Journal of Plant Sciences
2015; 3(6): 294-302
Published online November 14, 2015 (http://www.sciencepublishinggroup.com/j/jps)
doi: 10.11648/j.jps.20150306.12
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

Proline and Betaine Upregulated Glutathione Dependent Detoxification Enzymes in Tolerant Maize Seedlings under Saline Stress

Md. Motiar Rohman[1], M. R. Molla[2], Md. Mahfuzur Rahman[1], Asgar Ahmed[1], Arindam Biswas[1, 3], Mohammad Amiruzzaman[2]

[1]Molecular Breeding Lab, Plant Breeding Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh
[2]Molecular Biology Lab, Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh
[3]RARS, Bangladesh Agricultural Research Institute, Jessore, Bangladesh

Email address:

motiar_1@yahoo.com (M. M. Rohman), rezwanbt@gmail.com (M. R. Molla), r.gitla@gmail.com (Md. M. Rahman), asgar.gene@gmail.com (A. Ahmed), barindam19@yahoo.com (A. Biswas), amiruzzaman95@yahoo.com (M. Amiruzzaman)

To cite this article:

Md. Motiar Rohman, M. R. Molla, Md. Mahfuzur Rahman, Asgar Ahmed, Arindam Biswas, Mohammad Amiruzzaman. Proline and Betaine Upregulated Glutathione Dependent Detoxification Enzymes in Tolerant Maize Seedlings under Saline Stress. *Journal of Plant Sciences*. Vol. 3, No. 6, 2015, pp. 294-302. doi: 10.11648/j.jps.20150306.12

Abstract: Glutathione *S*-transferases (GSTs, EC: 2.5.1.18) and Glyoxalases [Glyoxalase-I (Gly-I, EC: 4.4.1.5) and glyoxalase-II (Gly-II, EC: 3.1.2.6)] are major glutathione dependent detoxification as well as important antioxidants enzymes in plants. On the other hand, proline and betaine are important osmoprotectants in plants under abiotic stresses including salinity. In this study, roles of GST, Gly-I, Gly-II and glutathione were investigated on cytotoxic metabolites in presence of 15 mM proline and betaine under 16 dSm^{-1} salinity in leaves of seedlings of a saline tolerant maize inbred CZ-10 to understand the underlying saline tolerant mechanism. The salinity stress increased the contents of H_2O_2, melondialdehyde (MDA), methylglyoxal (MG) along with decreased reduced glutathione (GSH) and glutathione redox state over control. The activities of GST and Gly-I increased under saline stress. However, activity of Gly-II decreased with stress duration. The application of proline and betaine in saline water reduced the contents of H_2O_2, MDA and MG. Conversely, proline and betaine increased the activities of GST, Gly-I and Gly-II, and GSH and glutathione-redox state over salinity stress. The western blotting of the soluble protein also suggested the accumulation of maize GST in leaf under salinity stress. The accumulation of GST along with reduced contents of H_2O_2 and MDA suggested its detoxification roles on organic hydroperoxides under saline stress. The higher activities of Gly-I and Gly-II concurrently with lower content of MG indicated their protective roles from cytotoxic MG. Considering all, this study concluded that both proline and betaine provided protective roles in maize seedlings under salinity stress by maintaining GSH and its related detoxification enzymes.

Keywords: Maize, Salinity, Glutathione *S*-transferase, Glyoxalases, Protective Role, Proline, Betaine

1. Introduction

Salinity is one of the most important abiotic stress factors limiting plant growth and productivity of crops and thus, increased soil salinity has become an increasingly important topic. High exogenous salt concentrations cause ionic imbalance in the cells resulting in ion toxicity and osmotic stress [1, 2]. Osmotic stress induced by salinity produced reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($OH^{\bullet}$) and concomitantly hydrogen peroxide (H_2O_2) [3, 4, 5] and methylglyoxal (MG) [6, 7] in plant cells. ROS are highly reactive and toxic to plants and can lead to cell death by causing damage to proteins, lipids, DNA and carbohydrates [5, 8]. At the same time, MG can react with and modify other molecules including DNA and proteins [6], whereas proteins being one of the major targets of ROS. Therefore ROS and MG are highly toxic and must be detoxified by cellular responses, if the plant is to survive and grow [9].

Proline and betaine are the most common compatible solutes that contribute to osmotic adjustment, stabilization and protection of membranes, proteins and enzymes [10, 11, 12]) from damaging effects of salt/osmotic stresses. In addition to

their roles as osmoprotectants, proline and betaine might perform a protective function by scavenging ROS [11, 13]. Exogenous proline and betaine improve salt tolerance by upregulating stress-protective proteins [14] and reducing oxidation of lipid membranes [1, 12].

Plants possess both non-enzymatic and enzymatic antioxidant defense systems to protect their cells against ROS [9, 15]. Among the non-enzymatic antioxidants, reduced glutathione (GSH) is the most abundant low molecular weight thiol in plants and plays an important role in the detoxification of ROS and MG [9]. Among the GSH dependent enzyme, glutathione *S*-transferases (GSTs) are an ancient and diverse group of multi-functional proteins that are widely distributed amongst living organisms. They can function as GSH transferases, GSH-dependent peroxidases, GSH-dependent isomerases and GSH-dependent oxidoreductases [16], as well as functioning as non-enzymatic carrier proteins and antioxidant recycling [17]. Up to 90 genes encoding GSTs are transcribed in different plant species, most of which are differentially induced by stress, and they play important parts in enzymatic thiol-dependent ROS scavenging mechanisms [18, 19, 20]. Importantly, after discovering of GST in maize in 1970, large number of studies was reported on vacuolar sequestration of endogenous substrates into vacuole [21, 22, 23, 24, 25]. However, reports on response of maize GST under saline stress is very limited. On the other hand, in plants, the MG is detoxified mainly by glyoxalase system [6] which consists of two enzymes: glyoxalase-I (Gly-I) and glyoxalase-II (Gly-II). Gly-I uses reduced GSH to convert MG into S-D-lactoylglutathione (SLG). Then Gly-II converts SLG to D-lactate and one molecule of reduced glutathione is recycled back into the system [26]. A large number of research group reported the role of glyoxalases in plant responses to salt stress [6, 7, 27, 28, 29]. Proline and betaine also reported to increase glyoxalaes [30, 31, 32], but this type of findings are limited in maize. In this report, the protective roles of GST, glyoxalases and GSH in maize seedlings in presence of proline and betaine under saline stress will be discussed.

