OSMOTIC STRESS INDUCED CHANGES IN CHLOROPHYLL BIOSYNTHESIS AND ANTIOXIDATIVE SYSTEM IN LIGHT GROWN MAIZE LEAVES

Swati Tiwary, Rekha Gadre and Meeta Jain*

School of Biochemistry, Devi Ahilya University, Takshashila Campus, Khandwa Road, Indore (M.P.) – 452 017, India.

(Received on Date: 2nd November 2015 Date of Acceptance: 11th December 2015)

ABSTRACT

Supply of 0.2 to 1.0M sorbitol decreased the total chlorophylls and carotenoids significantly in excised light grown maize leaf segments. δ- aminolevulinic acid (ALA) content was reduced at 1.0M sorbitol only. ALA synthesizing activity and δ- aminolevulinic acid de hydratase activity was inhibited significantly at higher concentrations of sorbitol. Significant R squared values on correlation was obtained between sorbitol concentration and these parameters. Proline and MDA content was increased with increasing concentration of sorbitol while H_2O_2 content decreased. The activity of ROS scavenging enzymes, catalase, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase (Gu-POX) and glutathione reductase (GR) enhanced with the supply of sorbitol. Anti-oxidants like total ascorbate (ASC) was enhanced whereas total glutathione (GSH) was decreased on sorbitol supplementation. It is suggested that sorbitol induced osmotic stress has an inhibitory effect on chlorophyll biosynthesis. Lesser degree of decrease in ALA content due to less inhibition of ALA synthesizing activity and also ALAD activity than total chlorophylls, by the supply of sorbitol indicates that some other enzymes of chlorophyll biosynthetic pathway may be contributing in addition to them in inhibiting the formation of chlorophylls. It seems that H_2O_2 level was maintained low by increased activities of CAT and Gu-POX and furthermore, enhanced APX and GR activities indicate that ASC- GSH cycle is operated at a rapid rate under oxidative stress.

***Keywords*:** Chlorophyll biosynthesis, Anti-oxidative system, Osmotic stress, Sorbitol, Maize leaves

No: of Tables: 1 **No: of Figures**: 3 **No: of References**: 33

INTRODUCTION

Plants natural environment is composed of a complex set of abiotic stresses and biotic stresses. Plant responses to these stresses are equally complex. Amongst abiotic stresses, osmotic stress, drought and salinity are the most severe problems in worldwide agricultural production (Saha et. al., 2010). Osmotic stress is a major abiotic factor limiting plant growth and development and hence productivity. Photosynthesis is a key phenomenon or process which contributes substantially to the plant growth and development. However, osmotic stress hampers the process of photosynthesis in most plants by altering the ultra structure of various pigments and metabolites including enzymes. Chlorophyll biosynthesis is a vital phenomenon in plants starts from amino acid, glutamic acid and involves many steps (Pattanayak and Triparthy, 2011; Biswal et.al., 2012). It is known that water stress caused reduction in chlorophyll biosynthesis during seedling stage which may be due to down regulation of gene expression, protein abundance or post translational modification of several enzymes involved in tetrapyrrole metabolism (Dalal and Tripathy 2012; Turan and Tripathy 2014). Adverse effects of osmotic stress on chlorophyll biosynthesis ultimately result in reduced photosynthetic activity. Earlier steps of the pathway are common with biosynthesis of other tetra pyrrole derivatives like heme, phyto chromes, phyco bilins etc. The synthesis of 5-amino levulinic acid (ALA) is the first committed step of chlorophyll bio synthesis, and is therefore a key control point in the regulation of chlorophyll formation. It is synthesized from glutamate by the activity of glutamyl – tRNA reductase (GluTR) and glutamate 1-semialdehyde aminotransferase (GSAT). The enzyme, 5-amino levulinic acid dehydratase ((EC 4.2.1.24, ALAD), one of the regulatory enzymes of the pathway, catalyzes the asymmetric condensation of two molecules of ALA leading to the formation of the basic unit of tetrapyrroles, the porphobilinogen (Sarangthem et. al., 2011). Oxidative stress is a serious consequence of water deficit/drought in plants with enhanced generation of ROS due to disruption of cellular homeostasis. In plants, ROS are formed by the inevitable leakage of electrons onto O_2 from the electron transport activities of chloroplasts, mitochondria, and plasma membranes or as a byproduct of various metabolic pathways localized in different cellular compartments. The enhanced production of ROS during environmental stresses can pose a threat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death pathway and ultimately leading to death of the cells. Scavenging or detoxification of excess ROS is achieved by an efficient anti oxidative system comprising of the non enzymatic as well as enzymatic antioxidants. The enzymatic antioxidants include superoxide dismutase (EC 1.15.1.1, SOD), catalase (EC 1.11.1.6, CAT), guaiacol peroxidase (EC 1.11.1.7, Gu-POX), ascorbate peroxidase (EC 1.11.1.11, APX) and glutathione

reductase (EC 1.6.4.2, GR). Ascorbate (ASC), glutathione (GSH), proline, malondialdehyde (MDA) serve as potent non-enzymatic antioxidants within the cell. Sorbitol, a six carbon sugar alcohol, has been used in water stress studies in plants (Jain et al. 2010).The main objective of this study was to understand the impact of osmotic stress on chlorophyll biosynthesis and antioxidative system in maize leaf segments.

MATERIALS

Plant material. Sterilized seeds of Zea mays L.cv. Ganga safed-2 were raised in continuous light for 7-8 d at 25 ± 3 °C. They were watered with half strength Hoagland solution containing 5 mM ammonium nitrate. For analysis of biochemical parameters, excised segments of primary leaves were treated with different concentrations of sorbitol (0.0 - 1.0M) in continuous light supplied with fluorescent tubes for 24 h at 25 ± 3°C. Treated leaf segments were thoroughly washed with distilled water prior to analysis.

