Biosaline Agriculture and High Salinity Tolerance
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Growth enhancement in two potential cereal crops, maize and wheat, by exogenous application of glycinebetaine

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Abstract

Ameliorative effect of exogenously applied glycinebetaine (GB) on growth, photosynthetic and antioxidant capacities of two potential cereals wheat (cv. S-24) and maize (cv. Golden) grown under salt stress was assessed in two different independent experiments. Plants of maize were grown at 0 or 10 dS/m NaCl, while those of wheat were subjected to 2.17 or 14.67 dS/m NaCl salinity. Different levels of GB, i.e., 0 (unsprayed), 50 and 100 mM (in 0.10% Tween-20 solution) were applied as a foliar spray to both wheat and maize plants at the vegetative growth stage. Salt stress reduced the growth and yield of both maize and wheat plants. However, salt-induced reduction in growth and yield of both maize and wheat was ameliorated by exogenous application of GB, but this enhancement effect was more in wheat than that in maize. Furthermore, this GB-induced growth and yield enhancement was positively associated with increased endogenous GB, photosynthetic capacity, and superoxide dismutase (SOD) activity. Although exogenous application of GB improved photosynthetic capacity of both maize and wheat by increasing stomatal conductance, and thus favoring higher CO_2 fixation rate, this effect seemed to be partial in maize. In addition, the GB-induced reduction in transpiration rate in wheat compared with that in maize was found to be an additional factor that might have contributed to a better growth and yield of wheat under salt stress. The activity of only SOD was enhanced by GB application in both maize and wheat under saline conditions. Thus, it is likely that both applied GB and intrinsic SOD scavenged reactive oxygen species in these potential cereals under saline conditions. In view of all these findings, it can be concluded that the adverse effects of salt stress on cereals such as maize and wheat can be alleviated by the exogenous application of GB, which in turn enhances photosynthetic capacity and modulates activities of antioxidant enzymes. Furthermore, effectiveness of GB application on regulation of photosynthetic and antioxidant capacities was found to be species specific.

Introduction

Soil salinity is one of the major environmental stresses causing substantial crop losses worldwide. According to an estimate, salinity reduced the average yield of major crops by more than 50% [1]. In view of rapidly increasing world population, crop production must increase substantially if food security is to be ensured, which is already considerably low for meeting world demands (http://www.unfpa.org/swp/2007/english/introduction.html). It is estimated that there is a need to increase productivity by 20% in the developed countries and by 60% in the developing countries [2]. Thus, reduction in soil salinization and increase in the salt tolerance of crops, particularly that of cereals, the demand for which is growing at 2% per year with growing urban population [2–5].

Hexaploid wheat is one of the world‘s most important cereal crop and it is grown under wide range of climatic conditions, particularly in Pakistan, India, China, the United Kingdom, the United States, Turkey, Australia, Russia, Germany and France (http://www.fao.org/statistics/yearbook/vol_1_1/site_en.asp?page=production). Due to rapid urbanization (http://www.unfpa.org/swp/2007/english/introduction.html) and economic growth, dramatic changes occur in dietary patterns, which have resulted in an increase demand for wheat production. It is calculated that wheat demand worldwide will increase by 40% from 552 metric ton in 1993 to 775 metric ton in 2020 [6]. Similarly, demand for maize is increasing day by day. According to projections, the demand of maize will increase from 526 metric tons to 784 metric tons from 1993 to 2020, particularly in developing countries [7]. In view of this alarming situation, different effective measures need to be adopted to improve crop productivity, particularly in salt-affected soils where crop productivity is reduced by more than 50%.

A plethora of information is available in the literature on salinity tolerance of potential agricultural crops at cellular level as well as on whole plant level, on the basis of which a number of strategies have been devised to overcome the salinity problem [4, 5, 8–10]. These include screening and conventional breeding, wide crossing, and, more recently, marker-assisted selection and the use of transgenic plants. A number of researchers still emphasize the use of conventional selection and breeding, with the help of advanced molecular biology techniques, to improve crop salt tolerance [4, 5, 8–11]. However, complex interactions between stress factors and various molecular, biochemical and physiological phenomena make it difficult to achieve the desired degree of salt tolerance [5, 8]. Thus, some alternative approaches need to be adopted to overcome the problem.

