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Effect of Salinity on Growth, Xylem Structure and Anatomical Characteristics of Soybean

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Abstract

This research was conducted in order to evaluation the salinity stress effect on growth parameters and stem anatomical changes of soybean grown under controlled conditions. Soybean seeds were surface sterilized and then sown into plastic pots filled up with perlite and vermiculite. Seeds were irrigated with Broughton and Dilworth solution daily. At full folded cotyledons stage (5 day after sowing), salinity stress was induced by adding NaCl into nutrition solution with final concentration of 0, 25, 50 and 100 mM. Thirty days after sowing plants were harvested and growth parameters and anatomical changes were evaluated. The results showed that, salinity stress was significantly decreased shoot and root weight either fresh weight or dry weight, in addition, total plant weight, plant height and leaf number were decreased due to salinity stress. Interestingly, leaf area was not affected by salinity stress. Stem microscopic study demonstrated that, salinity stress significantly increased cutin mass and trichome density on epidermal cells. On the other hand, cortex thickness was decreased because of salinity stress while xylem thickness had upward increase when soybean plants were grown under salinity stress especially high level of salinity. Additionally, there were changed in xylem formation and arrangement in stressed plants.

Keywords: anatomical characteristic, growth, salinity stress, soybean

Introduction

One of the most widespread agricultural problems in arid and semiarid regions is soil salinity, which makes fields unproductive and decreases crop yield. All natural waters contain soluble salts. The concentration of the salts determines whether the water is of high quality or of low quality. Salinity becomes a concern when an excessive amount or concentration of soluble salts occurs in the soil or water. In general, NaCl is the most common salt. It has been reported that salinity limits plant growth and productivity (Ashraf and Foolad, 2006; Ghazi and Al-Karaki, 2006). Salinity stress decreases photosynthetic capacity, due to the osmotic stress and partial closure of stomata (Drew *et al.*, 1990). Salt stress also affects phytohormones which are naturally occurring organic substances, influencing physiological processes at low concentrations either in distant tissues to which they are transported or in the tissue where synthesis occurred (Davies, 1995). It is evident that there are big changes in morphology and anatomy of plants growing in saline soils. The effect of salinity on root (An *et al.*, 2003) and leaf anatomy (Hu and Schmidhalter, 2001; Kiliç *et al.*, 2007) of plants had already been reported in previous works. Many researchers reported that with an increase in salinity there was a decrease in the development of the xylem. Pimmongkol *et al.* (2002) stated that the width of vascular bundles and diameters of rice stems decreased in NaCl medium. Junghans *et al.* (2006) showed that high salt concentrations reduced the cambial activity in *Populus euphratica*. Salinity causes reduced total leaf area (Awang *et al.*, 1993), and increased leaf thickness (Raafat *et al.*, 1991). Salinity also reduces development of vascular tissue (Belda and Ho, 1993), increases trichome density and decreases or has no effect on stomatal density (Ludders and Kaminski, 1991).

Soybean is one of the most important fabaceae plants. It is also considered as a good source of vegetable protein and oil since it has the highest level of protein in comparison with the other leguminous plants (Moussa, 2004). In soybean, salinity stress inhibits seed germination and seedling growth, reduces nodulation, and decreases biomass accumulation and yield (Essa, 2002). Unfortunately, there are fewer studies on the effect of salinity on stems anatomy of soybean. Thus, we investigated the effect of NaCl induced stress on growth attributes and stem anatomical characteristics of soybean. The objectives of this study were to determine whether these traits were affected differently when the soybean plants were grown under salinity stress.

Materials and methods

Seeds of the soybean [*Glycine max* (L.) Merr.] c.v. 'L17' were surface sterilized in hydrogen peroxide/ethanol solution (10 ml of 30% H_2O_2 and 75 ml of 96% ethanol filled up to 100 ml with sterile distilled water) and rinsed several times with sterile water. The surface sterilised soybean seeds were sown into plastic pots filled up with 1:1 autoclaved perlite and vermiculite at depth of 1-2 cm. Four seeds were

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sown in each pot. The pots were placed into a growth cabinet (L/D=16/8 h, T=28/25°C), and watered with full strength of Broughton and Dilworth solution containing 8 mM KNO_3 (Broughton and Dilworth, 1971).