METHODOLOGY

Estimation of pigment content. Leaf tissue was extracted with 80% acetone in cold. The extract was centrifuged and the absorbance of clear supernatant was measured at 646, 663 and 470 nm. The chl a, chl b and carotenoid contents were calculated using equation of Lichtenthaler and Welburn.

Measurement of ALA content

ALA was extracted from the leaf tissue by the method of Tewari and Triparthy (1998) and the content was estimated by ethyl aceto acetate condensation method, using modified Ehrlich reagent (Mauzerall and Granick, 1956).

ALA synthesizing activity was determined according to the method of Tewari and Triparthy (1998). ALAD activity was assayed colorimetrically by estimating the amount of porphobilinogen formed by using Ehrlich reagent. Extraction and assay of ALAD were carried out according to the procedure described in Jain and Gadre (2007). One unit of enzyme activity is defined as 1nmol of PBG formed per hour.

Proline content was estimated spectrophotometrically by Bates et al. (1973).

Lipid peroxidation was measured by estimating thiobarbutaric acid reactive substances (TBARS) using the method described by Vaidyanathan et al. (2003). The absorbance was read at 532 nm. The value for the non specific absorbance was read at 600 nm and the lipid peroxides were calculated as nmole of malondialdehyde formed using extinction coefficient (ε 532 = 156 × $10^3 M^{-1}$ cm^{-1}).

Hydrogen peroxide content was determined by the method of Jana and Choudhary (1981). The intensity of yellow colour of the supernatant was measured at 410 nm. The H_2O_2 content was calculated with the help of standard curve prepared using H_2O_2.

Assay of Enzymatic antioxidants. Superoxide dismutase activity was assayed by using photochemical

nitroblue tetrazolium (NBT) reduction method as described by Beyer and Fridovich (1987). The increase in absorbance due to formazone formation was read at 560 nm. Under the described conditions, the increase in absorbance without the enzyme extract was taken as 100% and the enzyme activity was calculated by determining the percentage inhibition per min; 50% inhibition was taken as equivalent to one unit of SOD activity. Catalase activity was assayed in terms of decrease in absorbance at 240 nm spectrophotometrically according to the method of Aebi (1984). The reaction was started by addition of 0.2 ml of enzyme extract and decrease in absorbance was followed at 240 nm for 3 min after starting the reaction. The catalase activity was calculated by using an extinction coefficient (ε = 39.4$mM^{-1}cm^{-1}$). One unit of enzyme activity was defined as 1nmol H_2O_2 decomposed min^{-1}.Guaiacol peroxidase activity was measured spectrophotometrically by the method described by Chance and Maehly (1955). The initial and final absorbance was recorded at 475 nm for 2 min Enzyme activity was calculated using extinction coefficient 26.6 $mM^{-1}cm^{-1}$. The Gu-POX activity was expressed as nmole guaiacol oxidized min^{-1}.Ascorbate peroxidase activity was determined spectrophotometrically by the method of Nakona and Asada (1981). The decrease in absorbance of solution was recorded at 290 nm for 3 min after starting the reaction. The enzyme activity was calculated by using an extinction coefficient of 2.8 $mM^{-1}cm^{-1}$. One unit of enzyme activity was defined as 1nmol ascorbate oxidized min^{-1}. Glutathione reductase activity was assayed by the method of Rao et al. (1996). Decrease in absorbance was recorded at 340 nm for 10 min Enzyme activity was calculated using extinction coefficient of 6.2 $mM^{-1}cm^{-1}$. The GR activity was expressed as 1 n mole NADPH oxidized min^{-1}.

Estimation of Non – enzymatic Antioxidants: Total ascorbate was determined by the method of Gossett et al., by measuring absorbance 525 nm in the spectrophotometer Shimadzu UV-1800A. The total ascorbate content was calculated with the help of standard curve prepared in the range 60-600 nmole of ASC. Reduced glutathione content was determined using Ellman's reagent as described by Tukendrof and Rauser (1990). The absorbance was measured at 412 nm. The reduced glutathione content was calculated with the help of standard curve prepared in the range 20-200 nmole GSH.

Statistical Analysis: Data presented in the study are average of at least four independent experiments with ± S.E. Significance of difference obtained for various treatments was tested by Student's t test. Correlation was analyzed using Microsoft Excel X-Y scatter.

RESULTS AND DISCUSSION

Osmotic stress effect on chlorophyll Bio Synthesis: Treatment of leaf segments obtained from light grown maize seedlings with different concentrations of sorbitol decreased the total chlorophylls and carotenoids in a concentration dependent manner (Table 1). Further, the decrease at each concentration of

sorbitol was to almost the same extent for both the pigments. The R squared values obtained from correlation analysis were found to be significant, being 0.863 with total chlorophylls and 0.956 with carotenoids (Figure 1). Supply of 1.0M sorbitol to leaf segments decreased the ALA content slightly, while, it remained almost same at other concentrations of sorbitol. Unlike to the ALA content, concentration dependent and substantial increase in proline content was noticed with increasing concentration of sorbitol (Table 1). There was almost 1.7 fold increase in proline level at 1.0M sorbitol treatment. Correlation analysis yielded highly significant R squared value of 0.902 with proline and insignificant being 0.401 with ALA content (Figure 1). However, decline in ALA content was to a lesser degree than the decrease in total chlorophylls (Table 1). Reduced chlorophyll synthesis due to decreased accumulation of chlorophyll biosynthetic intermediates, that is, GSA, 5-ALA, Mg-protoporphyrin IX monomethylester and proto chlorophyllide has been reported in rice seedlings under water stress conditions (Dalal and Tripathy 2012; Turan and Tripathy 2014). Further, impairment of ALA synthesis in chill and heat stress conditions was suggested to be partially responsible for inhibition of chlorophyll synthesis in *Cucumis sativa* seedlings (Tewari and Tripathy 1998). ALA synthesizing activity is inhibited from 0.6 -1.0M sorbitol only (Table 1). Supplementation of 0.4 - 1.0M sorbitol to light grown leaf segments inhibited the ALAD activity significantly in a concentration dependent manner showing increased inhibition of activity with increasing concentration of sorbitol (Table 1). Amongst these, inhibition of ALAD activity is most prominent. Thus, it reveals that inhibition of ALAD activity mainly contributes in inhibiting chlorophyll biosynthesis rather than ALA synthesizing activity. Correlation analysis yielded highly significant R square value of 0.878 with ALA synthesizing activity and 0.965 with ALAD (Figure 1).

