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INTERACTIVE EFFECT OF SALINITY AND ASCORBIC ACID ON *BRASSICA RAPA L.* PLANTS

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ABSTRACT
Excessive soil salinity is a major factor resulting in the low productivity of cultivated crops. Salinity causes oxidative stress in plants by enhancing production of reactive oxygen species. Ascorbic Acid is an efficient antioxidant which is essential for tolerance. However, Antioxidant responses of plants to salinity vary considerably in *Brassica Rapa L.* plant. The present study was conducted to investigate the effects of Ascorbic acid on *Brassica Rapa L.* growing under saline conditions. Different concentrations of Ascorbic acid were applied (50 mM, 100 mM). Salt toxicity significantly reduced growth of *Brassica Rapa L.* plants. Significant decrease in root, shoot length, protein content and antioxidant enzyme wasalleviated by the application of Ascorbic acid. Among the concentrations, 100 mM was more effective in reducing the salinity stress. Thus, it can be concluded that Ascorbic acid ameliorated the effect of salinity.

KEY WORDS: *Brassica Rapa L.*, Sodium chloride, Ascorbic acid.

INTRODUCTION

Seed germination is usually the most critical stage in seedling establishment which shows sensitivity to drought and salt tolerance. Sodium decreases soil permeability but increases compactness, which reduces the flow of water to the plants possibly affecting germination. Chloride is absorbed through roots and increases the mobility of heavy metals, such as iron and cadmium, in soil (Sadeghian and Yavari, 2004). Ascorbic acid (AsA), the water-soluble antioxidant molecule is an essential compound for plants and act as a modulator of plant development through regulation of photosynthesis, hormone biosynthesis and regeneration of other antioxidants (Pastori *et al.*, 2003). AsA is also implicated in the control of cell division, cell expansion, growth and development including flowering, senescence and root development and also regulates defense response and survival of plants under abiotic and biotic stress. AsA, its redox couple AsA/DHA and related enzymes (MDHAR, DHAR and APX) together form an AsA redox system to efficiently protect plants from oxidative stress caused by exogenous and endogenously generated reactive oxygen species (ROS) and its products (Akram *et al.*, 2017).The family Brassicaceae (Cruciferae) consists of 350 genera and about 3,500 species, and includes several genera like Camelina, Crambe, Sinapis, Thlaspi and Brassica. Turnip (*Brassica rapa L.)* is rich in vitamins, minerals (such as calcium, potassium, iron, copper, magnesium and complex mixture of photochemical possessing antioxidant activity. Various studies have reported that salt stress significantly affected the germination and early seedling growth resulting in growth retardation and reduction in fruit size, and consequently yield (Mohammad *et al.*, 2014; Tatatabaei and Larijani, 2016). However, exogenous application of AsA stimulates the germination percentage and early seedling growth in variety of plants *e.g.*, wheat (Afzal *et al.*, 2006), bean (Azooz and Al-Fredan, 2009), pea (Burguieres *et al.*, 2007), tomato (Barh *et al.*, 2008) and sorghum (Arafa *et al.*, 2009). There are very few studies regarding the effect application of AsA on Turnip seedlings grown in both normal and saline condition and their effect on the antioxidant levels. The present study was carried to study the effect of ascorbic acid in counteracting salt stress by estimating protein, phenol and total antioxidant content in turnip.

MATERIALS & METHODS

Brassica rapa L. seeds were obtained from local nursery and were surface sterilized with teepol detergent for 5 min and then washed three times under running tap water. The seeds were then treated with 2-3% fungicide for 5 min to prevent fungal infection and are washed three times with sterile distilled water. After washing, the seeds were placed in three ascorbic acid concentrations (conc.) of 0, 50 and 100mgL^{-1} for one day. Following this, seeds were transfered to a sterilized petri dish at the bottom of which a filter paper was placed. All petri dishes had sodium chloride solution in concentration of 50mM and 100mM. Seed germination was observed daily with fresh salt solution added to the Petri dishes to maintain moisture levels. After 10 days, seedling vigour and metabolic activity at the stage of second leaf opening were assayed. Seedling vigour was determined using the percent seed germination, seedling stem and root length, seedling fresh and dry weight and seedling index. Dry weights were determined after drying the plant tissue to a constant weight in a hot air oven at 85^0C for 12 hours.

Preparation of Homogenate: The germinated seeds were homogenized in distilled water. The supernatant was

collected as a sample for testing of various parameters and debris was discarded.

Statistical analysis: Statistical analysis was based on one-way analysis of variance (ANOVA). The effects of heavy metal treatment were considered statistically significant when $p \leq 0.05$.

Protein Estimation: The protein concentration determination was done by the Lowry protein assay method.

Estimation of Total Phenolics by modified Folins-Ciocalteau method: The hydroxyl (-OH) group of phenolic compounds reduce the phosphomolybdic acid to molybdenum blue in the presence of an alkaline medium (present in Folin's reagent). The blue coloured complex was then spectrophotometrically measured at wavelength 760nm.

Estimation of Antioxidant activity by FRAP assay: The antioxidants present in the sample reduce $Fe3^{+}$ to $Fe^{2+.}$ This ion conjugated with the ferricyanide ion to form a Prussian blue coloured product, which was spectrophotometrically measured at wavelength 700nm.

