Effect of Salicylic acid, 24-Epibrassinolide and Jasmonic acid in modulating morpho-physiological and biochemical constituents in *Glycine max L. merill* under salt stress

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ABSTRACT *Salinity is an environmental constrain having negative impact on plant growth and productivity. Foliar application of phytohormones has higher efficiency in alleviating the adverse effect of salt stress. Therefore, Present investigation was carried out to elucidate the mechanism of salinity tolerance by exogenous application of salicylic acid, 24-epibrassinolide, jasmonic acid on Soybean (Pusa-9712) under 0 and 150 mM concentration of NaCl. Chlorophyll and Carotenoids and total soluble proteins were estimated. The level of lipid peroxidation was measured by estimating themalondialdehyde (MDA) present.Proline content was measured by the method of Bates et al. (1973). The plants exposed to salt exhibited a significant decline in growth parameters, photosynthetic pigments, protein content while MDA and proline contents increased considerably. Application of different PGRs increased the plant growth by reducing the adverse effects of salinity. JA is the most efficient to alleviate the adverse effect of salt stress at extremely low concentration.*

Keywords: *Soybean, Salicylic acid, Jasmonic acid, 24-Epibrassinolide, Proline, Lipid peroxidation, Salt stress*

Introduction

Salinity and sodicity constraints have been resulting in low crop yields are and poor overall environmental services, which affects the livelihood of people dependent on soil and water resources of marginal lands (Qadir et al. 2007). Kaya *et al*. (2003) estimated that about one-third of world's cultivated land is affected by salinity. Hence, soybean (*Glycine max* L.) though, one of the most important commercialand nutritious crops, as it containshigh protein and oil content (Yaklich*et al.*, 2002) is no exception to this. Among different cultivars of soybean a wide genetic variability exists (Shereen and Ansari, 2001). Nevertheless, under any type of environmental stresses such as soil salinity soybean production may be limited (Ghassemi-Golezani*et al*., 2009). Salinity limits the productivity of agricultural crops, with adverse effects on germination, plant vigour and crop yield (Munns and Tester 2008).Parida and Das (2005) reported that salt stress affect plant survival, biomass, plant height, plant morphology and affect the capacity of a plant to collect water and nutrients

Salinity can cause hyperionic or hyperosmotic effects on plants leading to membranedisorganization, increase in reactive oxygen species(ROS) levels and metabolic toxicity (Jaleel*et al*., 2007). The greatest effect of salinity is the inhibitionof crop growth by the reduced hormone deliveryfrom root to leaves (Jaleel*et al*., 2007).Salt stress is also known to affect several physiological processes includingphotosynthesis. For example, In *Brassica spp*. (Nazir*et al*., 2001) and wheat (Raza*et al*., 2006) reported a significant reduction in photosynthesis. Sabir*et al*.(2009) reported that reduction in photosynthesis depends on decrease in chlorophyll contents,leaf area, net photosynthetic rate, stomatal conductance andsubstomatal CO_2concentrations. Reduction in net assimilation may originate due to decreased efficiency of ribulose-1, 5biphosphate (RuBP) carboxylase. To protect the cell against the injurious effects of salt stress compatible osmoprotectant metabolites like proline, glycine-betaine and soluble sugars are produced in plants (Chelli-Chaabouni*et al*., 2010).

Variousstrategieshave also been developed for reducing deleterious effects of salinity. One of these strategies is to employ different types of phytohormonesin reducing the severity of salinity on crops (Houimli*et al*., 2008).Besides, conventional phytohormones, a new class of plant hormones viz. salicylic acid (SA), jasmonic acid (JA) and epibrassinolide (Ebl) are recognized as the potent phytohormones and stress alleviators against various biotic and abiotic stresses.The mitigating effects of salicylic acid have been documented in inducing salt tolerance in many crops (El- Tayeb, 2005; Gunes*et al*., 2005).SA enhances leaf area and dry matter production in lemongrass (Ghaderi et al., 2015), Soybean (Kuchlan et al., 2017). Application of SA at physiological concentration exerted a significant effect on plant growth and metabolism and thus acted as one of the plant growth regulating substances (Kalarani*et al*., 2002).In biotic and abiotic

stresses, the elevating effect of SA on cell membrane stability can also be related to the activity of antioxidant enzymes (Pawlowski et al. 2016)

