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ORIGINAL ARTICLE

Salinity induced damage overwhelmed by the treatment of brassinosteroids in *Zea mays* seedlings

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ABSTRACT
The present study was carried out to investigate the stress extenuation role of 24-epibrassinolide (EBL) by analyzing the growth parameters (root/ shoot length, fresh and dry weight), photosynthetic pigments (total chlorophyll, carotenoid, anthocyanin and xanthophyll), malondialdehyde content (MDA), confocal studies, protein content, activity of antioxidative enzymes: superoxide dismutase (SOD), ascorbate peroxidase (APOX), glutathione reductase (GR) and total sugars (quantitatively and qualitatively) in ten days old seedlings of Zea mays L. var. DKC 9106 subjected to different salt concentrations (0,40,60,80,100mM). Growth parameters and pigments were found to be decreased under salt stress whereas MDA content, protein content, activities of antioxidative enzymes and sugar content were enhanced. Further application of EBL decreased the MDA content and improved the growth, protein content and activities of antioxidative enzymes, pigment level and total sugars content in salt stressed seedlings which reveals that application of BRs overcome the toxic effects of salt stress.
Keywords: *salt stress, brassinosteroids, maize, oxidative damage, antioxidative enzymes, photosynthetic pigments, confocal studies*

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Abbreviations:
BRs Brassinosteroids
EBL 24-Epibrassinolide
MDA Malondialdehyde content
SOD Superoxide dismutase
GR Glutathione reductase
APOX Ascorbate peroxidase
ROS Reactive Oxygen Species
ANOVA Analysis of Variances

INTRODUCTION

Plants are often exposed to various types of abiotic and biotic stresses during their life cycle. These stresses affected the survival, yield and biomass production of food crops which poses major threat to the food security [1]. Among numerous stresses, salinity stress is the most damaging stress and causes worldwide reduction of agriculture yield and production. It has been estimated that 6% of the world's land and 30% of the world's irrigated areas are already facing the salinity problems [2]. Salinity occurs through natural or human induced processes that result in the accumulation of dissolved salts which inhibit the plant growth [3]. Natural salt induced processes include weathering of mineral rocks, inadequate rainfall and drainage, climatic change, evapotranspiration process and intrusion of saline water into rivers [4], whereas human salinity induced processes involve the poor quality of water used for irrigation, chemicals released from industries accumulated in the soil, overgrazing and deforestation [5] etc. The salt whether comes from any process, poses adverse impact on plant life by interfering with

their normal functioning. It enters through the root system to the plant and affects plant growth and development processes such as seed germination, growth, flowering and fruit set [6]. In addition to this, major processes such as photosynthesis, nutrition, protein synthesis, nucleic acid synthesis and lipid metabolism are also affected by salt stress in plants [7]. Excessive Na^+ generated due to salinity stress interfere with other ions and restricts the uptake of essential nutrients like K^+, Mg^{2+}, Ca^{2+} and N which leads to nutritional deficiencies [8]. Other consequence of salt stress includes the overproduction of reactive oxygen species (ROS) which leads to oxidative stress. The most common ROS are singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet-}$) and hydroxyl radicals ($^{\bullet}OH$) [9]. During normal metabolism, plants constantly produced ROS essentially from photosynthesis, photorespiration and respiration phenomenon, whereas under severity of stress, production of ROS exceeds from the limits and causes damage to biological molecules such as lipids, proteins, carbohydrate and DNA [10]. Lipids are the important constituent of membranes. Free radicals generated due to overproduction of ROS steals electron from the lipids and causes oxidative degradation of lipids which diminished the cellular functions and membrane stability [11]. In order to overcome the salinity induced damage, plants adopted several morphological, physiological and biochemical changes which helps the plants to flourish under mild saline condition [12], whereas under severity of stress, plants fail to maintain the favorable conditions and in most cases leads to death of the plant. Development of salinity tolerance through the exogenous application of different types of chemicals like plant growth regulators seems to be effective strategy in increasing the growth and yield of many crops grown under saline conditions [13, 14].

Of various plant growth regulators, BRs are first natural polyhydroxysteroidal plant hormones having significant growth promoting activity [15]. BRs are considered as hormones with multiple effects, as they influence various types of processes like growth, germination, flowering, leaf senescence, rhizogenesis, photosynthetic activities, protein synthesis, nucleic acid synthesis, changes in activities of antioxidative enzymes, reproductive and vascular development, membrane polarization, proton pumping and photo and skotomorphogenesis processs etc [16-18]. Despite of regulating the miscellaneous physiological and morphogenetic responses in plants, BRs plays significant role in amelioration of various biotic and abiotic stresses [19, 20]. The protective effects of BRs against different types of stresses have been already reported in crops like rice, radish, tomato and potato [21-24] etc.

Maize is third most important staple food crop belongs to the family *Poaceace* and economically grown crop in mediterranean regions. The demand of maize is increasing day by day due to its high nutritional value and used in formation of biodegradable plastics and biofuel production. It has high nutritional value and plays an important role in people's nutrition. Nutritionally, maize contains 60 to 68% starch and 7to 15% protein, contains a high percentage of essential amino acids and sources of Vitamin-A, riboflavin and rich in phosphorous and potash and contains 1.2 to 5.7 % edible oil. Salinity stress is a major constraint to this crop which adversely limits its production. In view of the above, as well as the wide occurrence and economic importance of maize crop, the aim of present work was to investigate the stress ameliorative role of EBL against salt stress in ten days old seedlings of *Zea mays* by analyzing various morphological and biochemical parameters.

MATERIAL AND METHODS

Seed treatments and growth conditions

Seeds of *Zea mays* were surface sterilized with 0.01% mercuric chloride ($HgCl_2$) for 2 minutes. Seeds were rinsed in sterile distilled water and soaked in different concentrations of EBL for 12 hours. Different concentrations of EBL used for presoaking treatment were 10^{-10}, 10^{-8} and 10^{-6}M for both the hormones. Seedlings were allowed to grow under controlled conditions of seed germinator (25±0.5°C, 16:8 h Light: dark photoperiod) for ten days in different concentrations of NaCl (0, 40, 60, 80, 100 mM). Test solutions were supplied on alternative days up to 10 days and control seedlings were supplied with distilled water.

Growth parameters

Three replicates of each treatment and six seedlings per treatment were taken on tenth day for analyzing the growth parameters. Roots and shoots were separated accordingly. The length and fresh weight of roots and shoots were measured. The seedlings were oven-dried at 70°C for 24 h to determine their dry weights.

MDA content

MDA content was assessed by the method of Heath and Packer [25]. 1g of seedlings was extracted in 3 ml of 0.1% (w/v) trichloroacetic acid and centrifuged at 5000 rpm. 3ml of supernatant was mixed with 3 ml of 20% (w/v) trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid. This mixture was heated at

95^{0}C for 30 min and immediately cooled on ice. The absorbance of the supernatant was taken spectophotometrically at 532 nm and 600 nm.

Confocal studies

Confocal microscopic studies were performed to visualize the oxidative damage due to salt stress (100mM). Roots were cut from tip and washed with water. Washed root tips were stained in the propidium iodide (25 µM) dye. After 30 minutes of staining, slides of stained roots were prepared. Stained root tips were placed carefully on glass slide with a drop of water, then covered with a cover slip and observed under confocal microscope at 20X magnification. He-Ne gas laser is used for propidium iodide for the excitation of electrons at 543.5 nm wavelength.

Photosynthetic Pigments

Chlorophyll Content

Chlorophyll content was determined by the method of Arnon [26]. One gram shoot material was homogenized in 1.5 ml of 80% acetone, followed by centrifugation at 13,000 rpm for 10 minutes. After centrifugation, absorbance of supernatant was recorded at 645 and 663 nm to determine chlorophyll **a**, **b** and total chlorophyll against 80% acetone as blank

Carotenoid Content

Carotenoid content was estimated by the method of Maclachlan and Zalik [27]. 1g fresh shoot tissue was homogenized in chilled pestle and mortar using 80 % acetone. Then centrifugation was carried out for 20 minutes at 13,000 rpm. The supernatant was collected and absorbance was taken at 480 and 510nm.

