ESTABLISHMENT OF DOSE DEPENDENT RESPONSES OF BRASSINOSTEROIDS AND PROLINE AGAINST SALINITY STRESS IN *BRASSICA JUNCEA*

THESIS
SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy
IN
BOTANY

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DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)
2013

Dedicated to my parents

For their endless love, support and encouragement

ANNEXURE-I

CANDIDATE'S DECLARATION

I, **Arif Shafi Wani**, Department of Botany, certify that the work embodied in this Ph.D. thesis is my own bonafide work carried out by me under the supervision of **Prof. Aqil Ahmad** at Aligarh Muslim University, Aligarh. The matter embodied in this Ph.D. thesis has not been submitted for the award of any other degree.

I declare that I have faithfully acknowledged, given credit to and referred to the research workers whenever their works have been cited in the text and the body of the thesis. I further certify that I have not willfully lifted up some others work, para, text, data, results, etc. reported in the journals, books, magazines, reports, dissertations, thesis, etc., or available at web-sites and included them in this Ph.D. thesis and cited as my own work.

Date: 24-07-13

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CERTIFICATE FROM THE SUPERVISOR

I endorse the statement of **Mr. Arif Shafi Wani** (Annexure-I) and certify that the thesis entitled, **"Establishment of dose dependent responses of brassinosteroids and proline against salinity stress in *Brassica juncea*"**, submitted for the degree of **Doctor of Philosophy in Botany** is a faithful record of the bonafide research work carried out at the Aligarh Muslim University, Aligarh, India by him under my guidance and supervision and that no part of it has been submitted for any other degree or diploma.

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This is to certify that **Mr. Arif Shafi Wani,** Department of Botany has satisfactorily completed the course work/comprehensive examination and pre-submission seminar requirement which is part of his Ph.D. programme.

(Prof. Firoz Mohammad)
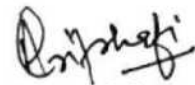
ANNEXURE-III

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(Arif Shafi Wani)

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Introduction

INTRODUCTION

Brassica juncea (L.) Czern & Coss is the member of the family Brassicaceae. It is chiefly grown in China, India, Pakistan, Canada, France, Poland and Germany. It is an annual herb attaining the height up to 1 m. The branches are long, erect or patent. Lower leaves are pinnately divided, stalked, 10-20 cm long, toothed. Upper leaves are alternate, stalkless or nearly so, not clasping, smaller than lower leaves, lance-shaped to linear, mostly non-toothed. Flowers are in branched clusters, yellow in color. Fruit is a narrow pod, round with a beak and net-veined. It is an important oilseed crop known for its oil content, edible and medicinal uses. It is reported to be used as anodyne, apertif, diuretic, emetic, rubefacient, and stimulant, folk remedy for arthritis, foot ache, lumbago and rheumatism (Duke and Wain, 1981; Mishra *et al.*, 2012). The seed is a warming stimulant with antibiotic effects. The leaves, seeds and the stem of mustard are edible. These are rich in vitamin A and vitamin K.

The special feature of mustard is phytoremediation. It is cultivated to remove heavy metals from the soil in hazardous waste sites because it has higher tolerance for these chemicals and can store the heavy metals. The plant is then harvested and disposed-off properly. It also prevents erosion of soil from these sites preventing further contamination. India ranks second in the world with regard to the production of *Brassicas* (Afroz *et al.*, 2005) and supplies nearly 7% of the world's edible oil (Khan *et al.*, 2002). However, production of rapeseed still remains insufficient to fulfill the daily requirements of the people (Khan *et al.*, 2002). The insufficient economic yield can be attributed to various biotic and abiotic stresses among which salinity has emerged as one of the serious problems limiting productivity of agricultural crops as well as claiming substantial farmable area (Allakhverdiev *et al.*, 2000; Al-Karaki, 2001; Koca *et al.*, 2007). About 20% of the world's cultivated land area and 50% of all irrigated lands are affected by salinity (Moud and Maghsoudi, 2008). High salinity level causes ionic imbalance due to excess accumulation of Na^+ and Cl^- in the cells thus reducing the uptake of essential mineral nutrients, such as K^+, Ca^{2+} and Mn^{2+} thus affecting the normal physiology of plants, both at the cellular as well as whole plant levels (Bayuelo-Jimenez *et al.*, 2003). The excess amount of Na^+ ions in cells cause enzyme inhibition and metabolic dysfunction such as degradation of photosynthetic pigments (Chaves *et al.*, 2009). Salinity stress causes decrease in the stomatal conductance (Parida *et al.*, 2004), internal CO_2 pressure and stomatal

opening that affect gaseous exchange which result in the inhibition of photosynthesis in the salt affected plants (Iyenger and Reddy, 1996). This decrease in photosynthesis under saline conditions is considered as one of the most important factor responsible for reduced plant growth and the productivity (Manikandan and Desingh, 2009).

Plants have been classified as glycophytes or halophytes according to their capacity to grow on high salt medium. Glycophytes are the plants which cannot tolerate salt stress that include most of the cultivated plants (Sairam and Tyagi, 2004). The salinity stress has deleterious effects on growth which are associated with: (a) low osmotic potential of soil solution (water stress), (b) nutritional imbalance, (c) specific ion effect (salt effect) or (d) a combination of these factors (Marschner, 1995). With the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy and lipid metabolism are adversely affected. The earliest response is a reduction in the leaf surface expansion rate followed by its cessation as stress intensifies, but growth resumes when the stress is relieved (Parida and Das, 2005). Soil salinity causes a lower rate of photosynthesis by decreasing the chlorophyll content, the activity of rubisco (Soussi *et al.*, 1998) and the closure of stomata thereby, decreases partial CO_2 pressure (Bethke and Drew, 1992). Salinity reduces plant productivity first by reducing plant growth during the phase of osmotic stress and subsequently by inducing leaf senescence during the phase of toxicity when excessive salt is accumulated in transpiring leaves (Munns, 2002). All of these cause adverse pleotropic effects on plants.

Recently, brassinosteroids (BRs) have emerged as a new paradigm in the category of phytohormones. These are the class of poly-hydroxysteroids that have been recognized as sixth class of plant hormones. Till now, about 69 BRs have been isolated from plants (Bajguz, 2010a). Like other plant hormones (auxins, gibberellins, cytokinins, ethylene and abscisic acid), BRs act at very low concentration to control numerous processes associated with plant growth and development (Friedrichsen and Chory, 2001; Bajguz and Hayat, 2009). BRs have been implicated in a wide range of physiological and molecular responses in plants. They have the ability to cause cell elongation and cell division in stem, inhibit root growth, promote xylem differentiation, and abscission of plant organs (Mandava, 1988; Nemhauser *et al.*, 2004). They have also been noted to control several other process in plants, such as

induced synthesis of nucleic acids and of proteins (Khripach *et al.*, 2003), activation of several enzymes (Hasan *et al.*, 2008), photosynthesis (Hayat *et al.*, 2007a) and increased fruit set (Fu *et al.*, 2008; Ali *et al.*, 2006). Apart from this, BRs also have the ability to confer tolerance against osmotic stress (Sairam, 1994; Ahmed *et al.*, 2011a; Vardhini and Rao, 2003), temperature stress (Fariduddin *et al.*, 2011), salinity (Hayat *et al.*, 2007b; Ali *et al.*, 2007a), and various heavy metal stress like, cadmium (Hayat *et al.*, 2007a, 2010a), nickel (Alam *et al.*, 2007; Yusuf *et al.*, 2011), aluminium (Ali *et al.*, 2008a), copper (Fariduddin *et al.*, 2009a) and nitrosative stress (Hayat *et al.*, 2010b).

On being exposed to stressful conditions, plants accumulate an array of metabolites, particularly amino acids that have traditionally been considered as precursors as well as constituents of all proteins. Proline, an amino acid, plays a highly beneficial role in plants, exposed to various stress conditions. Besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule. Stressful environment results in an overproduction of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance; stabilizing membranes thereby preventing electrolyte leakage; and bringing concentrations of reactive oxygen species (ROS) within normal ranges, thus preventing oxidative burst in plants. Proline, when supplied exogenously at lower concentrations to plants exposed to various biotic and abiotic stresses, results in stress mitigation thereby enhancing growth and activating other physiological processes of plants. Exogenous proline maintains the turgidity of the cells and also enhances photosynthesis during the times of stress. Lower concentrations of proline are known to provide tolerance against the damaging effects of salinity, drought, irradiance or heavy metal stress (Ashraf and Foolad, 2007). Moreover, the exogenous proline improves the activity of antioxidative enzymes viz. CAT, POX, SOD, etc. and also the enzymes of nitrogen metabolism (Hoque *et al.*, 2007a).

Keeping in view the above recognized roles, assigned to BRs and proline and the ever increasing salinity stress in soil, the present studies were designed with an objective to relate the changes in growth, photosynthetic parameters and the level of antioxidative enzymes, in salinized plants of *Brassica juncea* (L.) Czern & Coss with the BRs and proline induced resistance. The hypothesis that is put to trail is that the

application of brassinosteroids and proline will ameliorate the toxic effects of salinity on the growth and yield of the test plant, *Brassica juncea* (L.) Czern & Coss, which is widely cultivated throughout the world and is accepted by the local farmers as a cash crop.

The following objectives were kept in mind while planning the experiments:

1. To establish the tolerant and resistant levels of salinity on mustard.

2. To observe the effect of foliage applied HBL/EBL on mustard plants.

3. To screen out the best concentration of proline for foliar spray to the given mustard cultivars.

4. To observe the impact of leaf applied HBL/EBL on mustard plants, raised in the soil amended with three doses of NaCl.

5. To observe the response of mustard plants, raised in the soil treated with three doses of NaCl, to the leaf applied proline.

6. To observe the cumulative effect of BR and proline on mustard plants, raised in the soil treated with three doses of NaCl.

7. To select the growth, physiological and biochemical traits showing maximum response to the treatment that may be designated as a marker to forecast the growth and crop productivity, or to ensure corrective measures.

Review of Literature

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REVIEW OF LITERATURE

2.1 SALT STRESS

Various environmental factors negatively affect plant growth and development and finally the biological yield of the crop, at harvest. These factors include salinity, drought, heavy metal toxicities and temperature extremes which limit the crop productivity worldwide. Salinity is one of the most important abiotic stress, limiting crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation may be insufficient for leaching (Zhao *et al.,* 2007). Soil salinity can be defined as the concentration of dissolved mineral salts in soil solution as a unit of volume or weight basis (Ghassemi *et al.,* 1995) whereas, the sodicity expresses the presence of sodium, attached (exchangeable) to clay particles (plates) of the soil matrix. Sodic soils, by definition, contain excessive concentrations of exchangeable sodium (Bernstein, 1975). Sodicity differs from salinity by being specific to only one salt (sodium) rather than a range of salts and it is a measure of ions on clay surfaces rather than in the solution. Because sodium chloride (NaCl) is the dominant salt in alkaline soils, therefore sodium exists in the soil solution as well as on clay surfaces. Consequently, salinity and sodicity usually occur together. Soil salinity stresses the plants in two ways: higher concentrations of salts in the soil make it harder for roots to extract water (osmotic stress), and secondly high salt level within the plant may be harmful (specific ion toxicity) (Munns and Tester, 2008; Hussain *et al.,* 2008). According to the Food and Agriculture Organization (FAO) Land and Nutrition Management Service (2008), over 6% of the world's land is affected by either salinity or sodicity which accounts for more than 800 million ha of land. Low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices are among the major contributors to the increasing salinity. Secondary salinization, in particular, exacerbates the problem where once productive agricultural lands are becoming unfit to cultivation due to poor quality irrigation water. Salt stress has various damaging effects on plant physiological processes such as increased respiration rate, ion toxicity (Sudhir and Murthy, 2004), changes in C and N metabolism (Kim *et al.,* 2004a), mineral distribution, membrane instability (Marschner, 1986), membrane permeability (Gupta *et al.,* 2002) and decreased

efficiency of photosynthesis (Hayat *et al.,* 2010c), reduced leaf area, dry mass and stomatal conductance which ultimately lead to decrease in the plant productivity.

2.1.1 Salt stress and plant growth

Salinity stress has a major impact on plant growth and development. Processes such as seed germination, seedling growth and vigour, vegetative growth, are adversely affected by high salt concentration, that ultimately cause poor plant growth (Sairam and Tyagi, 2004). Plant growth responds to salinity in two phases: a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves. Salt stress is a condition where excessive salts in soil solution cause inhibition of plant growth or even their death. The effects of salt stress on the plant growth of various species are summarised in the table 1.

Table 1: Effect of salt stress on plant growth in various plant species

Plant species	Response	References
Brassica juncea	Significantly decreased the growth	Hayat *et al.,* 2011a; Ahmad *et al.,* 2012
Lycopersicon esculentum	Growth in the plants, subjected to saline stress was reduced	Hayat *et al.,* 2010c; Tantaway *et al.,* 2009
Juglans regia	Fresh shoot weight, dry shoot and root weight decreased with increasing salt stress	Akca and Samsunlu, 2012
Ocimum basilicum	Fresh weight decreased significantly with an increase in salinity level	Heidari, 2012
Cajanus cajan	Salt stress caused significant reduction in the growth of the plants	Amuthavalli and Sivasankaramoorthy, 2012
Eleusine coracana	Reduction in the growth of stressed plants	Manikenda and Desingh, 2009
Solanum tuberosum	Reduction in the growth of stressed plants	Daneshmand *et al.,* 2010
Kochia scoparia	Dry matter reduction at 23.1 dsm^{-1} level of saline stress	Nabati *et al.,* 2011

Plant species	Response	References
Helianthus annuus	Salt induced reduction in growth of the plants	Akram *et al.*, 2009; Akram and Ashraf, 2011
Populous alba	The growth of the plants was reduced	Imada and Tamai, 2009
Fragaria ananassa	Salt induced reduction in plant growth	Keutgen and Pawelzik, 2009
Morus alba	Decrease in the growth of the plants, subjected to salt stress	Ahmad and Sharma, 2010
Abelmoschus esculentus	Significantly reduced growth of the plants	Saleem *et al.*, 2011
Panicum miliaceum	Reduction in the growth of the plants	Sabir *et al.*, 2011

2.1.2 Salt stress and photosynthesis

Growth of the plants is dependent on the level of photosynthates and, therefore, environmental stresses affecting photosynthesis also affect growth (Taiz and Zeiger, 1998; Manikandan and Desingh, 2009). A positive relationship between photosynthetic capacity and growth has already been reported for various plants, grown under saline conditions e.g., *Abelmoschus esculentus* (Saleem *et al.*, 2011), *Triticum aestivum* (James *et al.*, 2002), *Zea mays* (Crosbie and Pearce, 1982), *Asparagus officinalis* (Faville *et al.*, 1999), *Gossypium hirsutum* (Pettigrew and Meredith, 1994), *Phaseolus vulgaris* (Seemann and Critchley, 1985) and *Cynodon dactylon* (Akram *et al.*, 2007).

Iyengar and Reddy (1996) attributed the decrease in photosynthetic rate to salinity stress induced following factors:

1. Dehydration of cell membranes which reduce their permeability to CO_2 due to osmotic stress caused by high salt concentration in soil and water inactivating the photosynthetic electron transport via shrinkage of intercellular spaces.

2. Specific ion toxicity caused particularly by Na^+ and Cl^- ions. Cl^- inhibits photosynthetic rate through its inhibition of NO_3-N uptake by the roots (Banuls *et al.*, 1991).

3. The reduction in CO_2 supply because of the closure of stomata results in restricting the availability of CO_2 for carboxylation reactions (Brugnoli and Bjorkman, 1992) and also minimizes the loss of water through transpiration and this affects light-harvesting and energy-conversion systems thus leading to decrease in chloroplast activity.

4. Enhanced leaf senescence, induced by salinity

5. Changes in enzyme activity, induced by the alterations in cytoplasmic structure

6. Negative feedback by reduced sink activity.

The reduction in photosynthetic rate and its related attributes has been observed in various crops which is summarised in table 2.