Recently, some rapid and economically feasible shotgun approaches have been proposed to alleviate the adverse effects of salt stress [10, 12]. A number of studies have emphasized that exogenous application of osmoprotectants is a useful approach in inducing salt tolerance in crops [12]. Of the different compatible solutes known, glycinebetaine (GB) is relatively more important as it is capable of promoting plant growth and yields under normal or stress conditions due to its osmoprotective influence on photosynthetic machinery [13–15], and regulation of antioxidant capacity and ion homeostasis [16, 17]. However, the detailed physiological basis of how GB regulates these phenomena is not clearly understood. It is therefore necessary to confirm and further elucidate the mode of action of GB in plants under stress conditions. In the present study, GB was exogenously applied as a foliar spray to assess up to what extent exogenously applied GB could mitigate the adverse effects of salt stress on plant growth and yields of the commonly grown cereal crops, wheat and maize.

Materials and methods

In our work, the influence of exogenously applied GB on maize and wheat grown under saline conditions was assessed in two independent experiments. Field grown 9-day-old wheat plants were subjected to 2.17 or 14.67 dS/m NaCl salinity for 47 days after which GB treatments (0, i.e., no spray; 50 and 100 mM GB in 0.1% Tween-20 solution) were applied as a foliar spray. At 40 days after the exogenous application of GB (when plants were 96 days old or at the initiation of the boot stage), nine plants from each treatment (three plants/replicate) were uprooted and washed with distilled water. After drying with filter paper, roots were carefully removed and all plant parts were dried at 65°C until constant dry weight. Before harvest, photosynthetic capacity, GB, proline, and activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were measured in the second leaf from the top of each plant following standard laboratory protocols.

The experiment with maize was conducted in a similar manner except with different cultural and growth conditions. Fourteen-day-old maize plants grown in plastic pots filled with sands were subjected to 0 or 10 dS/m NaCl salinity. When plants were 3 weeks old, GB treatments (0, 50 and 100 mM GB in 0.1% Tween-20 solution) were applied as a foliar spray. At 2 weeks after the exogenous application of GB, two plants from each replicate were harvested and their fresh weights recorded. After oven-drying at 65°C dry weights recorded. Before harvest, photosynthetic capacity, GB, proline, and activities of SOD, CAT, and POD were measured in the second leaf from top of each plant following standard laboratory protocols.

Influence of exogenously applied GB on wheat

The growth and grain yield of wheat plants were improved due to exogenous application of GB under saline conditions (Fig. 1a–d). This growth and yield enhancement in the salt-stressed plants of wheat was positively associated with enhanced endogenous level of GB, resulting from GB application (Fig. 2a, b). Thus, higher endogenous levels of GB in wheat due to exogenous application of GB can be related to enhanced salt tolerance. These findings are similar to some earlier studies in which it has been observed that exogenous application of GB alleviated the adverse effects of salt stress on the growth and/or yield of different crops, e.g., wheat [18, 19], tomato [20], and rape [14].

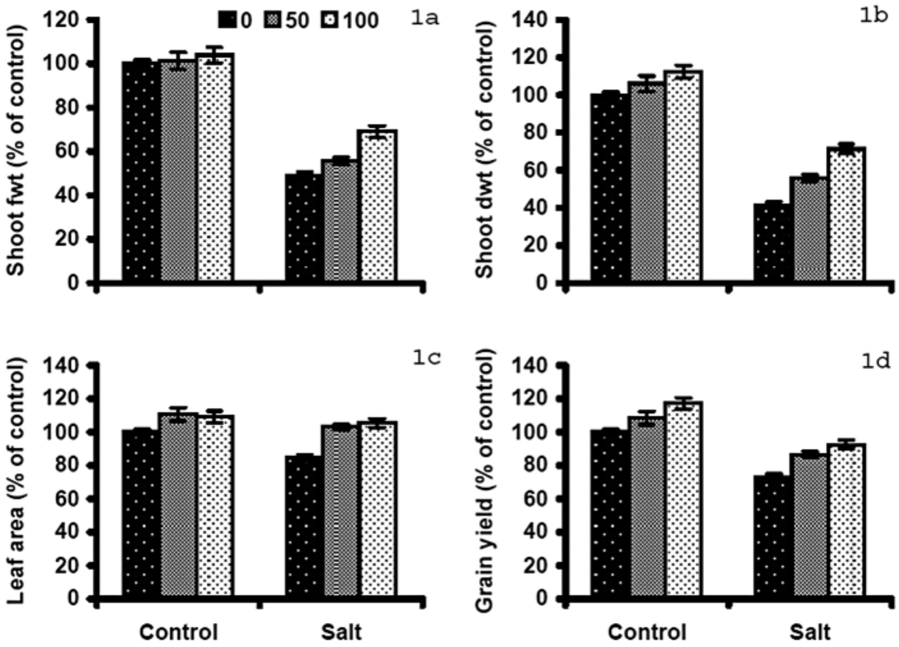

Figure 1. Fresh and dry weight of shoot, leaf area and grain yield of wheat when different amounts of glycinebetaine (GB) were exogenously applied to salt-stressed or non-stressed plants (0, no spray; 50, 50 m*M* GB; and 100, 100 m*M* GB foliar spray).