Anthocyanin content

Total anthocyanin content was estimated by method given by Macinelli [28]. One gram fresh plant tissue was homogenized in chilled pestle and mortar with 3ml of extraction mixture (methanol: water: HCl, 79:20:1). The homogenized material was then centrifuged for 20 minutes at 13,000 rpm for 20 minutes at 4^{o}C. Later on, supernatant was collected and absorbance was taken at 530 and 657nm.

Xanthophyll Content

Xanthophyll content was estimated by the method purposed by Lawrence [29]. Dried plant material (0.05g) was homogenized with 30 ml of extraction mixture in 100 ml of flask. Then flask was refluxed on water bath at 56^{o}C, followed by cooling. After cooling flask containing samples were kept in dark for 1 hour then pipette 30ml of hexane into the flask and shake for 1 minute. After that volume was made up with 10 % sodium sulphate solution and kept for 1 hour. The upper phase was collected in 50 ml volumetric flask and the volume was made up by hexane and measured spectrophotometrically at 474 nm.

Estimation of antioxidative enzymes activities and protein content

One gram of shoot material was homogenized in 3ml of 100 mM potassium phosphate buffer (pH 7.0) in an ice chilled pestle and mortar. The homogenate was allowed to centrifuge at 15,000 *g* for 20 min at 4^{0}C and the supernatant was collected to estimate the enzymes activities and protein content.

Protein content

Protein content in the seedlings was estimated by following the method purposed by Lowry et al [30]. 0.1ml of the sample and standard were pipetted into a series of test tubes. 1.0 ml was made up in all test tubes with distilled water. The intensity of blue color developed in the reaction mixture was read at 550 nm and amount of protein were calculated from the graph made by taking known concentrations of the standard protein.

Antioxidative enzymes activities

Superoxide dismutase activity (SOD)

The activity of SOD was determined according to the method of Kono [31]. For total SOD assay, 3.0 ml reaction mixture contained 50 mM sodium carbonate (pH 10.2), 24 µM NBT, 0.1 mM EDTA, 1 mM hydroxylamine hydrochloride, 0.03% (v/v) Triton X-100 and 70 µl of enzyme extract in cuvette. The absorbance was recorded at 560 nm for 2 minutes.

Glutathione reductase activity (GR)

GR activity was determined by using the method of Carlberg and Mannervik [32]. Three ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.6), 1 mM GSSG, 0.5 mM EDTA, 0.1 mM NADPH and 100 µl enzymes extract. The reaction was initiated by addition of 0.1mM NADPH and GR activity was determined by the oxidation of NADPH at 340 nm for 1 min.

Ascorbate peroxidase (APOX)

APOX activity was determined as described by Nakano and Asada [33]. Three ml reaction mixture contained 50 mM Phosphate buffer (pH 7.0), 0.5 mM ascorbate, 1.0 mM H_2O_2 and 100 µl enzyme extract.

The H_2O_2 dependent oxidation of ascorbate was followed by monitoring the decrease in absorbance at 290 nm.

Quantitative and Qualitative estimation of total sugars

Quantitative analysis of sugars through anthrone method

Total sugars were quantitatively detected by the method given by Scott and Melvin [34]. In 25 mg of plant sample, 1.25 ml of 2.5 N HCl was added and cooled it to room temperature. Na_2CO_3 was added to neutralize it and made final volume to 25 ml. Then 4 ml of anthrone reagent was added to 1 ml of the supernatant. It was heated for 8-10 minutes in boiling water bath. After cooling the reaction mixture, the optical density of dark green colour appeared was taken at 630 nm on UV-VIS PC Based Double Beam Spectrophotometer (Systronics 2202). A graph of absorbance v/s concentration for standard solutions of glucose was plotted and the amount of total sugars in sample was calculated from it.

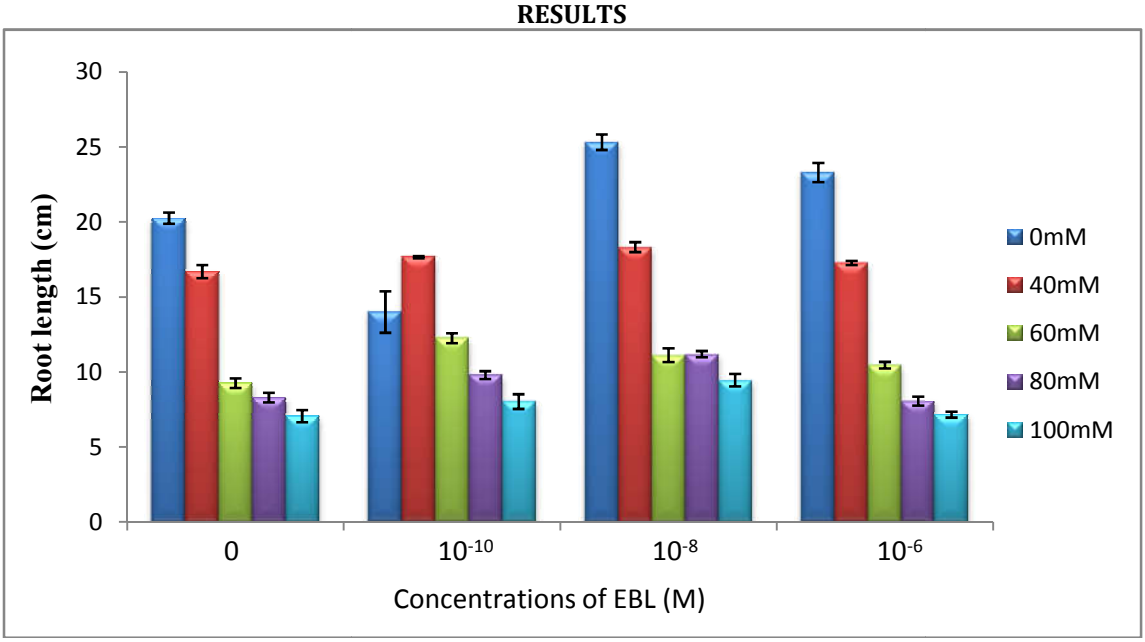
Qualitative analysis of sugars through HPLC

Seedlings were dried in hot air oven at 80^0C and crushed to make fine powder. 2 g powered sample were mixed with 5 ml of DDW and leave for 12 h at room temperature. After 12 h incubation, samples were filtered through the 0.22 micron pore sized filter paper. 20 µl supernatant was used for the analysis of sugars by HPLC. The conditions maintained were: 0.5 ml/min flow rate, 20 min sample run, 80ºC column compartment (oven temperature), Refractive Index detector and mobile phase was MilliQ water. Injections of the standard solutions were preceded by two injections of MilliQ water. The chromatographs were obtained for each sample and presence of sugars was correlated with the peaks of standards.

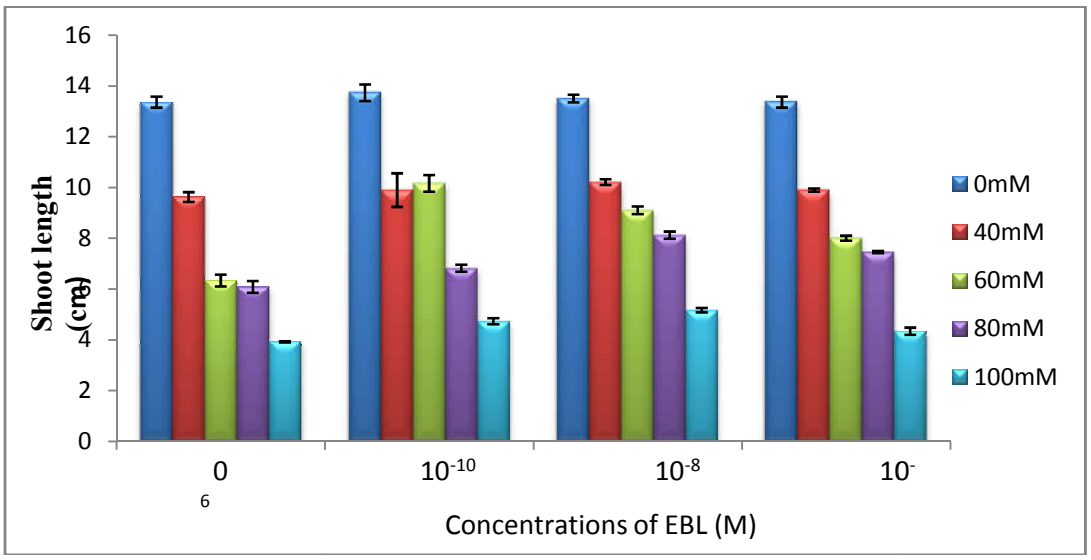
Statistical analysis

Three repetitions were designed for each experiment and data was expressed as mean ± SE value. Two way analysis of variance (ANOVA) was performed and data was presented at significance of $p \leq 0.05$

