Anatomical Characteristics of Root and Stem of Ascorbic Acid Presoaked *Vicia faba* Seeds Grown Under Salt Stress

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Research Article

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ABSTRACT

The present work aimed to investigate changes in anatomical characteristics of root and stem in NaCl-stressed bean plants, and assessing the role of ascorbic acid to alleviate these changes. The pot experiment was carried out to study the response of presoaked faba bean seeds (*Vicia faba* cv. Misr 2) in freshly prepared ascorbic acid (50 ppm ≈ 0.3 mM; as recommended dose as described by or distilled water (control) for 4 hrs at natural environmental conditions, salinity caused a deeply tear of the outermost layers of the root in both periderm and cortex zones. In addition, NaCl treatments caused an increase in root cortex width at 150 mM NaCl compared with control. NaCl caused a highly significant decrease in xylem width of either treated plants or untreated with ascorbic acid, but the response was more prominently at high dose of NaCl. NaCl caused a highly significant decrease in xylem width and stem area of salinized plants either treated or not with ascorbic acid, especially at 100 mM NaCl.

INTRODUCTION

The over salinity of the soil is one of the most factors that limit the unfold of plants in their natural habitats. It's an associated ever-increasing drawback in arid and semi-arid regions [1]. Estimate that arid and semi-arid lands represent around four-hundredth of the earth's space. Salinity stress negatively disturbs basic plant metabolic processes, which successively leads to yield reduction moving agricultural production, primarily in coastal areas. Thanks to the storage of salt within the soil through tidal currents, agricultural land will become unproductive. High NaCl concentrations in irrigation water or soil cause serious economic losses from dry land as a result of salinity stress reduces the expansion and productivity of the many crop species [2,3]. Many physiological and organic chemistry processes let plants deal with excessive salt concentrations in saline soils. It's essential to research the anatomical and metabolite-derived characteristics stricken by salt stress, like cell wall depolarization, disturbed ionic balance, diffusion adjustment, nutrient deficiency, virulent accumulation of Na^+ in tissues, and therefore the physiological and chemical changes ensuing from interactions between multiple stresses [4]. Many researchers have targeted anatomical, ecological, physiological, and molecular modifications in crops underneath the strain of salinity [5-7]. They found that whole-plant metabolism is reprogrammed to extend stress tolerance, which comes with trade-offs and reduced growth rates throughout the following stages of crops' development. Ascorbic acids ($C_6H_8O_6$) is present in all living plant cells, the largest amounts being usually in the leaves and flowers, i.e., in actively growing parts.

To use active vitamins to beat the forceful effects of salinity on seed germination and spermatophyte growth in addition to some metabolic mechanisms. Presowing seed treatment of responsive cultivars with vitamins may so be exploited to boost grain yield at harvest [8]. Moreover, according to that below sure conditions, the exogenous application of vitamins to plants stimulate their growth, Thus, excluding their main role as coenzymes, it's not unbelievable that vitamins might also play different freelance roles within the organic chemistry processes of plants, repairing the injurious effects of unfavorable conditions. The water-soluble vitamins will be scavenged the reactive O species that square measure terribly harmful to plant growth. It's a product of D-glucose metabolism that affects some biological process cycle activities in higher plants and plays a very important role within the electron transport system. Many studies have shown that water-soluble vitamin plays a very important role in rising plant tolerance to abiotic stress.

Beans (*Vicia faba*) are thought-about the primary legume crop in Egypt of the cultivable space. Total yield and consumption as inexperienced and dry seeds are consumed in human feed as a result of the plant has high levels of macromolecule (18%), carbohydrates (58%), vitamins, and different minerals. additionally to the development of soil texture and its fertility, plant seeds are thought-about a valuable supply of energy and proteins [9].

So far, the literary references indicated no hint for work the response of *Vicia faba* to vitamin C. Therefore, this work geared toward foremost work changes in crop yield, seed contents, and macromolecule pattern in NaCl-stressed bean plants, second assessing the role of vitamin C to alleviate these changes, third finding an evidence for such moderating role, and eventually finding a suggested dose for treating *Vicia faba* with vitamin C.

MATERIALS AND METHODS

Sand-clay soil ½ v/v (EC of 1:5 soil extract at 25ºC=0.58 m mohs cm^{-1}, pH of 1:5 soil suspension=7.8) was used, the soil was mixed thoroughly to assure complete and uniform distribution (25 cm diameter, 35 cm depth, 5.5 Kg soil pot-1). Faba seeds were divided into 3 groups (0.0, 100 and 150 mM NaCl). Each was classified into 2 subdivisions (0.0 and 50 ppm ascorbic acid). Fifteen seeds were sown per pot, and then gradually thinned to five before the end of the season. The sowing date was Nov., 2003 and experiment was conducted for about 5 months. Pots were irrigated with the above NaCl concentrations, to slightly lesser than the field capacity level, whenever they needed, but with equal amounts.