Table 2: Effect of salt stress on various photosynthetic attributes in different plant species

Plant species	Response	References
Solanum melongena	Photosynthetic gas-exchange parameters (P_N, g_s, E and C_i) were significantly reduced due to salt stress	Wu *et al.*, 2012; Shaheen *et al.*, 2012
Iris lactea	Photosynthetic characteristics dropped significantly in response to salt stress	Wen-Yuan *et al.*, 2012
Cucumis sativus	Salt stress caused severe reduction of net photosynthetic rate	Shu *et al.*, 2012
Brassica juncea	Salt stress significantly decreased the net photosynthetic rate, transpiration rate and stomatal conductance	Ahmad *et al.*, 2012; Hayat *et al.*, 2011a
Lycopersicon esculentum	Salt stress either applied through soil or as seed soaking significantly decreased the net photosynthetic rate and its related attributes	Hayat *et al.*, 2010c
Chenopodium quinoa	The net photosynthesis decreased under high salinity	Eisa *et al.*, 2012

Plant species	Response	References
Aster tripolium	The net photosynthetic rate greatly decreased by salt stress	Geissler *et al.*, 2009
Laguncularia racemosa	Salinity decreased the P_N due to stomatal closure and low leaf internal CO_2 conductance	Sobrado, 2005
Vicia faba	Salinity decreased P_N due to stomatal closure and other non-stomatal effects like ultra-structural damage and decrease in chlorophyll content	Melesse and Caesar, 2008
Phaseolus vulgaris	Photosynthetic rate and stomatal conductance decreased gradually with salinity in salt-sensitive species	Bayuelo-Jimenez *et al.*, 2012
Phragmites australis	The P_N, g_s and E decreased significantly with an increase in NaCl concentration	Gorai *et al.*, 2010
Catharanthus roseus	The chlorophyll content, photosynthetic activity and transpiration rate declined with the increase in salinity	Elfeky *et al.*, 2007
Shepherdia argentea	Plants treated with high level of salinity suffered from photo-inhibition which lead to lowest P_N, g_s and C_i values	Qin *et al.*, 2010
Triticum aestivum	P_N, g_s, and E were reduced in response to NaCl	Sharma *et al.*, 2005
Jatropha curcas	The salt stress caused reduction in leaf gas exchange parameters	Nascimento da Silva *et al.*, 2011
Pisum sativum	The salt stress caused decrease in the stomatal conductance	Hernandez and Almansa, 2002
Atriplex portulacoides	Reduction in P_N was accompanied by lower g_s and C_i values in response to salt stress	Redondo-Gomez *et al.*, 2007

Plant species	Response	References
Triticum aestivum and *Hordeum Vulgare*	Increase in NaCl concentration in the nutrient solution resulted in stomatal closure and decreased net CO_2 assimilation rate	Dulai *et al.*, 2011

2.1.3 Salt stress and the level of ions and nutrient content

The salt ions like Na^+, Cl^-, SO_4^{-2} present in the soil compete with the uptake of other nutrient ions like K^+, Ca^{2+} etc. which results in the nutritional disorder and eventually leads to reduce the quality and yield of plants (Grattan and Grieve, 1999). Higher NaCl concentration has been reported to increase the level of Na^+ and Cl^- ions and decrease those of Ca^{2+}, K^+ and Mg^{2+} in various plants (Bayuelo-Jimenez *et al.*, 2003). In the plant cells under the normal conditions (non-saline), there is 100 to 200 mM K^+ and 1 to 10 mM Na^+, an environment in which the enzymes function optimally. However, the higher ratio of Na^+ to K^+ and accumulation of total salts at an elevated level inactivate these enzymes and inhibit protein synthesis. Moreover, Na^+ displaces Ca^{2+} from the cotton root hair plasma membrane, resulting in the change in membrane permeability that can be noticed by the leakage of K^+ from the cells (Cramer *et al.*, 1985). Decrease in the content of Ca^{2+} and Mg^{2+} in the leaves of *Brugueira parviflora* has also been reported under salt accumulation (Parida *et al.*, 2004). Plant acquisition and utilization of necessary nutrients particularly that of K^+ and Ca^{2+} may also impair under saline conditions (e.g. ion deficiency) causing changes in the ratios of K^+/Na^+ and Ca^{2+}/Na^+, thus further lowering the growth and productivity of plants (Zhu *et al.*, 2001). Some ions also serve as buffer to counter the effect of salinity on the accumulation of other ions. For example, when excess Ca^{2+} or NH_4 is added to the growth medium containing high salinity, growth and nutrient accumulation can be stimulated, compared to the control (Cramer, 2002).

2.1.4 Salt stress and plant water relations

As the salinity increases in the soil, its water potential decreases (Meloni *et al.*, 2001; Gulzar *et al.*, 2003) which reduces water uptake leading to slower growth (Munns *et al.*, 2006). The plants ultimately get stunted (Wang and Nil, 2000), where plant height, fresh and dry mass of leaves, stem, and roots and yield are reduced (Ali Dinar *et al.*, 1999; Chartzoulakis and Klapaki, 2000). However, at low or moderate salt

concentration, plants adjust osmotically by accumulating solutes, thereby lowering the water potential and maintain a potential gradient for the influx of water. Leaf water potential decreases in response to salt stress in *Chenopodium quinoa* Willd. (Eisa *et al.*, 2012), *Shepherdia argentea* (Qin *et al.*, 2010) and *Iris lacteal* (Wen-Yuan *et al.*, 2012). It has been reported that significant decrease in relative water content (RWC) occurred in response to salt stress in *Beta vulgaris* (Ghoulam *et al.*, 2002) and *Brassica* species (Singh *et al.*, 2010). Water use efficiency (WUE) also decreased with increasing levels of NaCl in *Thymus vulgaris* L. (Najafian *et al.*, 2009) and *Brassica juncea* (Hayat *et al.*, 2011a).

2.1.5 Salt stress and antioxidant system in plants

Salinity and drought stress are well known to induce oxidative stress through the production of superoxide radicals by the process of Mehler reaction. These free radicals initiate the chain of reactions that produce more harmful oxygen radicals (Hsu and Kao, 2003). These reactive oxygen species (ROS) are continuously generated during normal metabolic processes in mitochondria, peroxisomes and cytoplasm which disturb normal metabolism through oxidative damage of lipids, proteins, and nucleic acids when produced in excess (Hernandez *et al.*, 2001; Ahmad *et al.*, 2010). Plants throughout their life are prone to oxidative damage caused by environmental factors due to their sessile nature (Hippeli and Elstner, 1996). There is a constant need for efficient mechanisms to compensate the possible oxidative damage to cellular components. Plants have evolved efficient systems for ROS removal, which include specific ROS-scavenging antioxidative enzymes and small non-enzymatic molecules that act as ROS scavengers such as ascorbate, glutathione, α-tocopherol, flavonoids, anthocyanines, polyphenolic compounds and carotenoids.

To overcome salt-mediated oxidative stress, plants detoxify ROS by up-regulating antioxidative enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPX). In plants, superoxide dismutase scavenges superoxide anions and converts them to hydrogen peroxide (Alscher *et al.*, 2002). Catalase, the second line of defence, converts lethal hydrogen peroxide to water and molecular oxygen. Another versatile antioxidant enzyme is ascorbate peroxidase which utilizes ascorbate (AsA) as electron donor and scavenges H_2O_2 in water-water and ascorbate glutathione

cycles. Hydrogen peroxide is reduced to water by APX and thus plays a vital role in cell defense mechanism (Kangasjarvi *et al.*, 2008; Ashraf, 2009). GR catalyses the NADPH-dependent reduction of oxidized glutathione (GSSG) to its reduced (GSH) form (Meister, 1988). GR activity is thought to increase the ratio of $NADP^+/NADPH$. The $NADP^+$ accepts electrons from the photosynthetic electron transport chain (Bishop, 1971). Thus, the flow of electrons to O_2 and the formation of $O_2^{\cdot-}$ can be minimized. The harmonized activities of the multiple forms of these enzymes in different subcellular compartments achieve a balance between the rate of formation and removal of ROS, and sustain H_2O_2 at the required levels for cell signalling. It is now widely accepted that the degree of oxidative cellular damage in plants, exposed to abiotic stress is controlled by the operative capacity of the antioxidative systems (Turkan *et al.*, 2005). A correlation between antioxidant capacity and salinity tolerance has been reported in several plant species such as *Oryza sativa* (Demiral and Turkan, 2005), *Beta vulgaris* (Bor *et al.*, 2003), *Lycopersicon esculentum* (Hayat *et al.*, 2010c), *Sesamum indicum* (Koca *et al.*, 2007), *Portulaca oleracea* (Yazici *et al.*, 2007), *Plantago maritima* (Sekmen *et al.*, 2007), *Brassica napus* (Ashraf and Ali, 2008), *Populus cathayana* (Yang *et al.*, 2009a), *Helianthus annuus* (Noreen *et al.*, 2009), *Panicum miliaceum* (Sabir *et al.*, 2011), *Triticum aestivum* (Ashraf *et al.*, 2010a), and *Carthamus tinctorius* (Siddiqi, 2010). Furthermore, transgenic plants overexpressing ROS-scavenging enzymes, such as SOD (Alscher *et al.*, 2002), APX (Wang *et al.*, 1999), GR (Foyer *et al.*, 1995) and GPX (Roxas *et al.*, 1997, 2000) showed enhanced tolerance to osmotic, temperature, photo-inhibition and oxidative stresses. Proline accumulation is believed to improve adaptation of plants to salt and drought stresses by scavenging free radicals and stabilizing membranes to maintain the conformation of proteins under stress conditions (Chen and Dickman, 2005). Proline is also reported to play a significant role in reducing the photo-damages of thylakoid membranes by scavenging the superoxide radicals (Ashraf and Foolad, 2007; Banu *et al.*, 2009). Proline accumulation under dehydrated conditions is mainly due to increased biosynthesis and decreased degradation. Enhanced synthesis of proline under drought or salinity conditions has been involved in the alleviation of the stress in various plants such as *Cynodon dactylon* (Hameed and Ashraf, 2008), *Pisum sativum* (Noreen and Ashraf, 2009), *Brassica juncea* (Hayat *et al.*, 2011a), *Saccharum officinarum* (Chaum and Kirdmanee, 2009), *Panicum miliaceum* (Sabir *et al.*, 2011),

Lycopersicon esculentum (Hayat *et al.*, 2010c). The ROS regulatory pathways are very flexible and degree of redundancy and cross talk between different branches of ROS network, as well as the way in which the network senses and the components through which ROS signals transmit need further investigation.

2.1.6 Salt stress and yield

Abiotic stresses are the major factors for reducing the crop yield (Munns and Tester, 2008; Reynolds and Tuberosa, 2008). Specifically, salt stress has been reported to cause substantial yield losses in the agriculture world-wide (Ashraf *et al.*, 2008). Bray and his co-workers (2000) reported that salinity and drought reduce the yield potential of annual crops by 51-82%. Similarly, Ashraf *et al.* (2008) reported that high salt level in soils cause a significant reduction in the yield of a wide variety of crops world over. Different yield components (pod number per plant, seeds per pod and seed weight) of *Vigna radiata* were significantly affected by salinity stress (Nahar and Hasanuzzaman, 2009). These components were negatively correlated with salinity levels. In *Oryza sativa*, grain yield was lost significantly by different salinity levels (Hasanuzzaman *et al.*, 2009; Mahmood *et al.*, 2009). Furthermore, Rad *et al.* (2012) reported that the salinity levels significantly decreased the filled panicle length, number of filled grains per filled panicle, number of spikelets per filled panicle and total number of spikelets per panicles in *Oryza sativa*. The salt stress reduced umbel number per plant, 1000 seed weight and see yield in *Foeniculum vulgar* Mill. (Rahimi *et al.*, 2012), grain yield in *Phaseolus vulgaris* (Ghassemi-Golezani *et al.*, 2012) and *Triticum aestivum* (Asgari *et al.*, 2012). This reduction may be attributed to low production, expansion, senescence and physiologically less active green foliage (Wahid *et al.*, 1997), and reduced photosynthetic rate (Seemann and Critchley, 1985). Moreover, reduced viability of pollen under stress condition could result in the failure of seed set (Abdullah *et al.*, 2001).These reports suggest that salt stress is one of the key challenges to crop production, and thus some specific means should be devised to improve crop productivity on saline soils and it should be given a major research priority. Although a multitude of means have already been suggested to improve crop salt tolerance, an integrated approach comprising conventional breeding and molecular marker-assisted breeding techniques seems to be more effective in improving crop salt tolerance (Munns and Tester, 2008).

2.2 PLANT GROWTH REGULATORS

Plant growth regulators (PGRs) have been used to promote plant growth and productivity under various stress conditions (Arora *et al.*, 2008; He and Zhu, 2008). The endogenous concentrations of various phytohormones are influenced by numerous stimuli (Wang *et al.*, 2005), thereby modify various signal transduction pathways. Such alterations cause serious metabolic disorders leading to the inhibition of plant growth and development under stress conditions (Lerner and Amzallag, 1994). It has been reported that salt-stress causes reduced synthesis and also degradation of phytohormones (Kuiper *et al.*, 1988). Under environmental stress, however, exogenous application of PGRs, either through the seed treatment before planting or to the growing plants, may overcome much of their internal deficiency and may lead to healthy growth (Ashraf *et al.*, 2008). Therefore, exogenous application of many natural and synthetic hormones appears to improve plant salt tolerance, or at least partially reduce the salt-induced harmful effects. However, till date it is not clear that how the exogenously applied PGRs improve plant tolerance, against stress. Like many other PGRs, brassinosteroids (BRs) and proline play vital roles in promoting growth and development of plants, exposed to saline conditions, by modulating a number of metabolic phenomenons. The application of BRs ameliorates the inhibitory effects of several abiotic stresses, including salt-stress, in different plant species (Jason *et al.*, 2006; Ali *et al.*, 2007a). Like BRs, exogenously applied proline also plays an important role in enhancing plant tolerance to various stresses including the salt stress (Yancey, 1994; Yan *et al.*, 2000). Any approach which reduces the adverse effects of salt stress on agricultural productivity will be a boon to the agriculturalists and farmers worldwide. In this article we review how the exogenous application of BRs and proline ameliorate the inhibitory effects of salt stress. We will also discuss the possible mechanisms of action of these exogenously applied substances to the salt stressed plants.

2.2.1 Brassinosteroids

Brassinosteroids represent a new group of phytohormones with wide occurrence in the plant kingdom. BRs are a class of polyhydroxy steroidal lactones that play essential roles in plant development (Hayat *et al.*, 2010d). They also play a significant role in the amelioration of various biotic and abiotic stresses such as cold, salt, oxidative damage, temperature, heavy metals and pathogen attack.

Mitchel *et al.* (1970) screened the pollens of nearly 60 plant species out of which extracts of about 30 species were the rich source for growth promoting substances. These growth promoting substances were named as brassins (defined as crude lipid extract from rapeseed pollen). In 1972, Mitchel and Gregory showed that brassins enhanced the seed vigour and crop productivity. In order to isolate the active component in brassin, 500 pounds of bee collected rape seed pollen was extracted and purified. The resulting 10 mg pure crystalline material was identified as active component of brassins and named as brassinolide (Grove *et al.*, 1979). The first plant steroidal hormone brassinolide (BL) was later named a brassinosteroid (Rao *et al.*, 2002). After the discovery of BL, second steroidal hormone castesterone was discovered (Yokota *et al.*, 1982). Since then numerous analogues have been discovered and isolated from various plant species out of which approximately 70 are fully characterised till date (Rao *et al.*, 2002; Bajguz and Tretyn, 2003) in 37 angiosperms (9 monocots and 28 dicots) and 5 gymnosperms (Bajguz 2007).

