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ALLEVIATIVE EFFECTS OF EXOGENOUS SALICYLIC ACID ON SOME ANTIOXIDATIVE ENZYMES OF *PISUM SATIVUM* L. UNDER SODIUM FLUORIDE STRESS

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ABSTRACT: The aim of the present study was to examine the effect of salicylic acid on the salinity tolerance of two varieties (RKS-510 and Classic) of pea (*Pisum sativum* L.*)*. Sodium fluoride (NaF), in concentrations of 50, 100, 150, and 200 ppm, was applied, in a pot experiment during the growth season 2015–2016, to the plants as a soil drench on a biweekly basis to create a stressed condition by its gradual accumulation causing toxic effects on the biochemical processes of the plants. Salicylic acid, in concentrations of 100, 200, and 300 ppm, was also given exogenously to the fluoride-stressed plants as a foliar application to examine its effect on two anti-oxidative enzymes, ascorbate peroxidase and peroxidase. Control plants were treated with tap water. At 44 days after sowing (DAS), the activity of ascorbate peroxidase was lowest in the control plants for both cultivars (controls: 0.47 and 0.45 µmol/min/g fresh weight for RKS-510 and Classic, respectively) and increased with increasing concentrations of NaF (50 ppm: 0.57 and 0.54; 100 ppm: 0.76 and 0.72; 150 ppm: 0.85 and 0.82; 200 ppm: 0.93 and 0.90 µmol/min/g fresh weight for RKS-510 and Classic, respectively). Similarly, the activity of peroxidase was lowest in the control plants for both cultivars (controls: 0.27 and 0.25 units of enzyme activity g^{-1} fresh weight min^{-1} for RKS-510 and Classic, respectively) and increased with increasing concentrations of NaF (50 ppm: 0.35 and 0.32; 100 ppm: 0.38 and 0.35; 150 ppm: 0.44 and 0.43; 200 ppm: 0.49 and 0.47 units of enzyme activity g^{-1} fresh weight min^{-1} for RKS-510 and Classic, respectively). Compared to the NaF-treated group, the foliar application of the application of both salicylic acid (foliar application) and NaF (soil drench) lowered the fluoride-induced stress resulting in lower activity levels for ascorbate peroxidase and peroxidase. However, at higher concentrations, salicylic acid itself may cause a high level of stress in plants and was less effective. We concluded that the foliar application of salicylic acid has an ameliorative effect on the impaired salinity tolerance in the RKS-510 and Classic cultivars of *Pisum sativum* L. produced by sodium fluoride stress.

Key word: Salicylic acid, Anti-oxidative enzymes, *Pisum sativum* L. Sodium fluoride stress

INTRODUCTION

Fluorine is the 13th most abundant element in the earth's crust and is a common environmental pollutant. It significantly affects various physiological processes of plants, such as decreased plant growth, chlorosis, leaf tip burning, and necrosis.

Salicylic acid (SA) is a naturally occurring water-soluble antioxidant phenolic compound produced in plants that enhances their growth. It is an important endogenous signaling molecule which modulates the responses of plants to environmental stresses. In 1839, a German scientist extracted salicylic acid from the herb meadow sweet (*Filipendula ulmaria* L.), which has also been named *Spiraea ulmaria* L. SA also occurs naturally in white willow (*Salix alba*). Due to its isolation from nature, it is commonly known as a "natural product." Salicylic acid acts as a

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signaling molecule for the regulation of several physio-biochemical processes in plants, such as photosynthesis. The growth and photosynthetic capacity of plants under saline stress and stress conditions is enhanced by the exogenous application of SA. Salicylic acid promotes salinity tolerance by accelerating the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX).[1] Moreover, it has been found that salicylic acid lowers the lethal effects of salinity by enhancing the relative water content and increasing the availability of nutrients in the roots and leaves of strawberry plants.[2] It also been reported that the pea plant contains antioxidants which lower the oxidation of free radicals.[3]