All seedlings were inoculated with *Bradyrhizobium japonicum*. The inoculant was produced by culturing *B. japonicum* in yeast extract-mannitol broth (YMB) in 250 ml flasks shaken at 150 rpm at 28°C. The five days old plants were inoculated with a liquid YMB and watered with B&D solution containing 2 mM KNO_3 supplemented with 25, 50 and 100 mM NaCl. Control plants were maintained in a NaCl-free solution. Two times a week, the plants were also watered with distilled water to prevent of salt accumulation. An excess of drain water was removed from the saucers. After 30 days after sowing the soybean plants were removed from the pots. The roots were gently washed with water to remove all perlites and vermiculites and then shoot and root were detached and weighted. Number of leaves was counted and also leaf area was measured by leaf area meter (Delta-T Devices LTD, England), afterwards the samples were dried at 70°C for 24 h to calculate dry weight. Stem sample of each treatment was taken and fixed into 70% ethanol for anatomical assay. Stem sections were cut with a razor blade and placed in distilled water and observed under fluorescence microscopy. Olympus microscope equipped with a digital camera was used; the study was conducted in Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

The experiment was structured following a Completely Randomized Design (CRD) with three replications. For all variables, analysis of variance (ANOVA) was performed. The significance of differences among treatment means were compared by Duncan's Multiple Range Test (DMRT).

Results and discussion

The analysis of variance showed a significant effect ($p < 0.01$) on all traits due to salinity stress. The results are shown in Tab. 1. In addition, comparison of means by Duncan's Multiple Range Test demonstrated that, salinity stress decreased root weight either fresh weight or dry weight (Tab. 2). Decrease in root weight was parallel with enhancement of NaCl concentration in nutrition solution. The highest root weights (fresh or dry weight) were obtained in control treatment while the lowest one was observed in 100mM NaCl treatment (Tab. 2). Similar results were achieved when shoot fresh weight and then shoot dry weight were measured. The highest and the lowest shoot weights were related to control treatment and 100mM NaCl treatment, respectively (Tab. 2). Salinity stress was diminished plant growth and significantly decreased total dry weight. There was downward decrease in shoot weight because of deterrent effect of salinity on plant height (Tab. 2). Salt stress adversely affects the growth and development of crops, and the results of our study confirm that all growth variables of soybean drastically decreased with NaCl treatment. It has been reported that the plants had the reduction in their fresh weights because of the proportional increase in Na^+ concentration, which could imply that an ionic effect was being manifested. It is also assumed that in addition to toxic effects of NaCl, higher concentration of salt reduces the water potential in the medium which hinders water absorption and thus reduces plant growth. Sagi *et al.* (1997) also found the adverse effects of salinity stress on shoot and root growth. It has been reported that, decline in plant biomass may be due to excessive accumulation of NaCl in chloroplasts of soybean, which affects growth rate, and is often associated with a decrease in the electron transport activities of photosynthesis (Kirst, 1989) and inhibition of PSII activity (Kao *et al.*, 2003). In general, salinity reduces leaf number, leaf area, shoot and root dry weight, leading to low yields (Essa, 2002; Hamdy *et al.*, 1993; Li *et al.*, 2006; Sharifi *et al.*, 2007).

There was no significant difference between control and 25 mM NaCl treatments on plant height, while increase of NaCl concentration from 25 mM to 50 mM and finally to 100 mM dramatically decreased plant height (Tab. 2). Reduction in growth attributes especially plant height may be due to changes in plant-water relationships under salt stress, which suppress meristem activity as well as cell elongation (Dorgham, 1991). Data reported by Essa (2002) showed that reduction of 4% in plant height and in dry weight, at 45 days after planting of soybean under increase of soil salinity.

Leaf number was not affected by 25 and 50 mM NaCl concentrations so that there was not significant difference between those treatments in compare with control but increase of salinity to 100 mM significantly decreased leaf number, which confirms the results of Ünlükara *et al.* (2008). Inhibition of the formation of leaf primordia under salinity stress could be the probable reason for low leaf number. Iyenger *et al.* (1977) reported that saline irrigation water reduced the number of leaves.

Also there was no significant difference among salinity treatments on leaf area (Tab. 2). Our results are inconsistence with Gosset and Lucas (1994) who reported that NaCl highly reduced total leaf area; it seems that plant height was more sensitive to salinity than leaf number or leaf area expansion.

Pearson correlations between growth parameters are given in Tab. 3. Briefly, there were positive and significant correlations between all traits except leaf number and root dry weight.

Microscopic results showed that salt induced increment in cutin synthesis on epidermal cells. Enhancement of salinity level increased cutin layer thickness in compare with control treatment. Cutin layer is distinguishable by a bright and orange layer on the epidermal cells under florescence microscope (Fig. 1 and Fig. 2). The plant cuticle is a lipidic layer of cutin that covers essentially all aerial organs

Tab. 1. Analysis of variance on soybean growth parameters affected by salt stress

Sources of variation	df	Root fresh weight	Root dry weight	Shoot fresh weight	Shoot dry weight	Total dry weight	Plant height	Leaf number	Leaf area
Treatment	3	0.817**	0.323**	1.780**	1.060**	2.549**	134.000**	0.666**	409.888ns
Error	8	0.004	0.018	0.003	0.016	0.033	2.333	0.083	16.000
CV (%)		2.76	34.57	2.21	17.64	16.35	4.09	7.87	2.77

