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Mitigation effects of glycinebetaine on oxidative stress and some key growth parameters of maize exposed to salt stress

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Abstract: The aim of the current study was to investigate the effects of glycinebetaine (GB) on oxidative stress and some key growth parameters in maize (*Zea mays* L. 'DK 647 F1') grown under saline conditions. Maize seedlings were grown in pots containing perlite. The experiment was designed in a 2 × 2 factorial arrangement with 2 levels (0 and 100 mM) of sodium chloride (NaCl) and 2 levels (25 and 50 mM) of GB sprayed onto leaves of maize seedlings. Saline stress caused a considerable decline in total dry matter, chlorophyll content, relative water content (RWC), peroxidase (POD; EC. 1.11.1.7), and catalase (CAT; EC. 1.11.1.6); however, it increased proline, polyphenol oxidase (PPO; 1.10.3.1), and electrolyte leakage. Foliar application of both GB doses mitigated the deleterious effects of salinity stress to variable extents on the key growth parameters tested. As expected, sodium (Na^+) concentrations were higher in the tissues of plants grown under saline conditions, and the GB treatments significantly reduced Na^+ concentration in the plant tissues. Salinity stress reduced both calcium (Ca^{2+}) and potassium (K^+) in the leaves, and GB treatments increased concentrations of both elements in plant tissues; however, their levels were still lower than control values. In particular, foliar application of 50 mM GB mitigated some of the deleterious effects of salt stress by improving proline, Ca^{2+}, and K^+ levels and maintaining membrane permeability.

Key words: Antioxidant enzyme, nutrient acquisition, salt stress, salt tolerance, *Zea mays*

1. Introduction

Salinity is an abiotic stress factor that deleteriously affects the development, growth, and productivity of crops (Foolad et al. 2003a, 2003b; Ashraf and Harris 2004). High salt concentrations cause oxidative stress by producing reactive oxygen species (ROS) (Cramer et al. 1994). These ROS are highly reactive and may disrupt growth physiology through oxidative damage to protein, nucleic acids, and lipids (Namjooyan et al. 2012). However, crops have developed strategies to mitigate the deleterious effects of salinity through the production of antioxidant enzymes (Dionisio-Sese and Tobita 1998; Tavallali et al. 2010). Salt-tolerant plants have an ability to maintain ion and water movement and may have developed an antioxidant system to remove ROS effectively (Rout and Shaw 2001). This system allows plants to grow under salinity conditions by holding ROS to a minimum range (Masood et al. 2006). The hydrogen peroxide produced by salinity stress can be scavenged by peroxidase (POD) enzyme (Dionisio-Sese and Tobita 1998).

Coping with salinity stress in order to sustain food production is a major issue in many arid and semiarid regions globally. Although conventional plant breeding methods have changed over to the use of physiological selection criteria, this is time-consuming and relies on genetic variability that is already present (Heuer 2003). Plants synthesize and accumulate organic compounds in the cytosol and organelles when they are exposed to salt stress (Ashraf and Harris 2004; Bartels and Sunkar 2005; Sairam et al. 2006). Compatible osmolytes are accumulated and function as protein or enzyme activities in the cytoplasm. Currently, researchers pay more attention to glycinebetaine (GB) as an osmoprotectant substance in higher plants. The ability of compatible solutes, and GB in particular, to regulate net fluxes of Na^+ and K^+ across the plasma membrane has been reported at the cellular level, both in response to NaCl (Cuin and Shabala 2005, 2007a) and ROS (Cuin and Shabala 2007b) treatments; these effects were attributed to GB control over both depolarization-activated outward-rectifying K^+ selective and ROS-activated nonselective channels. Moreover, the ability of GB to act as a molecular chaperon protecting photosystem II against oxidative stress has recently been shown in the halophyte species *Chenopodium quinoa* (Shabala et al. 2012)

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The principal objective of this study was to test whether the exogenous application of GB as a foliar spray could reduce salinity stress in maize. This study also aimed to investigate the results of the interactive effects of GB and NaCl on oxidative damage, antioxidant systems, and the distribution of Na, K, and Ca in the leaves and roots of maize seedlings.

2. Materials and methods

2.1. Plant culture and treatments

This experiment was carried out in a growth chamber with maize (*Zea mays* L. 'DK 647 F1') grown in pots containing perlite. Depending on the exact plant height, photosynthetic photon flux density above the plant was 355–390 μmol m^{-2} s^{-1}. Temperature and humidity of the growth chamber were maintained at 27 ± 2 °C and 65%–70%, respectively. The maize seeds were kept in 0.1% (w/v) sodium dodecyl sulfate (SDS) solution for 20 min for sterilization and rinsed with deionized water. After drying the seeds at 28 °C for 6 h, 5 seeds pot^{-1} were sown in plastic pots containing 8 L of perlite. They were thinned to 3 plants pot^{-1} and grown for 5 more weeks after germination. Plants were allowed to grow 1 week further to allow enough root growth prior to starting different treatments. A 2 × 2 factorial arrangement was designed with 2 levels of NaCl (0 and 100 mM) and 2 levels of GB (25 and 50 mM) sprayed onto the leaves of maize seedlings. Plants were sprayed with 50 mL GB pot^{-1} (Sigma Aldrich, Japan) once a week for 5 weeks. Further details on the composition of nutrients used were given by Kaya et al. (2010). In a randomized block design, 4 replications were used for each treatment and 6 plants were used for each replicate (i.e. 24 plants $treatment^{-1}$).

