Available online: www.notulaebotanicae.ro
Print ISSN 0255-965X; Electronic 1842-4309
Not Bot Horti Agrobo, 2014, 42(1):73-80

Exogenous Proline and Betaine-induced Upregulation of Glutathione Transferase and Glyoxalase I in Lentil (*Lens culinaris*) under Drought Stress

Md. Rezwan MOLLA[1], M. Rawshan ALI[2], Mirza HASANUZZAMAN[3], Mahamud Hossain AL-MAMUN[4], Asgar AHMED[2], M.A.N. NAZIM-UD-DOWLA[1], Md. Motiar ROHMAN[2]*

[1]*Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh*
[2]*Molecular Breeding Laboratory, Plant breeding Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh*
[3]*Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh; motiar_1@yahoo.com (*corresponding author)*
[4]*Olericulture Division, Horticultural Research Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh*

Abstract

This study was conducted to investigate the proline (Pro) and betaine (Bet) driven modulation of glutathione *S*-transferase (GST) and glyoxalase I (Gly I) in drought stressed lentil seedlings. Among the seven lentil varieties tested, BARI mosur 2 was found have higher activities of GST and Gly 1 which was selected for further time-course investigation. Drought stress resulted in a significant increase in glutathione (GSH), glutathione disulfide (GSSG) and hydrogen peroxide (H_2O_2) contents and GST and Gly I activities at different period of the stress, while the GST activity decreased significantly at one and three days drought. However, exogenous application of 15 mM Bet or Pro with drought stress resulted in an increase in GSH content, maintenance of high activities of GST and Gly I as compared to the control and mostly also drought stressed plants, with a concomitant decrease in GSSG content and H_2O_2 level suggesting important role in protecting cell from the toxic effects of reactive oxygen species (ROS) and methyl glyoxal (MG) detoxification system. The application of Pro and Bet with the stress period revealed that they have important role in upregulating the homeostasis in lentil under stress condition. However, Pro exhibited better protection than Bet under drought stress. These findings suggest that both Pro and Bet provided a protective role in drought induced oxidative stress by reducing H_2O_2 levels and by increasing the antioxidant defense systems.

Keywords: pulse crops, antioxidant defense, water stress, reactive oxygen species

Introduction

Plants are constantly challenged by various abiotic stresses, such as drought, salinity, cold, high temperature, chemical toxicity, high light intensity may 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 ($O_2^{\bullet-}$, $^1O_2$, $OH^{\cdot}$, $OH_2^{\bullet}$) 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 (Blokhina *et al.*, 2003). Osmotic stress caused by drought is one of the major abiotic factors limiting crop productivity because it affects almost all plant functions.

To counteract osmotic stress, many plants accumulate several kinds of compatible solutes such as Pro (Pro), betaine (Bet), sugars and polyols. Among them, Pro and Bet are the most common compatible solutes that contribute to osmotic adjustment (Greenway and Munns, 1980; Rhodes and Hanson, 1993; Hasegawa *et al.*, 2000; Ashraf and Foolad, 2007). In addition to their roles as osmoprotectants, Pro and Bet might perform a protective function by scavenging reactive oxygen species (ROS) (Hayat *et al.*, 2012). Both Pro and Bet improve salt tolerance in tobacco BY-2 cells by increasing the activity of enzymes involved in the antioxidant defense system (Hoque *et al.*, 2007a, b). Reactive oxygen species are highly reactive and toxic to plants and can lead to cell death by causing damage to proteins, lipids, DNA and carbohydrates (Noctor and Foyer, 1998; Apel and Hirt, 2004). Plants possess both enzymatic and non-enzymatic antioxidant defense systems to protect their cells against ROS (Noctor and Foyer, 1998;

Apel and Hirt, 2004). Thiol or thiol-containing compounds are chemically the most active groups found in cells and are known to act as antioxidants, and participate in the detoxification of xenobiotics. Reduced glutathione (GSH) is the most abundant low molecular weight thiol in plants and plays an important role in the detoxification of ROS (Noctor and Foyer, 1998; Noctor *et al.*, 2002; Smirnoff, 2005; Gill *et al.*, 2013). Glutathione dependent enzymes, glutathione *S*-transferases (GSTs; EC 2.5.1.18) and Glyoxalase I (Gly-1) play important role in stress combating through homeostatic and detoxification system. GSTs are the most studied multifunctional stress-inducible family enzyme which catalyze the conjugation of the tripeptide glutathione (*γ*-Glu-Cys-Gly; GSH) to a variety of electrophilic compounds to direct them to specific sites both intra- and extra-cellularly. Recently, these two enzyme systems have been reported as selection criteria for stress tolerant genotypes (Hefny and Abdul-Kader, 2007). On the other hand, pulse crop including lentil (*Lens culinaris*) is important source of sulphur which have important role in GSH metabolism. Since lentil is an important pulse crop and is sensitive to drought, for better understanding the mechanism of homeostatic role, this study was designed to examine the regulatory activities of the GST and Gly-I in lentil under draught condition with supplement of Pro and Bet.

