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Alleviation of Adverse Effects of Salt Stress in Brassica (*Brassica campestris*) by Pre-Sowing Seed Treatment with Ascorbic Acid

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Abstract: The present study was conducted to investigate the effects of ascorbic acid applied through seed soaking on *Brassica* plants growing under saline conditions. Different concentrations of ascorbic acid (0, 50, 100 mg/L) were applied through seed soaking on *Brassica campestris* s' plants. Salt toxicity significantly reduced the growth of *Brassica* plants. Ascorbic acid acts as an antioxidant and ameliorated the effect of salinity. Application of ascorbic acid through seed soaking enhanced the growth of plants, increased the shoot, root fresh and dry weight. Sodium toxicity was reduced by excluding the sodium from the roots. Chlorophylls contents in *Brassica campestris* s' plants were increased by applying the ascorbic acid as seed soaking. Among all these concentrations (0, 50, 100 mg/L) of ascorbic acid, 100 mg/L was more effective in reducing the salinity stress in *Brassica campestris* s' plants.

Key words: Ascorbic acid · Salinity · *Brassica campestris*

INTRODUCTIONS

Salinity stress is a major problem to reduce the production of different crops. About 82% yield of potential crops has been decreased [1]. Salt stress disturbed many physiological and biochemical changes in different crops and reduced the plant growth and yield [2] Stomatal limitation can adversely effect the crop production under saline conditions [3]. Salinity stress induce the production of reactive oxygen species in crops is responsible for various stress induced changes in crops [4]. Different types of antioxidants compounds are produced to scavenge ROS, synthesized in plants. Synthesis of these compounds is activated by different types of antioxidant enzymes [5]. Antioxidant enzymes activation is achieved through breeding of different crops species [6]. It has been observed though different studies that exogenous application of ascorbic could mitigate the effect of salinity in different crops [7, 8].

Oil seed crops are very important for human food and they have gained third position among crops next to cereals and legumes [9]. About 40 different plant species, including some trees, have been used for the commercial production of fatty oils [10]. All parts of some species are used as food including root (Swedes, turnips) stems (kohlrabi), leaves (cabbage, Brussels sprouts) flowers (cauliflower) and seeds (including mustard seed, oil seed rape).

The present study was conducted to investigate the effect of ascorbic acid applied through seed soaking on Brassica growing under saline conditions.

MATERIALS AND METHODS

An experiment was conducted in the Department of Biological Sciences University of Sargodha, Pakistan during the year, 2008. Different levels 0, 50, 100 mg/ L of ascorbic acid were applied exogenously as pre sowing seed treatment to determine whether ascorbic acid application could induce the salt tolerance in *Brassica campestris* s' plants. It consists of three replications. The experiment was laid out in a completely randomized design with two factor factorial arrangement. The seeds of these cultivars were obtained from Ayub Agricultural Institute Faisalabad, Pakistan and sown in pots. Each pot filled with 8 kg well mixed soil.

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Different salinity levels were adjusted in accordance with saturation percentage of soil. Data were collected related to growth, physiological and biochemical attributes of the *Brassica campestris* s' plants under saline and non- saline conditions.

Data Collection: Data for the following parameters were collected from the experimental pots containing *Brassica campestris* s' plants.

Shoot fresh and dry weight, Shoot and root length, chlorophyll a, b and total (mg/g f.wt) and Na^+, K^+, (mg/g d.wt) from the root and shoot of the *Brassica campestris* s' plants.

Chlorophyll Contents: The chlorophyll a and b of *Brassica campestris*'s plants were determined with the procedure as described by Arnon [11]. The fresh leaves of the *Brassica* campestris s' plants were chopped into 0.5 cm segments and chlorophyll was extracted with 80% acetone by keeping overnight at -10°C. The extract of leaves was centrifuged at 14000×g for 5 min. and the absorbance of the supernatant was read at 480, 645 and 663nm using a spectrophotometer (IRMECO U2020). The chlorophyll a and b were calculated by the following formulas:

$$\text{Chl.a} = [12.7(\text{OD663}) - 2.69\ (\text{OD645})] \times V\ /\ 1000 \times W$$
$$\text{Chl.b} = [22.9(\text{OD645}) - 4.68\ (\text{OD663})] \times V\ /\ 1000 \times W$$

V = Volume of the extract (ml)
W = Weight of the fresh leaf tissue (g)

Determination of ions. Sodium and potassium concentration were determined form the digested material of shoot and root of *Brassica campestris* s' plants [12] using flame spectrophotometer (Jenewa-7).

Statistical Analysis: The data were analyzed by analysis of variance technique [13] and the differences for various attributes were compared by using Least Significant Differences test at 0.05 level of probably.

RESULTS

Data in Table 1 indicated that the shoot fresh and dry weight of *Brassica* was reduced by salt stress. Application of ascorbic acid as seed soaking increased the shoot fresh and dry weight under saline and non-saline conditions. 100 mg L^{-1} ascorbic acid applied as seed soaking increased the shoot fresh and dry weight under saline and non-saline conditions as compared to the other concentration of the ascorbic acid. Similarly low salinity significantly reduced the root fresh and dry weight of *Brassica campestris* s' plants. 50 mg L^{-1} ascorbic acid applied as seed soaking increased the root dry weight under saline conditions. Shoot and root length was also affected by the application of salt stress. 100 mg L^{-1}ascorbic acid increased the shoot length and 50 mg L^{-1} ascorbic acid applied as seed soaking increased the root length under saline and non saline conditions. Also data in Table 1 showed that the chlorophyll a, b and total chlorophyll were affected by the application of salinity stress. However, application of ascorbic acid increased the chlorophyll b and total chlorophyll by the application of ascorbic acid under saline and non-saline conditions. The results presented in Table 1 indicated that the shoot and root potassium uptake was effected by the application of salinity stress. Application of ascorbic acid as seed soaking increased the uptake of potassium under both saline and non-saline conditions of the *Brassica campestris* s' plants. Uptake of sodium was enhanced in *Brassica* under saline and non-saline conditions.