Brassinosteroids, a recent classof plant hormone not only play an eminent role in various physiological and biochemical processes in plantsbut has also attracted increasing attention in studies addressing to accommodative response to environmental stresses, such as heavy metal (Ali *et al*., 2008), salt (Ali *et al*., 2007), temperature (Wilen*et al*., 1995), drought (Zhang *et al*., 2008).Endogenous growth substances like jasmonic acid and its methyl ester are also considered as a new class of hormones which is identified in many plant species. They affect a wide variety of physiological and developmental responses (Parthier*et al.*, 1992). Jasmonates antagonistically regulate the expression of salt stress inducible stress inducible proteins, associated with salt stress in rice (Moons *et al.*, 1997). Strategies to ensure food security for increasing population emphasize on mitigating thedeleteriouseffects of salinity on crop plants. A number of studies reported role of various PGRs in reducing the effects of different stresses on plants. Still little is known about the physiological significance of SA, JA, EBL andbrassinosteroids on soybean. Accordingly, the study tested the hypothesis: (i) foliar application of various phytohormones help the soybean plant in developing salt tolerance, and (ii)phytohormoneseffect the morpho-physiological characteristics in soybean plant.

Material and Methods

Plant material and growth conditions

Seeds of Soybean variety (Pusa- 9712) were obtained from CCS Haryana Agriculture University, Hissar. Seeds were surface sterilized. The experiment was set up in the experimental cage of Department of Botany, Kurukshetra University, Kurukshetra. Five seeds per pot were 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

The seedlings were thinned to two plants per pot after three weeks and each treatment consisted of three replications in a complete randomized design (CRD). After establishment of seedling 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 elucidate the effect of exogenous application of these hormones on plants. The plants were sampled at 75thDAS to assess various morpho-physological and biochemical parameters.

Morphological parameters

Ten plants at random were selected from each treatment. Shoot and root length was measured from the top of the plant to the base of the root using meter scale and expressed in centimeters. Number of leaves and branching were counted manually on each branch of the plant and expressed as number of leaves and branching per plant. Plants under each treatment were taken out carefully from the bags after washing away the sand adhering to roots with tap water and then were separated into their component i.e. leaves, stem, root. Then all the separated plant parts are blotted with filter paper in order to remove moisture from their surface. Then their fresh weight was determined with the help of an electronic balance. All the plant parts were dried in labeled paper envelopes in an oven at 60º C till a constant weight was attained. Their dry weight was measured with the help of an electronic balance.

Biochemical constituents

Estimation of chlorophylls and carotenoids

Chlorophyll (Chl) content was estimated with the formula of Arnon (1949) and Carotenoid level was calculated by the method of Holden (1965). Leaf sample (200 mg) was crushed in chilled 80% acetone (AR grade) with 20 mg of $CaCO_3$ and centrifuged at 3000 g for 5 min. 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 (Systronics, Double beam spectrophotometer 2203).

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 in 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.

Measurement of Lipid peroxidation

In leaf sample, the level of lipid peroxidation was measured by estimating themalondialdehyde (MDA) present (Heath and Packer, 1968). Now, leaf samples (0.2 g) were homogenized in 3 mL of 5 0 mM phosphate buffer (pH 7.0). The homogenate was centrifuged for 15 minutes at 15000 rpm. To 1.0 mL aliquot of the supernatant, 2.0 mL of 0.5 % thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) was added. The mixture was heated at 95°C for 30 min In a water bath and then cooled in an ice bath. The absorbance of the supernatant was recorded at 532 nm after centrifugation at 10000 rpm for 10 min. The value of each sample for nonspecific absorption was recorded at 600 nm and then subtracted from the absorbance recorded at 532 nm.

