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Biochemical adaptation of plants (monocot and dicot) under low light levels

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Abstract: This study was carried out in wire house conditions, in order to assess the effect of low light levels on plant growth, pigment concentration, antioxidant component in black gram (*Vigna mungo* L.) and maize (*Zea mays* L.). Growth of black gram plants was more negatively affected than maize under low irradiance levels. In black gram, fresh weight (FW) decreases by 43% at 185µmol m^{-2} s^{-1}, while it increases by 3% in maize plants. Pigments synthesis was greater in black gram when exposed to low irradiance levels. On other hand, in maize synthesis of these pigments significantly decreases with decrease in irradiance levels. It was also found that increased malondialdehyde content and electrolyte leakage percentage were due to rudimentary development of membrane under low light levels. As the irradiance levels decreased from 343 to 43 µmol m^{-2} s^{-1} synthesis of non-protein thiol was found to be decreased steadily.

Key words: *Vigna mungo*, *Zea mays*, Irradiance, Superoxide dismutase, malondialdehyde content

Introduction

Sun light is the key factor in photosynthesis, a process vital for life on earth. Besides photosynthesis, it helps in the synthesis of melanin and vitamin D in animals. It also helps in the development and differentiation. Beside beneficial role, high intensities of light perform several degenerative roles by the production of reactive oxygen species. Light rays produce excited states in molecules due to the absorption of one or more photons. Excited-states molecules can react with adjacent molecules and excite them. So a number of chain reactions, producing reactive oxygen species are generated. High irradiance of light on plants reduces the capacity of photosynthesis and cause oxidative stress through the formation of reactive oxygen species (ROS). These include superoxide radicals (O^{-2}), singlet oxygen, hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$), which cause tissue injury (Foyer *et al.,* 1994). Plants have evolved various protective mechanisms to eliminate or reduce ROS. These ROS species are highly toxic for biomolecules such as lipid, protein and nucleic acid through lipid peroxidation, protein denaturing and DNA mutation (Scandalios, 1993; Van Breusegen *et al*., 2003; Quiles and Lopez, 2004; Foyer and Noctor, 2005; Guo *et al*., 2006 and Moller *et al*., 2007). When plants exposed to high light intensities, high or low temperature, ozone or air pollution, more reactive oxygen species are produced than the scavenging mechanisms can detoxify (Alscher, 1997). Moreover ROS can also be formed in metabolic pathways. The chloroplast posses an elaborate system for scavenging ROS, comprises both enzymatic and non-enzymatic compound (Foyer *et al*. 1994 and Asada, 1996). Ascorbate peroxidase (APX) scavenges the hydrogen peroxide generated by the action of SOD, thereby preventing the chemical formation of other toxic oxygen species (Asada, 1994). As described by Elstner (1991), there are at least four sites within the chloroplast that can activate oxygen. In addition, one of the most damaging effect of activated oxygen and their product in cells is the peroxidation of membrane lipids which lead to ion leakage (Gratao *et al.,* 2005 and Lee *et al.,* 2007). Evidences suggest that membranes are one of the primary sites of cell and organelle injuries (Candan and Tarhan, 2003). This is because ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids. Besides, antioxidative responses plants display the ability to adjust their performance by alteration in morphology and physiology in response to environmental variations (Sultan, 1995). Although under normal conditions, production and destruction of ROS are well regulated in cell metabolism (Mittler, 2002). When a plant is encountered with harsh conditions, ROS production tends to overcome scavenging system and consequently oxidative stress dominates. Varying intensities of light affect plant growth and development. Reduction in chlorophyll content, due to breakdown of the structural integrity of chloroplast on exposure to excess light was found in some experiments (Rhizopaulou and Mitrakos, 1991; Valladares and Pearcy, 1998). Given the role of increased chlorophyll content in improving light harvesting capacity, the increased chlorophyll content in shade leaves has been interpreted as an adaptation to low light environment. Light affects the net photosynthesis of plants subsequently plant height, fresh weight and dry weight as well. Plants growing in high light intensities in their native habitat have a high capacity for photosynthesis at a saturative light intensity and show lower rates of net-photosynthesis than shaded plants at low light intensities. This paper explores adaptation to different irradiance levels, in context to the focusing on two different groups of plants a dicot (black gram) and a monocot (maize).