2. Materials and Methods

2.1. Plant Materials and Stress Treatments

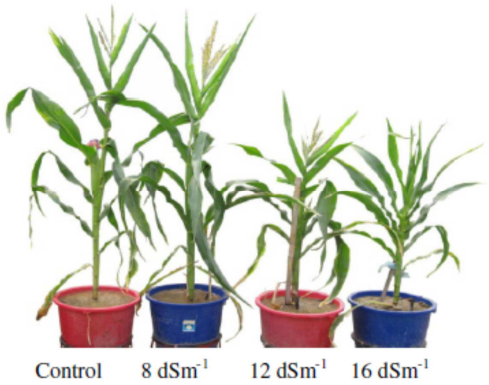

Fig. 1. *Performance of maize inbred CZ-10 under different concentrations of NaCl induced salinity.*

Twenty five maize inbreds were screened against different levels of salinity (8, 12 and 16 dSm^{-1} salinity). Among them CZ-10 survived whole life in all salinity levels (Fig. 1). Therefore, CZ-10 was termed as tolerant inbred and was selected as plant materials in this study. In this study, the seedlings were grown in Petridis (9 cm i.d.) and eight day old seedlings were subjected for imposing 16 dSm^{-1} salinity stress induced by NaCl with and without 15 mM proline and betaine. Data were recorded from fully expanded leaves after 1, 4 and 7 days of stress implementation and calculated from three independent experiments each containing at least two replications.

2.2. Extraction of Soluble Protein for GST and Glyoxalase Assay

Fresh leaves of 8-day old seedlings were extracted by homogenizing in an equal volume of 25 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, 1% (w/v) ascorbate and 10% (w/v) glycerol with mortar pestle. The homogenate was centrifuged at 11,500×g for 15 min, and the supernatant was used as a soluble protein solution for enzyme assay and Western blot analysis.

GST (EC: 2.5.1.18) activity was determined spectrophotometrically by the method of Rohman et al. [33]. 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 0.7 ml. 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}.

Gly-I (EC: 4.4.1.5) assay was carried out according to Yadav et al. [6]. Briefly, the assay mixture contained 100 mM Na-phosphate buffer (pH 7.0), 15 mM magnesium sulphate, 1.7 mM GSH, and 3.5 mM MG in a final volume of 0.8 ml. 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}.

Gly-II (EC: 3.1.2.6) activity was determined according to the method of Hoque et al. [32] by monitoring the formation of GSH at 412 nm for 1 min. The reaction mixture contained 100 mM Tris-HCl buffer (pH 7.2), 0.2 mM 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB), and 1 mM SLG in a final volume of 1 ml. The reaction was started by the addition of SLG, and the activity was calculated using the extinction coefficient of 13.6 mM^{-1} cm^{-1}.

2.3. Extraction and Measurement of Glutathione

Second leaves (0.5 g fresh weight) were homogenized in 3 ml 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 ascorbate and glutathione.

The glutathione pool was assayed according to Yu et al. [34],

utilizing 0.4 ml of aliquots of supernatant neutralized with 0.6 ml of 0.5 M K-phosphate buffer (pH 7.0). Based on enzymatic recycling, glutathione is oxidized by DTNB and reduced by NADPH in the presence of glutathione reductase (GR), and glutathione content is evaluated by the rate of absorbance changes at 412 nm of 2-nitro-5-thiobenzoic acid (NTB) generated from the reduction of DTNB. Oxidized glutathione (GSSG) was determined after removal of GSH by 2-vinylpyridine derivatization. Specific standard curves with GSH and GSSG were used.

2.4. Measurement of H_2O_2

H_2O_2 was assayed according to the method described by Yu et al. [34]. H_2O_2 was extracted by homogenizing 0.5 g of leaf tissue with 3 ml of 50 mM K-phosphate buffer (pH 6.5) at 4°C. The homogenate was centrifuged at 11,500×*g* for 15 min. The supernatant (3 ml) was mixed with 1 ml of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v), and the mixture was then centrifuged at 11,500×*g* for 15 min at room temperature. The optical absorption of the supernatant was measured spectrophotometrically at 410 nm to determine the H_2O_2 content (ϵ= 0.28 μM^{-1} cm^{-1}) and expressed as micromoles per gram fresh weight.

2.5. 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 [35]. Briefly, the leaf tissue (0.5 g) was homogenized in 3 ml 5% (w/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at 11,500×*g* for 10 min. The supernatant (1 ml) 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 11,500×*g* 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 nanomole of MDA per gram fresh weight.

2.6. Measurement of MG

Leaf tissue (0.3 g) was extracted in 3 ml of 0.5M perchloric acid. After incubating for 15 min on ice, the mixture was centrifuged at 4°C at 11,000×*g* for 10 min. A colored supernatant was obtained in some plant extracts that was decolorized by adding charcoal (10 mg/ml), kept for 15 min at room temperature, and centrifuged at 11,000×*g* for 10 min. Before using this supernatant for MG assay, it was neutralized by keeping for 15 min with saturated solution of potassium carbonate at room temperature and centrifuged again at 11,000×*g* for 10 min. Neutralized supernatant was used for MG estimation following the method of Wild et al. [36]. An aqueous 500 mM N-acetyl-L-cysteine solution (pH 7.0) was freshly prepared. The reaction was started by adding 20 µl of the N-acetyl-L-cysteine solution (final concentration up to 10 mM), and the formation of the product N-α-acetyl-S-(1-hydroxy-2-oxo-prop-1-yl)cysteine was recorded at a wave length of 288 nm. MG was calculated from standard curve of MG.