Nitrogen-Phosphorus fertilizers were applied at rates of one g of urea/pot and 1.7 g of super-phosphate/pot, respectively. Phosphorus was added during soil preparation (before sowing). Nitrogen was applied after 6 weeks of sowing.

Plant material and growth conditions

Faba bean seeds (*Vicia faba* cv. Misr 2) were obtained from Gemmiza Agricultural Research Station, Gharbia, Egypt. The seeds were selected for uniformity of size and shape and surface sterilized (2.5% Sodium hypochlorite for 5 min.) and rinsed thoroughly in distilled water. The seeds were then soaked in freshly prepared ascorbic acid (50 ppm ≈ 0.3 mM; as recommended dose as described by or distilled water (control) for 4 hrs at natural environmental conditions.

Measurments

Anatomical studies in root and stem: After plant harvested directly (70 days old), a part of the stem at a distance of 16 cm from the stem-root connection region and the whole root in all treatments have preserved in a mixture of (alcohol 95% 50 ml+distilled water 35 ml+formaline 10 ml+glacial acetic acid 5 ml) for 24 hours then all samples washed with distilled water and transfer to alcohol 70% permanently. Anatomy in many regions (lower-middle-upper) in the root and in the stem part was carried out. At the age of 70 days, root and stem sections were prepared using the paraffin method microtoming [10] .The amount of the killing and fixing fluid was 10 times the volume of the studied material using Carnoy's fluid. Water was removed from the fixed tissue by dehydration in a series of alcohol, 30 minutes for each. Dehydrated specimens were cleared by passing through a series of a mixture of absolute alcohol and xylol 3:1; 1:1 and 1:3, 20 minutes for each, then pure xylol. The material was then infiltrated with paraffin, embedded, microtomed and stained by safranine and light green.

Statistical analysis

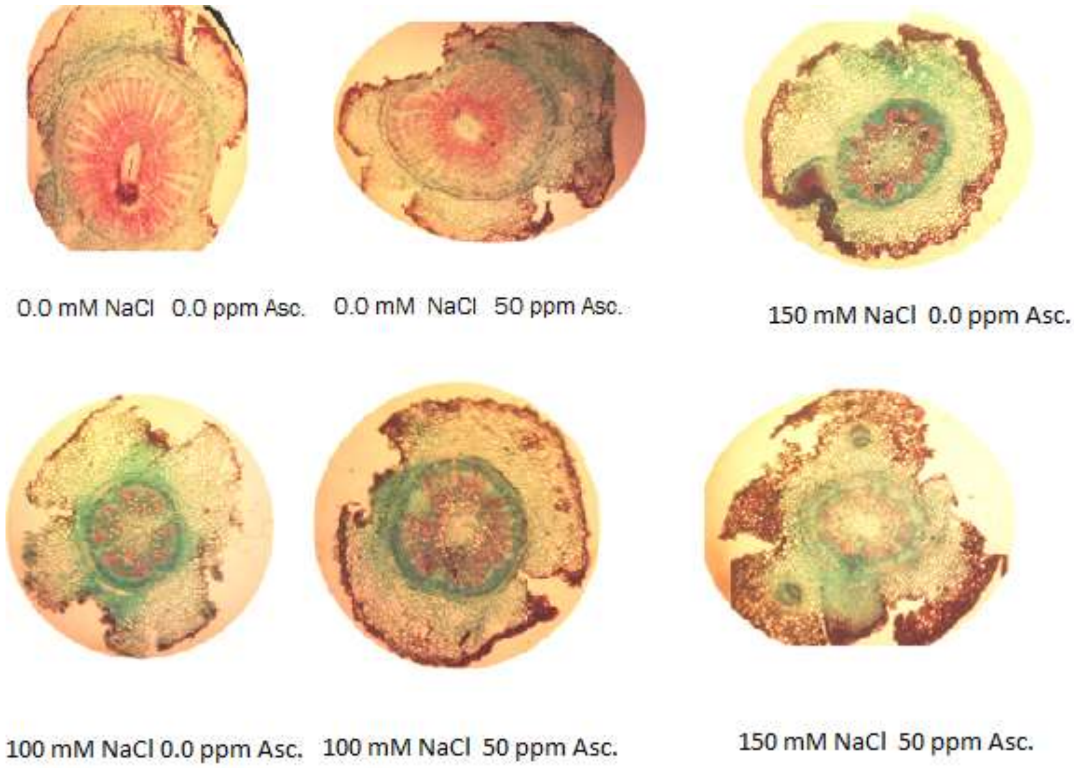
Data obtained were analyzed statistically to see the degree of significance between treatments. The strategy of two ways is that Analysis of Variance (ANOVA; factorial) was applied for all knowledge. The Least Significant Difference (LSD) at 5 % was used to compare means.