Materials and Methods

Uniform-sized black gram (*Vigna mungo* L. cv. PU-35) and maize (*Zea mays* L. cv. 4212) seeds were surface sterilized with 0.01% $HgCl_2$ for 5 min and rinsed extensively in running distilled water (d H_2O). Seeds were grown in clay pots which have

area 310cm^2 filled with soil and compost (3:1) under wire house conditions at 25±2ºC and 60% relative humidity. Light intensity in different pots was controlled by shading them with 0, 1, 2, 3 and 4 layer of muslin cloth to achieve 343, 185, 78, 46 and 40ìmolm^{-2}s^{-1} levels of photosynthetically active radiation, respectively. Plants were harvested after 35 days light treatment and used to various morphological and physiological studies.

Biomass estimation: At the end of experiment (35d) harvesting was done. The harvested plants were washed and rinsed with distilled water then blotted on the tissue and filter paper and root and shoot separated manually. They were kept in an oven at 65±5ºC for 48h and weighed on an electronic balance to determine the effect on dry biomass production.

Estimation of photosynthetic pigments: Chlorophyll was estimated by the method of Arnon (1949). Leaves were plucked and washed with distilled water and blotted. 100 mg leaves were taken and ground in 10 ml chilled acetone (85% v/v). Extract was centrifuged at 2000 rpm for 10 minutes. The absorbance of supernatant was read at 663, 645, 510 and 480 nm for chl a, chl b, chl T and carotenoids respectively using the double beam UV-VIS spectrophotometer UV5704SS. The content was expressed in mg g^{-1} fresh weight tissue.

Enzymes: -

Catalase: Catalase activity was assayed by the modified method of Bisht (1972).The reaction mixture for containing 0.01 mM phosphate buffer (pH 7.0) and 0.5mM H_2O_2 in 10 ml was incubated with suitable aliquot from the extract. The reaction was run for 5 minutes at room temperature (25ºC) and was stopped by the addition of 5ml 2N H_2SO_4. Corresponding zero hour blanks with added H_2SO_4 was also run. The mixture was titrated against 0.1 N $KMnO_4$ and the activity of catalase was expressed as µmol H_2O_2 decomposed/100 mg fresh weight tissue.

Peroxidase: The peroxidase activity was determined by the method of Luck (1963). The assay system for peroxidase contained 0.5 mM phosphate buffer (pH 6.0), 0.01% (v/v) H_2O_2, 5 mg p-phenylenediamine and extract in 8 ml. The reaction was run at 25^{0}C for 5 minute and stopped with 2ml 5N H_2SO_4. Blanks with added H_2SO_4 were also taken. After centrifugation, OD was measured at 485 nm on double beam UV-VIS spectrophotometer UV5704SS. The enzymes activity was measured as change in optical density per 100 mg fw.

Superoxide dismutase: The activity of SOD was assayed by the method of Beauchamp and Fridovich (1971) by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazoliun (NBT). The reaction mixture (3ml) contained 40mM phosphate buffer (PH 7.8), 13mM methionine, 75µM NBT, 2µM riboflavin, 0.1mM EDTA and a suitable aliquot of enzyme extract. Riboflavin was added in the end. The test tubes were shaken and reaction was allowed to run for 20 min by keeping them in sun light. The reduction in NBT was followed by reading the absorbance at 560nm using double beam UV-VIS spectrophotometer UV5704SS. Blanks were run in the same way but without illumination.

Lipid Peroxidation: The level of lipid peroxidation in plant tissue was measured as thiobarbituric acid reaction by the method of Heath and Packer (1971) in terms of malondialdehyde content. Fresh leaf tissue were homogenized in 20% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 3,500 rpm for 20 min. To a suitable aliquot, 1ml of 20% TCA containing 0.5 %(w/v) TBA was added. The mixture was heated at 95^{0}C for 30 min and then quickly cooled in ice bath. The contents were centrifuged at 10,000 X g for 15 min and the absorbance was measured at 532 nm using double beam UV-VIS spectrophotometer UV5704SS. Value for non – specific absorbance at 600 nm was subtracted. The concentration of MDA was calculated using an extinction coefficient of 155 Mm^{-1} cm^{-1} and expressed as nmol MDA content g^{-1} FW tissue.

Non-protein thiol: Non protein thiol group was estimated by the method of Ellman (1959). Leaves were plucked and washed with distilled water. 700mg plant tissue grinds in 6.67% sulphosalicylic acid. Extract was centrifuged at 13,000 rpm for 10 minute at 4ºC. Reaction mixture contains 5mM of EDTA, 0.6 mM of DTNB and 120mM of phosphate buffer. The absorbance of supernatant was read at 412nm using double beam UV-VIS spectrophotometer UV5704SS and NP-SH contents expressed as mmol g^{-1} FW tissue.