Some growth regulators like abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) have been implicated in imparting stress tolerance to plants.[4] Investigations show a role of SA in modulating plant responses to a wide range of oxidative stresses. Exogenous SA may act via the osmotic stress-induced stimulation of ABA synthesis and the accumulation of oxidative enzymes. Recently, numerous studies have indicated that the exogenous application of salicylic acid to stressed plants can potentially alleviate the toxic effects generated by salinity. An enhanced tolerance against salinity stress was observed in tomato plants raised from the seeds soaked in salicylic acid and was presumed to be due to the enhanced activation of some enzymes like aldose reductase and ascorbate peroxidase, and to the accumulation of certain osmolytes, such as proline.[5] The accumulation of large amounts of osmolytes is an adaptive response in plants exposed to stressful environments.

Pea, (*Pisum sativum* L.), is an edible leguminous seed crop for human nutrition. Its seeds contain 18–20% dry matter of which 5–8% is protein and 10–12% is carbohydrate. Pea is a cool-season annual plant generally used as a fresh vegetable, but its canning and freezing has also been introduced. Keeping the above production scenario and usefulness in mind, it is urgent that the production of pea is strengthened.

The aim of the present study was to examine the effect of salicylic acid on the salinity tolerance of two varieties (RKS-510 and Classic) of pea (*Pisum sativum* L.).

MATERIALS AND METHODS

A pot experiment was performed during the growth season from November 2015 to March 2016. It was conducted in a wire-house of the Botanical Garden, Quaid-e-Azam Campus, University of the Punjab, Lahore, Pakistan, which is located in the southern part of Lahore (74º21-00-E, 31º35-00-N), on a 21×15 m suburban habitation near fields. A randomized complete block design (RCBD) was used to design the whole experiment which was performed in triplicate. Two hybrid varieties were selected: variety RKS-510 obtained from Punjab Seeds Corporation and variety Classic obtained from Arain Seed Corporation, Lahore. Healthy seeds were selected to raise the plants. Unhealthy and infected seeds were carefully removed. Clay pots were each filled with 5 kg of a mixture of sandy and loamy soils, in a 1:3 ratio, with, in addition, some farm yard and leaf manure. The holes at the bottom of the pots were partially closed to prevent water loss. The pots were organized with reference to the

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particular variety, the respective treatment, and the replicate number. Seeds were soaked in clean tap water for 24 hours before sowing.

Various concentrations of sodium fluoride (NaF; 50, 100, 150, and 200 ppm) and salicylic acid (100, 200, and 300 ppm) were prepared by mixing measured amounts of solutes in distilled water according to the requirements necessary for producing the respective concentrations. The different dilutions of sodium fluoride and salicylic acid, in ppm, were then used to check the effect of salicylic acid on the two pea cultivars under sodium fluoride stress during the growth season 2015 to 2016. The sodium fluoride-induced stress condition was created by applying 150 mL of the various concentrations of NaF solution as a soil drench. To examine the effect of salicylic acid on the sodium fluoride-stressed plants, 6 mL of the various concentrations of salicylic acid was applied as an exogenous foliar spray by using a shower bottle. Control plants were treated by the administration of tap water. The treatments were applied on a biweekly basis throughout the growth season until their final harvest.

According to the method of Rao et al.,[6] APX activity (EC 1.11.1.11) was observed in leaf extracts by measuring the absorbance at 290 nm. By adding 25 µL of the crude enzyme preparation to 2 mL of a solution containing 50 mL potassium phosphate buffer pH 6.8, 20 mL H_2O_2, and 20 mL guaiacol, peroxidase (POX) activity was measured. After incubation at 30ºC for 10 min, the reaction was stopped by adding 0.5 mL 5% (v/v) H_2SO_4 and the absorbance was read at 480 nm.[7] One POX unit was defined as the change of 1.0 absorbance unit per mL enzymatic extract and expressed as units of enzyme activity per g fresh matter per min (UA g^{-1} FW min^{-1}). Data obtained from the pot experiment were then used to calculate the treatment mean, the standard error, and Duncan's Multiple Range Test, as described by Steel and Torrie.[8] For this purpose, the software package Costat (version 3.03) was employed using the computer facility of the laboratory.

