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Effect of salicylic acid on the anti-oxidative system of *Bt* and non - *Bt* cotton grown in semi-arid soils of Nizamabad

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Abstract

The effect of salicylic acid (SA) sprayed in three concentrations viz., 0.5 mM, 1.0mM and 3.0mM on the antioxidative system comprising of antioxidative enzymes [catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR) and super oxide dismutase (SOD)] as well as antioxidants like ascorbate (ASA) and reduced glutathione (GSH) were analyzed in two varieties of cotton (*Gossypium herbaceum* L.) viz., *Bt*- cotton and non-*Bt* plants grown in the semi-arid tropics of Nizamabad. The soil in Nizamabad district is saline and black soil wherein the plants usually experience drought and saline stresses. Application of all three concentrations of SA positively increased the activities of all the enzymes (catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and super oxide dismutase (SOD) in the two varieties of cotton compared to untreated control plants except peroxidase (POD) enzyme activity which was found decreased. Further SA was found to increase the contents of ascorbate (ASA) as well as reduced glutathione (GSH). The *Bt*-cotton variety showed better performance over non-*Bt* varieties. SA at 3.0 mM conc. was found most effective in increasing enzymes as well as antioxidants in both cotton varieties over 1.0mM, 0.5 mM and untreated controls. The promotion of enzyme contents as well as antioxidants in both cotton varieties is an indicator that SA mitigated the negative effect of the semi-arid soil conditions of Nizamabad district.

Keywords: Ascorbate (ASA); ascorbate peroxidase (APX); Bt-cotton; catalase (CAT); glutathione reductase (GR); non-Bt cotton; peroxidase (POD); reduced glutathione (GSH); salicylic acid (SA); super oxide dismutase (SOD).

1. Introduction

Salicylic acid is (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants [1, 2]. Plant growth regulators (PGRs) have an important role to play in mediating plant responses to abiotic stresses. SA plays a major role in the regulation of plant growth, development, and interaction with other organisms and defense responses to environmental stresses [3]. Salicylic acid (SA) acts as endogenous signal molecule that participates in the regulation of physiological processes in plants [4,7]. Many researchers clarified several beneficial effects of SA under abiotic stress conditions such as; can it play a significant role in plant water relations [5, 6], photosynthesis, growth rate and stomatal regulation [8], as well as ion uptake and transport [24] and membrane permeability [9].

Nizamabad district experiences a tropical dry and wet season with most of the rainfall in June to October. It usually experiences erratic rain fall. The soil is saline land black soil which is deep loamy to clay loam, moderately drained, neutral to alkaline in nature. The reduction of growth of many plants by salinity and drought usually effects on dry matter production, ionic relations, metabolic variations, physiological processes, water contents. The semi-arid condition directly poses a threat to the overall yield of the plants as they usually experience drought and saline stresses.

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Cotton (*Gossypium herbaceum* L.) is a commercially grown crop throughout India and is very responsive to environmental changes. Cotton is a fiber, oil and protein yielding crop grown in various parts of Telangana State of India. The ever changing environment is posing a severe threat on the growth and yield of cotton crop. The employment of growth promoting substances has been extensively used to enhance growth of cotton. The present study is focused on the effect of a plant growth regulator, salicylic acid on the antioxidative system of two varieties of cotton (*Bt* and non *Bt*) grown in semi-arid tropics of Nizamabad District of Telangana State in India.

2. Material and methods

Cotton (*Gossypium herbaceum* L.) seeds of *Bt* (NCS -863 Bt-2) and non-*Bt* (NCS 108-sunny) was procured from Nuziveedu Seed company Private Ltd., Gundlapochampally, Medchal, Rangareddy, Telangana State, India. Salicylic acid (SD–fine) was procured from Dwarakmai Enterprise, Hyderabad, Telangana State, India.

2.1. Growth of Cotton Varieties

The field was ploughed and levelled by garden workers in Botanical Garden, Telangana University. Individual plot (5 X 4 m) was prepared. The plot was uniformly mixed with 20 kg compost. The plants were grown in field (5m X 4m) length and width containing fresh sieved red soil mixed with well rotten farm yard manure in the ratio of soil. The seeds of cotton [*Bt* (NCS -863 Bt-2) and non-*Bt* (NCS 108-sunny)] were surface sterilized with 0.5% (v/v) sodium hypo chlorate and washed thoroughly with several changes of sterile distilled water. They were soaked for 24 h in sodium chloride supplemented and rhizobium inoculum. 20 seeds of *Bt* and non-*Bt* varieties were sown in each row keeping a gap of about 45cm X 40cm between each row in the field which was supplemented with farmyard manure. On the 7th day after the germination, only 15-16 healthy plants were retained in the soil.