Statistical analysis: The experimental data were tested for significance by using least significant difference (LSD) to compare means of different treatments that have an equal number of replications. All statistical test were performed with analysis tools from Microsoft office excel 2007.

Table-1: Effect of light intensity on plant height, fresh weight, dry weight, and moisture % in mature leaves of two different plants viz. black gram and maize observed at 35 days.

Light intensity (µmol m^{-2} s^{-1})	343	185	78	43	40	*	**
Black gram							
Plant height(cm)	22.50±0.28	29.00±0.57*	32.00±0.57**	30.00±0.28**	28.33±0.60*	4.43	7.32
Fresh weight (g)	4.60±0.12	2.60±0.15	1.24±0.08*	0.72±0.07**	0.61±0.05**	2.15	3.46
Dry weight(g)	0.71±0.04	0.30±0.02*	0.16±0.01**	0.14±0.005**	0.10±0.005**	0.312	0.517
Moisture %	83.27±0.17	86.13±0.33**	86.25±0.52**	84.97±0.55**	85.10±1.60*	1.48	2.46
Maize							
Plant height(cm)	39.66±0.88	66.00±0.28**	51.50±0.76*	38.33±0.60	30.66±0.44*	6.22	12.85
Fresh weight (g)	44.00±0.77	45.28±0.83	22.68±0.45*	11.00±0.37*	8.87±0.18*	19.59	39.59
Dry weight(g)	5.80±0.30	4.00±0.28	1.37±0.25*	0.82±0.04**	0.90±0.01**	2.76	4.58
Moisture %	84.33±0.88	90.00±1.15*	91.66±0.33**	93.00±0.57**	89.00±0.5*	4.13	6.85