RESULTS

At 44 days after sowing (DAS), treatment with NaF as a soil drench raised ascorbate peroxidase (APX) activity levels in a dose-related manner. The activity of APX was lowest in the tap water control plants for both cultivars (controls: 0.47 and 0.45 µmol/min/g fresh weight for RKS-510 and Classic, respectively) and increased with increasing concentrations of NaF (50 ppm: 0.57 and 0.54; 100 ppm: 0.76 and 0.72; 150 ppm: 0.85 and 0.82; 200 ppm: 0.93 and 0.90 µmol/min/g fresh weight for RKS-510 and Classic, respectively).

The salicylic acid lowered the stress of the NaF treatment so a smaller amount of enzyme was produced in the plants. Compared to the NaF-treated group, the application of both salicylic acid (foliar application) and NaF (soil drench) lowered the fluoride-induced stress, in both cultivars, resulting in lower APX activity levels (Figures 1 and 2).

At 44 DAS, the activity of the enzyme peroxidase (POX) was lowest in the control plants for both of the cultivars (controls: 0.27 and 0.25 units of enzyme activity g^{-1} fresh weight min^{-1} for RKS-510 and Classic, respectively) and increased with increasing concentrations of NaF (50 ppm: 0.35 and 0.32; 100 ppm: 0.38 and 0.35; 150 ppm: 0.44 and 0.43; 200 ppm: 0.49 and 0.47 units of enzyme activity g^{-1} fresh

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weight min^{-1} for RKS-510 and Classic, respectively). Compared to the NaF-treated group, the application of both salicylic acid (foliar application) and NaF (soil drench) lowered the fluoride-induced stress, in both cultivars, resulting in lower POX activity levels for (Figures 3 and 4).

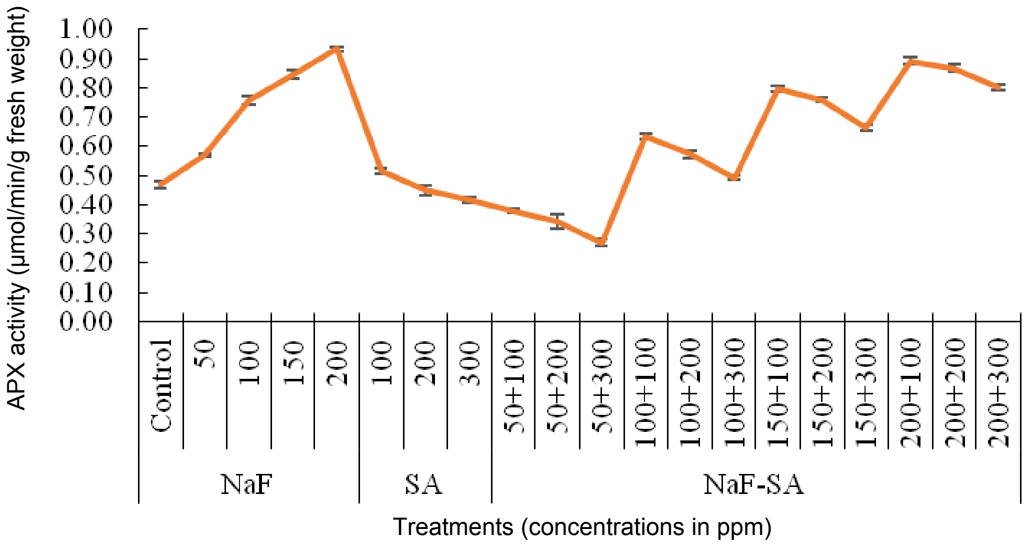

Figure 1. Showing ascorbate peroxidase (APX) activity in pea variety RKS-510 under different treatments of sodium fluoride and salicylic acid. NaF=sodium fluoride, SA=salicylic acid.