** significant at the 0.01 probability levels

Tab. 2. Means comparison of soybean growth parameters affected by salt stress

Treatments	Root fresh weight (g)	Root dry weight(g)	Shoot fresh weight(g)	Shoot dry weight(g)	Total dry weight(g)	Plant height (cm)	Leaf number	Leaf area (cm)
0 mM (Control)	2.94a	0.82a	3.49a	1.54a	2.36a	42.00a	4.00a	152.33a
25 mM NaCl	2.57b	0.45b	2.74b	0.76b	1.22b	42.33a	4.00a	150.66a
50 mM NaCl	2.11c	0.19c	2.15c	0.44c	0.63c	37.00b	3.66a	146.66a
100mM NaCl	1.74d	0.08c	1.71d	0.17d	0.25d	28.00c	3.00b	127.00a

Within each column followed by the same letter are not significantly differences ($p < 0.05$)

Tab. 3. Pearson correlations between growth parameters affected by salt stress

	Root fresh weight	Root dry weight	Shoot fresh weight	Shoot dry weight	Total dry weight	Plant height	Leaf number	Leaf area
Root fresh weight	1							
Root dry weight	0.92**	1						
Shoot fresh weight	0.97**	0.92**	1					
Shoot dry weight	0.94**	0.90**	0.96**	1				
Total dry weight	0.95**	0.96**	0.97**	0.98**	1			
Plant height	0.88**	0.72**	0.83**	0.75**	0.75**	1		
Leaf number	0.74**	0.52ns	0.75**	0.66*	0.62*	0.87**	1	
Leaf area	0.81**	0.68*	0.79**	0.70*	0.71**	0.96**	0.87**	1

*, ** significant at the 0.05 and 0.01 probability levels, respectively

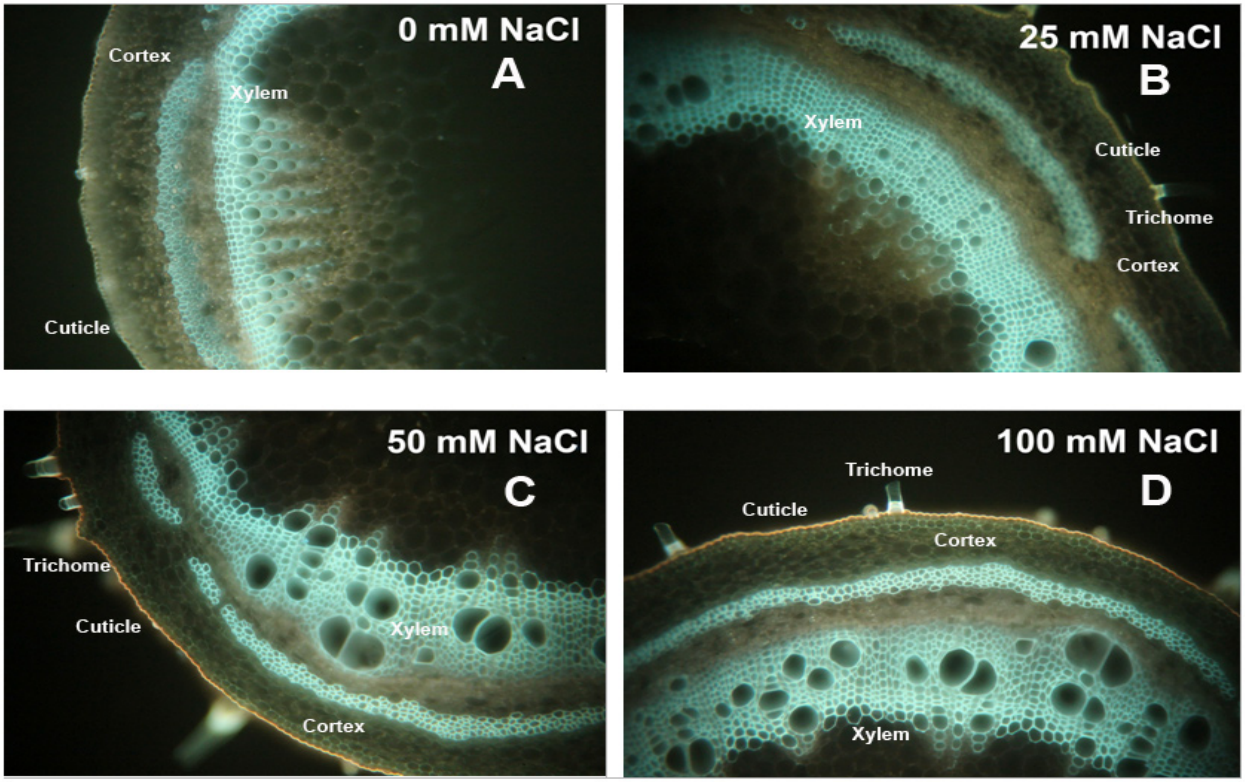

Fig. 1. Microscopic photos of stem sections treated by different concentration of NaCl

