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Modulation of Antioxidant System by Glutathione in Maize Seedlings under Salt Stress Conditions

Himani Chhatwal, Namarta Gupta, Meenakshi Goyal[1], Prinka Goyal, Gurjit Kaur Gill[1] and Nitika Garg

Department of Botany, [1]Department of Plant Breeding and Genetics,
Punjab Agricultural University, Ludhiana-141 004, India
E-mail: himani.chhatwal10@gmail.com

Abstract: Effect of glutathione on seed vigour parameters and antioxidant system at different salt stress levels was observed in maize . Seeds of maize (*Zea mays* L.) cv J 1006 were soaked for 4hrs in water (hydration), glutathione 100 and 500 ppm solutions and surface dried. The treated seeds were subjected to salt stress levels of 50 , 75 and 100 mM NaCl. Vigour and biochemical parameters were recorded from 10 days old seedlings. Seed treatments increased percent germination, seedling length and biomasss, proline content and activities of antioxidant enzymes *viz.* catalase, peroxidase and superoxide dismutase of seedlings whereas decreased the malondialdehyde content over untreated seeds. The glutathione 500 ppm was more effective in alleviating salt stress compared to 100 ppm. The findings conclude that glutathione 500 ppm will be useful in ameliorating the adverse effects of salt stress by using seed treatments of glutathione for growing maize in salt affected areas.

Keywords: Catalase, Glutathione, Maize, Peroxidase, salt stress, Superoxide dismutase

Maize (*Zea mays* L.) is third important cereal crop after wheat and rice. Besides being important as staple food crop in developing countries and is in demand due to use for animal feed and ethanol production. Being a moderately salt sensitive crop, it shows visible signs of salt stress including reduced root and shoot length. Restricted water absorption is the main reason for decreased seedling growth. Salinity is one of the major abiotic stresses that hinders the development of plant resulting in a variable yield loss. It is mainly because of osmotic stress, imbalance of ions, oxidative damage, negative impact of sodium (Na+) and chloride (Cl^-) ions accumulation (Hussain et al., 2018). Inhibition of germination index, seedling growth by salt stress is reported in sorghum (Chen et al., 2021). Salt stress is the major factor affecting the seedling growth and yield of the crop plants (Billah et al., 2017). Reduction in yield by salt stress is caused due to adverse effects on the physiology of the plants (Hemalatha et al., 2017). Besides salt stress reduces the early seedling growth of crop plants (Chuchra and Gupta 2020). The plant responds to these stresses by activating its defense system. There is a boost in the activity of antioxidant enzyme like catalase, peroxidase and superoxidedismutase (Bhattarai et al., 2020). Under salt stress, the membrane lipids are damaged due to lipid peroxidation by reactive oxygen species (Yu et al., 2020).

For adapting seeds to adverse environments, seed treatment is considered as an important technique as it helps in enhancing salt stress tolerance. Seed treatment is done by pretreating seeds with stimulants which increase the resistance to negative effect (Tanou et al., 2012). Amongst the non- protein thiol in plants, glutathione (GSH) is the is most prevalent and widely distributed in cellular components (Frrat et al., 2003) . Reduced glutathione detoxifies reactive oxygen species and controls the GSH dependent ROS detoxifying enzymes activity (Nahar et al., 2015). It regulates both enzymatic and non-enzymatic antioxidants and osmporotectants. Glutathione treatments improve SOD activity, thus stimulates the conversion of superoxide radical to H_2O_2, an important step in imparting protection to the cellular contents. Treatment with GSH recorded increased biomass of plants and yield (Pei et al., 2019). Taking into consideration the above findings, the present investigation was planned to mitigate the adverse effects of salinity in maize by seed treatments with glutathione.

MATERIAL AND METHODS

Plant material: The present investigation was done at, Punjab Agricultural University, Ludhiana. Seeds of maize (*Zea mays* L.) cv were used to study the effect of seed treatments on early seedling growth and activity of antioxidant enzymes under salt stress conditions. The seeds were sterilized with 0.1 % mercuric chloride and washed with distilled water to prevent any possibility of seed borne infection. The seeds were later soaked for 4 hrs in different seed treatment solutions and were exposed to salt stress. For salt stress stimulation, the germination paper in Petri plates(150mm) was moistened with solutions of different concentrations of NaCl (50, 75 and 100mM). The Petri plates

were kept at 25°C and relative humidity (RH) 80 ± 5 % in the seed germinator. Each treatment was replicated thrice and 20 seeds were used per replication.

