

Effect of Salinity Stress on Glutathione –S-Transferases (GSTs) of Maize

R. N. Remme^{1*}, N. A. Ivy², M. A. K. Mian³, M. Rohman⁴

^{1*} (Lecturer, Agrotechnology Discipline, Khulna University, Bangladesh)

² (Associate professor, Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh)

³ (Professor, Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh)

⁴ (Senior Scientific Officer, Plant Breeding Division, Bangladesh Agricultural Research Institute, Bangladesh)

Abstract: The present study was undertaken to explore the responses of maize GST to salinity. GST activities were found to induce with the increasing of salinity levels and duration of stress. At 3 day, 5 day and 7 day stress most of all the salinity concentration (4 dS/m, 8 dS/m and 12 dS/m) increased the GST activities over control. However 16 dS/m salinity decreased the activity over control. In foliar application of proline GST activities were increased gradually over control and NaCl treatment at 3 day, 5 day and 7 day stress in all the salinity level. In betaine application at 4 dS/m, 8 dS/m, 12 dS/m and 16 dS/m salinity also increased GST activity were gradually over control at 3 day, 5 day and 7 day stress. It also increases the GST activity during the stress period over NaCl stress in all the salinity levels, except 12 dS/m and 16 dS/m salinity at 3 day stress. When both proline and betaine applied together in stressed seedlings at 4 dS/m, 8 dS/m, 12 dS/m and 16 dS/m salinity increased the GST activity at 3 day, 5 day and 7 day stress over control. Though the combination of 15 mM proline and betaine decreased the activities at 3 day and 7 day stress at 4 dS/m, 8 dS/m and 12 dS/m at 3 day stress GST activity increased at other salinity stress in different stress period. To examine the detoxification role of maize GST, concentration of MDA (peroxidant product of poly unsaturated fatty acid) in the stressed maize seedlings was studied. In all the salinity levels, MDA contents increased over control. Supplementation of proline and betaine reduced the concentrations of MDA over salinity stresses. The simultaneous increased GST activities and lower MDA concentration suggested the detoxification role of maize GST on cytotoxic MDA.

Keywords: Glutathione –S-transferases, Maize, NaCl, Melondialdehyde

I. Introduction

Abiotic stresses, such as drought, salinity, cold, high temperature, chemical toxicity, high light intensity and oxidative stresses lead to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity and reduce crop yields by more than 50% worldwide (Wang *et al.*, 2002). Different abiotic stresses including salinity trigger in plants oxidative stress, generating the formation of reactive oxygen species (ROS). These species are partially reduced or activated derivatives of oxygen, comprising both free radical ($\cdot\text{O}_2$, $^1\text{O}_2$, $\cdot\text{OH}$, OH_2) and non-radical (H_2O_2) forms, leading to cellular damage, metabolic disorders and senescence processes, structural and functional loss of cell organelles, and eventually lead to death (Blokina *et al.*, 2003). To protect themselves against toxic oxygen intermediates and xenobiotics, various defense systems evolved within the plant that includes antioxidant enzymes. Plants possess an impressive array of antioxidant enzymes of which glutathione S-transferases are an important group. GSTs are a family of enzymes which catalyze the configuration of reduced GSH (tripeptide of γ -Glu-Cys-Gly) to a variety of electrophilic compounds to direct them to specific sites both intra- and extra-cellularly. GSTs are proposed to afford protection under various stress conditions by detoxifying endogenous plant toxins that accumulate as a consequence of increased oxidative stress (Marrs, 1996). In plant, GSTs, originally characterized by their ability to modify xenobiotics covalently by glutathionation, are rapidly induced by their substrates, particularly plant herbicides (Dean *et al.*, 2005). There has been increasing evidence indicating that GSTs may be implicated many other physiological processes. These include, stress response (Anderson and Davis, 2004), catalytic mechanism (Atkins *et al.*, 2003), protection of tissue from oxidative damage, lipid per oxidation (Yang *et al.*, 2001), ligandin functions (Axarli *et al.*, 2004), and protection of tissue from heavy metals (Marrs, 1996). After discovering of GSTs in 1970 in maize, they have been extensively studied for detoxification of xenobiotics, detoxification of endogenous cytotoxic substances (Marrs, 1996; Jepson *et al.*, 1997; Muller *et al.*, 2000; Dixon *et al.*, 2002; Rohman *et al.*, 2009). Though GSTs have been reported to have important roles under different biotic and abiotic stress environments, such information on maize GSTs are poorly available except drought; chilling tolerance (Abenavoli *et al.*, 2001). However, information on responses of maize GSTs to salinity responses is very limited.

There are evident that under stress condition, osmoprotectants like betaine, proline, glycine, jaesmonin acid, NO⁻ etc. are produced and among which proline and betaine are more common (Hossain and Fujita, 2010). It is reported that betaine and proline synthesis in maize under abiotic stress with protective roles (Ashraf and Foolad, 2007) and able to express antioxidant activity (Hossain and Fujita, 2010; Hoque *et al.*, 2007a). Considering all, this study was under taken to investigate the responses of maize GST to salinity.

II. Materials and Methods

2.1 Experimental site

This study was carried out in the Molecular Breeding Laboratory of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during the period from April, 2011 to August, 2012.

2.2 Plant materials

Seedlings of maize (BARI Hybrid Maize 7) were used as plant material.

2.3 Chemicals

All of the chemicals except Hydroxylapaptite, NaCl (Merck, Germany) were used manufactured from Sigma-Aldrich, USA.

2.4 Stress treatments

Maize seedlings were grown in plastic pots. Five days old seedlings were subjected for stress implementation. Salinity levels (4, 8 and 12 dS/m) were maintained by adding NaCl and water. A control treatment (with adding NaCl) was also growing under same condition. In NaCl stressed seedlings 15 mM proline and 15 mM betaine were applied as foliar spray twice daily. Digital EC meter was used to measured level of salinity, and the level was expressed at field capacity.

2.5 Preparation of soluble protein

To assay the activity level of GST in maize seedlings five gram of sample was homogenized in an equal volume of 50 mM potassium phosphate buffer (pH 7.0) containing 100 mM KCl, 1% (w/v) ascorbate and 10 % (w/v) glycerol with mortar and pestle. The homogenates were centrifuged at 11,500 x g for 10 min and the supernatant was used as a soluble protein solutions.

