Journal of Stress Physiology & Biochemistry, Vol. 9 No. 3 2013, pp. 262-271 ISSN 1997-0838

ORIGINAL ARTICLE

Effect of PEG-6000 Imposed Water Deficit on Chlorophyll Metabolism in Maize Leaves

Meeta Jain*, Mini Mittal and Rekha Gadre

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

*E-Mail: ***meetajainind@yahoo.com***

Received April 2, 2013

Drought stress is one of the major abiotic constraint limiting plant growth and productivity world wide. The current study was undertaken with the aim to investigate the effect of water deficit imposed by PEG-6000, on chlorophyll metabolism in maize leaves to work out the mechanistic details. Leaf segments prepared from primary leaves of etiolated maize seedlings were treated with varying concentrations of polyethylene glycol-6000 (PEG-6000; w/v- 5%, 10%, 20%, 30%) in continuous light of intensity 40 Wm^{-2} at 26±2 °C for 24 h in light chamber. The results demonstrate a concentration dependent decline in chlorophyll content with increasing concentration of polyethylene glycol-6000 (PEG-6000). Reduction in chlorophyll 'a' level was to a greater extent than the chlorophyll 'b'. The RNA content decreased in a concentration dependent manner with PEG, however, proline content increased significantly. Relative water content decreased significantly with the supply of 30% PEG only. A substantial decrease in chlorophyll synthesis due to significant reduction in ALA content and ALAD activity, with no change in chlorophyllase activity with the supply of PEG suggests that water deficit affects chlorophyll formation rather than its degradation.

Key words: Chlorophyll metabolism, PEG-6000, Zea mays

ORIGINAL ARTICLE

Effect of PEG-6000 Imposed Water Deficit on Chlorophyll Metabolism in Maize Leaves

Meeta Jain*, Mini Mittal and Rekha Gadre

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

*E-Mail: ***meetajainind@yahoo.com***

Received April 2, 2013

Drought stress is one of the major abiotic constraint limiting plant growth and productivity world wide. The current study was undertaken with the aim to investigate the effect of water deficit imposed by PEG-6000, on chlorophyll metabolism in maize leaves to work out the mechanistic details. Leaf segments prepared from primary leaves of etiolated maize seedlings were treated with varying concentrations of polyethylene glycol-6000 (PEG-6000; w/v- 5%, 10%, 20%, 30%) in continuous light of intensity 40 Wm^{-2} at 26±2 °C for 24 h in light chamber. The results demonstrate a concentration dependent decline in chlorophyll content with increasing concentration of polyethylene glycol-6000 (PEG-6000). Reduction in chlorophyll 'a' level was to a greater extent than the chlorophyll 'b'. The RNA content decreased in a concentration dependent manner with PEG, however, proline content increased significantly. Relative water content decreased significantly with the supply of 30% PEG only. A substantial decrease in chlorophyll synthesis due to significant reduction in ALA content and ALAD activity, with no change in chlorophyllase activity with the supply of PEG suggests that water deficit affects chlorophyll formation rather than its degradation.

Key words: Chlorophyll metabolism, PEG-6000, Zea mays

Plants are subjected to various abiotic stresses due to unfavourable environmental conditions that affect their growth, metabolism and productivity (Kaur and Gupta, 2005). Drought is one of the major abiotic stresses which is the most limiting factor for better plant performance and higher crop yield (Szilgyi, 2003; Hirt and Shinozaki, 2003). Several physiological processes are found to be affected by water stress, both at whole plant and cellular levels (Morgan, 1984). Inhibition of leaf growth is a primary whole plant response to water stress, which has been reported in maize, barley and rice seedlings (Lu and Neumann, 1998). Decrease in the percentage and rate of germination and seedling growth by polyethylene glycol (PEG) stress is observed in *Senna occidentalis* (Delachiave and de

Pinho, 2003) and Zea mays (Khayatnezhad *et al*, 2010). Reduction in chlorophyll level by water stress has been shown in a few systems (Albert and Thornber, 1977; Tomati *et al,* 1978). Drought stress also inhibits the photochemical activities and decreases the activities of enzymes of the Calvin cycle (Monakhova and Chernyadev, 2002). Exposure of plants to different stresses induce the overproduction of reactive oxygen species. As a consequence, plants evolved cellular adaptive responses, like up-regulation of oxidative stress protectors and accumulation of protective solutes (Horling *et al*, 2003). For drought stress induction, one of the most popular approaches involves the use of high molecular weight substances, such as, PEG (Turkan *et al*, 2005; Landjiva *et al*, 2008). It is known that PEG does not enter the cell wall space (Rubinstein, 1982) and PEG molecules with a molecular weight greater than 3000 are apparently not absorbed (Tarkow *et al*, 1996). In the present study, PEG-6000 was used for drought stress induction in maize leaves.

