A COMPARATIVE STUDY ON GROWTH OF AMARANTHUS CULTIVARS IN LIGHT AND SHADE ENVIRONMENTS

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Abstract

The growth and phytochemical constituents of *Amaranthus cruentus* L. and *Amaranthus palmeri* S. Wats. under light and shade conditions were studied. It found that growth of plants in light result more height, number of leaves, and biomass. It also noticed that the plants grow in shade accumulate less soluble protein, sugar, phenolics, anthocyanin and malonyldialdehyde. However, more accumulation of photosynthetic pigments was found in plants grow in shade. The results shows that *Amaranthus* varieties grow in open field will have better vegetative growth, biomass productivity and phytochemical constituents. Therefore, an optimized light management practice should be adopted to ensure the successful cultivation of *Amaranthus* cultivars in vertical farming.

Key words: light, shade, pigments, lipid peroxidation, biomolecules

Introduction

Light is the major factor affecting photosynthesis and biomass productivity of crops. However, light is one of the most variable components of plant environment. Light responses of plants differ based on the lightening environment, season, genotype, and cultivation practices and many others (Bayat et al. 2012). Plants have evolved several efficient protection mechanisms that make it possible for them to survive under unfavorable light and temperature conditions (Szymanska et al. 2017). Because of the urbanization process, vast urban spaces are located between the buildings, and there is increasing demand in vertical farming which is known as future of agriculture. Vertical farming decreases water usage about 95.0% because of recycling, and it also help to avoid usage of agrochemicals such as pesticides

(Benke and Tomkins, 2017). But the usage of artificial light is common in the course of vertical farming and therefore, optimizing light conditions for the plant growth helps in efficient usage of light during farming practices.

Light fluctuations not only affects plant morphology, physiology and microstructure but also has an important impact on crop productivity. This is mainly because plant growth requires an appropriate light intensity whereas excessively high or low intensity will retard photosynthesis in the plants. Shade not only influence the amount of light received by plants, but also changes other small environmental conditions affecting the plant growth such as temperature, humidity and carbon dioxide respectively (Wan et al. 2020). Shade imposes a limitation to biological productivity in plants although the extent of the limitation varies with the shade tolerance of the species and the nitrogen supply (Wan et al. 2020). Plants exhibits several well known shade avoidance responses, including accelerated stem extension growth, retarded leaf development and strengthened apical dominance during competition for light. Shade avoidance responses may improve plant fitness by increasing capture of the light under diverse environmental conditions. Stem elongation in particular has received considerable attention as an example of adaptive plasticity.

The plant *Amaranthus* belongs to the family of Amaranthaceae, with a worldwide distribution. It consists of about 60-70 species. *Amaranthus* is used as leafy vegetable, forage and ornamentals. Amaranthus is considered as one of the most promising food plant. It provides high quality protein, high amounts of unsaturated oils, dietary fiber and essential minerals. About 90.0 % vitamin C, 73.0 % vitamin A, 28.0 % calcium and 28.0 % iron of daily nutrient requirement can be obtained from one cup of cooked, boiled and drained *Amaranthus* leaves (Achigan-Dako et al. 2014). *Amaranthus* leaves contain a unique source of antioxidant pigments compared to other leafy vegetables. The plant grows well in a variety of soil conditions from clay to sandy loam, but cannot tolerate wet or water logged soil there by making a drought tolerant vegetable. The aim of this study is to evaluate the influence of light on the growth of two different varieties of *Amaranthus* in light and shade environment that will provide insights for vertical farming. Comparison of plant growth under shade and open field conditions with regard to crop productivity and phytochemical constituents were tested.

Materials and methods

Plant material

The seeds of *Amaranthus* used for the study was collected from seed market, Erattupetta. For this study *A. palmeri* and *A. crutentus* that are the edible species in the genus *Amaranthus*. These are edible and highly nutritious. *A. palmeri* and *A. crutetus* thrives in hot weather, and responds quickly to high levels of available nutrients. It can grow up to 2.0 m in height. The high protein content and amino acid composition give amaranth medicinal benefits. Different environmental conditions such as temperature, light, and soil have an influence on *Amaranthus* seed germination and thus affect grain yield. The selected plant varieties are cultivated in fertile soil. The soil used for this experiment was black soil. Black soil is enriched with minerals. Black soil is suitable for the cultivation of vegetables, and grains. Soil colour is due to the availability of organic matter content. The black colour of soil indicates that the soil has high organic matter content.