Percent germination (%): Final count of normal seedlings was recorded after 10 days and was expressed as germination percentage.

Seedling length (cm), seedling fresh and dry weight (mg): Average of five normal seedlings were taken. Root and shoot length was expressed in centimeter and fresh and dry weight in milligrams. For dry weight seedlings taken for fresh weight were oven dried at 65°C for 24 hours.

Measurement of antioxidant enzymes (CAT, POX, SOD) (ΔA/min/g FW): The known amount of seedlings was homogenized in 50 mM Na-phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone. The homogenate was filtered and then centrifuged at 4^{0}C for 20 min at 10,000g. Catalase activity was measured by following the decomposition of H_2O_2 at 240 in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 as described by Chance and Maehly (1955). Peroxidase activity was assayed by following the increase in absorbance at 470 nm due to guaiacol oxidation for 3 min (Chance and Maehly 1955). The assay mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol. Super oxide dismutase (SOD) was assayed (Marklund and Marklund 1974)

Measurement of malondialdehyde and proline contents: For MDA extraction, shoot samples were homogenized in 10 ml of 0.1% trichloroacetic acid (TCA) followed by centrifugation at 10000g and was estimated by method of Heath and Packer (1968). For proline extraction, 0.1 gm of seedlings were homogenised in sulphosalicylic acid (3%) followed by centrifugation at 3000 g and supernatant was used for proline estimation by the method of Bates et al. (1973)

Statistical analysis: Analysis of variance was carried out with software SAS 9.3 .

RESULTS AND DISCUSSION

Percent germination: Germination percentage in maize reduced as the salt concentration increased from 50 to 100 mM (Table 1). Increase in salt stress caused a reduction in germination percentage. Salt stress decreases the water absorption by decreasing the osmotic potential exterior to the seed thus cell expansion (Migahid et al., 2019). The findings are in agreement with the earlier study on sorghum genotypes, wheat and tomato where a decrease in percent germination was reported with increasing NaCl stress (Chuchra et al., 2020, Kaur et al., 2023, Singh et al., 2025a). In maize at all salt stress levels, GSH 100 and 500 ppm enhanced the germination percentage over control under salt stress conditions. GSH 500 ppm increased the germination

Table 1. Effect of seed treatments on percent germination, root and shoot length, fresh weight and dry weight in seedlings subjected to salt stress in maize

Salt stress (mM NaCl)	Treatments	Percent germination	Root length (cm)	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)
0	Control	90.0^{c}	10.24^{c}	6.42^{c}	401.23^{c}	42.67^{b}
	Hydropriming	91.67^{c}	10.37^{c}	6.40^{c}	415.34^{b}	45.67^{b}
	GSH 100 ppm	95.00^{b}	11.45^{b}	7.23^{b}	485.67^{a}	60.43^{a}
	GSH 500 ppm	98.33^{a}	12.85^{a}	9.10^{a}	483.66^{a}	62.02^{a}
50	Control	86.67^{c}	9.29^{c}	5.83^{c}	386.33^{d}	39.33^{b}
	Hydropriming	88.33^{c}	9.75^{c}	5.44^{c}	400.00^{c}	40.33^{b}
	GSH 100 ppm	93.33^{b}	10.94^{bc}	6.74^{b}	458.00^{a}	58.00^{a}
	GSH 500 ppm	95.00^{a}	12.10^{a}	8.56^{a}	419.33^{b}	61.01^{a}
75	Control	81.67^{c}	7.39^{c}	4.93^{d}	302.33^{d}	36.34^{c}
	Hydropriming	81.67^{c}	8.65^{b}	5.45^{cd}	341.33^{c}	33.00^{c}
	GSH 100 ppm	91.67^{b}	11.02^{a}	7.61b	378.67^{b}	46.67^{b}
	GSH 500 ppm	98.33^{a}	11.90^{a}	10.19^{a}	384.00^{b}	55.75^{a}
100	Control	61.33^{c}	6.73^{c}	3.41^{c}	138.33^{c}	30.67^{c}
	Hydropriming	55.00^{b}	6.66^{c}	4.57^{b}	172.67^{b}	33.68^{c}
	GSH 100 ppm	85.00^{a}	9.42^{b}	7.13^{a}	352.67^{a}	41.31^{b}
	GSH 500 ppm	85.67^{a}	11.24^{a}	7.22^{a}	350.33^{a}	49.14^{a}

GSH= Glutathione. Different small letters indicated that means are significantly different ($p \leq 0.05$).

percentage maximally at all salt stress levels. It enhanced the percentage of germination significantly from 86.67% control to 98.33% at 75 mM NaCl. However, hydration of seeds was either at par with the control at 50 and 75 mM or decreased the germination percent at 100mM.The effects of salt stress on maize seed germination were reduced by treating the seeds with glutathione which in turn increased the germination over untreated seeds.