Chlorophyll is the molecule that traps 'the most elusive of all powers' and thus acts as a photoreceptor. Entire pathway of chlorophyll biosynthesis is operated in plastids by a complex set of reactions involving many intermediates. δ-aminolevulinic acid (ALA) is the universal precursor of tetrapyrroles, which is synthesized from the intact carbon skeleton of glutamate and/or 2-oxoglutarate in plants. Condensation of two molecules of ALA to form porphobilinogen (PBG) is catalyzed by δ-ALA dehydratase (5-aminolevulinate-hydrolyase EC 4.2.1.24, ALAD). ALAD is a key enzyme of common biosynthetic pathway leading to formation of tetrapyrroles and plays a major role in the regulation of chlorophyll biosynthesis (Prasad *et al,* 1989; Padmaja *et al*, 1989; Prasad and Prasad, 1990). Chlorophyllase (chlorophyll- chlorophyllido-hydrolase, EC 3.1.1.14) degrades chlorophyll into chlorophyllide and phytol. It is one of first plant enzymes identified biochemically and its enzymatic activity is widespread in plant and algal species (Shioi and Sasa, 1986; Takamiya *et al*, 2000). Determination of chlorophyll content of plants is often accomplished to assess the impact of most environmental stresses, as the pigment content is linked to the visual symptoms and photosynthetic plant productivity. Hence, the present investigation was aimed to investigate the effect of water deficit imposed by PEG-6000, on chlorophyll metabolism in maize leaves to work out the mechanistic details.

MATERIALS AND METHODS

Seeds of *Zea mays* L. cv. Ganga Safed - 2 were surface sterilized with 0.1% $HgCl_2$ for 1-2 minutes and then washed thoroughly with tap and distilled water. Seeds were sown in small plastic pots containing acid washed sand for 6-7 days in continuous dark at 26 ± 2 °C. They were watered with ½ strength Hoagland's solution without N. For various experiments the first and fully elongated leaves were used.

Water stress conditions were simulated to polyethylene glycol-6000 at one of four concentrations: 0, 5, 10, 20 and 30%. The osmotic potentials of the solutions was measured using a water potential meter (Psypro Wescor Corporation, US).

PEG-6000 concentration	0%	5%	10%	20%	30%
OP (MPa)	0.00	-0.02	-0.27	-1.27	-1.80

Leaves were cut into small segments (0.5 cm^2) and were treated with varying concentrations of polyethylene glycol-6000 (PEG-6000; w/v); 5 %, 10 %, 20 % and 30 % in continuous light of intensity 30

Wm^{-2} at 26 ± 3 °C for 24 h in light chamber. Distilled water was used as control. At the end of treatment, leaf segments were thoroughly washed with distilled water prior to analysis.

The relative water content (RWC) was measured by the method of Barr and Weatherly (1962). After measuring the fresh weight and dry weight of treated leaf segments, RWC was calculated using the following equation:-

RWC (%) = (FW - DW) / (TW - DW) X 100

Where, FW is the fresh weight of the leaf segments; DW is the dry weight and, TW is the turgid weight of the leaf segments.

The Chl a, Chl b, and carotenoids were extracted in 80% acetone and their content was calculated in µg ml^{-1} using equation of Linchtenthaler and Welburn (1983).

Chl a (µg ml^{-1}) = 12.21 (A_{663}) - 2.81 (A_{646})

Chl b (µg ml^{-1}) = 12.21 (A_{646}) - 5.03 (A_{663})

Carotenoids (µg ml^{-1}) = [1000 (A_{470}) - 3.27 (Chl a) -104 (Chl b)] /227

Total RNA was extracted and estimated by the method of Webb and Levy (1958) using Orcinol reagent. The proline content was estimated as described by Bates *et al* (1973). Amino levulinic acid was extracted and estimated by the method of Tewari and Tripathy (1998).