Method of planting

The black soil collected was mixed with dried cow dung in a ratio of 2:1.Then the mixture is filled in four pots which are medium sized with a diameter of 14.0 cm. The *Amaranthus* seeds which were already sown maintained with care with regular water supply and by providing same environmental conditions are selected for the experiment. From the seedlings of two verities six seedlings from each variety are selected. From the six seedlings three of them from each verity are kept in shade (503.48 lux) and the remaining three are kept in full sun (50889.68 lux).The plants are watered regularly and observed the growth.

Measurement of growth parameters

The plant height, number of leaves, and the sun light intensity were determined in regular intervals. The plant height was measured from base to the apex using a centimeter scale. Biomass was quantified at the time of harvest using a weigh balance. These are the factors which indicate the growth of the plant.

Estimation of pigments

Chlorophyll and carotenoids quantified using leaf extract in acetone:dimethylsulphoxide (50:50). The optical density of leaf extract measured at 470.0, 646.0 and 663.0 nm and content of pigments estimated with the following formulae (Lichtenthaler and Wellburn,1983). Total chlorophyll = 20.2 (A646) + 8.02 (A663); Chlorophyll a = 12.21 (A663) - 2.81 (A646); Chlorophyll b = 20.13

(A646) - 5.03 (A663); Carotenoids = (1000 A470 - 3.27[chl a] – 104 [chl b])/227.

Estimation of phenolics

Total phenolics estimated from the methanol extract of dried leaf powder (McDonald et al. 2001). Leaf powder (1.0g) extracted with 10.0 ml methanol. To the extract, 5.0 ml of 0.1 % FolinCiocalteu reagent and 4.0 ml of 1.0 M sodium carbonate added. The reaction mixture incubated for 15.0 min. The amount of phenolics determined after measuring absorbance at 765.0 nm. Gallic acid dissolved in 50.0 % methanol used to make a standard graph, and the result represented as the equivalent of gallic acid**.**

Estimation of anthocyanin

Anthocyanin quantified from methanol/HCl/water (90:1:1) extract of leaf powder. The absorbance of the extracts read at 530 (A530) and 657(A657). Amount of anthocyanin calculated using formulae A530 - A 657 (Mancinelli, 1984). Amount of pigments expressed in cyanidin equivalents per gram fresh weight of leaf tissue.

Lipid peroxidation

Malondialdehyde (MDA) accumulates in plant cells as a result of peroxidation of lipids in membranes. Therefore, the extent of lipid peroxidation in leaves determined via estimation of MDA content (Heath and Packer,1968). Trichloroacetic acid (TCA) (4.0 ml) used to extract MDA from leaf tissues (500.0 mg).The extract mixed with 1.0 ml 2-thiobarbituric acid (0.5 %) and the mixture heated at 95.0 o C for 30min. The mixture cooled and centrifuge at 10000.0 rpm for 10 min. The supernatant subjected to the measurement of optical density at 532.0 and 600.0 nm (18). Extinction coefficient value of 155.0 mM−1 cm−1 used to calculate the MDA content in the sample, and the result expressed in nmol MDA per gram fresh weight.

Estimation of Total soluble sugar

Total carbohydrate content was measured using Phenol-Sulphuric acid method (Masuko et al. 2005). For this 100 mg of the sample weighed, and the soluble sugar extracted in double distilled water. The samples were centrifuged and then 1.0 ml aliquots were transferred to test tubes. Standards were prepared by mixing sucrose in double distilled water. The test solutions were mixed with 1.0 ml of 5.0 % phenol and 3.0 ml sulphuric acid and boiled for 20 minutes in a water bath. The absorbance of test solution read at 490.0 nm used for calculation of total soluble sugar.

Estimation of protein

Protein in the sample estimated using Bradford assay method. The plant tissues

were homogenized in 5.0 mL of 0.5 M Tris-HCl (pH 7.4) using a pestle and mortar. The crude extract was centrifuged at 8000 rpm for 10.0 min, and the supernatant was used to determine protein content by mixing with Bradford reagent and the absorbance read at 595.0 nm (Bradford,1976). Bovine serum albumin (BSA) was used as the standard.

