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Alleviation of adverse effects of salinity in Ber by foliar treatment with antioxidant

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Abstract

This two year experiment were carried out in earthen pots during 2018-20 under cage house, Department of Plant Physiology, S.K.N. College of Agriculture, Jobner. The *Ziziphus rotundifolia* root stock of ber planted in pots. The recommended doses of FYM, fertilizers and other inputs provided at proper time. These Plants were salt stressed by applying saline irrigation water of EC=Control (Tap water), EC= 8.0 and EC= 16.0 dSm⁻¹ as and when required after establishment in pots. The plants were foliar sprayed by three concentration of Ascobin i.e. 0, 400 and 800ppm. A significant increase were recorded in the proline content, cell membrane injury and hydrogen peroxide content in the plant leaves under increasing level of salinity as compared to control, whereas a significant decrease were noticed in these traits by the foliar spray of Ascobin up to 800ppm concentration. A significant increase were also noticed in ascorbic acid content and Superoxide Dismutase (SOD) Activity in the plant leaves under increasing level of salinity as compared to control with a significant increase also in these parameters by the foliar spray of Ascobin up to 800ppm concentration.

Keywords: Ber, salinity, Ascobin, proline, cell membrane injury, hydrogen peroxide and SOD

Introduction

Soil salinity is one of the most important abiotic stress and limiting factor for worldwide conventional agriculture. Salinity is one of the most brutal environmental factors limiting productivity of crop plants because most of crop plants are sensitive to salinity caused by high concentrations of salts in the soil. A considerable amount of land in the world is affected by salinity which is increasing day by day. More than 45 million hectares (M ha) of irrigated land which account to 20% of total land has been damaged by salt in the worldwide and 1.5 M ha are taken out of production each year due to high salinity levels in the soil (Munns and Tester, 2008) [7]. Salt stress is a serious problem in crop production in India.

Indian Jujube or ber is a common fruit endogenous to India. Its fruits are palatable and delicious with high concentration of vitamin A, B and B complexes. Ber leaves contain 10-19% crude protein with about 40% digestibility. The leaves are commonly used as a fodder for animals (Pareek, 1983) [8], compared to other agricultural and horticultural crops, Indian Jujube is known to grow successfully under a low erratic rainfall. Temperature extremes and saline soils with low fertility (Meena *et al.*, 2003) [5].

Ascobin (ascorbic acid and citric acid with ratio of 2:1) have auxinic and also synergistic effect on plant. Ascorbic acid has also synergistic effect on plant. Ascorbic acid is an important primary metabolite in plants that functions as antioxidant, an enzyme cofactor and a cell signaling modulator in a wide array of crucial physiological processes, including biosynthesis of the cell wall, secondary metabolites and phytohormones, stress tolerance, photoprotection, cell division and growth (Wolucka *et al.*, 2005) [11]. Antioxidant (such as ascorbic acid and citric acid) are designing chemicals when added in small quantities to plant, react rapidly with radical intermediates of an auto-oxidation chain and stop it from progressing (Khan *et al.*, 2006) [4]. This experiment was carried out to minimizing the adverse effects of salinity on ber by the foliar spray of ascobin.

Materials and Methods

The study was conducted in the cage house under natural conditions. Six-month-old plants of

Ziziphus rotundifolia grown in ceramic pots of 40x40 cm diameter were taken for study. The pots were filled with 20 kg of loamy sandy soil having a bulk density of 1.5 g cm⁻³, electric conductivity (EC) 0.6 dSm⁻¹, P^H 8.2, sodium absorption ratio 12.5 and CaCO₃ 0.14%. The field capacity and permanent wilting point of the soil were 11.8 and 2.8%, respectively. About 27 pots were used. The recommended doses of manures, fertilizers and other inputs were provided at the appropriate time. Salts used to prepare saline irrigation water: Chloride and sulphate in 3:1 ratio by using following salts; NaCl, NaSO₄, CaCl and MgCl₂. Saline water of EC Control (Tape water), 8.0 and 16.0 dSm⁻¹ were prepared and applied to plants. Two liter of the saline water was provided to each pot as and when required. The control plants were irrigated with tap water. The foliar spray of ascorbin at 0, 400 and 800 ppm concentration were applied on plants after 10, 20 and 30 days of start of saline irrigation. Observation was taken after 10 days of Ascorbin treatment in plants. The top most fully expanded leaves were sampled after 45 days of saline irrigation from the plants.