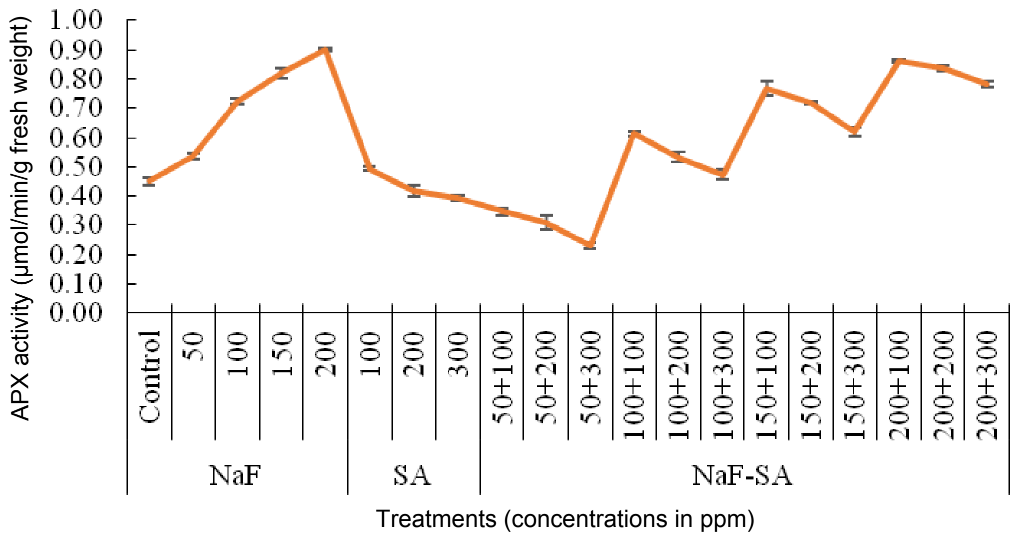

Figure 2. Showing ascorbate peroxidase (APX) activity in pea variety Classic under different treatments of sodium fluoride and salicylic acid. NaF=sodium fluoride, SA=salicylic acid.

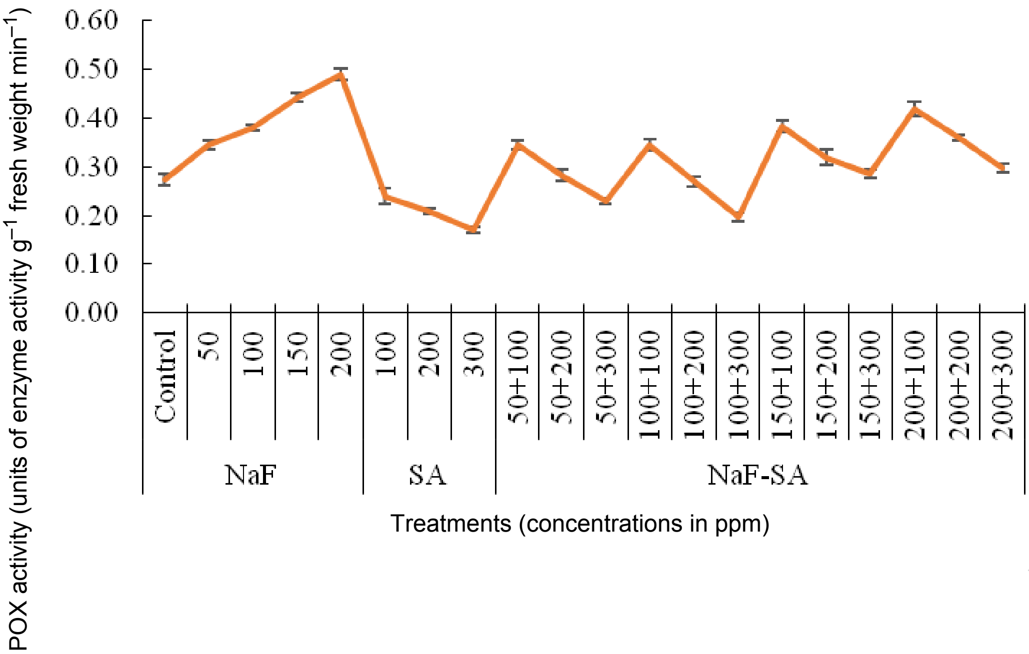

Figure 3. Showing peroxidase (POX) activity in pea variety RKS-510 under different treatments of sodium fluoride and salicylic acid. NaF=sodium fluoride, SA=salicylic acid.