RESULTS & DISCUSSION

In the present study, salinity stress due to Na^{+} and Cl^{-} ions accumulation caused inhibition of the uptake of essential nutrients such as K^{+} which resulted in significantly reduced ($p \leq 0.05$) germination of seeds, impaired root and shoot length as reported earlier in *Suaeda salsa* (Duan *et al*., 2007), *Solanum melongena*. In the present study on turnip, various factors such as reduced photosynthesis due to significant decrease in cholorophyll content ($p \leq 0.05$) or decreased levels of amylase ($p \leq 0.05$) could have affected the supply of carbohydrate to the growing meristematic cells resulted in significantly reduced growth of shoots and roots. The lowered water potential due to reduction of turgor in expanding tissues may also have affected root and shoot elongation (Alam *et al*., 2004). The significant decrease in total chlorophyll content observed in turnip in the present study could be due to salt stress induced Na^{+} toxicity or oxidative damage resulting in ROS synthesis. Mittler (2002) reported that salt stress causes breakdown of ultrastructure of chloroplasts including plastid envelop, thylakoids and photosynthetic apparatus. However, when ascorbic acid (AsA) (50mM, 100mM) was applied in turnip, there was significant increase in seed germination seedling vigour, plant seedling root length, shoot length, fresh weight and dry weight. Improved seedling fresh and dry weight might be due to increased cell division within the apical meristem of seedling shoots and roots due to enhanced IAA (indole acetic acid) and cytokinin levels in the plant tissues which enhance the plant growth (Sakhabutdinova *et al*., 2003). AsA resulted in increased photosynthetic efficiency due to significantly increased levels of chlorophyll (chlorophylls 'a' & 'b') probably by inhibiting activity of chlorophyllase, an enzyme degrading chlorophylls (Mishra and Sharma, 1994). Davey *et al*. (2000) reported that AsA acts as a substrate for ascorbate peroxidase (APX) to scavenge ROS produced in the thylakoid membranes.

TABLE I: Table showing effect of various conc of salinity (50mM , 100mM) and ascorbic acid (50mM, 100mM) on germination , root length , shoot length and total chlorophyll content

Ascorbic Acid (mg/L)	Salinity (mM NaCl)	Germination percentage (%)	Root Length (cm)	Shoot Length (cm)	Total Chlorophyll (mg/g)
0	50	50.5±3	2.8±.325	2.4±.345	1.338±0.030
50	50	55.8±1	3.2±.13	3.3±.43	1.643±0.060
100	50	58.7±2	3.5±.203	3.6±.93	1.807±0.048
p values		0.0239*	0.0289*	0.002*	0.017*
0	100	50±1	2.1±.43	2.03±.3	1.683±0.070
50	100	54.8±2.3	2.5 ±.32	2.4±.3	1.619±.347
100	100	56.5±3.1	2.7±.143	2.6±.3	1.988±.0569
p values		0.0247*	0.05*	0.0348*	0.043*

The application of ascorbic acid in turnip might have mitigated the salt stress by decreasing the conc. of sodium and increasing magnesium and potassium levels which are needed for enhanced chlorophyll synthesis by chloroplasts (Shaddad *et al*., 1999). In turnip, decreased protein content was observed with increasing amount of salt stress as reported earlier in *Bruguiera parviflora*. Decrease in protein content could be due to increased activity of acid and alkaline protease which hydrolyses proteins to release amino acids which help in osmotic adjustment, protection of cellular macro-molecules by maintaining cellular pH and scavenging of free radicals (Mansoor, 2000). The protein content in turnip showed a significant increase ($p \leq 0.05$) due to the application of ascorbic acid (AsA) which could be due to the expression of various new proteins. The exogenous application of AsA leads to the expression of cell wall loosening proteins, including expansins and XETs resulting in plant growth. During salt stress, AsA enzymes induces the expression of ascorbate peroxidases (APXs) that are known to dismutase H_2O_2 to water and molecular oxygen using AsA as an electron source (van Doorn and Ketsa, 2014). The increase in total antioxidant capacity in turnip after the application of AsA could be due to the regenerative nature of ascorbate which plays a key role in quenching intermediate/excited reactive forms of molecular oxygen either directly or through enzymatic catalysis (Ye *et al*., 2012). Since ascorbate is a part of enzymatic and non-enzymatic antioxidant defense system and thus contributes to ROS neutralization. The ascorbate glutathione also regulates plant development by maintaining ROS levels (Pignocchi *et al*., 2006).

The present study clearly indicates adverse effects of salt stress on the seed germination, seedling growth which was significantly improved by exogenous application of AsA.

However, the exact mechanism by which salinity inhibits growth is still not clear. The exogenous AsA being an important co-factor in photosynthetic enzymatic reactions treatment might have mitigated plant growth decline due to salt stress by effective synthesis of gibberellins and ethylene through specific AsA-dependant dioxygenase catalyzed reactions (Wang *et al*., 2013). It can be concluded that AsA may also suppressed the production of both O^{2-} and H_2O_2 with the help of enhanced antioxidant activity and also non enzymatically by inducing the synthesis of new phenolic compounds. However, further more field studies have to be carried out on different plant species to ascertain the role of ascorbic acid in mitigating salt stress.

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