Materials and Methods

Plant materials and stress treatments

Seeds of seven lentil (*Lens culinaris* L.) varieties viz. 'BARI' masur 1, 2, 3, 4, 5, 6 and 7 were collected from the Pulse Crop Research Division, Bangladesh Agricultural Research Institute. Seeds of uniform size were selected and surface-washed several times with sterile distilled water. The seeds were then sown in pot. Germinated seedlings were then allowed to grow under normal conditions (light, 100 μmol photon m^{-2} s^{-1}; temperature, 25±2 °C; RH, 65-70%) using Hogland solution as a source of nutrients. After 12 days, Lentil (BARI masur 2) seedlings were further grown without (control) and treated with 15 mM Pro and Bet solution at 1, 3, 5 and 7 day drought condition. The experiment was repeated three times under the same conditions.

Measurement of H_2O_2

H_2O_2 was assayed according to the method described by Yu *et al.*. (2003). H_2O_2 was extracted by homogenizing 0.5 g of leaf samples with 3 ml of 50 mM potassium-phosphate (K-P) buffer (pH 6.5) at 4 °C. The homogenate was centrifuged at 11500×*g* for 15 min. Three ml of supernatant was mixed with 1 ml of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v) and kept in room temperature for 10 min. After that the mixture was again centrifuged at 11500×*g* for 15 min. The optical absorption of the supernatant was measured spectrophotometrically (UV-1800, Simadzu, Japan) at 410 nm to determine the H_2O_2 content (ϵ=0.28 $\mu M^{-1}cm^{-1}$) and expressed as μmol g^{-1} fresh weight.

Extraction and measurement of glutathione

Fresh leaves with shoot were homogenized in ice-cold acidic extraction buffer (5% meta-phosphoric acid containing 1 mM EDTA) using a mortar and pestle. Homogenates were centrifuged at 11,500×*g* for 15 min at 4 °C and the supernatant was collected for analysis of glutathione.

The glutathione pool was assayed according to previously described methods (Yu *et al.*, 2003) with modifications (Paradiso *et al.*, 2008) utilizing 200 μl of aliquots of supernatant neutralized with 300 μl of 0.5 M K-P buffer (pH 7.0). Based on enzymatic recycling, GSH is oxidized by 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) and reduced by NADPH in the presence of GR, and glutathione content is evaluated by the rate of absorption changes at 412 nm of 2-nitro-5-thiobenzoic acid (NTB) generated from the reduction of DTNB. GSSG was determined after removal of GSH by 2-vinylpyridine derivatization. Standard curves with known concentrations of GSH and GSSG were used. The content of GSH was calculated by subtracting GSSG from total glutathione.

Determination of protein

The protein concentration of each sample was determined following the method of Bradford (1976) using BSA as a protein standard.

Enzyme extraction and assays

Using a pre-cooled mortar and pestle, 0.5 g of leaf tissue was homogenized in 1 ml of 50 mM ice-cold K-P buffer (pH 7.0) containing 100 mM KCl, 1 mM ascorbate, 5 mM *β*-mercaptoethanol and 10% (w/v) glycerol. The homogenates were centrifuged at 11500×*g* for 10 min and the supernatants were used for determination of enzyme activity. All procedures were performed at 0-4 °C.

GST (EC:2.5.1.18) activity was determined spectrophotometrically by the method of Rohman *et al.*, (2009) with some modifications. The reaction mixture contained 100 mM Tris-HCl buffer (pH 6.5), 1.5 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), and enzyme solution in a final volume of 700 μl. The enzyme reaction was initiated by the addition of CDNB and the increase in absorbance was measured at 340 nm for 1 min. The activity was calculated using the extinction coefficient of 9.6 $mM^{-1}cm^{-1}$.

Glyoxalase I (EC:4.4.1.5) assay was carried out according to Hasanuzzaman and Fujita (2013). Briefly, the assay mixture contained 100 mM K-P buffer (pH 7.0), 15 mM magnesium sulphate, 1.7 mM GSH and 3.5 mM MG in a final volume of 700 μl. The reaction was started by the addition of MG and the increase in absorbance was recorded at 240 nm for 1 min. The activity was calculated using the extinction coefficient of 3.37 mM^{-1} cm^{-1}.

Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) and the mean differences were compared by a Tukey's Test using SAS software (Addinsoft 2010). Differences at $p<0.05$ were considered significant.

Results and Discussion

Before stress implementation, the activities of GST and Gly I were compared among the lentil varieties. Among the

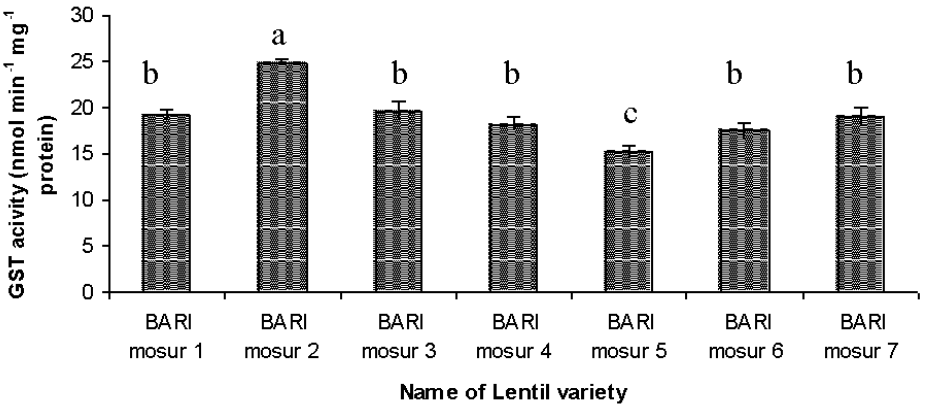

Fig. 1. Activities of glutathione *S*-transferase (GST) in lentil varieties under control condition

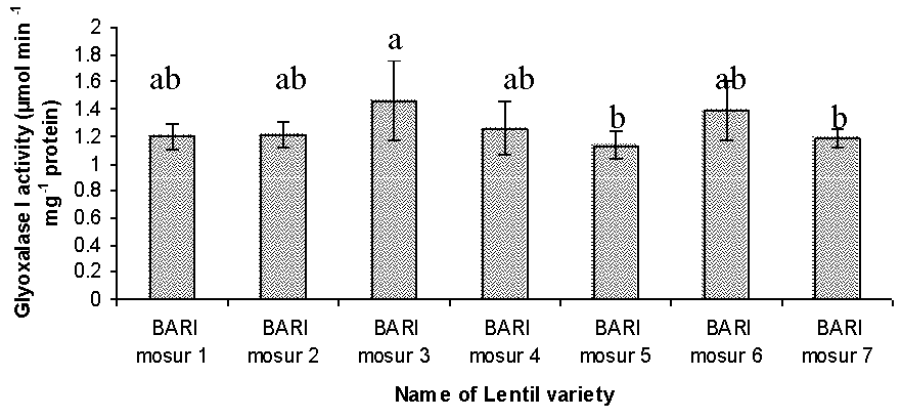

Fig. 2. Activities of glyoxalase I (Gly 1) in lentil varieties under control condition

lentil varieties, BARI mosur 2 exhibited the highest GST activity, while BARI masur 3 had the highest Gly I activity (Figs. 1 and 2). However, considering all, BARI masur 2 was selected for details study.

Activities of glutathione S-transferase

Incidences of drought stress in plants are might linked to osmoregulation problems and oxidative damage. Plant accumulates compatible solute such as Pro and Bet to mitigate the damaging effects of salt stress. Recent studies in plants have demonstrated that exogenous application of Bet or Pro at high concentration (1–20 mM) enhance tolerance to abiotic oxidative stress (Park *et al.*, 2006; Hoque *et al.*, 2007a, b; Huang *et al.*, 2009; Islam *et al.*, 2010; Hossain and Fujita, 2010). To check whether the upregulation of glutathione GST in lentil seedling plants in response to drought stress, its levels were measured in one, three, five and seven days seedlings under control as well as stressful conditions, It was found that GST activity decreased slightly (15 and 20%) in response to one and three days drought stress (Fig. 3). In contrary, GST activity increased sharply due to duration of stress followed by 24% at five days and 54% at seven days stress. Moreover, exogenous Pro and Bet exhibited further increase in GST activity by 61% and 41%, respectively, under five and seven days drought stress.