This group of polyhydroxy steroidal lactones have common 5α-cholestane skeleton which vary in their chemical structure and orientation of functional groups on the skeleton (Fujioka and Sakurai, 1997; Zullo and Adam, 2002). Several authors have reported the following criteria for an active BR: they must have a trans A/B ring system with a 5 α-hydrogen; must have a cis oriented hydroxyl groups at C22 and C23 plus a methyl or ethyl at C24. Moreover, the α-orientation at C22, C23 and C24 are more active than β-oriented groups (Arteca *et al.*, 1985; Takasuto *et al.*, 1983, Cutler, 1991). Conjugated forms of BRs especially with sugars or fatty acids have also been reported (Zullo and Adam, 2002; Zullo *et al.*, 2002).

2.2.1.1 Biosynthesis

Among all BRs identified till date, BL is the most biologically active compound and has been found in a large number of plant species (Kim *et al.*, 2008). BL, a 28 carbon molecule possess S-methyl group at C24 of the side chain of 5α- ergastane structure, which has been the focus of research on BRs. The site for BR biosynthesis in plants is endoplasmic reticulum. The precursors for BR biosynthesis are the sterols, namely campasterol, sitosterol and cholesterol (Kim *et al.*, 2004b; Taiz and Zeiger, 2006). Campasterol and sitosterol are abundantly found in plant membranes. However, the most common biosynthetic pathway originates from campasterol (derived from

isopentenyl disphosphate). Campesterol is converted to campestenol by involving DET2 (de-etiolated-2) enzyme, 5-α-reductase (Plate I). Campestenol is then converted to castasterone, the immediate precursor of BL through two analogue pathways (early and late C-6 oxidation pathway). In early C-6 oxidation pathway, the oxidation at C-6 of the B ring occurs before the hydroxylation at C22 and C23 of the side chain (Fujioka *et al.*, 2002) whereas in late, the oxidation at C6 occurs after the hydroxylation at the side chains and C2 of the ring A (Taiz and Zeiger, 2006). The scheme of BR biosynthesis is shown in the Plate I.

In addition to these two main pathways, several cross links occur between these branches at various steps forming intricate BR biosynthetic network. However, in addition to above pathways Divi and Krishna (2009) reported two other branching pathways viz early C22 oxidation pathway and a shortcut pathway from campesterol to 6-deoxytyphasterol involving C23 oxidation. The C22 and C23 hydroxylation and C6 oxidation reactions play a pivotal role in the regulation of BR biosynthesis. Therefore, the enzymes catalysing these reactions have gained much importance by researchers to study BR metabolism (Choe, 2006; Divi and Krishna, 2009).

2.2.1.2 Signalling

Since the discovery of BRs as a new class of phytohormones, extensive research has been carried out at molecular and biochemical levels to study BR signalling in plants. It has been revealed in *Arabidopsis thaliana* that BR signal transduction pathway starts from ligand perception on the cell membrane to gene expression in the nucleus. BRs are perceived by plasma membrane localised leucine rich repeat (LRR)-receptor like kinase (RLK) BRI1 (brassinosteriod insensitive 1). In general, BRI1 protein possesses three major domains with unique function in BR perception and receptor activation: a large extracellular domain, a small trans-membrane domain and intercellular kinase domain. Both LRR (located externally) and kinase (located internally) domains of BRI1 receptor are necessary for effective transmission of signals (Taiz and Zeiger, 2006). The auto-phosphorylation and association of BRI1 with second membrane localised receptor BAK1 makes it active after binding to BL. BAK1 is another receptor kinase which acts as a co-receptor with BRI1 (Cano-delgado *et al.*, 2004). Due to physical interactions and transphosphorylation of BAK1, it is required to positively regulate BRI1 (Chinchilla *et al.*, 2009). However it is still not clear whether BAK1 activates the BR receptor or link the receptor with
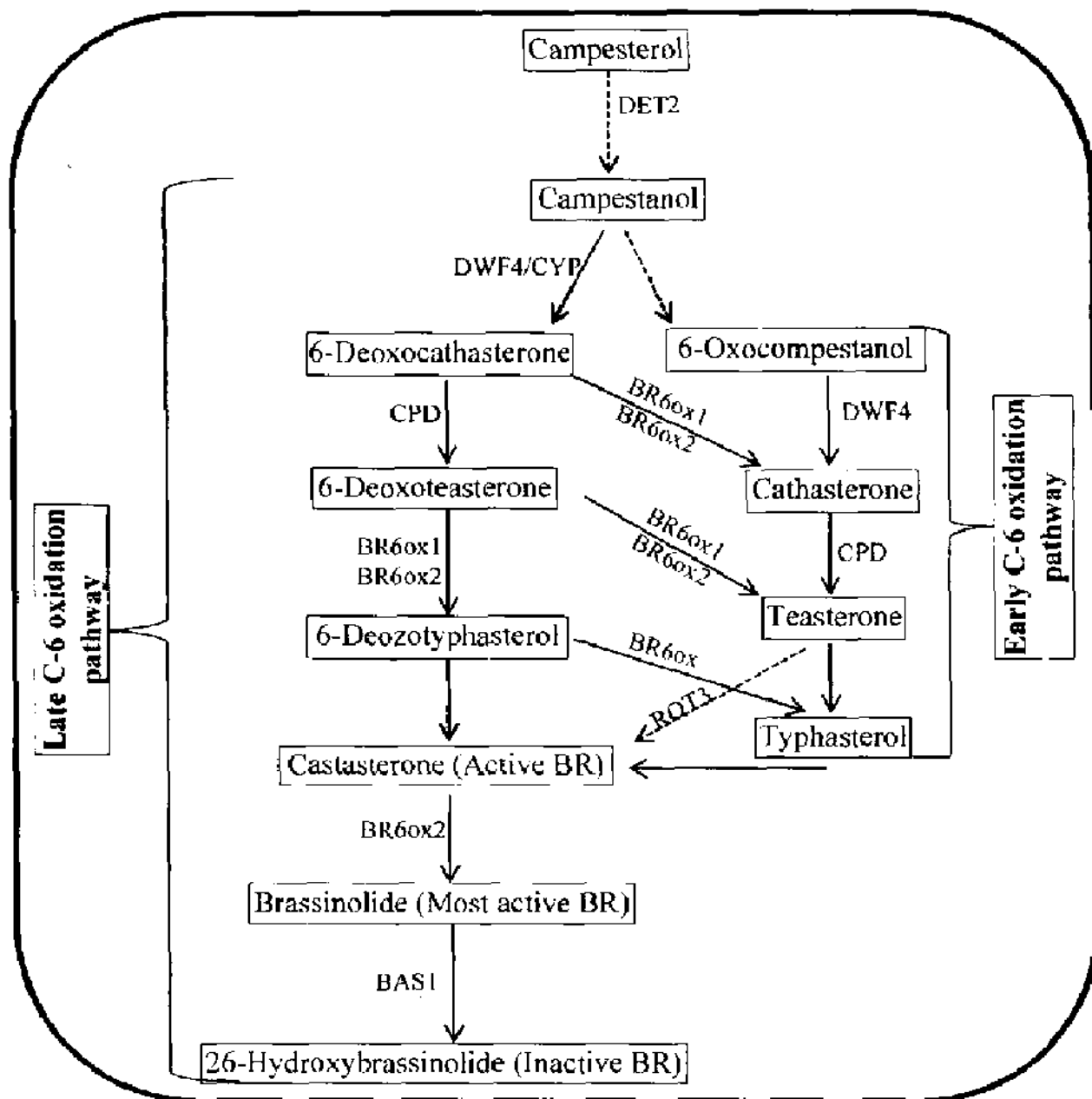
Plate I: Diagrammatic representation of brassinosteroids biosynthesis

DET2	DE-ETIOLATED-2
DWF4	DWARF4
CYP	Cytochrome P450
CPD	CONSTITUTIVE AND PHOTOMORPHOGENESIS AND DWARFISM
BR6ox	Brassinosteroid-6-oxidase
BAS1	phyB Activation-tagged Suppressor1
ROT3	ROTUNDIFOLIA3

downstream signalling cascades or promote receptor endocytosis (Vert, 2009). In plants BR-dependent activation of BRI1 preceded its association with BAK1 and BRI1 positively regulated BAK1 phosphorylation levels (Wang et al. 2008). BAK1 also transphosphorylates BRI1 increasing its kinase activity towards a specific substrate. However, Li and Jin (2007) reported that BR downstream signalling may be blocked by BKI1 (BRI1 kinase inhibitor 1), which inhibits the BRI1 with BAK1 association. In recent years, extensive research work on BR signalling led to the identification of three homologous plasma membrane bound BR-signalling kinases (BSK1, BSK2, and BSK3) which act as the substrates for BRI1 kinase (Tang *et al.,* 2008). These are actively involved in triggering BR signalling downstream of BRI1. The general pathway of BR signal transduction and its mediated cellular and phenotypic changes have been summarised in the Plate II.

In the absence of BRs, BRI1 remains in inactive state by the presence of BKI1. BIN2, a GSK3 kinase repressor protein which is present in nucleus, cytoplasm and plasma membrane phosphorylates two nuclear transcription factors, BZR1 (brassinozole-resistant 1) and BZR2/BES1 (bri1-EMS suppressor 1), thereby inhibits their activities. Therefore, BZR1 and BZR2/BES1 association with other proteins or transcription factors is inhibited making them non-functional transcription factors (Vert and Chory, 2006). This BZR1/BES1 phosphorylation catalysed by BIN2 not only prevents DNA binding but also enhances binding to the 14-3-3 proteins (phosphopeptide-binding proteins highly conserved in all eukaryotes) (Gampala *et al.,* 2007). However, after binding of BR to BRI1 receptor, phosphorylation of both BRI1 and BAK1 occurs which induces the BR response through the inactivation of BIN2. The inactivation of BIN2 results in the accumulation of biologically active form of BZR1 and BES1 (dephosphorylated). Dephosphorylation occurs due to the activity of BSU1 (bri1 suppressor 1). The dephosphorylated BES1 and BZR1 activate or inhibit BR-regulated genes. The dephosphorylated BES1 along with three BIM (BES1-interacting Myc-like 1) transcription factors bind to E-box motif (CANNTG) in the SAUR-AC1 promoter to trigger gene expression, whereas BZR1 recognizes the BR-response element [CGTG(T/C)G] and acts as a go-between in the feedback inhibition of a number of genes involved in BR biosynthesis (Li and Jin, 2007; Divi and Krishna, 2009).

2.2.1.3 Physiological roles

2.2.1.3.1 Brassinosteroids and seed germination

It is now well established that BRs promote seed germination like gibberellic acid (Leubner-Metzger, 2003). Seeds of several plant species have been found to contain endogenous BRs (Yokota *et al.*, 1996; Friebe *et al.*, 1999). BRs promote the rupture of endosperm in *Nicotiana tabacum* in dose dependent manner (Leubner-Metzger, 2001). It is proposed that BRs promote seed germination by directly enhancing the growth potential of the emerging embryo in a GA and βGLU I (I β-1,3-glucanase)-independent manner. It has been reported that BRs promote seed germination in wheat (Hayat and Ahmad, 2003), tobacco (Leubner-Metzger, 2001), tomato (Vardhini and Rao, 2000) and groundnut (Vardhini and Rao, 1997). Pre-treatment with brassinolide stimulated germination and seedling emergence of aged rice grains (Yamaguchi *et al.*, 1987). Seeds soaked in BRs showed an increase in α-amylase activity (Hayat and Ahmad, 2003). It has been shown that application of BRs enhanced the germination of certain parasitic angiosperms (Takeuchi *et al.*, 1995), cereals (Yamaguchi *et al.*, 1987) and *Arabidopsis* (Steber and McCourt, 2001). In *Arabidopsis* it was shown that BR signal reverses the ABA-induced dormancy thus stimulating the germination in *Arabidopsis* (Steber and McCourt, 2001).

Brassinosteroids have also been shown to rescue seed germination and seedling growth of *Arabidopsis thaliana* and *Brassica napus* under salt stress (Kagale *et al.*, 2007). Application of epi-brassinolide (EBL) resulted in the improved seed germination and seedling growth of *Eucalyptus camaldulensis* under saline stress (Sasse *et al.*, 1995). The ability of BRs to improve seed germination and seedling growth in various plant species under various abiotic stresses has been reviewed by Bajguz and Hayat (2009). Furthermore, there are reports demonstrating that BRs have crosstalk with other plant hormones. For instance, Hansen *et al.* (2009) demonstrated that BRs stimulate ethylene evolution in *Arabidopsis* by stabilizing ACS protein. BRs have also been shown to play a regulatory role in root development by interacting with auxin (Mussig *et al.*, 2003; Bao *et al.*, 2004). Zhang *et al.* (2007) reported that BRs improved the germination ability, root length, root vigour, root dry weight and shoot fresh and dry weight under high salinity doses.
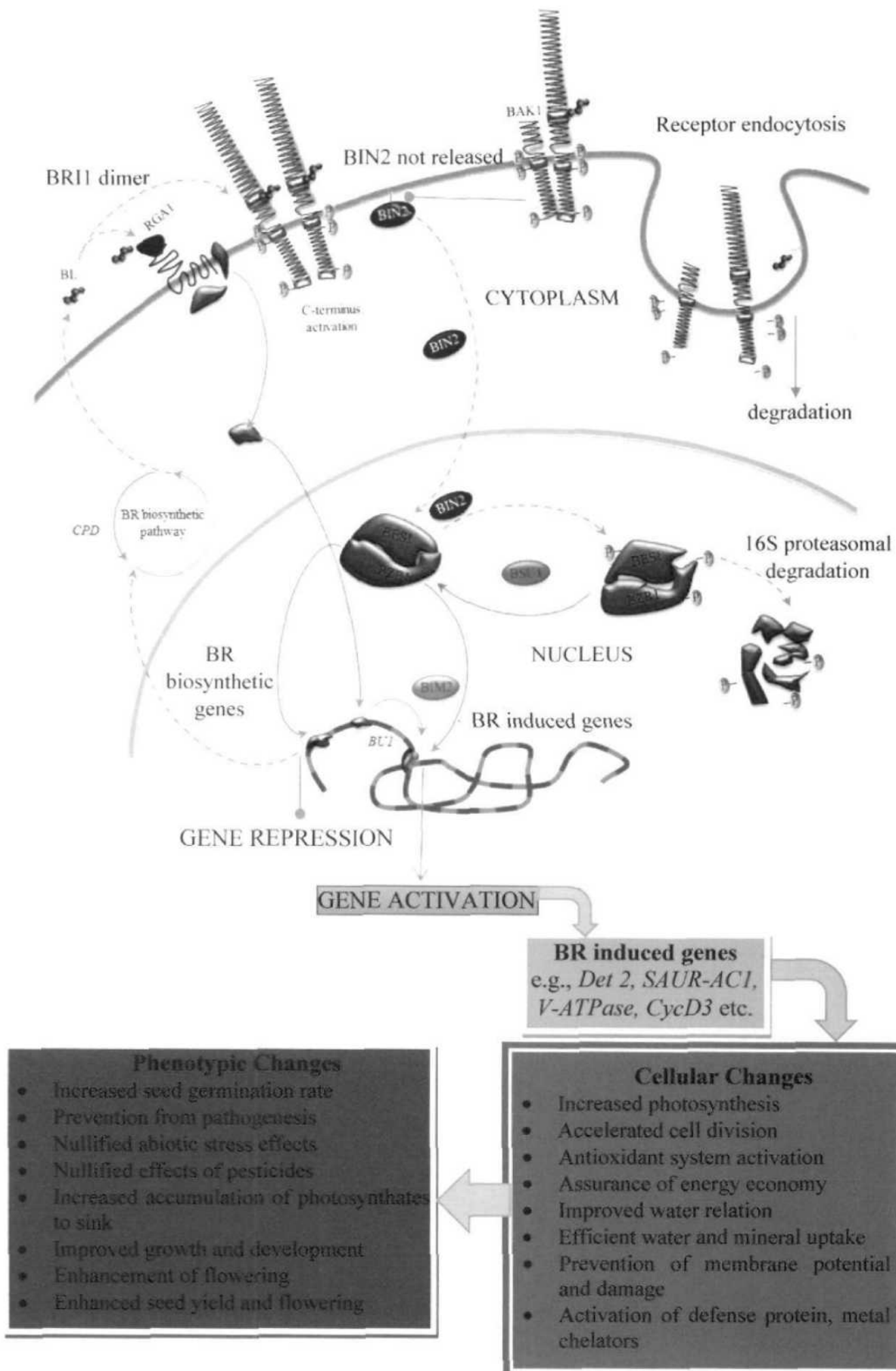
Plate II Signal transduction pathway of Brassinosteroids and BR mediated cellular and phenotypic changes

2.2.1.3.2 Brassinosteroids and photosynthesis

The main source of carbon assimilation in plants is photosynthesis. Increasing either photosynthetic efficiency or total plant photosynthetic capacity (delaying leaf senescence) results in the enhanced photo-assimilate production (van Camp, 2005). It is now well documented that brassinosteroids increase the net photosynthetic rate and the related attributes in various plant species (Hayat *et al.*, 2010d). There is a lot of literature available where the effects of BRs on photosynthesis and its related attributes has been examined but a lot of diversity exits among these observations. The information regarding the effect of brassinosteroids on photosynthesis and relates attributes has been summed up in the table 3.