RESULTS



1(a)



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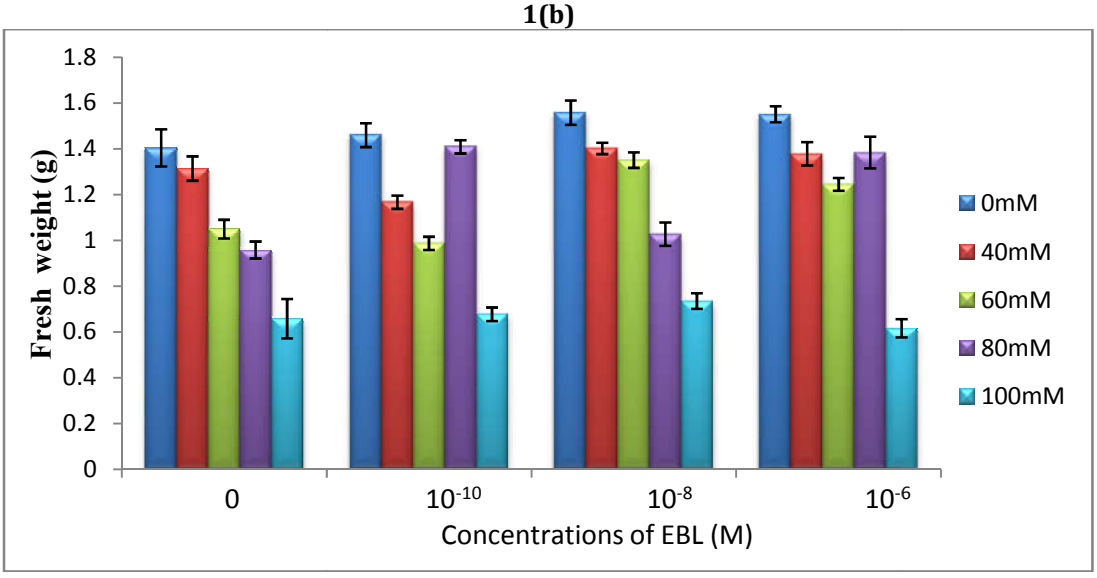

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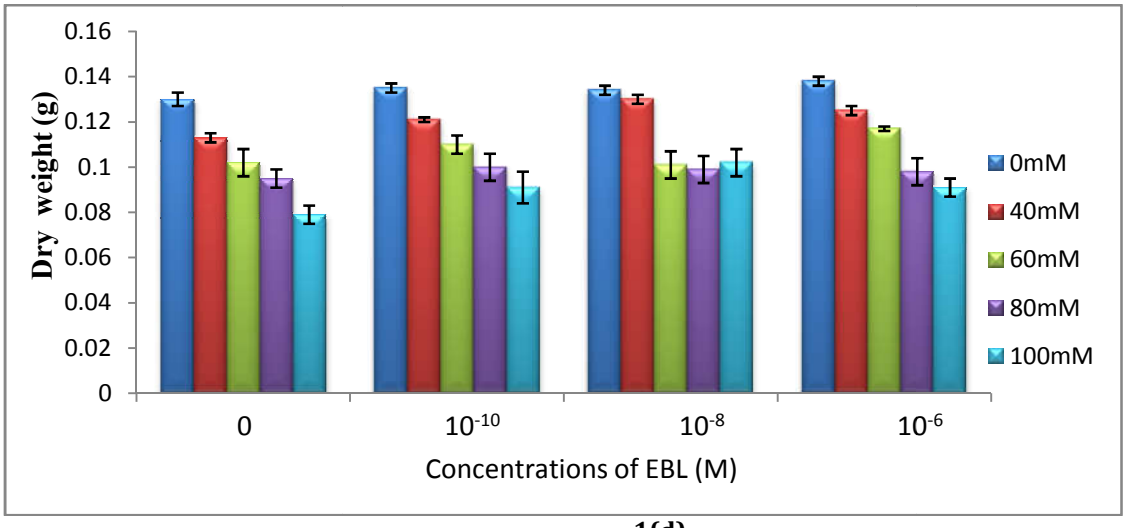
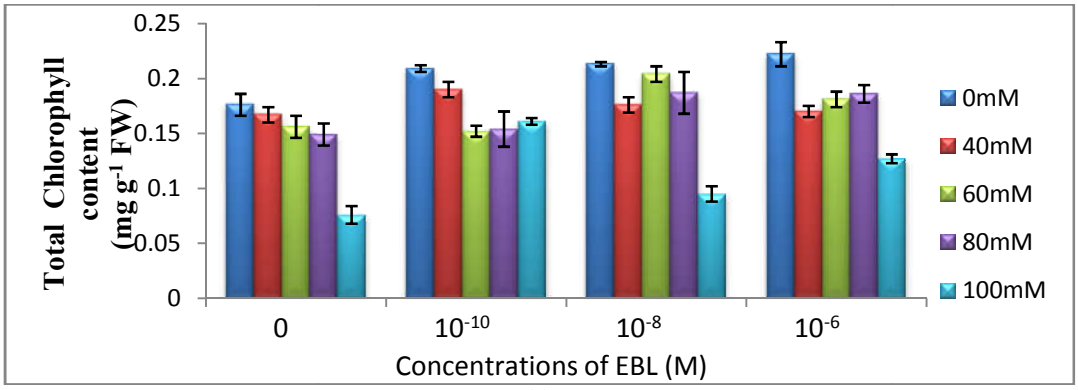


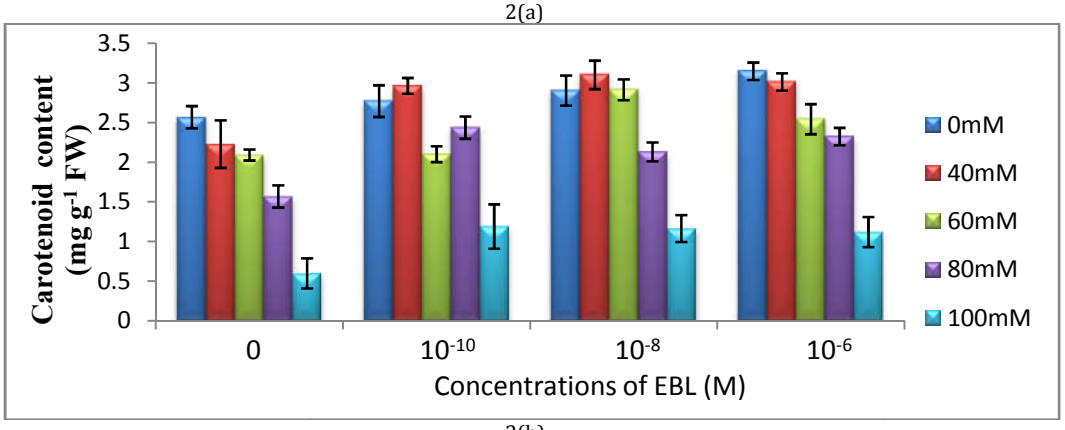
1(d)

Fig1. Effect of EBL on (a) root length, (b) shoot length, (c) fresh weight and (d) dry weight (d) of 10 days old seedlings of *Zea mays* subjected to salt stress.