RESULTS

The root

The root anatomy of *Vicia faba* cv. Misr 2 showed a normal secondary growth thickening after 70 days of growth. The root anatomy of either control or treated plants showed the following anatomical characters: periderm occupied the outermost region of the root followed by the cortex, which consists of 4 to 5 layers of parenchyma cells and the innermost layer is differentiated as an endodermis. The secondary vascular tissue occupied a wide area and the central primary xylem (tetrarch) gets embedded in the secondary xylem. The vascular bundles are regular and form a continuous cylinder. The continuous growth of the xylem-phloem ray parenchyma appeared to be separated the vascular bundle into a number of vascular strands. The central pith zone was occupied by parenchyma cells as shown in Figure 1.

Figure 1. Cross sections of 70-day old *Vicia faba* root (x30) as affected by NaCl level (0.0, 100 and 150 mM) and presoaking in 50 ppm ascorbic acid(Asc).



The anatomical changes, induced in root tissues by NaCl treatments, as shown in Figure 1 appeared that salinity caused a deep tear of the outermost layers of the root in both periderm and cortex zones, and the ascorbic acid application did not change the effects of salinity. In addition, showed that NaCl treatments caused an increase in cortex width at 150 mM NaCl compared with control. Two new vascular bundles were appeared outside the secondary vascular strands by 150 mM NaCl combined with 50 ppm ascorbic acid treatments. At 100 mM NaCl, a slight decrease in cortex width was observed. Ascorbic acid treatment furthered the same effects of salinity with both salinity levels.

NaCl caused a highly significant decrease in xylem width of either treated plants or untreated with ascorbic acid, but the response was more prominently at the high dose of NaCl. NaCl caused a highly significant increase in pith radius; application of ascorbic acid to salinized plants partially decreased the pith radius compared with their corresponding controls. Salt stress caused a highly significant decrease in the root radius and subsequently the area. Ascorbic acid application furthered the effects of salinity at 100 mM NaCl, but an appreciable increase was observed at 150 mM NaCl.

The stem

The stem anatomy of *Vicia faba* cv. Misr 2 showed a normal secondary thickening after 70 days of growth. The normal secondary thickening was due to the normal position and normal activity of the vascular cambium. The stem anatomy showed one layer of epidermal cells followed by a narrow area of 6 to 8 cortical parenchyma cells, the vascular bundles were arranged in one ring forming a complete cylinder with a wide hollow pith cavity, bounded by parenchyma cells that are in the center, appears the anatomical characteristics of *Vicia faba* stem at 70 days as affected by different NaCl concentrations and presoaking in 50 ppm ascorbic acid. Data showed that NaCl treatments caused a non-significant increase in cortex width of the plants either treated or not with ascorbic acid. The salt-treated plants showed an increase in cortical width. NaCl caused a highly significant decrease in xylem width and stem area of salinized plants either treated or not with ascorbic acid, especially at 100 mM NaCl.

DISCUSSION

According to the effect of salinity on the anatomical behavior, the results further showed that NaCl treatments caused an increase in root cortex width at 150 mM NaCl compared with control. This could be due to an increase in the number of parenchyma rows rather than cell size. At 100 mM NaCl, a slight decrease in cortex width was observed. Ascorbic acid treatment led to the same effects of salinity with both salinity levels. The data showed that NaCl treatment caused a non-significant increase in stem cortex width in either the plant treated or not with ascorbic acid. The salt-treated plants showed an increase in cortical width due to the larger size of parenchyma cells rather than their number.

NaCl caused a highly significant decrease of xylem width, in both root and stem, in either the plants treated or not with ascorbic acid. Responses to salinity may be expressed as anatomical and/or cytological changes. Such changes can differ from one organ to another and/or at different levels of organization. Changes in submicroscopic structures due to exposure of plants to sub-lethal levels of salinity are investigated. The shape and size of cortical cells were becoming smaller and more irregular with increasing NaCl concentration from 0 to 400 mM.

CONCLUSION

Two new vascular bundles were appeared outside the root secondary vascular strands by 150 mM NaCl combined with 50 ppm ascorbic acid. This may be considered as a special defense of the plant against salinity stress. Increasing the number of vascular bundles could play an important role in water absorption from the soil. The present wok demonstrated that the root cortex and xylem width were increased by ascorbic acid application compared with its salinized corresponding controls. Two new vascular bundles were appeared outside the root secondary vascular strands by 150 mM NaCl combined with 50 ppm ascorbic acid treatment.

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CONFLICT OF INTEREST

I hereby declare that I have no pecuniary or other personal interest, direct or indirect, in any matter that raises or may raise a conflict with my duties as a researcher and lecturer in faculty of science, botany and microbiology department, Damanhur University

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