Table 3: Effect of brassinosteroids on various photosynthetic attributes in different plant species

BR treatments	Mode of application	Plant species	Responses	References
BL	Foliar spray	*Vigna radiata*	Improved P_N, gs, Ci, Carboxylation efficiency and Fv/Fm	Hayat *et al.*, 2010e
HBL	Foliar spray	*Cucumis sativus*	Alleviated the loss of P_N, F_v/F_m, Φ_{PSII}, q_P under pesticide stress	Xia *et al.*, 2009
HBL	Foliar Spray	*Triticum aestivum*	Significant increase in photosynthetic rate	Sairam, 1994
BL	Foliar spray	*Triticum aestivum*	Increased activity and the content of Rubisco	Braun and Wild, 1984
HBL	Seed soaking /Foliar spray	*Triticum aestivum*	Significantly improved net photosynthetic rate	Sairam, 1994
HBL	Foliar Spray	*Brassica juncea*	Increase in photosynthetic rate	Hayat *et al.*, 2000,2001; Fariduddin *et al.*, 2009b
HBL	Seed soaking	*Lycopersicon esculentum*	Enhancement in photosynthetic rate	Hayat *et al.*, 2010a

BR treatments	Mode of application	Plant species	Responses	References
BL	Seed soaking	*Raphanus sativus*	Showed positive effect on net photosynthetic rate and stomatal conductance	Anuradha and Rao, 2009
EBL in association with GA$_3$	Seed soaking	Spinach	Increase in photosynthetic capacity	Liang *et al.*, 1998
BL	Foliar spray	*Glycine max*	Improved $^{14}CO_2$ assimilation and partitioning, P_N, F_v/F_m, Rubisco activity, phosphoenol-pyruvate carboxylase activity	Zhang *et al.*, 2008
HBL	Foliar spray	*Triticum aestivum*	Increased Fv/Fm	Yusuf *et al.*, 2011
EBL	Foliar spray	*Lycopersicon esculentum*	Alleviated the depressions caused by phenanthrene and pyrene toxicity with a sharp improvement in the activity of photosynthetic machinery	Ahammed *et al.*, 2012
BRs	Foliar spray	*Lycopersicon esculentum*	Improved the activity of photosynthetic machinery	Hasan *et al.*, 2011
EBL	Folair spray	*Lycopersicon esculentum*	Alleviated the decrease of leaf P_N and Fv/Fm	Wang *et al.* 2010
EBL	Foliar spray	*Cucumis sativus*	Increased photosynthetic parameters	Yu *et al.*, 2004
EBL	Seed soaking	*Vicia faba*	Strongly improved P_N, F_v/F_m, F_v'/F_m', q_P, NPQ, ETR, under stress	Pinol and Simón, 2009
EBL	Foliar spray	*Lycopersicon esculentum*	Alleviated the heat induced loss of P_N, C_i, g_s, F_v/F_m, F_v'/F_m', Φ_{PSII}, q_P, NPQ	Ogweno *et al.* 2008

BR treatments	Mode of application	Plant species	Responses	References
FBL	Foliar spray	*Capsicum annuum*	No significant effect on F_v/F_m	Houimli *et al.*, 2008
EBL, HBL	Seed soaking/ Foliar spray	*Oryza sativa*	Improved P_N, C_i, g_s values	Farooq *et al.*, 2009
EBL	Incubation with BRs	*Cucumis sativus*	Stimulated **net** photosynthetic rate	Kang *et al.*, 2009
HBL or EBL	Foliar spray	*Lycopersicon esculentum*	Increased all the photosynthetic attributes	Hayat *et al.*, 2011b
EBL or HBL	Foliar spray	*Coleus forskohlii*	Increased the rate of photosynthesis	Swamy and Rao, 2011

2.2.1.3.3 Brassinosteroids and chlorophyll content

Plentiful of literature is available on the inductive effect of BRs on the chlorophyll content in various plant species. However, the chlorophyll level in the leaves of plants to some extent depends on the mode of BR application. The effect of exogenously applied BRs on chlorophyll content in various crops is given in table 4.

Table 4: Effect of brassinosteroids on chlorophyll content in different plant species

BR treatments	Mode of application	Plant species	Responses	References
HBL	Foliar spray	*Brassica juncea*	Increase in chlorophyll content	Hayat *et al.*, 2001
HBL	Foliar spray	*Vigna radiata*	Enhancement in chl a, b content	Bhatia and Kaur, 1997
EBL	Foliar spray	*Cucumis sativus*	Increase in chlorophyll content	Yu *et al.*, 2004
HBL	Seed soaking	*Vigna radiata*	Chlorophyll content increased	Fariduddin *et al.*, 2008
BL	Incubation in water with BRs	*Zea mays*	Chlorophyll content increased	He *et al.*, 1991
HBL	Foliar spray	*Triticum aestivum*	Higher SPAD value of chlorophyll in Ni stressed and non-stressed plants	Yusuf *et al.*, 2011

BR treatments	Mode of application	Plant species	Responses	References
HBL/EBL	Foliar spray	*Lycopersicon esculentum*	Increase in SPAD value of chlorophyll	Hayat *et al.*, 2011b
HBL	Foliar spray	*Glycine max*	Increase in chlorophyll content	Cevahir *et al.*, 2008
EBL	Incubation with BRs	*Wolffia arrhiza*	Enhancement in chlorophyll content	Bajguz and Asami, 2005
EBL/HBL	Foliar spray	*Coleus forskohlii*	Increase in the levels of chlorophyll pigments	Swamy and Rao, 2011

2.2.1.3.4 Brassinosteroids and cell differentiation

Research studies conducted in various plant species so far have shown that BRs play an active role in vascular tissue differentiation. Low concentration of BRs in the medium resulted in a 10-fold increase in xylem differentiation within 24 hrs (Castle *et al.*, 2003). It was also observed that BR increased cell numbers indicating its role in cell division and differentiation (Clouse and Zurek, 1991). Zurek *et al.* (1994) showed that BRs play a key role in xylem differentiation in soybean epicotyls. Microscopic studies in BR-deficient mutants have revealed that endogenous BRs play an active role in vascular differentiation (Szekeres *et al.*, 1996). It was also reported that BR-deficient *Arabidopsis* mutant *dwf7* showed an increase in the phloem cells at the cost of xylem cells and the number of vascular bundles decreased with irregular spacing between them (Choe *et al.*, 1999). BRs have been shown to be involved in the transition phase between stages II and III in *Zinnia elegans* where secondary wall formation and cell death occurs (Fukuda, 1997). Exogenous application of BRs overcame the inhibitory effects of uniconazole (a putative BR biosynthesis inhibitor) which inhibits differentiation of mesophyll cells into tracheary elements in *Zinnia elegans* (Iwasaki and Shibaoka, 1991). Yamamoto *et al.* (1997) reported that uniconazole suppressed the transcription of genes related to differentiation but could be recovered by BR application. This study suggests that BR are synthesised prior to development of secondary cell wall and cell death and possibly induces entry into this stage (Yamamoto *et al.*, 1997).

2.2.1.3.5 Brassinosteroids and plant stress tolerance

BRs play a crucial role in promoting plant tolerance to a wide range of biotic and abiotic stresses. The application of BRs to improve crop growth and yield under various stress conditions such as drought, salinity, extreme temperatures, nutrient deficiency and toxicity, is well documented (Khripach *et al.*, 2000). It has been reported that BRs alleviated the toxic effects of copper in *Brassica juncea* (Sharma and Bhardwaj, 2007). The supplementation of EBL to radish seedlings (*Raphanus sativus* L.) reduced lead toxicity and enhanced the growth (Anuradha and Rao, 2007). The activities of antioxidant enzymes (CAT, APX, GPX, and SOD) showed an increase in brassinosteroid treated Pb-stressed seedlings which prove the role of BRs in stress tolerance. Brassinosteroids have the ability to regulate the uptake of ions into plant cells which can be used to reduce the accumulation of heavy metals and radioactive elements in plants. Moreover, BRs also minimize the toxic effects and symptoms generated by excess quantity of heavy metals (Bajguz, 2010b). BRs are also known to alleviate the adverse effects of salt-stress on growth, pigmentation, and nitrate reductase activity in rice (Anuradha and Rao, 2003). BRs reduce herbicidal injury in rice (Hamada, 1986). Terbutryn induced reduction in growth was overcome by higher dose of EBL in *Vicia faba* (Pinol and Simon, 2009). EBL was found successful in mitigating the toxic effects of Terbutryn on chlorophyll fluorescence and photosynthetic machinery (Pinol and Simon, 2009). BRs improved seed germination and seedling growth of *Zea mays* (He *et al.*, 1991) and *Cucumis sativus* (Khripach *et al.*, 1999) under chilling stress. Application of 24-epiBL minimally increased freezing tolerance of brome grass (*Bromus inermis*) cells at 3–5 °C, but markedly enhanced cell viability following the exposure to high (40–45 °C) temperature stress. More foliar application of EBL protected tomato plants against polychlorinated biphenyl stress by decreasing harmful ROS accumulation and lipid peroxidation through the induction of antioxidant enzymes which resulted in increased biomass, photosynthetic capacity, chlorophyll contents and alleviation of photo-inhibition by enhancing Fv/Fm, ΦPSII and qP (Ahammed *et al.*, 2013).

2.2.1.3.6 Brassinosteroids and reactive oxygen species

It is evident that BRs are involved in the regulation of reactive oxygen species (ROS) metabolism because they regulate the expression of many antioxidant genes which increase the activity of antioxidant enzymes, like superoxide dismutase (SOD),

peroxidase (POX) and catalase (CAT) (Cao *et al.*, 2005; Ogweno *et al.*, 2008). However, it is still not clear whether BRs directly or indirectly play this role to alter plant responses against oxidative stress (Cao *et al.*, 2005). However, both BRs and ROS act as secondary messengers for the induction and regulation of antioxidant systems in plants under stress (Mazorra *et al.*, 2002), but their association in stress-signal transduction is still unknown. However, detailed research work is going on in various laboratories to know the mechanisms by which BRs (endogenous or exogenous) regulate the stress responses in plants through ROS metabolism.

2.2.1.3.7 Exogenous application of BRs to enhance plant salt tolerance

Various studies on exogenous application of BRs in different plant species have reported a substantially improved plant growth and development, under various stress and non-stress conditions (Cao *et al.*, 2005; Houimli *et al.*, 2008; Hayat *et al.*, 2010e). BRs can be exogenously applied through seed soaking (seed priming), root treatment and/or foliar spray. Out of these, seed soaking and foliar spray are most commonly used.

2.2.1.3.7.1 Foliar application

Like many other organic and inorganic compounds, aqueous solutions of BRs could be applied to the leaves of a variety of agricultural crops. The foliar spray of BRs has produced encouraging results in terms of improving plant stress tolerance, including tolerance to salinity stress (Table 5). Foliar application of 24-epibrassinolide to pepper plants induces tolerance against salinity stress (Samira *et al.*, 2012). Similarly, the follow up treatment of EBL to salinity stressed pepper plants significantly improved relative growth rate, net CO_2 assimilation, stomatal conductance, transpiration and water use efficiency (Samira *et al.*, 2012). Application of EBL increased photosynthesis by increasing stomatal conductance in both control and salt stressed plants (Samira *et al.*, 2012). Application of 24- EBR as foliar spray increased biomass of wheat plants both under saline and non-saline conditions (Shahhaz and Ashraf, 2007).

The activity of key antioxidant enzymes, particularly under salt-stress, can be altered through the exogenous application of BRs (Zhang *et al.*, 2007; Shahbaz *et al.*, 2008). The catalase activity increased by the foliar application of 28-HBL or 24-EBR in *Arachis hypogaea* L. (Vardhini and Rao, 2000) and *Brassica juncea* L. (Hayat *et al.*, 2000). The effect of foliar application of 24-EBR on the antioxidant system of two

wheat cultivars differing in salt tolerance was studied by Shahbaz *et al.* (2008) where it overcome the salinity stress in both the cultivars by increasing POD and CAT activities. It can be inferred from these results that foliar applications of BRs has a potential in reducing the toxic effects of salt-stress in different plant species.

Table 5: Effect of foliar applied brassinosteroids on various parameters in different plant species, subjected to salinity (NaCl) stress.

Treatment	Plant species	Responses	References
EBL	*Triticum aestivum*	Improved P_N, C_i, C_i/C_a, g_S, under stress conditions	Qayyum *et al.*, 2007
EBL	*Capsicum annuum*	Increased plant growth, RWC and photosynthetic pigments but decreased electrolyte leakage	Houimli *et al.*, 2010
EBL	*Triticum aestivum*	Significantly ameliorated the loss of P_N, C_i, C_i/C_a, g_S, F_v/F_m under salinity stress	Shahbaz *et al.*, 2008
EBL	*Triticum aestivum*	Improved shoot fresh and dry weight	Shahbaz and Ashraf, 2007
EBL	*Lactuca sativa* L. var. Crispa	Increased fresh and dry weight of root and shoot as well as stem diameter, under salt stress. It also reduced leaf electrolyte leakage and has determined lower values of leaf electrolyte leakage than non-treated ones.	Ekinci *et al.*, 2012
HBL	*Triticum aestivum* L.	Significantly overcome the depressive effect of saline water at all the levels of growth.	Eleiwa *et al.*, 2011

Treatment	Plant species	Responses	References
BL	*Vigna Sinensis*	Increased fresh weight, dry weight and length of root and shoot, antioxidant enzymes activity, but decreased lipid peroxidation	El-Mashad and Mohamed, 2012
EBL	*Capsicum annuum*	Increased relative growth rate, net assimilation rate and leaf area, photosynthesis, stomatal conductance	Samira *et al.*, 2012

2.2.1.3.7.2 Application through rooting media

Harmful effects of salt stress on general physiology of various plant species can be overcome by the application of BRs through root. The ameliorative effect of root fed BRs on salinity induced changes in various crop species is summed up in table 6.

Table 6: Effect of brassinosteroids through rooting medium on various parameters in different plant species subjected to salinity (NaCl) stress

Treatment	Plant species	Responses	References
EBL	Rapeseed	Increased germination and seedling growth	Kagale *et al.*, 2007
EBL	*Sorghum bicolor*	Modified initiation, duration and intensity of leaf blade and leaf sheath, and perturbed leaf index	Amzallag, 2004
BR analogue (BioBras-16)	*Oryza sativa*	Increased activity of CAT, SOD, GR, and APX	Nunez *et al.*, 2003
EBR	*Solanum melongena* L.	Alleviated growth reduction and also significantly increased chlorophyll content and photosynthetic attributes.	Wu *et al.*, 2012
EBL	Cyanophyta	Improved growth	Saygideger and Deniz, 2008

The observations suggest that BRs ameliorate the harmful effects of the stress by the modulation of the involved physiological and biochemical processes. However, manifestation of such effects requires the adjustment of BR concentration and growth stage of the plant. This assessment is similar to that of traditional PGRs effects on plant growth and development, which often exhibit a bell shaped dose/response curve (Arteca, 1995), demonstrating that they are potentially harmful at higher concentrations (Oh and Clouse, 1998). There are various other problems of using BRs in rooting medium e.g., use of BRs in soil is not feasible as BR is degraded by soil microorganisms (Ashraf *et al.*, 2008) therefore needs careful attention.