Results

Analysis of plant growth

Growth, development, productivity and postharvest quality of any crop largely depend on the interaction between the plant and the environmental conditions under which they are grown. There was a distinct difference in sunlight intensity for the shaded plants as compared to plant grown in open field .The shade used in this experiment manipulated micro climatic properties and influenced growth and yield of *Amaranthus*. *A. palmeri* and *A. cruentus* are the two verities selected for the experiment. The growth and development of these two varieties in the open environment was particularly same throughout the study period. The average light intensity of environment plants was 50889.68 lux and for shaded plants was 503.48 lux (Table1). The plant growth parameters during the study period were given in table 1.The height of plants in shaded and open environment is observed. *A. palmeri* has the higher growth in open environment (Table 1, Figure.1A-D). While comparing with sunlight exposed light plants, the height of plants in the shade environment decreased about 83.0 % in the case of *A. palmeri* and 72.0 % in the case of *A. cruentus* respectively. There was highly significant effect of light on number of leaves. The number of leaves was more in plants grown in light. *A. palmeri* had about 68.0 % and *A. cruentus* had about 72.0 % more no. of leaves (Table. 1). Plant biomass is the stored chemical energy from the sun. Plant produces biomass through photosynthesis. Photosynthesis is a light dependent process, so the plant biomass recorded more about 97.0 % in *A. palmeri* and 94.0 % *in A. cruentus* when grown in light (Table. 1).

Photosynthetic pigments

Chlorophyll is an important photosynthetic pigment to the plant, largely determining the photosynthetic capacity and hence plant growth. High light environment may impart stress on the synthesis of photosynthetic pigments. The contents of total chlorophyll, chlorophyll a, and chlorophyll b are given in the Figure.2A-C. It found that chlorophyll increased up to 32.0 % *A. palmeri* and 65.0 % *in A. cruentus* respectively when grown shade. There was also a slight increase in chlorophyll a/b ratio (0.8 - 4.0 %) among plant grown in shade (Figure.2D).

Carotenoids are lipid - soluble pigments, which can be found in fruits and vegetables that help to avoid photo oxidative stress in plants (Ngamwonglumert et al, 2019). It found that carotenoids content increases up to 13.0 - 30.0 % when the plants grow shade (Fig. 3A).

Changes in molecules with antioxidant properties

Anthocyanins are coloured water soluble pigments belonging to the phenolics class. This pigment is responsible for the colours of red, purple and blue in fruits and vegetables. Anthocyanin content was more in *A. cruentus* compared to *A. palmeri* (Figure. 3B). Plant grown in light showed about 12.0 - 16.0 % more anthocyanin content than shaded plants. Phenolics are aromatic benzene ring compounds with one or more hydroxyl groups. These compounds mainly produced for protection against abiotic and biotic stress. Phenolics play important role in plant metabolism, particularly in the biosynthesis of liginin and pigment (Bhattacharya et. al. 2010). In the present study, phenolics content was more about 40.0 -50.0 % among plants grown in light (Figure. 3C).

Lipid peroxidation

Membrane lipid peroxidation is detected by measuring malondialdehyde (MDA). Malondialdehyde is a widely used marker of oxidative lipid injury caused by environmental stress (Kongw et al. 2016). Malondialdehyde content was more in *A. crutenus* which was grown in light (Figure. 3D). The decrease in MDA content was 50.0 - 70.0 % among plant grown in shade.

Changes in cellular macromolecules

Sugar is produced in plants as a result of photosynthesis. Therefore sugar content in plants also represents the rate of photosynthesis. Total soluble sugar was about 60.0% more in plants grown in light (Figure. 4A).Plants store protein in vegetative cells to provide carbon, nitrogen and sulphur resources for subsequent growth and development. The protein content was about 50 - 60.0 % more in plants grown light (Figure.4B).

Discussion

Shade can influence plant growth and development by changing the plant niche. The important visual effect of shade on plant growth is change in morphology such as height and number of leaves. Moderate shade found to increase stem elongation and specific leaf area in plants (Brainard et al. 2005). Also, plants responded to shade with a decrease in number of main leaves (Brainard et al. 2005).

This kind of changes were reported to be the results of action of plant hormones such as gibberllic acid and ABA (Jha et al. 2010). So the decrease in plant height and number of leaves found in plants grow in shade was the outcome of difference in hormonal activity.