Proline content

Proline content was measured by the method of Bates *et al*. (1973). 200 mg of leaf sample was homogenized in 2 ml of 3% sulfosalicylic acid. The homogenate was centrifuged for 15 min at 10, 000 rpm in a Remi centrifuge (R-8C). Free proline in the supernatant was treated with 2 ml of 3% glacial acetic acid (GAA) and 2 ml of acid ninhydrin. Reaction mixture in the test tubes was kept in boiling water bath for 1 hour at 95º C. Reaction was terminated in the ice bath and color complex was extracted in 4 ml of pure toluene. The absorbance was recorded at 520 nm in a UV- Vis spectrophotometer.

Statistical analysis

A mean of three readings was taken in every replication. In biochemical estimation, three aliquots were used for each replication. Statistical analysis was done using Statistical Packages for Social Sciences (SPSS) version 16.0. One-way ANOVA was used to test whether there was a significant difference in various estimations.

Results

Morphological parameter

Morphological parameters viz. shoot and root length, fresh and dry weight of whole plant, no. of branching/plant, and number of leaves/plant were recorded. Salt stress caused a significant reduction in almost all the studied growth character of *G. max* (Tables 1, 2). Shoot and root lengthwere found to be declined by about 16.8%&25.9% as compared to control of non-stressed plant at 75th DAS. However, plants sprayed with SA, JA and EBL mitigated the inhibitory effect of salt stress on the shoot and root length. The obtained data suggested that foliar application of three PGRs significantly enhanced shoot and root length in salt stressed as well as non-stressed soybean plants as compared to those of untreated ones. From the data it was revealed that under stressed condition, JA (0.5 µM) recorded highest shoot length followed by SA (10^{-6}M) and EBL (10^{-7}M) and it was 34.5%, 27.7% and 20.5 % respectively whereas root length was found to be increased by about 49.1%, 44.5%, 23.5 % respectively.

Fresh and dry weight of whole plant decreased by about 15.4 & 16.9 % respectively as compared to control of non-stressed plant at 75thDAS. However, application of (JA, EBL, and SA) increased fresh and dry weight of whole plant but this increase was lower as compared to control of non-stressed plants. Under stressed condition, JA (0.5 µM) recorded highest fresh weight followed by SA (10^{-6}M), EBL (10^{-7}M) and it was 52.9, 47.1 and 23.1% where as dry weight was found to be increased by 60.3, 51.9, 37.6 % over control respectively.

The no. of branching and no. of leaves per plant decreased significantly by 27.9 &27.6% respectively as compared to control of non-stressed plant. However, foliar application of (JA, EBL, and SA) increased number of branching and no. of leaves per plant but this increase was lower as compared to control of non-stressed plants. Among these groups, the highest number of branching and leaves per plant was recorded in JA (0.5 µM) followed by SA ((10^{-6}M) and EBL (10^{-7}M) and it was by 42.5, 39.7, 13.6 respectivelywhere as no. of leaves was found to be increased by 30.1, 22.0, 16.5 % over control respectively.

Biochemical constituents

Photosynthetic pigments-

Salinity stress significantly decreased the photosynthetic pigments (Chl. a, Chl. b, Total Chl. and carotenoids) by 30.3 %, 30.6 %, 30.3 %, and 34.9 % respectively in comparison to non-treated plants at 75th DAS after sowing. However, foliar application with different PGRs (SA, 24-EBL, JA) maintained the chlorophyll content under both stressed and non-stressed condition. Among the groups treated with PGRs under salt-stressed condition, JA exhibited highest increment in (chl.a, Chl. b, Total Chl. and carotenoids) followed by SA and EBL but it does not exceed the control. Under salt stressed, foliar spray of JA (0.5 µM), SA (10^{-6} M), EBL (10^{-7} M)

increased the Chl. a by 44.0, 39.3, 29.5 per percent however foliar spray of JA1, SA3, EBL2 increased the Chl. bby 43.3, 38.9, 28.3percent over control. Total chlorophyll content was found to increase by about 43.8, 39.1, 28.8 % and carotenoids by about 42.3, 34.4, 30.1 % at JA (0.5 µM), SA (10^{-6} M) and EBL (10^{-7} M) respectively. Among the PGRs used, JA showed better results in the improvement of photosynthetic pigments in soybean under salt stress condition.