2.6 Measurement of lipid peroxidation

The level of lipid peroxidation was measured by estimating MDA, a decomposition product of the peroxidized polyunsaturated fatty acid component of the membrane lipid, using thiobarbituric acid (TBA) as the reactive material following the method of Heath and Packer (1968) with slight modifications. The seedlings (2.5 g) were homogenized in 15 ml 5% (w/v) trichloroacetic acid (TCA) and the homogenate was centrifuged at 11,500 rpm for 10 min. One ml supernatant was mixed with 4 ml of TBA reagent (0.5% of TBA in 20% TCA). The reaction mixture was heated at 95°C for 30 min in a water bath and then quickly cooled in an ice bath and centrifuged at 11,500g for 15 min. The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed as nmol of MDA g⁻¹ fresh weight.

2.7 Data analysis

Data generated from this study were analyzed by MSTAT software where need. The graphs were prepared in MS Excel, 2007.

III. Results and Discussion

Since proline and betaine, the most common osmolytes synthesis under abiotic stresses with protective roles through over expression of antioxidant machinery including GST, to examine the responses of maize GST was tested under saline stress supplemented with proline and betaine. The activities changes in different level under different treatments. The activity also changed differently over duration of stresses.

3.1 Changes in GST activities at 4 dS/m salinity

At 4dS/m salinity, GST activity increased over control but it was statistically insignificant at 3 day stress. It increased the activity 10.54% over control from 199.11 to 220.10 nmol/min/mg protein (Fig. 1). In foliar application of proline and betaine at 15 mM concentration the activity increased significantly by proline and betaine individually over control. However, the combined application of proline and betaine under this level of salinity the activity slightly increased over control, but slightly lower than the activity level under 4 dS/m salinity. The activity levels increased in proline and betaine supplemented NaCl treated maize seedlings were

19.58% and 18.06%, respectively over control and 8.18 and 6.80%, respectively over NaCl (Table 1) at this stage.

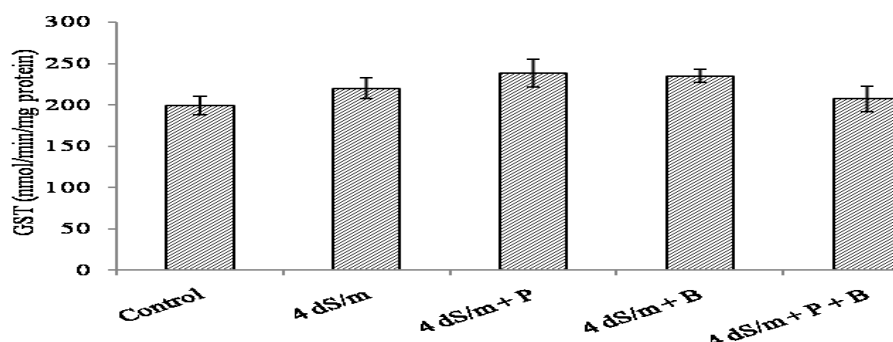


Fig. 1. Changes in the activity of GST in maize seedlings under 4 dS/m salinity stress supplemented with proline and betaine at 3 day stress.

At 5 day of stress under 4 dS/m salinity, the activities of GST increased more than 3 day stress (Fig. 2 and Table 1). At this stage, the activity increased 16.33% to 230.98% nmol/min/mg protein over control which was statistically significant. The foliar application of proline and betaine increased the activities over control and 4 dS/m salinity. However, the seedlings treated with both proline and betaine slightly (6.38%) increased the activity over NaCl treatment. Supplementation of proline and betaine increased activity by 53.66% and 39.77%, respectively over control, and 32.09% and 20.15%, respectively over NaCl treatment.

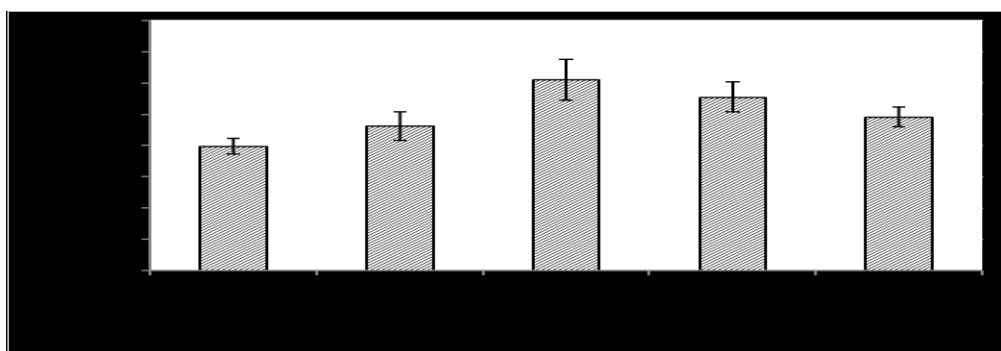


Fig. 2. Changes in the activity of GST in maize seedlings under 4 dS/m salinity stress supplemented with proline and betaine at 5 day stress

At 7 day of stress under 4 dS/m salinity, the activities of GST also increased more than 5 day stress (Fig. 2 and Table 1) and the activity was found to increase significantly over control at 7 day stress over control. The activity increased 34.03% to 270.20 nmol/min/mg protein from 201.59 nmol/min/mg protein. The sole application of proline and betaine increased the activities significantly over control and 4 dS/m salinity at this stage. The exogenous application of proline and betaine increased activity by 58.99% and 51.40%, respectively over control, and 18.62% and 12.95%, respectively over NaCl treatment. However, though the combined application proline and betaine increased the activity (28.48%), it decreased 4.15% activity over NaCl treatment in maize seedlings.

