DOI : 10.35124/bca.2019.19.2.3873

Biochem. Cell. Arch. Vol. 19, No. 2, pp. 3873-3882, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

IMPROVING PHOTOSYNTHETIC PIGMENTS AND ANTIOXIDANT ACTIVITIES OF SOYBEAN UNDER STRESS AND NON-STRESS CONDITION BY FOLIAR SPRAY OF SALICYLIC ACID, 24-EPIBRASSINOLIDE, JASMONIC ACID

Meenakshi Sheokand[1]*, Somveer Jakhar[2] and Sunidhi Chopra[3]

[1,2]Department of Botany, Kurukshetra University, Kurukshetra, India.
[3]Department of Biotechnology, Dyal Singh College, Karnal, India.
*e-mail: meenakshisheokand86@gmail.com

(Received 26 March 2018, Revised 14 July 2018, Accepted 25 January 2019)

ABSTRACT : Salinity is one of the major anthropogenic and desperate environmental factors limiting the productivity because most of the crop plants are susceptibile to salinity and the area affected by it increasing day by day. The objective of this reserach to elucidate the potential ameliorative effect of salicylic acid, 24-epibrassinolide, jasmonic acid in leaves of Soybean (Pusa-9712) under 0 and 150 mM concentration of NaCl. To check the efficacy of these bio-regulators foliar spray of SA (10^{-6}M), 24-EBL (10^{-7} M), JA (0.5 µM) was done fifteen days before taking the sample. After spraying sampling was done at three successive stage 45 (vegetative), 60 (flowering) and 75 (pod filling) stage. Results depicted that salt stressed exhibited a significant decline in photosynthetic pigments, protein content, while MDA and proline content considerably increased under both control and stressed plants Furthermore, application of these plant bio-regulators significantly enhanced the plant growth by reducing the adverse effects of salinity on the studied parameters. Plants when applied with (0.5 µM, 10^{-6}M SA, 10^{-7}M EBL) increased the SOD (47.4%, 35.9%, 21.1%), POD (35.6%, 29.2%, 20.6%), CAT (49.9, 32.6, 28.2), proline (125.5%, 97.6%, 79.1%) in salt stressed plant as compared to control at 60 DAS, respectively. Along with increasing in antioxidant activities application of these phytohormone (0.5 µM, 10^{-6}M SA, 10^{-7}M EBL) also reduced the MDA content by (36.4%, 30.2%, 26.4%) in salt stressed plant as compared to control at 45 DAS, respectively. Overall, from results it was concluded that JA, SA, 24-EBL found to be effective in ameliorating the adverse effect of salt stress at extremely low concentration. So, order of the effectiveness is found to be (JA> SA>EBL).

Key words : Soybean, Salicylic acid, jasmonic acid, 24-Brassinolide, jasmonic acid, foliar spray.

INTRODUCTION

With the onset of global warming, land salinization is becoming a most crucial problem due to increasing scarcity of fresh water, improper land irrigation and fertilization practices. Currently, there are about one billion square hectometers of saline alkali soil which is accounting for 7.6% of total land area in world (Yu *et al*, 1998). Jamil *et al* (2011) reported that near about 50% of the arable land would be salinized by the year 2050. Ion-toxicity, osmotic stress and production of excess ROS (reactive oxygen species) in plant cell such as superoxide radicals, hydrogen peroxide, hydroxyl anion and singlet oxygen (O_2) that damage lipids, protein, DNA are caused by salt stress (Yasar *et al*, 2006). Due to osmotic disturbance and ionic stress salinity disturb plant morpho-physiological and biochemical processes ultimately affect plant growth (Vinocur and Altman, 2005). Many attempts have been made to improve tolerance against salinity, one of them is foliar application of new recent bioregulators *viz.* salicylic acid (SA), jasmonic acid (JA), epibrassinolide (EBL). These bioregulators are recognized as stress alleviators against various type of biotic and abiotic stresses. Along with increasing the resistance against any type of stress application of these bioregulators also enhanced the production of many crop plants.

Salicylic acid is naturally occurring plant hormone acting as an important signaling molecule which adds to tolerance under any type of stresses. Salicylic acid participates in regulation of many physiological processes in plants such as stomatal closure, ion uptake and transport, inhibition of ethylene biosynthesis, transpiration, stress tolerance, membrane permeability photosynthesis and growth (Ashraf *et al*, 2010). Under saline and non-saline condition application of SA enhanced the growth

of wheat as well as increase in photosynthesizing tissue *i.e.* leaves (Dhaliwal *et al*, 1997; Zhou *et al*, 1998). SA could alleviate adverse effects of salinity via promotion of seedling growth, restoring plant growth and promoting accumulation of proline, ABA, IAA and cytokinin as reported by Ashraf *et al* (2010). Application of SA in low doses had the best effect on growth of wheat and cowpea as compared to other higher concentration (Afshari *et al*, 2013). Various physiological and biochemical processes of plants including photosynthesis, antioxidant capacity and ion homeostasis, adversely affected by salt stress however application of SA induced changes in these biochemical or physiological processes (Ashraf, 2004).

Brassinosteriods, a group of steroidal substances first isolated from the pollen of *Brassica napus* (Grove *et al*, 1979) as the sixth group of plant hormones. Several studies have established that BRs influence seed germination, plant growth, nitrogen fixation, senescence, leaf abscission, increased yield, fruit ripening and enhanced tolerance against various abotic/biotic stress like chilling, drought, thermal, heavy metals, pesticide, salt and diseases (fungal, viral and bacterial infection (Ali *et al*, 2007; Hayat *et al*, 2007). The adverse effect of salt stress on seed germination and growth (Anuradha and Rao, 2001) and root growth (Amzallag and Goloubinoff, 2003) has been mitigated by exogenously applied brassinosteriods. Exogenous application of BRs may influence a range of diverse processes of growth and development in plants (Montoya, 2005). Bajguz and Hayat (2009) reported that BRs also help to overcome stresses provoked by low or high temperature, drought, salt, infection, pesticides and heavy metals.

Jasmonates are known to regulate a wide range of plant processes such as growth and development including defense against abiotic and biotic stresses (Browse, 2009). Jasmonates are important cellular regulators which are involved in diverse developmental processes such as seed germination, root growth, gravitropism, trichome formation, embryo development, sex determination, fertility, seedling development, tuber formation, leaf movement, fruit ripening and leaf senescence (Creelman and Mulpuri, 2002; Wasternack and Hause, 2002). Therefore, present investigation was undertaken to assess the effects of different PGR's through their foliar application on the biochemical and antioxidant activities of soybean.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of soybean variety (Pusa-9712) were obtained from CCS Haryana Agriculture University, Hissar. The experiment was set up in the experimental cage of Department of Botany, Kurukshetra University. Five seeds per pot were surface sterilized and sown in earthen pots (30 cm diameter) lined with polythene having 5.0 kg of dune sand grown under natural light conditions during kharif season. The temperature conditions were 35±2°C and 24±2°C, during days and nights respectively; with mean relative humidity of 82 ± 5%.

