ROLE OF EXOGENOUS SALICYLIC ACID APPLICATIONS FOR SALT TOLERANCE IN VIOLET

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ABSTRACT

Role of exogenous applications of salicylic acid (SA) under NaCl stress in violet (Viola odorata L.) was investigated in soil filled earthen pots during 2009-10 at University of Gujrat, Pakistan. There were three treatments comprising control, 50-mol m^{-3} NaCl, and NaCl (50-mol m^{-3}) + SA (30-mg l^{-1}). NaCl significantly reduced the plant and root lengths, plant fresh and dry weights. In contrast, NaCl did not show any adverse effect on plants when supplemented SA. SA treated violet plants under NaCl salinity strongly reduced accumulations of Na^+, K^+, Ca^{2+} and Cl^- and glycine betaine and total soluble carbohydrates as compared to NaCl treatments. Higher N and relative water contents (RWC) was noted in T_2 (NaCl+SA) but it reduced in T_1 (NaCl) as compared to control. It was concluded that SA could be used as a potential growth regulator to improve salt tolerance in plants.

Key Words: **Exogenous, salicylic acid, growth, ion contents, salt tolerance, violet**

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INTRODUCTION

The violet (*Viola odorata* L.) is a low-growing perennial herb. Mainly it is propagated through seed and rootstocks. Based on traditional lore, the leaves and flowers of violet are regarded as antiseptic and expectorant (Kowalchik and Hylton, 1998). Violet leaves are also considered good remedy traditionally in bronchitious, mucus, coughs, asthma, cancer of breast, stomach, lungs and digestive tract (Bown, 1995). When plants are exposed to salt stress, they adapt their metabolism in order to cope with the changed environment. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly (Hasegawa *et al.* 2000). Salicylic acid (SA) plays an important role in the defense response to pathogen attack and stresses in plant species (Shakirova *et al.* 2003). Several studies also supported a major role of SA in modulating the plant response to several abiotic stresses including salt and water stress (Yalpani *et al.* 1994; Senaratna *et al.* 2000). Treating mustard seedlings with SA improved their thermotolerance and heat acclimation (Dat *et al.* 1998). In maize plants, pre-treatment with SA induced the production of antioxidant enzymes, which in turn increased chilling and salt tolerance (Janda *et al.* 1999). The objective of the present study was to assess the role of exogenous salicylic acid applications for salt tolerance in violet.

MATERIALS AND METHODS

Seeds of violet (*Viola odorata* L.) were obtained from Shakarganj Botanic Garden, Jhang-Pakistan. Seeds were surface sterilized by dipping in 10% sodium hypochlorite solution for 10 min, then rinsed with sterilized distilled water and air-dried at an ambient temperature of 32°C in the laboratory. Following treatments of NaCl salinity and SA were applied 21-days after germination. There was a total of 30-pots comprising 10-pots for each treatment.

T_0= Control (without any treatment)
T_1= NaCl 50-mol m^{-3}
T_2= NaCl 50-mol m^{-3} + SA (30-mg l^{-1})
Salinity levels were developed as:

1 mole of NaCl	= 58.5 g NaCl dissolved in 1 liter water
1 mol m^{-3}	= 0.0585 g NaCl dissolved in 1 liter water
10 mol m^{-3} (1 dS/m)	= 0.00585 g NaCl dissolved in 1 liter water
50 mol m^{-3} (5 dS/m)	= 0.00583 X 50 g NaCl per pot=0.292 g/pot

NaCl was applied in soil media and SA was applied as foliar spray. Experiments were laid out in Completely Randomized Design (CRD) with ten replicates. Plants were harvested after 120-days of treatment and following studies were made.