Salt stress impositions caused decrease of root and shoot length of *Zea mays* seedlings. Salinity stress decreased the root length (7.06 cm) and shoot length (3.92 cm) under 100 mM NaCl concentration as compared to control seedlings (root length: 20.26 cm; shoot length: 10.36 cm) (Fig. 1a, b). Similarly, fresh weight (0.65 g) and dry weight (to 0.07 g) was observed to decrease maximum under 100mM NaCl concentration as compared to control seedlings (fresh weight: 1.40 g; dry weight: 0.13 g) (Fig. 1c, d).
Pre-soaking treatments of EBL considerably increased the root/shoot length and fresh/dry weight of seedlings. Application of 10^{-8} M EBL and 10^{-10}M EBL showed maximum increase of root length (25.33 cm) and shoot length (13.73 cm) respectively in comparison to control seedlings (root length: 20.26 cm; shoot length: 10.36 cm). Under pre-soaking treatment of 10^{-8}M EBL and 40mM NaCl concentration, root length (18.33 cm) and shoot length (10.21 cm) was observed to increase maximum as compared to 40mM NaCl concentration only (root length: 16.7 cm; shoot length: 9.62 cm) (Fig. 1a, b). Furthermore, fresh and dry weight of the seedlings was also observed to be enhanced significantly by the implications of EBL treatments. Under 10^{-10}M EBL presoaking treatment and 80 mM NaCl concentration, fresh biomass (1.40 g) was found to elevate as compared to 80 mM NaCl treatment only (0.95 g) (Fig.1c). A similar trend was observed for dry biomass of seedlings under different concentrations of NaCl. 10^{-8} M dose of EBL was noticed to be most effective in enhancing morphological parameters of *Zea mays* seedlings grown under NaCl stress.



2(a)



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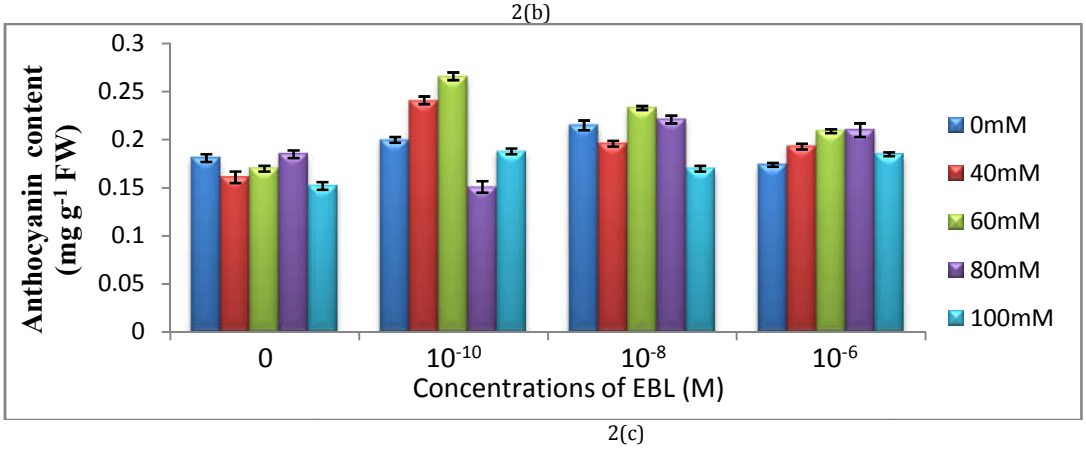

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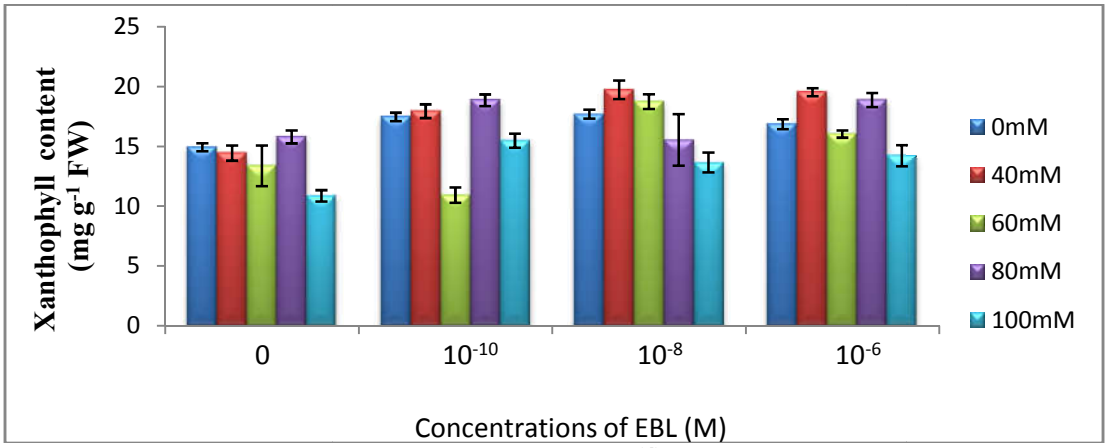


2(d)

Fig.2 Effect of EBL on (a), total chlorophyll, (b) carotenoid, (c) anthocyanin and (d) xanthophyll content of ten days old seedlings subjected to salt stress.

Photosynthetic pigments play an important role in stress protection. Photosynthesis is the basic fundamental process of plant life. Maximum decreased of chlorophyll content was observed under 100mM NaCl concentration. Carotenoid, anthocyanin and xanthophyll content was also found to decrease with increasing salt stress.

Total chlorophyll content (0.204 mg g^{-1} FW) was increased with the pre-soaking treatment of 10^{-8} M EBL and under 60mM NaCl concentration as compared to 60 mM NaCl concentration only (0.156 mg g^{-1} FW) (Fig. 2a). Carotenoid content was significantly enhanced with the pretreatment of 10^{-6} M EBL (3.14 mg g^{-1} FW) in comparison to control (2.57 mg g^{-1} FW). Similarly pretreatment of 10^{-8}M EBL and under 40mM NaCl concentration, maximum improved carotenoid content (3.10 mg g^{-1} FW) was observed in comparison to 40mM NaCl concentration only (2.23 mg g^{-1} FW) (Fig. 2b). Pretreatment of 10^{-8}M EBL showed the improved anthocyanin content (0.21 mg g^{-1} FW) in comparison to control (0.18 mg g^{-1} FW) (Fig. 2c). On the other hand, under 60mM NaCl concentration, pre-soaking treatment of 10^{-10}M EBL maximally enhanced the anthocyanin content (0.26 mg g^{-1} FW) in comparison to 60mM NaCl concentration only (0.17 mg g^{-1} FW). Significant increase of xanthophyll content was observed with the pre-soaking treatment of 10^{-8}M EBL (17.69 mg g^{-1} FW) in comparison to control (14.92 mg g^{-1} FW). Treatment of 10^{-8} M EBL and under 40mM NaCl concentration, increased xanthophyll content (19.73 mg g^{-1} FW) was observed as compared to 40 mM NaCl concentration only (14.43 mg g^{-1} FW) (Fig. 2d).