Treatments

After three weeks, the seedlings were thinned to two plants per pot and each treatment consisted of three replications in a complete randomized design (CRD). After establishment of seedling soil salinity was provided in form of final concentration (150 mM). Fifteen days before taking the sample, foliage of the plants was sprayed uniformly either with double distilled water (control), or with different phytohormones like SA, 24-EBL, JA (10^{-6} M, 10^{-7} M, 0.5 µM) to elu-cidate the effect of these exogenous application of these hormones on plants. The plants were sampled at 45^{th}, 60^{th}, 75^{th} DAS to assess various biochemical and antioxidant activities of soybean plant.

Estimation of chlorophylls and carotenoids

Leaf sample (200 mg) was ground in chilled 80% acetone (ARgrade) with 20 mg of $CaCO_3$ and centrifuged at 3000 g for 5min. Absorbance of the filtrate was recorded at 645 and 663 nm for chlorophylls and at 480 and 510 nm for carotenoids depending upon respective peaks in their absorption spectra using a UV-Visible spectrophotometer (Specord-205, Analytic-Jena, Germany). Chlorophyll (Chl) amount was estimated with the formula of Arnon (1949). Carotenoid level was calculated by the method of Holden (1965).

Estimation of total soluble protein

Total soluble proteins were estimated according to the method described by Bradford (1976) using Coomassie Brilliant Blue G-250. For a minute fifty mg of fresh leaf tissue (earlier stored in a freezer) was dropped boiling 80% ethanol (EtOH) on a water bath. At room temperature the tissue along with EtOH was homogenized. Now, the extract was centrifuged at 10,000 g for 5 min. With 5% perchloric acid the residue was re-extracted followed by centrifugation at 10,000 rpm for 5 min. Five-mL of 1N NaOH was added to the residue and maintained in warm water (40-50°C) with regular shaking for 30 min. The clear supernatant was used for further analysis.

Lipid peroxidation (MDA content)

The level of lipid peroxidation was measured in terms

of MDA content (Heath and Packer, 1968). Two hundred mg leaf sample was homogenized in 2 ml 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 8000 rpm for 20 min in a Remi centrifuge (R-8C). To 0.5 ml aliquot of the supernatant, 2 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 90ºC for 30 min in the water bath and then quickly cooled in an ice water bath. After centrifugation at 8000 rpm for 10 min the absorbance of each sample at 600 nm was also recorded and substracted from the absorption recorded at 532 nm. The concentration of MDA, an end product of lipid peroxidation was calculated in accordance to its extinction coefficient of 155 per mM per cm.

Superoxide dismutase (SOD) activity

Fifty milligram of fresh leaves crushed in 2 ml of 0.1 M EDTA- phosphate buffer of pH 7.8 containing 1.74 gm K_2HPO_4 (Mol. Wt. 174.18) and 3.72 gm of EDTA (Mol. Wt. 372.2) and the final volume was raised to 100 ml double distilled water (DDW). This was centrifuged at 15000 rpm and resultant supernatant was used as crude extract. The reaction mixture was prepared by adding 0.1 ml of crude extract followed by 0.9 ml of DDW, 0.5 ml of 300 mM Na_2Co_3 (pH 10.2), 0.5 ml of 378 µM p-nitroblue tetrazolium chloride (NBT), 0.5 ml of 78 mM L- methionine and 0.5 ml of 7.8 µM riboflavin. The final reaction mixture was 3 ml. The reaction was carried out in similar test tubes at 25ºC for 15 min. in 100 µmol photon per m^2 per's PFD from fluorescent lamp. The initial rate of reaction as measured by the difference in increase in absorbance at 560 nm in the presence and absence of extract was proportional to the amount of enzyme which under the experimental condition caused a 50% inhibition o the reaction observed in the absence enzyme (Giannopolities and Ries, 1977).

Guaiacol peroxidase (GPOX) activity

The method of Maehly (1954) method was employed to assay guaiacol peroxidase activity. The reaction mixture having 2 ml enzyme aliquot, 2ml 0.2 M phosphate buffer (pH 7.0) and 2 ml 20 mM guaiacol reagent (0.22 ml guaiacol in 100 ml DW, was prepared 24 hours before carrying out the estimation) was taken in a cuvette. The cuvette was placed in the spectrophotometer. The wavelength was adjusted to 470 nm and OD was noted. 2 drops of 10 µM hydrogen peroxide (0.4 ml H_2SO_4 of 20 volumes in 9.6 ml DW) was added to the reaction mixture in the cuvette and again the OD was noted after 30 seconds. The difference in OD before and after adding H_2O_2 to the reaction mixture was used to calculate peroxidase activity. Peroxidase activity was expressed as difference in OD/1 min/µg protein.

Catalase (CAT) activity

Catalase activity was estimated according to the method of Aebi (1984). In the test tube reaction mixture was prepared by adding 1.5 ml of 50mM HEPES buffer, 1.2 ml of 150 mM H_2O_2 and 30 µl plant extract. In the mixture without enzyme, no crude extract was added, instead of it 50 µl 50 mM HEPAS buffer was added. The change in absorbance was read at 490 nm in the test tube cuvette using a UV-Vis spectrophotometer (Systronic, Double beam spectrophotometer 2203). Specific activity of catalase was expressed in terms of per mg protein. Protein was estimated from the same extract following the procedure of Bradford (1976) with Coomassie blue dye G-250 at 595 nm using UV-Vis spectrophotometer.

RESULTS

Chlorophyll content

Chlorophyll-a

Chlorophyll-a (Chl.a) decreased significantly as compared to control in soybean plants under salt stress on all growth stages and it was 37.7, 48.7 and 30.3 per cent as compared to control of non-stressed plant on 45th, 60th and 75th DAS respectively (Table 1). On the other hand, application of SA, JA and EBL maintained the chl.a as compared to salt stress alone, but it does not exceed the control. Among the groups treated with PGRs under salt-stressed condition, JA1 exhibited highest percent increment in chl.a followed by SA3 and EBL2 and it was 60.0, 54.5 and 46.9 per cent over control respectively on 45th DAS. Similarly, on 60th DAS, salt-stressed plants treated with JA1 exhibited highest chl.a followed by SA3 and EBL2 and it was 52.7, 45.2 and 34.9 per cent over control respectively. Among the PGRs used, JA showed better results in the improvement in chl.a of soybean under salt stress condition.