2.2. Determination of some key growth parameters

Leaf relative water content (RWC) was measured according to the method of Weatherley and Barrs (1962). Chlorophyll determination was by the method of Kaya et al. (2010), and concentrations of chlorophyll were calculated using the formula given by Strain and Svec (1966).

Proline determination was done using the procedure proposed by Bates et al. (1973), and details were as described by Kaya et al. (2010). The absorbance of extracted solution was read at 520 nm with a Shimadzu UV 1601 spectrometer. Proline concentration was calculated based on the absorbance values obtained from appropriate proline standards. Determination of electrolyte leakage was done using methods proposed by Dionisio-Sese and Tobita (1998), and further details provided by Kaya et al. (2010) are also available.

2.3. Protein content and enzyme determination

Bovine serum albumin V was used as a standard to determine protein content in the enzyme extracts according to the method proposed by Bradford (1976).

Catalase activity was determined according to the method proposed by Kraus and Austin-Fletcher (1994), which is based on the consumption of hydrogen peroxide at 405 nm, and POD activity was assayed according to the method described by Chance and Maehly (1955). POD activity was expressed as change occurring in absorbance min^{-1} mg^{-1} protein. PPO activity was determined according to the method of Zauberman et al. (1991).

2.4. Nutrient analysis and dry weight

For determinations of dry weights, 3 plants were randomly selected from each replicate, and shoots and roots of the selected plants were first separated and then dried in an oven for 2 days. Chemical analyses of Na, Ca, and K were assayed based on the method of Chapman and Pratt (1982). Sodium, Ca, and K were analyzed using inductively coupled plasma. A 2-way analysis of variance was performed on all data, and the least significant difference was calculated at $P \leq 0.05$.

3. Results

3.1. Relative water content and plant growth

Salinity stress reduced the RWC and dry weights of shoots and roots compared to unstressed control plants (Table 1). Both treatments of GB elevated RWC and shoot and root dry weights in plants grown at salinity stress to a level lower than the control, but GB was not significantly effective on unstressed plants. Salinity stress resulted in a decrease in the concentration of chlorophyll in maize seedlings (Table 2). Exogenous application of GA increased the chlorophyll levels of plants under salinity stress.

3.2. Proline and membrane permeability

Salinity stress increased proline content in leaves of maize seedlings (Table 2). GB application caused further accumulation in proline levels of maize plants under salinity compared to the untreated salt-stressed group. Salinity stress impaired membrane permeability by inducing increased electrolyte leakage in leaves. GB treatments partly maintained membrane permeability by lowering electrolyte leakage (Table 2).

3.3. Enzyme activities

Salinity significantly increased PPO activity, but reduced POD and CAT activities in maize plants (Table 3). These results clearly show that the effects of salinity stress on enzyme activities are different. POD and PPO activities decreased in salt-stressed plants under foliar application of GB; however, GB treatment increased the CAT activity in salt-stressed plants.

Table 1. Relative water content (RWC), shoot, root, total plant dry weight, and root:shoot ratio in maize plants grown under high saline conditions in the presence of GB applied foliarly (values followed by different letters in the same column are significantly different at P ≤ 0.05).

Treatments	RWC (%)	Shoot (g plant^{-1})	Root (g plant^{-1})	Root:shoot ratio	Total plant (g plant^{-1})
C	86 a	8.76 a	1.18 a	0.135 c	9.94 a
C + GB1	87 a	8.79 a	1.19 a	0.135 c	9.98 a
C + GB2	89 a	8.90 a	1.20 a	0.135 c	10.10 a
S	68 c	4.75 d	1.06 c	0.223 a	5.81 c
S + GB1	74 b	6.73 c	1.12 b	0.166 b	7.85 b
S + GB2	75 b	7.01 b	1.12 b	0.160 b	8.13 b
Interactions S × GB	*	*	*	*	*

*P < 0.05**;** C: Control treatment (nutrient solution alone); S: 100 mM NaCl; GB1 and GB2: 25 and 50 mM GB, respectively.

