Bulgarian Journal of Agricultural Science, 24 (No 3) 2018, 437–442
Agricultural Academy

USING CHITOSAN TO IMPROVE GROWTH OF MAIZE CULTIVARS UNDER SALINITY CONDITIONS

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Abstract

Al-Tawaha, A.R., M.A. Turk, A.R.M. Al-Tawaha, M.H. Alu'datt, M.Wedyan, E. Al-D. M. Al-Ramamneh and A.T. Hoang, 2018. Using chitosan to improve growth of maize cultivars under salinity conditions. *Bulg. J. Agric. Sci.*, 24 (3): 437–442

Chitosan has been broadly used in agriculture to reduce harmful effects on plants during unfavorable conditions as well as to enhance plant growth. Two experiments were conducted to (i) determine the ability of chitosan to alleviate the deleterious effects of salinity on two maize cultivars, (ii) analyze the effects of different levels of chitosan application on the growth of maize under salinity stress. Results indicated that salinity has negative effects on shoot and root growth, where a significant decrease was observed in shoot and root dry weight, plant height and leaf areas for both maize genotypes at the 4-leaf stage. Foliar application of chitosan improved plant growth for non-saline treated plants and ameliorated the adverse effects of salinity on shoot and root growth more in SR03 than in Pioneer 3906 maize genotypes. Results also indicated that transpiration rates were neither affected by salinity level nor by chitosan application at any rate.

Key words: hydroponic; natural elicitors; transpiration rate

Introduction

Maize is one of the most important cereal crops cultivated across the world under diverse agro-ecological regions and farming schemes. In many parts of the world, the maize and other crops their productivity is limited by environmental factors such as salinity and drought (Musallam et al., 2004; Abebe et al., 2005; Tawaha and Al-Ghzawi, 2013; Tawaha et al., 2017; Altunlu et al., 2017). It is estimated that globally, the total area of saline soil is 830 Million hectares (Martinez-Beltran and Manzur, 2005) of which about 20% of salt affected lands are of irrigated ones (Pitman and Läuchli, 2002). Salinity affects plants in two ways, the first of which is osmotic effect which reduces the ability of plants to absorb water and retard growth while the second effect is ion toxicity and in this phase, salts may enter the transpiration system and injure leaf cells which also causes reduced cell division and cell expansion (Munns, 2005).

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Elicitors such as chitosan are biotic factors that can elicit a positive response in crops, typically a defense-related response such as the buildup of bioflavonoids (Al-Tawaha et al., 2005; Al-Tawaha et al., 2006). Accordingly, natural elicitors such as chitin have been used not only to enhance crop tolerance towards numerous pathogens (Al-Tawaha et al., 2005; Al-Tawaha et al., 2006), but also to enhance the attentiveness of useful bioflavonoids in numerous plant varieties e.g. Kim et al. (2005). Chitosan or Chitin is the most common natural polysaccharide present in cell walls of fungi, crabs, shrimp, insect exoskeletons, parasitic nematode eggs and gut linings. Many scientists have reported that chitin has potentials to enhance plant growth as well as it can control plant disease (Al-Tawaha et al., 2005; Al-Tawaha et al., 2006; Gornik et al., 2008). In a study conducted in Jordan by Tawaha and Al-Ghzawi (2013), it was inferred that when lentil seeds were primed with 30 mg/Lchitosan, it resulted in highest germination percentage, hypocotyl length, radical length, hypocotyl dry weight and radical dry weight. Further, in corn plant, Guan et al. (2009) found that seed priming with chitosan improved shoot and root length, shoot and root dry weights. Similar results were reported by Zhou et al. (2002) who indicated that chitosan application in peanut seeds can enhance germination percentage levels, energy, lipase activity, gibberellic acid (GA_3) and indole acetic acid (IAA). The objective of our study was to (i) determine the ability of chitosan to alleviate the deleterious effects of salinity on two maize cultivars.(ii) analyze the effects of different levels of chitosan application on the growth of maize under salinity stress.