2.2.1.3.7.3 Application through pre-sowing seed treatment

It is well documented that pre-sowing treatment of seeds with plant growth regulators improves the seed germination rate both under stress and non-stress conditions (Ashraf and Foolad, 2005). The seed soaking treatment also known as shotgun approach (Ashraf *et al.*, 2008) that not only improves seed germination rate and seed uniformity but it also improves seedling establishment and crop performance under field conditions. There are a number of reports where BRs have been employed as a shotgun approach in various plant species grown in varied conditions which have been summed up in table 7.

Table 7: Effect of brassinosteroids through pre-sowing treatment on various parameters in different plant species subjected to salinity (NaCl) stress

Treatment	Plant species	Responses	References
BL, EBL, HBL	*Oryza sativa*	Improved growth, restored pigment levels and increased nitrate reductase activity	Anuradha and Rao, 2003
HBL	*Triticum aestivum*	Enhanced activity of SOD, POD, CAT, APX, and GR. Improved protein content but decreased lipid peroxidation	Arora *et al.*, 2008
EBL, HBL	*Oryza sativa*	Improved germination and seedling growth and enhanced the levels of nucleic acids and soluble proteins	Anuradha and Rao, 2001

Treatment	Plant species	Responses	References
BRs	*Phaseolus vulgaris, Hordeum vulgare*	Enhanced growth, betaine level, and chlorophyll content	Abd El-Fattah, 2007
HBL	*Cicer arietinum*	Improved activity of leaf NR and CA, and increased dry biomass, leaf nodule number, nodule fresh and dry weight	Ali *et al.,* 2007a
EBL	*Oryza sativa*	Improved seedling growth, soluble protein content, and activity of APX. Reduced lipid peroxidation and oxidative damage	Ozdemir *et al.,* 2004
EBL	*Hordeum vulgare*	Improved germination percentage, radicle elongation, seedling fresh weight	Kilic *et al.,* 2007
BL	*Medicago sativa*	Improved germination percentage, vigor index, fresh and dry weight of shoot and root, root length, root vigor and activity of POD, SOD, CAT, while reduced malondialdehyde content	Zhang *et al.,* 2007
EBL	*Hordeum vulgare*	Improved germination percentage, radicle elongation, seedling fresh weight	Cavusoglu and Kabar, 2008
EBL	*Pisum sativum* L.	Increased germination, embryo axis length, growth and activity of antioxidant enzymes	Shahid *et al.,* 2011
EBL	*Lactuca sativa* L. var. Crispa	Increased fresh and dry weight of shoot and root, stem diameter.	Ekinci *et al.,* 2012

It may be summarised that BR applied as shotgun approach enhances seed germination and also ameliorates the oxidative stress in plants, induced by salt stress. BRs applied as a shotgun approach can therefore be a used as an effective tool to improve growth and crop production of plants subjected to saline conditions.

2.2.1.3.7.4 Comparison of different modes of BR application

The available literature on BR application revealed that feeding the hormone through seed soaking or foliar spray is much more efficient and economical than root application. Furthermore, the efficacy of foliar spray depends upon the stage of plant growth and the BR concentration used. Most of the research work suggests that foliar spray of BR at early stages of plant growth is more effective than at later stages (Zhang *et al.*, 2006; Ali *et al.*, 2008b). For spraying the solution on large scale, it is necessary to gather the information on proper formulation of the solution (Khripach *et al.*, 2000). The spraying solution should contain additives which will make easy the spreading of the active substance (BR) on the leaf surface (Akram *et al.*, 2009). These additives delay the drying of the leaf and help in quick diffusion of BR through the cell walls.

In case of pre-sowing seed treatment, the efficacy depends not only on the BR concentration but also on the duration of soaking in the BR solution. It can be also noted that the effectiveness of BR application may also depend on plant cultivars, climatic conditions, types of soil, and level of applied fertilizers. For instance, the effectiveness of BR on rice differed substantially at different temperatures and light conditions (Kamuro and Takatsuto, 1999). Therefore, it is imperative that, prior to field application on a large scale, the proper application protocols and BR concentrations for target growing conditions and plant species are to be determined.

2.2.1.3.8 Brassinosteroids and crop yield

It is now well evident that BRs increase the yield of crops in various species. Application of brassinosteroids significantly increased yields of potato, mustard, rice and cotton (Ramraj *et al.*, 1997), *Lens culinaris* (Hayat and Ahmad, 2003), *Vigna radiata* (Fariduddin *et al.*, 2003) and that of corn. tobacco, watermelon, cucumber and grape (Ikekawa and Zhao, 1991). In addition to this there have been reports that confirm the efficacy of BRs in enhancing the yield of many other plant species (Hayat *et al.*, 2010d). However, the promoting effects of BRs get partially lost when the experiment is shifted from growth chamber to field possibly due to unstable natural

conditions. It is reported that BL, EBL, HBL and TS303 increase yield in specific plants in field conditions (Maharjan, 2012). The discovery of BR analogues which are biologically active, stable, and absorbable in plant tissues, can result in high quantity and quality production of wide varieties of crops. However, modulating endogenous BR activity by direct manipulation of genes involved in either BR biosynthesis or signalling could allow for better crop yield and plant performance in a uniform and predictable manner rather than adopting the top dressing of plants.

2.2.2 Proline

In order to cope with the environmental stress faced by the plants throughout their life cycle, plants have evolved certain adaptive mechanisms. One such mechanism includes the accumulation of large quantities of compatible solutes (low molecular mass compounds) such as proline for the osmotic adjustment of the cells (Hsu *et al.*, 2003; Kavi Kishore *et al.*, 2005). Proline is an essential amino acid which is ubiquitous in all the plants where it has multiple metabolic roles (Szabados and Savoure, 2009). Under stress conditions it is known to scavenge free radicals, buffer redox potential, stabilize subcellular structures such as protiens and cell membranes (Ashraf and Foolad, 2007). In addition proline has a role of osmolyte for osmotic adjustment (Ahmed *et al.*, 2010). During stress recovery it can be metabolised which provides sufficient reducing agents for mitochondrial oxidative phosphorylation and ATP generation for repairing stress induced changes (Hare and Cress, 1997; Hare *et al.*, 1998).

Proline accumulation normally occurs in cytoplasm where it functions as molecular chaperons stabilizing the structure of proteins and its accumulation, buffer cytosolic pH and maintains cell redox status. It has also been proposed that its accumulation may be part of stress signal, influencing adaptive responses. The accumulation of proline was first reported in rye grass during wilting (Kemble and MacPherson, 1954). Numerous studies have shown that proline content increases under different stress conditions in plants. Proline accumulation has been reported during conditions of drought (Choudhary *et al.*, 2005), high salinity (Yoshiba *et al.*, 1995), high light and UV irradiation (Saradhi *et al.*, 1995), heavy metals (Schat *et al.*, 1997), oxidative stress (Yang *et al.*, 2009b) and in response to biotic stress (Haudecoeur *et al.*, 2009).

2.2.2.1 Metabolism

Since the last 40 years proline metabolism has been studied but still there is a little knowledge about the signalling pathways involved in its regulation. It is reported that compartmentalisation of proline between cytoplasm, mitochondria and chloroplast is of critical importance in addition to regulation and catabolic pathways. Biosynthetic pathway of proline has been outlined in *Escherichia coli* by Vogel and Davis (1952). There are two pathways for the proline biosynthesis in plants namely glutamate pathway and orinithine pathway (Plate III). The glutamate pathway accounts for major proline accumulation during osmotic stress. The proline is synthesized from glutamatic acid via intermediate Δ'-pyrroline-5-carboxylate (P5C). The reaction is being catalysed by Δ'-pyrroline-5-carboxylate synthetase (P5CS) and Δ'-pyrroline-5-carboxylate reductase (P5CR) (Sekhar *et al.*, 2007) (Plate III). P5CS is encoded by two genes whereas P5CR is encoded by only one in most plant species (Armengaud *et al.*, 2004). Proline catabolism occurs in mitochondria by chronological action of proline dehydrogenase or proline oxidase (PDH or POX) producing P5C. Further P5C dehydrogenase (P5CDH) converts P5C to glutamate. Two genes encode PDH, whereas a single P5CDH gene has been identified in *Arabidopsis* and tobacco (*Nicotiana tabacum*) (Deuschle *et al.*, 2001; Ribarits *et al.*, 2007). PDH transcription is activated by rehydration and proline but repressed by dehydration, thus preventing proline degradation during abiotic stress (Kiyosue *et al.*, 1996; Verbruggen *et al.*, 1996). In an alternative pathway proline can be synthesized from ornithine which is transaminated to P5C by orinithine-δ-aminotransferase (Verbruggen and Hermans 2008). It has been suggested that ornithine pathway is important during seedling development and in some plants for stress-induced proline accumulation (Armengaud *et al.*, 2004; Xue *et al.*, 2009). Accumulation of proline has been suggested to contribute to stress tolerance in many ways. As a molecular chaperon proline is able to maintain the protein integrity and enhances the activity of different enzymes (Rajendrakumar *et al.*, 1994). Numerous studies have reported proline as an antioxidant suggesting its role as ROS scavenger and singlet oxygen quencher (Matysik *et al.*, 2002).

The endogenous proline content can be maintained by biosynthesis, catabolism and transport between inter and intra cellular compartments. The enzymes (P5CS and P5CR) involved in the biosynthesis of proline from glutamate are localised

in cytoplasm or chloroplast whereas those (PDH and P5CDH) involved in the proline catabolism back to glutamate are compartmentalised in the mitochondria.

It has been repoted that the increased proline accumulation in detached rice leaves exposed to Cu stress was due to proteolysis and increased activities of P5CR or OAT (Chen *et al.*, 2001). It is also indicated that this proline synthesis and accumulation was mediated by ABA in deatched rice leaves exposed to Cu stress (Chen *et al.*, 2001). However, Zhang *et al.* (2008) reported that proline accumulation under Cu stress is associated with nitric oxide (NO) generation in *Chlamydomonas reinhardtii*. The authors suggested that the NO generation was involved in proline accumulation and signalling under Cu stress based on the fact that the application of sodium nitroprusside (NO donor) increased the activity and transcript of P5CS in Cu treated algea which was blocked if NO scavenger instead of NO donor was used. Moreover, proline works as a ROS detoxifying agent rather than increasing the antiosidative defence system in *Senedesmus* under Cu or Zn stress (Tripathi and Gaur, 2004).

2.2.2.2 Physiological role

2.2.2.2.1 Proline and plant growth

Plants on being exposed to abiotic stress, loose their growth, however, it can be overcome through osmoprotection by the exogenous proline application (Yancey, 1994; Aggarwal *et al.*, 2011; Abd El-Samad *et al.*, 2011). When added to the culture medium proline at low concentrations effectively alleviated the decline in fresh weight in *Arachis hypogea* subjected to salinity stress (Jain *et al.*, 2001). In a similar study, Gerdakaneh *et al.*, 2011 reported that exogenous proline application to Murashige and Skoog medium improved the growth of strawberry callus, under osmotic stress. Exogenous application of proline to immature embryos of *Zea mays* stimulated the somatic embryogenesis (Claparols *et al.*, 1993). Exogenous application of proline as a pre-sowing seed treatment increased the growth of mustard plants (Wani *et al.*, 2012). The exogenous proline application improved the growth of maize plants (Ali *et al.*, 2007b), seed germination of *Arabidopsis thaliana* (Hare et al. 2003), plant growth (Fedina *et al.*, 1993) and crop productivity (Itai and Paleg, 1982) under stress conditions. Proline application enhanced the vase life of *Rosa hybrida* through the alleviation of oxidative stress by improving Mn-SOD activity and reduced glutathione content (Kumar *et al.*, 2010). After stress, proline pools supply a reducing
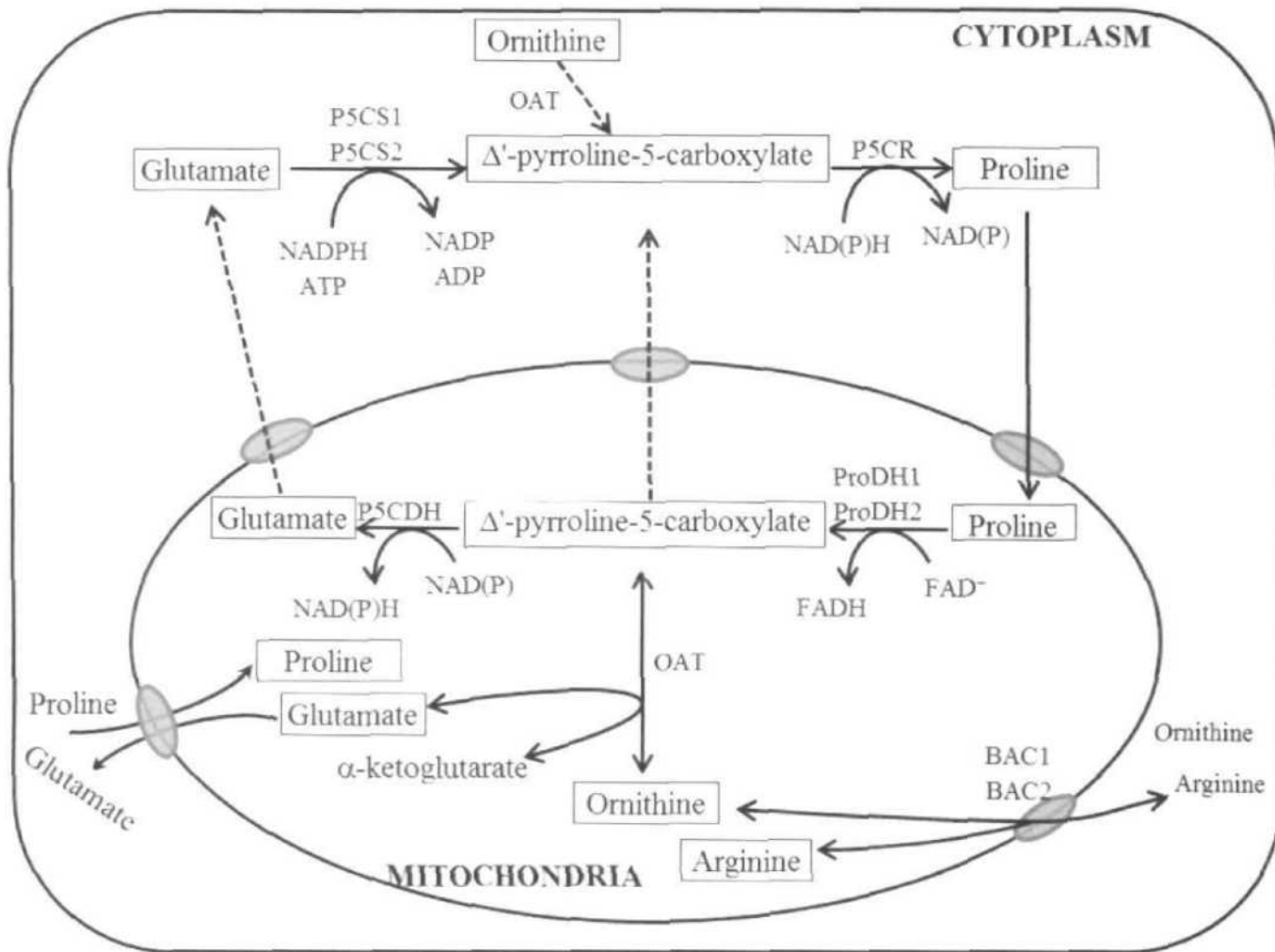
Plate III: Proline metabolism in plants

P5CS	Δ^1-Pyrroline-5-Carboxylate Synthetase
P5CR	Δ^1-Pyrroline-5-Carboxylate Reductase
ProDH	Proline Dehydrogenase
P5CDH	Δ^1-Pyrroline-5-Carboxylate Dehydrogenase
OAT	Ornithine δ-aminotransferase
BAC	Basic Amino Acid Carrier

potential for mitochondria through the oxidation of proline by PDH and P5CDH, provide electrons for the respiratory chain and therefore contribute to energy supply for resumed growth (Hare and Cress, 1997; Kavi Kishor *et al.*, 2005).

