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Loss of Pigments and Higher Methylglyoxal Contents in Leaves of Maize Seedlings Under Salinity Are Associated with Saline Susceptibility

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Abstract: Salinity is the most detrimental stress which impairs the growth and development of plants. Seven days old seedlings of two contrast genotypes of maize (*Zea mays* L.), two tolerant genotypes (9120 and Super Gold) and two susceptible genotypes (Pacific 984 and PS999), were subjected to 12 dSm^{-1} salinity stress for five days and contents of chlorophyll (*Chl*), carotenoid (Car), methylglyoxal (MG) as well as activities of glyoxalase I (Gly I) and glyoxalase II (Gly II) were investigated in fully expanded leaves. Loss of *Chl* and Car contents were higher in the susceptible genotypes compared to those in tolerant genotypes. Production of MG was also higher in the susceptible genotypes, Pacific 984 and PS999, compared to that in tolerant ones under salinity stress. Under salinity, Pacific 984 showed 105 and 91% higher MG over 9120 and Super Gold, respectively, while PS999 showed 75 and 63% higher MG over 9120 and Super Gold, respectively. On the other hand, both of the tolerant genotypes showed higher Gly I and Gly II activities as compared to susceptible genotypes which played important role in reducing cytotoxic MG in tolerant genotypes.

Keywords: Salinity, Maize, Pigments, Methylglyoxal Detoxification

1. Introduction

Lower productivity of crops is attributed with the occurrence of many environmental stresses like salinity, drought, flooding, heavy metal toxicity, high or low temperature, ozone, UV-radiations etc., which causes serious threat to the crop production [1]. More than 50% yield is reduced as a result of abiotic stresses [2, 3]. In Bangladesh, among the total area 14.4 million ha of lands, salinity occupies 20% coastal area and 30% net cultivable area which spreads inside upto 150 km from the coast [4]. Salinity impairs growth and development of crops through nutrient imbalance, membrane damage, enzymatic inhibition, altered levels of growth regulators and metabolic dysfunction, including photosynthesis which eventually pointers to plant death [5, 6].

Methylglyoxal (MG), a cyto-toxic compound, is found in plants under salt stress [7, 8]. It reacts with molecules including DNA and proteins and alters these [7]. MG can be produced as an intermediate compound of glycolysis pathway or by leakage of 1, 2-enediolate [9]. It can also be produced by the activity of MG synthase [10]. Excess accumulation of MG inhibits cell proliferation, degrades cellular protein, DNA by altering amino acids and inactivates antioxidant defense system [10]. Therefore, they must be modified or removed to secured organelles like vacuole. MG is detoxified through glyoxalase system [7]. This system comprises of two enzymes, glyoxalase I (Gly I) and glyoxalase II (Gly II). Importance of glyoalases has been reported to show considerable tolerance against oxidative stress in plants [11, 12]. The role of glyoxalase pathway in

plant against salt stress was also reported in a large number of studies in different plants [7, 8, 11, 13, 14, 15]. However, information on glyoxalases in maize is very limited. On the other hand, pigment like Chlorophyll (*Chl*) and Carotenoid (Car) are also very sensitive to salinity. Therefore, in this study, we conducted this experiment, to examine the effect of salinity on MG detoxification, *Chl* and Car in maize.

2. Materials and Methods

2.1. Site, Plant Materials and Stress Treatments

The experiment was conducted at Molecular Breeding Laboratory, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Seven days old seedlings of relative saline tolerant maize genotypes (9120 and Super Gold) and susceptible genotypes (Pacific 894 and PS999 were grown on hydroponic condition on Hoagland solution containing 12 dSm^{-1} NaCl induced salinity where. Control plants were grown with Hoagland solution only. Data were taken from fully expanded leavesafter 5days of stress.