Protein content

Salt stress caused a significant reduction in leaf protein content as compared to control of non- stressed plant (Fig1). On the other hand, foliar application with different PGRs (SA, JA and EBL) retained the protein content not only in salt stressed but also under optimum condition. Among the groups treated with PGRs under salt-stressed condition, JA (0.5 µM)exhibited highest percent increment in leaf protein content followed by SA (10^{-6} M) and EBL (10^{-7} M) and it was 36.0, 25.9 and 18.7% over control respectively on 75th DAS. However, under non- stressed condition plants treated with SA (10^{-6} M) exhibited highest leaf protein followed by EBL (10^{-7} M) and JA (0.5 µM) and it was 21.4, 19.0 and 7.1% over control respectively. Among the PGRs used in this study, SA showed better results in the improvement in total protein content of soybean under non-stressed condition whereas under salt stressed condition JA found to be more effective as compared to SA, EBL.

Lipid peroxidation (MDA content)

Our data revealed that as plants matured, they 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). Under stressed condition, level of lipid peroxidation increased by about 18.6 % as compared to control of non-stressed plant. Among the groups treated with PGRs under salt-stressed condition, JA (0.5 µM) exhibited highest percent declined in lipid peroxidation followed by SA (10^{-6} M) and EBL (10^{-7}M) and it was 22.0, 13.9 and 11.6 per cent over control respectively on 75th DAS. However, under non-stressed condition plant treated with SA (10^{-6} M) exhibited highest declined in lipid peroxidation followed by EBL (10^{-7} M) and JA (0.5 µM) and it was 28.2, 19.3 and 7.58 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 stress dramatically induced the accumulation of proline in the leaves of soybean plant. It leads to a significant increase in the proline content (32.5 %) over control in non-stressed plant at 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, JA (0.5 µM) exhibited highest increased in proline content followed by SA (10^{-6} M) and EBL (10^{-7} M) and it was 92.4, 118.6 and 69.7 per cent over control respectively on 75th DAS. However, under non-stressed condition plants treated with SA (10^{-6} M) exhibited highest proline content followed by EBL (10^{-7} M) and JA (0.5 µM)and it was 99.4, 80.3 and 71.7 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.

Discussion

Plant hormones regulate plant growth and development by affecting a wide range of cellular, developmental and physiological response. PGRs are chemical messengers produced in one part of plant and translocated to the other parts, where they play critical roles in regulating plant responses to any type of stress at extremely low concentration. Salicylic acid (SA), jasmonic acid (JA) and brassinosteroids (BRs) are growth regulators which participate in regulation of many physiological processes.Our results exhibited that foliar spray of SA exerted stimulatory effects on vegetative growth parameters of soybean plant compared with control at all the sampling stages. Application of SA led to increase in the plant height, root length, number of branching and leaves per plant along with increase in the dry weight also. Regarding foliar application of SA, our results are similar to those described by Salarizdah*et al.* (2012) on canola, Dawood*et al.* (2012) on sunflower. In the present study, foliar application of SA improved plant height in soybean cultivar. These results are consistent with those of Pakar*et al*. (2014) who demonstrated that positive effect on plant height was related to foliar applied SA.

Growth parameters were found to enhance especially at 10^{-7} M of 24-EBL as compared to control under both saline and non-saline condition in tested crop. Brassinosteriods when applied exogenously have unique growth promoting activity (Mandava*et al*., 1981). In the present study foliar spray of 24-EBL on soybean plant stimulated the positive response in growth parameters (shoot length, root length, fresh weight and dry weight). Vardhini and Rao (1999) analyzed induction of growth responses by BRs to changes in macromolecules such as nucleic acid and protein.

Foliar application of plant growth regulators (SA, 24-EBL, JA) resulted in significant increasein chlorophyll content in soybean plant. The stimulatory effect of SA on photosynthetic pigment in our study is in agreement with those obtained by Barakat and Nassar (2011) on wheat and Saeidnjad*et al*. (2012) on maize. In another study on *Brassica juncea*, Fariduddin*et al*. (2011) also reported that foliar spraying of SA enhanced the net photosynthetic rate, intracellular Co_2, water use efficiency, stomata conductance along with transpiration rate. Similar to our findings exogenous application of SA increased the cholorophyll content of soybean leaves as reported by (Khan *et al*., 2003). Under salinity stress, application of JA enhanced chlorophyll contents and in turn rate of photosynthesis as reported in soybean (Soad, 2007) and *Brassica napus* (Kaur*et al*., 2013). It has been suggested that treatment of JA increases active cytokinin concentration which increase the chlorophyll accumulation as reported in potato plants (Kovac and Ravnikar, 1994). BRs under salt stress lead to the accumulation of chlorophyll (Anuradha and Rao, 2003; Ali *et al*., 2007). It was further demonstrated that exogenous application of BRs removed the inhibitory effect of salt stress on photosynthetic pigments.