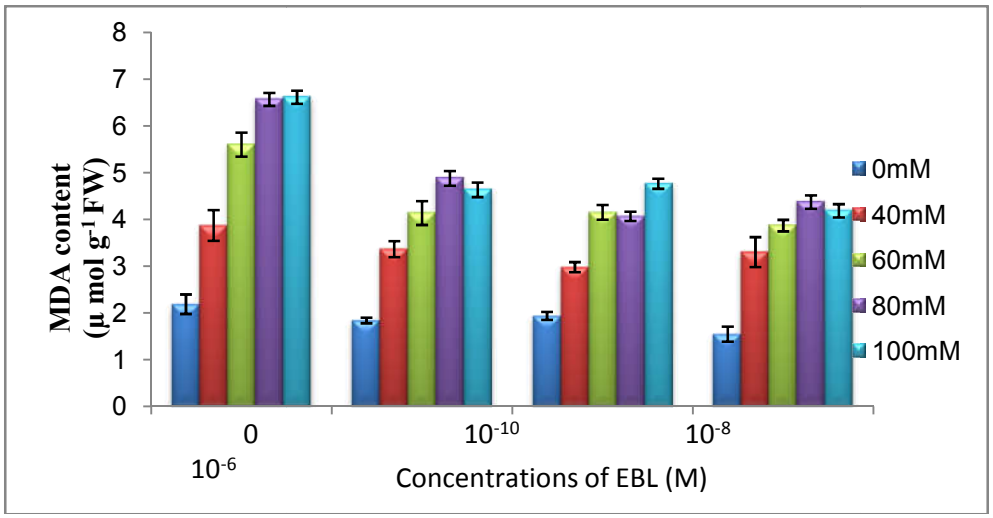
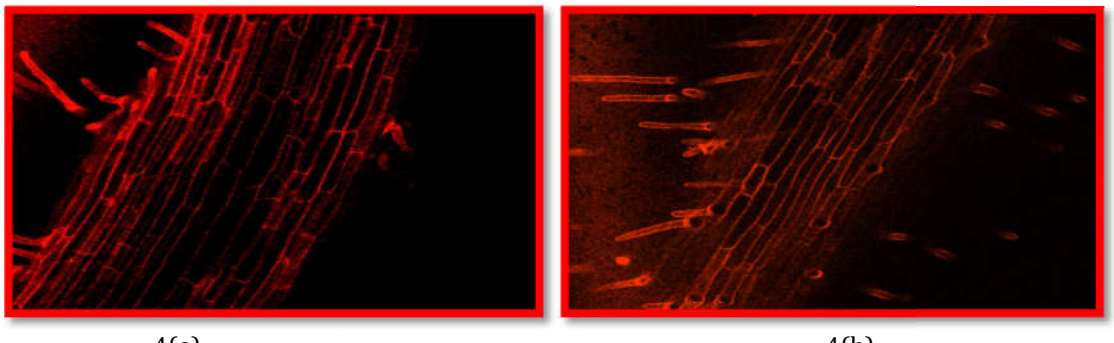
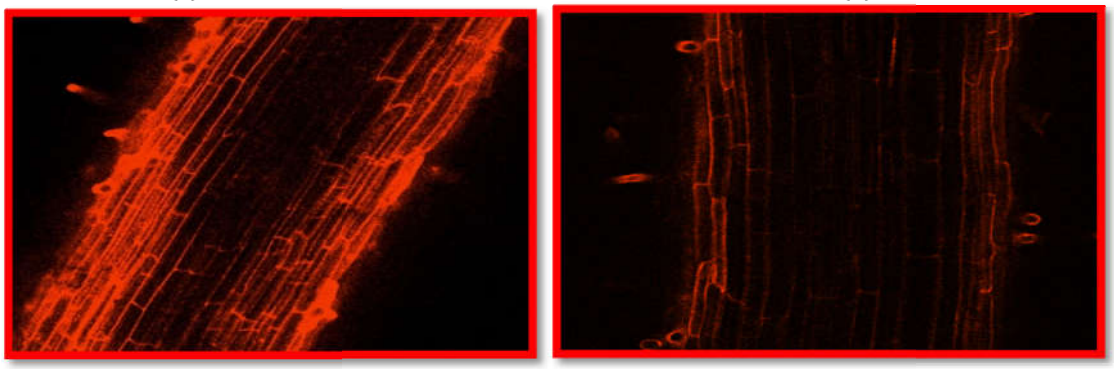


Fig.3 Effect of EBL on MDA content of ten days old seedlings subjected to salt stress.



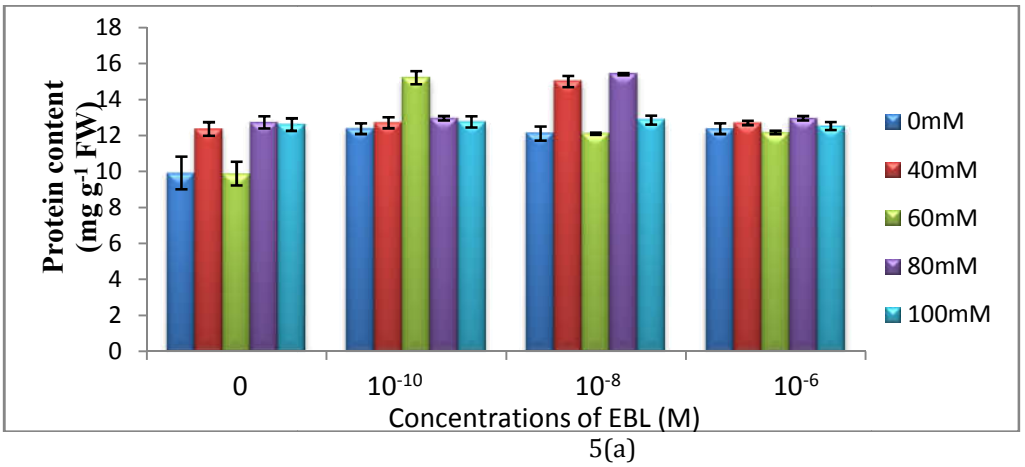
4(a) 4(b)



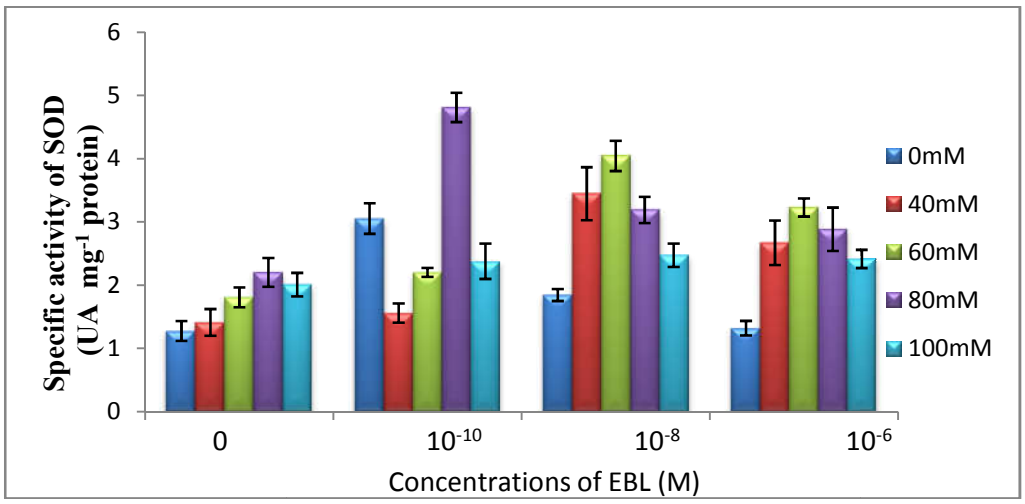
4(c) 4(d)

Fig. 4(a-d) Confocal microscopic images. (a) Untreated root cells (b) root cells treated with 10^{-8}M EBL (c) 100 mM NaCl stressed root cells (d) root cells treated with 10^{-8}M EBL and subjected to 100mM NaCl stress.

Salt stress significantly enhanced the MDA content (3.021 times) as compared to control (Fig. 3). Maximum increase in MDA content was observed at 100 mM salt stress and this increase in MDA content was further reduced significantly by EBL alone presoaking treatments. Pretreatments of 10^{-6} M EBL alone results the maximum decrease of MDA content (1.54 µmol g^{-1} FW) as compared to control (2.18 µmol g^{-1} FW). Similarly pre-soaking treatment of EBL (10^{-8}M) and 40mM NaCl concentration, showed the decrease in MDA level (2.98 µmol g^{-1} FW) as compared to 40mM NaCl concentration only (3.87 µmol g^{-1} FW). Similar results were confirmed by confocal microscopy where root cells were stained with propidium iodide (PI) staining dye. PI is a nucleic acid binding dye that is excluded from the viable and intact cells. However in non viable cells, it penetrates inside the damaged cell and stained the nucleic acid and cytoplasm. In fig (4a), the outline of intact and viable cells appears stained (red) which indicated that no damage had been occured while in fig (4c), the cells had been treated under 100 mM NaCl were assumed to be damaged on account of intense stain which could penetrate through the plasmamembrane and bind to DNA. However presowing treatment of EBL under 100mM NaCl concentration (fig 4d) showed less damage as compared to only salt concentration (fig 4c).