2.2. Chlorophyll and Carotenoid Determination

For *Chl* and Car estimation, extraction and determination were performed according to the method of Arnon [16]. Five hundred milligrams (mg) of fresh leaf material (from each treatment) was ground with 10 ml of 80% acetone at 4°C and centrifuged at 5000 × *g* for 10 minutes at 4°C. The absorbance was read at 645, 663 and 470 nm for *Chla*, *Chlb* and Car, respectively, with a spectrophotometer (UV-1800, Shimadzu, Japan) against 80% acetone as blank. *Chl* and Car were calculated using following formulas and expressed in $mg\ g^{-1}$ fresh weight (FW).

$$Chla\ (mg\ g^{-1}) = (0.0127) \times (A_{663}) - (0.00269) \times (A_{645})$$

$$Chlb\ (mg\ g^{-1}) = (0.0229) \times (A_{645}) - (0.00468) \times (A_{663})$$

$$Car\ (mg\ g^{-1}) = A_{470} + (0.114 \times A_{663} - 0.638 \times A_{645})$$

2.3. Measurement of MG

About 0.3 g leaf tissue 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. Supernatant was decolorized by adding charcoal (10 $mg\ ml^{-1}$), kept for 15 min at room temperature, and then centrifuged at 11,000 × *g* for 10 min at 20°C. Before using this supernatant for MG assay, it was neutralized by incubating for 15 min with saturated potassium Carbonate solution at room temperature and centrifuged again at 11,000 × *g* for 10 min. Neutralized supernatant was used for MG estimation following the method of Rohman *et al.* [17] using N-acetyl-L-cysteine.

2.4. Gly-I (EC: 4.4.1.5)

Gly-I assay was carried out according to Yadav *et al.* [7]. Briefly, the assay mixture contained 100 mM K-P buffer (pH 7.0), 15 mM magnesium sulfate, 1.7 mM GSH, and 3.5 mM MG in a final volume of 0.7 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 $mM^{-1}\ cm^{-1}$.

2.5. Gly-II (EC: 3.1.2.6)

Gly-II activity was determined according to the method of Principato *et al.* [18] 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'-Dithiobis(2-nitrobenzoic acid (DTNB), and 1 mM *S*-D-lactoylglutathione (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 $mM^{-1}\ cm^{-1}$.

2.6. Extraction of Soluble Protein

Total soluble protein was extracted from fresh leaves of maize seedlings 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 a mortar and pestle. The homogenate was centrifuged at 11,500 × *g* for 15 min at 4°C, and the supernatant was used for enzyme assay.

2.7. Determination of Protein

The protein concentration in the leaf extracts was determined according to the method of Bradford [19] using BSA as a protein standard.

2.8. Statistical Analysis

All data obtained was analyzed by SAS (SAS Institute Inc. Cary, NC, USA, Version 9.3) program following complete randomized design (CRD) and the mean differences were compared by Tukey's tests. Differences at the P=0.05 level were used as a test of significance.

3. Results and Discussion

3.1. Effect of Salinity on Chlorophyll and Carotenoid Contents

Higher losses in *Chl* and Car contents were found in susceptible genotypes as compared to tolerant ones (Table 1). As compared to respective control, 26%, 15%, 30% and 28% decreases in *Chla* and 35%, 22%, 48% and 50% losses in *Chlb* were recorded in 9120, Super Gold, Pacific 984 and PS999, respectively. Higher loss in total *Chl* was also observed in susceptible genotypes. Importantly ratio of *Chla* to *Chlb* (a/b) was also higher in susceptible genotypes. The ratio was increased by 13%, 8%, 36% and 43% in 9120, Super Gold, Pacific 984 and PS999, respectively. In case of Car content, 23%, 11%, 50% and 30% decreases were observed in salinity treated seedlings of 9120, Super Gold, Pacific 984 and PS999, respectively, when compared to their respective control.