SA was supplied to the plants as foliar spray at three different concentration levels viz., 0.5 mM, 1.0 mM and 3.0 mM on 40^{th}, $50^{th}$and 60^{th} day (from the day of sowing). The extraction of enzymes was done at 4°C. Five grams of freshly plucked cotton leaves were macerated in a mortar using a pestle with 0.1 M phosphate buffer pH = 7 for around fifteen minutes employing a magnetic stirrer. This homogenate was carefully filtered through Whatman No.41 filter paper and then centrifuged for twenty minutes at 2,500 rpm in a Remi - Refrigiratory Cooling Centrifuge and used for assaying CAT, POD and PPO activities.

2.1.1. Catalase (E.C. 1.11.1.6.)

CAT activity was assayed by the method of Aebi [10]. The reaction mixture contained enzyme extract, hydrogen peroxide and phosphate buffer (pH = 7). The reaction was stopped by adding conc. sulphuric acid and the residual hydrogen peroxide was titrated with potassium permanganate. The activity was calculated by the following formula:

$$C = 25/2 \times 0.0017 \times v/w$$

where w = fresh weight of tissue in g, v = difference in the titre value between the blank and the sample.

2.1.2. Peroxidase (1.11.1.7)

POD activity was assayed by adopting the method of Kar and Mishra [11]. The assay mixture for POD activity contained phosphate buffer (pH = 7), pyragallol, hydrogen peroxide and enzyme extract. After incubation, the reaction was stopped by adding conc. sulphuric acid. The amount of purpurogallin formed was estimated by measuring the absorbance at 420 nm.

2.2. Ascorbate peroxidase activity (apx: e.c 1.11.11).

0.5 g of Fresh cotton leaves were macerated in a mortar using a pestle in 50 mM Tris-hydrochloric acid (pH 7.5) by adding 40 mM phenyl methyl sulfonyl fluoride (PMSF, 0.2 mM EDTA and 2% (w/v) polyvinyl pyropyrolidone (PVPP). This extract was then centrifuged for twenty minutes at 15,000 X g. The supernatant was used for assaying the ascorbate peroxidase enzyme.

Ascorbate peroxidase (APX) activity in cotton leaves was determined following the procedure given by Nakano and Asada [13]. The reaction mixture comprised of 1.5 ml of 50 mM sodium phosphate buffer (pH 7), 0.2 mM EDTA, 0.5 ml of 0.5 mM ascorbic acid, 0.5 ml 0.5 mM hydrogen peroxide and 0.5 ml of enzyme extract. The activity of APX was measured as the decrease in absorbance at 290 nm for one minute. The amount of ascorbic acid oxidized was estimated from the extinction coefficient of 2.6 $mM^{-1}cm^{-1}$. Lowry et al. (1951) was used to measure the amount of protein present in the enzyme extract.

2.3. Superoxide dismutase (E.C. 1.15.1.1.)

One gram of the leaf material was homogenized in 5ml of 50 mM phosphate buffer (pH= 7.0) containing 1% poly vinyl pyrrolidine. The homogenate was filtered and centrifuged at 15000 × *g* for 10 min. The supernatant obtained was used as the enzyme extract. All steps in the preparation of the enzyme extract were carried at 0-4°C. An aliquot of 0.1ml was used for the determination of protein content by using Lowry *et al*. [12] method. The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) as per the procedure of Beauchamp and Fridovich [14]. The absorption was measured at 560nm under the above conditions. The increase in the absorbance in the absence of the enzyme was taken as 100% and 50% of the inhibited activity was taken equivalent to one unit of SOD activity. SOD activity was expressed as U/mg protein.

2.4. Glutathione reductase (E.C. 1.6.4.2)

The extraction and assay for glutathione reductase (GR) in sorghum leaves was carried out according to Smith *et al*. [15]. One gram of leaf material was homogenized with a mortar and pestle using 5ml of 0.1 M potassium phosphate buffer (pH=7.5) containing 0.5 mM EDTA. The brie was filtered through cheese cloth and the filtrate was centrifuged for 10 min for 20,000 × *g*. The supernatant was used as enzyme extract. An aliquot of 0.1ml was used for the determination of protein content by using Lowry *et al*. [12] method. The increase in the absorbance at 415 nm was continuously monitored for 5 min. The rate of the enzyme activity was calculated using standard curve prepared by known amounts of glutathione. GR activity was expressed as µmoles of reduced DTNB/min/mg protein.