5(a)



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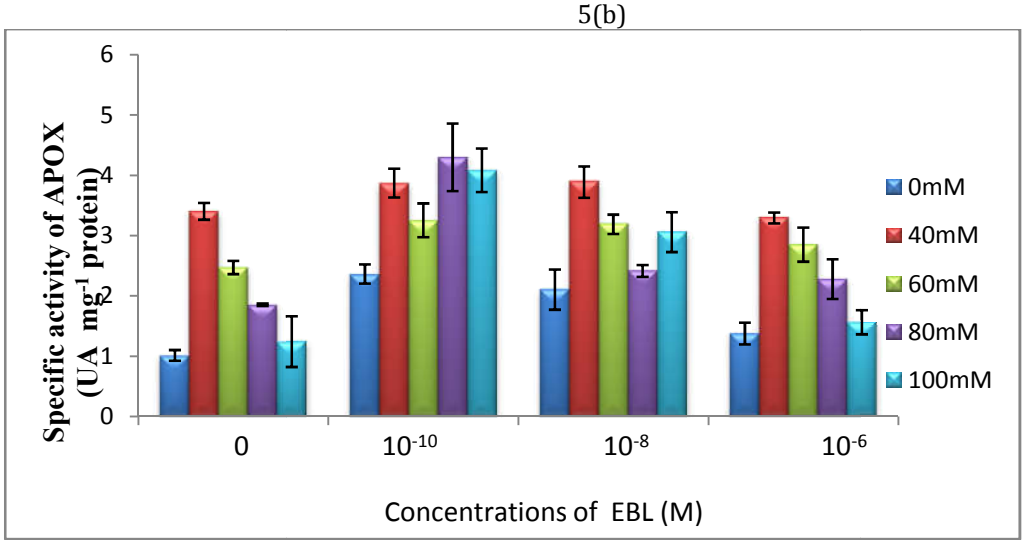

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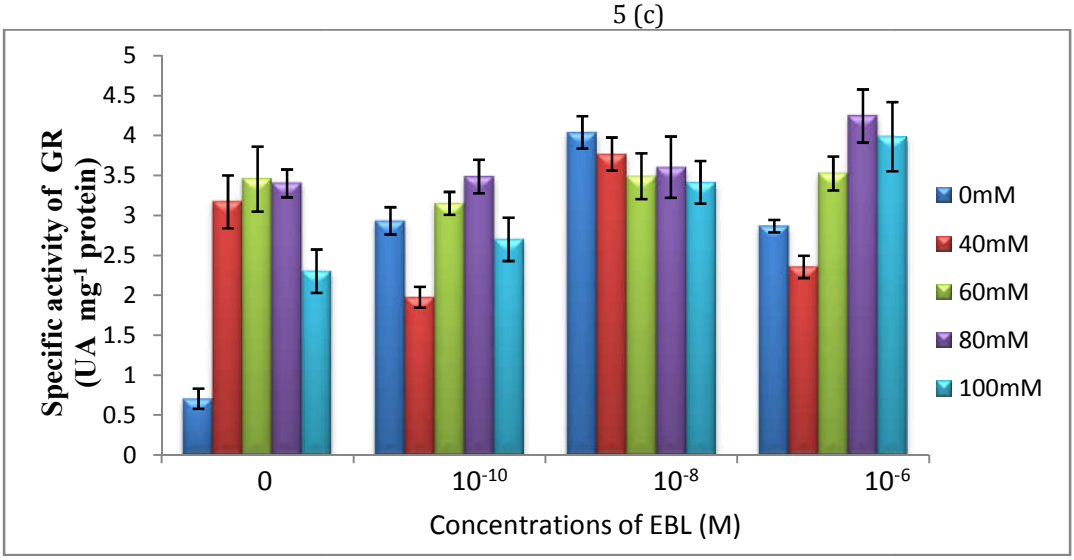

5(d)

Fig.5 Effect of EBL on (a) protein content, (b) SOD activity, (c) APOX activity and (d) GR activity of ten days old seedlings subjected to salt stress.

The observations on salt stressed seedlings revealed that protein content was increased with increasing concentrations of salt stress except at 60 mM salt concentration where protein content was decreased in comparison to control. Maximum increase of protein content (4.72 mg g^{-1} FW) was observed under 80

mM NaCl concentration as compared to control (3.91 mg g^{-1} FW). Pretreatment of EBL showed the enhancement of protein content as compared to control (Fig. 5a). Further seedlings raised from seeds pretreated with 10^{-8}M EBL and under 80 mM NaCl concentration showed the maximum increase of protein content (5.41 mg g^{-1} FW) as compared to 80 mM concentration only (Fig. 5a).

Antioxidative enzymes were also observed to enhance in *Zea mays* seedlings with the increasing concentrations of NaCl. Presowing treatment of EBL significantly overcame the salt stress by enhancing the activities of antioxidative enzymes. 10^{-10}M EBL treatment significantly increased the activity of SOD (3.05 U mg^{-1} protein) and APOX (2.36 U mg^{-1} protein) as compared to SOD (1.27 U mg^{-1} protein) and APOX (1.01 U mg^{-1} protein) activity in control seedlings (Fig. 5b, c). With the pre-treatment of 10^{-10}M EBL and under 80mM NaCl concentration, SOD activity was found to maximally (4.81 U mg^{-1} protein) enhanced as compared to 80mM NaCl concentration only (2.20 U mg^{-1} protein) (Fig. 5b). Pretreatment of 10^{-10} M EBL enhanced the APOX activity under 80mM NaCl concentration (4.30 U mg^{-1} protein) (Fig. 5c). Activity of GR was also found to enhance under salt stress as well as with the pretreatment of EBL (Fig. 5d). Maximum enhancement in activity of GR (4.04 U mg^{-1} protein) was observed with the application of 10^{-8}M EBL in comparison to control (GR: 0.70 U mg^{-1} protein). Further pretreatment of 10^{-6}M EBL under 80mM NaCl concentration, showed the enhanced activity of GR (4.24 U mg^{-1} protein) as compared to 80mM NaCl concentration only (3.40 U mg^{-1} protein).

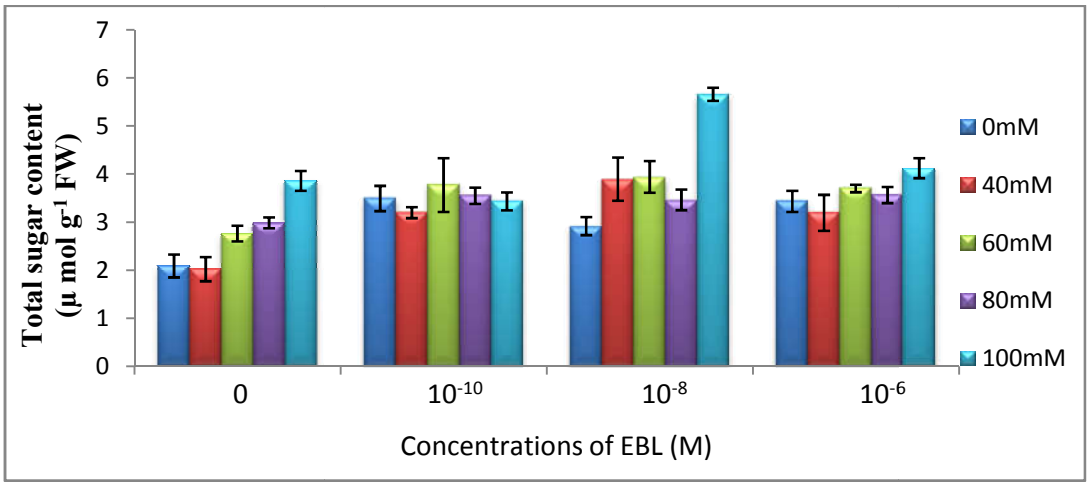

Fig.6 Effect of EBL on total sugar content of ten days old seedlings subjected to salt stress.