The plant GSTs are a large and diverse group of enzymes that catalyze the conjugation of electrophilic xenobiotic substrates with the GSH and it is associated with responses to various forms of abiotic stress and confers stress tolerance in plants (Hossain *et al.*, 2006; Dixon *et al.*, 2010). In our study, GST activity in lentil seedlings profoundly increased in response to drought stress (Fig. 3). More importantly, drought stress seedlings supplemented with Pro and Bet also increased the activities further. This enhanced activity of GST decreased the levels of H_2O_2 and lipid peroxidation in lentil seedlings under drought stress condition (Fig. 4). Similar increases in GST activity after Pro and Bet supplementation under stress were observed by other researchers (Hasanuzzaman and Fujita, 2011). The higher level of GSTs activities in lentil seedlings suggested that the enzymes are capable of detoxifying a maximum amount of toxins in the presence of sufficient GSH. Beside detoxification, they also play important role in other physiological functions such as GSH peroxidase activity (Bartling *et al.*, 1993; Mannervik and Danielson, 1988) and intracellular binding and transport of phytochemicals (Edwards *et al.*, 2000). However, the fact that exogenous Pro and Bet increased GST activities and maintained higher GSH level indicates that both Pro and Bet might play a significant role in GSH biosynthesis and metabolism. Transgenic tobacco plants overexpressing both GST and

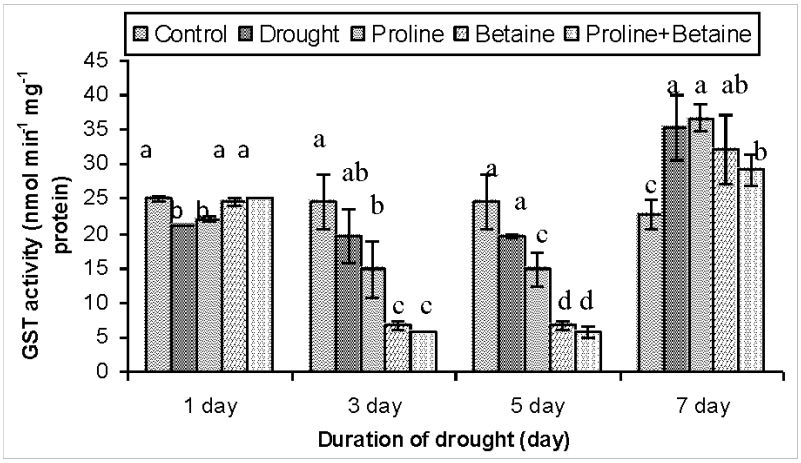

Fig. 3. Activities of glutathione *S*-transferase (GST) in lentil cv. BARI mosur 2 under control and stress condition. Fifteen mM of Proline (P) and betaine (B) was treated as foliar spray twice a day. Data were measured in soluble protein extracts of seedling after 1, 3, 5 and 7 days of stress implementation. Values represent the means ± SE from three independent experiments.

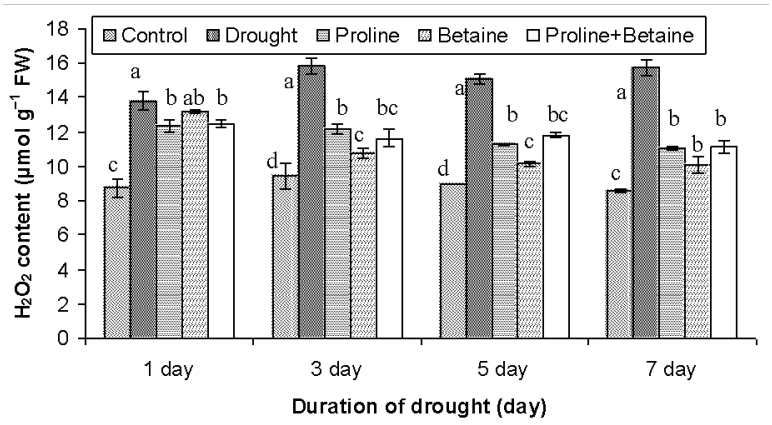

Fig. 4. Content of Hydrogen peroxide (H_2O_2) in lentil cv. BARI mosur 2 under control and stress condition. Fifteen mM of Proline (P) and betaine (B) was treated as foliar spray twice a day. Data were measured in soluble protein extracts of seedling after 1, 3, 5 and 7 days of stress implementation. Values represent the means ± SE from three independent experiments

GPX also displayed improved tolerance to salinity and chilling stress (Roxas *et al.*, 1997).