2.5. Estimation of ascorbic acid (AsA)

The content of ascorbic acid (AsA) was estimated following Hodges et al. [16] method. 0.2 Grams of Fresh cotton leaves were macerated in a mortar using a pestle with 5 ml of 5% (v/v) m-phosphoric acid. This homogenate was centrifuged for around fifteen minutes at 12,000 X g. The reaction mixture for estimation of ascorbic acid (AsA) consisted of 0.1 ml supernatant, 0.5 ml of 100 mM potassium dihydrogen phosphate buffer (pH 7.4) in 5 mM EDTA and 0.2 ml of ddH_2O which were mixed and allowed to stand at room temperature for fifteen minutes. 0.8 ml 10% (w/ v) trichloroacetic acid, 0.8 ml 44% (v/v) o-phosphoric acid, 0.8 ml a, a´-dipyridyl in 70% (v/v) ethyl alcohol and 0.4 ml 30 g l^{-1} ferric chloride were added one by one and thoroughly mixed and incubated for 40 ºC for 1 hr. The optical density (O.D.) of the mixture at measured at 525 nm.

2.6. Estimation of reduced glutathione (GSH)

The amount of reduced glutathione (GSH) was determined adopting Hissin and Hilf [17] method. One gram of cotton leaf material was macerated in a mortar using a pestle with 10 ml phosphate-EDTA (0.1M sodium phosphate -0.005M EDTA; pH 8.2) and then centrifuged for thirty minutes at 25,000 x g and 40 ^{0}C. The reaction mixture for estimation of GSH comprised of 450 µl of cold phosphate EDTA buffer (pH=8), 50 µl of supernatant and mixed thoroughly. Then, aliquots of 25 and 50 µl was taken into 5 ml test tube and made up to 100 µl with cold glass distilled water. 1.8 ml of phosphate EDTA buffer (1.8 ml) was added to the test tube and mixed. 100 µl of freshly prepared O-phthalaldehyde (OPT: 5 mg OPT/ 5ml in distilled methyl alcohol) solution was added and mixed thoroughly. This mixture was allowed to stand at room temperature around 25^{0}C for fifteen minutes. The fluorescence was measured at an excitation and emission wavelength of 350 and 420 nm using JASCO, FP-750 Spectroflourimeter. GSH Standard (1 mg of GSH was dissolved in 5 ml of ice cold 0.1M sodium phosphate -0.005M EDTA (pH = 8) which was diluted to 10 ml using buffer and stored at 4°C which were run simultaneously. The standard range was around 0.4-2µg.

The values were presented as mean ± S.E. of 5 replicates

3. Results

The effect of 0.5 mM, 1.0mM and 3.0 mM of SA on the catalase(CAT) activity in the leaves of two varieties of cotton (*Bt* and non *Bt*) grown in semi-arid tropics of Nizamabad is shown in Table 1. Exogenous application of SA at three concentrations caused substantial increase in the levels of catalase activity in cotton plants. 3.0mM conc. of SA particularly proved to be highly efficient in accounting highest levels of catalase activity over 1.0mM and 0.5mM as well as control plants.

The effect of 0.5 mM, 1.0mM and 3.0 mM of SA on the content of peroxidase (POD)activity in the leaves of two varieties of cotton (*Bt* and non *Bt*) grown in semi-arid tropics of Nizamabad is shown in Table 1. Exogenous application of SA at three concentrations caused substantial decrease in the levels of peroxidase activity in cotton plants. 3.0mM conc. of SA particularly proved to be poorly efficient in accounting lowest levels of peroxidase activity over 1.0mM and 0.5mM as well as control plants.