Chlorophyll-b

Chlorophyll-b (Chl.b) exhibited a rapid decline under salt stress compared to control of non-stressed plants on all growth stages and it was 47.0, 44.3 and 30.2 per cent over control on 45th, 60th and 75th DAS respectively (Table 2). On the other hand, application of SA, JA and EBL increased chl.b as compared to salt stress alone, but it does not exceed the control. Among the groups treated with PGRs, JA1 exhibited highest percent increment in chl.b followed by SA3 and EBL2 under salt-stressed condition and it was 64.8, 55.5 and 44.4 per cent over control respectively on 45th DAS. Similarly, on 60th DAS, salt-stressed plants treated with JA1 exhibited

highest chl.b followed by SA3 and EBL2 and it was 65.2, 57.9 and 42.0 per cent over control respectively. Among the PGRs used, JA showed better results in the improvement in chl.b of soybean under salt stress condition.

Total chlorophyll content

Total chlorophyll (Total chl.) content exhibited a rapid decline under salt stress as compared to control of non-stressed plant on all growth stages and it was 43.8, 47.4, and 30.3 per cent over control on 45th, 60th and 75th DAS, respectively (Table 3). On the other hand, application of SA, JA and EBL increased Total chl. as compared to salt stress alone, but it does not exceed the control of non-stressed plant. Among the groups treated with PGRs, JA1 exhibited highest percent increment in Total chl. followed by SA3 and EBL2 and it was 61.8, 54.8 and 49.4 per cent over control respectively on 45th DAS. Similarly, on 60th DAS, salt-stressed plants treated with JA1 exhibited highest Total chl. followed by SA3 and EBL2 and it was 54.4, 49.3 and 37.2 per cent over control, respectively. Among the PGRs used, JA showed better results in the improvement in Total chl. of soybean under salt stress condition.

Carotenoid content

Salt stress caused a significant reduction in carotenoids as compared to control on all growth stages and it was 49.0, 30.6 and 34.9 per cent over control on 45th, 60th and 75th DAS respectively (Table 4). On the other hand, application of SA, JA and EBL increased carotenoids contents as compared to salt stress alone, but it does not exceed the control of non-stressed plant. There was found non-significant difference among these PGRs on 45th DAS. Among the groups treated with PGRs, JA1 exhibited highest percent increment in carotenoids content under salt stress followed by SA3 and EBL2 and it was 60.7, 44.3 and 29.1 per cent over control respectively on 45th DAS. Similarly, on 60th DAS, salt-stressed plants treated with JA1 exhibited highest carotenoids content followed by SA3 and EBL2 and it was 41.8, 37.5 and 28.4 per cent over control respectively. Among the PGRs used, JA showed better results in the improvement in carotenoids content of soybean under salt stress condition.

Biochemical constituents

Protein content

Salt stress caused a significant reduction in leaf protein content as compared to control on all growth stages and it was 28.3, 34.7, and 27.5 per cent over control on 45th, 60th and 75th DAS, respectively (Fig. 1). On the other hand, application of SA, JA and EBL increased protein content as compared to salt stress alone, but it does not exceed the control. Among the groups treated with PGRs under salt-stressed condition, JA1 exhibited highest percent increment in leaf protein content followed by SA3 and EBL2 and it was 50.4, 35.3 and 27.8 per cent over control respectively on 45th DAS. Similarly, on 60th DAS, salt-stressed plants treated with JA1 exhibited highest leaf protein followed by SA3 and EBL2 and it was 37.8, 34.0 and 28.1 per cent over control respectively. Among the PGRs used, JA showed better results in the improvement in total protein content of soybean under salt stress condition.

Lipid peroxidation (MDA content)

Our data revealed that as plants matured, there exhibited a significant increase in lipid peroxidation by increasing malondialdehyde (MDA) content, the end product of lipid peroxidation in leaves of soybean plants (Fig. 2). Among the groups treated with PGRs under salt-stressed condition, JA1 exhibited highest percent declined in lipid peroxidation followed by SA3 and EBL2 and it was 36.4, 30.2 and 24.6 per cent over control respectively on 45th DAS. Similarly, on 60th DAS, salt-stressed plants treated with JA1 exhibited highest declined in lipid peroxidation followed by SA3 and EBL2 and it was 47.6, 36.0 and 22.7 per cent over control respectively. Among the PGRs used, JA showed better results in the lipid peroxidation of soybean under salt stress condition

Proline content

Salt stressed dramatically induced the accumulation of proline in the leaves of soybean plant. Its leads to a significant increased in the proline content on all growth stages and it was 62.4, 54.6 and 47.8 per cent over control of non-stressed plant on 45th, 60th and 75th DAS respectively (Fig. 3). Furthermore, application of SA, JA and EBL increased the proline content under stressed as well as non-stressed condition. Among the groups treated with PGRs under salt-stressed condition, JA1 exhibited highest increased in proline content followed by SA3 and EBL2 and it was125.5, 97.6 and 79.1 per cent over control respectively on 60th DAS. Similarly, on 75th DAS, salt-stressed plants treated with JA1 exhibited highest proline content followed by SA3 and EBL2 and it was 92.4, 118.6 and 69.7 per cent over control respectively. Among the PGRs used, JA showed better results in the improvement of proline content of soybean under salt stress condition.

Enzymatic antioxidants

Superoxide dismutase (SOD) activity

Salt stress caused a significant increased in the SOD activity as compared to control on all growth stages and it was 42.5, 32.6 and 25.5 per cent over control of non-

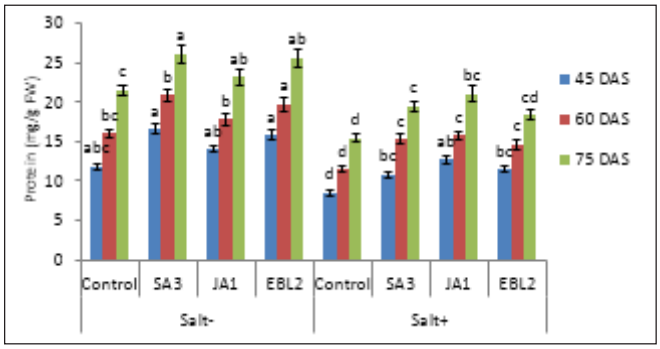

Fig. 1 : Effect of SA, JA and EBL on protein content in the leaves of soybean plant at 45, 60, 75 DAS under stress and non-stress condition.