5-Amino levulinic acid dehydratase (ALAD) activity was assayed by estimating colorimetrically the amount of porphobilinogen formed by using modified Ehrlich's reagent (Mauzerall and Granik, 1956) according to the method mentioned in Jain and Gadre (2004). The unit of enzyme activity is expressed as the number of nmoles PBG formed h^{-1}.

The enzyme chlorophyllase was extracted and assayed according to the method of Nag *et. al* (1981). The chlorophyllase activity was expressed as % of chlorophyll degraded h^{-1}.

The data presented in the text are the average values of at least four replicate experiments with ± standard errors. One way ANOVA was performed and F values i.e. Calculated variance ratio is given in Tables.

RESULTS

Supply of 30 % PEG to excised maize leaf segments from etiolated seedlings decreased the relative water content significantly, but it was reduced slightly by 20 % PEG and remained almost same by 5 and 10 % PEG (Table 1A).

When leaf segments were treated with 5 to 30 % PEG, a concentration dependent decrease in RNA, but increase in proline content was observed (Table 1B).

Supply of 5 to 30 % PEG to maize leaf segments decreased the total chlorophyll content and carotenoids in a concentration dependent manner; however, former was decreased to a greater extent than the latter (Table 2A).

Treatment of maize leaf segments with 5 to 30 % PEG to impose water deficit caused significant decline in chlorophyll 'a' level, while, chlorophyll 'b' content decreased to a lesser extent (Table 2B).

Incubation of maize leaf segments with different concentrations of PEG decreased the ALA content as well as ALAD activity significantly, while chlorophyllase activity remained unaffected, (Table 3). Decrease in ALA content was more severe at each concentration of PEG than the inhibition of ALAD activity.

Table 1A. Effect of water deficit on relative water content (RWC) in excised etiolated maize leaf segments during greening

PEG concentration (%)	Fresh weight (mg)	Dry weight (mg)	RWC (%)
00	206 ± 5.13 (100)	17 ± 1.20 (100)	103 ± 0.88 (100)
05	205 ± 5.23 (99)	17 ± 0.33 (100)	102 ± 1.20 (99)
10	202 ± 4.91 (98)	17 ± 0.57 (100)	105 ± 1.66 (102)
20	190 ± 2.02 (92)	17 ± 1.45 (100)	96 ± 0.88 (93)
30	127 ± 8.70 (61)	15 ± 1.60 (88)	83 ± 2.02 (80)
F value	1.96 (5.96)	1.26 (5.96)	0.33 (5.96)

Critical variance ratio from F-distribution table at 5 % level of significance is given in parentheses.

Leaf segments from maize seedlings grown in continuous dark were floated on distilled water and different concentrations of PEG 6000 for 24 h in continuous light of intensity 30 Wm^{-2} at 26 ± 3°C.

Values relative to control are given in parentheses.

Table 1B. Effect of water deficit on proline and RNA content in excised etiolated maize leaf segments during greening

PEG concentration (%)	Proline content (mg g^{-1} fr.wt.)	Total RNA (mg g^{-1}fr.wt.)
00	100 ± 11 (100)	7.58 ± 0.24 (100)
05	150 ± 12 (150)	6.92 ± 0.17 (91)
10	167 ± 13 (167)	6.31 ± 0.11 (83)
20	251 ± 17 (251)	4.67 ± 0.12 (61)
30	297 ± 25 (297)	3.57 ± 0.44 (47)
F value	3.05 (5.63)	43.86 (5.63)

Critical variance ratio from F-distribution table at 5 % level of significance is given in parentheses.

Leaf segments from maize seedlings grown in continuous dark were floated on distilled water and different concentrations of PEG 6000 for 24 h in continuous light of intensity 30 Wm^{-2} at 26 ± 3°C.

Values relative to control are given in parentheses.