2.7. Determination of Protein

The protein concentration in the leaf extracts was determined according to the method of Bradford [37] using BSA as a protein standard.

2.8. Extraction of Crude Protein for GST Purification

Eighty gram of maize seedlings was homogenized in an equal volume of 25 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, 1% (w/v) ascorbate and 10% (w/v) glycerol with Waring blender. The homogenates squeezed in a nylon cloth and was centrifuged at 11500×*g* for 15 min, and the supernatant was used as crude enzyme solution.

2.9. DEAE-Cellulose Chromatography

Proteins were precipitated by ammonium sulfate at 65% saturation from the supernatant and centrifuged at 11,500×*g* for 10 minutes. The proteins were dialyzed against 10 mM Tris-HCl buffer (pH 8) containing 0.01% (w/v) β-mercaptoethanol and 1 mM EDTA (buffer A) overnight to completely remove low molecular inhibitors. The dialyzate (crude enzyme solution) was applied to a column (1.77 cm i.d. × 20 cm) of DEAE-cellulose (DE-52, Whatman, UK) that had been equilibrated with buffer A and eluted with a linear gradient of 0 to 0.4 M KCl in 750 ml of buffer A. The high active eluted peak at around 91.67 mM KCl was collected for further purification.

2.10. Hydroxyapatite Chromatography

The pooled high active GST fractions separated by DEAE-cellulose column chromatography, was applied on a hydroxyapatite column (1.5 cm i.d. × 5.5 cm) that had been equilibrated with buffer A. The column was eluted with a 300 ml linear gradient of potassium phosphate buffer (K-P buffer; 0-20 mM, pH 7.0) in buffer A. The high active fraction (5 ml) was found to elute which was collected and further purified by affinity chromatography.

2.11. Affinity Chromatography

The collected sample was applied to a column (0.76 cm i.d. × 4.0 cm) of *S*-hexyl glutathione agarose (Sigma, St. Louis, MO) that had been equilibrated with 10 mM Tris-HCl buffer (pH 8.0) containing 0.01% (v/v) β-mercaptoethanol (buffer B). The column was washed with buffer B containing 0.2 M KCl and eluted with buffer B containing 1.2 mM *S*-hexyl glutathione. The high active protein fractions eluted with *S*-hexyl glutathione were combined and dialyzed against buffer B and the dialyzate was used as the purified GST.

2.12. Rabbit Treatment for Antibody Production

A rabbit (weighing about 2.5 kg) received subcutaneous injections of a 0.5 mg of purified GST protein in Freund's complete adjuvant at several sites. After two weeks, the rabbit was given a first booster injection of 0.5 mg of the purified GST protein in incomplete adjuvant, and then a second booster injection of 0.5 mg of the purified protein in incomplete adjuvant was given two weeks after the first booster injection. Blood was taken from the ear vein one week after the second booster injection.

2.13. SDS-PAGE and Western Blotting

To check the accumulation of GST, SDS-PAGE was done in 12.5% (w/v) gel containing 0.1% (w/v) SDS by the method of Laemmli [38] followed by western blotting following the protocol of Perkin Elmer Life Science Inc., USA).

3. Results and Discussion

The CDNB conjugated GST activities were observed to increase with duration under NaCl induced salinity stress (Fig. 2A). The activity increased only 15% over control at 1 day stress which increased to 65% and 59%, at 4 day and 7 day, respectively. The maize seedlings pretreated with 15 mM of proline increased the GST activities over control (44%, 90% and 63% at 1, 4 and 7 day of stress, respectively) and NaCl stress (25%, 20% and 9% at 1, 4 and 7 day stress, respectively) (Fig. 2A). Betaine treated seedlings also showed induced the GST activities (26%, 37% and 40%, at 1, 4 and 7 day stress, respectively). Since GST activity increased strongly under salinity stress in presence and absence of proline and betaine, maize GST was separated by a DEAE-cellulose chromatography. Three GST peaks were found to elute at 91.7, 162.5 and 234.1 mM KCl accounting for 92.2%, 6.2% and 1.6% of total activity. The GST eluted at 91.7 mM KCl was farther purified by hydroxyapatite and affinity chromatography as described in materials and methods. The purified GST was used to prepare polyclonal antibody in a rabbit blood serum. The western blot analysis of soluble protein extract with the antibody also showed significant accumulation of the GST in saline stressed seedlings with or without proline and betaine treatment (Fig. 2B).