Osmotic stress effect on MDA and H_2O_2 content: Water stress conditions favor the formation of active oxygen species which can cause irreversible damage to the cell. In order to improve drought stress tolerance in crops, it is essential to identify salient components of antioxidative defense which are induced under drought stress and may have role in conferring drought tolerance. Therefore, to examine the relationship between imposition of drought stress by sorbitol and induction of oxidative stress in maize leaf tissue, the effects of sorbitol induced osmotic stress was studied on antioxidative system. The results demonstrate a concentration dependent increase in the MDA content of the stressed leaf tissue treated with 0.6 – 1.0M sorbitol (Figure 2). The occurrence of MDA, a secondary end product of the oxidation of polyunsaturated fatty acids of the membrane is considered a useful index of general lipid peroxidation (Smirnoff, 1993). Thus, possibility of membrane injury is likely due to oxidative damage induced by sorbitol. Plants have an internal protective enzymatic and non- enzymatic cleanup system, which is fine and elaborative enough to avoid injuries of active oxygen, thus guaranteeing normal cellular function (Wang et al. 2002). Concentration

dependent decline in the H_2O_2 content was noted with increasing osmotic stress induced by supplying different concentration of sorbitol (Figure2). Supply of 0.6, 0.8 and 1.0M sorbitol to light grown maize leaf segments increased the MDA content in a concentration dependent manner; however, there was no change at lower concentration of sorbitol, i.e. at 0.2 M and 0.4 M (Figure 4a). Correlation analysis between sorbitol concentration and H_2O_2 content and MDA content yielded highly significant R squared values of 0.946 and 0.924 respectively (Figure 3). However, increased production of H_2O_2 with increasing sorbitol treatment has been reported in pea leaves (Jain and Raghavendra, 2007).

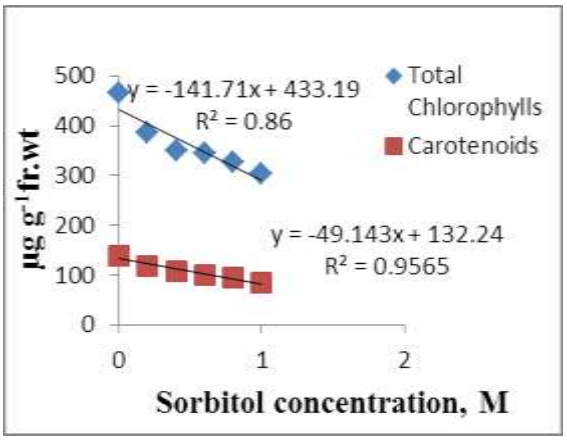
Table 1. Osmotic stress effect on total chlorophylls, carotenoids, ALA content, proline content, ALA synthesizing activity and ALAD activity in maize leaf segments.

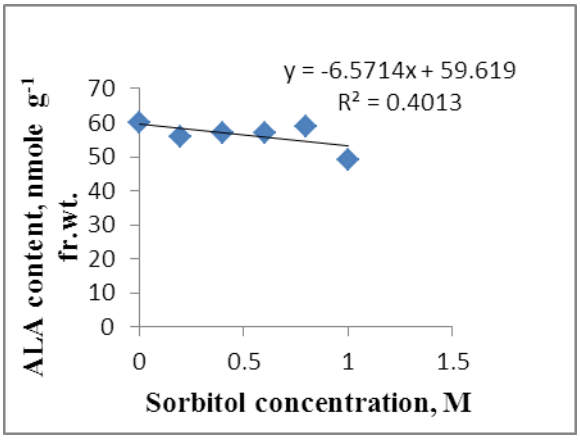
Leaf segments from light grown maize seedlings were treated with varying concentration of sorbitol in continuous light for 24 h at 25 ± 2 °C.

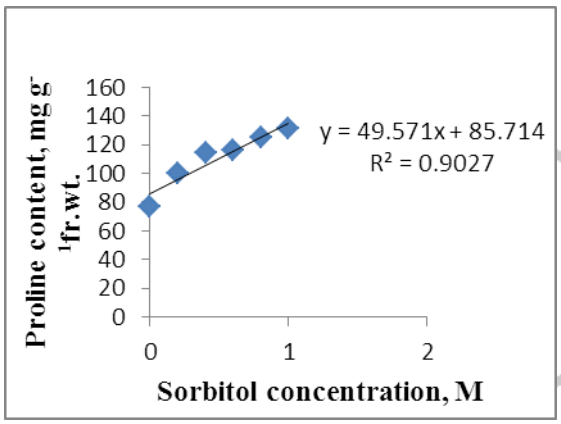
Sorbitol Conc. M	Total Chlorophylls µg g^{-1} fr.wt.	Carotenoids µg g^{-1} fr.wt.	ALA content nmole g^{-1}fr.wt.	Proline content mg g^{-1} fr.wt.	ALA synthesizing activity nmole ALA formed $h^{-1}g^{-1}$ fr.wt.	ALAD activity units g^{-1} fr.wt.
0.0	465 ± 20 (100)	138 ± 14 (100)	60 ± 3 (100)	77 ± 0.4 (100)	52 ± 5 (100)	177 ± 10 (100)
0.2	385 ± 30* (83)	119 ± 11 (86)	56 ± 4 (93)	100 ± 0.2*** (130)	48 ± 1* (92)	172 ± 2 (97)
0.4	350 ± 23** (75)	108 ± 7 (78)	57 ± 9 (95)	114 ± 0.4*** (148)	47 ± 0.4*** (90)	145 ± 3*** (82)
0.6	345 ± 21** (74)	101 ± 7 (73)	57 ± 1 (95)	116 ± 0.8 *** (151)	44 ± 0.6*** (85)	114 ± 4 *** (64)
0.8	326 ± 19***(70)	95 ± 5* (69)	59 ± 13 (98)	125 ± 0.7*** (162)	41 ± 0.3*** (79)	88 ± 3 *** (50)
1.0	303 ± 6*** (65)	85 ± 5** (62)	49 ± 10 (82)	131 ± 0.5*** (170)	38 ± 0.5*** (73)	81 ± 4 *** (46)