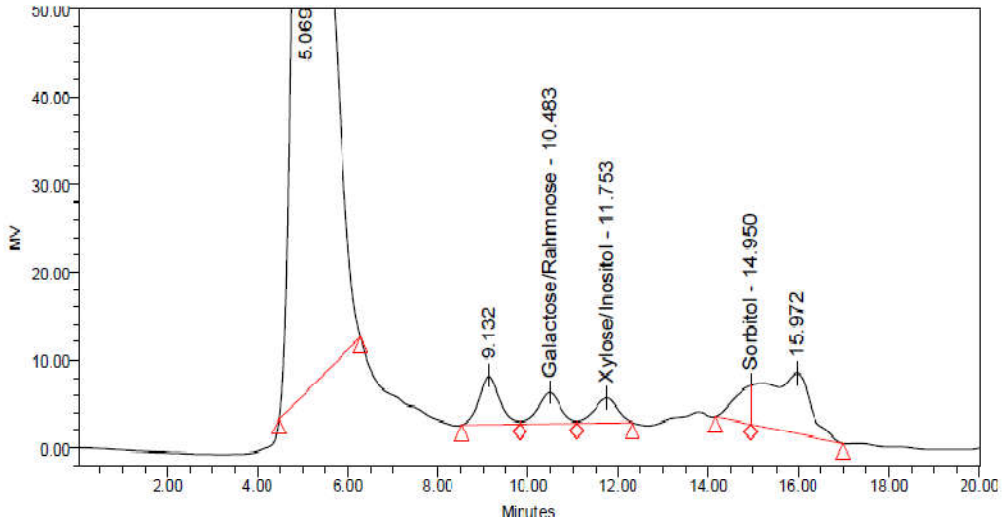

Fig. 6(a) Qualitative analysis of sugars presents in 10 days old control *Zea mays* seedlings

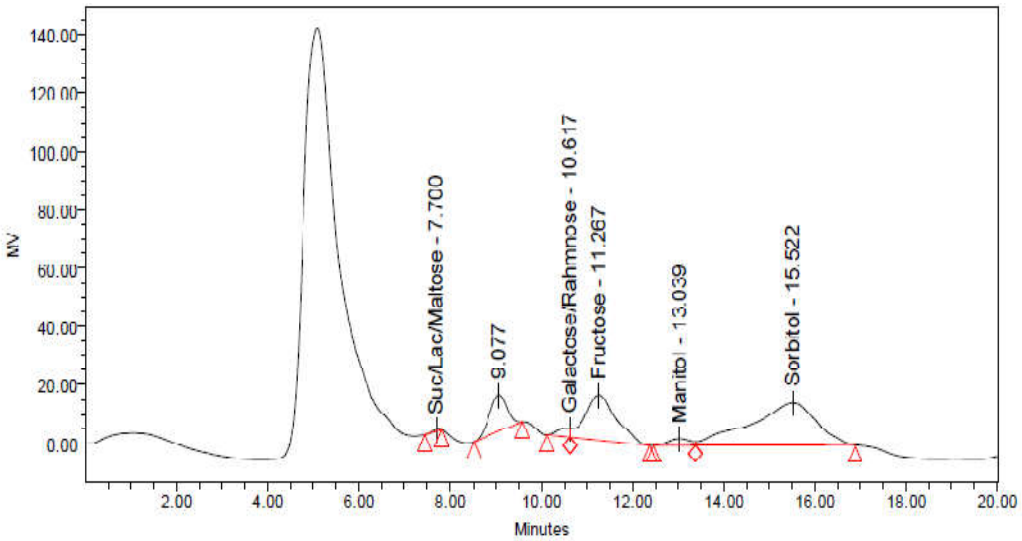

Fig. 6(b) Effect of EBL on qualitative analysis of sugars presents in 10 days old *Zea mays* seedlings

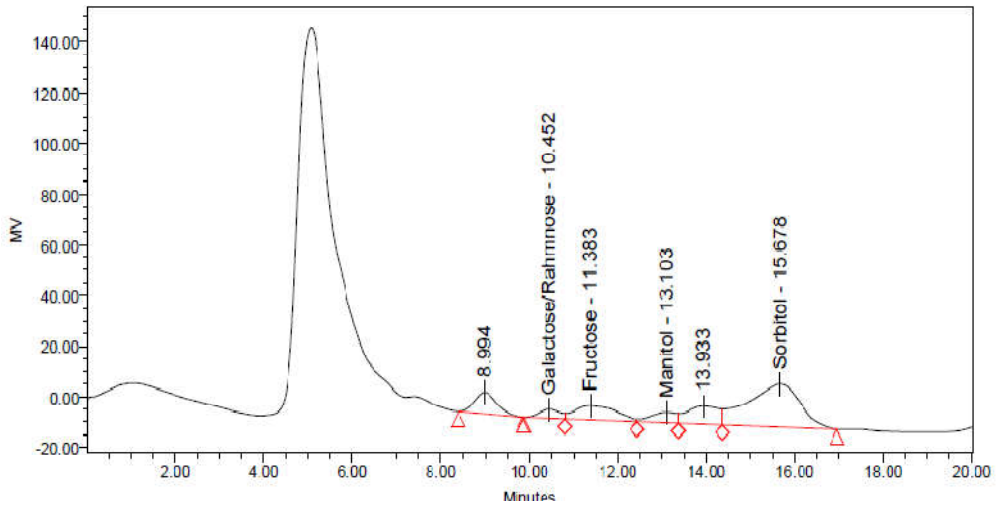

Fig. 6 (c) Qualitative analysis of sugars in 10 days old *Zea mays* seedlings subjected to 100 mM NaCl stress

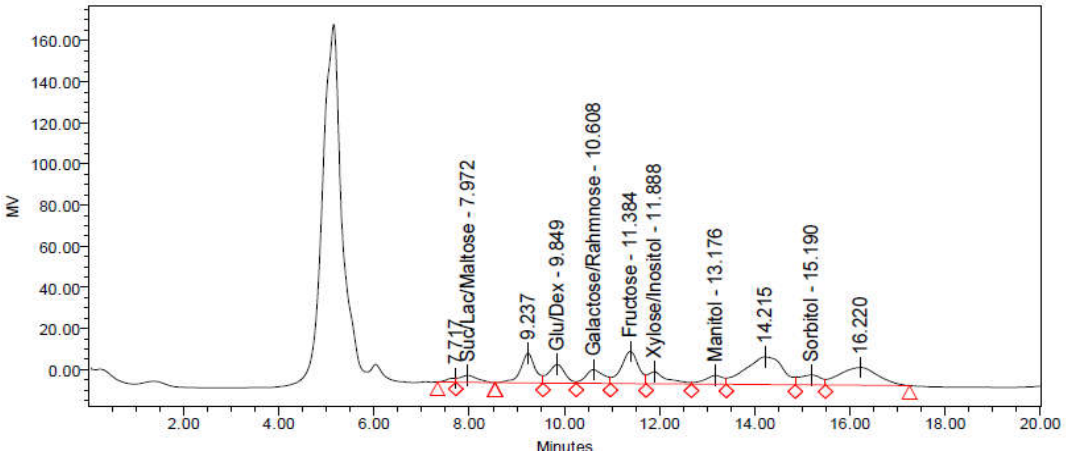

Fig. 6(d) Effect of EBL on qualitative analysis of sugars in 10 days old *Zea mays* seedlings subjected to 100 mM NaCl stress

Sugars content was determined both quantitatively by spectrophotometrically and qualitatively by HPLC. Sugars content were found to be increased with increasing concentration of salt stress in comparison to control. Maximum increase of sugar content (3.86 μ mol g^{-1} FW) was observed under 100 mM salt concentration as compared to control seedlings (2.09 μ mol g^{-1} FW) (Fig. 6). 10^{-10}M EBL (3.49 μmol g^{-1}FW) dose resulted in increase of total sugar content as compared to control (2.09 μmol g^{-1}FW). Approximately 1.46 times (from 5.66 μmol g^{-1}FW) enhancement in total sugar content was observed in 100mM NaCl concentration when pre-sowing treatment of 10^{-8} M EBL was given to seedlings as compared to 100mM NaCl concentration only (3.86 μmol g^{-1}FW) (Fig. 6). Similar results were confirmed qualitatively through the HPLC. Qualitatively determined sugars content was also observed to be increased in response to salt stress (100mM). In control seedlings distinct peaks of galactose/rahmnose, xylose/inositol and sorbitol were observed (Fig.6a), whereas seedlings treated with 10^{-8} M EBL alone showed the peaks of suc/lac/maltose, galactose/rahmnose, fructose mannitol and sorbitol (Fig.6b). During 100 mM NaCl stress four distinct peaks for sugars namely galactose/rahmnose, fructose, mannitol and sorbitol (Fig.6c) were recorded as compared to control (Fig.6a). Seed pre-soaking treatments of EBL (10^{-8} M) to 100mM NaCl stress seedlings resulted in accumulation of sugars such as suc/lac/maltose, glucose/dextrose and xylose/inositol in addition to peaks of galactose/rahmnose, fructose, mannitol and sorbitol recorded under 100mM NaCl concentration only (Fig.6d).