Level of hydrogen peroxide

A significant increase in the H_2O_2 level was observed in Lentil in response to drought stress and compared to control the levels was 69 and 84% higher at five and seven day drought treatment respectively (Fig. 4). Proline and Bet supplemented drought-stressed seedlings also showed continual increase in H_2O_2 content. However, the level of H_2O_2 content was significantly lower than the seedlings treated with drought alone after different days of treatment. After seven days of treatment Pro and Bet supplemented drought-stressed seedlings showed 30 % and 36 % decrease in H_2O_2 content as compared to the seedlings subjected to drought stress without Pro and Bet.

Glutathione contents

Reduced glutathione (GSH) is a key component of the antioxidant network that scavenges ROS either directly or indirectly by participating in the ascorbate glutathione cycle (Noctor and Foyer, 1998; Smirnoff, 2005).

The central role of GSH in the antioxidant defense system is due to its ability to regenerate ascorbate through reduction of dehydroascorbate via the ascorbate glutathione cycle (Noctor and Foyer, 1998). It is known that (GSH) is essential for effective scavenging of toxic compounds (such as H_2O_2 and organic H_2O_2) and for maintenance of other antioxidants.

In addition, GSH plays a vital role in the antioxidant defense system as well as the glyoxalase system by acting as a substrate or cofactor for certain enzymes like glutathione peroxidase (GPX), GST and Gly I and thus participate in removal of ROS, methylglyoxal (MG) and other endogenous toxic compounds (Noctor *et al.*, 2012).

To understand the GSH-dependent protective mechanisms offered by Pro and Bet in plant responses to oxidative stress, GSH level and its associated enzyme activities induced by Pro and Bet under drought stress are discussed. In this study, slightly decreases in GSH contents were observed in response to one day drought stress, as

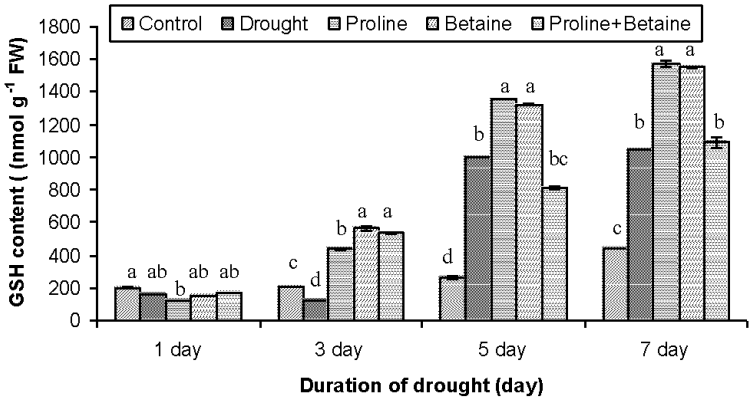

Fig. 5. Content of reduced glutathione (GSH) in lentil cv. BARI mosur 2 under control and stress condition. Fifteen mM of Proline (P) and betaine (B) was treated as foliar spray twice a day. Data were measured in soluble protein extracts of seedling after 1, 3, 5 and 7 days of stress implementation. Values represent the means ± SE from three independent experiments

compared to the control (Fig. 5). On the contrary, the amount of GSH increased dramatically after supplementation of Pro and Bet with extent of drought stress. The increased level of the GSH pool is generally regarded as a protective response against oxidative stress (May and Leaver, 1993; Xiang and Oliver, 1998), although defense against stress situations sometimes occurs irrespective of the GSH concentration (Potters *et al.*, 2004). The seedlings exposed to seven days of drought stress showed highest amount of GSH (1569.90 nmol/g FW) in presence of Pro compared to their respective control (Fig. 5). An increase in GSH content due to Pro pretreatment and lower lipid peroxidation has recently been reported (Xu *et al.*, 2009). Therefore, both Pro and Bet might play a significant role in maintaining higher GSH level either through efficient recycling or by modulating higher GSH synthesis (Kocsy *et al.*, 2005; Hossain and Fujita, 2010).