Table 1 Effect of salicylic acid (SA) on the antioxidative enzymes of two varieties of cotton (*Bt* and non *Bt*) plants grown in semi-arid tropics of Nizamabad

Varieties	Treatments	Catalase (CAT) (Enzyme Units)	Peroxidase (POD) (Enzyme Units)	Ascorbate Peroxidase (APX)(ASA/mg-1/min/protein)*	Glutathione Reductase(GR) DTNB/min/mg/ protein)*	Superoxide Dismutase (SOD)(U/mg protein)*
Non Bt-cotton	0.5 mM SA	25.20 ± 1.11	0.598 ± 0.03	0.694 ± 0.02	24.90 ± 0.42	11.20 ± 0. 42
	1.0 mM SA	26.71 ± 0.75	0.572 ± 0.06	0.737 ± 0.03	26.30 ± 0.28	13.20 ± 0.28
	3.0 mM SA	27.86 ± 0.78	0.494 ± 0.07	0.837 ± 0.04	21.10 ± 0.54	14.80 ± 2.46
	Control	23.50 ± 0.23	0.699 ± 0.08	0.681 ± 0.05	28.60 ± 0.32	9.40 ± 2.38
Bt cotton	0.5 mM SA	24.39 ± 0.29	0.501 ± 0.08	0.688 ± 0.03	22.60 ± 0.92	10.40 ± 2.08
	1.0 mM SA	25.90 ± 0.84	0.489 ± 0.08	0.724 ± 0.07	24.70 ± 0.81	12.20 ± 1.80
	3.0 mM SA	26.70 ± 0.61	0.473 ± 0.04	0.784 ± 0.05	27.50 ± 0.072	13.80 ± 1.56
	Control	21.80 ± 0.45	0.548 ± 0.09	0.578 ± 0.04	19.90 ± 0.81	8.83 ± 1.28

The values were presented as mean ± S.E. of 5 replicates.

The influence of SA on the activity of ascorbate peroxidase (APX) in the leaves of two varieties of cotton (*Bt* and non *Bt*) grown in semi-arid tropics of Nizamabad cotton plants is shown in Table 1. The exogenous application of SA more increased the ascorbate peroxidase activity.3.0mM conc. of SA was caused which an enhancement of over 1.0mM and 0.5mM as well as control plants.

The effect of SA on glutathione reductase activity content in the leaves of both the varieties of cotton (*Bt* and non *Bt*) grown in semi-arid tropics of Nizamabad control is shown in Table 1. Supplementation of SA to cotton plants as foliar spray registered good improvement of glutathione reductase activity compared to untreated plants. Among all the three concentrations utilized, the effect of 3.0mM was marginally more effective than 1.0mM and 0.5mM as well as control plants.

The study on the role of salicylic acid (SA) on the superoxide dismutase activity of two varieties of cotton (*Bt* and non *Bt*) grown in semi-arid tropics of Nizamabad cotton plants is shown in Table 1. The results obtained in the present study clearly indicate substantial increase in the superoxide dismutase activity of cotton plants. All the three concentrations of SA viz., 0.5mM, 1.0 mM and 3.0 mM increased in the superoxide dismutase activity of both the *Bt* and non-*Bt* varieties of cotton plants grown in semi-arid soils of Nizamabad over control plants. SA at 3.0mM was found most effective in substantial increase in the superoxide dismutase activity compared to the other two concentrations as well as control plants.

The effect of SA on both antioxidants like ascorbic acid (AsA) and reduced glutathione (GSH) contents in the leaves of both the varieties of cotton (*Bt* and non *Bt*) grown in semi-arid tropics of Nizamabad control is shown in Table 2. Supplementation of SA to cotton plants as foliar spray registered good improvement of both antioxidants like ascorbic acid (AsA) and reduced glutathione (GSH) contents compared to untreated plants. Among all the three concentrations utilized, the effect of 3.0mM was marginally more effective than 1.0mM and 0.5mM as well as control plants in both antioxidants like ascorbic acid (AsA) and reduced glutathione (GSH).

Table 2 Effect of salicylic acid (SA) on the antioxidants of two varieties of cotton (*Bt* and non *Bt*) plants grown in semi-arid tropics of Nizamabad

Varieties	Treatments	Ascorbate (ASA) (mg g-^{1}fr.wt.)	Reduced Glutathione (GSH) (mg g-^{1}fr.wt.)
Non Bt-cotton	0.5 mM SA	686 ± 9.57	21.30 ± 1.01
	1.0 mM SA	724 ± 11.37	22.8 ± 2.21
	3.0 mM SA	784 ± 11.37	24.7 ± 1.08
	Control	634 ± 11.75	19.04 ± 2.24
Bt cotton	0.5 mM SA	678 ± 12.61	22.10 ± 3.43
	1.0 mM SA	794 ± 10.45	24.70 ± 1.21
	3.0 mM SA	778 ± 10.98	26.10 ± 3.37
	Control	634 ± 11.75	20.08 ± 1.24

The values were presented as mean ± S.E. of 5 replicates.