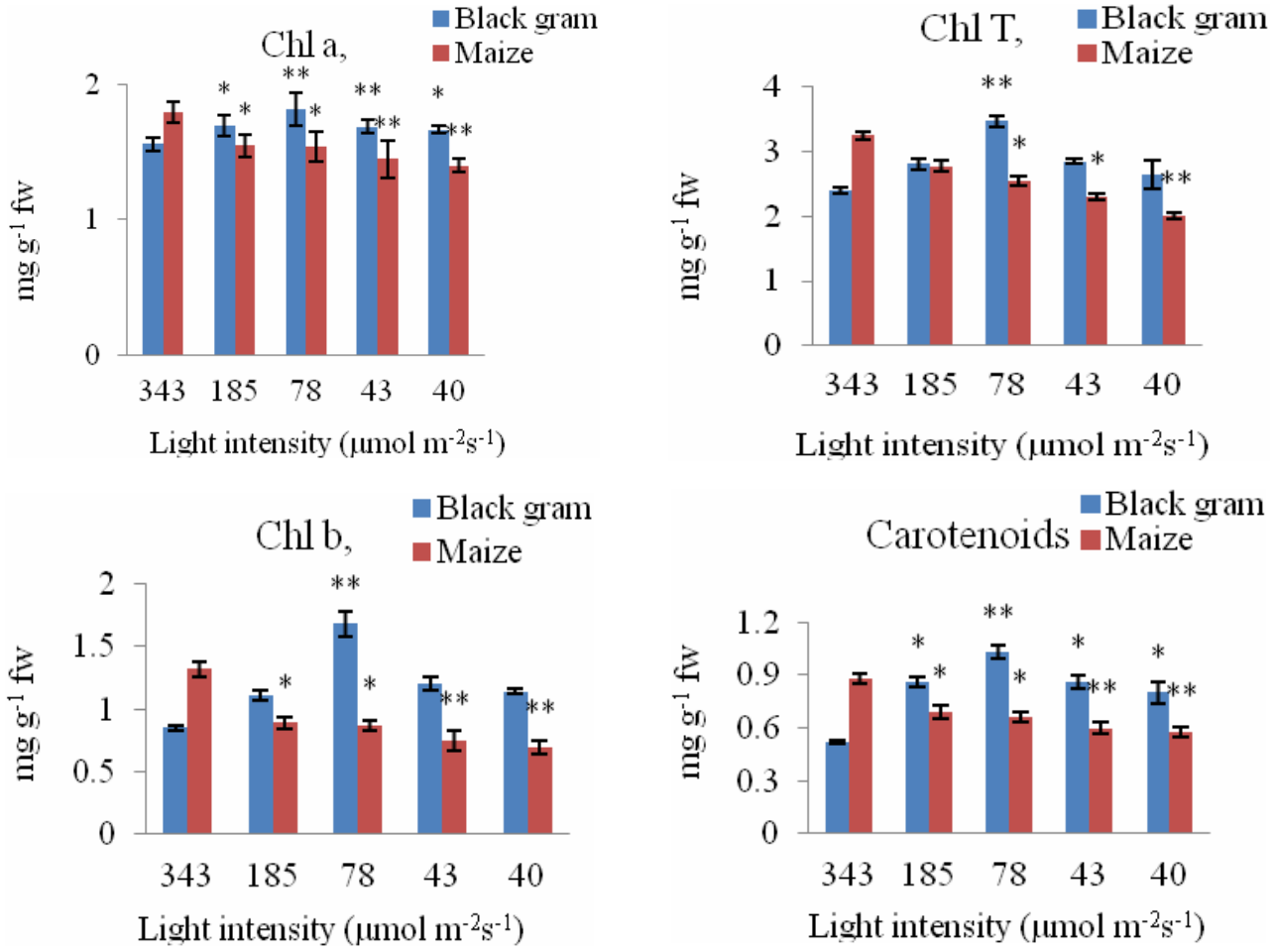

Fig. 1: Effect of light intensity on Chl T, chl a, chl b and carotenoids contents in mature leaves of two different plants viz. black gram and maize observed at 35 days (Bars represent SE. *- value significant at P<0.05 and **- value significant at P<0.01 levels)

Results and Discussion

Most common etiolating growth was observed in black gram than maize exposed to low shades of sun irradiance. In black gram plant height was significantly (P<0.01) increased by 42 and 33% exposed to 78 and 46 µmolm^{-2}s^{-1}, while in maize plants height significantly (P<0.01) increased by 66.4% exposed to 185µmolm^{-2}s^{-1} and decreased by 23% at 40µmol m^{-2}s^{-1} compared to plant exposed to normal sun irradiance 343 µmol m^{-2} s^{-1} (table 1). Fresh weight of black gram plant decreased by 43.5% exposed to 185µmol m^{-2} s^{-1}, while at the same exposure FW of maize plants increased by 3.0% compared to plants exposed to 343µmol m^{-2} s^{-1} (table-1). Dry biomass was reduced in both plants exposed to different shades of irradiance. But reduction in dry biomass in black gram was so high as compared to maize. These findings suggested that leaves acclimated to each irradiance level are those best adapted to that level, in so far as they appear to have the highest rate of leaf photosynthesis under those conditions Bjorkman (1972). These results suggested that monocot (maize) was more adopted than the dicot (black gram) in response to shady irradiance.

The contents of total chl, chl a, chl b and carotenoids were significantly modified under low light irradiance. The effect of low irradiance on pigment synthesis in black gram and maize plants has been given in figure 1. In black gram, pigment synthesis was higher under low irradiance levels. While in maize it was decreased. Total chl, chl a, chl b and carotenoids synthesis were significantly increased in leaves of black gram exposed to 78µmol m^{-2} s^{-1} compared to fully exposed (343µmol m^{-2} s^{-1}) plants. While in maize plant synthesis of these pigments significantly decreased by 38, 22, 48 and 34% at 40µmol m^{-2} s^{-1} sun irradiance respectively. Result of pigment synthesis obtained in black gram was in accordance with that of coffee plants (Marcelo, 2010). In black gram increased synthesis of pigments under low irradiance levels could be due to enhanced nitrogen fixation by root rhizobium and low photo-oxidation of chl molecules. Carotenoids synthesis was also found to be higher in shaded conditions. Thus either chlorophyll or carotenoids synthesis might increase acclimation to low irradiation. According to Marcelo (2010) nitrogen application enhanced pigments synthesis in coffee plants. While in this experiment required nitrogen, necessary for proper synthesis of pigment is not available under low irradiance of light which led to low synthesis of pigments in maize plants. Results showed that there was a progressive increase in the chlorophyll *a/b* ratio with decrease in light intensity in maize plants while similar