Table 1: Effect of presowing seed treatment with different levels of ascorbic acid on shoot fresh and dry weight, root fresh and dry weight, shoot and root length, chlorophyll a, b and total, shoot, root potassium sodium and potassium concentration under saline and non-saline conditions

Salinity Levels (mmol)	0			50			100		
Ascorbic acid levels (mg/L)	0	50	100	0	50	100	0	50	100
Shoot fresh weight (g)	1.22±0.12	1.71±0.12	1.3±0.11	0.5±0.13	1.11±0.11	1.05±0.2	0.68±0.12	1.11±0.05	1.4±0.07
Shoot dry weight (g)	0.18±0.0014	0.16±0.0014	0.15±0.001	0.038±0.004	0.14±0.002	0.15±0.0012	0.038±0.003	0.14±0.004	0.16±0.004
Root fresh weight (g)	0.086±0.004	0.137±0.02	0.14±0.01	0.07±0.003	0.09±0.001	0.07±0.002	0.08±0.014	0.08±0.01	0.08±0.01
Root dry weight (g)	0.004±0.001	0.008±0.002	0.007±0.004	0.007±0.001	0.007±0.001	0.009±0.001	0.006±0.001	0.005±0.001	0.005±0.002
Shoot length (cm)	20.33±1.2	19.45±2	21.45±3.2	15.4±2.2	17.4±2.1	16.0±2.3	15.45±3.2	14.75±2.1	16.54±1.4
Root length (cm)	5.4±0.71	6.7±0.21	6.36±0.8	5.6±0.23	5.5±0.41	5.0±0.31	4.7±2.0	5.9±1.5	5.7±0.42
Chlorophyll a (mg/g. f.wt)	0.33±01	0.57±0.02	0.37±0.02	0.33±0.012	0.57±0.021	0.37±0.001	0.53±0.002	0.43±0.012	0.34±0.001
Chlorophyll b (mg/g f.wt)	0.46±0.01	0.38±0.02	0.24±0.02	0.12±0.03	0.26±0.02	0.34±0.021	0.14±0.03	0.22±0.021	0.32±0.01
Total Chlorophyll (mg/g f.wt)	0.79±0.0	0.96±0.2	0.62±0.3	0.46±0.1	0.84±0.04	0.71±0.2	0.67±0.01	0.69±0.01	0.66±0.01
Shoot Na (mg/g d.wt)	29.83±2.3	31.0±1.4	25.7±2.0	37.4±2.0	35.4±1.4	35.4±1.3	38±1.3	32±1.2	34.5±2.0
Root Na mg/ g d.wt)	23±1.2	27.5±2.0	22.0±1.4	28±1.4	32±1.2	35.2±2.0	35±1.3	30.2±2.0	31.2±2.1
Shoot K(mg/ g d.wt)	22±2.1	26±1.2	25±1.0	9.16±2.0	25.5±1.2	26.5±2.0	8.56±2.0	10.75±1.4	17.16±2.3
Root K (mg/ g d.wt)	6.87±1.32	5.4±2.0	3.5±1.2	2.33±1.0	1.96±1.0	6.16±1.2	5.16±0.4	2.83±0.9	5.16±0.6

Application of ascorbic acid reduced the uptake of sodium in Brassica in both saline and non-saline conditions. However, 100 mg L^{-1}ascorbic acid was more effective in reducing the shoot and root sodium in Brassica plants under saline and non-saline conditions as compared to the other concentration of the ascorbic acid (Table 1).

DISCUSSION

Soil salinity is the major environmental factors that reduce the plants growth. The effects on plants growth due to soil salinity depends on low water potential, nutritional imbalance caused by the interference of salinity toxicity as well as the production of reactive oxygen species. Germination rate, fresh and dry weight, chlorophyll contents has been increased by the exogenously application of ascorbic acid [14]. The ascorbic acid plays a remarkable role in case of seed germination, cell growth under salinity due to its antioxidants properties. The chlorophyll contents reduction in leaves occur in plants grown at 100 mM NaCl [15]. These reductions could be attributed to the effect of salinity that causes inhibition of synthesis of chlorophyll or accelerating its degradation [16]. Other studies indicated that salt tolerant cultivars have higher activity of antioxidant system than salt sensitive cultivars of a same species [17, 18]. Of all these strategies, exogenous application of antioxidants has gained a considerable ground as a shotgun approach to ameliorate the adverse effects of salt stress [19, 20].

It is evident that effectiveness of the ascorbic acid applied exogenously depends on the concentration and mode of application such as 50 mg $^{L-1}$ was effective in promoting the growth of tomato, turnip and rape [21]. Similarly 100 mg L^{-1} is the most effective concentration of ascorbic acid in reducing the effect of salinity [22].

From the above discussion it is concluded that ascorbic acid can mitigate the effect of salinity by applying as a seed soaking agent.

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