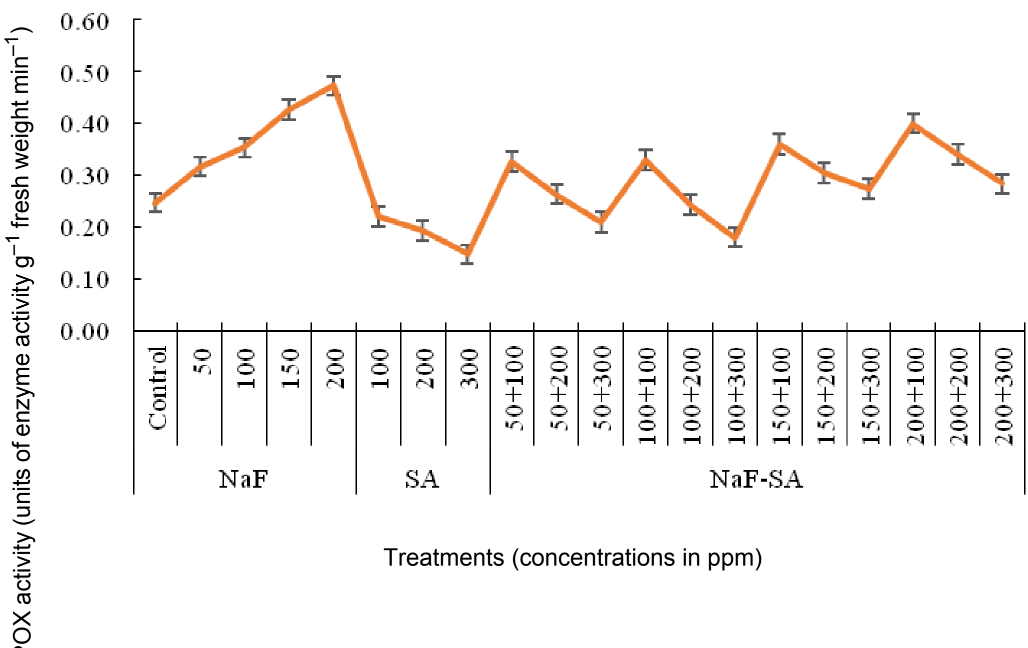

Figure 4. Showing peroxidase (POX) activity in pea variety Classic under different treatments of sodium fluoride and salicylic acid. NaF=sodium fluoride, SA=salicylic acid.

DISCUSSION

Fluorine is a widespread element in nature and the contamination of the natural environment with fluorine compounds has become a global problem.[9] Fluorine in the environment originates from both industrial activities, e.g., brick kilns, and natural minerals, especially fluorite, apatite, muscovite, and biotite.[10] Numerous investigations have shown that a high fluorine content, adversely affects the growth and development of plants,[11] causing leaf damage[12] or decreasing the content of chlorophyll in the leaves.[13] Many kinds of fluorides are poisonous and present in acidic soils. These soils frequently have moderate to high levels of fluoride thus causing a stressed condition for plants. *Pisum sativum* L. is commonly used as fresh peas and dry pulses. Economically, it has been grown for the canning industry. In the present study, the impacts of fluoride stress on the productivity of the pea plant was determined by applying various concentrations of NaF.

Ascorbate peroxidase (APX) and peroxidase (POX) enzyme activities tend to increase successively with the increase in sodium fluoride stress while the exogenous application of salicylic acid caused a reduction in the activity of both enzymes. Similar to the results of the present study, is the finding of Tuna et al. that the antioxidant enzymes POX and APX increased in plants grown under salt stress but decreased with the application of SA.[14] In barley, the APX activity was increased significantly under salt stress.[15] APX activity is generally increased with elevated salinity levels.[16] The tolerance capacity of plants subjected to fluoride stress increases due to the stress-induced higher enzyme production.[17]