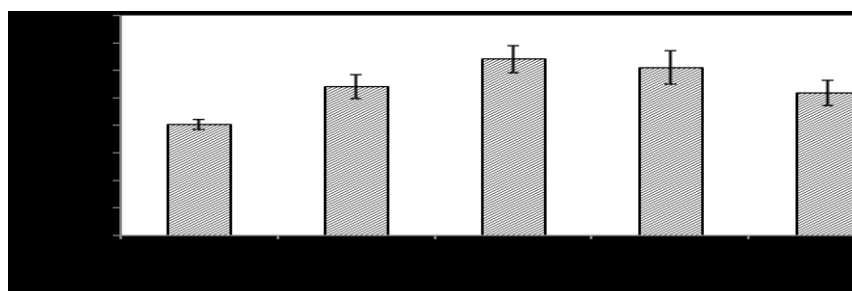


Fig. 3. Changes in the activity of GST in maize seedlings under 4 dS/m salinity stress supplemented with proline and betaine at 7 day stress

Table 1. Percentage of activity changed over control and 4 dS/m NaCl treatment in maize seedlings supplemented with proline (P) and betaine (B)

Treatment	% activity increased over control			% activity increased over NaCl		
	3 day	5 day	7 day	3 day	5 day	7 day
4 dS/m	10.54	16.33	34.03	-	-	-
4 dS/m + P	19.58	53.66	58.99	8.18	32.09	18.62
4 dS/m + B	18.06	39.77	51.40	6.80	20.15	12.95
4 dS/m + P + B	4.08	23.75	28.48	-5.85	6.38	-4.15

3.2 Changes in GST activities at 8 dS/m salinity

At 8 dS/ salinity GST activities were found to be changed over duration of stress (Fig. 4 and Table 2). At this salinity level the activity increased 12.92% over control in 3 day stressed seedlings, however, at this stage; the increased level was not significantly higher than the control. Among the osmolytes, only in proline supplemented NaCl stressed seedlings, the activity significantly increased over control (27.85%), but over NaCl it only increased 13.52% activity only (Table 2). Though betaine increased the activities by 16.71% and 3.61% over control and NaCl treated seedlings, in the combined application of the osmolites, the activity remained almost similar.

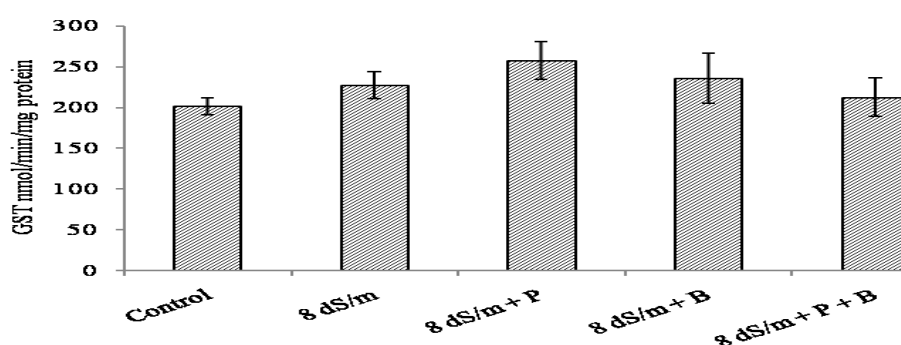


Fig. 4. Changes in the activity of GST in maize seedlings under 8 dS/m salinity stress supplemented with proline and betaine at 3 day stress

During the stress period, 5 day stress resulted in the highest induction of GST activity under 8 dS/m salinity (Fig. 5 and Table 2). At this stage, the 8 dS/ m salinity increased 31.05% activity from 192.47 to 252.24 nmol/min/mg protein, which was statistically significantly higher than control. The foliar single spray of 15 mM proline and betaine and their combined application increased the activity level increased significantly (79.62%, 46.78% and 31.48%, respectively) over control under this salinity level. Interestingly, proline magnificently enhanced the activity of 8 dS/m from 270.70 to 315.20 nmol/min/mg protein which increased 1.8 fold activities suggesting the protective role of GST through activating its activity by proline. Though the betaine increased the activity slightly over NaCl, the activity level remained similar in combined application of proline and betaine at this stage.

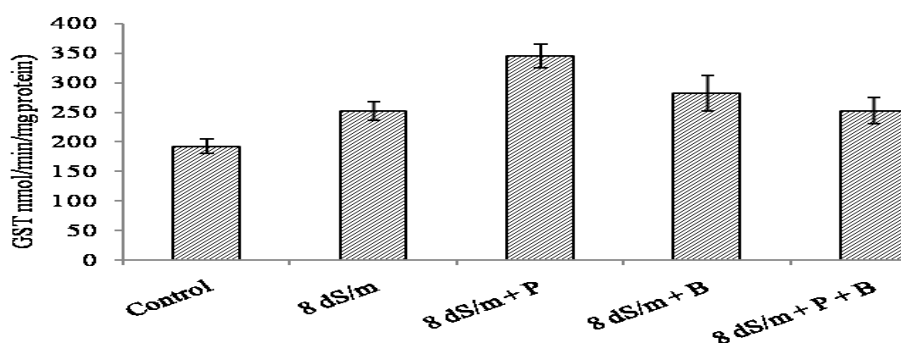


Fig. 5. Changes in the activity of GST in maize seedlings under 8 dS/m salinity stress supplemented with proline and betaine at 5 day stress

At 7 day of stress, the GST activities were observed to be decrease that the activities at 5 day stress in the osmolites supplemented seedlings; however, the NaCl slightly increased the activity (Table 2). From the figure 6 we found that the activity significantly increased (33.68%) over control. At this stage, only proline could able to increase the activity significantly over control and NaCl treatment (55.65% and 16.44%, respectively). On the other hand, the activity in betaine supplemented seedlings did not able to increase the

activity over NaCl, where, the activity level by combined of the osmolites remains statistically similar to control (Fig. 6).

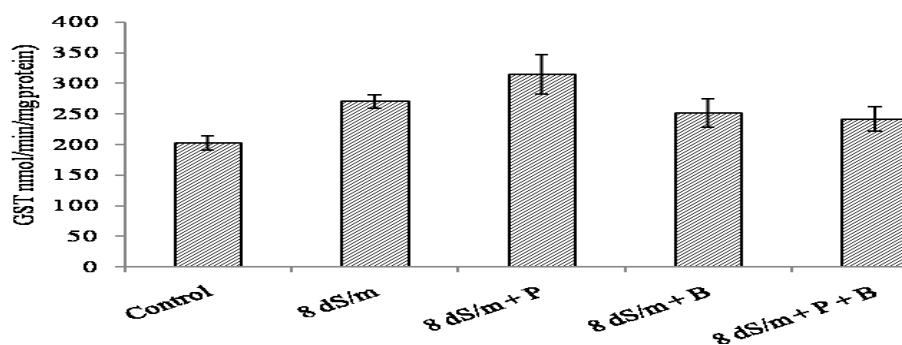


Fig. 6. Changes in the activity of GST in maize seedlings under 8 dS/m salinity stress supplemented with proline and betaine at 7 day stress

Table 2. Percentage of activity changed over control and 8 dS/m NaCl treatment in maize seedlings supplemented with proline and betaine

Treatment	% activity increased over control			% activity increased over NaCl		
	3 day	5 day	7 day	3 day	5 day	7 day
8 dS/m	12.92	31.05	33.68	-	-	-
8 dS/m + P	27.85	79.62	55.65	13.52	37.06	16.44
8 dS/m + B	16.71	46.78	24.54	3.61	12.00	-6.83
8 dS/m + P + B	5.47	31.41	19.51	-4.49	0.27	-10.60

3.3 Changes in GST activities at 12 dS/m salinity

At 3 day stress, 12 dS/m salinity increased the activity significantly over control (Fig. 7). It increased 21.74%. The individual application of proline and betaine in NaCl induced salinity increased the activity significantly (42.15% and 20.05%, respectively), where GST activity increased significantly in only proline supplemented maize seedlings over NaCl treatment. It increased 16.94% activity. Though the application of both proline and betaine increased the activity slightly over untreated control, the activity levels were statistically similar.