GSH is a redox molecule that could help in seedling establishment by enhancing the reductive status of seed. This molecule helped in combating oxidative stress that might increase seedling growth by stimulation of cell repair mechanisms and activation of germination and antioxidant enzymes (Martínez-Ballesta et al., 2020). There was a marked decrease in seedling length and weight under salt stress. The seed treatments with GSH 100 and 500 ppm improved the root and shoot length of salt stressed seedlings. Under salt stress conditions GSH 500 ppm increased the root and shoot length maximally. It increased the root length significantly from 9.29 cm (control) to 11.24 cm (100 mM NaCl). Similarly increased the shoot length significantly from 5.83 cm (control) to 7.22 cm (100 mM NaCl). GSH 100 and 500 ppm improved the fresh and dry weight compared to the control under salt stress conditions. GSH 500 ppm increased the fresh weight maximally from 350.33 to 386.33 mg at 100 mM NaCl. GSH increased the dry weight from 39.33 mg (control) to 49.14 mg at 100 mM NaCl. For dry weight, amongst all treatments, GSH 500 ppm showed the most significant improvement, especially at 50 mM and 75 mM. Thus, the salt induced length inhibition could be reverted back by glutathione treatment. Roots and shoots act as an important marker of plant's response to salt stress as the roots tend to be in direct contact with soil. Salt stress induces negative effects in the germinating seeds, salinity stress impairs seedling growth and development (Liang et al., 2018). Similar results were reported in sorghum where increasing levels of salt stress reduced root and shoot growth (Dehnavi et al., 2020). In the present experiment, glutathione application significantly improved root and shoot length, thus considerably reversing the damaging effect of salt stress. Glutathione modulates the growth and development, cell proliferation and thus exogenous application of glutathione seems promising in enhancing plant's abiotic stress tolerance (Kumar et al., 2019). Salt stress severely affects the fresh weight and dry weight of plants. Fresh weight and dry weight decreased with the increase in salt stress. The reduction in biomass occurs because of the slowed down metabolic mechanisms and thus decrease in the growth of plants under increasing salt stress levels (Kamran et al., 2020). Similar inhibitory effects of salt stress have reported in maize (Pei et al., 2019) and oats (Iqbal et al*.,* 2020). In the present study, GSH 100 and 500 ppm effectively increased seedling biomass of maize compared to the control. Ibrahim et al. (2017) reported similar findings in the salt sensitive cultivar of *Gossypium hirsutum*, which recorded improved biomass after treatment with glutathione under salt stress. External application of GSH, increased the endogenous GSH levels which could promote tolerance to many stresses in plants (Pei et al., 2019). Thus the damaging effect of salt stress may be reduced by decrease in the seedling growth.

Antioxidant enzyme activities: The increase in the activities of enzymatic antioxidants (CAT, POX, SOD) in maize seedlings under the effect of salt stress was observed (Tables 2). The activities were further enhanced significantly upon treatment with GSH 100 and 500 ppm. The activity of catalase increased from 50 to 100mM NaCl as GSH 500ppm maximally enhanced the catalase activity over the untreated control and GSH 100ppm at all the stress levels. Similarly peroxidase and superoxide dismutase were increased as the concentration of NaCl increased from 50 to 100mM NaCl when compared to the unstressed control. The treatment with GSH 100 and 500ppm significantly enhanced the activities of peroxidase and superoxide dismutase compared to the untreated control. The most injurious effect of salt stress in plants is the oxidative stress caused by the reactive oxygen species (ROS). In the present study, the levels of CAT, SOD and POX showed an increase with increasing salt stress. Similar results were observed in faba bean and wheat under salt stress where H_2O_2 increased which lead to an increase in the levels of CAT and SOD (Alzahrani et al., 2019, Singh et al., 2025b). This stress impairs the cellular membranes, damages proteins and organelles, especially of mitochondria, peroxisomes, chloroplast and affects cell's integrity (Mushtaq et al., 2020). In study, the activities of the antioxidant enzymes showed a significant increase with glutathione application compared to the control. GSH 500 ppm showed the maximum increase at all stress levels. Similar results were also reported in sunflower where seed treatment with GSH resulted in considerable improvement in SOD, CAT and POX enzyme activity under salt stress (Asmaa et al., 2020).