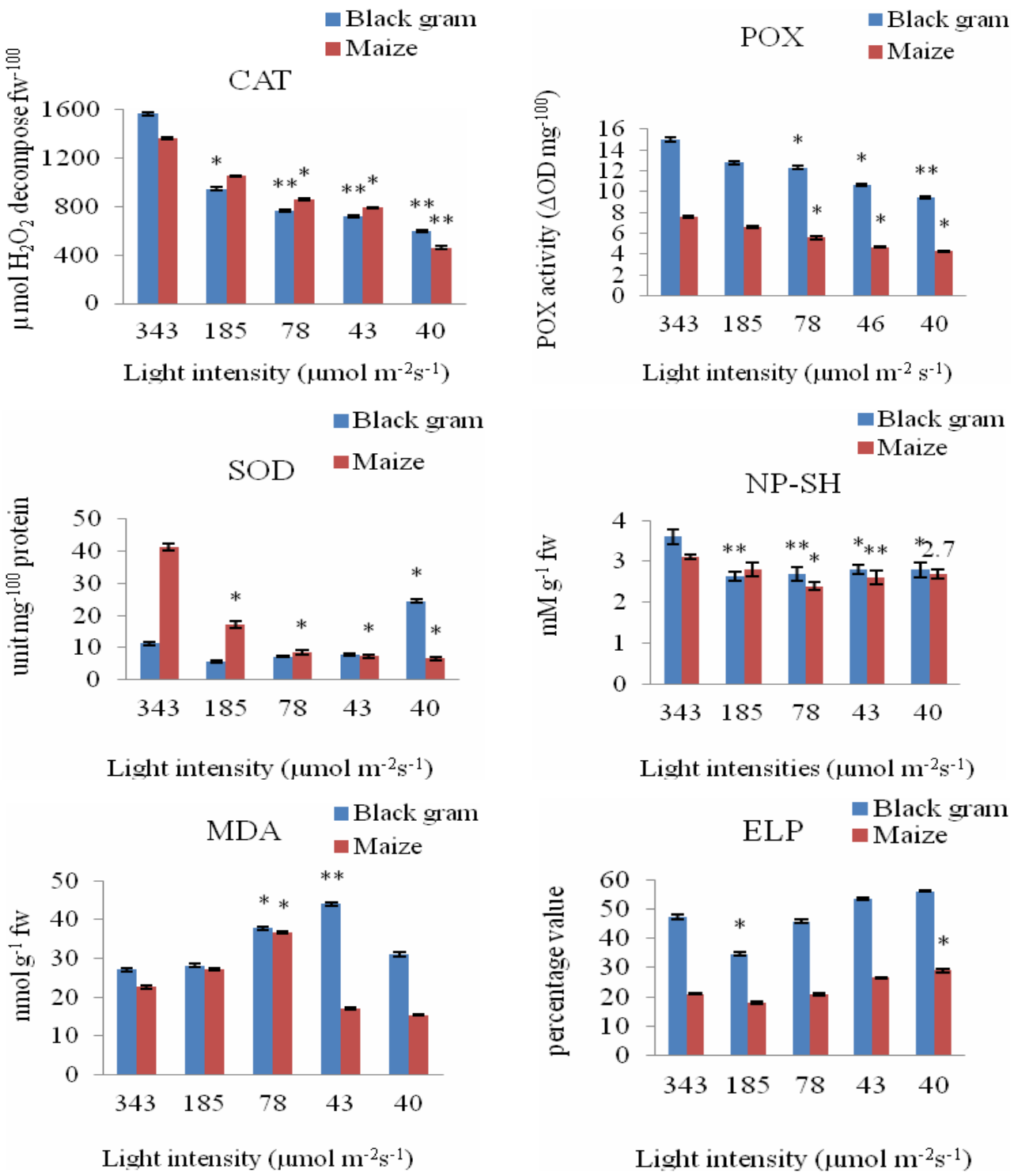

Fig. 2: Effect of light intensity on activity of CAT, POX, SOD, NP-SH, MDA and ELP in mature leaves of two different plants viz. black gram and maize observed at 35 days (Bars represent SE. *- value significant at P<0.05 and **- value significant at P<0.01 levels)

results were obtained in black gram exposed to lower doses of light. Previous investigations of light acclimation in higher plants showed that higher chlorophyll *a/ b* ratios were correlated with higher rates of electron transport and photophosphorylation per unit of total chlorophyll (Anderson and Osmond, 1987), which lead to the over production of reactive oxygen species and one of the main reason of oxidative damage under low light condition.