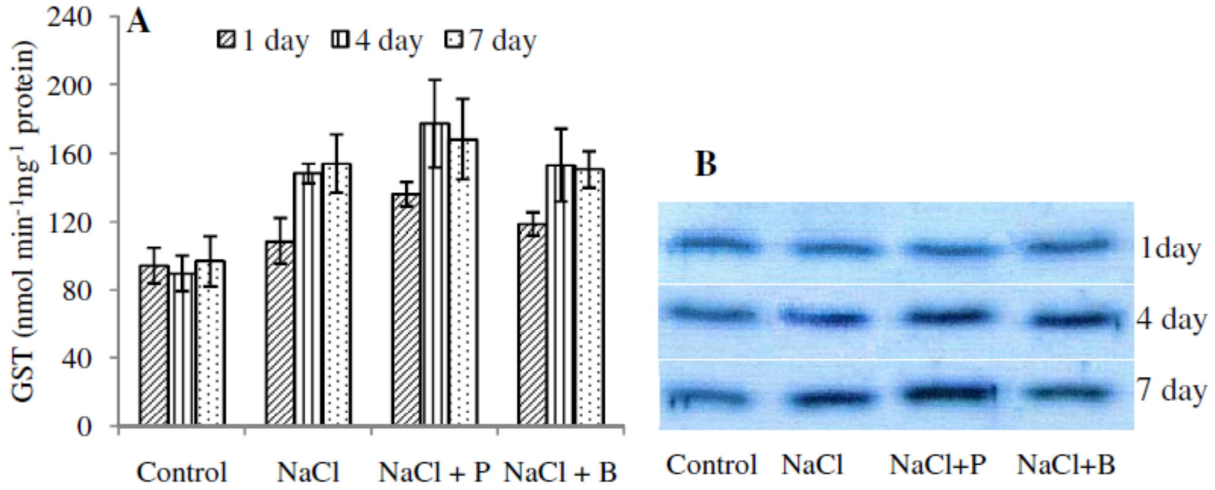

Fig. 2. *Activities of GSTs (A) and accumulation of dominant GST (B) in maize seedlings induced by proline (P) and betaine (B) under 16 dSm^{-1} salinity stress. The activity of each enzyme was expressed in nmolmin^{-1}mg^{-1} protein. Values represent the mean ± SE from three independent experiments.*

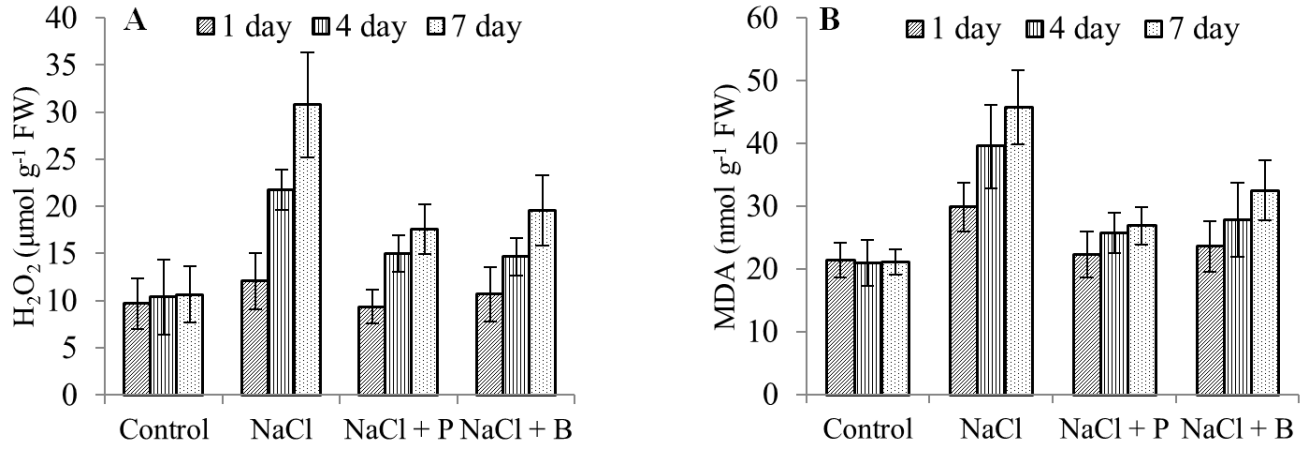

Fig. 3. *Contents of H_2O_2 (A) and MDA (A) in maize seedlings induced by proline (P) and betaine (B) under 16 dSm^{-1} salinity stress. Values represent the mean ± SE from three independent experiments.*

The level of H_2O_2 contents sharply increased with duration of salinity stress in leaves of maize seedlings compared to the untreated control (Fig. 3A) and the highest increase (2.9 folds) was observed at the 7 day of stress. Both proline and betaine treated seedlings maintained the levels of H_2O_2 contents lower (23%, 31% and 43% by proline and 12%, 33% and 36% by betaine at 1, 4 and 7 day stress, respectively) compared to the seedlings subjected to saline stress without treatment. The lipid peroxidation levels in leaf tissues, measured as the MDA content, are represented in Fig. 3B. Like H_2O_2, MDA contents increased remarkably with stress duration. At 7 day of stress, the level was 2.2 fold higher in NaCl stressed seedlings compared to control. Importantly, application of proline and betaine successfully alleviated the H_2O_2 levels (25%, 35% and 41% by proline and 21%, 30% and 29% by betaine at 1, 4 and 7 day stress, respectively).

GSTs are an ancient and diverse group of multi-functional proteins that are widely distributed amongst living organisms. Originally defined solely as enzymes that catalyze conjugation of the tripeptide glutathione (GSH) to an electrophilic substrate [21], it is now clear that GSTs catalyse a variety of reactions. Early plant GST research focused on the role of GSTs in herbicide resistance and vacuolar sequestration of anthocyanins in maize [39]. In the present study, the induced GST activity and accumulation of GST (Fig. 2A, B) under salinity might play important physiological role like vacuolar sequestration of maize flavonoids like anthocyanin as anthocyanin has been reported to accumulate under saline stress [40]. On the other hand, high activity might be associated with recycling and stabilizing flavonoid [17, 41] to protect cell from toxic effect.