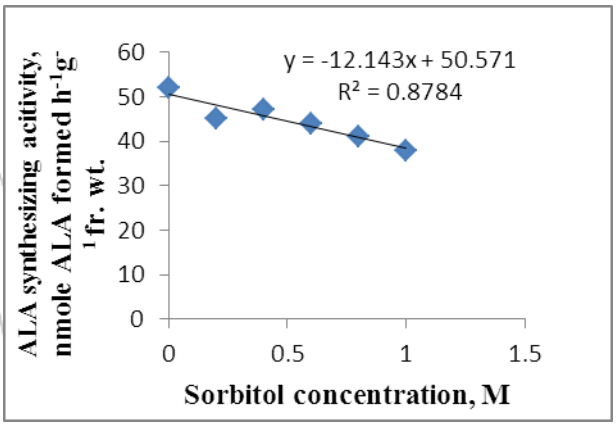
Values relative to control are given in parentheses. Level of significance: 'p' values < 0.05 *, < 0.01 **, < 0.001 *** compared with control.

Figure 1. Correlation analysis of sorbitol concentration and total chlorophylls, carotenoids, ALA content, proline content, ALA synthesizing activity and ALAD activity









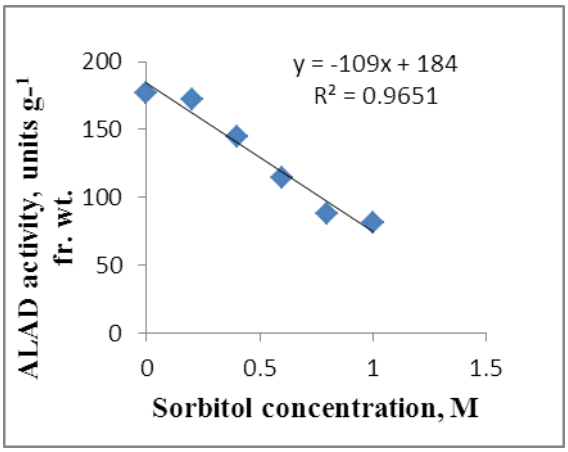
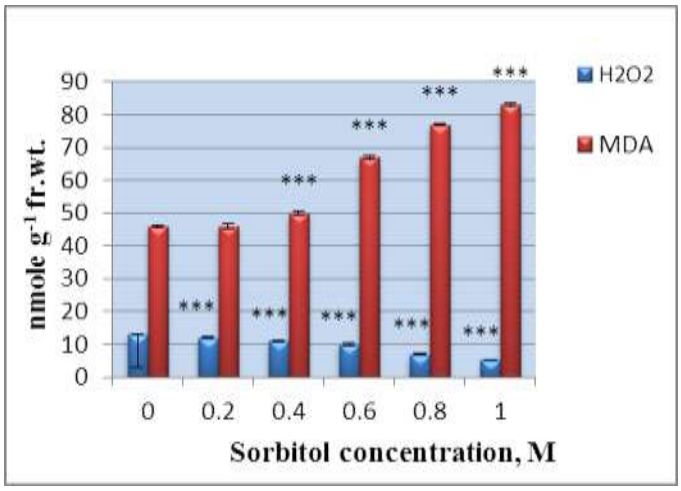
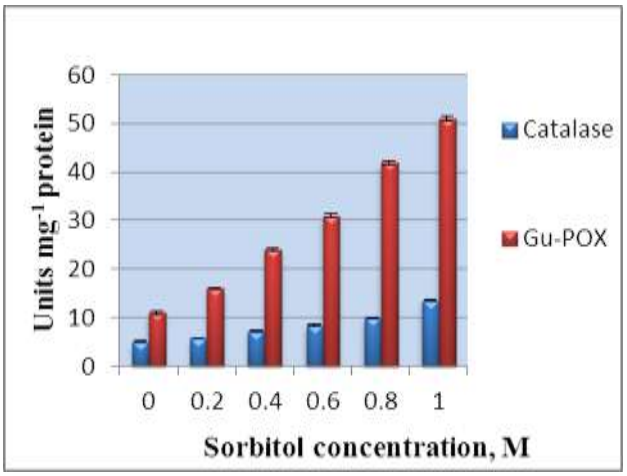
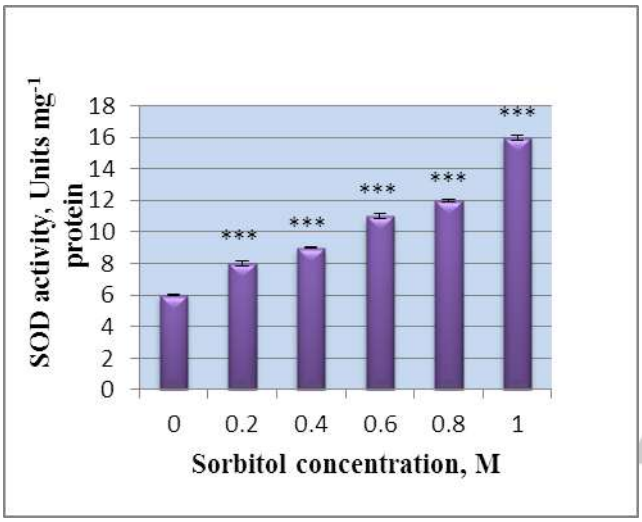
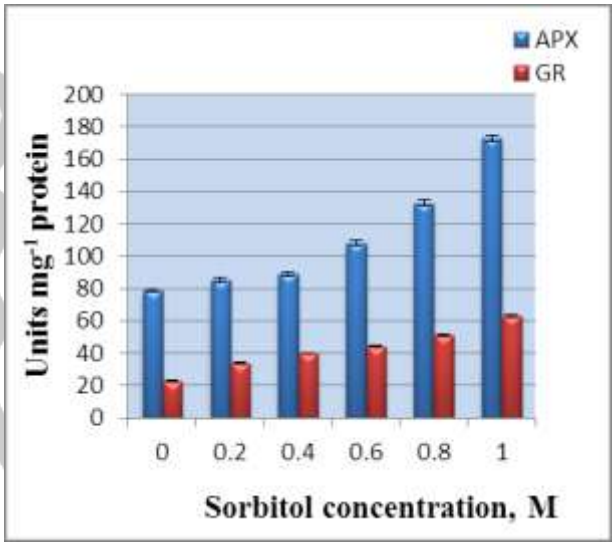