From previous reports (e.g., [21]), it is evident that GB-induced increase in salt tolerance is associated with improved photosynthetic capacity of most crops under saline conditions (Fig. 3a). The same was demonstrated in wheat in our present study. The GB-induced increase in photosynthetic capacity in wheat may have been due to stomatal or non-stomatal limitations (Fig. 3a–e), which are major controlling factors of photosynthetic rate [22, 23]. Furthermore, increase in photosynthesis was found to be primarily due to an increase in stomatal conductance, which caused higher CO_2 diffusion inside the leaf, thereby favoring higher photosynthetic rate [23]. These results are similar to those of Mäkela et al. [14, 20] in which increase in salt tolerance of field-grown tomatos due to GB application was linked with increased net CO2 assimilation rate and stomatal conductance under salt or water stress. However, the mechanism by which GB application reversed to some extent salt-induced injurious effects on photosynthesis through stomatal conductance was not clear [14, 20]. More importantly, rate of transpiration with GB application decreased in wheat under saline conditions, resulting in improved water-use efficiency (WUE). Another possibility for the GB-induced enhancement in photosynthetic rate could be a protective effect of GB on photosynthetic pigments of wheat. However, in the present study, only chlorophyll ‘b’ content

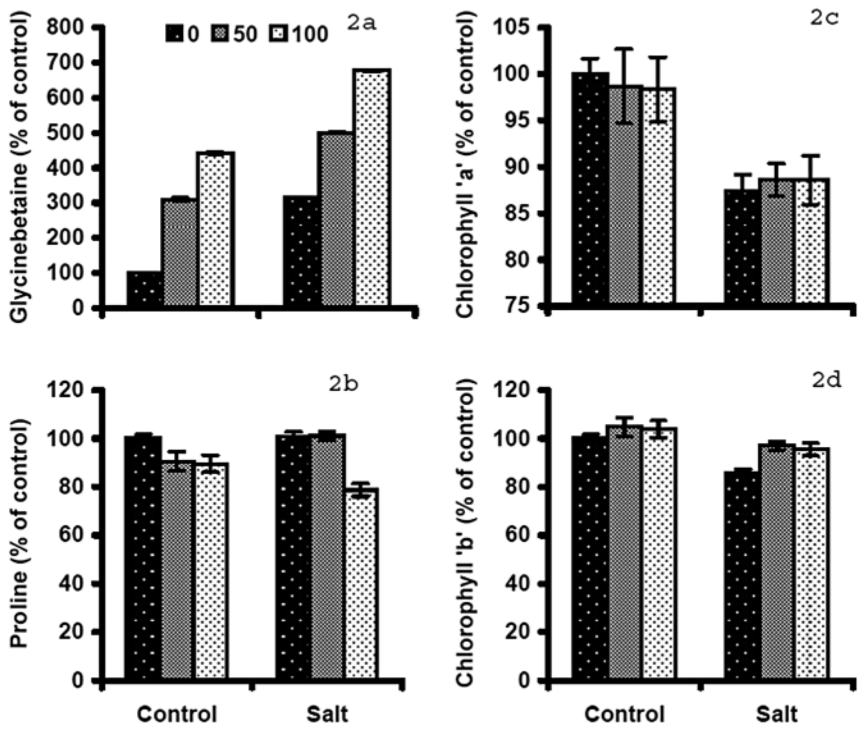

Figure 2. Leaf GB, proline, chlorophyll 'a' and 'b' of wheat when different amounts of GB were exogenously applied to salt-stressed or non-stressed plants (0, no spray; 50, 50 m*M* GB; and 100, 100 m*M* GB foliar spray).

was improved under saline conditions as a result of GB application (Fig. 2c, d). As chlorophyll 'b' is mainly associated with PS-II antenna, GB-induced improvement in Chl 'b' concentration might have been due to structural/conformational changes in the PS-II antennae, as suggested by Kocheva et al. [24]. This could be one of the additional factors causing an increase in photosynthetic capacity due to exogenously applied GB.

