the plants when both light and nutrient were limiting. The data obtained from the study were first tested between normality and assumption of constant variance. A two-way analysis of variance (ANOVA) was carried out considering both factors (light and nutrient) as sources of variation to investigate the effects of full light and full nutrient (FLFN), full light and partial nutrient (FLPN), Partial light and full nutrient (PLFN) and partial light and partial nutrient (PLPN) on the parameters studied in *Lycopersicum esculentum*.

Keywords: growth, light, nutrient, pigment, stress, tomato

Introduction

Crop plants are often exposed to various environmental stresses which severely affect soil productivity and crop production, worldwide. Bray *et al.* (2000) estimated that the contribution of environmental stress factors to loss of food production was becoming increasingly important. Survival and productivity of crop plants exposed to environmental stresses are dependent on their ability to develop adaptive mechanisms to avoid or tolerate stress. Accumulating evidence suggests that the mineral nutritional status of plants greatly affects their ability to adapt to adverse environmental conditions.

Photo-oxidative damage, i.e. light dependent generation of reactive oxygen species (ROS) in chloroplasts, is the key process involved in cell damage and cell death in plants exposed to environmental stress factors (Foyers *et al.*, 1997; Asada 2000; Foyer and Noctor, 2005). High light intensity may induce severe photo-oxidative damage to chloroplasts, and consequently cause decrease in the yield capacity of plants. The mineral nutritional status of plants greatly influences photosynthetic electron transport and CO₂ fixation in various ways (Marshner, 1995; Mengel and

Kirkby, 2001). Impairment of the mineral nutrition of plants can, therefore, be accompanied by an enhanced potential for photo-oxidative damage and this threat can be especially serious when plants are simultaneously exposed to an environmental stress.

The problem faced by plants under conditions of high solar radiation and temperature is energy absorption by the leaves, which can easily raise the temperature of the leaf by 5°C or more above ambient. Productivity of crop plants exposed to environmental stresses is dependent on the availability to develop adaptive mechanisms to avoid and tolerate stress (Willits and Peet, 2001). The quantum efficiency of photosynthesis of a plant is largely reduced (photoinhibition) when it is exposed to excess light level (Sudhir *et al.*, 2005).

Nutrient stress can occur as a result of the form in which nutrient exist; the process by which they become available to the plant; the content of the soil solution and pH (Hale and Orcutt, 1987). Since light and temperature are closely related and the developmental stages closely related to temperature over a period of time, then light has an important role to play in the developmental stages of plants. Thompson *et al.* (1988), observed that at medium irradiance and high nutrient levels, leaf expansion,

chlorophyll content and photosynthesis were optimal. A plant acclimatizes to a given irradiance and nutrient availability by physiological adjustment, which serves to increase carbon gains (Thompson *et al.*, 1988).

Among the most important molecules for plant function are the pigments. Chlorophyll is the primary pigment in plants; it is a porphyrin that absorbs red and blue wavelengths of light while reflecting green. It is the presence and relative abundance of chlorophyll that gives plants their green colour. All chlorophylls serve as the primary means that plants use to intercept light in order to fuel photosynthesis.

Carotenoids are red, orange, or yellow tetraterpenoids. They function as accessory pigments, helping to fuel photosynthesis by gathering wavelengths of light not readily absorbed by chlorophyll. The most familiar carotenoids are carotene (an orange pigment found in carrots), lutein (a yellow pigment found in fruits and vegetables), and lycopene (the red pigment responsible for the colour of tomatoes). Carotenoids have been shown to act as antioxidants and to promote healthy eyesight in humans.

This study is aimed at contributing to the existing literature on light and nutrient as they operate independently and/or interact together to affect the growth and the yield of tomato plants. It is also aimed at highlighting some of the morphological and physiological changes that occur in tomato in response to light and nutrient stress. The interactive effects of nutrient and light stress on pigment accumulation will also be highlighted.

Studies till date have focused on the impact of one single environmental stress event, for example, water deficits or heat shock (Reddy *et al.*, 2004; Camejo *et al.*, 2005). The combined effect of more than one type of stress (e.g. light + nutrient) on plant metabolism has received less attention.

In the present study, the effect of nutrient and light stress on growth parameters like shoot height, number of leaves, leaf area and plant biomass were compared. Effect of light and nutrient stress on photosynthetic pigments like chlorophylls and carotenoids were also compared.