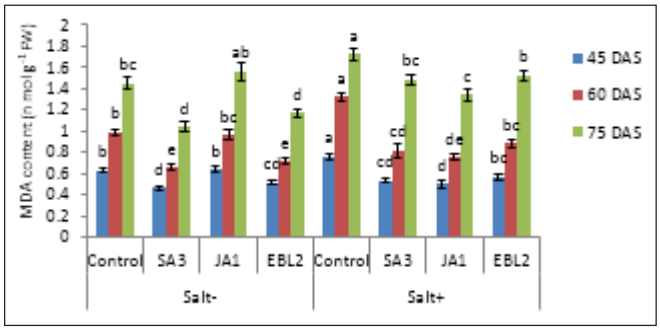

Fig. 2 : Effect of SA, JA and EBL on MDA content in the leaves of soybean plant at 45, 60,75 DAS understress and non-stress condition.

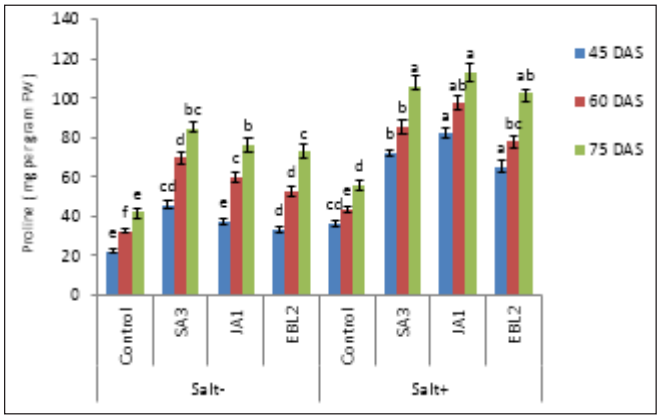

Fig. 3 : Effect of SA, JA and EBL on proline content in the leaves of soybean plant at 45, 60,75 DAS under stress and non-stress condition.

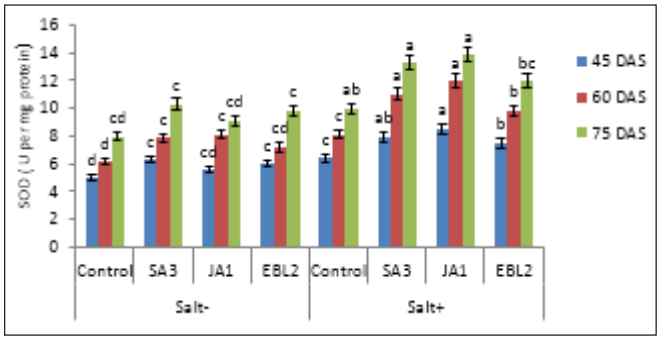

Fig. 4 : Effect of SA, JA and EBL on SOD activtiy in the leaves of soybean plant at 45, 60, 75 DAS under stress and non-stress condition.

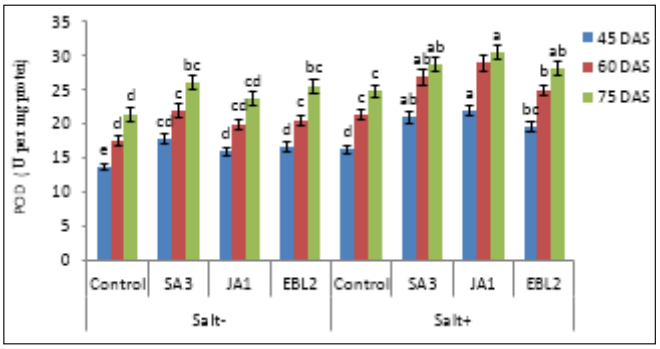

Fig. 5 : Effect of SA, JA and EBL on POD activityin the leaves of Soybean plant at 45, 60, 75 DAS under stress and non-stress condition.

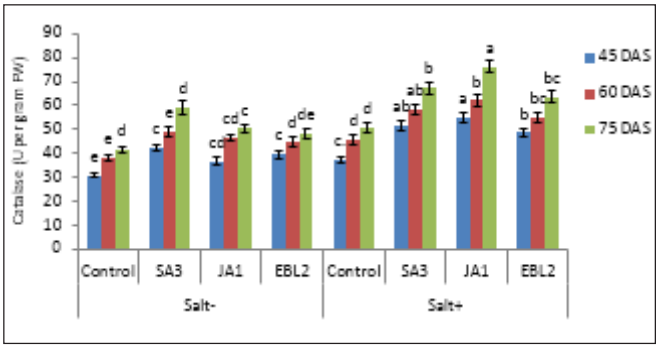

Fig. 6 : Effect of SA, JA and EBL on catalase activity in the leaves of Soybean plant at 45, 60, 75 DAS under stress and non-stress condition.

stressed on 45th, 60th and 75th DAS, respectively (Fig. 4). Furthermore, application of SA, JA and EBL increased the SOD activity under stressed as well as non-stressed condition. Among the groups treated with PGRs under salt-stressed condition, JA1 exhibited highest increased in SOD activity followed by SA3 and EBL2 and it was 47.4, 35.9 and 21.1 per cent over control respectively on 60th DAS. Similarly, on 75th DAS, salt-stressed plants treated with JA1 exhibited highest SOD activity followed by SA3 and EBL2 and it was 38.8, 33.2 and 20.1 per cent over control respectively. Among the PGRs used, JA showed better results in the improvement of SOD activity of soybean under salt stress condition.

Peroxidase (POD) activity

Salt stress caused a significant increased in the POD activity on all growth stages and it was 18.9, 21.6 and 15.4 per cent over control of non-stressed on 45th, 60th and 75th DAS respectively (Fig. 5). Furthermore, application of SA, JA and EBL increased the superoxide POD activity under salt-stressed condition. Among the groups treated with PGRs under non-stressed condition, JA1 exhibited highest increased in POD activity followed by SA3 and EBL2 and it was 35.6, 29.2 and 20.6 per cent over control respectively on 45th DAS. Similarly, on 60th DAS, salt-stressed plants treated with JA1 exhibited

Table 1 : Influence of SA(10^{-6}M), EBL(10^{-7}M) and JA (0.5 µM) on the changes in Chlorophyll a (mg/g FW) ± S.E. in *Glycine max* L. at 45, 60 and 75 DAS under stress and non- stress condition.