2.2.2.2.2 Proline and various enzymes and metabolites

There are many reports indicating the effects of proline on the activity of various enzymes. These reports have been listed in the table 8.

Table 8: Effect of proline on various enzymes and metabolites

Plant species	Responses	References
Zea mays	Protection of enzymes against cold stress by maintaining their 3D structure	Schobert, 1977; Paleg *et al.*, 1981
Glycine max	Faster import of proline across symbiosome membrane, which is used as substrate by bacteroids to enhance the nitrogenase activity in drought stressed nodules	Pedersen *et al.*, 1996
Vicia faba	Increased the contents of soluble sugars and proteins during salinity stress	Gadallah, 1999
Pancratium maritimum	Alleviated salt stress by protecting enzymes	Khedr *et al.*, 2003
Nicotiana tobaccum	Alleviated salt stress by reducing lipid peroxidation	Okuma *et al.*, 2004
Nicotiana tobaccum	Enhanced CAT, POX and SOD activities	Hoque *et al.*, 2007
Vigna radiata	Increased phenolic contents during exposure to chilling stress.	Posmyk and Janas, 2007
Phaseolus vulgaris	Alleviated the selenium stress by increasing enzymatic and non-enzymatic antioxidants	Aggarwal *et al.*, 2011
Brassica juncea	Increased the activity of CAT, POX and SOD enzymes	Wani *et al.*, 2012

2.2.2.2.3 Proline and photosynthesis

It is well documented that exogenous application of proline protects plants against stress by stabilizing complex II electron transport (Hamilton and Heckathorn, 2001), membranes and proteins (Holmstrom *et al.,* 2000) and enzymes such as Rubisco (Makela *et al.,* 2000). Compared with other osmolytes such as glycine betaine, exogenously applied proline effectively alleviates NaCl-generated stress in tobacco cells (Ashraf and Foolad, 2007). Both upper and lower stomata in *Vicia faba* · responded to different concentrations of proline supplied exogenously either to detached leaves or to intact leaves (Rajagopal, 1981). The stomata on abaxial surface exhibited higher resistance than those on adaxial surface on being treated with . proline. Furthermore, lower concentrations of exogenous proline were even more effective in increasing stomatal resistance than that of ABA spray (Rajagopal, 1981). It is also observed that exogenously applied proline maintained turgidity in leaves of barley and wheat undergoing stress (Rajagopal and Sinha, 1980). It is reported that seed soaking of *Brassica* seeds in lower concentration of proline solution increased the net photosynthetic rate and related attributes in non-stress conditions (Wani *et al.,* 2012). Photosynthetic rate and related parameters in maize plants subjected to water stress were improved by the foliar application of proline (Ali *et al.,* 2007b).

2.2.2.2.4 Proline and abiotic stress tolerance

There is sufficient literature available on the role of proline in abiotic stress tolerance. The effects of proline under various stresses is summarised in table 9.

Table 9: Effect of proline on plants under various abiotic stresses

Plant species	Stress	Responses	References
Hordeum vulgare	UV-B radiations	Reduced chlorophyll/carotenoid ratio, oxygen evolution rate and photochemical efficiency of PS II, and increased proline accumulation	Fedina *et al.,* 2003
Vigna radiata	Chilling stress	Hydropriming of seeds with proline increased germination rate at low temperature and protect *Vigna radiata* seedlings against chilling injury.	Posmyk and Janas, 2007

Plant species	Stress	Responses	References
Hordeum dsitichum	Water stress	Improvement in the crop productivity	Itai and Paleg, 1982
Cucumis sativus	Water stress	Improvement in the crop productivity	Itai and Paleg, 1982
Solanum	Frost stress	Increased frost tolerance	van Swaaij *et al.*, 1985
Saccharomyces cerevisiae	Cd and Zn stress	Protection of activity of glucose-6-phosphate dehydrogenase and nitrate reductase *in vitro* against the heavy metal stress	Sharma *et al.*, 1998
Chlorella vulgaris	Cu, Cr, Ni and Zn	Counteract lipid peroxidation as well as K^+ efflux	Mehta and Gaur, 1999
Phaseolus vulgaris	Selenium	Increased the endogenous level of proline that antagonised the toxic effects of stress through the improvement in seedling growth	Aggarwal *et al.*, 2011
Nicotiana tobaccum	Cd	Alleviated the damaging effects of oxidative stress by the up-regulation of the antioxidant defense system in plants.	Islam *et al.*, 2009
Scenedesmus armatus	Cd, Mn, Ni	Alleviated the toxic effects of heavy metal stress by increasing the activity of antioxidant enzymes which was reflected in improved growth	el-Enany and Issa, 2001

2.2.2.2.5 Exogenous application to enhance plant salt tolerance

The exogenous application of proline plays an important role in enhancing the plant tolerance against various stresses. This enhancement in tolerance can either be through osmoprotection (Handa *et al.*, 1986; Yancey, 1994) or cryoprotection (Santarius 1992). The effects of exogenous application of proline in various plant species grown under saline conditions are summarized in table 10.

Table 10: Impact of proline in various plant species grown under salt stress

Plant species	Responses	References
Glycine max	Increased production of SOD and POX in stressed plants	Yan *et al.*, 2000; Hua and Guo 2002
Allenrolfea occidentalis	Proline neutralized the increased ethylene production in stressed plants	Chrominski *et al.*, 1989
Hordeum vulgare	Proline caused a decrease in shoot Na^+ and Cl^- accumulation in embryo culture cells	Lone *et al.*, 1987
Allium cepa	Proline resulted in mitigating the effect of NaCl on cell membrane disruption	Mansour, 1998
Nicotiana tabacum	Proline promoted the growth of suspension cells under salt stress without maintaining a high ratio of K^+/Na^+	Krishnamurthy and Bhagwat, 1993
Nicotiana tabacum	Proline up regulated the antioxidant system providing protection against NaCl-induced oxidative damage to the plants	Hoque *et al.*, 2007
Oryza sativa	Proline reduced Na^+/K^+ ratio, increased endogenous proline content and transcript levels of P5CS and P5CR in stressed plants	Nounjan *et al.*, 2012
Oryza sativa	Proline suppressed Na^+-induced apoplastic uptake and Na^+ accumulation while slightly increased K^+ content, thereby increased K^+/Na^+ ratio under saline conditions.	Sobahan *et al.*, 2009
Zea mays	Proline application increased the tolerance of plants to salinity stress	Abd El-Samad *et al.*, 2011
Oryza sativa	Pre-treatment of rice seeds and then grown in different NaCl concentrations counteracted the adverse effects of salt	Deivanai *et al.*, 2011
Zea mays	Proline as foliar spray counteracted the NaCl-induced growth inhibition	Ali *et al.*, 2007b

Plant species	Responses	References
Zea mays	Proline as foliar spray counteracted the NaCl-induced growth inhibition	Ali *et al.*, 2007b
Brassica napus L.	Proline applied through rooting medium alleviated the adverse effects of NaCl on seed germination and seedling growth	Athar *et al.*, 2009
Triticum durum L.	Exogenous proline application in combination with *Azotobacter vinelandii* inoculation had significantly positive effect on seed germination under NaCl stress	Silini *et al.*, 2012
Olea europaea	Mitigated the reduction of growth and photosynthetic activity under salt stress and also improved antioxidant system under salt stress	Ahmed *et al.*, 2010
Oryza sativa L.	The exogenous proline increased fresh and dry weight, reduced the Na^+/K^+ ratio, increased endogenous proline content and transcript levels of P5CS and P5CR under salt stress	Nounjan *et al.*, 2012
Cucumis melo L.	Exogenous application of proline increased fresh and dry mass, increased P_N, Fv/Fm, and Chl content, reduced the O_2^- level and the H_2O_2 content under salt stress	Yan *et al.*, 2011

2.2.2.2.6 Proline and oxidative stress

Plants regularly synthesize reactive oxygen species (ROS) as a by-product of various metabolic pathways (Foyer and Harbinson, 1994). ROS play a significant role in providing protection against harmful pathogens (Doke, 1997; Bolwell *et al.*, 2002). They are also important in treachery elements formation, lignification and several other developmental processes (Jacobson, 1996; Teichmann, 2001; Fath *et al.*, 2002). Reports indicate that proline is responsible for scavenging ROS and other free radicals (Hong *et al.*, 2000; Okuma *et al.*, 2004; Chen and Dickmann, 2005). Proline, when

applied exogenously to roots of *Arabidopsis*, resulted in the reduction of ROS level, indicating the ROS scavenging potential of proline (Cuin and Shabala, 2007). Further, exogenous proline application reduced ROS-induced K^+ efflux (Cuin and Shabala, 2007). Hoque *et al.* (2007) reported that the activities of antioxidant enzymes viz. CAT, POX and SOD enhanced significantly when proline was applied in tobacco suspension cultures, exposed to salinity stress.

Another important defense system of plants that protect cells against the destructive ROS (i.e., those generated in response to stress) is the ascorbate-glutathione (ASC-GSH) cycle (Noctor and Foyer, 1998). Exogenous proline application up-regulates the activity of enzymes in ASC-GSH cycle. The activity of APX (ascorbate peroxidase), MDHAR (monohydro ascorbate reductase) and DHAR (dihydro ascorbate reductase) enzymes, which are the components of ASC-GSH cycle, was significantly enhanced by proline application in tobacco cultures, exposed to salinity stress (Hoque *et al.*, 2007). Kaul *et al.* (2008), using in vitro studies, showed that exogenously applied L-proline proved to be a potent free radical (particularly ROS) scavenger. Hong *et al.* (2000) concluded that the role of proline as a free radical scavenger is more important in alleviating stress than as a simple osmolyte.

2.2.2.3 Proline toxicity in plants

Despite the reported protective roles of proline, it can be a problem if over-accumulated or applied at excessive concentrations to the plants. Such negative effects of proline were observed in *Lycopersicon esculentum* where an imbalance in inorganic ions was observed (Heuer, 2003). Exogenous application of proline at a low concentration (e.g., 30 mM) ameliorated the adverse effects of salinity on early seedling growth in *Oryza sativa*, whereas at higher concentrations (40-50 mM) generated poor plant growth (Roy *et al.*, 1993). In *Arabidopsis*, lower concentration of proline at hypocotyl enhanced shoot organogenesis whereas higher concentration inhibited growth, under *in vitro* conditions (Hare *et al.*, 2001). At lower concentrations, proline activated a cycle of cytosolic proline synthesis from glutamate and mitochondrial proline degradation, which simultaneously provided $NADP^+$ to drive cytosolic purine biosynthesis and reducing equivalents for mitochondrial ADP phosphorylation (Hare, 1998). However, at higher concentrations feedback inhibition of P5CS (Garcia-Rios *et al.*, 1997; Zhang *et al.*, 1995) blocked the biosynthetic

portion of this cycle and thereby inhibits organogenesis, as observed in *Arabidopsis* (Hare *et al.*, 2001). The exogenous proline when applied at higher concentration inhibited growth and proline biosynthesis in suspension culture of *Distichlis spicata* (Rodriguez and Heyser, 1988). Nanjo *et al.* (2003) evaluated proline toxicity in *Arabidopsis* T-DNA tagged mutant *pdh* that was defective in pro dehydrogenase (At ProDH), responsible for catalysing the first step of proline catabolism. This *pdh* mutant was hypersensitive to exogenous L-proline at concentrations <10 mM whereas the wild type grew normally at such concentrations.

2.3 CONCLUSION AND FUTURE PROSPECTS

The exogenous application of BRs and proline has opened a new perspective for the development of resistance in plants, under adverse conditions. Their exogenous application increases plant growth and development but the response varies with the plant species and the stage of development. Therefore, before any commercial recommendation is made for exogenous application of BRs and proline to increase plant tolerance to stress, it is important to fix the optimum concentration and appropriate stage of growth for each plant species.

Since, the discovery of brassinosteroids the mechanism involving steroid perception at cell surface to alterations in gene expression which leads to developmental changes is now much clearly understood (Clouse, 2011). The pleiotropic effects of BRs on physiology of plants appear to be due to BZR1 and BES1 which regulate the genes to amplify the BR signals. While BES1 and BZR1 appear to be the predominant modulators of BR-regulated gene expression, it is possible that additional, undiscovered transcription factors may be involved in some specific BR responses therefore, searches in future for these proteins may be productive. Plants accumulate proline which plays an important role in mediating osmotic adjustment and subcellular structure protection under adverse growth conditions. But not all plants accumulate proline in sufficient amounts to overcome the adverse conditions. Different approaches have been made to increase the concentration of proline in plants grown under various abiotic stresses. Some achievements have been made in introducing genes for the production of proline in naturally non-accumulating and low-accumulating plant species. Exogenous application of proline to the plants is a short-cut method, under stress conditions to enhance their tolerance.

A detailed study at the biochemical and genetic level to uncover various steps of BRs or proline signal transduction pathways in plants under salt-stress appears to be a promising area of future research. Furthermore, an improved knowledge of the mechanisms of action of exogenously applied BRs or proline will certainly promote their efficient use in crop production under stressful conditions.

Materials and Methods

CONTENTS

MATERIALS AND METHODS

3.1 Proposed study

To achieve the objectives drafted in chapter one, six pot experiments were conducted to explore the effects of two brassinosteroids (28-homobrassinolide and 24-epibrassinolide) and/or proline on mustard plants [*Brassica juncea* (L.) Czern & Coss] cv. Varuna and RH-30 grown under different levels of salt (NaCl) stress at selected stages of growth during 2009-2012.

3.2 Seeds

The authentic seeds of *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30 were procured from National Seed Corporation Ltd., Pusa, New Delhi, India. Prior to setting up of each experiment, healthy and uniform size seeds were tested for their per cent viability. Healthy and uniform sized seeds were surface sterilized with 0.01% mercuric chloride solution for 5 min, followed by rinsing the seeds with double distilled water (DDW), at least thrice, to remove even the traces of adhered mercuric chloride to the seed surface.

3.3 Preparation of pots

Experimental earthen pots (25×25 cm) were filled with an equal quantity of sandy loam soil mixed with farmyard manure, in a ratio of 6:1. A uniform basal starter dose of inorganic fertilizers (urea, single superphosphate and muriate of potash) was added at a rate of 40 mg, 138 mg and 26 mg respectively, per kg of the soil to each pot to maintain the fertility of the soil. The pots were arranged in a simple randomized design, in the net house of Department of Botany, Aligarh Muslim University, Aligarh, India.

3.4 Hormones and preparation of solutions

28-homobrassinolide (HBL) and 24-epibrassinolide (EBL) were purchased from Sigma-Aldrich India Ltd Chemicals, USA. Stock solutions (10^{-4} M) of both HBL and EBL were prepared by dissolving required quantity of the hormone in 5 cm^3 of ethanol, in a 100 cm^3 volumetric flasks and final volume was maintained up to the mark with DDW. The required lower concentration of HBL or EBL (10^{-8} M) was prepared by diluting the stock solution. Surfactant "Tween-20" (0.5 cm^3) was added to each above flask, holding HBL/EBL solution, prior to making up the volume to 100 cm^3 by using DDW.