Growth Attributes and Ion Contents

Plants were uprooted carefully and washed in distilled water. Plant and root length was measured with the help of scale meter. Shoot fresh weight (g) was noted by electric balance. Plant samples were placed in oven at 75°C. After 4-days shoot and root dry weight (g/pot) was calculated with the help of electric balance. Dried plant material was finely ground and digested with a nitric-perchloric mixture (1:1). In leaves and roots ion contents of Na^+ and K^+ were determined by emission spectrophotometry and Ca^{2+} by atomic absorption spectrophotometry (Allan, 1969). Total nitrogen was estimated by Kjeldhal procedure (Bremner, 1965). Chloride was extracted by stirring ground-dried samples with 0.1 M $NaNO_3$ for 30 minutes. After extract clarification with activated coal, it was added 13.2 mM Hg $(SCN)_2$ in methanol and 20.2% (w/v) $Fe(NO_3)_3$ (4 + 1) and absorbance was determined at 460 nm (Gaines *et al*. 1984).

Total Soluble Carbohydrates (µg g^{-1} DW)

Total soluble carbohydrates (TSC) concentrations were determined according to method of Cahi and Brun (1978). Samples of 100 mg of roots and leaves were homogenized with 10 mL of extracting solution (glacial acetic acid: methanol: water, 1:4:5, v/v/v). The homogenate was centrifuged for 10 min at 3,000 rpm and the supernatant was decanted. The residue was re-suspended in 10 mL of extracting solution and centrifuged another 5 min at 3,000 rpm. The supernatant was decanted, combined with the original extract and made up to 50 mL with water. For measurement of TSC, a phenol-sulfuric acid assay was used as described by Dubois *et al.* (1956). A volume of 0.5 mL of 5% (v/v) phenol solution and 2.5 mL of concentrated sulfuric acid were added to 0.5 mL aliquots. The mixture was shaken, heated in a boiling water-bath for 20 min and cooled to room temperature. The absorption was then determined by spectrophotometry at 490 nm (Shimadzu spectrophotometer, Duisburg, Germany).

Leaf Relative Water Contents (%)

The leaf relative water contents (RWC) were calculated according to Beadle *et al.* (1993) using the equation:

RWC (%)= [(FW - DW)/ (TW - DW)] 100

Where FW is fresh weight, DW is dry weight, and TW is turgid weight.

Glycine betaine and Proline (µg g^{-1} DW)

Glycinebetaine was extracted by stirring finely ground-dried samples of roots with demineralized water at 100ºC for one hour. Glycinebetaine contents were determined spectro-photometrically after reaction with KI-I_2 at 365 nm (Grieve and Grattan, 1983). Proline was also determined spectro-photometrically following the ninhydrin method described by Bates *et al.* (1973) using L-proline as a standard. Approximately 300 mg of dry tissue was homogenized in 10 mL of 3% (w/v) aqueous sulphosalicylic acid and filtered. In 2 mL of the filtrate, 2 mL of acid ninhydrin was added, followed by the addition of 2 mL of glacial acetic acid and boiled for 60 min. The mixture was extracted with toluene, and the free proline was quantified spectro-photometrically at 520 nm from the organic phase using a Shimadzu spectrophotometer (Duisburg, Germany).

Isolation of Salicylic Acid (SA)

Salicylic acid was measured before NaCl treatments in all three propagated types (seed, roots and direct regenerated) of violet plants by the method described by Meuwly and Métraux (1993). Violet cells were collected by filtration under vacuum through four layers of Miracloth, snap frozen in liquid nitrogen, and kept at -80°C until the extraction was performed. SA was extracted from cells (0.5 g fresh weight of frozen tissue), separated by HPLC, and quantified by spectrofluorescence monitoring at 407 nm emissions.

Statistical Analysis

Analysis of variance (ANOVA) technique employed for carrying out statistical analysis of data collected (Steel and Torrie, 1980). The means values were compared with Least Significant Difference (LSD) Test, following Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Growth Attributes

In the 50 mol m^{-3} NaCl application plant height, root length, plant fresh and dry weights were reduced in comparison to the control (Table I). Effect of salt stress was non-significant in NaCl treated plants

supplemented with SA (T_2). It is apparent from the table I that plants supplemented with SA under NaCl showed statistically equal measurements for morphological attributes i.e. plant height, root lengths, plant fresh and dry weights as compared control. SA assisted the violet plants to eradicate the effect of NaCl stress.