Salinity level	Treatments	Vegetative stage V5 (45 DAS)	Flowering stage R1 (60 DAS)	Pod stage R3 (75 DAS)
(0 mM)	Control	2.12 ± 0.06^{b}	2.85 ± 0.09^{ab}	3.44 ± 0.11^{b}
	SA3 (10^{-6} M)	3.12 ± 0.09^{a}	3.94± 0.13^{a}	4.42 ± 0.15^{a}
	JA1 (0.5 µM)	2.29± 0.07^{ab}	3.14 ± 0.10^{b}	3.76 ± 0.11^{c}
	EBL2 (10^{-7} M)	2.94± 0.11^{a}	3.72± 0.15^{a}	4.12± 0.18^{b}
(150 mM)	Control	1.32 ± 0.04^{c}	1.46 ± 0.05^{d}	2.28 ± 0.08^{d}
	SA3 (10^{-6} M)	2.04± 0.08^{bc}	2.12± 0.06^{c}	3.24± 0.12^{bc}
	JA1 (0.5 µM)	2.12± 0.06^{b}	2.23± 0.08^{c}	3.36± 0.15^{ab}
	EBL2 (10^{-7} M)	1.94± 0.13^{c}	1.97± 0.07^{bc}	3.02 ± 0.10^{c}

Data are means of ± SE of three replicate. Means followed by the same letter for each tested parameter are not significantly different ($P < 0.05$).

Table 2 : Influence of SA(10^{-6}M), EBL(10^{-7}M) and JA (0.5 µM) on the changes in Chlorophyll b (mg/g FW) ± S.E. in *Glycine max* L. at 45, 60 and 75 DAS under salt stress condition.

Salinity level	Treatments	Vegetative stage V5 (45 DAS)	Flowering stage R1 (60 DAS)	Pod stage R3 (75 DAS)
(0 mM)	Control	1.02± 0.03^{b}	1.24± 0.04^{d}	1.65± 0.05^{ab}
	SA3 (10^{-6} M)	1.43± 0.05^{a}	1.90 ± 0.08^{a}	2.04± 0.08^{a}
	JA1 (0.5 µM)	1.10± 0.12^{b}	1.48± 0.05^{c}	1.72± 0.06^{bc}
	EBL2 (10^{-7} M)	1.36± 0.04^{a}	1.74± 0.05^{b}	1.98± 0.08^{a}
(150 mM)	Control	0.54± 0.01^{d}	0.69± 0.02^{e}	1.12± 0.02^{d}
	SA3 (10^{-6} M)	0.84± 0.02^{c}	1.09± 0.04^{de}	1.42± 0.05^{bc}
	JA1 (0.5 µM)	0.89± 0.02^{c}	1.14± 0.03^{d}	1.59± 0.06^{ab}
	EBL2 (10^{-7} M)	0.78± 0.03^{c}	0.98 ± 0.02^{e}	1.34 ± 0.06^{c}

Data are means of ± SE of three replicate. Means followed by the same letter for each tested parameter are not significantly different ($P< 0.05$).

highest SOD activity followed by SA3 and EBL2 and it was 35.7, 25.8 and 17.3 per cent over control respectively. Among the PGRs used, JA showed better results in the improvement of POD activity of soybean under salt stress condition.

Catalase (CAT) activity

Salt stress caused a significant increased in the catalase activity (CAT) on all growth stages and it was 45.3, 37.6 and 29.2 per cent over control of non-stressed plant on 45th, 60th and 75th DAS, respectively (Fig. 5). Furthermore, application of SA, JA and EBL increased the CAT activity under stressed condition. Among the groups treated with PGRs under salt-stressed condition, JA1 exhibited highest increased in CAT activity followed by SA3 and EBL2 and it was 47.7, 38.8 and 30.9 per cent over control respectively on 45th DAS. Similarly, on 75th DAS, salt-stressed plants treated with JA1 exhibited highest CAT activity followed by SA3 and EBL2 and it was 49.9, 32.6 and 25.2 per cent over control, respectively. Among the PGRs used, JA showed better results in the improvement of CAT activity of soybean under salt stress condition.

DISCUSSION

Phytoharmone are natural growth regulators and when applied in small amounts, they bring rapid changes in the phenotypes of the plant and also influence the plant growth and development right from seed germination to senescence either by enhancing or by stimulating the natural growth regulatory system. In present study, chloroplast pigments (a & b) drastically reduced (Tables 1, 2, 3, 4) under salt stress, as our results agree with earlier work (Kaya *et al*, 2001). This reduction in chloroplast pigments was found to be maintained by application of SA, JA and BRs in the present research. Photosynthesis which is a major contributing factor for plant growth and yield (Natr and Lawlor, 2005) might have been affected due to SA application. In *Brassica juncea* exogenous application of SA enhance the net

Table 3 : Influence of SA(10^{-6}M), EBL(10^{-7}M) and JA (0.5 µM) on the changes in total chlorophyll (mg/g FW) ± S.E. in *Glycine max* L. at 45, 60 and 75 DAS under stress and non-stress condition.

Salinity level	Treatments	Vegetative stage V5 (45 DAS)	Flowering stage R1 (60 DAS)	Pod stage R3 (75 DAS)
(0 mM)	Control	3.31± 0.11^{b}	4.09± 0.13^{c}	5.09± 0.16^{b}
	SA3 (10^{-6} M)	4.55± 0.18^{a}	5.84± 0.23^{a}	6.46± 0.26^{a}
	JA1 (0.5 µM)	3.39± 0.13^{b}	4.62± 0.18^{b}	5.48± 0.16^{b}
	EBL2 (10^{-7} M)	4.30± 0.12^{a}	5.46± 0.22^{a}	6.10± 0.24^{a}
(150 mM)	Control	1.86± 0.06^{d}	2.15± 0.06^{e}	3.40± 0.11^{c}
	SA3 (10^{-6} M)	2.88± 0.11^{c}	3.21± 0.12^{d}	4.66± 0.19^{b}
	JA1 (0.5 µM)	3.01± 0.10^{bc}	3.37± 0.13^{d}	4.95± 0.19^{ab}
	EBL2 (10^{-7} M)	2.78± 0.09^{c}	2.95± 0.11^{d}	4.36± 0.18^{b}

Data are means of ± SE of three replicate. Means followed by the same letter for each tested parameter are not significantly different (P< 0.05).

Table 4 : Influence of SA(10^{-6}M), EBL(10^{-7}M) and JA (0.5 µM) on the changes in Carotenoids (mg/g FW) ± S.E. in *Glycine max* L. at 45, 60 and 75 DAS under stress and non-stress condition.