Exogenous application of SA maintained the protein content not only under salt stress condition but also under optimum conditions. Our observation are similar to those of Kumar *et al*. (1999) who reported that total soluble protein content was found to be enhanced in soybean plants when sprayed with SA and this increase might be due to enhanced activity of nitrate reductasedue to SA application.BRs regulate plant growth and development through promoting cell elongation and division (Fridman and Savaldi-Goldstein, 2013). Treatment with BRs also increased the protein content under saline as well as non-saline soybean. Enhancement of protein content provoked the transcription and translation processes of specific stress tolerance genes by BRs (Kagale*et al*., 2007). In rice seedling, exogenous application of BRs overcame the salinity stress by enhancing the synthesis of proteins (Anuradha and Rao, 2001). In another report it was observed that application of JA, significantly enhanced protein content in *Cajanuscajan* both in presence and absence of oxidative stress, (Sharma *et al*., 2013). Similar results were found in JA treated plants such as soybean (Anderson, 1981), rice (Rakwal and Komastu, 2001) and peanut (Kumari*et al*., 2006).

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 enhancewith the maturity of plants,and under salinity stress(Fig.2). However foliar spray of PGRs 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. However, in salt treated seedlings exogenous application of SA declines the MDA content. In SA treated plants, similar reduction in MDA content was also observed elsewhere (Hayat *et al*., 2010; Alam*et al*., 2013).Similarly, 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 *Lycopersiconesculentum* (Slathia*et al*., 2012). Bandurska*et al*. (2003) reported that application of JA protects membrane from damage by various stress factors by declining the MDA content.

Proline concentration was found to increase in plants under salt stressand helps in membrane stabilization and protein synthesis.It also traps free radicals,acts as cytoplasmic osmoticum and potent non-enzymatic antioxidant. Against salinity stress,proline is the one of the most important component which develops adaptation in plants (Abbaspour, 2012).Pre-treatment of plants with SA also contributed in accumulation of this amino acid under stress possibly through maintaining an enhancement level of ABA in the plants (Ervin, 2005). Rohwar*et al*. (2008) reported that proline content was highest in drought stressed shoot tips supplemented to MeJA. Similarly, Anjum*et al*. (2011) asserted that application of MeJA in drought stressed soybean plant enhanced the proline content and also helped to maintain relative water content with respective to control plant. Application of BR increasedprolinecontent which in turn results in increased tolerance against salt manifested in terms of improved growth and photosynthesis. Hence enhanced proline content is a common metabolic response in higher plants against salinity stress.

Conclusion

The resultof this study proves both hypotheses and confirms that used PGRs have significant effectin developing tolerance against salinity stress.Foliar spray with different PGRs (SA, 24-EBL, JA) can help to increase the tolerance of this crop by maintaining the growth character and biochemical constituents of soybean plants.

Application of JA protects membrane from damage by various stress factors by declining the MDA content.

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Table 1

Influence of SA(10^{-6}M), EBL(10^{-7} M) and JA (0.5 µM) on the changes in Shoot length(cm), root length (cm), no. of branching/plant ± S.E. in *Glycine max* L. at 75 DAS under salt stress condition