Glutathione disulfide content

Drought stress significantly increased the content of GSSG in lentil seedlings (Fig. 6). On the other hand, the seedlings in response to one day drought stress which were supplemented with Pro and Bet maintained the GSSG levels lower than the seedlings that were grown under drought condition without Pro and Bet supplementation. At seven days after supplementation, drought stress caused a significant decrease in the amount of GSSG, whereas, importantly, both Pro and Bet could not significantly contributed to the maintenance of the drought-induced redox state. Our results indicated that an increase GSH biosynthesis is an immediate response to drought stress and a drastic reduction of GSH concentration at seven days due to high drought stress and simultaneous increase in GSSG (Fig. 1a-C), a true indication of oxidative stress. The formation of GSSG in drought-treated seedlings might be due to the reaction of GSH with oxyradicals generated due to oxidative stress or due to enhancement of GPX activity that decompose H_2O_2 (Aravind and Prasad, 2005; Shalata *et al.*, 2001) as well as dehydroascorbate reductase (DHAR) activity (Hoque *et al.*, 2007b) which required GSH to regenerate ascorbate from dehydroascorbate. Proline and Bet supplemented drought stressed seedlings maintained higher GSH level and glutathione redox state with simultaneous decrease in the oxidized GSSG (Figs. 5 and 6).

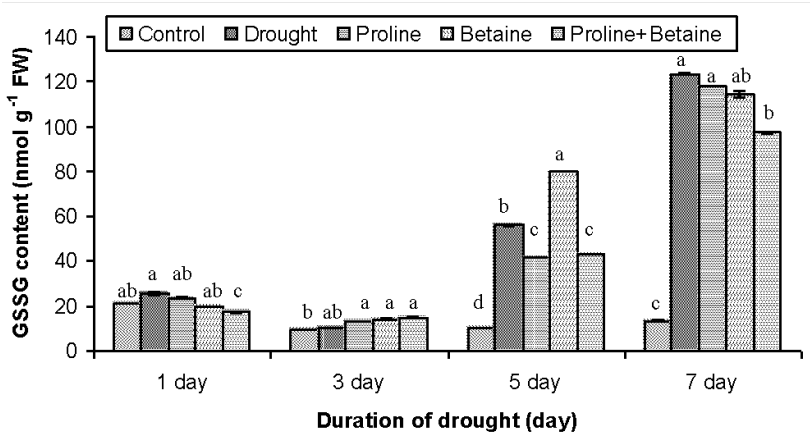

Fig. 6. Content of oxidized glutathione (GSSG) in lentil cv. BARI mosur 2 under control and stress condition. Fifteen mM of Proline (P) and betaine (B) was treated as foliar spray twice a day. Data were measured in soluble protein extracts of seedling after 1, 3, 5 and 7 days of stress implementation. Values represent the means ± SE from three independent experiments

Activity of glyoxalase I

Glyoxalase I could be expected to be a house-keeping protein present in all cells. Although up-regulation of glyoxalase I activity in response to salt, water deficit, ABA and heavy metal stress treatments has been reported earlier in plant (Espartero *et al.*, 1995; Veena *et al*., 1999; Yadav *et al.*, 2005). It is conceivable that an elevated level of glyoxalase I activity is required to remove MG, a toxic and unavoidable by-product of triosephosphate metabolism produced in ample amounts under normal and various stressful conditions. In this study, dramatic increase in Gly I activity was observed in response to drought stress. As shown in Fig. 7, drought treatment for three days resulted in 455% increase in Gly I activities compared to control. However, exogenous Pro and Bet application in drought stressed seedlings caused more increase in the activities of Gly I compared to drought alone. Upon three days of drought treatment with Pro and Bet the activity increased by 773 and 573%, respectively compared to control (Fig. 7). Application of Pro with drought treatment at three days five and seven days enhanced the activity of Gly I compared to drought treatment with Bet or combination of Pro and Bet (Fig. 7).

Increase in Gly I activity during stress tolerance may also indicate active metabolic status of the cell, in which cell division and growth are compromised in order to conserve energy for mobilization of resources towards stress tolerance and defense strategies. The gene expression profile of Gly I also showed true reflection of possible changes in activity levels due to different abiotic stresses. Moreover, overexpression of glyoxalase genes involved in the regulation of glutathione homeostasis (namely, GR, GST / GPX) in transgenic plants has been shown to result in an increased tolerance against oxidative stress (Broadbent *et al.*, 1995; Noctor *et al.*, 1998; Roxas *et al.*, 1997; Yadav *et al.*, 2005).