Salinity level	Treatments	Vegetative stage V5 (45 DAS)	Flowering stage R1 (60 DAS)	Pod stage R3 (75 DAS)
(0 mM)	Control	1.55± 0.05^{b}	2.38± 0.08^{ab}	2.92± 0.12^{c}
	SA3 (10^{-6} M)	2.17± 0.08^{a}	3.12± 0.13^{a}	3.42± 0.20^{a}
	JA1 (0.5 µM)	1.67± 0.06^{b}	2.24± 0.07^{c}	2.58± 0.18^{b}
	EBL2 (10^{-7} M)	2.06± 0.06^{a}	2.98± 0.13^{a}	3.12± 0.16^{ab}
(150 mM)	Control	0.79± 0.02^{cd}	1.65± 0.05^{d}	1.84± 0.07^{e}
	SA3 (10^{-6} M)	0.84± 0.03^{bc}	2.27± 0.10^{bc}	2.42± 0.10^{d}
	JA1 (0.5 µM)	0.89± 0.04^{c}	2.34± 0.08^{b}	3.26± 0.11^{cd}
	EBL2 (10^{-7} M)	0.78± 0.03^{cd}	2.12 ± 0.11^{c}	2.98± 0.09^{d}

Data are means of ± SE of three replicate. Means followed by the same letter for each tested parameter are not significantly different (P< 0.05).

photosynthetic rate, water use efficiency, intercellular CO_2, stomatal conductance and transpiration rate (Fariduddin *et al*, 2003). Maity and Bera (2009) also revealed that foliar application of SA influences different physiological and biochemical aspects of green plant via increasing in assimilation rate which showed increment in chloroplast content and Hill reaction activity in the leaf. Foliar application of BRs has been shown to increase the total chlorophyll content and hence net photosynthetic rate in *Brassica juncea* (Fariduddin *et al*, 2009); rice (Farooq *et al*, 2009); wheat (Sairam *et al*, 2005). Our findings are in conformity of earlier researches (Yu *et al*, 2004), which supported that application of BRs to the non-stressed plants significantly increased the pigment content. JA treatment could recover the salt induced defects on seedling development and photosynthetic activity in several cultivar crops (Javid *et al*, 2011). Similarly, foliar application of JA improved carotenoid synthesis in presence of drought stress in marigold plants (Sedghi *et al*, 2012).

According to Levitt (1980) under salt stress decline in the activity of protein content might be due to the reducing availability of amino acid and deformation of enzyme that are necessary in the synthesis of amino acid and protein. Further in another study, wheat leaves when treated with SA provided a significant protection to the enzyme nitrate reductase thereby keeping the normal level of protein (Singh and Usha, 2003). Chandra *et al* (2007) reported that application of SA increased the total soluble protein in cowpea plants. EI-Tayeb (2005) studied on maize conducted that foliar application of SA under saline condition increases the protein and amino acid content.

Exogenous application of BRs has been found to be enhanced protein content in normal plant as well as those subjected to different kind of stress (Behnamina *et al*, 2009). Application of BRs significantly increased the growth of the plants further, which are associated with enhanced levels of DNA, RNA, soluble proteins and

carbohydrate (Vardhini and Rao *et al*, 1998).

Malondialdehyde (MDA) is the end product of lipid peroxidation which leads to severe damage to various biological macromolecules. In the present study, MDA content was found to be enhanced as plants matured (Fig. 4.4, 4.5, 4.6, 4.17) and under salinity stress, elevated levels of MDA was observed (Figs 4.22). However, foliar spray of PGRs alone or in combination lowered the MDA content considerably both under stressed and non-stressed condition. Our results are in agreement with those of Bor *et al* (2003), who reported that salt stress increase the lipid peroxidation in the leaves of two beet species. In SA treated plants, reduction in MDA content was also observed elsewhere (Alam *et al*, 2013). Application of BRs regulated MDA content may be due to the scavenging of ROS and thus declined the membrane destruction caused due to peroxidation of lipids (Cao *et al*, 2005). Our results co-relate with those of EBL, in which application of EBL mitigated the adverse effect of salt by reducing the MDA content in *Lycopersicon esculentum* (Slathia *et al*, 2012). Zhang *et al* (2007) reported that seed priming with BL improved the seedling growth in *Medicago sativa* when subjected to salt stress by reducing the MDA content. However application of JA is very much dose dependent along with absence or presence of any type of stress factors.

Proline, a potent non-enzymatic antioxidant increased in plants when subjected to salt stress (Fig. 4.23) in the present study. Against salinity stress proline is the one of the most important component of the adaptation of plants (Abbaspour, 2012) and pretreatment with SA also contributed to accumulation of this amino acid under stress possibly through maintaining an enhancement level of ABA in the plants (Ervin, 2005). Present study is in conformity with that of Hayat *et al* (2010), who reported that application of SA enhanced the endogenous level of the proline content in leaves. Priming with SA in chickpea plant under saline condition caused a considerable increasing in proline content (Asadi *et al*, 2013). The elevation in the proline content on exogenous application of EBL may be due to the activation of the genes of proline biosynthetic pathway (Ozdemir *et al*, 2004). Our results are co-relate with an earlier work, who demonstrated that exogenous application of 24-EBL increase the proline content in plants (Vardhini and Rao, 2003). Due to application of BR increase in the pool of proline resulted in increased in tolerance against salt further which manifested in terms of improved growth and photosynthesis.

From the present work, it was evaluated that antioxidant enzyme activities of soybean plant were increased in response to different concentration phytohormone. Plants have the potential to neutralize the ROS generated under oxidative stress by synthesizing the antioxidant activities like SOD, POD and CAT (Seckin *et al*, 2010) as well as some non-enzymatic antioxidant activities ascorbic acid, tocopherols, carotenoids and flavonoids. Experimentally it has been proved that exogenous application of BRs could enhance the expression of antioxidant genes and increase the activities of some enzymes including superoxide dismutase, peroxidase and catalase (Xia *et al*, 2009). In rice grown under salinity higher activity of antioxidant system were observed by (Nunez *et al*, 2003). Our results are in conformity with those of earlier work which showed that exogenous application of BRs modified the antioxidant enzyme activity (Arora *et al*, 2008). Similarly under water deficient enhancement in POD activities in soybean plant due to application of BRs has been reported (Zhang *et al*, 2004). An increase in POX activity in response to the application of BRs has also been reported (Arora *et al*, 2010). Senaratna *et al* (2000) reported that SA confers tolerance to pepper plants and the tolerance was associated with changes in antioxidant system. Catalase found to be a major enzyme in salicylic acid induced stress tolerance, which varies according to the time of its assay and intensity of stress (Chen *et al*, 1993). Present study co-relate with that application of JA which activate the enzymatic and non-enzymatic antioxidant of *Wolffia arrhiza* (Piotrowska *et al*, 2009). These results are in agreement with Majid *et al* (2006), who found that MeJA increased the production of several antioxidant enzymes. Exogenous application of MeJA under drought stress in case of strawberry and maize seedling mitigates the ROS effects (Norastehnia *et al*, 2006).