Salinity level	Treatments	Shoot length (cm)	Root length (cm)	Number of branching/plant
(0 mM)	Control	49.54 ± 1.72^{b}	15.72 ± 0.54^{c}	8.52 ± 0.34^{bc}
	SA (10^{-6} M)	59.26 ± 2.40^{a}	21.60 ± 0.87^{a}	11.47 ± 0.46^{a}
	JA (0.5 µM)	54.47 ± 2.52^{ab}	16.84± 0.58^{bc}	9.24 ± 0.31^{b}
	EBL (10^{-7}M)	56.74 ± 2.30^{ab}	19.43 ± 0.79^{b}	10.74 ± 0.43^{ab}
(150 mM)	Control	41.20 ± 1.43^{c}	11.64 ± 0.40^{d}	6.14 ± 0.20^{e}
	SA (10^{-6} M)	52.60 ± 2.44^{ab}	16.82± 0.68^{c}	7.58± 0.30^{cd}
	JA (0.5 µM)	57.40 ± 2.33^{a}	17.36 ± 0.70^{bc}	7.75 ± 0.31^{cd}
	EBL (10^{-7}M)	49.72 ± 2.02^{b}	15.76± 0.54^{c}	6.98 ± 0.27^{de}

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 no. of leaves/plant, fresh weight (cm), dry weight (cm) ± S.E. in *Glycine max* L. at 75 DAS under salt stress condition

Salinity Level	Treatments	No. of leaves Per plant	Fresh weight (gms)	Dry weight (gms)
(0 mM)	Control	59.72 ± 2.41^{cd}	9.32 ± 0.37^{c}	1.78 ± 0.05^{d}
	SA (10^{-6} M)	70.43 ± 2.84^{a}	12.95 ± 0.52^{a}	2.65 ± 0.08^{a}
	JA (0.5 µM)	62.20 ± 2.51^{bc}	10.67± 0.43^{bc}	2.13 ± 0.08^{c}
	EBL (10^{-7}M)	67.76 ± 3.13^{ab}	11.92 ± 0.28^{ab}	2.38 ± 0.10^{bc}
(150 mM)	Control	43.18 ± 1.49^{f}	7.74 ± 0.26^{d}	1.54 ± 0.05^{d}
	SA (10^{-6} M)	52.72 ± 2.13^{de}	11.39 ± 0.45^{b}	2.34 ± 0.09^{bc}
	JA (0.5 µM)	56.18 ± 2.27^{cde}	11.84 ± 0.61^{ab}	2.47 ± 0.08^{ab}
	EBL (10^{-7}M)	50.32 ± 2.03^{ef}	9.54 ± 0.44^{c}	2.12 ± 0.08^{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 3

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

Salinity level	**Treatments**	**Chl. a**	**Chl. b**	**Total chl.**	**Carotenoids**
(0 mM)	Control	3.36 ± 0.11^{b}	1.62± 0.05^{ab}	4.98± 0.16^{b}	3.52± 0.12^{c}
	SA (10^{-6} M)	4.38± 0.15^{a}	2.14± 0.08^{a}	6.52± 0.26^{a}	4.42± 0.20^{a}
	JA (0.5 µM)	3.24± 0.11^{c}	1.56± 0.06^{bc}	4.80± 0.16^{b}	3.94± 0.18^{b}

	EBL (10^{-7} M)	4.12± 0.18^{b}	1.98± 0.08^{a}	6.10± 0.24^{a}	4.24± 0.16^{ab}
	Control	2.34 ± 0.08^{d}	1.13± 0.02^{d}	3.47± 0.11^{c}	2.29± 0.07^{e}
	SA (10^{-6} M)	3.26± 0.12^{bc}	1.57± 0.05^{bc}	4.83± 0.19^{b}	3.08± 0.10^{d}
(150 mM)	JA (0.5 µM)	3.37± 0.15^{ab}	1.62± 0.06^{ab}	4.99± 0.19^{ab}	3.26± 0.11^{cd}
	EBL (10^{-7} M)	3.02 ± 0.10^{c}	1.45 ± 0.06^{c}	4.47± 0.18^{b}	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)