Conclusion

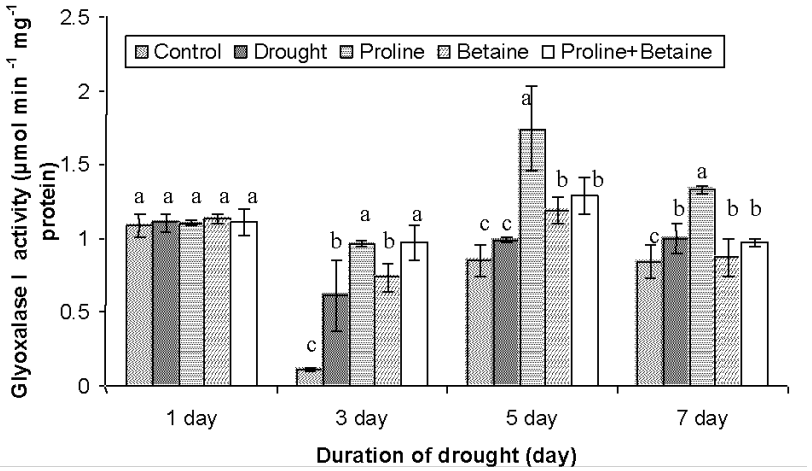

Fig. 7. Activities of glyoxalase I (Gly I) in lentil cv. BARI mosur 2 under control and stress condition. Fifteen mM of Proline (P) and betaine (B) was treated as foliar spray twice a day. Data were measured in soluble protein extracts of seedling after 1, 3, 5 and 7 days of stress implementation. Values represent the means ± SE from three independent experiments

In conclusion, drought stress induces a severe oxidative stress in lentil seedlings where the antioxidant defense and glyoxalase systems seemingly fail to combat with the stress-induced oxidative damage. Exogenous applications of Pro or Bet showed enhance tolerance to oxidative damage by enhancing ROS and MG detoxification systems. These findings suggest that both Pro and Bet provide protective effects against drought induced oxidative stress by reducing H_2O_2 and by increasing the antioxidant defense and glyoxalase systems. However, Pro provided better protective roles that Bet. Therefore, it was concluded that GST and glyoxalase system mediated detoxification and antioxidant activities played important roles with supplementation of Pro and Bet.

References

Apel K, Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:73-99.

Aravind PA, Prasad NV (2005). Modulation of cadmium induced oxidative stress in *Ceratophyllum demersum* by zinc involves ascorbate-glutathione cycle and glutathione metabolism. Plant Physiol Biochem 45:107-116.

Ashraf M, Foolad MR (2007). Roles of glycinebetaine and praline in improving plant abiotic stress resistance. Environ Exp Bot 59:206-16.

Bartling D, Radzio R, Steiner U, Weiler EW (1993). A glutathione *S*-transferase with glutathione-peroxidase activity from *Arabidopsis thaliana*: Molecular cloning and functional characterization. Eur J Biochem 216:579-586.

Blokhina O, Virolainen E, Gagerstedt KV (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 91:179-194.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254.

Broadbent P, Creissen GP, Kular B, Wellburn AR, Mullineaux P (1995). Oxidative stress responses in transgenic tobacco containing altered levels of glutathione reductase activity. Plant J 8: 247-255.

Dixon DP, Skipsey M, Edwards R (2010). Roles for glutathione transferases in plant secondary metabolism. Phytochem 71:338-350.

Edwards R, Dixon DP, Walbot V (2000). Plant glutathione *S*-transferases: Enzymes with multiple functions in sickness and health. Trends Plant Sci 5:193-198.

Espartero J, Aguayo IS, Pardo JM (1995). Molecular characterization of glyoxalase I from a higher plant; upregulation by stress. Plant Mol Biol 29:1223-1233.

Gill SS, Anjum NA, Hasanuzzaman M, Gill R, Trivedi DK, Ahmad I, Pereira, Tuteja N (2013) Glutathione reductase and glutathione: a boon in disguise for plant abiotic stress defense operations. Plant Physiol Biochem 70:20-212.

Greenway H, Munns R (1980). Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31:149-90.

Hasanuzzaman M, Fujita M (2011). Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biol Trace Elem Res 143:1758-1776.

Hasanuzzaman M, Fujita M (2013). Exogenous sodium nitroprusside alleviate arsenic-induced oxidative stress in wheat seedlings by enhancing antioxidant defense and glyoxalase system. Ecotoxicology 22:584-596.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51:463-99.

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012). Role of proline under changing environments: A review. Plant Signal Behav 7:1-11.

Hefny M, Abdel-Kader DZ (2007). Antioxidant-Enzyme System as Selection Criteria for Salt Tolerance in Forage Sorghum Genotypes (*Sorghum bicolor* L. Moench. Int J Plant Breed Genet 1:38-53.