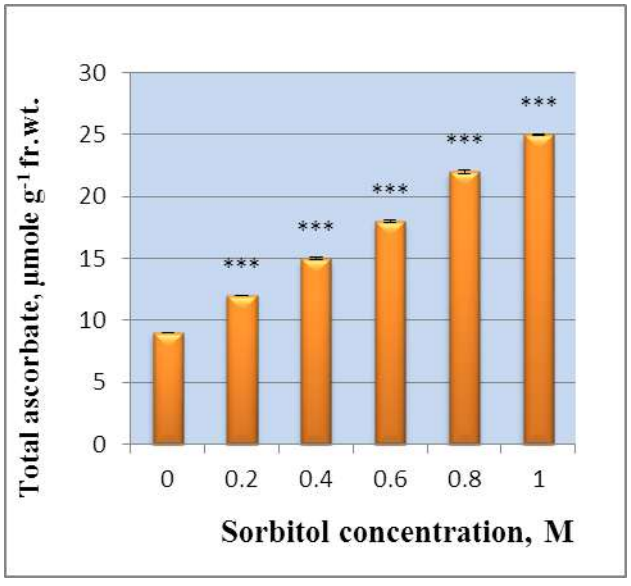
Figure 2. Osmotic stress effect on H_2O_2, MDA Content, Catalase, Gu-POX, APX, GR, total ascorbate and GSH content.











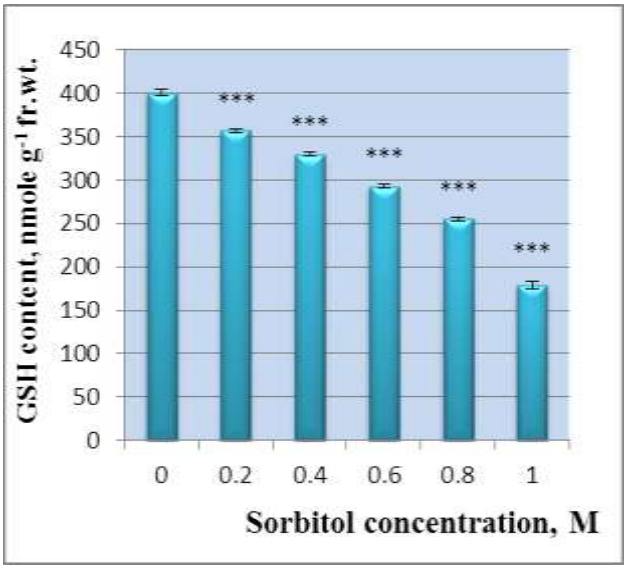
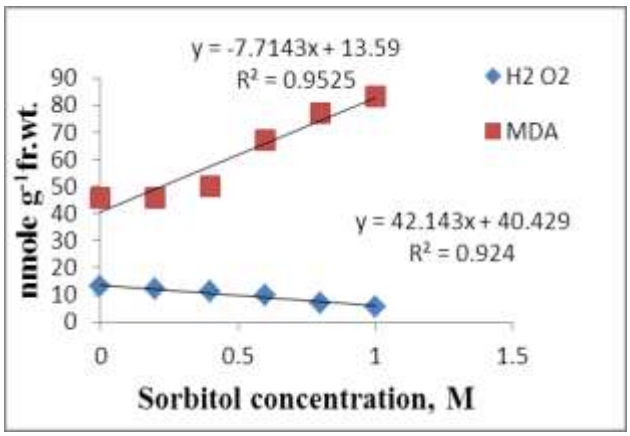
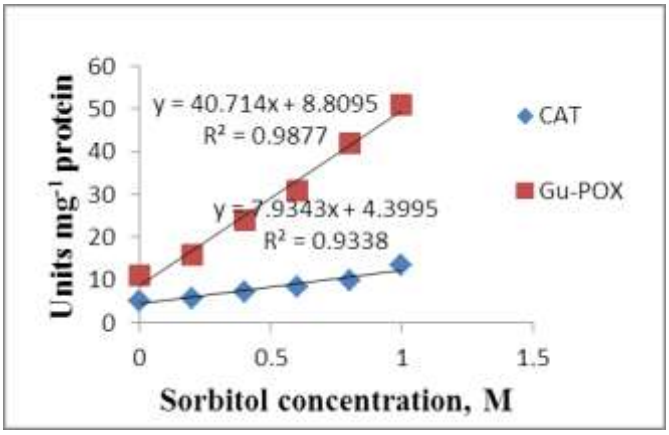
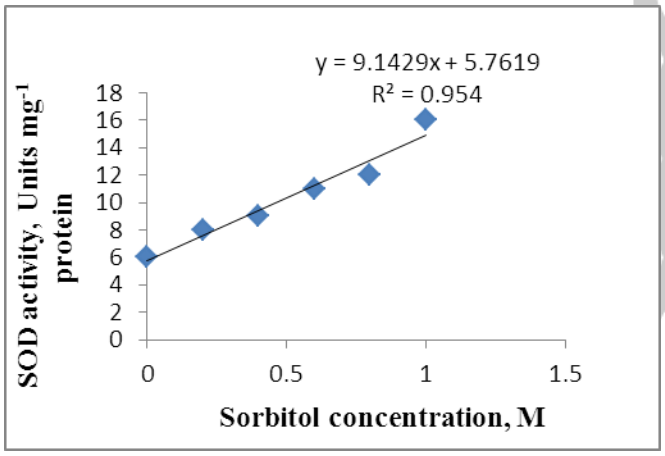
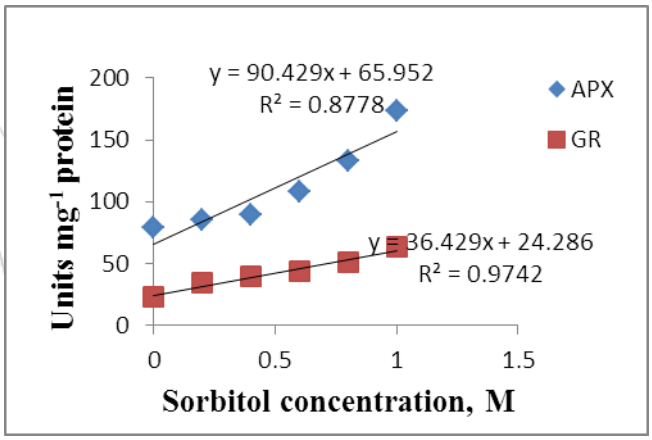