Materials and Methods

First experiment: Two hybrid maize varieties; a salt-sensitive, Pioneer 3906 and a highly salt-resistant, SR03, were grown in a growth chamber in hydroponic conditions. Seeds of Pioneer 3906 and SR03 were soaked in aerated 0.001 mol/L $CaSO_4$ solution for one day and were allowed to germinate between two layers of the filter paper at 28ºC for three days in the dark. The nutrient solution is composed of 0.0025 mol/L $Ca(NO_3)_2$, 0.001 mol/L K_2SO_4, 0.0002 mol/L KH_2PO_4, 0.0006 mol/L $MgSO_4$, 0.005 mol/L $CaCl_2$, 0.001 mol/L NaCl, 0.001 mol/L H_3BO_4, 0.002 mol/L $MnSO_4$, 0.0005 mol/L $ZnSO_4$, 0.0003 mol/L $CuSO_4$, 0.00005 mol/L $(NH_4)_6Mo_7O_{24}$ and 0.002 mol/L Fe–EDTA (Fortmeier and Schubert, 1995). The nutrient solution was changed two times a week followed by every two days once the plants reached four-leaf stage so that continuous nutrient is supplied. Maize plants of each hybrid were subjected to the following treatments: T1: 0 mol/L NaCl (Control); T2: 0.1 mol/L NaCl; T3: 10mg/L Chitosan application; T4: 0.1 mol/L NaCl + 10mg/L Chitosan. Chitosan solution was prepared by dissolving in HCl and subsequent pH adjustment to 6 using NaOH. Five ml of the 10 mg $L^{-1}$of chitosan has been applied to each pot, containing 4 plants. The experiment was organized in randomized complete blocks design with four replicates. The main plots were assigned to the two hybrids of maize, whereas the sub plots were assigned for salinity and chitosan application.

Second experiment: Seeds of the maize hybrid SR03 were soaked in aerated 1 mol/L $CaSO_4$ solution for one day then allowed to germinate and the resulted seedlings were established in hydroponic conditions as described above in experiment one. Seedlings were supplemented with the nutrient solution using the same schedule as described in the previous experiment. Plants of the maize hybrid SR03 were grown in 4.5 L plastic pots and divided into two groups, the first of which were subjected to 0.15 mol/L NaCl, whereas the second group were control grown without exposure to salinity. To each group of plants foliar-form of chitosan was applied at 30, 60, and 120 mg/L when maize reached four-leaf stage. Periodic change of nutrient solution was applied as mentioned in previous experiment to evade nutrient reduction. Besides, for each treatment plants received 10 ml of chitosan applied to their root system. The experiment was organized in a randomized complete block design (RCBD) with four replicates. For both experiments, NaCl was applied when the full nutrient concentration was reached. The verified levels of NaCl were 0.001 mol/L for controls and 0.15 mol/L for salt treatments. NaCl concentration was enhanced in a stepwise fashion by 0.025 mol/L increments at daily intervals.

Measured variables: Root fresh weight (g), root dry weight (g), shoot fresh weight (g), shoot dry weight (g), plant height (g), leaf area (cm^2) and transpiration rate were recorded.

Statistical analysis: The analysis of variance and mean separation according to Least Significant Differences (LSD) were performed using statistical computer program Mstatc for Randomized Complete Block Design (RCBD) as described by Steel and Torrie (1980). Means were compared at 0.05 probability level. Histograms and standard error bars were drawn according to Dytham (1990).

Results and Discussion

Plant biomass and growth relations

Results indicated that salinity has negative effects on root and shoot growth and at 0.1 mol/L NaCl significantly decreased root and shoot dry weights, and plant height of maize genotypes compared to non-treated control in the first experiment (Figures 1-4). The adverse effects of salinity stress were previously reported for many crops (Kurth et al. 1986, Zidan et

al. 1990, Neumann 1995, Reinhardt and Rost 1995, Sanderson et al., 1997, Ramos et al., 2004, Othman et al. 2006, Ashraf et al.2008, Schubert et al. 2009, Tawaha and AL-Ghzawi 2013). The results obtained here is in line with earlier results obtained from using similar genotypes (Beltag et al. 2006, Geilfus et al. 2010). Foliar application of chitosan improved plant growth than untreated plants and increased root dry weight as well as shoot dry weight for SR03, but not for Pioneer 3906 plant when it reached 4-leaf stage (Figsures 1and 2). Chitosan also increased root dry weight in SR03- and Pioneer 3906- saline treated plants, although effect was more evident with SR03 plants (Figure 1). However, the effect of chitosan in enhancing shoot dry weight was significantly ($P \leq 0.05$) observed in SR03- but not for Pioneer 3906- saline treated plants (Figure 2). The two maize genotypes varied in height, with SR03 genotype, being the tallest and Pioneer 3906, being the shortest one. However, while SR03-saline treated plants did not show significant response ($P \leq 0.05$) to chitosan application in terms of their height, Pioneer 3906- saline treated plants showed a significant decrease in height (Figure 3). Overall, chitosan application ameliorated the effects of salinity stress in SR03 more than in Pioneer 3906 emphasizing the differences existed between genotypes with regard to salt stress tolerance (Figures 1-4).