Materials and Method

Seeds of *Lycopersicon esculentum* (Ife No 1 Variety) that were utilized in this experiment were collected from Osun state Ministry of Agriculture, Oshogbo, Osun state, Nigeria. Since the work was on the effect of nutrient and light, sand was used for the germination of the seedlings because it does not contain any mineral element except for the SiO₂ that is present in it. Sixty plastic pots which were 21 cm in height and 24 cm in diameter were gotten. Five holes of equal diameter were bored at the bottom of each of the pots to allow for proper drainage of excess water during the course of the experiment. The pots were filled near brim with the sand that had already been demineralized by washing it in 1N HCl and then rinsing it with tap water to a pH of 7. Seeds of *Lycopersicon esculentum* were planted in a nursery and fifteen days after planting, the seedlings were transplanted at the rate of four seedlings per plastic pot. The plants were then divided into four groups containing fifteen pots each. The plants in groups 1 and 2 were made to receive direct sunlight by putting them in the open space, with group 1 receiving 100 ml of complete nutrient solution

everyday while group 2 plants only received 100 ml of complete nutrient solution once in every four days. Plants in group 3 and 4 were placed under a shade provided by the Tecoma stans tree with group 3 plants receiving 100 ml of complete nutrient solution everyday while plants in group 4 were given 100 ml of complete nutrient solution once in four days (Adelusi and Aileme, 2006). The four groups were represented as Full Light Full Nutrient (FLFN) for group 1 plants; Full Light Partial Nutrient (FLPN) for group 2 plants; Partial Light Full Nutrient (PLFN) for group 3 plants and Partial Light Partial Nutrient (PLPN) for group 4 plants. The mean monthly intensities of light under the shade was found to be approximately 16300 lux while that of the direct sunlight was found to be approximately 48400 lux. The complete nutrient solution was prepared according to the modified Long Ashton Formula (Hewitt, 1952).

Samplings were done at weekly intervals, starting from the fifteenth day after planting to the 78th day. Plants were randomly picked from the pots in each of the four treatments. Three replicates were used for each parameter. A transparent metric ruler was used to measure the shoot height from the top of the soil to the terminal end. The leaf length and width were also measured and used to calculate the leaf area. Total number of leaves per plant was noted. For the dry weight determination, plants were randomly harvested and the soil attached to their root was washed off. The plants were then dried in a Gallenkamp oven at 80 °C until a constant weight was achieved. After cooling, the dry weights were taken on a weighing balance.

For chlorophyll determination, 5g of tomato leaves were ground in 20 ml of 80% (v/v) acetone using a mortar and pestle. The brei was filtered using a Whatman's No 1 filter paper. The pigment quantities in the acetone extract was determined on a digital spectrophotometer at wavelengths of 664 nm and 647 nm. Chlorophylls 'a' and 'b' and also the total chlorophyll content were determined using the formula according to Combs *et al.* (1985).

$$\text{Chlorophyll 'a' } (\mu\text{M}) = 13.19A_{664} - 2.57A_{647}$$

$$\text{Chlorophyll 'b' } (\mu\text{M}) = 22.10A_{647} - 5.26A_{664}$$

$$\text{Total chlorophyll } (\mu\text{M}) = 7.93A_{664} + 19.53A_{647}$$

A₆₆₄ represent the absorbance at wavelength 664 nm while A₆₄₇ represents the absorbance at wavelength 647 nm.

For the carotenoids (carotene and xanthophyll), 5 g of tomato leaves was ground in 20 ml of 80% (v/v) acetone using a mortar and pestle. The brei was filtered through a Whatman's No 1 filter paper. 25 ml of petroleum ether was placed in a separating funnel and the acetone extract of the pigment was added. The funnel was gently rotated, releasing the pressure periodically. 35 ml of distilled water was gently poured down the sides of the funnel and the funnel was rotated until the upper layer was very green. The two layers were then allowed to separate before drawing off the lower acetone-water layer. The petroleum ether fraction was washed with 25 ml of distilled water at three consecutive times and discarded each time. This removed any trace of acetone that might remain within the petroleum ether fraction. 25 ml of 92% (v/v) methanol was added to the petroleum ether fraction, rotated and then separated into upper and lower fractions (carotene and xanthophyll). The absorbance of both fractions were determined using digital Spectrophotometer. Petroleum ether and diethyl ether served as blanks (Machlis and Torrey, 1956).