3.5 Preparation of proline solution

Stock solution (0.1M) of proline was prepared by dissolving the required quantity of proline in DDW in a 100 cm^3 volumetric flask and making up to the volume by using DDW. Concentrations of 10, 20 and 30 mM were prepared by diluting the stock solution. Surfactant "Tween-20" (0.5 cm^3) was added to each above flask prior to making up the final volume to 100 cm^3 by using DDW.

3.6 NaCl treatments

The required quantity of NaCl (0.77, 1.54 or 2.31 g/kg) was added to the soil to generate the electrical conductivity of 2.8, 4.2 or 5.6 dsm^{-1} respectively. The electrical conductivity measured in the control was 1.4 dsm^{-1}.

3.7 Experiment 1

This experiment was performed during the winter season of 2009-10, under simple randomized block design to study the sensitivity of *Brassica juncea* (L.) Czern & Coss cultivars to varying doses of NaCl (2.8, 4.2, or 5.6 dsm^{-1}) applied through the soil.

The surface sterilized seeds of *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30 were sown in earthen pots (25× 25 cm) at the rate of 10 seeds per pot. A uniform basal dose of N, P, and K was added to the soil at the time of sowing (refer to 3.2). Thinning was done on 7th day after sowing (DAS), leaving three plants per pot. Each treatment was represented by 5 pots. Irrigation was done with tap water as and when required. A selected number of plants were randomly sampled at 30 and 60 DAS to evaluate the following characteristics:

1.   Root and shoot length per plant

2.   Root and shoot fresh and dry mass per plant

3.   Leaf area

4.   Chlorophyll value (SPAD level)

5.   Leaf electrolyte leakage

6.   Leaf water potential

7.   Net photosynthetic rate

8.   Stomatal conductance

9.   Internal CO_2 concentration

10.   Transpiration rate

11.   Maximum quantum yield of photosystem II

12. Leaf nitrate reductase activity

13. Leaf carbonic anhydrase activity

14. Leaf catalase activity

15. Leaf peroxidase activity

16. Leaf superoxide dismutase activity

17. Leaf proline content

The remaining plants were allowed to grow up to maturity (120 DAS) and were harvested to study the following yield characteristics:

1. Number of pods per plant

2. Number of seeds per pod

3. Seed yield per plant

4. 100 seed mass

3.8 Experiment 2

This experiment was set up according to simple randomized block design during the winter season of 2009-10 to study the effect of foliar application of HBL (10^{-8} M) or EBL (10^{-8} M) on *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. All the agronomic and cultural practices remained the same as described in Experiment 1. Three plants per pot were maintained after thinning and each treatment was represented by five pots. Foliage of 29 days old seedlings was treated in the following manner:

a) Sprayed with DDW, used as control.

b) Sprayed with the aqueous solution of 0.5% Tween 20.

c) Sprayed with the aqueous solution of 5% ethanol.

d) Sprayed with the aqueous solution of HBL (10^{-8}M).

e) Sprayed with the aqueous solution of EBL (10^{-8}M).

Each plant was sprayed thrice at an interval of 10 min. The nozzle of the sprayer was adjusted in such a way that it pumped out about 1 cm^3 of the solution in a single spray, therefore each plant received about 3 cm^3 (15 µl Tween-20, 150 µl ethanol, 0.0148 µg HBL or 0.0144 µg EBL) of each solution. Irrigation was done with tap water as and when required. A selected number of plants were sampled at vegetative stages (30 and 60 DAS) and the characteristics studied were the same as in Experiment 1. The remaining plants were allowed to grow up to maturity and were harvested after 120 DAS, to study the yield characteristics listed in Experiment 1.

3.9 Experiment 3

In the winter season of 2009-10, this experiment was conducted under simple randomized block design to study the impact of foliar application of different concentrations of proline (0, 10, 20, or 30 mM) on *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30 seedlings. All the agronomic and cultural practices remained the same as described in Experiment 1. Three plants per pot were maintained after thinning and each of the following treatments was replicated 5 times. The foliage of the seedlings (29 days old) were sprayed under the following scheme:

a)   Sprayed with DDW, used as control.

b)   Sprayed with the aqueous solution of 10 mM of proline.

c)   Sprayed with the aqueous solution of 20 mM of proline.

d)   Sprayed with the aqueous solution of 30 mM of proline.

Each plant was sprayed thrice with an interval of 10 min between each spray. The nozzle of the sprayer was adjusted in such a way that it pumped out about 1 cm^3 (1.15, 2.30 or 3.45 mg proline) of the solution in a single spray. Irrigation was done with tap water as and when required. The vegetative selected number of plants were sampled at 30 and 60 DAS and the characteristics studied were the same as in Experiment 1. The remaining plants were allowed to grow up to maturity and were harvested after 120 DAS to study the yield characteristics as mentioned in Experiment 1.

3.10 Experiment 4

This experiment were conducted during the winter season of 2010-11 under simple randomized block design to study the interactive effects of NaCl and brassinosteroids (HBL or EBL) on the growth and productivity of *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. All the agronomic and cultural practices were maintained the same as described in Experiment 1. Each treatment had five replicates (pots) and each pot was left with three plants after the thinning. The scheme of the treatments is presented in Table 1 where NaCl was supplemented into the soil at the time of sowing and the brassinosteroids were applied to the foliage of 29 day old seedlings.

Table 1. Scheme of treatment for experiment 4

Treatments	NaCl added to soil to generate following electrical conductivities (dsm^{-1})	Solution applied to the foliage (10^{-8} M)
Control	Nil	DDW
A	Nil	HBL
B	Nil	EBL
C	2.8	DDW
D	4.2	DDW
E	5.6	DDW
F	2.8	HBL
G	4.2	HBL
H	5.6	HBL
I	2.8	EBL
J	4.2	EBL
K	5.6	EBL

Each plant was sprinkled three times with an interval of 10 min. The nozzle of the sprayer was adjusted in such a way that it pumped out 1 cm^3 (approx.) in each spray. Therefore, every plant received about 3 cm^3 of DDW or HBL (0.0148 μg) or EBL (0.0144 μg) solution. A group of plants was sampled at 30 and 60 DAS to assess the parameters as studied in Experiment 1 and the remaining plants were allowed to grow up to maturity and were harvested after 120 DAS to study the yield characteristics as mentioned in Experiment 1.

3.11 Experiment 5

This experiment was conducted during the winter season of 2010-11 under simple randomized block design to study the interactive effects of salinity and proline on the growth and the yield of *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. The stage of the plants (29 DAS) and concentration of proline (20 mM) was selected on the basis of Experiment 3. All the agronomic and cultural practices remained the same as described in Experiment 1. Three plants were maintained in each pot, after thinning and each of the following treatments was replicated five times. The scheme of the treatments is presented in Table 2. The NaCl was applied to the soil at the time of sowing and the proline was sprayed to the foliage of 29 days old seedlings.

Table 2. Scheme of treatment for experiment 5

Treatments	NaCl added to soil to generate following electrical conductivities (dsm^{-1})	Solution applied to the foliage, 29 DAS
Control	-	DDW
A	-	Proline (20 mM)
B	2.8	DDW
C	4.2	DDW
D	5.6	DDW
E	2.8	Proline (20 mM)
F	4.2	Proline (20 mM)
G	5.6	Proline (20 mM)

Each plant was sprayed three times with the gap of 10 min. The nozzle of the sprayer was adjusted in such a way that it pumped out 1 cm^3 (2.30 mg proline) in one sprinkle. Therefore, each plant received 3 cm^3 of DDW or proline solution. The plants, in required number, were sampled at 30 and 60 DAS to assess the vegetative parameters, as studied in Experiment 1 and the remaining plants were allowed to grow up to maturity and were harvested after 120 DAS to study the yield characteristics as mentioned in Experiment 1.

3.12 Experiment 6

This experiment was conducted during the winter season of 2011-12 under simple randomized block design to study the interactive effects of EBL (selected on the basis of Experiment 4) and proline on the NaCl induced changes in *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. The concentration of proline was also selected on the basis of the observations of Experiment 3. All the agronomic and cultural practices remained the same as described in Experiment 1. Three plants were maintained in each pot after thinning and each treatment was replicated five times.

Each plant was sprayed to its foliage with 20 mM proline, 28 DAS and/or 10^{-8} M EBL, 29 DAS to elucidate the interactive effects of proline and/or EBL on soil applied NaCl induced changes in *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. The scheme of the treatment is presented in Table 3.

Table 3. Scheme of treatment for experiment 6

Treatments	NaCl added to soil to generate following electrical conductivities (dsm⁻¹)	Solution applied to the foliage, 28 DAS	Solution applied to the foliage, 29 DAS
Control	-	DDW	DDW
A	-	Proline (20 mM)	-
B	-	-	EBL (10^{-8} M)
C	-	Proline (20 mM)	EBL (10^{-8} M)
D	2.8	-	-
E	4.2	-	-
F	5.6	-	-
G	2.8	Proline (20 mM)	EBL (10^{-8} M)
H	4.2	Proline (20 mM)	EBL (10^{-8} M)
I	5.6	Proline (20 mM)	EBL (10^{-8} M)

Each plant received foliar application three times at the stages mentioned with an interval of 10 min. The nozzle of the sprayer was allowed to pump out 1 cm³ (approx.) of the solution in each spray. Therefore, each plant received 3 cm³ of DDW/proline (6.90 mg) /EBL(0.0144 µg) solution. A selected number of plants were sampled at 30 and 60 DAS to assess the same vegetative parameters as studied in Experiment 1. The rest of the plants in each treatment were allowed to mature to be harvested at 120 DAS to study the yield characteristics as mentioned in Experiment 1.

3.13 Parameters

The details of the methods executed to assess each parameter (pp. 42-43) are described below:

3.13.1 Growth parameters

3.13.1.1 Length of shoot and root per plant

The plants along with the soil were removed from each pot to get the intact roots and dipped in a bucket filled with tap water. The plants were gently stirred to remove adhering soil particles. This was followed by washing the roots under running tap water. The length of root and shoot was measured by using a meter scale.

3.13.1.2 Fresh and dry mass of shoot and root

The washed plants were gently soaked by using blotting sheets to remove the adhering water. The root and shoot of each plant were separated and weighed on an electronic balance to record their respective fresh mass. Plant roots and shoots were

subsequently transferred to an oven run at $70°C$ and left for 48 h after which they were weighed separately to record their dry mass.

3.13.1.3 Leaf area

It was determined by gravimetric method where the leaf area of randomly selected leaves from each treatment, was determined by tracing their outline on the graph sheet.

3.13.2 Chlorophyll content (SPAD value)

The chlorophyll content (SPAD value), at each selected stage, was measured in fully expanded leaves of the plants by using Minolta chlorophyll meter (SPAD-502; Konica Minolta Sensing Inc. Japan).

3.13.3 Electrolyte leakage

The total inorganic ions leaked out of the leaves were measured by the method described by Sullivan and Ross (1979).

Twenty leaf discs were taken in a boiling tube containing 10 cm^3 of deionized water. The contents were heated at $45°C$ (EC_a) and $55°C$ (EC_b) for 30 min each in a water bath and respective EC was measured by a conductivity meter. The contents in the boiling tube were again boiled at $100°C$ for 10 min and the EC (EC_c) was again recorded. The per cent electrolyte leakage was calculated by putting the values in the following formula:

$$\text{Electrolyte leakage (\%)} = \frac{EC_b - EC_a}{EC_c} \times 100$$

3.13.4 Leaf water potential

Leaf water potential (LWP), at each selected stage, was measured in fresh, detached leaves of the plants by using PSYPRO, water potential system (WESCOR Inc. Longman, USA).

3.13.5 Photosynthesis and related attributes

Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and internal CO_2 concentration (C_i) at each selected stage, was measured in fully expanded leaves of the plants by using portable photosynthesis system (LI-COR 6400, LI-COR, Lincoln, NE, USA). The air temperature, relative humidity, CO_2 concentration and PPFD were maintained at $25°C$, 85%, 600 $\mu mol\ mol^{-1}$ and 800 $\mu mol\ mol^{-2}\ s^{-1}$, respectively. All the measurements were made between 11:00 to 12:00 hours under the clear sun light.

3.13.6 Maximum quantum yield of Photosystem II

Maximum quantum yield of Photosystem II (Fv/Fm) was measured by using Leaf Chamber Fluormeter (LI-COR 6400-40, Portable photosynthesis system, LI-COR, Lincoln, NE, USA). All the measurements were carried out at a photosynthetic photon flux density (PPFD) of 1500 μmol m^{-2} s^{-1}, with a constant airflow rate of 500 μmol s^{-1}. The sampled leaves were dark adapted for 30 min prior to each measurement of Fv/Fm.

3.13.7 Biochemical analysis

3.13.7.1 Leaf nitrate reductase (NR) activity

The activity of nitrate reductase (E.C.1.6.6.1) was measured following the method laid down by Jaworski (1971), in fresh leaf samples.

The sampled leaves were cut into small pieces (1 cm^2). 200 mg of these chopped leaves was weighed and transferred to plastic vials. To each vial 2.5 cm^3 of phosphate buffer (pH 7.5) (Appendix 1.1) and 0.5 cm^3 of potassium nitrate solution (Appendix 1.2) were added, followed by the addition of 2.5 cm^3 of 5% isopropanol (Appendix 1.3). These vials were incubated in a BOD incubator for 2 h at 30 = 2°C in dark. Incubated mixture (0.4 cm^3) was taken in a test tube to which 0.3 cm^3 each of sulphanilamide solution (Appendix 1.4) and N-1-napthyl-ethylendiamin hydrochloride (NED-HCl) (Appendix 1.5) were added. The test tube was left for 20 min, for maximum color development. The mixture was diluted to 5 cm^3 by using DDW. The absorbance was read at 540 nm on spectrophotometer (Spectronic-20D, Milton Roy, USA). A blank was run simultaneously with each sample. Standard curve was plotted by using known graded concentrations of NaNO$_2$ (sodium nitrite) solution. The absorbance of each sample was compared with that of the calibration curve and nitrate reductase (NR) activity [n mole NO$_2$ g^{-1} (FM) s^{-1}] was calculated.

3.13.7.2 Leaf carbonic anhydrase (CA) activity

The carbonic anhydrase (CA) activity (E.C.4.2.1.1) in fresh leaf samples was measured by following the method described by Dwivedi and Randhawa (1974).

The fresh leaf samples were cut into small pieces at a temperature below 25°C. 200 mg of these leaf pieces was weighed and transferred to a petriplate. The leaf pieces were further cuts into fine pieces in 10 cm^3 of 0.2M cysteine hydrochloride (Appendix 2.1) and were left at 4°C for 20 min and then filtered. The filtrate was transferred to a test tube containing 4 cm^3 of phosphate buffer of pH 6.8 (Appendix

2.2). To this test tube, 4 cm^3 of 0.2M sodium bicarbonate (Appendix 2.3) solution and 0.2 cm^3 of 0.002% bromothymol blue (Appendix 2.4) were added. The test tube was shaken gently and left at 4°C for 20 min. CO_2 liberated by the catalytic action of carbonic anhydrase on $NaHCO_3$ was estimated by titrating the reaction mixture against 0.05N HCl (Appendix 2.5) using methyl red as an indicator (Appendix 2.6). The volume of HCl used to develop light purple colour, persisting for at least five seconds was noted. A blank consisting of all the above components of the reaction mixture except the leaf sample was run simultaneously with each set of the samples. The activity of enzyme was calculated by putting the values in the formula:

$$\text{Carbonic anhydrase activity} = \frac{V \times 22 \times N}{W} \quad [\text{mol } (CO_2) \text{ kg}^{-1} \text{ (leaf fresh mass) s}^{-1}]$$

V = Difference in volume (cm^3 of HCl used, in control and test sample titrations)

22 = Equivalent weight of CO_2

N = Normality of HCl

W = Fresh mass of tissue used

3.13.7.3 Estimation of antioxidant enzymes

500 mg of fresh leaf tissue was homogenized in 5 cm^3 of 50 mM phosphate buffer (pH 7.0) containing 1% polyvinyl pyrollidone. The homogenate was centrifuged at 15,000 x g for 10 min at 5°C and the supernatant obtained was used as an extract for the estimation of catalase, peroxidase and superoxide dismutase activity.