Malondialdehyde and proline content: Malondialdehyde content was upregulated with the increasing levels of salt stress (Table 3). Both the concentrations of glutathione decreased the MDA content as compared to the control under salt stress. GSH 500ppm was more effective. Increase in MDA levels with the increase in salt stress and is because the most harmful effect of salt stress is the destruction of lipids by chain reactions and generation of lipid radicals which then damage many other biomolecules (Mushtaq et

Table 2. Influence of seed treatments on Peroxidase (A/min/g FW) and Superoxide dismutase (A/min/g FW) in maize (*Zea mays* L.) seedlings under salt stress conditions

Salt stress level/Treatment	0 mM NaCl (No stress)	50 mM NaCl	75 mM NaCl	100 mM NaCl
Catalase (ΔA/min/g FW)				
Control	32.17±0.67	40.22±0.41	58.60±1.63	69.87±0.50
Hydration	38.34±0.42	43.82±1.91	65.03±0.66	76.31±1.57
GSH 100 ppm	48.76±1.06	60.93±1.75	84.42±1.13	84.96±1.46
GSH 500 ppm	66.79±0.23	77.10±0.50	96.42±0.67	107.66±1.92
CD (p=0.05)	2.24	2.34	2.70	2.71
Peroxidase (ΔA/min/g FW)				
Control	0.01±0.00	0.02±0.00	0.04±0.00	0.06±0.00
Hydration	0.01±0.00	0.03±0.00	0.03±0.00	0.06±0.00
GSH 100 ppm	0.01±0.00	0.03±0.00	0.04±0.00	0.06±0.00
GSH 500 ppm	0.03±0.00	0.04±0.00	0.05±0.00	0.07±0.00
CD (p=0.05)	0.0039	0.0041	0.0040	0.0044
Superoxide dismutase (ΔA/min/g FW)				
Control	81.56±3.11	88.36±2.69	96.57±2.12	108.67±1.10
Hydration	88.32±3.55	93.95±1.55	104.18±0.68	114.99±3.15
GSH 100 ppm	121.65±1.22	131.42±2.25	130.30±1.98	136.49±3.17
GSH 500 ppm	134.23±1.45	147.67±3.75	156.06±5.45	150.57±1.52
CD (p=0.05)	1.03	4.33	5.16	3.80

al., 2020). Similar results were also reported in sweet pepper in which MDA levels increased by 2-fold when exposed to salt stress (Abdelaal et al., 2020). Glutathione plays a crucial role in maintaining the equilibrium between oxidation and antioxidation. Salt stressed mung bean seedlings also showed a decrease in MDA levels due to improved antioxidant enzymes by GSH application (Nahar et al., 2015).

Proline concentration showed a marked increase in the maize seedlings when seeds were grown in an exposure to salt stress (Table 3). Both concentrations of glutathione improved the proline content. However GSH 500ppm recorded the maximum increase in proline content when compared to untreated but stressed control. Proline content increased in salt stress exposed plants of both the cultivars. Proline protects the protein integrity by functioning as a molecular chaperone. To maintain the redox balance, it also acts as quencher of singlet oxygen species (Mansour et al., 2017). Similar results were also observed in mustard genotypes in which salt stress significantly elevated the proline content (Hossain et al., 2020) and in *Cicer arietinum* in which exogenous GSH treatment increased the accumulation of proline under salt stress (Sadak et al., 2017).

AUTHORS' CONTRIBUTION

N. Gupta: Conceptualization of research, designing of the experiments; H. Chhatwal: Execution of experiments, data collection, analysis of data and interpretation, preparation of the manuscript; G Kaur Conceptualization of research, designing of the experiments and supervised the manuscript; M. Goyal: Supervised the manuscript; P. Goyal: Supervised the manuscript; N. Garg Helped in editing the manuscript.

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