Shade could greatly alter the contents of the leaf photosynthetic pigments of which total chlorophyll and chlorophyll a/b ratio were important indicators for assessing shade tolerance in plants (Zhao et al. 2012). Carotenoids are accessory pigments which help to dissipate excess light energy via nonphotochemical quenching (Rosas-Saavedra and Stange, 2016). Shade tolerant plants had high total chlorophyll contents and carotenoids (Zhao et al. 2012). In the present study, plant grown in shade had more chlorophyll, chlorophyll a/b ratio and carotenoids. However, the surface area of leaves drastically decreased among plant grows in shade. It reported that the sun plants will have more chlorophyll especially with regard to chlorophyll a and carotenoids (Atanasova et al. 2003). But the contradictory result observed in the present study was the outcome of decrease in plant growth which in turn leads to more accumulation of metabolites in unit volume.

Plant phenolics had pivotal role in abiotic stress tolerance in plants (Dai and Mumper, 2010). The biosynthesis of phenolic acids in plants occurs through the shikimate-phenylpropanoid pathway using aromatic amino acids such as L-phenylalanine and L-tyrosine, respectively (Cheynier et al. 2013). Light signal is critical for the expression of genes responsible for enzymes involved in the synthesis of phenolics (Cheynier et al. 2013). This compound mainly helps to defense against ultraviolet radiation and biotic stress respectively. Also, phenolics play an important role in determination of market value of plant produce because these compounds contribute to the bitterness and astringency of plant based products. Phenolics are rich in hydroxyl groups, and hence they act as antioxidants which scavenge reactive oxygen species. So it is concluded that plant grow in shade accumulate less phenolics because of retardation of biosynthetic pathway of phenolics which require light.

The plants grown in shade especially *A. crutenus* showed had fading of red colour. Anthocyanins impart red colour in plants (Kayesh et al. 2013). These pigments are produced via the phenyl propanoid pathway and the accumulation of anthocyanin occurs during exposure to light (Kayesh et al. 2013).This pigment is a water soluble flavanoid. The synthesis of anthocyanin is under control of cordial gene expression and environmental factors. Anthocyanin helped to overcome abiotic stress such as highlight. This molecule act as a scavenger of reactive oxygen species. However, light could regulate related anthocyanin biosynthetic enzyme

activities according to a certain light stress tolerance mechanisms (Khandaker et al. 2010). So the decrease in anthocyanin among plants grow in shade was the outcome of delay in switching of genes involved in anthocyanin biosynthetic pathways.

Malonyldialdehyde (MDA) is an indicator of abiotic stress in plants. This compound is formed as a result of lipid peroxidation in plants. The degradation of arachidonic acids and polyunsaturated fatty acid via activity of reactive oxygen species results formation of MDA in plants. An increase in antioxidant activity decrease accumulation of MDA in plant (Pietryczuk and Czerpak, 2012).But the contradictory result observed in the present study was the outcome of difference in metabolic status of the plant. The MDA accumulation in sun plants was not in toxic levels even though more MDA accumulated in these plants compared with plants rear in shade. Exposure to relatively high intensity of light triggered MDA production in Amaranthus grows in sunlight where synthesis of bioactive compounds having antioxidant capacity prevented light stress (Ping et al. 2015). This resulted efficient photosynthesis which in turn help to produce more cellular macromolceule. Due to greater penetration of sunlight in the amaranths planted in the light, they were able to perform better photosynthesis even though photosynthetic pigment content decreased as results of photoprotective mechanisms and mobilization of photosynthates for biosynthesis of cellular metabolites such as sugar and protein involved in plant development (Poorter et al. 2015, Sebastian et al. 2015). The results of present study indicate that the sunlight intensity in the light has a great effect on the growth of *Amaranthus* cultivars. The plant growth parameters such as biomass, plant height, and leaf number decreased when *A. palmeri* and *A. crutenus* were grown shade. Therefore, it is also clear that shade will negatively influence the crop productivity *Amaranthus*.