In the second experiment, Chitosan application at 30 to 120 mg/L significantly ($P \leq 0.05$) promoted root dry weight as well as shoot dry weight than untreated plants under non-saline conditions (Table 1). The significantly highest root dry weight was recorded when maize plants were treated with 120 mg/L chitosan at 4-leaf stage (17.1 g). Application of chitosan at the three levels tested promoted shoot dry weight of maize plants, with significant effects being observed for shoot dry weight (31.8, 32.3 and 31.7 g corresponding for chitosan at 30, 60, and 120 mg L^{-1}, respectively) than untreated plants (28.3 g). Salinity also adversely affected root and shoot growth in the second experiment (Table 1). A significant decrease in root dry weight (13.0 g,) as well as in shoot dry weight (17.3 g) was recorded under severe salinity stress (0.15 mol/L NaCl) than in untreated plants (15.7, 28.3 g corresponding to root dry weight and shoot dry weight, respectively) (Table 1). Although effects were not significant, chitosan application decreased the unfavorable effect of salinity on root dry weight of maize plant when used at 30 to 60 mg/L (Table 1). Plants treated with 0.15 mol/L NaCl,

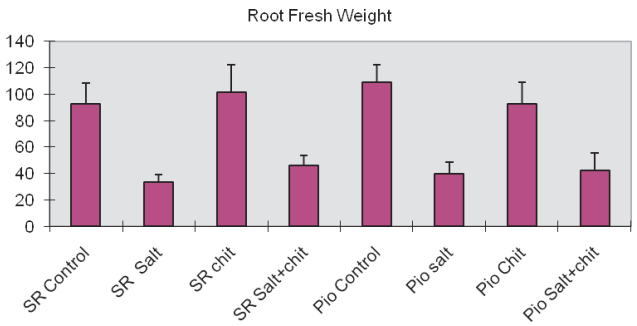

Fig. 1. Root fresh weight for two maize genotype subjected to slat stress and chitosan application

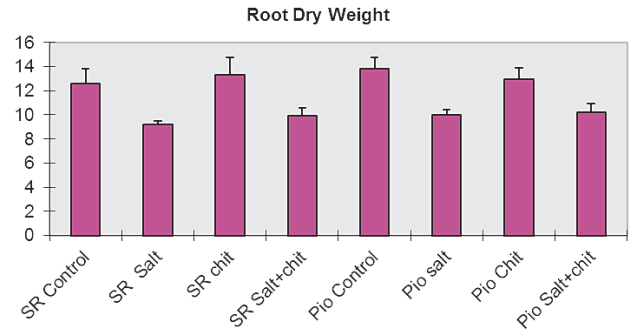

Fig. 2. Root dry weight for two maize genotype subjected to slat stress and chitosan application

Fig. 3. Shoot fresh weight for two maize genotype subjected to slat stress and chitosan application

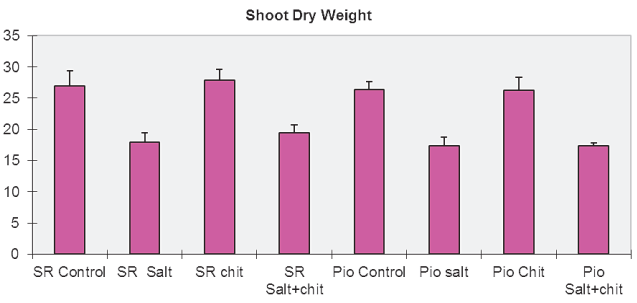

Fig. 4. Shoot dry weight for two maize genotype subjected to slat stress and chitosan application

Table 1
Effect of chitosan application and NaCl on shoot and root dry weight, plant height, leaf area and transpiration rate of maize plants

Treatments	Shoot dry weight (g)	Root dry Weight (g)	Plant height (cm)	Leaf area (cm^2)	Transpiration rate. (ml cm^{-2} d^{-1})
Control	28.28 b	15.70 b	81.94 b	494.20 a	0.31 a
Chitosan (30 mg/L)	31.77 a	16.33 ab	84.06 ab	532.10 a	0.26 a
Chitosan (60 mg/L)	32.30 a	16.60 ab	84.38 ab	610.30 a	0.26 a
Chitosan (120 mg/L)	31.67 a	17.08 a	84.81 a	560.90 a	0.26 a
NaCl (0.15 mol/L)	17.30 d	13.00 c	60.06 d	286.90 b	0.20 a
NaCl (0.15 mol/L) + Chitosan (30 mg/L)	19.75 cd	13.20 c	62.00 cd	270.90 b	0.21 a
NaCl (0.15 mol/L) + Chitosan (60 mg/L)	22.48 c	13.73 c	63.81 c	313.50 b	0.20 a
NaCl (0.15 mol/L + Chitosan (120 mg/L)	17.27 d	12.82 c	60.92 d	262.60 b	0.23 a
LSD (0.05)	3.04	1.12	2.73	164.00	0.15

then recieving chitosan at 60 mg/L had roots that was 13.7 g dry weight compared to roots of saline- but not chitosan-treated plants (13.0 g dry weight) (Table 1). The effects of chitosan were significant (P≤0.05) on improvement of shoot dry weight in maize plants grown under saline conditions, with the highest response being observed for plants treated with chitosan at 60 mg/L(22.5 g), although at the same level of significance to using chitosan at 30 or 120 mg L^{-1}, than in saline- but not chitosan- treated plants (17.3 g) (Table 1).