4. Discussion

The role of SA in positively monitoring the antioxidant system in plants subjected to various abiotic stresses like salt, water deficit conditions usually result in formation of Reactive Oxygen Species (ROS) like superoxide radicals , hydroxyl radicals, hydrogen peroxide etc. cause oxidative stress [18]. PGRs usually reduce/deactivate the ROS by activating the antioxidants, antioxidative enzymes etc. and exogenous application of SA enhanced the efficiency of antioxidant system in plants [19].

Noreen et al. [20] also found that exogenous foliar applied SA enhanced antioxidant capacity in salt stressed sunflower. Treatment with SA induced salt tolerance by increased induction of antioxidant enzymes and decreased H_2O_2 content [21]. He and Zhu [22] observed that alleviation of NaCl toxicity by SA was related to decreased Na contents, increased K and Mg levels in shoots and roots and increased SOD, CAT, GPX and DHAR activities as well AsA and glutathione levels. Exogenous application of SA as foliar spray mitigated salt stress in *Brassica juncea* L. increased CAT, POD and SOD activities [23] and application of SA reduced peroxidase activity in *Ocimum basilicum* L. [24].

Ascorbic acid/Ascorbate (AsA) is reported to be the main component of the ascorbate - glutathione cycle [25]. Glutathione is present as reduced GSH and is an integral part of the antioxidative system of the plants [26]. The enzymatic role of GR is mainly to reduce the substrate GSSG (oxidized form) to the reduced GSH in plants subjected to oxidative stress and it is an established fact that GSH plays a key role in salt tolerance and its availability is influenced both by assimilation and PGRs action which was reported in earlier studies [27].

5. Conclusion

The present study reveals that application of SA to *Bt* and non-*Bt* varieties of cotton plants grown in semi-arid soils of Nizamabad cotton plants as foliar spray was found to be very profound in increasing the CAT, APX, SOD, GR activities but decreasing POD activity in both the varieties (*Bt* and non *Bt*) of cotton though the effect was more in *Bt*- cotton grown in semi- arid tropics of Nizamabad.

The soils of Nizamabad are saline and dry in nature inhibiting the growth of plants. The foliar treatment of SA in *Bt* and non-*Bt* cotton plants was reflected with increased catalase activity (CAT) and reduced peroxidase (POD) activity. Though catalase and peroxidase possess similar prosthetic group (iron porphyrin), it is not clear why the activity of these two oxidizing enzymes showed different trends to each other. The exogenous application of SA –treatment to *Bt* cotton and non –*Bt* cotton plants grown in the semi-arid tropical region of Nizamabad exhibited increased glutathione reductase (GR), ascorbate peroxidase (APX) and superoxide dismutase(SOD) activities. The present study gives an insight that application of SA overcame the negative effect of the semi-arid conditions of the soil (reflected in the control

plants) and promoted the antioxidative enzyme activities of both *Bt* and non-*Bt* varieties of cotton though the effect was more pronounced in *Bt* variety.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of Interest.

References

[1] Afzal I, Basra, Iqbal SA. The effect of seed soaking with plant growth regulators on seedling vigor of wheat under salinity stress. Journal of Stress Physiology and Biochemistry. 2005; 1(1): 6-14.

[2] Alyemeni MN, Hayat Q, Wijaya L, Hayat S. Effect of salicylic acid on the growth, photosynthetic efficiency and enzyme activities of leguminous plant under cadmium stress. Notulae Botanicae Horti Agrobotanici. 2014; 42(2).

[3] Wittwer SH. Phytohormones and chemical regulators in agriculture. In: Phytohormones and related compounds. A comprehensive treatise. eds. Letham PS, Goodwin PB. and Higgins TJV. Elsevier, North Holland Biomedical Press, Amsterdam/Oxford/NewYork. 1978; 599-615.

[4] Durner J, Klessig DF. Inhibition of ascorbate peroxidase by salicylic acid and 2, 6 dichloroisonicotinic acid, two inducers of plant defense responses. Proceedings of National Academy of Sciences USA. 1995; 92: 11312-16.