The activity of SOD responded to light treatments, but response was more significant in leaf of maize than black gram (Fig- 2). In maize plants SOD activity gradually was found to be decreased as the

intensity of light decreased. But in black gram lowest value of SOD was noticed at 185 µmol $m^{-2}s^{-1}$, which was increased to significant ($p<0.05$) limit at 40 µmol $m^{-2}s^{-1}$. The increased activity of SOD in black gram correlated with low light oxidative tolerance (Sen Gupta *et al*. 1993; Scandalios 1993). Dionisio-Sese and Tobita (1998) reported that increased SOD activity is effective in improving plant's tolerance to environmental stresses, while Low SOD activity in maize leaves may lead to more super oxide radical ($O_2^{"}$) accumulation and the occurrence of Haber-Weiss reaction, which in turn results in highly toxic levels of hydroxyl radical. On the other hand, $O_2^{"}$ radical can decrease the activity of POX (Kono and Fridovich, 1983). Our results suggested that maize leaf was more acclimated to shade than black gram.

The activity of POX in both plants decreased as the light intensity was decreased (Fig 2). In maize, activity of POX decreased with decrease in sun irradiance and reached to 4.3 unit mg^{-1} which was 38% less than activity of POX in plant exposed 343µmol$m^{-2}s^{-1}$. Similar trend of POX activity was observed in black gram. This could be due to reduced synthesis of lignin under low irradiance which leads to low level of POX activity. This result was in accordance with that on soybean (Andersson-Gunneras *et al*. 2006). The relationship between lignin deposition and the POX activity was shown in light grown chick-pea stem (Angelini *et al*., 1990). Participation of POX in lignification process is quite obvious from many studies on the influence of biotic or abiotic stress factors on plants, e.g. wounding, attack of pathogen (Angelini *et al.* 1993 and Rea *et al.* 1998). More intensive production of hydrogen peroxide could be expected on the basis higher POX and CAT activity in light grown seedlings. According to Angelini *et al.* (1993), plant grown under low irradiance showed lower rate of lignin synthesis as well as low level of POX activity. It was concluded that under low levels of irradiance reduced activity of POX associated with weakening of plants defense system.

Activity of CAT decreased in both plants with decrease in irradiance levels (fig. 2). Plants grown at low irradiance had lower respiration rates than those acclimated to other irradiance levels (Bjorkman *et al.* 1972). Similarly at low irradiance levels plants had lower rate of photo-respiration thereby decreased in the H_2O_2 production which led to decreased activity of CAT.

The peroxidation reactions differ among the fatty acids depending on the number and position of the double bonds on the acyl chain. Oxidation of unsaturated fatty acids by singlet oxygen produces distinctly different products such as MDA (Bradley and Min, 1992). MDA is a common product of lipid peroxidation and a sensitive diagnostic index of oxidative injury (Janero, 1990). In this respect increase in lipid peroxidation was reported in many plants under various environmental stresses (Moran *et al*., 1994 and Prassad 1996). In the present study, increase in membrane damage was observed on decreasing irradiance levels of sun light (fig. 2). MDA content, significantly ($p<0.01$) increased in leaves of black gram plants by 62% at 46µmol m^{-2} s^{-1}, while in maize maximum value of MDA content 36.6 nmol g^{-1} fw was observed at 78µmol m^{-2} s^{-1} sun irradiance. Increase in MDA content under low irradiance suggested the accumulation of reactive oxygen species. This was supported by the results obtained in pigment concentration.

ELP also suggested membrane damage which was increased on decreasing irradiance levels in both plants (fig. 2). In black gram, ELP increased by 18.4% at 48µmol $m^{-2}s^{-1}$ while in maize leaves its value gradually increases on decreasing irradiance and significantly ($p<0.05$) increased by 38% at 48 µmol m^{-2} s^{-1}. In present study, NP-SH synthesis showed decreasing trend under decreasing levels of sun irradiance. In black gram, NP-SH synthesis significantly ($p<0.01$) was decreased by 26.6 and 25.0% at 185 and 78µmol m^{-2} s^{-1} compared to plants exposed to normal sun irradiance. In conclusion, our data indicated a correlation between pigment syntheses, ROS production and cellular damage provoked by low level of sun irradiance. The considerable increase in pigment concentration in black gram and better growth performance in maize under 185 µmol m^{-2} s^{-1} light level provide a knowledge that somewhat shady environment is better for these crops than full sun light. While very low light conditions create oxidative damage through intense production and accumulation of reactive oxygen species.

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