Statistical analysis was performed using Statistical Analysis System (SAS) software version 9.1 (SAS, 2003). The data were first tested between normality and assumption of constant variance. A two-way analysis of variance (ANOVA) was carried out considering both factors (light and nutrient) as sources of variation to investigate the effect of light and nutrient stress on the growth parameters as well as pigment accumulation of the tomato plant. Post hoc testing was carried out using Duncan Multiple Range Test (DMRT) to separate the significance means at 0.05, 0.01 and 0.001 confidence limit (alpha level) for the mean.

Since all the plants in the four different regimes were subjected to the same conditions, except that some were light stressed while others were nutrient stressed, any observed differences in the parameters investigated between the stressed and unstressed plant can therefore be attributed to the effect of light and nutrient stress which were the only variable introduced into the experiment.

Result

The shoot height of plants in all the four treatments increased from the beginning to the end of the experimental period with the plants in the partial light recording the higher shoot height than those in the full light (Fig. 1). The highest shoot height was recorded in PLPN plants while the lowest shoot height was recorded by the FLFN plants. The result of the ANOVA carried out showed that there was no significant effect of light and also of nutrient on the shoot height of tomato plants ($P > 0.05$). There is also no interactive effect of light and nutrient on the shoot height of tomato ($P > 0.05$).

There was a gradual but steady increase in the number of leaves in all the treatments (fig.2) from the beginning to the end of the experiment. The FLFN plants however recorded a sharp decrease in number of leaves on the 50th day of the experiment. This was followed by a steady increase to the end of the experiment. The results of the ANOVA showed that light had a significant effect on the number of tomato leaves ($p < 0.05$). It also showed that nutrient did not have any significant effect on the number of tomato leaves ($p > 0.05$). There was also no significant interactive effect of light and nutrient stress on the number of tomato leaves ($p > 0.05$).

There was an increment in leaf area of plants in all the four treatments from the beginning to the end of the

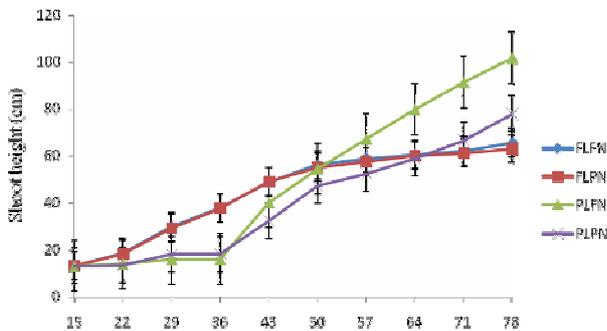


Fig. 1. Effect of light and nutrient stress on the shoot height

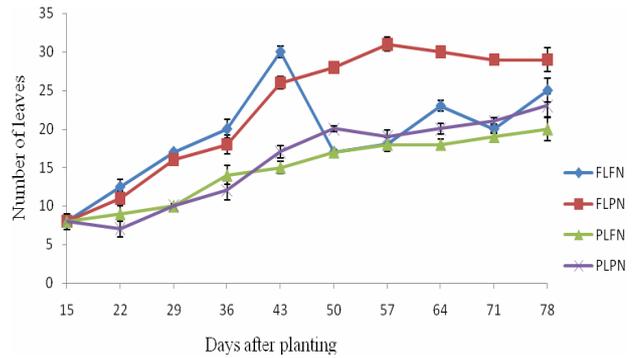


Fig. 2. Effect of light and nutrient stress on the number of leaves

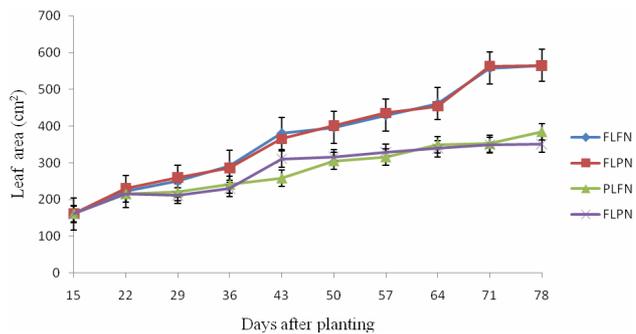


Fig. 3. Effect of light and nutrient stress on the leaf area

experimental period (Fig. 3). The plants in the full light recorded an approximately equal leaf area throughout the experimental period, while the plants in the partial light also recorded an approximately equal leaf area throughout the course of the experiment. The result of the ANOVA showed that light has a significant effect on the leaf area ($p < 0.05$). Nutrient however did not have any significant effect on the leaf area ($p > 0.05$). In addition, there was no significant interactive effect of light and nutrient on the leaf area of tomato ($p > 0.05$).