[5] Durner J Klessig DF. Salicylic acid is a modulator of tobacco and mammalian catalases. Journal of Biochemistry. 1996; 271(45): 28492-501.

[6] Slaymaker DH, Navarre DA, Clark D, Del-Pozo O, Martin GB, Klessig DF. The tobacco salicylic acid-binding protein 3 (sabp3) is the chloroplast carbonic anhydrase, which exhibits antioxidant activity and plays a role in the hypersensitive defense response. Proceedings of National Academy of Sciences USA. 2002; 99: 11640-11645.

[7] Davies PJ. The plant hormones: Their nature, occurrence, and functions. In: Davies, P.J. Ed. Plant Hormones, Springer, Netherlands. 2010; 1-15.

[8] Kang HM, Saltveit ME. Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. Physiologia Plantarum. 2002; 115: 571-576.

[9] Mohammed AR, Tarpley L. Impact of high night-time temperature on respiration, membrane stability, antioxidant capacity, and yield of rice plants. Crop Science. 2009; 49: 313–322.

[10] Aebi H, Catalase. In: Bergmeyer H. Ed. Methods in enzymatic analysis. Vol 2, New York: Academic Press. 1974; 674-84.

[11] Kar M, Mishra D. Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. Plant Physiology. 1976; 57: 315-319.

[12] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 1951; 193: 265–275.

[13] Nakano Y, Asad K. Hydrogen peroxide is scavenged by ascorbate specific peroxidise in spinach chloroplasts. Plant Cell Physiology. 1981; 22: 867-880.

[14] Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 1971; 44:276-287.

[15] Smith IK, Vie Heller TL, Thorne CA. Assay of glutathione reductase in crude tissue homogenates using 5, 5‟-dithiobis (2-benzoic acid). Annals of Biochemistry. 1998; 175(2): 408-413.

[16] Hodges DM, Andrews CJ, Jhonson DA, Hamilton RI. Antioxidant composed response to chilling stress in differentially sensitive inbred maize lines. Physiologiae Plantarum. 1996; 98:685-692.

[17] Hissin PJ, Hilf R. A flurometric method for determination of oxidized and reduced glutathionein tissues. Annals of Biochemistry. 1976; 74(1):214-226.

[18] Panda SK, Sinha LB, Khan MH. Does aluminium phytotoxicity induce oxidative stress in green gram (Vigna radiata). Bulgarian Journal of Plant Physiology. 2003; 29:77-86.

[19] He YL, Liu YL, Chen Q, Bian AH. Thermo tolerance related to antioxidation induced by salicylic acid and heat hardening in tall Fescue seedlings. Journal of Plant Physiology and Molecular Biology. 2002; 28: 89-95.

[20] Noreen S, Ashraf M, Akram NA. Does exogenous application of salicylic acid improve growth and some key physiological attributes in sunflower plants subjected to salt stress? Journal of Applied Botany and Food Quality.2011; 84:169–77.

[21] Erdal S, Aydın M, Genisel M1, Taspınar MS, Dumlupinar R, Kaya O, Gorcek Z. Effects of salicylic acid on wheat salt sensitivity. African Journal of Biotechnology. 2011; 10 (30): 5713-5718.

[22] He Y, Zhu ZJ. Exogenous salicylic acid alleviates NaCl toxicity and increases antioxidative enzyme activity in *Lycopersicon esculentum*. Biologia Plantarum. 2008; 52: 792-795.

[23] Yusuf M, Hasan SA, Ali B, Hayat S, Fariduddin Q, Ahmad A. Effect of salicylic acid on salinity induced changes in *Brassica juncea*. Journal of Integrative Plant Biology. 2008; 50: 1196-1202.

[24] Karalija E, Paric A. Effects of salicylic acid foliar application on growth and antioxidant potential of basil (*Ocimum basilicum* L.). Biologica Nyssana. 2017; 8(2): 145-150.

[25] Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 2010; 48: 909–930.

[26] Jimenez JA, Hernandezm G, Pastorim LA, del Rio F, Sevilla F. Role of the ascorbate glutathione cycle of mitochondria and peroxisomes in the senescence of pea leaves. Plant Physiology. 1998;118: 1327-1335.

[27] Anjum NA, Ahmad I, Mohmooda I, Pacheco M, Duarte AC, Pereira E. Modulation of glutathione and its related enzymes in plants‟ responses to toxic metals and metalloids - A review. Environmental and Experimental Botany. 2012; 75:307-24.