Ion Contents

Results obtained regarding ion contents as Na^+, K^+, Ca^{2+}, Cl^- (ppm) and N (%) are given in (Table I). Impact of NaCl stress was highly significant for ions accumulations in violet plants. Na^+, K^+ and Cl^- concentrations were higher in T_1 (50-mol m^{-3} NaCl) over T_0 (control) both in roots and leaves. It was noted that concentrations of these ions were higher in leaves as compared to roots. In contrasts, SA supplemented plants under NaCl showed non-significant effect for accumulation of these ions. N contents were reduced in T_1, while T_2 plants showed higher N contents both in roots and leaves than control, while plants supplemented with SA showed no significant effect of NaCl on Ca^{2+} accumulation (Table I).

Table-I ***Comparison of means of exogenous salicylic acid applications for salt tolerance in violet***

Attribute	T_0 (0 mol m^{-3})	T_1 (50 mol m^{-3} NaCl)	T_2 (50 mol m^{-3} NaCl + SA)	LSD at 5%
Plant hieght (cm)	49.9±2.4 a	25.4±1.6 b	47.1±3.6 a	4.8
Root length (cm)	10.1±1.1 a	8.9+1.1 b	10.4±1.4 a	2.9
Plant fresh weight (g)	11.0 +1.7a	8.1±0.07 b	10.8±1.9 a	1.6
Plant dry weight (g)	6.2±1.2 a	3.9±0.09 b	6.1±0.09 a	1.4
Na^+ (ppm) in roots	17.2±1.4 b	26.6+1.8 a	16.8+1.5 b	3.5
Na^+ (ppm) in leaves	34.5±3.2 b	72.2+1.2 a	32.1±2.5 b	3.1
K^+(ppm) in roots	866.2+4.3 b	1033.2±2.9a	845.2±2.1 c	15.9
K^+(ppm) in leaves	621.1±3.3 b	721.6±2.4 a	632.0±2.5 b	21.5
Ca^{2+} (ppm) in roots	140.1+6.5 a	50.0±1.12 b	137.2±1.6 a	10.2
Ca^{2+} (ppm) in leaves	39.7±5.2 a	32.6+1.9 b	41.2+2.2 a	3.6
Cl^- (ppm) in roots	140.3±4.9 b	276.7±1.3 a	136.5±1.6 b	10.9
Cl^- (ppm) in leaves	209.2±2.1 b	328.2+1.6 a	202.9±1.4 b	18.7
N (%)in roots	1.6+0.6 b	1.1±0.7 c	2.1±0.1 a	0.51
N (%)in leaves	2.4+1.02 b	1.2+0.4 c	2.9±0.2a	0.32
TSC (µg g^{-1} DW) in roots	745±4.6 b	970±3.4 a	743±5.6 b	6.6
TSC (µg g^{-1} DW) in roots	633±2.3 b	720 ±4.1 a	621±4.4 b	5.3

Different small letters indicate significant difference among treatments within each row (p=0.05)

Total Soluble Carbohydrates (µg g^{-1} DW)

Total soluble carbohydrates (TSC) concentrations in roots and leaves increased sharply in relation to the salt stress (T_1) as root length significantly greater under salt stress, while it had non-significant effect in T_2 supplemented with SA under NaCl (Table I). It probably reflected the maintenance or even induction of root elongation at low water potentials, which could be considered as an adaptive response to salinity in plants.

Relative Water Contents (%)

Salt stress lowered the relative water contents (RWC) significantly under NaCl stress (T_1). It decreased below to 60% RWC. On the other hand treatment T_2 (SA + NaCl) had constant RWC as control that was above 75% (Fig. 1). This reduction in RWC might be resulted in decline of plant growth attributes.

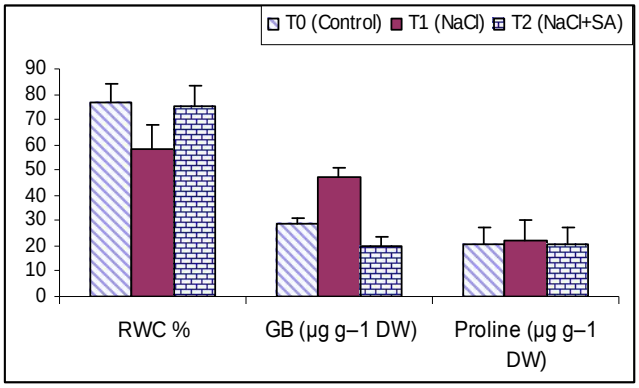

Fig. 1. Effect of SA in violet under salt stress on relative water content (%) glycine betaine and proline (µg g^{-1} DW)