Plants in the full light recorded higher dry weights than those under the shade irrespective of the nutrient levels. The plants under the shade recorded approximately equal dry weights throughout the experimental period. The result of the ANOVA showed that light had a significant effect on plant biomass ($p < 0.001$). Nutrient however did not have any significant effect on plant biomass ($p > 0.05$). There was also no significant interactive effect of light and nutrient on the plant biomass ($p > 0.05$).

The accumulation of *chlorophyll a*, *chlorophyll b*, total chlorophyll, carotene and xanthophylls in the plants were similar, in that they did not follow any particular pattern throughout the experiment (Figs. 5, 6, 7, 8 and 9). However, the PLPN plants recorded the highest value of these pigments, followed by the PLFN plants while the FLFN plants recorded the least accumulation of *chlorophyll a*, *chlorophyll b*, total chlorophyll, carotene and xanthophylls in the plants did not follow any particular pattern (Figs. 4, 5, 6, 7, 8 and Tabs. 1 and 2). It could however be seen that *chl b* was higher in the shade than in full sunlight. The result of the ANOVA shows that there is a significant effect of light on *chlorophyll b*, total chlorophyll and xanthophyll

accumulation in the plants ($p < 0.05$). It however showed that there was no significant effect of light on *chlorophyll a* and carotene accumulation in the plants. The result also shows that there was a significant effect of nutrient on *chlorophyll a* and total chlorophyll accumulation in the plants. It however shows that there was no significant effect of nutrient on *chlorophyll b*, carotene and xanthophyll accumulation in the plants ($p > 0.05$). There was also no significant interactive effect of light and nutrient stress on all the parameters ($p > 0.05$).

Discussion

The higher plant heights in the shade was in agreement with the findings of Warrington *et al.* (1988); Niinemets (1999) who found an increase in stem elongation and a reduction in leaf dry mass per area as a response of plants to conditions of low photo flux density. The results were also in agreement with the findings of Barber and Anderson, (1992) who found that under the conditions of low light intensity, plants generally bear longer internodes and are less