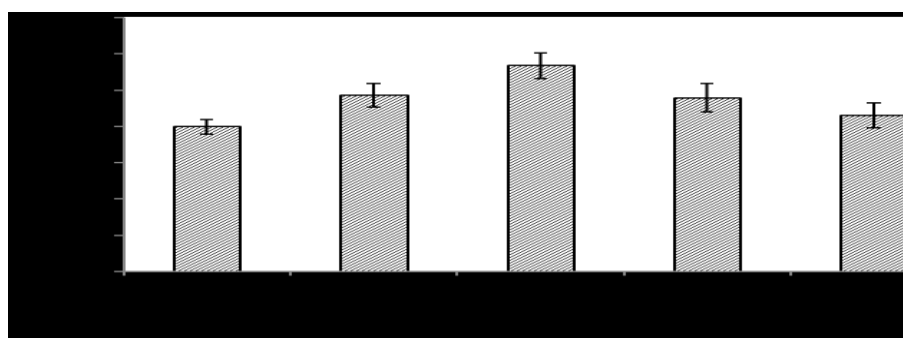


Fig. 7. Changes in the activity of GST in maize seedlings under 12 dS/m salinity stress supplemented with proline and betaine at 3 day stress

Though at 5 day stress at 12 dS/m salinity showed higher GST activities than 3 day stress, the activity levels were lower than those of 5 day stressed seedlings under 8 dS/m salinity (Fig. 8 and Table 3). All the treatments increased the activity significantly over control (Fig. 8). The 12 dS/m salinity increased 24.76% activity suggesting its inducibility under salinity stress. This activity was found to be further augmented in presence of 15 mM proline, while, it increased the activity (23.87%) significantly over NaCl.

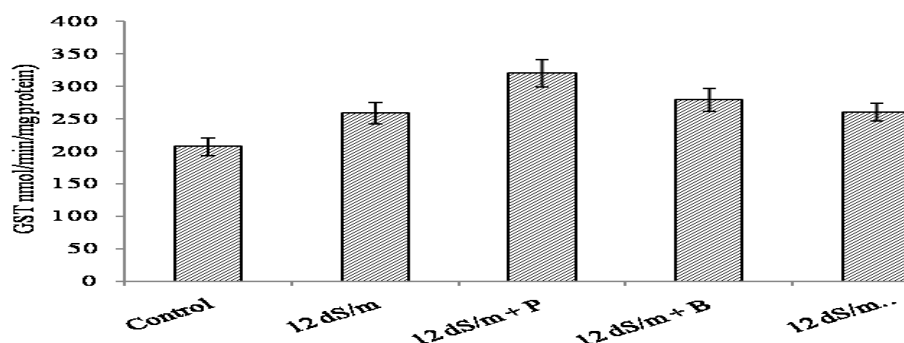


Fig. 8 Changes in the activity of GST in maize seedlings under 12 dS/m salinity stress supplemented with proline and betaine at 5 day stress

At 7 day stress, 12 dS/m salinity the GST activity was found to increase slightly (5.25%) over control, but statistically insignificant (Fig. 9 and Table 3). Proline alone and in combination with betaine increased the activity over control (38.71% and 24.32%, respectively) and NaCl (31.80% and 18.12%, respectively). The activity increased by betaine alone was significantly higher (22.68%) than the untreated control.

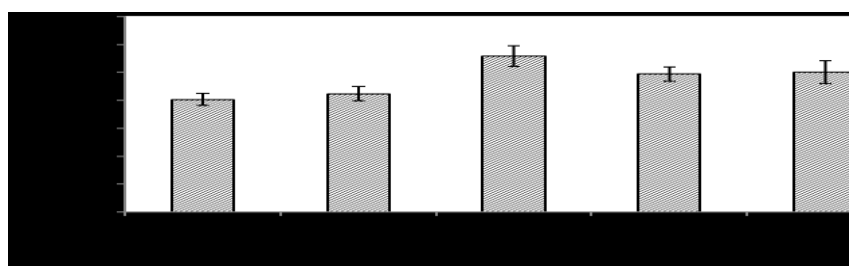


Fig. 9. Changes in the activity of GST in maize seedlings under 12 dS/m salinity stress supplemented with proline and betaine at 7 day stress

Table 3. Percentage of activity changed over control and 12 dS/m NaCl treatment in maize seedlings supplemented with proline and betaine

Treatment	% activity increased over control			% activity increased over NaCl		
	3 day	5 day	7 day	3 day	5 day	7 day
12 dS/m	21.74	24.76	5.25			
12 dS/m + P	42.15	54.54	38.71	16.94	23.87	31.80
12 dS/m + B	20.05	34.75	22.68	-1.39	8.01	16.57
12 dS/m + P + B	7.88	25.51	24.32	-11.38	0.60	18.12

3.4 Changes in GST activities at 16 dS/m salinity

At 3 day stress under 16 dS/m salinity increased the activity (18.72%) but it was statistically similar to the activity level of untreated control (Fig. 10 and Table 4). Though the application of proline at 15 mM remained the activity slightly (10.40%) under 16 dS/m salinity, it was insignificant over NaCl. Therefore, at this stage, the lower rate of increased activity due to toxic effect of high salinity as well as the detrimental effect of ROS produced under salinity stress.