Table 2. Chlorophyll a (Chl a), chlorophyll b (Chl b) (mg kg^{-1}), membrane permeability (%), and proline content (μmol g^{-1} fresh weight) of maize plants grown under high saline conditions in the presence of GB applied foliarly (values followed by different letters in the same column are significantly different at P ≤ 0.05).

Treatments	Chl a	Chl b	Membrane permeability	Proline
C	1276 a	898 a	13.23 d	0.48 c
C + GB1	1287 a	908 c	13.45 d	0.49 c
C + GB2	1265 a	902 b	14.45 d	0.42 d
S	898 d	587 d	36.54 a	0.74 b
S + GB1	980 c	698 c	27.68 b	0.86 a
S + GB2	1078 b	756 b	24.38 c	0.89 a
Interaction S × GB	**	*	*	*

*P < 0.05; **P < 0.01; C: Control treatment (nutrient solution alone); S: 100 mM NaCl; GB1 and GB2: 25 and 50 mM GB, respectively.

Table 3. Polyphenol oxidase (PPO: unit × 100 mg^{-1} protein), catalase (CAT: unit × 100 mg^{-1} protein), and peroxidase (POD: ΔA_{470} min^{-1} mg^{-1} protein) levels in maize plants grown under high saline conditions in the presence of GB applied foliarly (values followed by different letters in the same column are significantly different at P ≤ 0.05).

Treatments	PPO	POD	CAT
C	76.21 f	1.89 a	1.92 a
C + GB1	84.95 e	1.47 c	0.75 d
C + GB2	136.94 b	1.47 c	1.07 c
S	184.80 a	1.67 b	1.16 c
S + GB1	97.03 d	1.29 d	1.46 b
S + GB2	112.81 c	1.50 c	1.36 b

C: Control treatment (nutrient solution alone); S: 100 mM NaCl; GB1 and GB2: 25 and 50 mM GB, respectively.

3.4. Ion contents

In the present experiment, the Na level was significantly higher in the leaves and roots of maize seedlings grown at salinity stress compared to unstressed plants. GB induces a greater decrease in Na^+ in the roots and leaves (Table 4).

The K^+ and Ca^{2+} concentrations were lower in the leaves of maize seedlings grown at salt stress (Table 4). GB increased both K^+ and Ca^{2+} concentrations in the shoots of plants grown at salt stress (Table 4).

4. Discussion

4.1. RWC and plant growth

The electrolytes in saline solution first induce an imbalance in water potential between the symplast and apoplast, and this causes decreased turgor, which can lead to growth reduction (Bohnert et al. 1995). Lower RWC indicates a loss of turgor, which results in restricted water availability for cell enlargement (Katerji et al. 1997). Similar reports are available in the literature for a broad range of plant species grown under saline conditions (Srivastava et al. 1998; Thind and Malik 1988;). Lower leaf RWC may be due to lower water availability under saline conditions (Shalhevet 1993), or plant roots could lose their ability to reabsorb water lost through transpiration (Gadallah 2000).

GB may serve as an osmoprotective to prevent cell damage from dehydration (Yancey et al. 1982; Chen et al. 2000). It has been reported that GB prevents NaCl-induced K^+ leak (Cuin and Shabala 2005) and, thus, indirectly aids water retention in plant tissues.

Salinity stress may cause decreased plant growth (Dodd and Donavan 1999; Ephron et al. 1999). Salt compartmentation and osmotic adjustment in salt-tolerant plants can result in the continued growth of plants at salinity stress (Volkmar et al. 1998). When GB is applied externally it is rapidly absorbed by leaves and moves to other organs so that it can improve stress tolerance in plants (Makela et al. 1998a). Moreover, GB naturally synthesizes in plants and does not normally break down in plants (Bray et al. 2000). As a result it can easily be extracted from high-producing plants such as sugar beets relatively inexpensively (Rhodes and Hanson 1993). This may render the application of GB an economically feasible solution for mitigating deleterious effects of salinity stress on plant growth and yield. For example, this has been demonstrated in rice (Harinasut et al. 1996; Lutts 2000) and tomato plants (Makela et al. 1998a, 1998b).

The reduction of chlorophyll content through salinity stress may be due to the formation of proteolytic enzymes such as chlorophyllase, which causes the degradation of chlorophyll (Sabater and Rodriguez 1978); or salinity stress may induce damage to the photosynthetic apparatus (Yasseen 1983). In the present experiment, exogenous GA application restored the chlorophyll levels of plants at salinity stress. Similar findings were found by Makela et al. (2000), who reported that foliar-applied GB improved chlorophyll content in tomato plants under salinity.

4.2. Proline and membrane permeability

One of the responses of plants to salinity stress is to accumulate proline, and this has been shown by many researchers. At the cellular level, accumulation of proline may regulate osmotic adjustment (Perez-Alfocea et al. 1993). Proline acts as a major supply of energy, which helps to improve salinity tolerance (Chandrasekhar and Sandhyarani 1996).