Table 1. *Chl and Car contents in maize seedlings under salinity stress. Values represent the mean ± SE from three independent experiments. Value with asterisk mark is significantly different from respective control at P=0.05.*

Genotype	Treatment	*Chla*	*Chlb*	*Chl* (*a*+*b*)	*a*/*b*	Car
9120	Control	1.281±0.035	1.200±0.061	2.481±0.096	1.068	0.204±0.031
	Salinity	0.936*±0.025	0.776*±0.038	1.713*±0.239	1.206	0.155*±0.019
Super Gold	Control	1.071±0.11	1.033±0.043	2.105±0.153	1.037	0.161±0.037
	Salinity	0.907±0.002	0.809±0.003	1.715±0.005	1.121	0.143±0.026
Pacific 984	Control	1.123±0.02	1.154±0.024	2.277±0.044	0.973	0.211±0.013
	Salinity	0.786*±0.085	0.596*±0.148	1.382*±0.233	1.318*	0.106*±0.024
PS999	Control	1.226±0.063	1.003±0.131	2.229±0.068	1.222	0.166±0.011
	Salinity	0.880*±0.011	0.502*±0.19	1.382*±0.201	1.751*	0.117*±0.011

From this result, it is clear that loss of *Chl* was always higher in the susceptible genotypes compared to the tolerant genotypes (Table 1). The photosynthetic pigments reduce through decreasing the cellular water potentials, ion imbalance and disturbance in the electron transport chain [20]. According to Sakr *et al.* [21] salinity stress reduced the Chlorophyll contents through increasing activity of chlorophyllase enzyme or disrupting the fine structure of Chloroplast and instabilizing the pigment protein complexes by ions. This result is supported by some former findings in maize [22] and rice [23]. Lower loss of *Chl* content in 9120 and Super Gold as compare to Pacific 984 and PS999 might also be due to lower loss of RWC [17]. The ratio of *Chl* (a/b) as well as total *Chl* (*a*+*b*) loss was also higher in susceptible genotypes (Table 1). This imbalance might limit the nitrogen distribution more in theses genotypes resulting in higher breakdown in *Chl* contents. Nitrogen limitation in *Bignoniaceae* increased *Chl* (a/b) ratio along with higher loss of total *Chl* (*a*+*b*) was reported by Kitajima and Hogan [24]. Another report in wheat also reported increased ratio in response to drought condition [25] (Ashraf *et al.* 1994).

Like *Chl* content, Car was also decreased comparatively higher in the susceptible genotypes compared to the tolerant ones (Table 1). Genotypes with higher *Chl* and Car content may serve as a criterion of salt tolerance [26]. Higher *Chl* and Car contents found in the tolerant genotypes were also reported earlier [27, 28].

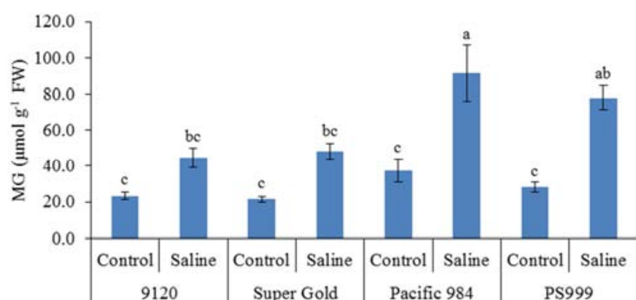

Fig. 1. *Contents of MG in leaves of maize seedlings of four different genotypes under salt stress. Values represent the mean ± SE from three independent experiments. Bars with the same letters are not significantly different at P=0.05.*

3.2. Effect of Salinity on MG detoxification System

Methylglyoxal content was substantially increased by salinity in all the genotypes (Fig. 1), but the content in susceptible genotypes was significantly higher as compared to tolerant ones. As compared to control, 9120, Super Gold, Pacific 984 and PS999 showed 91%, 125%, 145% and 174% higher MG, respectively, under salinity. Under salinity, Pacific 984 showed 105 and 91% higher MG over 9120 and Super Gold, respectively, while PS999 showed 75 and 63% higher MG over 9120 and Super Gold, respectively.