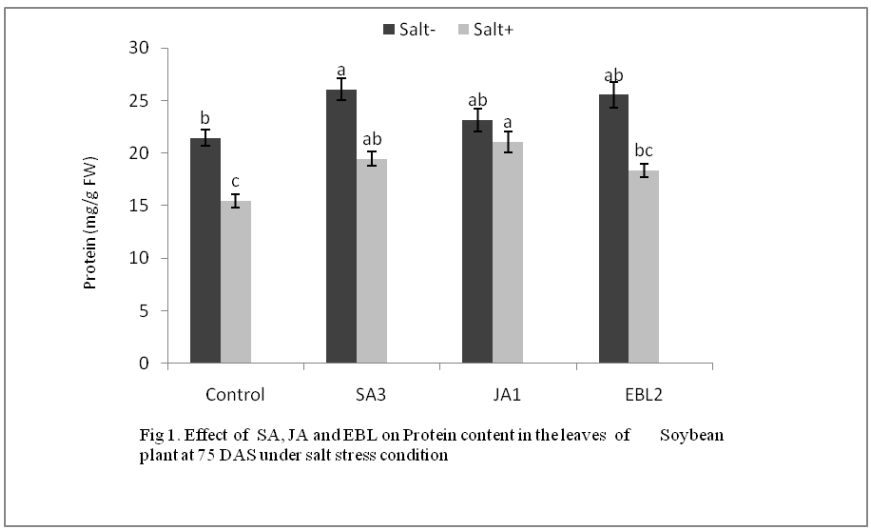

Fig 1. Effect of SA, JA and EBL on Protein content in the leaves of Soybean plant at 75 DAS under salt stress condition

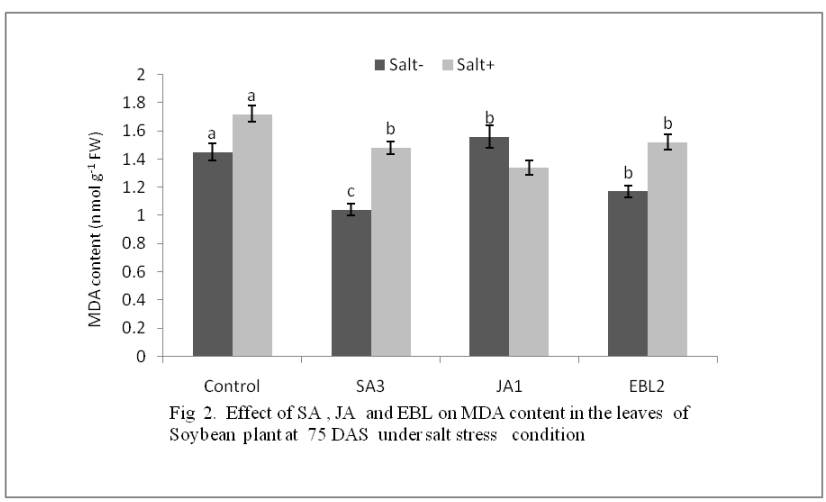

Fig 2. Effect of SA , JA and EBL on MDA content in the leaves of Soybean plant at 75 DAS under salt stress condition

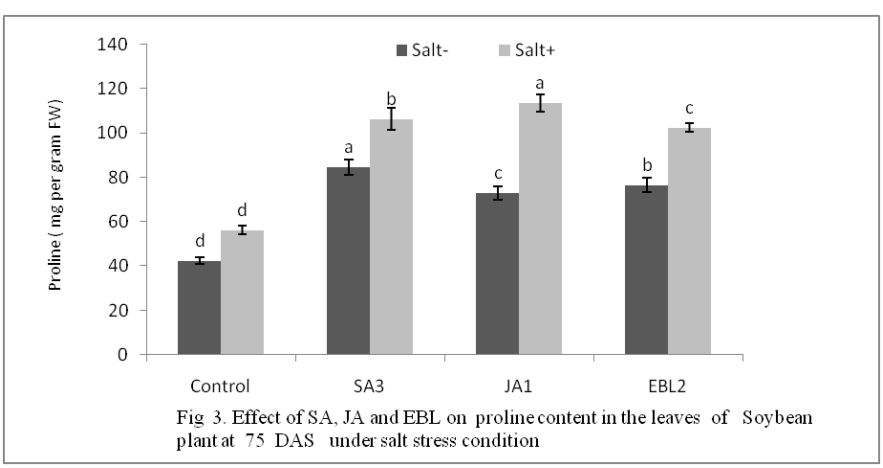

Fig 3. Effect of SA, JA and EBL on proline content in the leaves of Soybean plant at 75 DAS under salt stress condition