Accumulation in proline levels with GB application in maize plants grown at salinity stress indicates that proline accumulation through GB probably reduced the degree of stress damage to a greater extent than in the salt-stressed group alone. Similar results were also reported in rice plants (Demiral and Türkan 2006).

Table 4. Sodium, Ca^{2+}, and K^+ (g kg^{-1} dry matter) in leaves and roots of maize plants grown under high saline conditions in the presence of GB applied foliarly (values followed by different letters in the same column are significantly different at $P \leq 0.05$).

Treatments	Leaves				Roots		
	Na^+	Ca^{2+}	K^+	Na^+:K^+	Na^+	Ca^{2+}	K^+
C	0.68 d	6.6 b	34 b	0.020 d	2.4 c	6.6 b	4.8 a
C + GB1	0.75 d	7.1 a	38 a	0.020 d	1.8 d	6.9 ab	4.8 a
C + GB2	0.72 d	7.3 a	40 a	0.018 d	2.0 cd	7.0 a	4.9 a
S	7.50 a	4.5 d	22 d	0.341 a	6.4 a	3.1 c	2.8 c
S + GB1	6.40 b	5.9 c	30 c	0.213 b	4.3 b	2.7 cd	3.8 b
S + GB2	5.52 c	6.4 b	32 bc	0.173 c	4.1 b	3.0 cd	4.0 b

C: Control treatment (nutrient solution alone); S: 100 mM NaCl; GB1 and GB2: 25 and 50 mM GB, respectively.

Salinity stress leads to irreversible damage of the cell membrane (Mundree et al. 2002). Membrane injury due to salt stress may be related to increased production of ROS (Shalata et al. 2001), and highly toxic ROS must be scavenged in order to maintain normal plant growth (Demiral and Türkan 2004). Increased electrolyte leakage observed in the present study at salinity stress could be partly due to decreased chlorophyll concentration (Kaya et al. 2001). Electrolyte leakage from cells exposed to oxidative stress may occur not only as a result of nonspecific oxidation of the plasma membrane phospholipids; it may result from the direct activation of ion-permeable channels by ROS species (Demidchik et al. 2003; Demidchik et al. 2010). GB protects plants against salt stress by maintaining osmotic adjustment and stabilizing membranes (Mansour 1998).

4.3. Enzyme activities

Antioxidant enzyme activities increase under salinity stress in crops such as pea (Hernandez et al. 1999) and wheat shoot (Meneguzzo et al. 1999; Sairam and Srivastava 2002). Manchandia et al. (1999) reported that salinity stress resulted in great increases in POD activity. POD activity is higher in tolerant plant species, and this probably enables tolerant plants to grow much better under oxidative stress (Scalet et al. 1995); sensitive plants have no such strategy (Peters et al. 1989). Many researchers have shown that high POD activity is highly linked with reduction in plant growth (MacAdam et al. 1992; Zheng and Van Huystee 1992). Salinity stress resulted in both increased and decreased CAT activities in the roots of salt-tolerant and salt-sensitive tomato cultivars, respectively (Shalata et al. 2001).

Foliar application of GB partly reduced POD activities in salt-stressed plants. GB must overcome the deleterious effects of oxidative stress by, for example, activating or stabilizing ROS-scavenging enzymes and/or repressing the production of ROS by an unknown mechanism (Chen and Murata 2008).

4.4. Ion contents

As expected, Na concentration was significantly higher in both the leaves and roots of maize seedlings grown at salinity stress. These results are generally in agreement with reports for other plant species, e.g., rice (Asch et al. 1999) and tomato (Kaya et al. 2001). Glycophytes have an ability to restrict sodium uptake or partition sodium in older leaves, which are eventually sacrificed (Cheeseman 1988).

Lutts (2000) reported that GB minimized Na accumulation in rice grown at salinity stress. Compartmentalization and uptake of ions are essential for plants grown at normal and saline conditions (Adams et al. 1992). The beneficiary effects of GB in lowering Na and enhancing K in the shoot might be due to the GB-induced production of supplement vacuoles in the root cells. This causes greater Na^+ accumulation in the root and a decrease in its transportation to the shoot. Similarly, foliar application of GB resulted in reduced Na^+ accumulation and maintenance of the K^+ concentration in the shoot of rice plants growing under saline condition (Lutts et al. 1999).

One of the effects of salinity on mineral nutrition was to reduce Ca^{2+} in plants. It is well known that salinity stress induces calcium deficiencies in various plant species such as tomato (Lopeza and Sattia 1996; Navarro et al. 2000) and strawberry (Kaya et al. 2002). The results strongly suggest that GB ameliorates the growth of maize seedlings under salt stress by enhancing antioxidant enzyme activities, maintaining membrane permeability, and improving concentrations of K^+ and Ca^{2+} in the plants.

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