Activity of GlyI in maize leaves was noticeably increased in salinity stressed tolerant maize seedlings compared to control seedlings (Fig. 2A). However, in susceptible genotypes, the activity remained almost similar. Salinity increased Gly I activity by 42%, 26%, 7% and 10% in 9120, Super Gold, Pacific 984 and PS999, respectively, over respective control.

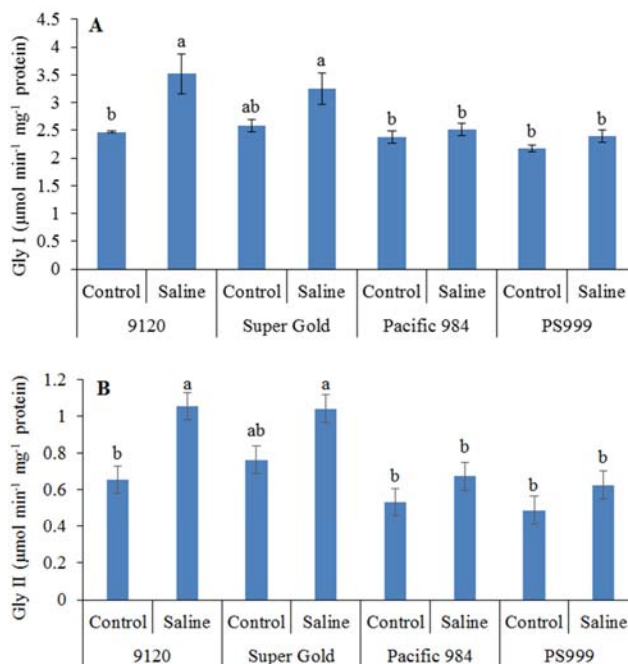

Fig. 2. *Activities of Gly I (A) and Gly II (B) in leaves of maize seedlings of four different genotypes under salt stress. Values represent the mean ± SE from three independent experiments. Bars with the same letters are not significantly different at P=0.05.*

Like Gly I, activity of Gly II in maize leaves was increased in salt stressed tolerant maize seedlings compared to control seedlings (Fig. 2B). However, this activity was also increased in susceptible genotypes. In 9120, Super Gold, Pacific 984 and PS999, the Gly II activity was increased by 61%, 37%,

27% and 28%, correspondingly, over the respective control under salinity stress.

Methylglyoxal (MG) is a cyto-toxic oxo-aldehyde which is produced during physiological process in higher plant cells. This is produced enormously as an intermediate when exposed to salinity stress and it can be detoxified through the glyoxalase system [29]. Present experiment exhibited that salinity stress caused significant accumulation of MG in all genotypes, the content being significantly higher in the susceptible genotypes Pacific 984 and PS999 (Fig. 2). Gly I converts MG to S-D-lactoyl glutathione (SLG) while Gly II catalyzes the hydrolytic reaction through which lactic acid and free GSH are liberated [14, 30, 31]. In this experiment, both activities of Gly I and Gly II were increased significantly in the tolerant genotypes under salt stress (Fig. 2) that suggest a role of glyoxalase system in detoxifying excess amount of MG under salinity stress. These result indicated glyoxalase mediated detoxification of MG in tolerant maize genotypes 9120 and Super Gold is much consistence which ultimately conferred tolerance in these genotypes. Similarly results were also found in some previous reports in different plants [11, 12, 32, 33].

4. Conclusion

Considering all, salinity caused higher degradation of *Chl* and Car in susceptible maize genotypes. Comparatively higher amount of MG accumulation in the susceptible genotypes is an important cause of their susceptibility to salinity. Higher activities of Gly I and Gly II played important role in maintenance of MG content relatively lower in tolerant genotypes under salinity.

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