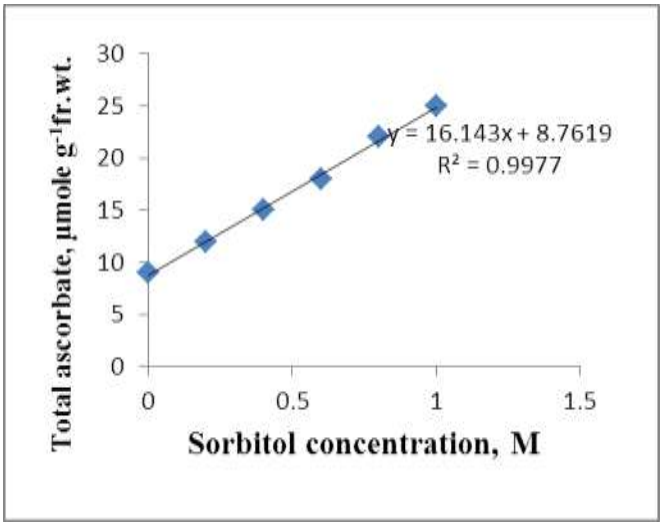
Figure 3. Correlation analysis of sorbitol concentration and H_2O_2, MDA Content, Catalase, Gu-POX, APX, GR, total ascorbate and GSH content.

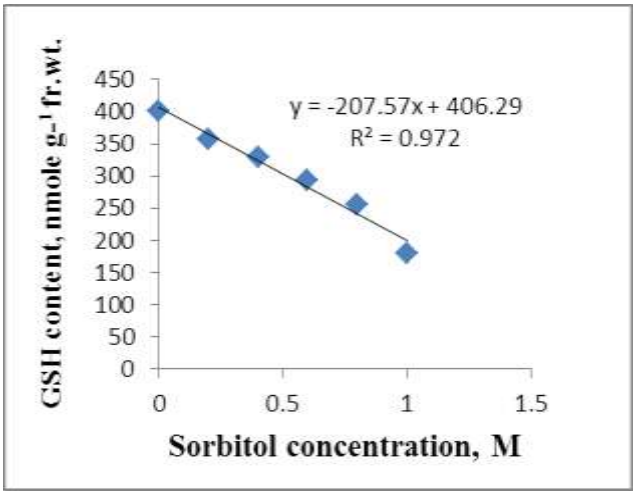












Osmotic stress effect on enzymatic antioxidants:

Catalase activity enhanced with increasing concentration of sorbitol. There was substantial increase in the enzyme activity, more than 250 fold at 1.0M concentration of sorbitol (Figure 2). Concentration dependent increase in the Gu-POX activity was noted with increasing osmotic stress by supplying different concentration of sorbitol, however, prominent increase at higher concentration was observed (Figure 2). Correlation analysis between sorbitol concentration and catalase activity yielded highly significant R squared values of 0.933 and 0.987 with catalase and guaiacol peroxidase respectively (Figure 3). Catalase and peroxidase enzymes are involved in detoxification of H_2O_2. Hence, it is very much likely that the increased activities of these enzymes under osmotic stress rapidly and efficiently scavenging H_2O_2 from the system which leads to decline in H_2O_2 content. Higher Gu- POX activity and decline in the concentration of H_2O_2 under mild drought stress has been shown in rice seedlings (Sharma and Dubey, 2005).