In addition to being induced by xenobiotic-type stresses, plant GST expression is activated by abiotic stress like chilling [42], hypoxic stress [43], dehydration [44, 45], wounding [46], pathogen attack [47], ethylene [48], auxin [21], H_2O_2[49] and the defense signal salicylic acid [49]. GSTs have been shown to possess GST activity towards 4-hydroxy-2-nonenal (HNE) [50], a naturally occurring lipid peroxidation product that can cause oxidation and alkylation of proteins and DNA. Potentially, GST activity allows GSTs to detoxify electrophilic compounds by catalyzing their conjugation to GSH, while GSH peroxidase (GPX) activity allows GSTs to directly detoxify lipid and DNA peroxidation products [51]. It is also possible that the induced GST activities (Fig. 2A, B) could detoxify HNE as well as MDA, another natural lipid peroxidation product, under stress condition. In this study, we found to accumulate H_2O_2 and MDA under salinity stress concurrently with high GST activity while presence of proline and betaine boosted up the GST activity in the seedlings along with lower H_2O_2 and MDA contents (Fig. 2 and 3) suggested it's detoxification role by conjugation or directly detoxification via GPX activity. An increase in the level of H_2O_2 and lipid peroxidation in saline-treated seedlings resulted in increased oxidative damage probably due to impairment of the antioxidant defense system [52, 53]. Conversely, a decreased level of H_2O_2 and MDA in both proline and betaine treated seedlings (Fig. 3A, B) suggested that both proline and betaine protect against saline-dependent oxidative damage by enhancing antioxidant defense [1, 30, 31, 54, 55].

Saline stress caused changes in GSH and GSSG contents in maize seedlings (Fig. 4A, B). The GSH content decreased as the stress period increased (24% at 4 day and 38% at 7 day stress). On the contrary, GSSG contents increased enormously with stress duration (2.73 fold at 4 day and 5.73 fold at 7 day) resulting in significant decrease in the glutathione redox state. Importantly, both proline and betaine significantly contributed to maintain the NaCl mediated reduced GSH redox state (Fig. 4C).

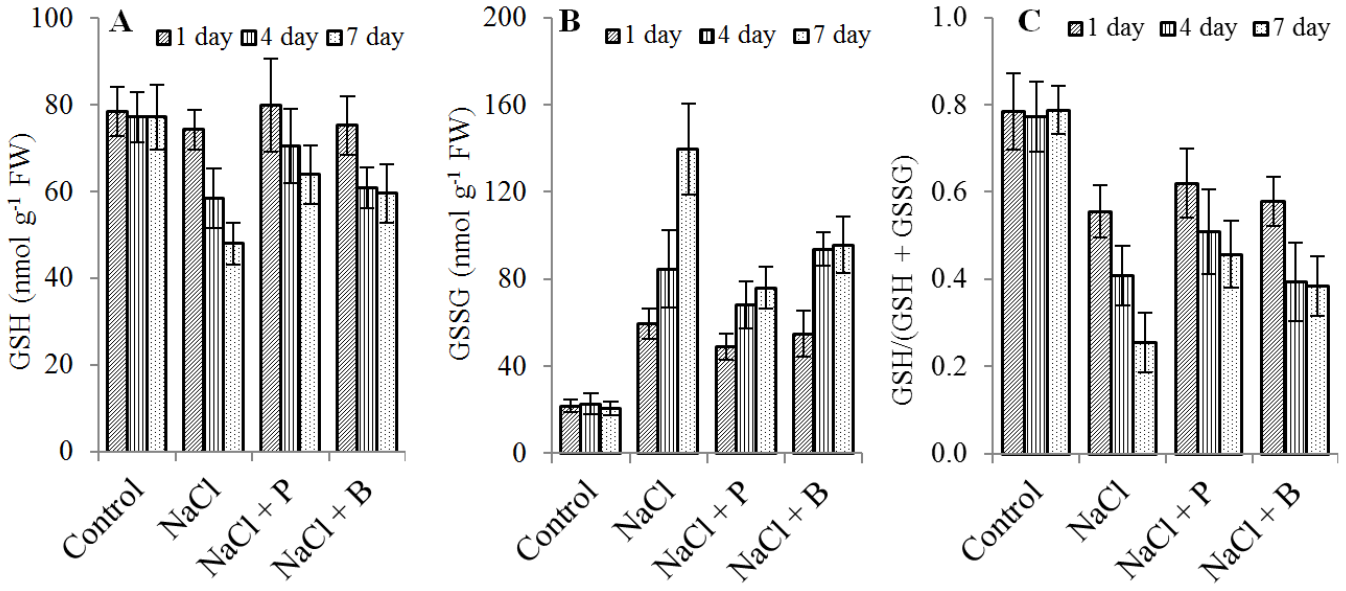

Fig. 4. *Contents of GSH (A) and GSSG (B), and glutathione redox state [GSH/(GSH+GSSG) ratio] (C) in maize seedlings induced by proline (P) and betaine (B) in x axis under 16 dSm^{-1} salinity stress. Values represent the mean ± SE from three independent experiments.*

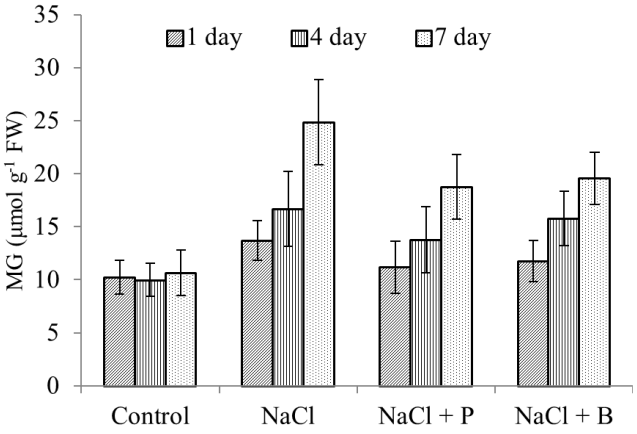

Fig. 5. *MG in maize seedlings induced by proline (P) and betaine (B) under 16 dSm^{-1} salinity stress. Values represent the mean ± SE from three independent experiments.*

Reduced glutathione is the most important antioxidant in plant that scavenges ROS either directly or indirectly by participating in the ascorbate-glutathione cycle [8, 56]. It can regenerate ascorbate through reduction of dehydroascorbate via the ascorbate-glutathione cycle [8]. Although defense against stress situations sometimes occurs irrespective of the GSH concentration [9], the increased level of GSH pool is generally regarded as a protective response against oxidative stress [57], In this study, salt stress decreased in GSH with stress duration, contrary, caused a significant increase in GSSG levels (Fig. 4A, B). Under salt stress, proline and betaine not only increased GSH contents remarkably, but also decreased the level of GSSG under salinity stress, suggesting the contribution of proline and betaine in maintenance of the glutathione redox state under salinity. Improved GSH contents and glutathione redox state have also been reported previously [32, 58].