Table 2A. Effect of water deficit on total chlorophylls and carotenoids in excised etiolated maize leaf segments during greening

PEG concentration (%)	Total chlorophylls (µg ml^{-1})	Carotenoids (µg ml^{-1})
00	19.38 ± 1.71 (100)	5.89 ± 0.40 (100)
05	12.26 ± 0.56 (63)	4.16 ± 0.69 (70)
10	11.33 ± 1.05 (58)	3.86 ± 0.49 (65)
20	9.46 ± 0.77 (48)	3.23 ± 0.41 (55)
30	6.53 ± 1.10 (33)	3.06 ± 0.26 (52)
F value	17.75 (5.63)	6.40 (5.63)

Critical variance ratio from F-distribution table at 5 % level of significance is given in parentheses.

Leaf segments from maize seedlings grown in continuous dark were floated on distilled water and different concentrations of PEG 6000 for 24 h in continuous light of intensity 30 Wm^{-2} at 26 ± 3°C.

Values relative to control are given in parentheses.

Table 2B. Effect of water deficit on chlorophyll 'a', chlorophyll 'b' and chlorophyll a/b ratio in excised etiolated maize leaf segments during greening

PEG concentration (%)	Chl a (µg ml^{-1})	Chl b (µg ml^{-1})	Chl a /b ratio
00	15.75 ± 1.37 (100)	3.64 ±0.76 (100)	5.16 ± 0.99 (100)
05	9.71 ± 0.58 (62)	2.54 ± 0.19 (70)	3.89 ± 0.42 (75)
10	8.29 ± 0.64 (53)	3.03 ± 0.73 (83)	3.38 ± 0.50 (65)
20	6.92 ± 0.47 (44)	2.53 ± 0.60 (69)	3.61 ± 0.69 (70)
30	4.44 ± 0.40 (28)	2.08 ± 0.81 (57)	3.48 ± 0.63 (67)
F value	20.40 (5.63)	00.88 (5.63)	03.93 (5.63)

Critical variance ratio from F-distribution table at 5 % level of significance is given in parentheses.

Leaf segments from maize seedlings grown in continuous dark were floated on distilled water and different concentrations of PEG 6000 for 24 h in continuous light of intensity 30 Wm^{-2} at 26 ± 3°C.

Values relative to control are given in parentheses.

Table 3. Effect of water deficit on ALA content, ALAD and chlorophyllase activities in excised etiolated maize leaf segments during greening

PEG concentration (%)	ALA content (nmole g^{-1} fr.wt.)	ALAD (nmole PBG formed h^{-1} g^{-1} fr.wt.)	Chlorophyllase (% Chlorophyll degraded h^{-1})
00	437 ± 39 (100)	218 ± 34 (100)	95 ± 4 (100)
05	373 ± 25 (85)	205 ± 43 (94)	91 ± 5 (96)
10	315 ± 21 (72)	179 ± 32 (82)	94 ± 7 (99)
20	264 ± 21 (60)	166 ± 30 (76)	93 ± 5 (97)
30	192 ± 19 (44)	113 ± 22 (52)	95 ± 5 (100)
F value	13.47 (5.63)	18.34 (5.63)	00.59 (5.63)

Critical variance ratio from F-distribution table at 5 % level of significance is given in parentheses.

Leaf segments from maize seedlings grown in continuous dark were floated on distilled water and different concentrations of PEG 6000 for 24 h in continuous light of intensity 30 Wm^{-2} at 26 ± 3°C.

Values relative to control are given in parentheses.

DISCUSSION

The RWC is considered to be a measure of plant water status, reflecting the metabolic activity in tissues and used as most meaningful index of dehydration tolerance (Sinclair and Ludlow, 1986). In the present study, incubation of leaf segments with high concentration of PEG decreased the RWC (Table 1A). Decline in RWC due to PEG induced water stress has also been reported in barley leaves (Yuan *et al*, 2005) and tomato (Zgallai, 2005) and pigeonpea (Kumar *et. al*, 2011) plants. Environmental biotic and abiotic stress could evoke compensatory metabolic changes through modification and modulation of various biochemical parameters. The present data indicate a pronounced reduction in RNA content with increasing water deficit (Table 1B). Reduced RNA synthesis with increased water stress has been reported by He *et al* (1999) in wheat leaves; they also suggested upregulation of chloroplast RNAase as one of the possible reasons causing the degradation of RNA. Furthermore, many