Incubation of leaf segments obtained from light grown maize plants with different concentration of sorbitol accelerated the activities of antioxidative enzymes, SOD, APX and GR (Figure 2). There was gradual increase in the APX activity noticed with increasing concentration of sorbitol; with SOD and GR, the enzyme activity increased substantially with increasing supply of sorbitol (Figure 2). The R squared values obtained from correlation analysis for all the enzymes were found to be highly significant, being 0.954 with SOD, 0.877 wit APX and 0.974 with GR (Figure 3). The enzyme superoxide dismutases play a prime role in protecting cells against oxidative stress since they dismutate $O_2^{\bullet -}$ to H_2O_2 and O_2. Increased SOD activity is observed in our study with increasing osmotic stress by sorbitol supplementation, which could be either due to increased production of ROS or a protective measure adopted by maize leaves against oxidative damage. Increased activities of SOD iso enzymes have been shown under drought stress in rice seedlings (Sharma and Dubey, 2005). Lower SOD activity has been noted in NaCl stressed seedlings of rice variety (Vaidyanathan *et al*., 2003). As oxidative stress is regarded as a key component in the injury of plants to abiotic stresses, the enzymes of ascorbate-glutathione cycle constitute important components of antioxidative defense system of plants. In the present study, two major enzymes of the ASC – GSH cycle, APX and GR were investigated in relation to osmotic stress imposed by sorbitol. Ascorbate peroxidase prevents the accumulation of excess H_2O_2 in cells via this pathway (Foyer and Halliwell, 1976) and GR mitigate the oxidative stress by increasing the regeneration of potential antioxidants ASC and GSH from their oxidized forms. Substantial increase in the APX and GR activities was observed with increasing concentration of sorbitol (Figure 2). This implies that apart from SOD enzyme for detoxification of $O_2^{\bullet -}$, other antioxidative enzymes, such as, APX and

GR are also playing a role. Enhanced activities of all the enzyme of ASC- GSH cycle has been shown in *O. sativa* seedlings under drought stress (Sharma and Dubey, 2005). Further, an increase in chloroplastic APX and its enhanced expression in cytosol as well as in cellular organelles has been observed in drought stressed *Spinacia oleracea* seedlings with a concomitant decrease in H_2O_2 concentration (Yoshimura, 2000).

Osmotic stress effect on non-enzymatic antioxidants: Treatment of light grown leaf segments with varying concentration of sorbitol increased the total ascorbate content substantially with increasing concentration of sorbitol (Figure 2). Glutathione content of the leaf segments decreased gradually with increased supply of sorbitol (Figure 2). There was almost 50% reduction in the GSH content resulted with supply of 1.0M sorbitol. Highly significant R squared values 0.997 with total ascorbate and 0.972 with GSH content were obtained on applying correlation analysis between sorbitol concentration and these parameters (Figure 3). The compounds ascorbate and glutathione are key non-enzymatic antioxidants that play an essential role in protecting plants against oxidative damage. Both compounds are involved in the maintenance of redox status of cells; react directly and neutralize $^1O_2$ and $OH^\bullet$ (Dalton, 1993). ASC eliminates ROS through multiple mechanisms and also maintains membrane - bound antioxidant a- tocopherol in the reduced state and directly eliminates H_2O_2 through APX activity. Ascorbate content has been shown to be higher in leaves of tolerant maize genotypes in response to drought (Chugh et al. 2011). The drought induced reduction in GSH content indicate that a large amount of constitutive GSH counteract the harmful effects of drought. In agreement with the present study, a partial degradation of the constitutive GSH was reported in *Sporobolus stapfianus* leaves subjected to dehydration (Sgherri et al. 1994) and in sunflower plants under severe drought (Sgherri et al. 1995). Drought stress can either increase or decrease GSH content (Gong et al. 2005). In my study, the decrease in GSH concentration with osmotic stress in leaf segments may partly be attributed to decreased rate of GSH synthesis or increased rate of its degradation as suggested by Noctor and Foyer, 1998.

CONCLUSIONS

The present study reveals that the sorbitol induced osmotic stress has an inhibitory effect on chlorophyll biosynthesis in light grown maize leaf segments. Lesser degree of decrease in ALA content due to less inhibition of ALA synthesizing activity and also ALAD activity than total chlorophylls, by the supply of sorbitol indicates that some other enzymes of chlorophyll biosynthetic pathway may be contributing in addition to them in inhibiting the formation of chlorophylls. It seems that H_2O_2 level under the conditions of oxidative stress was maintained low by increased activities of H_2O_2 scavenging enzyme, CAT and Gu-POX. Further, enhanced APX and GR activities indicate that ASC- GSH cycle is operated at a rapid rate under oxidative stress. Thus, our study suggests that Ganga

safed-2 maize variety is osmotic tolerant due to its strong defense mechanism towards osmotic stress; however, it needs further investigation.

ACKNOWLEDGMENT

Financial support to MJ by MPCST, Bhopal is gratefully acknowledged.

REFERENCES

Aebi I. I. "Catalase *in vitro*". Methods in Enzymology. 105: 121-126. (1984).

Bates L. S., Waldren R. P. and Teari D. "Rapid determination of free proline for water stress studies". Plant Soil. 39: 205-207. (1973).

Beyer W. F. and Fridovich I. "Assaying for superoide desmutase activity: some large consequences of minor changes in conditions". Anal. Biochem. 161: 559-566. (1987).

Biswal A.K., Pattanayak G.K., Pandey S.S., Leelavathi S., Reddy V.S., Govindjee, Tripathy B.C. "Light intensity-dependent modulation of chlorophyll b biosynthesis and photosynthesis by overexpression of chlorophyllide a oxygenase (CAO) in tobacco". Plant Physiol. 159: 433–449. (2012).