It is now evident that under saline conditions plants up-regulate antioxidant enzymes to detoxify salt-induced reactive oxygen species (ROS). A better antioxidant system can protect the plants from the adverse effects of salt stress [16, 25–27]. However, in the present study, the activity of only SOD was enhanced by GB application under saline conditions (Fig. 4a–c). From these results, it is possible that both SOD and GB scavenged ROS in wheat. Similarly, Demiral and Turkan [16], in a study on rice, found that 15 mM GB applied to the roots enhanced the SOD activity under saline conditions. These results are also in agreement with those of Shalata and Tal [28], who reported constitutively higher SOD and ascorbic acid peroxidase (APX) activities in

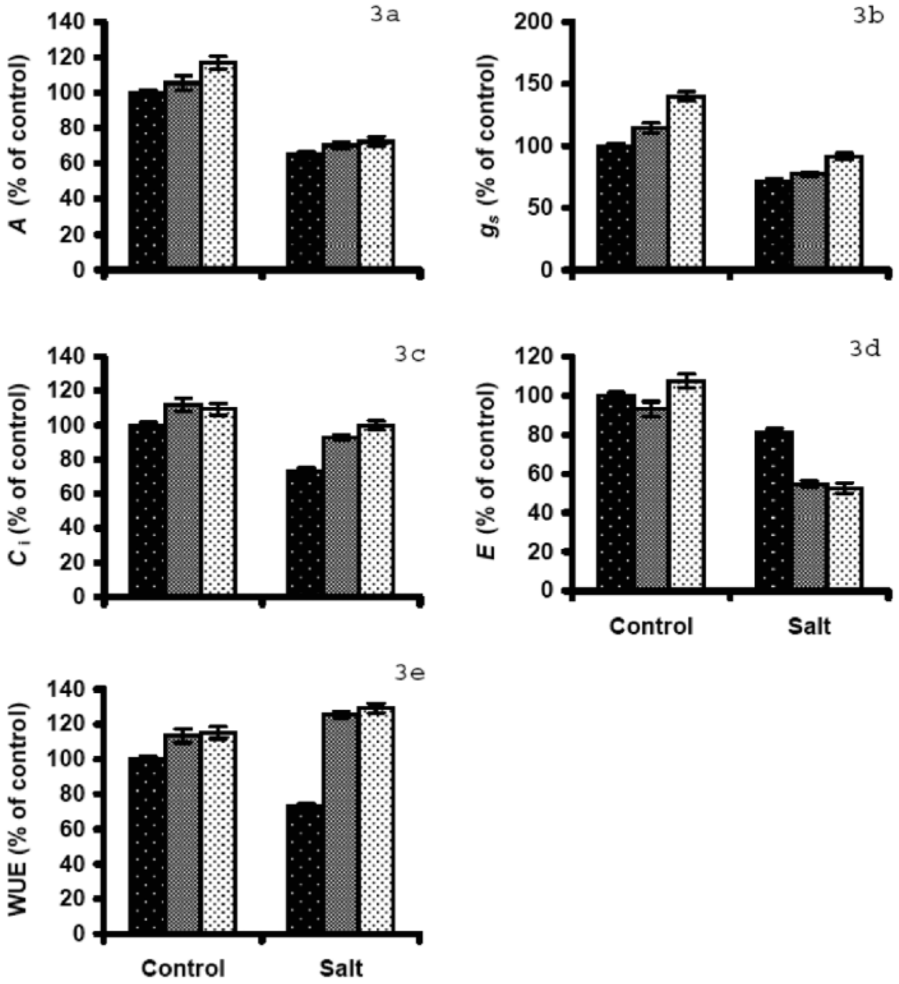

Figure 3. Net CO_2 assimilation rate (A), transpiration rate (E), sub-stomatal CO_2 (C_i), stomatal conductance (g_s), water use efficiency (WUE measured as A/E) of wheat when different levels of GB were exogenously applied to salt-stressed or non-stressed plants (0, no spray; 50, 50 m*M* GB; and 100, 100 m*M* GB foliar spray).

wild salt-tolerant tomato plants. If we draw parallels between the endogenous level of GB and antioxidant activities of each enzyme, it is evident that endogenous level of GB may have a significant protective effects on membranes by decreasing the levels of ROS in wheat plants, which thereby results in lower activities of other antioxidant enzymes (CAT and POD) under salt stress, because GB is known to scavenge hydroxyl radicals [29].