researchers showed that the ribosomes and the proportion of polyribosomes decreased remarkably during water stress (Mason *et al*, 1988; Scott *et al,* 1979). It is known that ribosomes cluster on mRNAs to protect them from degradation. So, disruption of ribosomes may be another reason for enhanced mRNA degradation. Proline accumulation under stress has been linked with its role as an osmolyte by contributing towards osmotic adjustment between cytoplasm and vacuoles (See Delauney and Verma, 1993). Moreover, because of its zwitter ionic and highly hydrophilic character it has a role as an osmoticum and acts as a compatible solute for plants subjected to low water potential and other environmental stresses (Sampras *et al,* 1995). Water deficit imposition resulted in an increased proline level and there is about three fold rise in proline content at highest concentration of PEG used (Table 1B). Increase in the free proline content during water stress condition due to PEG has also been shown in pigeonpea (Kumar *et al*, 2011).

Photosynthetic pigments determine the physiological status of the plants. The change in the chl a/b ratio provides further information about modification process taking place in the photosynthetic apparatus. In our study, the experiments indicate the concentration dependent reduction in total chlorophylls as well as carotenoids in maize leaf segments subjected to water deficit with more pronounced effect for chlorophylls (Table 2A). Decrease in the total chlorophyll content by PEG 6000 (-5 bar) has also been noticed by Pratap and Sharma (2010) in black gram and Guo *et al* (2013) in wheat seedlings. Unlike this, increase in chlorophyll content has been demonstrated in graminaceous chlorophyllic cell lines of grass *Bouteloua gracilis* exposed to different concentrations of PEG 8000 (Garcia Valenzuela *et al*, 2005). The marked reduction of total chlorophylls in water deficit leaves was due to decrease in both the chl a and chl b contents but chl a was decreased to a greater extent than chl b (Table 2B). Reduction in chlorophyll content due to water stress is also reported by Zayed and Zeid (1997/98) in mung bean seedlings and by Jeyaramraja *et al* (2005) in tea leaves. Decrease in level of chlorophyll and increase in proline content by water deficit suggest that glutamate availability for chlorophyll biosynthesis could be checked under water stress, as both require it for biosynthesis.

PEG-6000 imposed water deficit has affected the activities of enzymes of chlorophyll metabolism also. Thus, ALA content and ALAD activity are severely inhibited while chlorophyllase activity remains almost same (Table 3). Salinity has been found to enhance the chlorophyllase activity in pigeonpea and *Gingellay* which results in lowering of chlorophyll content (Rao and Rao, 1981). ALA, the key compound in the chlorophyll biosynthetic pathway is synthesized via Beale's pathway from the intact carbon skeleton of 5 carbon compounds, glutamate and/or 2-oxoglutarate. Hence, it is possible that glutamate, being precursor of proline is not made available for ALA synthesis. Increased synthesis of glutamate for proline accumulation has been suggested in groundnut cotyledons during saline stress (Satakopan *et al*, 1989). Further, decreased ALA content may be responsible for reduced ALAD activity. Thus, a pronounced decrease in chlorophyll biosynthesis due to decline in ALA content and inhibition of ALAD activity suggests that water deficit imposed by PEG-6000 affects chlorophyll formation rather than its degradation.

REFERENCES

Albert, R.S. and Thornber, J.P. (1977) Water stress effect on the content and organization of chlorophyll in mesophyll and bundle sheath chloroplast. *Plant Physiol.,* **59**: 351-353.

Barr, H.D. and Weatherly, P.E. (1962) A re-examination of the relative turgidity technique for estimating water deficit in leaves. *Aust. J. Biol. Sci*., **15**: 413-428.

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

Delachiave, M.E.A. and de Pinho, S.Z. (2003) Germination of *Senna occidentalis link: S*eed at different osmotic potential levels. *Brazilian Archives Biol. Technol*., **46(2)**: 163-166.

Delauney, A.J. and Verma, D.P.S. (1993) Proline biosynthesis and osmoregulation in plants. *Plant J*., **4**: 215-223.