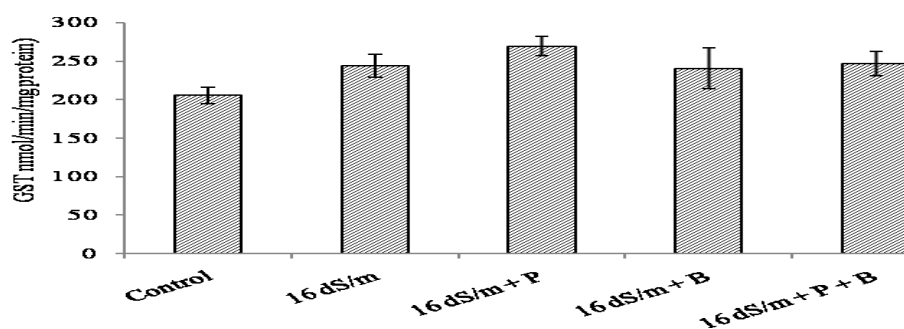


Fig. 10. Changes in the activity of GST in maize seedlings under 16 dS/m salinity stress supplemented with proline and betaine at 3 day stress

At 5 day of stress, though all the treatment increased the activity significantly over control, the only application of proline maintained the activity significantly higher over 16 dS/m salinity (Fig. 11). This level of salinity increased 14.96% activity over control which was statistically similar to the activity levels under salinity with betaine and salinity with proline plus betaine (Table 4). Proline maintained the activities 51.07% and 31.41% higher over control and 16 dS/m salinity at this stage.

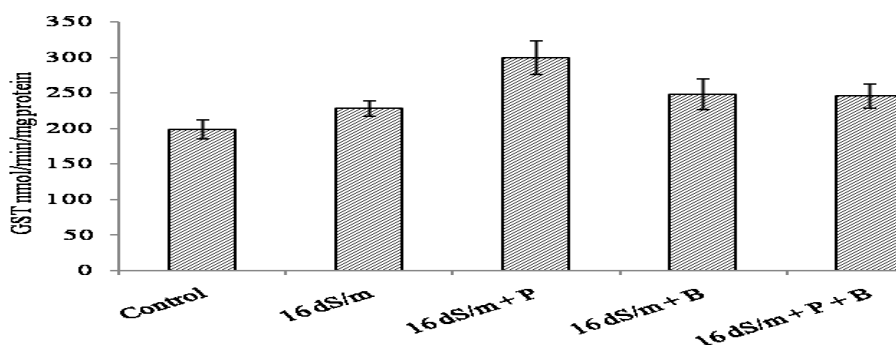


Fig. 11. Changes in the activity of GST in maize seedlings under 16 dS/m salinity stress supplemented with proline and betaine at 5 day stress

At 7 day stress the GST activity was found to be down-regulated over control at 16 dS/m salinity and both proline and betaine maintained the activities significantly higher over NaCl. GST activities increased 24.77% and 58.97% over control and NaCl, respectively in proline treated seedlings at this stage. Interestingly, at this high salinity stress betaine also maintained the activity higher over salinity stress alone (Fig. 12 and Table 4).

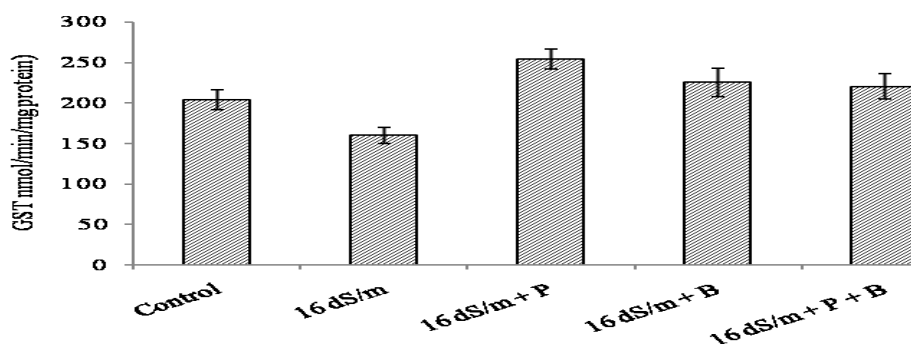


Fig. 12. Changes in the activity of GST in maize seedlings under 16 dS/m salinity stress supplemented with proline and betaine at 7 day stress

Table 4. Percentage of activity changed over control and 16 dS/m NaCl treatment in maize seedlings supplemented with proline and betaine

Treatment	% activity increased over control			% activity increased over NaCl		
	3 day	5 day	7 day	3 day	5 day	7 day
16 dS/m	18.72	14.96	-21.52			
16 dS/m + P	31.06	51.07	24.77	10.40	31.41	58.97
16 dS/m + B	16.97	25.04	10.65	-1.47	8.76	40.98
16 dS/m + P + B	20.08	23.68	8.23	1.15	7.58	37.90

In this study, the GST activities increased with increasing concentration of salinity and duration of stress (Fig. 1-12) which suggested that GST could be induced to eliminate the toxic lipid peroxidises and H₂O₂, as production of these compounds are common phenomenon under abiotic stresses including NaCl (Awasthi *et al.*, 2003b; Yang *et al.*, 2003; Alexios *et al.*, 1999; Hossain and Fujita, 2010). Here GST could important role in protecting cells through antioxidant and detoxification activities. At higher concentration and at 7 day stress, the decreased GST activities might be due to loss of cellular membrane integrity due to accumulation of abundant lipid peroxidation products like 4-Hydroxynonenal (HNE) and MDA (Awasthi *et al.*, 2003b; Hossain and Fujita, 2010). Under saline stress upregulation of GST activities also reported by Hoque *et al.*, (2007a) in tobacco, Rohman *et al.*, (2009) in onion. Recently, Csiszár *et al.* (2011) reported the higher GST activities in tomato plant

under salinity stress suggesting its stress tolerant. Csiszár *et al.* (2011) also reported that supplementation of salicylic acid enhance the activity under this saline condition.

Proline and betaine two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals. In this study, the foliar application of proline at 3 day, 5 day and 7 day stress of 4 dS/m, 8 dS/m, 12 dS/m and 16 dS/m salinity the GST activities increased over control and over NaCl treatment. Ketchum *et al.* (1991), Ober and Sharp (1994), Sharp *et al.* (1994) found the similar result. It may be due to drought or salinity stress in plants, proline accumulation normally occurs in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions, protein compatible hydrotrope (Srinivas and Balasubramanian, 1995), alleviating cytoplasmic acidosis, and maintaining appropriate NADP+/NADPH ratios compatible with metabolism (Hare and Cress, 1997). Furthermore, proline is known to induce expression of salt stress responsive genes, which possess proline responsive elements (e.g. *PRE*, *ACTCAT*) in their promoters (Satoh *et al.*, 2002; Oono *et al.*, 2003; Chinnusamy *et al.*, 2005).