CONCLUSION

In conclusion, our results showed that although common soybean is a moderately sensitive plant against salinity stress. However foliar spray with different PGRs (SA, 24-EBL, JA) can help to increase the tolerance of this crop by maintaing the growth character and biochemical constituents of soybean plants.

REFERENCES

Abbaspour H (2012) Effect of salt stress on lipid peroxidation, antioxidative enzymes, and proline accumulation in pistachio plants. *J. Med. Plants Res.* **6**, 52-69.

Aebi H (1984) Catalase *in vitro*. *Met. Enzymol.* **105**, 121-126.

Afshari M, Shekari F, Azimkhani R, Habibi H and Fotokian V (2013) Effects of foliar application of salicylic acid on growth and physiological attributes of cowpea under water stress conditions. *Iran Agri. Res*. **32**(1), 56-69.

Alam M M, Hasanuzzaman M, Nahar K and Fujita M (2013)

Exogenous salicylic acid ameliorates short-term drought stress in mustard (*Brassica juncea* L.) seedlings by up-regulating the antioxidant defense and glyoxalase system. *Aust. J. Crop Sci.* **7**, 1053–1063.

Ali B, Hayat S and Ahmad A (2007) 28-Homobrassinolide ameliorates the salt stress in chickpea (*Cicer arietinum*). *Environ .Exp. Bot.* **59**, 33–41.

Amzallag G N and Goloubinoff P (2003) An Hsp90 inhibitor, geldanamycin, as a brassinosteroid antagonist: evidence from salt-exposed roots of *Vigna radiata*. *Plant Biol*. **5**, 143-150.

Anuradha S and Rao S S R (2001) Effect of brassinosteroids on salinity stress induced inhibition of seed germination and seedlings growth of rice (*Oryza sativa* L). *Plant Growth Regul*. **33**, 151-153.

Arnon D I (1949) Copper enzymes in isolated chloroplast: Polyphenoloxidase in *Beta vulgaris*. *J. Plant physiol*. **24**, 1-15.

Arora N, Bhardwaj R, Sharma P and Arora H K (2008) Effects of 28-homobrassinolide on growth, lipid peroxidation and antioxidative enzyme activities in seedlings of *Zea mays* L. under salinity stress. *Acta Physiol*. **11738**, 008-0188.

Arora P, Bhardwaj R and Kanwar M K (2010) 24-Epibrassinoliide regulated diminution of Cr metal toxicity in *Brassica juncea* L. *J. Plant Physiol.* **22**, 159–165.

Asadi M, Heidari M A, Kazemi M A and Filinejad R (2013) Salicylic acid induced changes in some physiological parameters in chickpea (*Cicer arietinum* L.) under salt stress. *J. Agric. Tech.* **9**(2), 311-316.

Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. *Flora* **199**, 361-376.

Ashraf M, Akram N A, Arteca R N and Foolad M R (2010) The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. *Crit. Rev. Plant Sci*. **29**, 162-190.

Bajguz A and Hayat S (2009) Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiol. Biochem.* **47**, 1–8.

Bates L S, Waldren R P and Teare I D (1973) Rapid determination of proline for water stress studies. *Plant Soil.* **39**, 205–207.

Behnamnia M, Kalantari K H M and Rezanejad F (2009) Exogenous application of brassinosteroid alleviates drought-induced oxidative stress in *Lycopersicon esculentum* L. *Gen. Appl. Plant Physiol*. **35**(1–2), 22–34.

Bor M, Ozdemir F and Turkan I (2003) The effect of salt stress on lipid peroxidation and Antioxidants in leaves of sugar beet (*Beta vulgaris* L.) and wild beet (*Beta maritime* L.). *Plant Sci*. **164**, 77-84.

Bradford M M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**(1), 248–254.

Browse J (2009) Jasmonate passes muster: a receptor and targets for the defense hormone. *Annu. Rev. Plant Biol.* **60**, 183–205.

Cao S, Xu Q, Cao Y, Quian K, Zhu Y, Bineng H, Zhao H and Kuai B (2005) Loss of function mutations in DET2 gene lead to an enhanced resistance to oxidative stress in *Arabidopsis*. *Physiol. Plant.* **123**, 57–66.

Chandra A, Anand A and Dubey A (2007) Effect of salicylic acid on morphological and biochemical attributes in cowpea. *J. Environ. Biol.* **28**, 193-196.

Chen Z, Silva H and Klessig R F (1993) Active oxygen species in the induction of plant systemic acquired resistance by SA. *Science* **262**, 1883–188.

Creelman R A and Mulpuri R (2002) The oxylipin pathway in *Arabidopsis*. In : CR Somerville E M Meyerozitz (eds). The Arabidopsis Book American Society of Plant Biologists, Rockville, MD, 1–24. *Current Sci*. 82, 1239-1245.

Dhaliwal H S, Malik R K, Gosal S S and Dhaliwal L S (1997) Studies on hardening of the propagated sugarcane (*Saccharum officinarum* L.) plant parameters and biochemical estimations. *Ann. Biol.* **13**, 15-20.

El-Tayeb M A (2005) Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul*. **45**, 215-224.

Ervin D (2005) Evaluation of Payments – Mid-term Evaluation of Rural Development Plans,' in Evaluating Agri-Environmental Policies: Design, Practice and Results, OECD, 101-102.

Fariduddin Q, Hayat S and Ahmad A (2003) Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. *Photosynthetica* **41**, 281–284.

Fariduddin Q, Khanam S, Hasan S A, Ali B, Hayat S and Ahmad A (2009) Effect of 28-homobrassinolide on the drought stress-induced changes in photosynthesis and antioxidant system of *Brassica juncea* L. *Acta Physiol. Plant* **31**, 889–897.

Farooq M, Basra S M, Wahid A and Rehman H (2009) Exogenously applied nitric oxide enhances the drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). *J. Agron Crop Sci.* **195**, 254-261.