The accumulation of MG increased in maize leaf under salinity stress (Fig 5). At 7 day of stress implementation, the concentration MG was 2.3 times higher in stress seedlings over control. Presence of proline and betaine in NaCl solution caused remarkable decrease in cytotoxic MG in the leaves. At 7 day, proline and betaine alleviated 31% and 25% of MG in leaves of NaCl stressed seedlings.

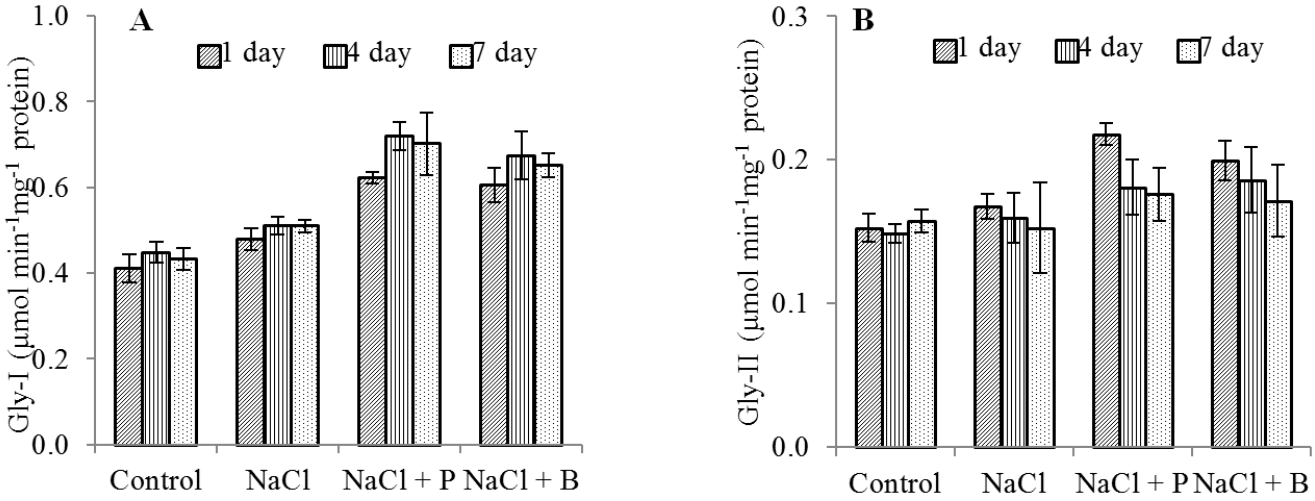

Fig. 6. *Activities of Gly-I (A) and Gly-II (B) in maize seedlings induced by proline (P) and betaine (B) under 16 dSm^{-1} salinity stress. The activity of each enzyme was expressed in µmolmin^{-1}mg^{-1}protein. Values represent the mean ± SE from three independent experiments.*

Salinity stress increased Gly-I activities by 17%, 14% and 18% over control at 1, 4 and 7 day stress, respectively, in maize seedlings (Fig. 6A). In presence of proline and betaine, the seedlings showed higher activity compared to salinity stress alone, where proline induced 30%, 41% and 38% higher activities at 1, 4 and 7 day stress, respectively. In case betaine treatment, 26%, 32% and 28% higher activities were obtained at 1, 4 and 7 day stress, respectively. Unlike Gly-I, Gly-II activity decreased with stress duration (Fig. 6B). At 1 day, proline and betaine increased Gly-II activities by 30% and 19%, respectively, over salinity stress. However, this level decreased with stress period, and at 4 and 7 day, the activities were increased by13% and 15%, respectively, by proline application and 17% and 12%, respectively, by betaine application.

The glyoxalase system consists of two enzymes (Gly-I and Gly-II) acts to convert the potential cytotoxic MG to non-toxic hydroxyacids such as lactate [9]. Gly-I use GSH to convert MG to SLG, while the hydrolytic reaction catalyzed by Gly-II liberates the lactic acid and free GSH. In several plant species, upregulation or overexpression of these enzymes increases tolerance to abiotic stresses [27, 29]. The present investigation demonstrated that saline stress caused accumulation of MG in maize cell (Fig. 5). Under salt stress, though Gly-I activity increased, Gly-II activity decreased with stress duration (Fig. 6). The decrease in Gly-II activities under salinity stress suggested that the detoxification of excess MG under salinity stress might slow the process in maize seedlings. In proline and betaine treated seedlings, both Gly-I and Gly-II increased along with lower contents of MG suggesting their

antioxidative role via increases in glyoxalase activities. Previously, Gly-I and Gly-II activities were reported to increase under sanity stress in presence of proline and betaine in rice (Hasanuzzaman et al. 2014) and mungbean (Hossain et al. 2011). However, in white cell of tobacco, betaine failed to maintain GLy-II activity under salinity [32].