On the other hand, betaine also mitigates adverse effects of salinity stress in some plant species (Måkelä *et al.*, 1998a, b). In this study, the foliar application of betaine at 3 day, 5 day and 7 day stress of 4 dS/m, 8 dS/m, 12 dS/m and 16 dS/m salinity the GST activities increased over control and over NaCl treatment; but only 8 dS/m salinity at 7 day stress, 12 dS/m at 3 day stress, 16 dS/m at 3 day stress it decreased the activity over NaCl treatment. Harinasut *et al.* (1996), Lutts (2000) found the similar result. In maize (*Zea mays*, L.), exogenously applied betaine improved growth, leaf water content, net photosynthesis, and the apparent quantum yield of photosynthesis of the salt-stressed plants (Yang and Lu, 2005). Betaine application, however, did not affect maximum efficiency of PSII photochemistry. The improvement in photosynthesis of salt-stressed maize plants by betaine application was suggested to be associated with improvements in stomatal conductance and actual PSII efficiency. Betaine-treated plants had significantly lower Na⁺ and higher K⁺ concentrations in the shoots, compared with untreated plants. Furthermore, salt-induced ultrastructural damages to the leaf, including swelling of thylakoids, disintegration of grana stacking and intergranal lamellae, and destruction of mitochondria, were prevented by betaine pre-treatment. These beneficiary effects in the shoot were suggested to be due to betaine-induced production of additional vacuoles in root cells, which resulted in a greater accumulation of Na⁺ in the root and a decrease in its transportation to the shoot. In the root, the most frequently observed ultra-structural change due to salt stress was an increase in the number and aggregation of mitochondria in the cytoplasm of the root tip and root cap cells. But at higher concentration and at higher day's duration, the decreased GST activities might be due to loss of cellular membrane integrity.

Both proline and betaine are reported to increase activities of antioxidant enzymes including GSTs (Hossain and Fujita, 2010; Hoque *et al.*, 2008) through activation. In this study, the exogenous application of both proline and betaine also activated GST in maize seedlings (Fig 1-12). In combination of both proline and betaine treatment 3 day, 5 day and 7 day stress of 4 dS/m, 8 dS/m, 12 dS/m and 16 dS/m salinity the GST activities increased over control and over NaCl treatment; but only 3 day and 7 day stress of 4 dS/m salinity, 3 day stress of 8 dS/m and 12 dS/m salinity decreased the activity over NaCl treatment. This may be due to in from maintaining the cell membrane integrity and photosynthesis system. They might keep the concentration of Na⁺ lower through creating vacuole.

Under abiotic stress production of that 4-Hydroxynonenal (HNE) and melondialdehyde (MDA) the major end products of lipid peroxidation which causes the membrane disruption (Awasthi *et al.*, 2004; Hossain and Fujita, 2010).

3.5 Changes in concentration of MDA in maize seedlings under salinity stress

Since GSTs are reported to detoxify indigenous cytotoxic compounds under stress condition (Marrs 1996). Recently Csiszár *et al.* (2011) reported the detoxification role of GSTs in melondialdehyde (MDA), the peroxidation product of polyunsaturated fatty acid. Therefore, in this study, the concentration of MDA in stressed maize seedlings to examine the detoxification role of maize GST. A linear increase was observed in the concentration of MDA in maize seedlings with duration of stress and concentration of salinity (Fig. 13). At 3 day stress, all of the salinity levels significantly increased the MDA contents, where, the concentration increased with the increasing salinity levels. At this level, content of MDA increased 31.53%, 37.77%, 39.74% and 45.40% over control at 4, 8, 12 and 16 dS/m salinity, respectively. This concentration further increased with duration. At 5 day stress, content of MDA increased 39.33%, 49.57%, 56.04% and 59.28% over control at 4, 8, 12 and 16 dS/m salinity, respectively, which further increased 59.90%, 63.05%, 73.02% and 77.27% over control at 4, 8, 12 and 16 dS/m salinity, respectively at seven day stress. Application proline and betaine were found to maintain the MDA concentration lower than the concentration at different salinity levels, though the concentrations were higher than the control (Fig. 13). Proline was found to reduce the MDA concentrations in

most of the cases. However, at higher concentrations betaine performed better. At 3 day stress, proline caused 9.24%, 12.25%, 11.68% and 12.14% reduction in MDA contents over 4, 8, 12 and 16 dS/m salinity, respectively, where, betaine caused 8.21%, 4.57%, 2.79% and 9.02% reduction and combination of proline and betaine caused 11.29%, 7.59%, 4.58% and 9.54% reduction. At 5 day stress, proline caused 14.50%, 18.94%, 22.37% and 25.17% reduction in MDA contents over 4, 8, 12 and 16 dS/m salinity, respectively, where, betaine caused 10.23%, 10.78%, 13.93% and 19.43% reduction and combination of proline and betaine caused 11.25%, 14.04%, 9.60% and 22.22% reduction. At 7 day stress, proline caused 20.05%, 24.91%, 22.80% and 19.84% reduction in MDA contents over 4, 8, 12 and 16 dS/m salinity respectively, where, betaine caused 15.41%, 15.00%, 18.57% and 27.63% reduction and combination of proline and betaine caused 15.40%, 17.81%, 20.51% and 18.69% reduction.