The results presented here, as well as those from some previous studies, clearly show that foliar application of GB improved the growth and yield

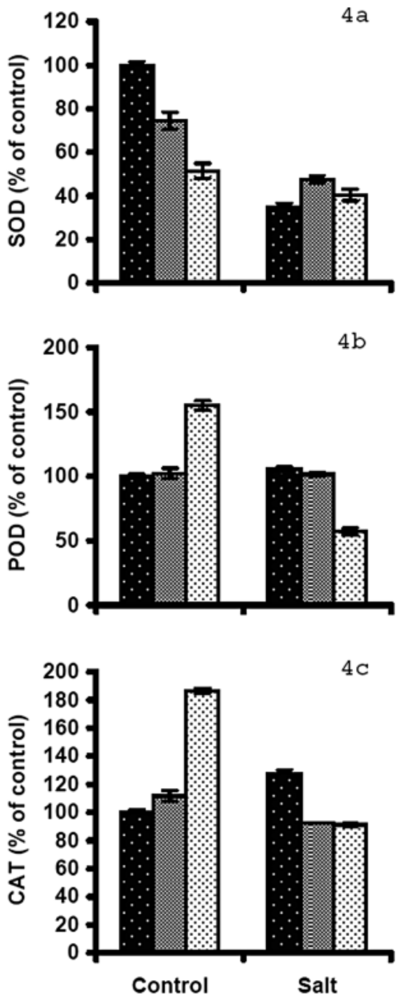

Figure 4. Activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) of wheat when different amounts of GB were exogenously applied to salt-stressed or non-stressed plants (0, no spray; 50, 50 m*M* GB; and 100, 100 m*M* GB foliar spray).

of wheat plants by improving photosynthetic capacity and alleviating the adverse effects of salt-induced oxidative stress.

Influence of exogenously applied GB on maize

Undoubtedly, in the present study, salt stress caused a reduction in growth and yield of maize; however, this reduction in growth and yield was com-

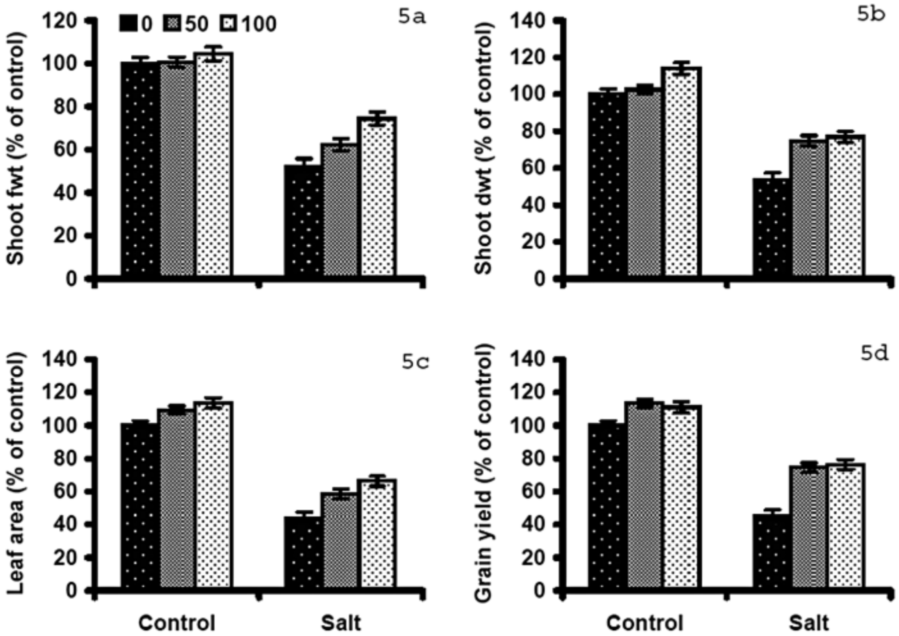

Figure 5. Fresh and dry weight of shoots, leaf area and grain yield of maize when different levels of GB were exogenously applied to salt-stressed or non-stressed plants (0, no spray; 50, 50 m*M* GB; and 100, 100 m*M* GB foliar spray).