Membrane stability index (MSI): Leaves of control and salt-treated plants were collected and thoroughly washed with distilled water. 200 mg of a leaf sample was placed in 25 ml of double distilled water at 40°C for 30 min and thereafter an electric conductivity (C1) was measured with conductivity meter. Subsequently, the same samples were placed on boiling water bath (100°C) for 10 min and their electric conductivity was recorded (C2). MSI was calculated as $MSI = [1 - \{C1/C2\}] \times 100$.

Determination of proline and protein: Leaves of plants were homogenized with 3% sulphosalicylic acid and the homogenate was centrifuged at 3,000 g for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 hr. and then absorbance at 520nm was determined. Contents of proline were expressed as µg/g fr. wt. of leaf (Bates, *et al.*, 1973) [1]. The protein of leaf samples were determined by the method of Bradford (1976) [2]. Protein content was expressed as mg/g fr. wt. of leaf.

Determination of Hydrogen peroxide

H₂O₂ was determined using the method outlined by (Velikova, *et al.* 2000) [10]. A proportion of fresh leaf sample (0.5 g) was homogenized in a chilled mortar and pestle with 5 mL of 0.1% trichloroacetic acid (TCA). After filtration, 1.0

mL supernatant was mixed with 0.5 mL phosphate buffer and 1 mL of 1 M potassium iodide. The sample mixtures were vortexed thoroughly and their absorbance read at 390 nm using a spectrophotometer. H₂O₂ was calculated from a standard curve developed by using tannic acid as standard.

Determination of Ascorbic acid

A fresh leaf sample (0.5 g) was homogenized in 10 mL of TCA (6%). Then 4 mL of the extract were mixed with 2 mL of dinitrophenyl hydrazine and then 1 drop of thiourea was added to the mixture. The mixture was boiled for 15 min and then allowed to cool to room temperature. Five mL of 80% H₂SO₄ were added to the mixture. Absorbances of all treated samples were read at 530 nm following (Mukherjee and Choudhuri, 1983) [6] and compared with standard curve drawn by using ascorbic acid ranges from 10–100 mg L⁻¹.

Determination of SOD activity

For enzyme extraction, a sample (0.5 g) of fresh leaf was homogenized with 10 mL of sodium phosphate buffer (pH 7.8). The extract supernatant obtained after centrifugation for 15 min at 15,000 rpm was preserved at -20 °C in an ultra-low freezer. Activities of superoxide dismutase (SOD) was recorded as follows: For measuring the activity of SOD enzyme, a reaction mixture was prepared (0.4 mL H₂O + 0.25 mL of phosphate buffer + 0.1 mL methionine + 0.1 mL triton-X + 0.5 mL NBT + 0.5 mL enzyme extract + 0.5 mL riboflavin) according to (van Rossum, *et al.* 2020) [9]. Then, the mixture was kept in the light for 15 min and the absorbance recorded at 560 nm.

Statistical analysis: There were three replication for each treatment. Statistical analysis of data was processed using completely randomized block design (Gomez and Gomez, 1984) [3].

Results and Discussion

This experiment entitled “Alleviation of Adverse Effects of Salt Stress in Ber by Foliar Treatment with Antioxidant” was conducted to study the alleviation of harmful effects of salt stress on ber plants by the foliar spray of Ascorbin. A significant increase were recorded in the proline content, cell membrane injury and hydrogen peroxide content in the plant leaves under increasing level of salinity as compared to control, whereas a significant decrease were noticed in these traits by the foliar spray of Ascorbin up to 800ppm concentration.