Glycine betaine (µg g^{-1} DW)

The osmotic adjustment would be accomplished by the accumulation of organic solutes as SA mitigates this osmotic effect. Among the organic solutes investigated, glycine betaine (GB) showed the highest absolute accumulation in response to salinity (T_1 treatment) that was above that 40-µg g^{-1} DW. In control GB was below 25-µg g^{-1} DW. In contrast, treatment T_2 (SA + NaCl) showed significant reduction in GB accumulations that was below 20-µg g^{-1} DW (Fig. 1).

Proline (µg g^{-1} DW)

Proline concentrations were statistically equal in all treatments Fig. 1. Contrary to its generally accepted role in many other plant species, proline did not seem to play an important role in the mechanism of salt tolerance. The significance of proline accumulation in osmotic adjustment is still debated and varies according to the plant species.

Salicylic Acid (µg g^{-1} FW)

Results for SA isolated are presented in (Fig. 2). It was noted that T_2 (SA + NaCl) had significantly higher accumulations of SA as compared to control. NaCl stress had non-significant effect on SA accumulations in violet (T_1) both in roots as well as leaves. Both control and T_1 had almost equal concentrations of SA.

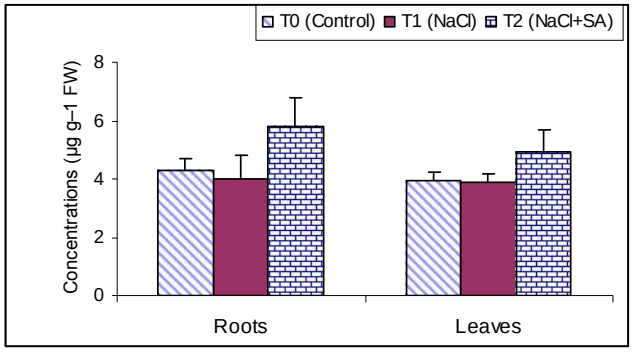

Fig. 2. Effect of exogenously applied SA on concentrations of SA in leaves and roots of violet under salt stress

Salt (NaCl) stress is among the factors most limiting to plant productivity (Shi *et al.* 2002). Plants exposed to salt stress adapt their metabolism in order to cope with the changed environment. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly (Hussain *et al*. 2007). The reason for growth reduction in violet could be due to water shortage and ionic toxicity caused by salinity. The decrease in plant growth may be due to decrease in turgor potential by water deficit conditions produced by high slats concentrations in the soil (Haung and Redmann, 1995). Assessment of pattern of accumulation of toxic ions concentrations in different plant parts is of vital importance to understand as to whether salt resistant or sensitive in toxic ions present in its growth medium. It also affects the enzyme activities of plants. Violet plants under NaCl salinity showed accumulations of Na^+, K^+, Ca^{2+} and Cl^- ions and changes in enzyme activities. Similar results of ion accumulations and enzymes activities have been earlier found in Atriplex by Khan *et al.* (2000). Similar results of accumulations of inorganic ions in salt sensitive and resistant pearl millet lines were described by Hussain *et al.* (2007). These results are also in accordance with Feitosa *et al*. (2001) and Meloni *et al*. (2001). Similar results for GB and proline under NaCl were found by many scientists as Heuer (2003) in tomato and in rice by Lutts (1996). SA plays an important role in the defense response to stresses (salts, water) in many plant species (Yalpani *et al.* 1994; Senaratna *et al.* 2000). Exogenously applications of SA helped to increase plant growth significantly in saline conditions (Setevens *et al.* 2007). Exogenously applications of SA strongly inhibited Na^+, K^+ and Cl^- and organic solute accumulations (GB and TSC) but stimulated N and RWC (Shirasu, 1997).

CONCLUSION

It was concluded that exogenous SA could be used as a potential growth regulator to improve plant salinity stress tolerance

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