pensated by foliar application of GB (Fig. 5a–d). These results are similar to some earlier findings in which foliar application of GB resulted in a significant improvement in salt tolerance of maize plants [21, 30]. Similarly, in rice, a marked improvement in salt tolerance was observed on exogenous application of GB [31, 32]. However, these changes in the growth of maize due to imposition of salt stress or foliarly applied GB were found to be associated with leaf growth, as biomass production is closely related to leaf area index (LAI) in different agricultural crops [33] and other vegetation types [34]. Exogenous application of GB also increased the endogenous level of GB of both salt-stressed and non-stressed plants of maize plants. It is widely accepted that GB protects higher plants against salt/osmotic stresses by stabilizing many functional units, like the oxygen-evolving PS-II complex [31], membranes [35], quaternary structures of complex proteins [13], and enzymes such as rubisco [36]. Furthermore, increased salt tolerance of maize may to be due to enhanced endogenous GB level caused by exogenously applied GB (Fig. 6a, b). Such a relationship between salt tolerance and endogenous GB level has been reported previously in different crops such as tomato and rape [20], kidney beans [37], and rice [16].

Photosynthesis in maize was reduced due to salt stress; however, foliarly applied GB ameliorated this inhibitory effect (Fig. 7a). It has already been

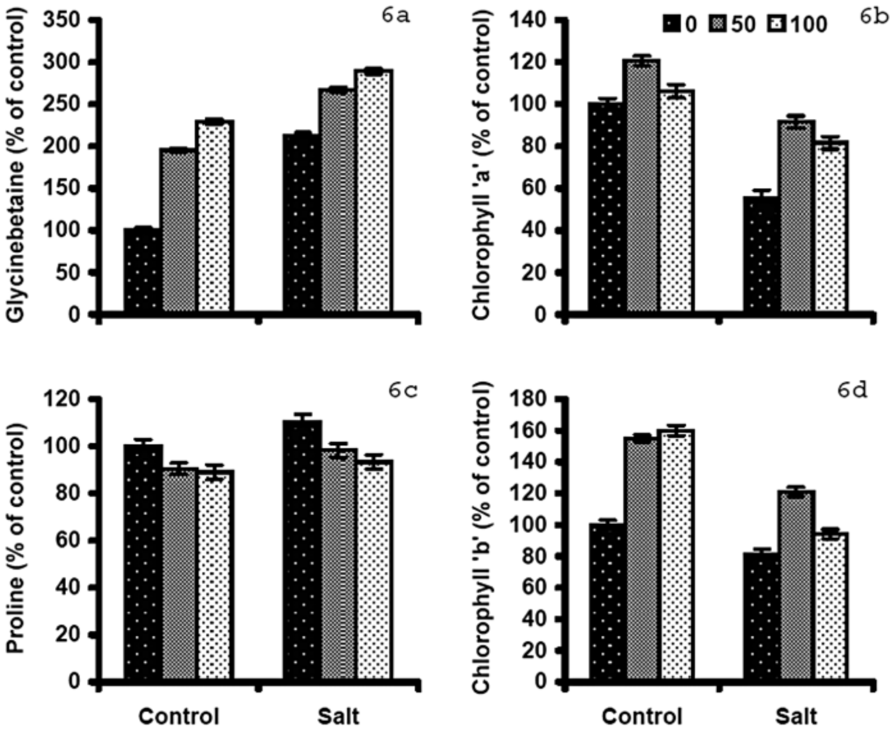

Figure 6. Leaf glycinebetaine, proline, chlorophyll 'a' and 'b' of maize when different levels of GB were exogenously applied to salt-stressed or non-stressed plants (0, No spray; 50, 50 mM GB; and 100, 100 m*M* GB foliar spray).

reported that the enhancement in net CO_2 assimilation rate due to foliarly applied GB is correlated with the degree of salt tolerance of different crops, e.g., in tomato [14] and wheat [15]. In the present study, foliar application of GB significantly increased the stomatal conductance in maize plants. Furthermore, net CO_2 assimilation rate (A) and stomatal conductance (g_s) showed a significant positive relationship. Similarly, A and g_s were also positively correlated with sub-stomatal CO_2 (C_i) (Fig. 7a–d). Foliar application of GB also improved WUE in salt-stressed plants (Fig. 7e). Furthermore, enhanced WUE in the salt-stressed maize plants due to GB application under saline conditions showed a positive relationship with growth and yield. These results can be related to those of Ashraf and Bashir [38] that demonstrated a positive relationship between WUE and grain yield of wheat.