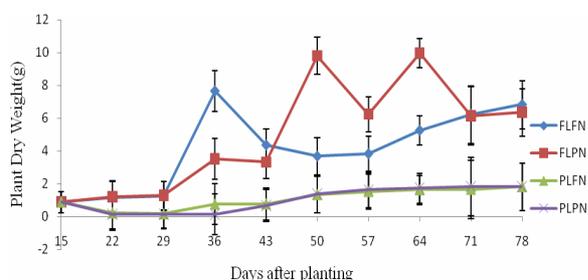


Fig. 4. Effect of light and nutrient stress on the dry weight of plants

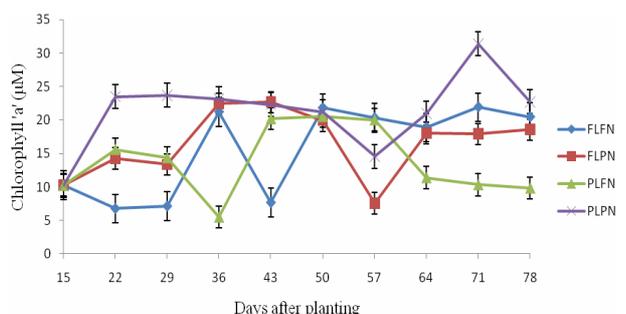


Fig. 5. Effect of light and nutrient stress on chlorophyll *a* content

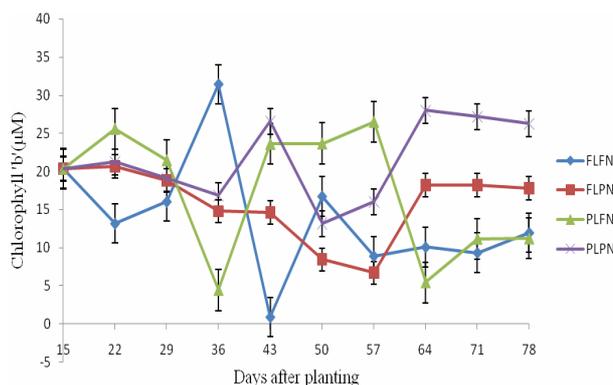


Fig. 6. Effect of light and nutrient stress on chlorophyll *b* content

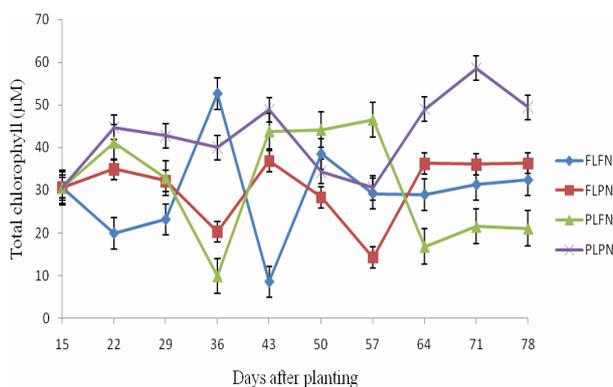


Fig. 7. Effect of light and nutrient stress on the total chlorophyll content

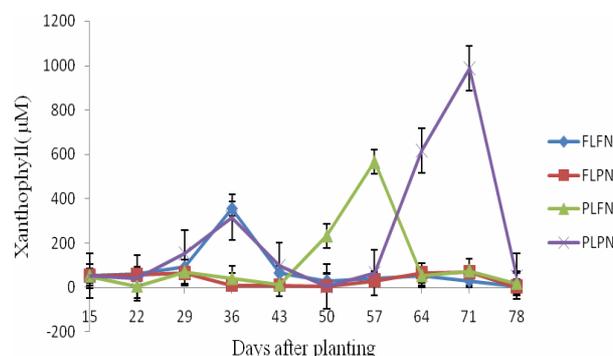


Fig. 8. Effect of light and nutrient stress on the xanthophyll accumulation

Tab.1. Result of Duncan Multiple Range Test (DMRT) for the parameters measured

Treatments	Shoot height (cm)	Number of leaves	Leaf area (cm ²)	Plant biomass (g)	Chlorophyll <i>a</i> (µM)	Chlorophyll <i>b</i> (µM)
FLFN	46.033±1.17 ^a	13.11±0.46 ^b	365.5±2.51 ^b	3.752±0.65 ^a	15.61±0.45 ^a	11.12±0.37 ^a
FLPN	43.421±1.12 ^a	13.45 ^b ±0.55	363.5±1.01 ^b	3.251±1.05 ^a	16.1±0.33 ^b	15.76±1.01 ^a
PLFN	45.531 ±2.02 ^a	15.04 ^a ±1.01	278.9±1.02 ^c	2.034±0.57 ^b	13.72±1.25 ^a	17.3 ±2.01 ^b
PLPN	44.367±1.90 ^a	15.87 ^a ±2.06	280.1±0.95 ^c	1.902±0.25 ^b	21.32±1.05 ^b	21.32±1.50 ^b

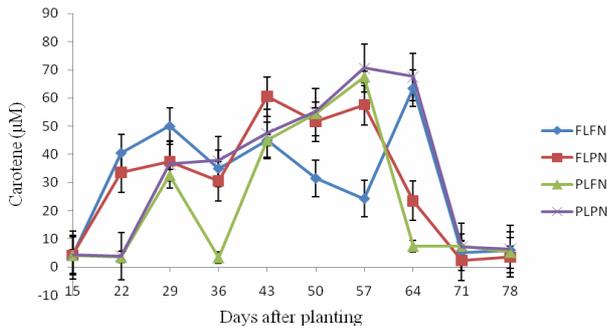


Fig. 9. Effect of light and nutrient stress on the carotene accumulation

Tab. 2. The accumulation of total chlorophyll, carotene and xanthophylls in different plants

Treatments	Total chlorophyll (µM)	Carotene (µM)	Xanthophyll (µM)
FLFN	26.50±2.35 ^a	30.53±0.54 ^a	79.14±2.10 ^a
FLPN	30.91±2.01 ^b	30.50±1.55 ^a	36.55±1.55 ^a
PLFN	30.30±1.54 ^b	23.10±2.01 ^a	108.9±2.55 ^b
PLPN	42.30±2.01 ^a	33.63±1.21 ^a	113.31±1.55 ^b