Garcia-Valenzuela, X., Garcia-Moya, E., Rasconcruz, Q., Estrella-Herrera, L. and Aguado-Santacruz, G.A. (2005) Chlorophyll accumulation is enhanced by osmotic stress in graminaceous chlorophyllic cells. J*. Plant Physiol*., **162**: 650-661.

Guo, R., Hao, W.P., Gong, D.Z., Zhong, X.L. and Gu, F.X. (2013) Effects of water stress on germination and growth of wheat, photosynthetic efficiency and accumulation of metabolites. Chapter 13 in book "Soil Processes and Current Trends in Quality Assessment" edited by Maria C. and Hernandez Soriano. 367-380.

He, J.X., An, L.Z., Lin, H.H. and Liang, H.G. (1999) Evidence for transcriptional and post-transcriptional control of protein synthesis in water stressed wheat leaves: a quantitative analysis of messenger and ribosomal RNA. *J. Plant Physiol* **155**: 63-69.

Hirt, H. and Shinozaki, K. (2003) Plant responses to abiotic stress. Springer-Verlag, Berlin Hiedelbergh.

Horling, F., Lamkemeyer, P., Konnig, J., Finkemeir, I., Kandlbinder, A., Baier, M. and Dietz, K. (2003) Divergent light, ascorbate and oxidative stress-dependent regulation of expression of the peroxiredoxin gene family in *Arabidopsis. Plant Physiol.,* **131**: 317-325.

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

Jeyaramraja, P.R., Meenakshi, S.N., Kumar, R.S., Joshi, S.D. and Ramasubramanian, B. (2005) W*ater deficit induced oxidative damage in tea (Camellia sinensis) plants, J. Plant Physiol*., **162**: 413-419.

Kaur, N. and Gupta, A.K. (2005) Signal transduction pathways under abiotic stresses in plant. *Current Sci*., **88(11)**: 1771-1778.

Khayatnezhad, M., Gholamin, R., Jamaati-e-Soramin, S. and Zabihi-e-Mahmoodabad, R. (2010) Effects of peg stress on Corn cultivars (*Zea mays* L.) at germination stage. *World Applied Sciences Journal*., **11(5)**: 504-506.

Kumar, R.R., Karajol, K. and Naik, G.R. (2011) Effect of polyethylene glycol induced water stress on physiological and biochemical responses in pigeonpea (Cajanus cajan L. Millsp.). *Recent Research in Science and Technology*., **3(1)**:148-152.

Landjeva, S., Neumann, K., Lohwasser, U and Borner, M. (2008) Molecular mapping of

genomic regions associated with wheat seedling growth under osmotic stress. *Biol. Plan.*, **52**: 259-266.

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

Lu, Z. and Neumann, P.M. (1998) Water-stressed maize, barley and rice seedlings show species diversity in mechanisms of leaf growth inhibition. *J. Exp. Bot*., **49**: 1945-1952.

Mason, H.S., Mullet, J.E. and Boyer, J.S. (1988) Polysomes, messenger RNA, and growth in soybean stems during development and water stress. *Plant Physiol*., **86**: 725-723.

Mauzerall, D, and Granick, S. (1956) The occurrence and determination of δ-aminolevulinic acid dehydratase and porphobilinogen in urine. *J. Biol. Chem*., **219**: 435-446.

Monakhova, O.F. and Chernyadev, I.I. (2002) Protective role of kartolin-4 in wheat plants exposed to soil drought. *Appl. Biochem. Microbiol*., **38**: 373-380.

Morgan, J.M. (1984) Osmoregulation and water stress in higher plants. *Annu. Rev. Plant Physiol*., **35**: 299-319.

Nag, P., Paul, A.K. and Mukherji, S. (1981) Heavy metal effects in plant tissues involving chlorophyll, chlorophyllase, Hill reaction activity and gel electrophoresis patterns of soluble ptoteins. *Ind. J. Exp. Biol*., **19**: 702-706.

Padmaja, K., Prasad, D.D.K. and Prasad, A.R.K. (1989) Effect of selenium on chlorophyll biosynthesis in mung bean seedlings. *Phytochemistry*, **28**: 3321-3328.