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Table. 1 Morphological changes of plants grow under different light intensity				
Parameter	Sunlight (50889.65 lux)		Shade (503.48 lux)	
	A. crutentus	*A. palmeri*	*A. crutentus*	*A. palmeri*
No of leaves	25.0 ± 1.0	22.0 ± 2.0	7.0 ± 1.0	7.0 ± 1.0
Plant height (cm)	30.0 ± 0.4	48.0 ± 0.7	8.2 ± 0.5	8.0 ± 0.9
Plant biomass (g)	11.52 ± 0.1	10.52 ± 0.2	0.62 ± 0.3	0.22 ± 0.2

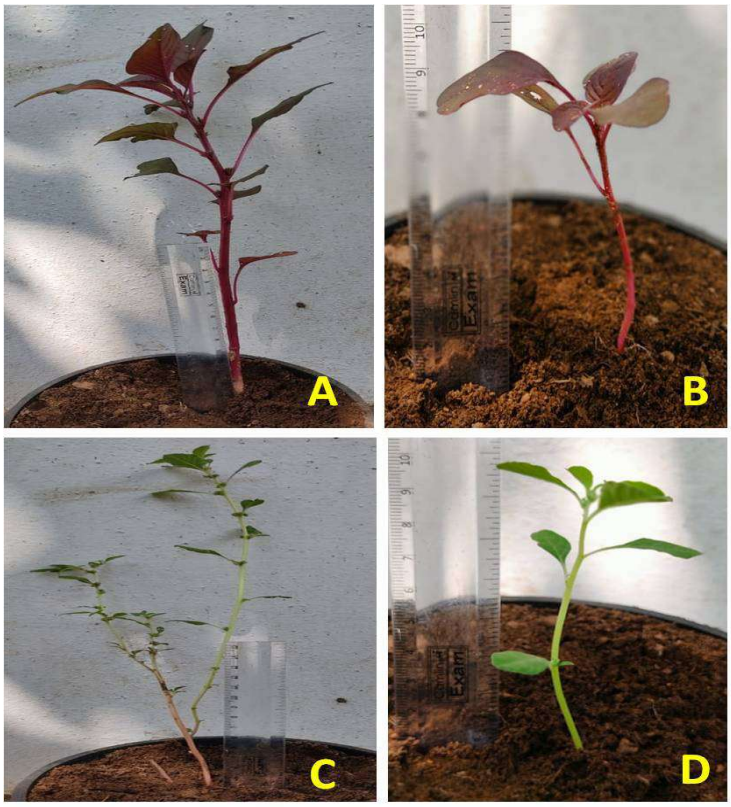

Figure.1

Growth responses of *Amaranthus* (*A. crutentus - purplish*, *A. palmeri-green*) grown in light (A, C) and shade (B, D).

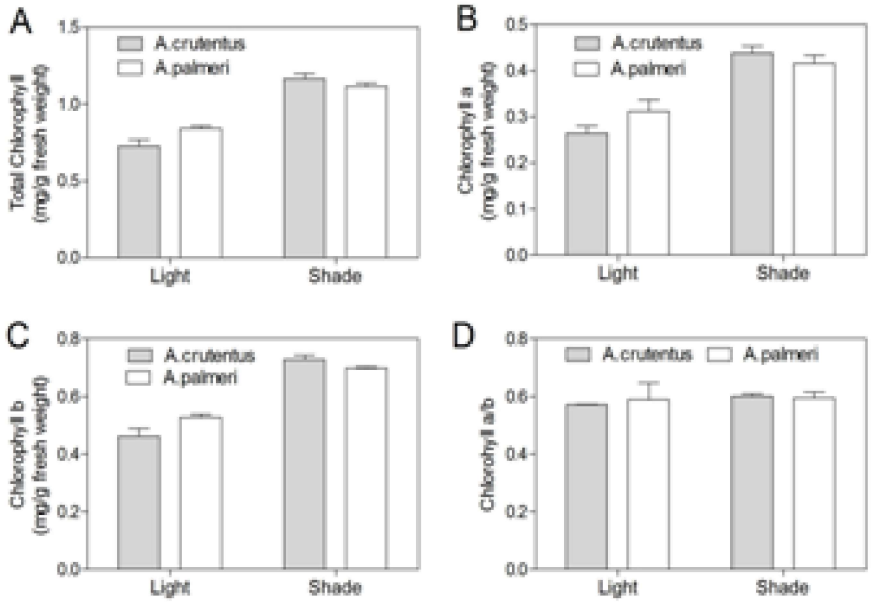

Figure. 2

Photosynthetic pigments content in *Amaranthus* (*A. crutentus, A. palmeri*) grown in light and shade environments A. Total chlorophyll B. Chlorophylla C.Chlorophyll b D. Ratio of chlorophyll a against chlorophyll b