Mean ± (SE) values followed by the same letter within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range

tough and more succulent than those in intense light. High light intensities may be excessive for optimum growth regulation and photosynthetic activity. Plant growth is related to the function of growth hormones, like auxins which is sensitive to high light intensity. The PLFN plants devoted more of their nutrient availability to stem extension, hence their higher shoot height than the PLPN plants. The survival of the plants under the shade depends on the efficiency with which they capture and utilize light. According to (Weiner *et al.*, 1990; Jurik, 1991; Aarssen, 1995; Berntson and Wayne, 2000), stem extension plays an important role in determining exposure of leaves to light, shading of competitors, and elevation of reproductive structures. Variation in temperature greatly affects plant growth and flowering. The plant height and internodes length increased as the light intensity decreased. These results were in agreement with the findings of Mortensen and Larsen (1989), who observed a decrease in shoot length at high light intensity. Shaded plants showed increased stem elongation which is considered to be due to photosynthetic limitation under low light condition.

The greater plant biomass of the plants in the sunlight compared to those of the plants under the shade contradicted the findings of Pooter *et al.* (1999) who found that plant dry weight decreases with increasing light intensity. Leaves in the full sunlight retained a relatively high photosynthetic rate irrespective of the nutrient level.

The lower leaf area in plants under the shade compared to plants in the full sunlight (FLFN and FLPN) was in agreement with the findings of Devkota *et al.* (2000) who found that morphological adaptation of plants to low light intensities results in longer and narrower leaves with higher specific leaf area to maximize light interception. Leaves of plants grown in full sunlight had increased leaf length, leaf width and consequently increased leaf area. The higher leaf area of PLFN plants compared to those of PLPN plants was

also in agreement with the findings of Aerts (1989), Oikawa *et al.* (2006). They found that plants grown at high nutrient availability generally produce larger but short-lived leaves with higher nitrogen concentrations per unit area than those at low nutrient availability, and with greater allocation to photosynthetic protein (Evans, 1989). Plants typically respond to shading by producing leaves with less mass per unit area (LMA) (Pooter and Evans, 1998), which enables greater light capture per unit mass (Hirose, 1995). Yet reductions in thickness and LMA may also lead to a reduction in leaf mechanical resistance, and therefore leaves may become more vulnerable to mechanical damage. This lower leaf area in the plants under the shade (PLFN and PLPN) also agreed with the findings of (Anten *et al.*, 2003) who found loss of leaf area under light-limited conditions.

Chlorophyll b is most abundant in the antennae of the light harvesting complex, whereas *Chlorophyll a* is concentrated around PSII. To capture as much light as possible, shade-grown plants typically have more light-harvesting complexes per unit area than do sun-grown plants that typically receive more light than needed. Therefore, it was not surprising that the *Chlorophyll b* content was higher in the shade-grown plants. The decrease in *Chlorophyll b* content in sun plants could be an indication of Chlorophyll destruction by excess irradiance. Lowest Chlorophyll ratio in sun plants is an indicator of senescence, stress and damage to the plant and the photosynthetic apparatus, which is expressed by faster break down of Chlorophyll than Carotenoids. These results corroborate many studies made with sun or high light and shade or low-light leaves. Lin *et al.*, (2009) clearly indicated that low light-grown plants are more susceptible to photoinhibition than high light-grown plants. The Chlorophyll content increase in the low intensity plants due to reduced photooxidation in lower light conditions. In case of *J. curcus*, where the ratio *a/b* increased in the low intensity plants due to less synthesis of *Chl a* than to the reduction of photooxidation of *Chl b* in the shade. It is notable that the conditions of both *Chl a* and *b* were observed to increase under low light conditions (Wijanarko *et al.*, 2007).

High light intensity has an effect on carotenoid biosynthesis. This explains the reason why the plants in the full sunlight recorded higher carotenoid than the plants under the shade. Carotenoids play an important role in light harvesting complex and photoreception of the photosystems. Several studies have shown that carotenoids are very important in protecting the photosynthetic apparatus against photodamage (Ort, 2001).

In conclusion, most variables analyzed in this study showed that growth of *Lycopersicum esculentum* was greatly enhanced under high light conditions as compared with its growth under the shade. There was a decrease in the total plant biomass and the number of leaves produced, with decreased irradiance. The accumulation of photosynthetic pigments studied (chlorophyll and carotenoid) was reduced when both light and nutrient were limiting. Since chlorophyll is a precursor for carotenoid formation, any factor that causes a reduction in the chlorophyll accumulation will be expected to affect carotene and xanthophyll accumulation.

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