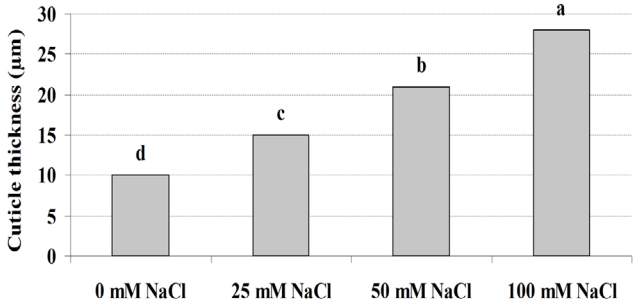

Fig. 2. Changes in stem cuticle thickness affected by different salinity stress. Within each column followed by the same letter are not significantly differences ($p < 0.05$)

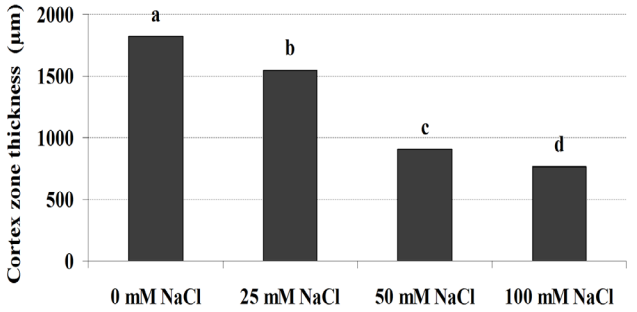

Fig. 3. Changes in stem cortex zone thickness affected by different salinity stress. Within each column followed by the same letter are not significantly differences ($p < 0.05$)

and functions to restrict transpiration. By this mechanism, the cuticle is thought to play a critical role in plant tolerance through its ability to postpone the onset of cellular dehydration during stress (Kosma and Jenks, 2007; Samuels *et al.*, 2008). Our results, which show a significant increase in cutin and an increase in the thickness of cuticle after salt stress, lead us to speculate that salinity involves synthesis of cutin.

Furthermore, we observed that, in salt stressed plants, number of trichomes was increased from epidermal stem cells. In the other word, increase of salinity level led to more trichomes on epidermal layer in compare with control plants (Data are not shown) (Fig. 1). There are several reports on increased trichome density under environmental stresses such as drought and salinity (Abernethy *et al.*, 1998; Aguirre-Medina *et al.*, 2002). Increase of trichome density may be a mechanism to increase of tolerance to salt stress. It was recently suggested that leaf glandular trichomes could contribute to the high salt tolerance by the excretion of ions (Gucci *et al.*, 1997).

Salinity induced structural changes in xylems in stems. In salt stressed plants, stems vascular cell thickness was much larger than control treatment; the salinity effect was concentration dependent. Generally, plants grown in saline solution showed higher thickness in cuticle, vascular tissues and vessel than unstressed plant while cortex zone thickness was decreased (Fig. 1 and Fig. 3). Cell walls are known to become lignified when cell expansion decreases,

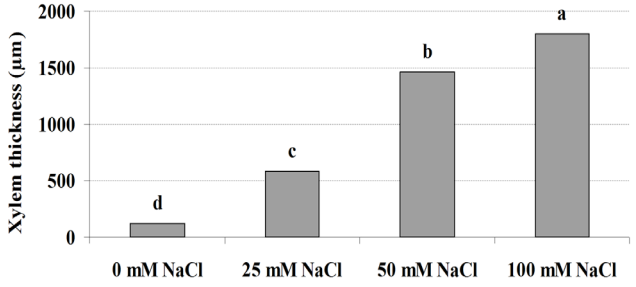

Fig. 4. Changes in stem xylem thickness affected by different salinity stress. Within each column followed by the same letter are not significantly differences ($p < 0.05$)

when the cell is under stress and when it differentiates to particular specialized tissues, notably the xylem (Christensen *et al.*, 1998). Salinity stress has been associated with a greater deposition of lignin in vascular tissues and/or xylem development. In bean-root vascular tissue, NaCl caused earlier and stronger lignifications, which has been suggested to be a factor that inhibits root growth and, consequently, represents an adaptation mechanism in resisting salinity-imposed stress (Cachorro *et al.*, 1993). In this study saline stress induced acceleration of the development of xylem and phenolic compounds in soybean stems (Fig. 1 and Fig. 4).

In conclusion, this study shows that salt stress decreases soybean growth and induces changes in anatomical characteristics such as increment of cutin synthesis on epidermal stem cells and also changes in xylem structure and lignification of them in soybean stems.

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