4. Conclusion

Taken as a whole, the results suggest that GST and glyoxalases play important role in surviving tolerant maize genotype through detoxification and antioxidant activities under salinity stress. However, activity of Gly-II was inhibited under salinity stress. The higher activity of GST along with lower MDA suggested its protective role through detoxification. Presence of proline and betaine in saline enhanced the activities of GST and glyoxalases as well as improved GSH homeostasis. However, the protective role by antioxidants is complex and involved many of the enzymatic and non-enzymatic antioxidants. Therefore, this study suggested more research in this regard.

Acknowledgement

The authors acknowledge Professor Dr. Masayuki Fujita, Kagawa University, Japan for his technical suggestion, and Improvement and Quality Seed Production of Rice, Wheat and maize Project, Ministry of Agriculture for financial support.

References

[1] Demiral T, Türkan I. 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Botany 53 (3): 247-257.

[2] Mandhania S, Madan S, Sawhney V. 2006. Antioxidant defense mechanism under salt stress in wheat seedlings. Biol. Plant 50 (2): 227-231.

[3] Misra N, Gupta AK. 2006. Interactive effects of sodium and calcium on proline metabolism in salt tolerant green gram cultivar. Am. J. Plant Physiol 1 (1): 1-12.

[4] 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: 463-499.

[5] Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant. Biol 55: 373-399.

[6] Yadav SK, Singla-Pareek SL, Reddy MK, Sopory SK. 2005a. Transgenic tobacco plants overexpressing glyoxalase enzymes resist an increase in methylglyoxal and maintain higher reduced glutathione levels under salinity stress. FEBS Lett 579 (27): 6265-6271.

[7] Yadav SK, Singla-Pareek SL, Ray M, Reddy MK, Sopory SK. 2005b. Methylglyoxal levels in plants under salinity stress are dependent on glyoxalase I and glutathione. Biochem. Biophys. Res. Commun 337 (1): 61-67.

[8] 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 (2): 454-484.

[9] Noctor G, Foyer CH. 1998. Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol 49: 249-79.

[10] McNeil SD, Nuccio ML, Hanson AD. 1999. Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. Plant Physiol 120 (4): 945-949.

[11] Okuma E, Soeda K, Tada M, Murata Y. 2000. Exogenous proline mitigates the inhibition of growth of *Nicotiana tabacum* cultured cells under saline conditions. Soil Sci. Plant Nutr 46 (1): 257-263.

[12] Ashraf M and Foolad MR. 2007. Roles of glycinebetaine and proline in improving plant abiotic resistance. Environ. Exp. Bot 59: 206-16.

[13] Okuma E, Murakami Y, Shimoishi Y, Tada M, Murata Y. 2004. Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. Soil Sci. Plant Nutr 50 (8): 301-1305.

[14] Hasanuzzaman M, Alam MM, Rahman A, Hasanuzzaman M, Nahar K, Fujita M. 2014. Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. Bio Med Res Intl. Vol. 2014, Article ID: 757219, doi:10.1155/2014/757219.

[15] Choudhury S, Panda P, Sahoo L, Panda SK. 2013. Reactive oxygen species signaling in plants under abiotic stress. Plant Signal. Behav 8(4): e23681.

[16] Edwards R, Dixon DP. 2005. Plant glutathione transferases. In Glutathione Transferases and Gamma-Glutamyl Transpeptidases (Sies, H. and Packer, L., eds). San Diego, CA: Elsevier Academic Press Inc, pp. 81169-186.

[17] Dixon DP, Steel PG, Edwards R. 2011. Roles for glutathione transferases in antioxidant recycling, Plant Signaling & Behavior 6 (8): 1223-1227.

[18] Chi Y, Cheng Y, Vanitha J, Kumar N, Ramamoorthy R, Ramachandran S, Jiang SY. 2011. Expansion mechanisms and functional divergence of the glutathione *S*-transferase family in Sorghum and other higher plants. DNA Res 18 (1):1-16.

[19] Chen JH, Jiang HW, Hsieh EJ, Chen HY, Chien CT, Hsieh HL, Lin TP. 2012. Drought and salt stress tolerance of an Arabidopsis glutathione *S*-transferase U17 knockout mutant are attributed to the combined effect of glutathione and abscisic acid. Plant Physiol 158 (1): 340-351.

[20] Zagorchev L, Seal CE, Kranner I, Odjakova M. 2013. A Central role for thiols in plant tolerance to abiotic stress. Int. J. Mol. Sci 14: 7405-7432.

[21] Marrs KA, Alfenito MR, Lloyd AM, Walbot V. 1995. A glutathione *S*-transferase involved in vacuolar transfer encoded by the maize gene Bronze-2. Nature 375 (6530): 397-400.

[22] Mueller LA, Goodman CD, Silady RA, Walbot V. 2000. AN9, a petunia glutathione *S*-transferase required for anthocyanin sequestration, is a flavonoid-binding protein. Plant Physiol 123 (4): 1561-1570.

[23] Kitamura S, Shikazono N, Tanaka A. 2004. TRANSPARENT TESTA 19 is involved in the accumulation of both anthocyanins and proanthocyanidins in Arabidopsis. Plant J 37 (1): 104-114.

[24] Alfenito MR, Souer E, Goodman CD, Buell R, Mol J, Koes R, Walbot V. 1998. Functional complementation of anthocyanin sequestration in the vacuole by widely divergent glutathione *S*-transferases. Plant Cell 10 (7): 1135-1149.

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

[26] Mustafiz A, Sahoo KK, Singla-Pareek SL, Sopory SK. 2010. Metabolic engineering of glyoxalase pathway for enhancing stress tolerance in plants. Methods Mol. Biol 639: 95-118.

[27] Singla-Pareek SL, Yadav SK, Pareek A, Reddy MK, Sopory SK. 2008. Enhancing salt tolerance in a crop plant by overexpression of glyoxalase II. Transgenic Res 17 (2): 171-180.