Hoque MA, Banu MNA, Okuma E, Amako K, Nakamura Y, Shimoishi Y, Murata Y (2007b). Exogenous praline and glycinebetaine ingresses NaCl-induced ascorbateglutathione cycle enzyme activities and proline improves salt tolerance more than glycinebetaine in tobacco Bright yellow-2 suspension- cultured cells. J Plant Physiol 164:553-561.

Hoque MA, Okuma E, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y (2007a). Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. J Plant Physiol 164:553-561.

Hossain MA, Fujita M (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 Plant 16:19-29.

Hossain MZ, Hossain MD, Fujita M (2006). Induction of pumpkin glutathione *S*-transferase by different stresses and its possible mechanisms. Biol Plant 50:210-218.

Huang Y, Bie Z, Liu Z, Zhen A, Wang W (2009). Protective role of proline against salt stress is partially related to the improvement of water status and peroxidase enzyme activity in cucumber. Soil Sci Plant Nutr 55:698-704.

Islam MM, Hoque MA, Okuma E, Banu MNA, Shimoishi Y, Nakamura Y, Murata Y (2010). Exogenous proline and glycinebetaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. J Plant Physiol 166: 1587-1597.

Kocsy G, Laurie R, Szalai G, Szilagyi V, Simon-Sarkadi L, Galiba G (2005). Genetic manipulation of proline levels affects antioxidants in soybean subjected to simultaneous drought and heat stresses. Physiol Plant 124:227-35.

Mannervik B, Danielson UH (1988). Glutathione transferases–structure and catalytic activity. CRC Crit Rev Biochem 23:283-337.

May MJ, Leaver CJ (1993). Oxidative stimulation of glutathione synthesis in *Arabidopsis thaliana* suspension cultures. Plant Physiol 103:621-627.

Noctor G, Foyer CH (1998). Ascorbate and glutathione: Keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49:249-279.

Noctor G, Gomez L, Vanacker H, Foyer CH (2002). Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signaling. J Exp Bot 53:1283-304.

Noctor G, Mhamdi A, Chaouch S, Han Y, Neukermans J, Marquez-Garcia B, Queval G, Foyer CH (2012). Glutathione in plants: an integrated overview. Plant Cell Environ 35:454-484.

Paradiso A, Berardino R, de Pinto M, di Toppi LS, Storelli FT, de Gara L (2008). Increase in ascorbate-glutathione metabolism as local and precocious systemic responses induced by cadmium in durum wheat plants. Plant Cell Physiol 49:362-374.

Park EJ, Jeknic Z, Chen THH (2006). Exogenous application of glycinebetaine increases chilling tolerance in tomato plants. Plant Cell Physiol 47:706-714.

Potters G, Horemans N, Bellone S, Caubergs RJ, Trost P, Guisez Y (2004). Dehydroascorbate influences the plant cell cycle through a glutathione-independent reduction mechanism. Plant Physiol 134:1479-1487.

Rhodes D, Hanson AD (1993). Quaternary ammonium and tertiary sulfonium compounds in higher plants. Annu Rev Plant Physiol Plant Mol Biol 44:357-84.

Rohman MM, Hossain MD, Suzuki T, Takada G, 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.

Roxas VP, Smith RK, Ellen ER, Allen RD (1997). Overexpression of glutathione *S*-transferase/ glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. Plant Cell Physiol 41:1229-1234.

Shalata A, Mittova V, Volokita M, Guy M, Tal M (2001). Response of the cultivated tomato and its wild salt tolerant

relative *Lycopersicon pennellii* to salt-dependent oxidative stress: the root antioxidative system. Physiol Plant 112:487-494.

Smirnoff N (2005). Antioxidants and reactive oxygen species in plants. Oxford: Blackwell Publishing.

Veena, Reddy VS, Sopory SK (1999). Glyoxalase I from *Brassica juncea*: molecular cloning, regulation and its over-expression confer tolerance in transgenic tobacco under stress. Plant J 17:385-395.

Xiang C, Oliver DJ (1998). Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in *Arabidopsis*. Plant Cell 10:1539-1550.

Xu J, Yin HX, Li X (2009). Protective effects of proline against cadmium toxicity in micropropagated hyperaccumulator, *Solanum nigrum* L. Plant Cell Rep 28:325-353.

Yadav SK, Singla-Pareek SL, Ray M, Reddy MK, Sopory SK (2005). Transgenic tobacco plants overexpressing glyoxalase enzymes resist an increase in methylglyoxal and maintain higher reduced glutathione levels under salinity stress. FEBS Lett 579:6265-6271.

Yu CW, Murphy TM, Lin CH (2003). Hydrogen peroxide-induces chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. Funct Plant Biol 30:955-963.