Although the relationship between A, g_s and C_i was significant, the pattern of increase in A, along with g_s and C_i was not consistent with increasing level of GB applied (Fig. 7a–d), indicating that this effect was partial. These findings are similar to those of Mäkela et al. [14, 36], who reported that exogenous application of GB increased photosynthetic capacity of tomato through

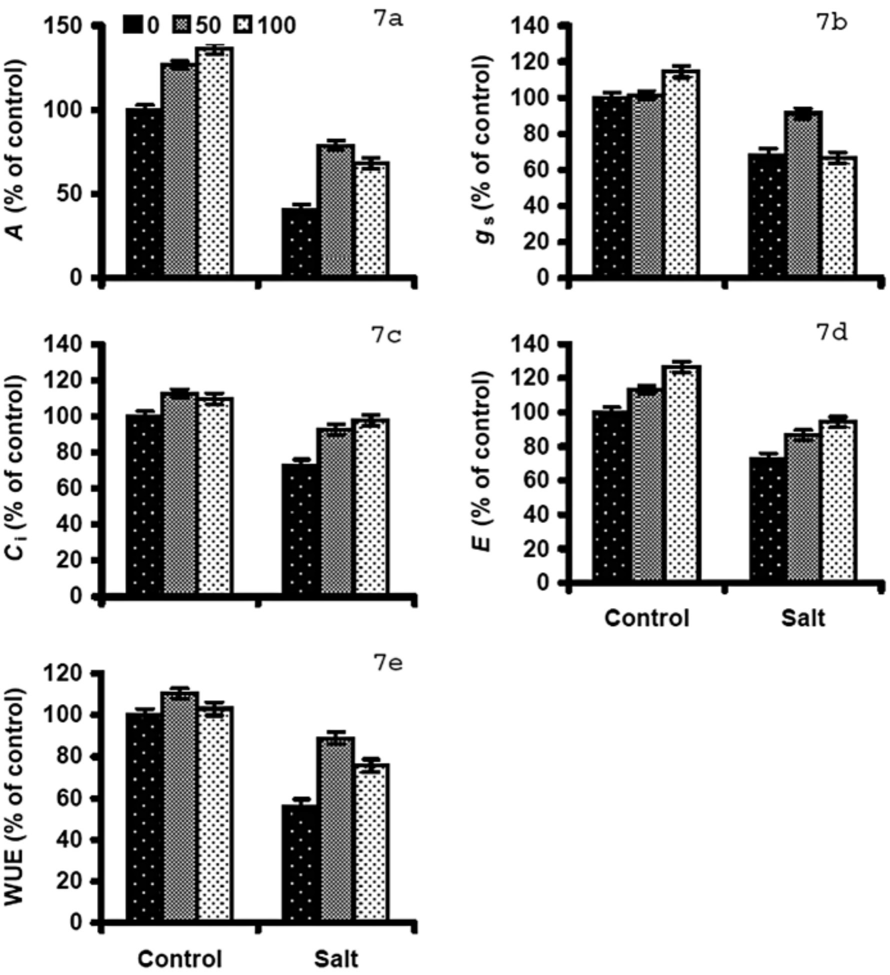

Figure 7. Net CO_2 assimilation rate (*A*), transpiration rate (*E*), sub-stomatal CO_2 (C_i), stomatal conductance (g_s), water use efficiency (WUE measured as *A*/*E*) of maize when different levels of GB were exogenously applied to salt-stressed or non-stressed plants (0, no spray; 50, 50 m*M* GB; and 100, 100 m*M* GB foliar spray).

stomatal limitations as well as by improving activity of rubisco. Likewise, Yang and Lu [21] reported that 10 mM GB applied to the roots enhanced salt tolerance of wheat plants by improving photosynthesis through stomatal conductance. In another study with maize, Yang and Lu [30] reported that GB improved photosystem-II (PS-II) efficiency in maize plants. This view was further supported by the beneficial effect that foliar application of GB had on photosynthetic pigments (chlorophyll '*a*' and '*b*') in the salt-stressed plants of maize (Fig. 6c, d). In addition, leaf chlorophyll 'a' of both cultivars