Prasad, D.D.K. and Prasad, A.R.K. (1990) Porphyrin metabolism in lead and mercury treated bajra (*Pennisetum typhoideum*) seedlings. *J. Biosci*., **15(4)**: 271-279.

Prasad, D.D.K., Santhi, G. and Prasad, A.R.K. (1989) Regulation of porphyrin biosynthesis by lead and mercury in mung bean (*Phaseolus vulgaris*) seedlings. *Biochem. Int*. **19(6)**: 1403-1417.

Pratap, V. and Sharma, Y.K. (2010) Impact of osmotic stress on seed germination and seedling growth in black gram (*Phaseolus mungo*) *Journal of Environmental Biology*, **31(5)**: 721-726.

Rao, G.G. and Rao, G.R. (1981) Pigment composition and chlorophyllase activity in pigeonpea (*Cajanus indicus*) and gingellay (*Sesamum indicum* L.) under NaCl salinity. *Indian J. Exp. Biol*., **19**: 768-770.

Rubinstein, B. (1982) Regulation of H^+ excreation. I. Effects of osmotic shock. *Plant Physiol.*, **99**: 355-360.

Sampras, Y., Bressan, R.A., Csonka, L.N., Garcia Rio, M.G., Paino, D., Urgo, M. and Rhodes, D. (1995) Proline accumulation during drought and salinity. In: Smirnoff N. (ed.), Environment and Plant Metabolism Flexibility and Acclimation, Bios Scientific publishers, Oxford, UK, 161-187.

Satakopan, U.N., Jayshree, H. and Srinivasan, R. (1989) A possible role for aminotransferases in proline accumulation in groundnut cotyledons during saline stress. *Sci. Cult.,* **55**: 415-418.

Scott, N.R., Munns, R. and Barlow, E.W.R. (1979) Polyribosomes content in young and aged wheat leaves subjected to drought. *J. Exp. Bot*., **30**: 905-911.

Shioi, Y. and Sasa, T. (1986) Purification of

solubilized chlorophyllase from Chlorella protothecoides. *Methods Enzymol*., **123**: 421-427.

Sinclair, T.R. and Ludlow, M.M. (1986) Influence of soil water supply on the plant water balance of four tropical grain legumes. *Aust. J. Plant Physiol*., **13**: 329-341.

Szilgyi, L. (2003) Influence of drought on seed yield components in common bean. *Bulg. J. Plant Physiol*., Special issue 320-330.

Takamiya, K.I., Tsuchiya, T. and Ohta, T. (2000) Degradation pathway(s) of chlorophyll: What has gene cloning revealed? *Trends Plant Sci*., **5**: 426-431.

Tarkow, H., Feist, W.C. and Southerland, C.F. (1996) Interaction of wood and polymeric materials Penetration versus molecular size. *Forest Prod*. J., **16**: 61-65.

Tewari, A.K. and Tripathy, B.C. (1998) Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in cucmber and wheat. *Plant Physiol*., **117**: 851-858.

Tomati, U., Veri, G. and Gall, E. (1978) Effect of water status on photosynthesis and nitrate reductase activity in maize plants. *Review in Agronomy*, **12**: 119-122.

Turkan, I., Bor, M., Ozdemir, F. and Koca, H. (2005) Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris L.* subjected to polyethylene glycol mediated water stress. *Plant Sci*., **168**: 223-231.

Webb, J.M. and Levy, H.B. (1958) In "Methods in biochemical Analysis." Ed. Glick, D., Interscience, NY, 61.

Yuan, S., Liu, W.J., Zhang, N.H., Wang, M.B., Liang, H.G. and Lin, H.H. (2005) Effects of water stress on major photosystem II gene expression and protein metabolism in barley leaves. *Physiol. Plant*., **125**: 464-473.

Zayed, M.A. and Zeid, I.M. (1997/98) Effect of water and salt stresses on growth, chlorophyll, mineral ions and organic solute contents and enzymes activity in mung bean seedlings. *Biologia Plantarum*, **40(3)**: 351-356.

Zgallai, H., Steppe, K. and Lemeur, R. (2005) Photosynthetic, physiological and biochemical responses of Tomato plants to Polyethylene Glycol- induced water deficit. *J. Integr. Plant Biol.,* **47(12)**: 1470-1478.