Table 1: Effect of Ascorbin on Physio-biochemical traits of *Ziziphus rotundifolia* under Salinity

Treatments	Physio-biochemical traits						Reactive Oxygen Species (ROS)		
	Proline (µg/g fw of leaf)			CMS (%)			Hydrogen peroxide (µmol g ⁻¹ fw)		
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
EC 0.3 Ascobin 0 PPM	146.2	126.2	136.2	78.6	70.4	74.5	3.7	3.52	3.61
EC 0.3 Ascobin 400 PPM	128.0	108.0	118.0	85.2	77.1	81.15	3.52	3.14	3.33
EC 0.3 Ascobin 800 PPM	105.2	95.2	100.2	92.1	83.9	88.2	2.56	2.38	2.47
EC 8.0 Ascobin 0 PPM	270.8	230.8	250.8	67.4	59.2	63.3	5.44	5.26	5.35
EC 8.0 Ascobin 400 PPM	240.4	210.4	225.4	71.0	62.8	66.9	4.87	4.69	4.78
EC 8.0 Ascobin 800 PPM	215	175.4	195.0	76.3	68.1	72.2	3.23	3.05	3.14
EC 16.0 Ascobin 0 PPM	412.5	372.5	392.5	61.2	55.0	58.1	7.86	7.68	7.77
EC 16.0 Ascobin 400 PPM	362.1	267.1	314.6	66.0	57.8	61.9	6.42	6.24	6.33
EC 16.0 Ascobin 800 PPM	307.3	267.3	287.3	70.8	62.6	66.7	5.12	4.94	5.03
CRD (P = 0.05)	12.4	10.4	11.4	6.72	4.72	5.72	0.23	0.19	0.21

The maximum decrease in these parameters were seen by the application of 800ppm concentration of Ascorbin as foliar spray.

Table 2: Effect of Ascobin on antioxidants content and activity in *ziziphus rotundifolia* under Salinity

Treatments	Non Enzymatic Antioxidants			Enzymatic Antioxidants		
	Ascorbic Acid (mg ascorbate g ⁻¹ fw of plant)			Superoxide Dismutase(SOD) Activity (Unit /min /mg protein)		
	2018	2019	Mean	2018	2019	Mean
EC 0.3 Ascobin 0 PPM	0.215	0.203	0.209	12.12	10.32	11.22
EC 0.3 Ascobin 400 PPM	0.234	0.222	0.228	14.31	12.51	13.41
EC 0.3 Ascobin 800 PPM	0.259	0.247	0.253	18.1	16.3	17.2
EC 8.0 Ascobin 0 PPM	0.325	0.313	0.319	22.26	20.46	21.36
EC 8.0 Ascobin 400 PPM	0.34	0.328	0.334	25.72	23.92	24.82
EC 8.0 Ascobin 800 PPM	0.365	0.353	0.359	28.56	26.76	27.66
EC 16.0 Ascobin 0 PPM	0.578	0.566	0.572	31.41	29.61	30.51
EC 16.0 Ascobin 400 PPM	0.592	0.58	0.586	34.21	32.41	33.31
EC 16.0 Ascobin 800 PPM	0.625	0.613	0.619	38.2	36.4	37.3
CRD (P = 0.05)	0.018	0.012	0.015	2.95	1.95	2.45

A significant increase were also noticed in ascorbic acid content and Superoxide Dismutase (SOD) Activity in the plant leaves under increasing level of salinity as compared to control with a significant increase also in these parameters by the foliar spray of Ascobin up to 800ppm concentration. This enzyme works as scavenging enzyme of hydrogen peroxide in plant leaves, which causes degradation of chlorophyll molecules and favors senescence in the plant leaves. It is concluding that the harmful effects of salt stress on ber plants may be alleviated by foliar application of Ascobin at 800 ppm concentration by the farmers under the field areas having saline irrigation water up to EC 16.0 dSm⁻¹.

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