Grove M D, Spencer G F, Rohwedder W K, Mandava N, Worley J F and Warthen J D, Steffens G L, Flippenanderson J L and Cook J C (1979) Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. *Nature* **281**, 216–217.

Hayat Q, Hayat S, Irfan M and Ahmad A (2010) Effect of exogenous salicylic acid under changing environment: A review. *Environ. Exp. Bot.* **68**, 14–25.

Hayat S, Ali B, Hasan S A and Ahmad A (2007) Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. *Environ. Exp. Bot.* **60**, 33–41.

Heath R L and Packer L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **125**, 189–198.

Holden M (1965) *Chemistry and biochemistry of plant pigments*. Goodwin T W (ed.). Academic Press, New York: 462-468.

Jamil A, Riaz S, Ashraf M and Foolad M R (2011) Gene expression profiling of plants under salt stress. *Crit. Rev. Plant Sci.* **30**(5), 435–458.

Javid M G, Sorooshzadeh A, Moradi F, Modarres Sanavy S A M and Allahdadi I (2011) The role of phytohormones in alleviating salt stress in crop plants. *Aust. J. Crop Sci.* **5**, 726–734.

Kaya C, Higgs D and Kirnak H (2001) Effects of supplementary phosphorus and potassium on physiological development and mineral nutrition of cucumber and pepper cultivars grown at high salinity (NaCl). *J. Plant Nutr*. **24**, 1457-1471.

Levitt J (1980) *Responses of plants to environmental stresses*. vol. **2**, Academic Press, New York.

Maehly A C (1954) Determination of peroxidase activity. In: *Methods*

of Biochemical Analysis. Glick D (ed.). Inter Science Publisher Inc; New York **1**, 385-386.

Maity U and Bera A K (2009) Effect of exogenous application of brassinolide and salicylic acid on certain physiological and biochemical aspects of green gram (*Vigna radiate* L. Wilczek). *Indian J. Agric. Res.* **43**, 194-199.

Majid N A and Akbar N (2006) A Possible Role for Methyl Jasmonate in Affecting Superoxide Dismutase and Catalase Activities under PQ-Induced Oxidative Stress in Maize Seedlings. *J. Biol. Sci.* **6**, 55-60.

Montoya T (2005) Patterns of Dwarf expression and brassinosteroid accumulation in tomato reveal the importance of brassinosteroid synthesis during fruit development. *Plant J.* **42**, 262–269.

Natr L and Lawlor D W (2005) Photosynthetic plant productivity. In: *Photosynthesis Handbook*. (2nd Ed) M. Pessarakli. C.R.C. Press New York, USA pp 501-524.

Norastehnia A, Sajedi R H and Nojavan-Asghari M (2006) Inhibitory effects of methyl jasmonate on seed germination in maize (*Zea mays*): effect on á-amylase activity and ethylene production. *Gen. Appl. Plant Physiol.* **33**, 13–23.

Nunez M, Mazzafera P, Mazorra L M, Siqueira W J and Zullo M A T (2003) Influence of brassinosteroid analogue on antioxidant enzymes in rice grown in culture medium with NaCl. *Biol. Plant* **47**, 67–70.

Ozdemir F, Bor M, Demiral T and Turkan I (2004) Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. *Plant Growth Regul*. **42**, 203-211.

Piotrowska A, Bajguz A, Godlewska-y³kiewicz B, Czerpak R and Kamin'ska M (2009) Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (Lemnaceae). *Environ. Exp. Bot.* **66**, 507–513.

Seckin B, Turkan I, Sekmen A H and Ozfidan C (2010) The role of antioxidant defense systems at differential salt tolerance of *Hordeum marinum* Huds. (sea barley grass) and *Hordeum vulgare* L. (cultivated barley). *Environ. Exp. Bot.* **69**, 76–85.

Sedghi M, Nemati A and Esmaielpour B (2012) Effect of seed priming on germination and seedling growth of two medicinal plants under salinity. *Emir J. Food Agric*. **22**(2), 130-139.

Senaratna T, Touchell D, Bunn E and Dixon K (2000) Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regulation.* **30**, 157-161.

Singh B and Usha K (2003) Salicylic acid induced physiological and biochemical changes in wheat.

Singh B and Usha K (2003) Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul.* **39**, 137–141.

Slathia S, Sharma A and Choudhary S P (2012) Influence of exogenously applied epibrassinolide and puterscine on protein content, antioxidant enzymes and lipid peroxidation in *Lycppersicon esculentum* under salinity stress. *Ann. J. Plant Sci.* **3**, 714-720.

Vardhini B V and Rao S S R (1998) Effect of brassinosteroids on growth, metabolite content and yield of *Arachis hypogaea*. *Phytochemistry* **48**, 927–930.

Vardhini B V and Rao S S R (2003) Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. *Plant Growth Regul.* **41**, 25–31.

Vinocur B and Altman A (2005) Recent advances in engineering plant tolerance to aboitic stress: achievements and limitations. *Curr. Opin. Biotechnol*. **16**, 123-132.

Wasternack C and Hause B (2002) Jasmonates and octadecanoids: signals in plant stress responses and plant development. *Progress in Nucleic Acid Research and Molecular Biology* **72**, 165–221.

Xia X J, Huang L F, Zhou Y H, Mao W H, Shi K, Wu J X, Asamim T, Chen Z X and Yu J Q (2009) Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus*. *Planta* **230**, 118–119.

Yasar F, Kusvuran S and Ellialtýolu S (2006) Determination of anti-oxidant activities in some melon (*Cucumis melo* L.) varieties and cultivars under salt stress. *J. Hortic. Sci. Biotechnol.* **81**(4), 627-630.

Yu S W and Tanag Z C (1998) *Plants Physiology and Molecular Biology*, Beijing: Science Press.

Yu J Q, Huang L F, Hu W H, Zhou Y H, Mao W H, Ye S F and Nogues S (2004) A role of brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. *J. Exp. Bot.* **55**, 1135-1143.

Zhang S, Hu J, Zhang Y, Xie X J and Knapp A (2004) Seed priming with brassinolide improves lucerne (*Medicago sativa* L.) seed germination and seedling growth in relation to physiological changes under salinity stress. *Aust. J. Agric. Res.* **58**, 811–815.

Zhou N, Tootle T L, Tsui F, Klessig D F and Glazebrook J (1998) PAD4 functions upstream from salicylic acid to control defense responses in *Arabidopsis*. *Plant Cell* **10**, 1021-1030.

Zhu J K (2001) Plant Salt Tolerance. *Trends Plant Sci.* **2**(6), 66–71.