Chance B. and Mahely A. C. "Assay of catalase and peroxidase". Methods in Enzymol. 2: 764-817. (1955).

Chugh V., Kaur N. and Gupta A. K. "Evaluation of oxidative stress tolerance in maize (*Zea mays* L.) seedlings in response to drought". Indian J Biochemistry and Biophysics. 48: 47-53. (2011).

Dalal V. K. and Tripathy B. C. "Modulation of chlorophyll biosynthesis by water stress in rice seedlings during chloroplast biogenesis". Plant, Cell & Environment. 35(9): 1685-1703. (2012).

Dalton D. A., Baird L. M. and Langeberg L. "Subcellular localization of oxygen defense enzymes in soybean (*Glycine max* L.) root nodules". Plant Physiology. 102(2): 481-489. (1993).

Foyer C. H. and Halliwell B. "The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism". Planta. 133: 21-25. (1976).

Gong O., Li P., Shisong M. A., Rupassara S. I. and Bhonert H. J. "Salinity stress adaptation competence in the extremophile *Thellungiella halohila* in comparision with its relative *Arabidiopsis thaliana*". The Plant J. 44: 826-839. (2005).

Gossett D. R., Millhon E. P., Cran L. M. "Antioxidant response to Nacl stress in salt - sensitive cultivars of cotton". Crop Sci. 34: 706-714. (1994).

Jain M. and Gadre R. (Puranik). "Inhibition of 5-aminolevulinic acid dehydratase activity by arsenic in excised etiolated maize leaf segments during greening". J. Plant Physiol. 161: 251-255. (2004).

Jain M. and Raghavendra A. S. "Effect of osmotic stress on production of hydrogen peroxide in pea (*Pisum sativum* L.) leaves and its relationship to shrinkage". J. Plant Sci. Res. 23(1-2): 87-90. (2007).

Jain M., Tiwary S. and Gadre R. "Sorbitol Induced Changes in Various Growth and Biochemical Parameters in Maize". Plant, Soil and Environment. 56(6): 263-267. (2010).

Jana S. and Choudhari M. A. "Glycolate metabolism of three submerged aquatic angiosperms during ageing". Aquat. Bot. 12: 345-354. (1981).

Lichtenthaler H. K. and Welburn A. R. "Determination of total carotenoids and chlorophylls a and b of extracts in different solvents". Biochem. Soc. Trans 11: 591-592. (1983).

Mauzerall D. and Granik S. "The occurrence and determination of δ-aminoleveulinic acid dehydratase and Porophobilinogen in urine". J. Biol. Chem. 219: 435-446. (1956).

Nakano Y. and Asada K. "Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast". Plant cell Physiol. 22: 867-880. (1981).

Noctor G. and Foyer C. H. "Ascorbate and glutathione: keeping active oxygen under control". Annu. Rev. Plant Physiol. Plant Mol. Biol. 49: 249-279. (1998).

Pattanayak G.K and Tripathy B.C. "Overexpression of protochlorophyllide oxidoreductase C regulates oxidative stress in Arabidopsis". PLOS ONE 6: e26532. (2011).

Rao M. V., Paliyath G. and Ormrod D. P. "Ultraviolet-B- and ozone- induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*". Plant Physiol. 110: 125-136. (1996).

Saha P., Chatterjee P. and Biswas A. K. "NaCl pretreatment alleviates salt stress by enhancement of antioxidant defence system and osmolytes accumulation in mung bean (*Vigina radiate*)". Indian Journal of experimental biology. 48: 593-600. (2010).

Sarangthem J., Jain M. and Gadre R. "Inhibition of δ-aminolevulinic acid dehydratase activity by cadmium in excised etiolated maize leaf segments during greening". Plant Soil Environ. 57(7): 332-337. (2011).

Sgherri C. L. M., Loggini B., Puliga S., Navari-Izzo F. "Antioxidant system in *Sporobolus stapfianus* changes in response to desiccation and rehydration". Phytochemistry. 33: 561-565. (1994).

Sgherri C. L. M., Navari-Izzo F. "Sunflower seedlings subjected to increasing water deficit stress: oxidative stress and defense mechanisms". Physiol. Plant. 93: 25-30. (1995).

Sharma P. and Dubey R. S. "Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectants". J. Plant Physiol. 162: 854-864. (2005).

Smirnoff N. "The role of active oxygen in the response of plants to water deficit and desiccation". New Phytol. 125: 27-58. (1993).

Tewari A. K. and Triparthy B. C. "Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in Cucumber and Wheat". Plant Physiol. 117: 851-858. (1998).

Tukendorf A. and Rauser W. E. "Changes in glutathione and phytochelatins in roots of maize seedlings exposed to cadmium". Plant science. 70: 155-166. (1990).

Turan S. and Triparthy B.C. "Salt-stress induced modulation of chlorophyll biosynthesis de-etiolation of rice seedlings". Physiologia Plantarum. 117:851-858. (2014).

Vaidyanathan H., Sivakumar P., Chakrabarty R. and Thomas G. "Scavenging of reactive oxygen species in NaCl stressed rice (*Oryza sativa* L.) - Differential response in salt tolerant and sensitive varieties". Plant Science. 165(6): 1411-1418. (2003).

Wang W. X., Pelah D., Alergand T., Shoseyov O., Altman A. "Characterization of SP1, a stress-responsive, boiling-soluble, homo-oligomeric protein from aspen (*Populus tremula* L.)". Plant Physiol. 130: 865-875. (2002).

Yoshimura K., Yabuta Y., Ishikawa T. and Sgigeoka S. "Expression of spinach ascorbate peroxidase isozymes in response to oxidative stress". Plant Physiol. 123: 223-234. (2000).