In the second experiment, for plants grown under non-saline conditions chitosan application at the three levels tested significantly increased height of maize plants, with the tallest plants being produced when subjected to chitosan at120 mg/L (84.8 cm) than in untreated plants (Table 1). In contrast, salt stress at 0.15 mol/L NaCl significantly (P≤0.05) reduced plant height (60.1 cm) than untreated plants (81.9 cm) (Table 1 and Figure 5). When applied to saline-stressed plants, chitosan at 60 mg/L increased plant height significantly than untreated ones (Table 1).

Leaf area and transpiration rate

The Leaf area per plant of the two maize genotypes was significantly (P≤0.05) reduced by 0.1 **mol/L** NaCl compared to non-treated control in the first experiment. The reduction in leaf area (Figure 6) for maize genotypes under salinity stress has been attributed to suppressed cell division. The results proved that reported by Mathur et al. (2006) whom concluded that the high concentrations of sodium chloride lead to decreased leaf area in bean plants. Similar results were reported in *Avena sativa* L. (Zhao et al., 2007) and *Fragaria x ananssa* L. (Yilmaz and Kina, 2008). The harmful effects of salt also affected photosynthesis due to decreased leaf area and chlorophyll content (Netondo et al., 2004). However, Chitosan increased leaf area in the two- saline treated genotypes of maize, with higher response being observed in SR03 plants (Figure 4). Moreover, leaf area increased significantly under non-saline conditions in both genotypes with chitosan application at 10mg/L concentration, when the plant has reached 4-leaf stage compared to untreated plants.

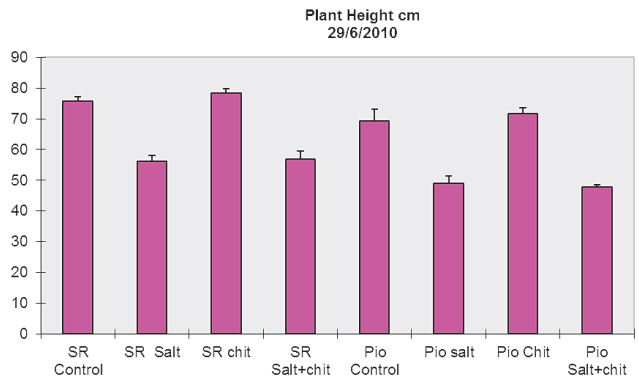

Fig. 5. Plant height (cm) for two maize genotype subjected to slat stress and chitosan application

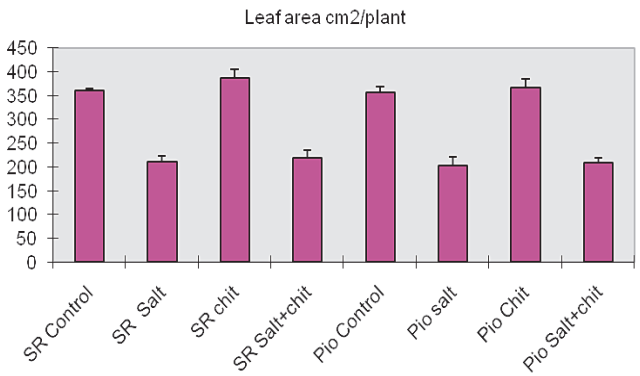

Fig. 6. Leaf area (cm^2/plant) for two maize genotype subjected to slat stress and chitosan application

In the second experiment, leaf area decreased significantly from 494.2 (cm^2) in control to 286.9 (cm^2) under 0.15 mol/L NaCl. Chitosan application at 30, 60 or 120 mg/L had no effects on leaf area when the plant reached 4-leaf stage whether plants were untreated or under saline stress. The transpiration rate was unaffected by salinity level or chitosan whatever level was used (Table 1).

Overall, chitosan enhances the growth of maize plant under control condition as well as under salinity stress. So it is suggested that natural elicitor could be a promising element that can be used to mitigate the damaging effects of salinity stress on growth and yield of maize plants. This observation is supported by the study results of El-Tantawy (2009) who reported that with chitosan application, the growth and development of tomato plants were enhanced. The study concluded that Chitosan is a promising candidate that could be used in future to lessen the harmful effects of salinity with regards to growth and yield of maize plants. The current study is the first study to report this phenomenon.

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Received November, 17, 2017; accepted for printing May, 9, 2018