[28] Saxena M, Bisht R, Roy SD, Sopory SK, Bhalla-Sarin N. 2005. Cloning and characterization of a mitochondrial glyoxalase II from *Brassica juncea* that is upregulated by NaCl, Zn and ABA. Biochem. Biophys. Res. Commun 336 (3): 813-819.

[29] Saxena M, Deb Roy S, Singla-Pareek S-L, Sopory SK, Bhalla-Sarin N. 2011. Overexpression of the glyoxalase II gene leads to enhanced salinity tolerance in *Brassica juncea*. The Open Plant Sci. J 5: 23-28.

[30] 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 (5): 553-61.

[31] Hoque MA, Banu MNA, Okuma E, Amako K, Nakamura Y, Shimoishi Y, Murata Y. 2007b. Exogenous proline and glycinebetaine ingresses 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 (11): 1457-1468.

[32] Hoque MA, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y. 2008. Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. J. Plant Physiol 165 (8): 813-824.

[33] Rohman MM, Uddin S, Fujita M. 2010. Up-regulation of onion bulb glutathione *S*-transferases (GSTs) by abiotic stresses: A comparative study between two differently sensitive GSTs to their physiological inhibitors. Plant Omics 3 (1): 28-34.

[34] 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 (9): 955-963.

[35] Heath RL, Packer L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys 125 (1): 189-198.

[36] Wild R, Ooi L, Srikanth V, Münch G. 2012. A quick, convenient and economical method for the reliable determination of methylglyoxal in millimolar concentrations: the N-acetyl-L-cysteine assay. Anal. Bioanal. Chem 403 (9): 2577-2581.

[37] 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.

[38] Laemmli, UK. 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature 227 (5259): 680-685.

[39] Edwards R, Dixon DP. 2000. The role of glutathione transferases in herbicide metabolism. In Herbicides and Their Mechanisms of Action (Cobb, A.H. and Kirkwood, R.C., eds). Sheffield: Sheffield Academic Press Ltd, pp. 8138-71.

[40] Fini A, Brunetti C, Ferdinando MD, Ferrini F, Tattini. 2011. Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. Plant Signal. Behav 6 (5): 709-711.

[41] 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 (2): 301-309.

[42] Seppanen MM, Cardi T, Hyokki MB, Pehu E. 2000. Characterisation and expression of cold-induced glutathione *S*-transferase in freezing tolerant *Solanum commersonii*, sensitive *S. tuberosum* and their interspecific somatic hybrids. Plant Sci 153 (2): 125-133.

[43] Moons, A. 2003. Osgstu3 and osgtu4, encoding tau class glutathione *S*-transferases, are heavy metal- and hypoxic stress-induced and differentially salt stress-responsive in rice roots. FEBS Lett 553 (3): 427-432.

[44] Kiyosue T, Yamaguchi-Shinozaki K, Shinozaki K. 1993. Characterization of two cDNAs (ERD11 and ERD13) for hydration-inducible genes that encode putative glutathione *S*-transferases in *Arabidopsis thaliana* L. FEBS Lett 335 (2): 189-192.

[45] Bianchi MW, Roux C, Vartanian N. 2002. Drought regulation of GST8, encoding the Arabidopsis homologue of ParC/Nt107 glutathione transferase/peroxidase. Physiol. Plant. 116 (1): 96-105.

[46] Vollenweider S, Weber H, Stolz S, Chetelat A, Farmer EE. 2000. Fatty acid ketodienes and fatty acid ketotrienes: Michael addition acceptors that accumulate in wounded and diseased *Arabidopsis* leaves. Plant J 24 (4): 467-476.

[47] Mauch F, Dudler R. 1993. Differential induction of distinct glutathione-*S*-transferases of wheat by xenobiotics and by pathogen attack. Plant Physiol 102 (4): 1193-1201.

[48] Zhou J, Goldsbrough PB. 1993. An Arabidopsis gene with homology to glutathione *S*-transferases is regulated by ethylene. Plant Mol. Biol 22: 517-523.

[49] Chen W, Chao G, Singh KB. 1996. The promoter of a H_2O_2 inducible, Arabidopsis glutathione *S*-transferase gene contains closely linked OBF- and OBP1-binding sites. Plant J 10 (6): 955-966.

[50] Gronwald JW, Plaisance KL. 1998. Isolation and characterization of glutathione *S*-transferase isozymes from sorghum. Plant Physiol. 117 (3): 877-892.

[51] Marrs KA. 1996. The functions and regulation of glutathione *S*-transferases in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol 47: 127-158.

[52] Chattopadhayay MK, Tiwari BS, Chattopadhyay G, Bose A, Sengupta DN, Ghosh B. 2002. Protective role of exogenous polyamines on salinity-stressed rice (*Oryza sativa*) plants. Physiol. Plant 116 (2): 192-199.

[53] Bor M, Ozdemir F, Turkan I. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of suger beet *Beta vulgaris* L. and *Beta maritime* L. Plant Sci 164 (1): 77-84.

[54] Banu MNA, Hoque MA, Watanble-Sugimoto M, Masuoka K, Nakamura Y and Shimoishi Y. 2009. Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress. J. Plant Physiol 166 (2): 146-156.

[55] Park EJ, Jeknic Z, Chen THH. 2006. Exogenous application of glycinebetaine increases chilling tolerance in tomato plants. Plant Cell Physiol 47 (6): 706-714.

[56] Smirnoff N. 2005. Antioxidants and reactive oxygen species in plants. 1st ed. Oxford: Blackwell Publishing Ltd. 302 p.

[57] May MJ, Leaver CJ.1993. Oxidative stimulation of glutathione synthesis in *Arabidopsis thaliana* suspension cultures. Plant Physiol 103: 621-7.