Table1. F-ratio of two way ANOVA of EBL treatments for morphological parameters (root/shoot length, fresh weight/ dry weight), photosynthetic pigments (Total chlorophyll, carotenoid, anthocyanin and xanthophyll content), MDA content, protein content, activities of antioxidative enzymes (SOD, APOX, GR) and total sugars content.

Source of variation	Treatment (EBL)	Dose (NaCl)	Treatment ×Dose	HSD
Root length	1347.5*	83.82*	10.79*	1.79
Shoot length	898.01*	80.01*	12.73*	1.01
Fresh weight	63.09*	15.17*	4.93*	0.42
Dry weight	60.07*	4.92*	1.20	0.02
Total Chlorophyll	108.70*	11.10*	9.97*	0.035
Carotenoid content	450.71*	87.84*	8.26*	0.37
Anthocyanin content	19.97*	30.95*	11.21*	0.043
Xanthophyll content	23.10*	29.88*	6.05*	4.12
MDA content	216.92*	73.92*	6.12*	0.96
Protein content	674.38*	11.03*	5.06*	0.82
SOD	21.79*	28.86*	12.06*	1.21
APOX	54.49*	63.21*	4.94*	1.06
GR	7.16*	13.96*	5.06*	1.71
Total sugars	91.16*	16.20*	5.69*	1.19

*Indicate statistically significant differences from control and salt treatment at $p \leq 0.05$

DISCUSSION

Salt stress is the most detrimental stress that harmfully affected the plant growth and development. Osmotic stress, ionic stress, oxidative stress, mechanical stress and nutritional stress are the main consequences of salt stress. The other effects of salt stress involves: reduced crop yield, soil erosion and ecological unbalancing that ultimately affected the agricultural production adversely. Osmotic stress induced due to salinity, decreased the osmotic potential of soil which causes the water deficiency [35], whereas ionic stress increased the Na^+ and Cl^- ions to such a toxic level that these ions interfere with other ions and causes the disruption of various cellular function by affecting enzymes, lipids, cell division, cell expansion, photosynthesis and pigment biosynthesis [36].

In the present study, salt stress affected the seedling growth by decreasing root length, shoot length, fresh weight and dry weight. However treatment of EBL along with salt stress overcomes the salt inhibited growth of seedlings. The growth stimulating effects of BRs on seedlings under salt stress conditions indicating the involvement of BRs in cell elongation and cell cycle progression [37] as well as in regulation of genes encoding xyloglucan endotransglu-cosylase/hydrolases (XTHs) which incorporates new xyloglucan into the growing cell wall [38]. Similarly improved seedling growth, fresh weight and dry weight with the application of BRs under water stress in radish seedlings had been observed by Mahesh et al. [39].

The growth reduction under salt stress was also manifested by alteration of photosynthetic pigment biosynthesis [40]. The content of total chlorophyll, carotenoid, anthocyanin and xanthophyll was found to be decreased under saline treatments. Saline stress slows down the production of photosynthetic pigments by causing degradation of the photosynthetic apparatus with the increased level of the toxic Na^+ ions [41]. Carotenoids are the constituents of thylakoid membranes and play important role in providing protection to chlorophyll molecules from photooxidation. Salt stress decreased the carotenoid content by inducing degradation of β-carotene [42]. Thus degradation of carotenoid synthesis may further cause the degradation of chlorophylls and ultimately results in pigment loss [43]. BRs application restored the normal level of pigments. In the present study application of BRs enhanced the pigments content. The restoration of normal levels of pigments chlorophyll **a**, **b** and carotenoids through the application of BRs application under stress suggests that there is positive influence of BRs on pigments. BRs enhanced the pigment level by inducing transcription and translation of the enzymes involved in chlorophyll biosynthesis [44]. Present observations were according to the finding of Ali et al. [45], who also found the enhanced pigment content in *Solanum lycopersicum* when subjected to cadmium stress.

Phytotoxicity from salt stress has been related to the production of ROS. Generations of ROS during stress leads to peroxidation of lipids. MDA is the end product of lipid peroxidation has been widely used to assess the salinity damage [46]. Under salt stress, MDA content was found to be enhanced due to generation of free radicals which results in disruption of cellular functioning by affecting lipids metabolism, physiochemical properties of cell membranes, alternations of ion transport and metabolic processes [47]. BRs treatment significantly overcomes the damage by decreasing the MDA content. Decreased MDA content with the application of BRs specified the role of BRs in overcoming the stress by scavenging of ROS and thus reduced the damage [48].

Thus in order to overcome the salinity induced damage, plants adopted various physiological mechanisms to minimize the accumulation of toxic ions in plant tissue including accumulation of compatible solutes, enhancement of activity of antioxidant enzymes to overcome the oxidative stress, portioning of toxic ions into apoplast and vacuole [36]. Among these mechanisms, activities of antioxidative enzymes and compatible solutes have been discussed in the present study. Increased ROS production during stress leads to oxidative stress because production and quenching of ROS becomes unbalanced and resulted in oxidative stress. In order to scavenge ROS, plant antioxidative system plays an important role to keep ROS under balance. Plants evolved a set of antioxidative enzymes which works in a coordination system to overcome the stress [49]. SOD is the first line of defense against ROS and it causes detoxification of $O_2^{\bullet-}$ to H_2O_2 and O_2 [50]. H_2O_2 is further removed by APOX of the ascorbate-glutathione antioxidant cycle in the chloroplast [51]. GR activity maintains the pool of glutathione in a reduced state, which further reduces the dehydroscorbate to ascorbate through the ascorbate-glutathione cycle [52]. In the present study significant increase in activity of SOD, APOX and GR was observed under salt stress. Application of EBL to salt stressed seedlings further stimulated the activities of antioxidative enzymes to overcome the stress. Enhanced activity of these antioxidative enzymes provides maximum tolerance against salinity [53]. Application of BRs ameliorated the oxidative stress generated due to stress by regulating the expression of genes which are involved in the induction of antioxidant systems [18]. Application of BRs improved the seedling growth by enhancing the activity of antioxidative enzymes in rice cultivar subjected to salt stress [21]. BRs significantly decreased harmful ROS accumulation through the induction of antioxidant enzymes activity in tomato plant under polychlorinated biphenyl oxidative stress which again strengthened the role of BRs in alleviation of oxidative damage [54]. BRs treatment enhanced the protein content with increase of salt concentration. Increasing protein content under salt stress involves the stimulation of activation of transcription and translational processes of specific stress tolerance genes by BRs [55].

Osmolytes plays a protective role in plant response to salt stress. Accumulation of various sugars in response to salinity stress has been observed in present study. Sugars play important role in providing tolerance against salinity stress by decreasing the cytoplasmic water potential [56]. Sugars act as cellular protector which accumulated in response to stress and scavenge ROS [57]. Further BRs application enhanced the sugars both quantitatively and qualitatively. Vardhini et al [58], reported increased carbohydrate fractions like reducing sugars and starch in the radish roots with the treatment of BRs which reveals that BRs induces the osmolytes accumulation during stress to counter the adverse effect of salt stress.

CONCLUSION

Application of BRs showed a stress protectant activity in alleviating the adverse effects of salt stress. The seed presoaking treatments of EBL improved maize seedling growth, pigments content, protein content, activities of antioxidative enzymes and sugars content under stress. 10^{-8}M EBL was found to be effective in overcoming the salt induced damage and provided tolerance against salinity stress which might be mediated through the cross talk of this hormone with other plant growth regulator in developing stress tolerance.

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