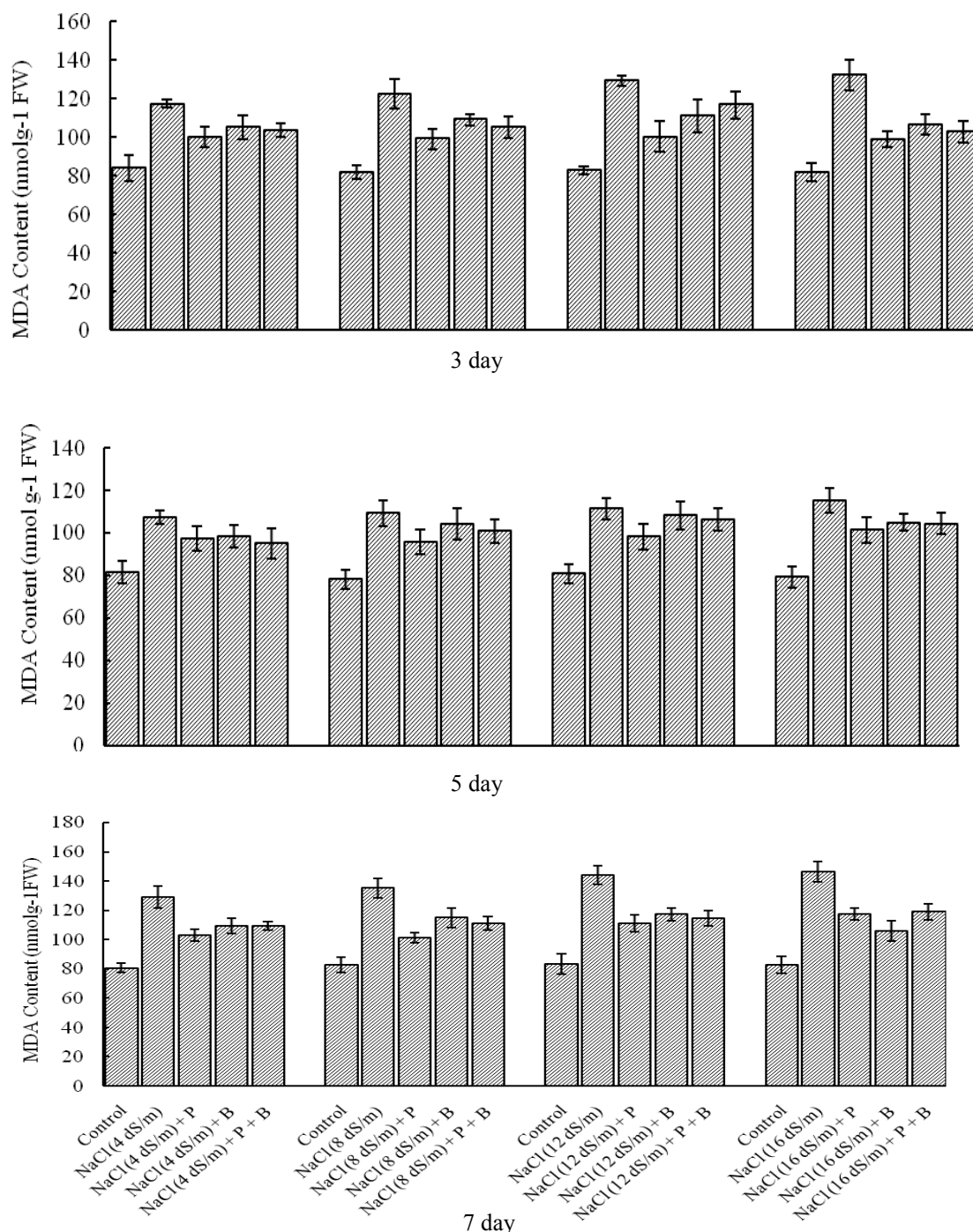


Fig. 13. Changes in MDA contents in maize seedlings under NaCl stress conditions supplemented with proline and betaine.

Importantly, the reduction in concentrations of MDA under salinity stress with supplementation of proline and betaine had correlation with the expression of GST activities. The maximum reduction of MDA caused in the seedlings where the highest expression of GST was observed. The simultaneous increase in MDA contents and GST activities indicated important biological relation between them under salinity stress. Recently, increased GST activity along with decreased content of MDA under salinity supplement with glycine and betaine has been reported (Hossain and Fujita, 2010; Hoque *et al.*, 2008). In this study, the decreased contents of MDA (Fig. 13) with increased GST activities (Fig. 1-12) in proline and betaine supplemented maize seedlings under NaCl stress suggesting the detoxification role of GST. These detoxifications might maintain the lower concentration of MDA confer better membrane integrity and photosynthesis (Csizsár *et al.*, 2011).

IV. Conclusion

GST activities changes in different levels with duration of stress and concentration of salinity. GST activity were increased gradually over control at 3 day, 5 day stress at 4 dS/m, 8 dS/m, 12 dS/m salinity level, but incase of 16 dS/m salinity at 7 day stress GST activity was found down regulated over control. In foliar application of proline GST activity were increased gradually over control and NaCl treatment. In betaine treated seedlings at 3 day, 5 day and 7 day stress of 4 dS/m, 8 dS/m, 12 dS/m and 16 dS/m GST activity were increased gradually over control, but only 8 dS/m salinity at 7 day stress, 12 dS/m at 3 day stress, 16 dS/m at 3 day stress it were decreased the activity over NaCl treatment. In combination of both proline and betaine treatment GST activity were increased gradually over control and NaCl treatment only at 3 day and 7 day stress of 4 dS/m salinity, 3 day of 8 dS/m salinity and 12 dS/m salinity decreased activity. Lower concentration of MDA suggested the detoxification role of maize GST.