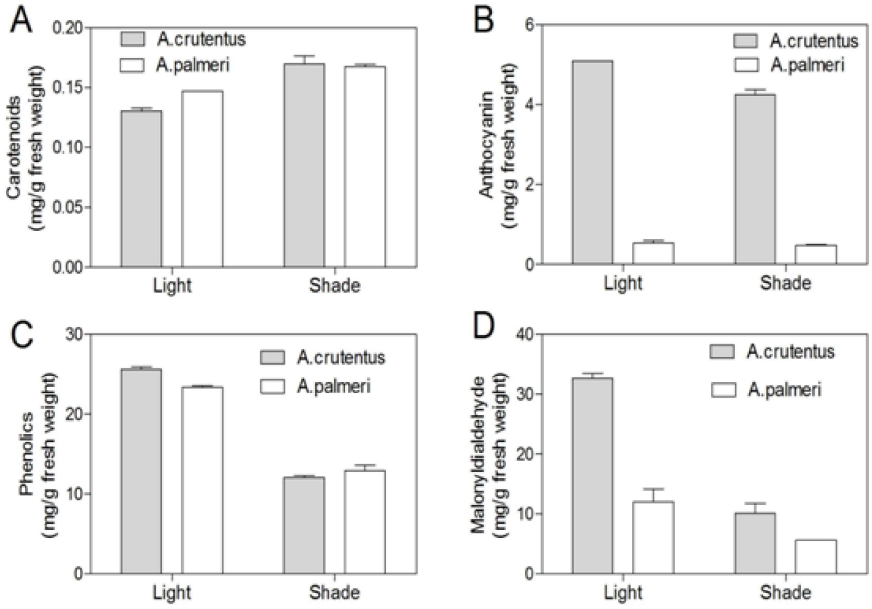

Figure. 3

Phytochemical constituents in *Amaranthus* (*A. crutentus, A. palmeri*) grown in light and shade environments A. Carotenoids B. Anthocyanin C. Phenolics D. Malonyldialdehyde

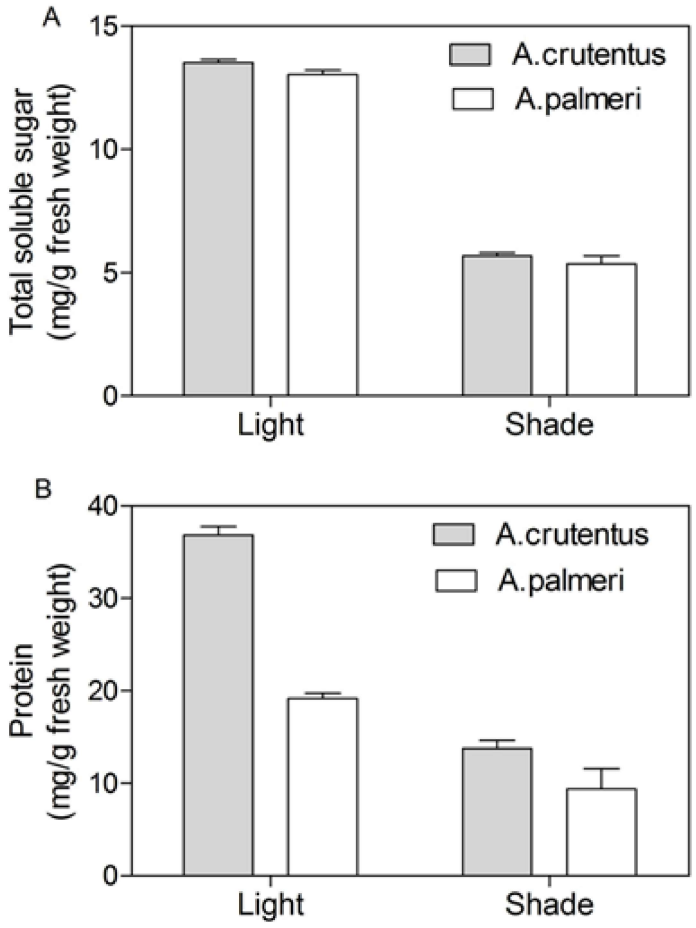

Figure. 4

Accumulation of cellular macromolecules in *Amaranthus* (*A. crutentus, A. palmeri*) grown in light and shade environments. A. Total soluble sugar B. Total soluble protein