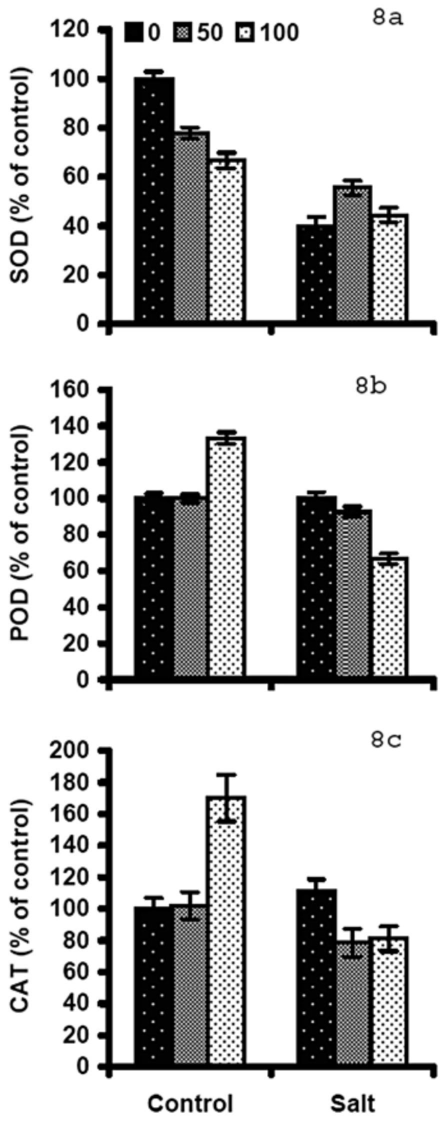

Figure 8. Activities of SOD, CAT and POD of maize when different levels of GB were exogenously applied to salt-stressed or non-stressed plants (0, no spray; 50, 50 m*M* GB; and 100, 100 m*M* GB foliar spray).

was positively correlated with *A*. A similar positive relationship between *A* and chlorophyll '*a*' has already been observed in sunflower [39], and wheat [15]. Thus, GB-induced enhancement of photosynthetic capacity was due to both stomatal and non-stomatal limitations.

Environmental stresses such as drought and salinity are known to increase the production of ROS, such as H_2O_2 (hydrogen peroxide), $O^{\bullet}{}_2^{-}$ (superoxide), $^1O_2$ (singlet oxygen) and $OH^{\bullet}$ (hydroxyl), by enhanced leakage of electrons from electron transport chains in the chloroplasts and mitochondria to molecular oxygen [40]. It is known that cytotoxic ROS can destroy normal metabolism through oxidative damage of lipids, proteins and nucleic acids [41]. Membrane injury induced by salt stress is related to an enhanced production of highly toxic ROS [42]. To scavenge these ROS, plants either synthesize different antioxidant compounds or activate antioxidant enzymes [42]. SOD is an important antioxidant enzyme [43, 44] because it is present in different cellular compartments such as chloroplasts, mitochondria, microsomes, glyoxysomes, oxysomes, apoplasts, and cytosol [44]. In the present study, salt stress caused the reduction in SOD activity of maize plants, but the exogenous application of GB enhanced its activity (Fig. 8a). In contrast, the activities of POD and CAT were inconsistently increased or decreased due to both salt stress and foliar application of GB (Fig. 8c, d). These results suggest that GB-induced enhancement in SOD activity may have protected photosynthetic machinery from salt-induced oxidative damage. These results can be related to those of Ma et al. [45] who found that GB-treated wheat plants that exhibited increased SOD and APX activities showed higher photosynthetic activity and water stress tolerance. In view of these findings, it is suggested that higher SOD activity in the salt-stressed plants of maize due to GB applied at the vegetative stage might be one of the additional factors in improving salt tolerance in maize cultivars as recently proposed by Cuin and Shabala [46].

From these results, it is clear that foliar application of GB was effective in ameliorating the adverse effects of salinity on photosynthesis and yield of maize plants. GB-induced enhancement in photosynthetic capacity of maize was found to be associated with both stomatal and non-stomatal limiting factors. Furthermore, exogenous application of GB also scavenged free radicals generated by salt stress directly and by enhancing SOD activity.

Conclusions

Although exogenous application of GB induced salt tolerance in potential cereals (wheat and maize) by enhancing photosynthetic and antioxidant capacities, its effect seems to be greater in wheat plants assessed as percent increase in growth and yield under saline conditions. However, GB-induced improvement in photosynthetic capacity in wheat was found to be mainly through stomatal limitations, while that in maize it was through both stomatal and non-stomatal factors, suggesting that the mechanism by which GB-induced salt tolerance is species specific. Furthermore, because naturally produced GB does not normally break down in plants [1], it can easily be extracted from high-producing plants such as sugar beets. According to an estimate, exogenous application of GB, with a cost of US$ 20–25 kg^{-1} ha^{-1}, the net benefit appears to be as high as US$ 580 ha^{-1} [47]. Thus, the easy extraction and its exogenous application make the use of GB an economically feasible approach to counteract adverse effects of environmental stresses on crop productivity.

Overall, exogenous application of GB is a promising means to improve growth and crop yield under salt stress.

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