References

- [1]. Abenavoli, M.R., D. Santis, C.M. Sidari, A. Sorgona, M. Badiani and G. Cacco. 2001. Influence of coumarin on the net nitrate uptake in durum wheat. *New Phytologist*. 150: 619-627.
- [2]. Alexios, N., Polidoros and J. G. Scandalios. 1999. Role of hydrogen peroxide and different classes of antioxidants in the regulation of catalase and glutathione S-transferase gene expression in maize (*Zea mays* L.). *Physiologia Plantarum*. 106: 112–120.
- [3]. Anderson, J.V. and D.G. Davis. 2004. Abiotic stress alters transcript profiles and activity of glutathione S-transferase, glutathione peroxidase, and glutathione reductase in *Euphorbia esula*. *Physiol Plant*. 120: 421-433.
- [4]. Ashraf, M. and M. R. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot*. 59:206-216.
- [5]. Atkins, W.M., W. Regina, W. Andrew and Y. H. Anthony. 2003. The catalytic mechanism of glutathione S-transferase (GSTs). *The Journal of Biological Chemistry*. 26: 267-268.
- [6]. Awasthi, Y.C., Y. Yang, N.K. Tiwari and S. Awasthi. 2004. Regulation of 4-hydroxynonenal-mediated signaling by glutathione S-transferases. *Free Radical Biology and Medicine*. 27(5): 607-619.
- [7]. Awasthi, S., S.S. Singhal, J. Singhal, Y. Yang, P. Zimniak, Y.C. Awasthi. 2003b. Role of RLIP76 in lung cancer doxorubicin resistance: III. Anti-RLIP76 antibodies trigger apoptosis in lung cancer cells and synergistically increase doxorubicin cytotoxicity. *Acta Biologica Szegediensis*. 56(1):69-70.
- [8]. Blokhina, O., E. Virolainen and K.V. Gagerstedt. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann Bot (Lond)*. 91: 179-194.
- [9]. Chinnusamy, V., A. Jagendorf and J. Zhu. 2005. Understanding and improving salt tolerance in plants. *Crop Sci*. 45:437–448.
- [10]. Csizsár, J., Z. Váry, E. Horváth, A. Gallé and I. Tari. 2011. Role of glutathione transferases in the improved acclimation to salt stress in salicylic acid-hardened tomato. *Acta Biologica Szegediensis*. 55(1):67-68.
- [11]. Dean, J.D., P.H. Goodwin and T. Hsiang. 2005. Induction of glutathione S-transferase genes of *Nicotiana benthamiana* following infection by *Colletotrichum destructivum* and *C. orbiculare* and involvement of one in resistance. *J. Exp. Bot*. 56: 1525-1533.
- [12]. Dixon, E.T., R. Mornet and M. Laloue. 2002. Mechanism of action of glutathione-dependent enzymes. *Adv. Enzymol. Relat. Areas Mol. Biol*. 59: 103–67.
- [13]. Hare, P.D. and W.A. Cress. 1997. Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regul*. 21: 79–102.
- [14]. Harinasut, P., K. Tsutsui, T. Takabe, M. Nomura and S. Kishitani. 1996. Exogenous glycine betaine accumulation and increased salt tolerance in rice seedlings. *Biosci. Biotechnol. Biochem*. 60, 366–368.
- [15]. Hoque, M.A., M.N.A. Banu, E. Okuma, K. Amako, Y. Nakamura and Y. Murata 2007a Exogenous proline and glycinebetaine increase NaCl-induced ascorbate-glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells. *J Plant Physiol*. 164: 1457-1468.
- [16]. Hossain, M.A and M. Fujita. 2010. Evidence for a role of exogenous glycinebetaine and proline in antioxidant defense and methylglyoxal detoxification systems in mung bean seedlings under salt stress. *Physiol. Mol. Biol. Plants*. 16(1) : 19-29.
- [17]. Jepson, I., V.J. Lay, D.C. Holt, S.W.J. Bright and A. J. Greenland. 1994. Cloning and characterization of maize herbicide safener-induced cDNAs encoding subunits of glutathione-S-transferase isoform-I, isoform-II and isoform-IV. *Plant Molecular Biology*. 26, 1855-1866.
- [18]. Ketchum, R.E.B., R.C. Warren, L.J. Klima, F. Lopez-Gutierrez, M.W. Nabors. 1991. The mechanism and regulation of proline accumulation in suspensioncultures of the halophytic grass *Distichlis spicata* L. *J. Plant Physiol*. 137:368–374.
- [19]. Lutts, S. 2000. Exogenous glycine betaine reduces sodium accumulation in salt-stressed rice plants. *Int. Rice Res. Notes* 25:39–40.
- [20]. M^aakela, P., K. Jokinen, M. Kontturi, S. Somersalo, 1998a. Foliar application of glycine betaine – a novel product from sugarbeet – as an approach to increase tomato yield. *Ind. Crops Prod*. 7: 139–148.
- [21]. M^aakela, P., P. Pelttonen-Sainio, K. Jokinen, E. Pehu and H. Setälä. 1998b. Effect of foliar application of glycine betaine on stomatal conductance, abscisic acid and solute concentrations in leaves of salt- and drought-stressed tomato. *Aust. J. Plant Physiol*. 25:655–663.

- [22]. Marrs, K.A. 1996. Functions and regulation of glutathione S-transferases in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 47: 127-158.
- [23]. Mueller, L.A., C.D. Goodman, R. A. Silady and V. Walbot. 2000. A petunia glutathione S-transferase required for anthocyanin sequestration, is a flavonoid-binding protein. *Plant Physiol.* 123: 1561-1570.
- [24]. Ober, E.S. and R.E. Sharp. 1994. Proline accumulation in maize (*Zea mays L.*) primary roots at low water potentials. I. Requirement for increased levels of abscisic acid. *Plant Physiol.* 105: 981–987.
- [25]. Oono, Y., C. Ooura, A. Rahman, E.T. Aspuria and H. Uchimiya. 2003. p-chlorophenoxyisobutyric acid impairs auxin response in *Arabidopsis* root. *Plant Physiol.* 133:1135–1147.
- [26]. Rohman, M.M., M.D. Hossain, T. Suzuki, G. Takada, and Fujita, M. 2009. Quercetin-4'-glucoside: a physiological inhibitor of the activities of dominant glutathione S-transferases in onion (*Allium cepa L.*) bulb. *Acta Physiol Plant.* 31: 301–309.
- [27]. Satoh, R., K., M. Nakashima and K. Shinozaki. 2002. A novel cis-acting element for proline- and hypoosmolarity-responsive expression of the ProDH gene encoding proline dehydrogenase in *Arabidopsis*. *Plant Physiol.* 130: 709–719.
- [28]. Sharp, R.E., Y. Wu., G.S. Voetberg and I.N. Saab. 1994. Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials. *J. Exp. Bot.* 45: 1717–1743.
- [29]. Srinivas, V. and D. Balasubramanian. 1995. Proline is a protein-compatible hydrotrope. *Plant Physiol.* 11: 2830–2833.
- [30]. Wang, L.P., Y.X. Zhao and H. Zhang. 2002. Cloning and sequencing of GST gene of *Suaeda salsa* and its expression characteristics. *J. Plant Physiol. Mol. Biol.* 28: 133-136.
- [31]. Yang, X. and C. Lu. 2005. Photosynthesis is improved by exogenous glycinebetaine in salt-stressed maize plants. *Physiol. Plant.* 124, 343–352.
- [32]. Yang, Y., J. Cheng, S.S. Singhal, M. Saini, U. Pandya, S. Awasthi and Y.C. Awasthi. 2001. Role of Glutathione S-transferase in protection against lipid peroxidation. *J. Biol. Chem.* 276: 19220-19230.
- [33]. Yang, Y., J. Cheng, S.S. Singhal, M. Saini, U. Pandya, S. Awasthi and Y.C. Awasthi. 2003. Studied on role of glutathione S-transferases in the regulation of lipid hydroperoxide levels reaffirm that GSTs play a key role in regulating lipid peroxidation by terminating the autocatalytic chain of lipid peroxidation. *Int J Oncol.* 21: 741–742.