In the present experiment, sodium fluoride was continuously given to the plants on a biweekly basis and this produced detrimental effects on the physiological processes. This is a matter of serious concern, from an agricultural view point, as Pakistan is an agrarian country and its soils are being contaminated with a toxic load of NaF from various sources. Though plants are silent suffers, they reveal their response in the form of an impaired growth pattern and reduced yield. However, the foliar application of salicylic acid proved very effective against the sodium fluoride stress. The exogenous application of salicylic acid successively reduced the effects of the sodium fluoride stress and enhanced the physiological parameters of *Pisum sativum*. However, at higher concentrations, salicylic acid itself may cause a high level of stress in plants and was less effective.

REFERENCES

1 Noreen S, Ashraf M, Hussain M, Jamil A. Exogenous application of salicylic acid enhances antioxidative capacity in salt stressed sunflower (*Helianthus annuus* L.) plants. Pak J Bot 2009; 41(1):473-9.

2 Karlidag H, Yildirim E, Turan M. Salicylic acid ameliorates the adverse effect of salt stress on strawberry. Scientia Agricola 2009;66(2):180-7.

3 Ismail A, Tan ST. Antioxidant property of some non-leafy vegetables. Nutrition and Food Science 2009;39:176-80.

4 Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: a review. Env Exp Bot 2010; 68:14-25.

5 Szepesi A, Csiszar J, Bajkan SZ, Gemes K, Horvath F, Erdei L, et al. Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt and osmotic stress. Acta Biol Szegediensis 2005;49:123-5.

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6 Rao MV, Paliyath G, Ormrod DP. Ultravoilet-B and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiol 1996;110(1):125-36.

7 Urbanek H, Kuzniak GE, Herka K. Elicitation of defense responses in bean leaves by *Botrytis cinerea* polygalacturonase. Acta Phys Plant 1991;13:43-50.

8 Steel RGD, Torrie JH. Principles and procedures of statistics. 2nd ed. New York: McGraw-Hill; 1980. pp. 336-76.

9 Kumar S, Puri A. A review of permissible limits of drinking water. Indian Journal Occup 2012;16(1):40-44.

10 Kinnunen H, Holopainen T, Raisanen LM, Karenlampi L. Fluoride in birch leaves, ground vegetation, litter and humus in the surroundings of a fertilizer plant and apatite mine in *Siilinjarvi*, eastern Finland. Bor Environ Res 2003;8:185-92.

11 Joshi M, Bhardwaj N. Effect of fluoride on growth parameters and its accumulation in *Triticum aestivum* var. Raj. 3675. Fluoride 2012;45(3 Pt2):297-301.

12 Doley D. Rapid quantitative assessment of visible injury to vegetation and visual amenity effects of fluoride air pollution. Environmental Monitoring and Assessment 2010;160(1-4):181-98.

13 Chakrabarti S, Patra PK, Mandal B, Mahato D. Effect of sodium fluoride on germination, seedling growth and biochemistry of bengal gram (*Cicer arietinum*). Fluoride 2012;45(3 Pt 2):257-62.

14 Tuna AL, Kaya C, Dikilitas M, Yokas I, Burun B, Altunlu H. Comparative effects of various salicylic acid derivatives on key growth parameters and some enzyme activities in salinity stressed maize (*Zea mays* L.) plants. Pak J Bot 2007;39(3):787-98.

15 Shi WM, Muramoto Y, Ueda A, Takabe T. Cloning of peroxisomal ascorbate peroxidase gene from barley and enhanced thermotolerance by overexpressing in *Arabidopsis thaliana*. 2007;273:23-7.

16 Seday U, Gulsen O, Uzun A, Toprak G. Response of citrus rootstocks to different salinity levels for morphological and antioxidative enzyme activities. Journal of Animal and Plant Sciences 2014;24(2):512-20.

17 Datta JK, Maitra A, Mondal NK, Banerjee A. Studies on the impact of fluoride toxicity on germination and seedling growth of gram seed (*Cicer arietinum* L. cv. Anuradha). Journal of Stress Physiology & Biochemistry 2012;8(1):194-202.