3.13.7.3.1 Leaf catalase (CAT) activity

The activity of catalase was measured by following the method of Chance and Maehly (1956).

The estimation of catalase was carried out by the permanganate titration method. 3 cm^3 of phosphate buffer (pH 6.8) (Appendix 3.1), 1 cm^3 of 0.1M H_2O_2 (Appendix 3.2) and 1 cm^3 of enzyme extract were mixed and this mixture was incubated at 25°C for 1 min. Then 10 cm^3 of 2% H_2SO_4 (Appendix 3.3) was added. The mixture was titrated against 0.1N potassium permanganate (Appendix 3.4) to find the residual H_2O_2 until a purple color persists for at least 15 s. Similarly, a control set was maintained in which the enzyme activity was stopped by the addition of H_2SO_4, prior to the addition of enzyme extract.

3.13.7.3.2 Leaf peroxidase (POX) activity

The activity of peroxidase was measured following the method of Chance and Maehly (1956).

To 3 cm^3 solution of pyrogallol phosphate buffer (Appendix 4.1), 0.1 cm^3 of enzyme extract and 0.5 cm^3 of 1% H_2O_2 were mixed in a cuvette and a change in absorbance, at 20s intervals for a period of 3 min was read at 420 nm on a spectrophotometer (Spectronic-20D, Milton Roy, USA). The control set was prepared by using all the above reagents, except the enzyme extract.

3.13.7.3.3 Leaf superoxide dismutase (SOD) activity

The activity of superoxide dismutase was measured by the method of Beauchamp and Fridovich (1971).

3 cm^3 of reaction mixture containing 1 cm^3 of 50 mM phosphate buffer (pH 7.8) (Appendix 5.1), 0.5 cm^3 of 13 mM methionine (Appendix 5.2), 0.5 cm^3 of 75 μM NBT (Appendix 5.3), 0.5 cm^3 of 2 μM riboflavin (Appendix 5.4), 0.5 cm^3 of 0.1 mM EDTA (Appendix 5.5) and 0.1 cm^3 of the enzyme extract was prepared. Riboflavin was added at last. The reaction mixture in the tubes were placed under 15 W fluorescent lamps for the initiation of reaction. After 10 min, the reaction was stopped by switching off the light. Non-illuminated reaction mixture was used as a blank. The absorbance was measured at 560 nm on a spectrophotometer (Spectronic-20D, Milton Roy, USA) and the SOD activity was expressed as unit g^{-1} fresh mass. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photo-reduction.

3.13.7.4 Proline content in leaves

The proline content in the fresh leaf samples was measured following the method described by Bates *et al.* (1973).

Fresh leaf sample (0.5 g) was homogenized in a mortar with 5 cm^3 of 3% sulfosalicylic acid (Appendix 6.1). The homogenate was filtered through Whatman No. 2 filter paper and collected in a test tube with two washings, each with 5 cm^3 of sulfosalicylic acid. 2 cm^3 each of glacial acetic acid and acid ninhydrin (Appendix 6.2) was added to 2 cm^3 of the above extract. This mixture was heated in a boiling water bath for 1 h. The reaction was terminated by transferring the test tube to ice-bath. 4 cm^3 of toluene was added to the reaction mixture with vigorous shaking for 20-30 s. The chromophore (toluene) layer was aspirated and warmed to room

temperature. The absorbance of red colour was read at 520 nm against a reagent blank. The amount of proline in the sample was calculated by using a standard curve prepared from pure proline (range 0.1–36 μ mol) and expressed on fresh mass basis of the sample.

$$\mu \text{ moles of proline } g^{-1} \text{ tissues} = \frac{\mu g \text{ proline } cm^{-3} \times cm^{-3} \text{ toluene}}{115.5} \times \frac{5}{g \text{ (sample)}}$$

Where, 115.5 is the molecular mass of proline.

3.13.8 Yield parameters

3.13.8.1 Number of pods per plant

At harvest (120 DAS), 15 plants (3 from each replicate) representing each treatment were randomly sampled and counted for the number of pods per plant.

3.13.8.2 Number of seeds per pod

25 pods from each treatment were randomly selected and crushed to get number of seeds per pod.

3.13.8.3 Seed yield per plant and 100 seed mass

The remaining pods from each treatment were crushed, cleaned and computed to assess seed yield per plant. 100 seeds were subsequently randomly picked and weighed to record 100 seed mass.

3.14 Statistical analysis

The experiment was conducted according to simple randomized block design. Each treatment was represented by five pots (15 plants). Data was statistically analysed by analysis of variance (*ANOVA*) using SPSS software version 17 for window (SPSS, Chicago, IL, USA). Least significant difference (LSD) was calculated and 'F' test was applied to assess the significance of the data at 5% level of probability.

Experimental Results

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EXPERIMENTAL RESULTS

4.1 Experiment 1

This experiment was performed to study the sensitivity of *Brassica juncea* (L.) Czern & Coss cultivars to varying doses of NaCl (2.8, 4.2, or 5.6 dsm^{-1}) applied through the soil during the winter season of 2009-10, under simple randomized block design. The surface sterilized seeds of *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30 were sown in earthen pots (25×25 cm) at the rate of 10 seeds per pot. A uniform basal dose of N, P, and K was added to the soil at the time of sowing (refer to 3.2). Thinning was done on 7th day after sowing (DAS), leaving three plants per pot. Each treatment was represented by 5 pots. Irrigation was done with tap water as and when required. The plants were randomly sampled at 30 and 60 DAS to achieve the following results.

4.1.1 Shoot and root length

As evident from table 1, the length of the shoot and that of the root increased as the growth advanced from 30 to 60 DAS in both the varieties (Varuna and RH-30). However, the values decreased when subjected to different levels of NaCl (2.8, 4.2, or 5.6 dsm^{-1}). Out of the three concentrations of NaCl, lowest concentration (2.8 dsm^{-1}) proved least toxic. Moreover, the highest concentration (5.6 dsm^{-1}) caused maximum damage at both the sampling stages. The intensity of loss caused by NaCl (5.6 dsm^{-1}) to shoot length of Varuna and RH-30 was 34% and 43%, whereas in case of root it was 40% and 56%, less than the respective controls, at 60 DAS. The damage was more prominent in variety RH-30 than Varuna, at both the sampling stages.

4.1.2 Shoot and root fresh mass

With the advancement of age from 30 to 60 days, the fresh mass of shoot and root increased (Table 2). The NaCl administered to the soil induced a significant reduction in the fresh mass of shoot and root in both the varieties (Varuna and RH-30) at 30 and 60 DAS. The highest concentration of NaCl (5.6 dsm^{-1}) proved toxic and decreased the values of fresh mass of shoot by 27% and 46% and that of root by 43% and 62% in Varuna and RH-30 respectively, compared with their respective control plants at 60 DAS. The severity of damage was more prominent in RH-30 than Varuna, at both the sampling stages.

Table 1: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on shoot and root length (cm) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Shoot length						Root length					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	15.16	14.33	14.75	70.04	68.00	69.02	16.00	15.47	15.74	24.32	22.57	23.45
NaCl (2.8 dsm^{-1})	12.12	10.60	11.36	60.93	54.40	57.67	11.52	10.05	10.79	19.94	17.15	18.55
NaCl (4.2 dsm^{-1})	9.85	8.74	9.30	54.63	48.20	51.42	9.28	6.49	7.89	16.53	13.09	14.81
NaCl (5.6 dsm^{-1})	9.20	7.59	8.40	46.22	38.48	42.35	7.20	6.03	6.61	14.59	9.93	12.26
Mean	11.58	10.32		57.96	52.27		11.00	9.51		18.85	15.69	

LSD at 5%	V = 0.36 (Sig)	V = 3.52 (Sig)	V = 0.38 (Sig)	V = 0.69 (Sig)
	T = 0.51 (Sig)	T = 4.98 (Sig)	T = 1.16 (Sig)	T = 0.98 (Sig)
	V x T = 0.72 (Sig)	V x T = NS	V x T = 1.64 (Sig)	V x T = 1.38 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 2: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on shoot and root fresh mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot fresh mass | | | | | | Root fresh mass | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	6.47	5.25	5.86	11.96	9.71	10.84	2.70	2.56	2.63	5.18	4.81	4.99
NaCl (2.8 dsm^{-1})	4.92	3.26	4.09	10.17	6.65	8.41	1.76	1.38	1.57	3.83	3.03	3.43
NaCl (4.2 dsm^{-1})	4.46	2.68	3.57	9.45	6.12	7.79	1.40	1.16	1.28	3.47	2.78	3.13
NaCl (5.6 dsm^{-1})	3.95	2.42	3.19	8.73	5.24	6.99	1.14	1.13	1.14	2.95	1.87	2.41
Mean	4.95	3.40		10.08	6.93		1.75	1.56		3.86	3.12	
LSD at 5%	V	= 0.38 (Sig)		V	= 0.86 (Sig)		V	= 0.14 (Sig)		V	= 0.48 (Sig)	
	T	= 0.54 (Sig)		T	= 1.22 (Sig)		T	= 0.20 (Sig)		T	= 0.68 (Sig)	
	V x T = NS			V x T = NS			V x T = 0.29 (Sig)			V x T = NS		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.1.3 Shoot and root dry mass

Both the varieties showed significant reduction in the shoot and root dry mass under the influence of NaCl, both at 30 and 60 DAS (Table 3). The highest concentration of NaCl (5.6 dsm^{-1}) generated values for shoot and root dry mass that were 30% and 56%; 28% and 47% less in Varuna and RH-30 respectively at 60 DAS, compared to their respective control plants. The variety RH-30 proved more sensitive to NaCl than Varuna.

4.1.4 Leaf area

As the growth advanced from 30 to 60 days, leaf area increased (Table 4). However, soil applied NaCl caused a significant loss in leaf area in both the varieties. The decrease in values was more prominent in RH-30 than in Varuna at both the samplings (30 and 60 DAS). The highest level of NaCl (5.6 dsm^{-1}) caused maximum decrease in both the varieties. In terms of percentage decrease, it was 33% and 46% in Varuna and RH-30 respectively, compared to their control plants at 60 DAS.

4.1.5 SPAD chlorophyll values

The plants supported in the soil amended with different concentrations of NaCl possessed significantly lower values of SPAD chlorophyll than the stress free, control plants (Table 4). Out of the tested NaCl concentrations (2.8, 4.2, or 5.6 dsm^{-1}), 5.6 dsm^{-1} was most toxic. This treatment decreased the SPAD chlorophyll values by 42% and 50% at 30 DAS and 32% and 37% at 60 DAS in Varuna and RH-30 respectively, compared with their control plants. This decrease was more prominent in RH-30 than Varuna, at both the sampling stages.

4.1.6 Electrolyte leakage

Unlike the other parameters, leaf electrolyte leakage decreased as growth advanced from 30 to 60 days in both varieties, Varuna and RH-30 (Table 5). However, the presence of NaCl in the soil caused a significant increase in the electrolyte leakage and increased further as the concentration of the salt increased. The NaCl (5.6 dsm^{-1}) increased the electrolyte leakage by 27% and 20% in Varuna and 32% and 26% in RH-30 at 30 and 60 DAS respectively, as compared to their respective controls. The maximum value for electrolyte leakage was observed in RH-30 raised with 5.6 dsm^{-1} of NaCl.

Table 3: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on shoot and root dry mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot dry mass | | | | | | Root dry mass | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	1.69	1.42	1.56	3.13	2.59	2.86	0.785	0.600	0.693	1.47	1.08	1.28
NaCl (2.8 dsm^{-1})	1.30	0.86	1.08	2.62	1.76	2.19	0.557	0.300	0.429	1.22	0.72	0.97
NaCl (4.2 dsm^{-1})	1.16	0.76	0.96	2.50	1.55	2.03	0.494	0.258	0.376	1.14	0.65	0.89
NaCl (5.6 dsm^{-1})	1.06	0.52	0.79	2.19	1.14	1.67	0.408	0.246	0.327	1.05	0.57	0.81
Mean	1.30	0.89		2.61	1.76		0.561	0.351		1.22	0.76	
LSD at 5%	V = 0.13 (Sig)			V = 0.05 (Sig)			V = 0.03 (Sig)			V = 0.09 (Sig)		
	T = 0.18 (Sig)			T = 0.07 (Sig)			T = 0.04 (Sig)			T = 0.12 (Sig)		
	V x T = 0.26 (Sig)			V x T= 0.10 (Sig)			V x T= 0.06 (Sig)			V x T= 0.18 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 4: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on leaf area (cm^2) and SPAD Chlorophyll values in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Leaf area | | | | | | SPAD Chlorophyll value | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	25.42	24.36	24.89	39.24	36.54	37.89	42.90	38.20	40.55	57.90	50.90	54.40
NaCl (2.8 dsm^{-1})	20.33	15.83	18.08	33.74	27.03	30.39	34.70	27.50	31.10	50.90	41.30	46.10
NaCl (4.2 dsm^{-1})	15.76	13.64	14.70	29.03	25.21	27.12	30.00	22.50	26.25	45.80	34.70	40.25
NaCl (5.6 dsm^{-1})	13.47	10.23	11.85	26.29	19.73	23.01	24.90	19.10	22.00	39.50	32.10	35.80
Mean	18.75	16.02		32.08	27.13		33.13	26.83		48.53	39.75	

LSD at 5%												
	V	=	0.90 (Sig)	V	=	1.49 (Sig)	V	=	0.27 (Sig)	V	=	0.50 (Sig)
	T	=	1.28 (Sig)	T	=	2.11 (Sig)	T	=	0.38 (Sig)	T	=	0.71 (Sig)
	V × T	=	1.81 (Sig)	V × T	=	NS	V × T	=	0.54 (Sig)	V × T =		1.01 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 5: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on electrolyte leakage (%) and leaf water potential (MPa) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Electrolyte leakage | | | | | | Leaf Water Potential | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	7.10	8.05	7.58	5.82	6.70	6.26	-0.64	-0.74	-0.69	-0.46	-0.54	-0.50
NaCl (2.8 dsm^{-1})	7.90	9.30	8.60	6.27	7.40	6.84	-0.74	-0.87	-0.81	-0.50	-0.61	-0.56
NaCl (4.2 dsm^{-1})	8.47	9.76	9.12	6.72	7.84	7.28	-0.81	-0.94	-0.88	-0.54	-0.67	-0.61
NaCl (5.6 dsm^{-1})	9.00	10.65	9.83	7.00	8.44	7.72	-0.86	-1.02	-0.94	-0.58	-0.72	-0.65
Mean	8.12	9.44		6.45	7.59		-0.76	-0.89		-0.52	-0.64	
LSD at 5%	V = 0.14 (Sig)			V = 0.10 (Sig)			V = 0.007 (Sig)			V = 0.01 (Sig)		
	T = 0.20 (Sig)			T = 0.15 (Sig)			T = 0.016 (Sig)			T = 0.01 (Sig)		
	V x T = 0.29 (Sig)			V x T = 0.21 (Sig)			V x T = 0.022 (Sig)			V x T = 0.02 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant
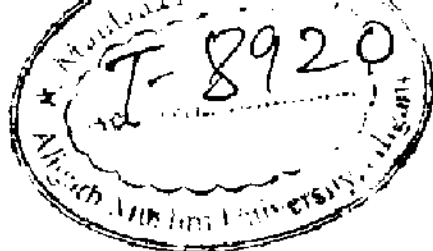
4.1.7 Leaf water potential

The leaf water potential increased as the growth progressed from 30 to 60 days in both the varieties (Table 5). The variety RH-30 possessed lower values for leaf water potential than Varuna, at both the sampling stages. However, the presence of NaCl in the soil decreased the values. The maximum loss was observed at 5.6 dsm^{-1} level of NaCl where in terms of per cent, it was 34% and 26% in Varuna and 38% and 33% in RH-30 at 30 and 60 DAS respectively, compared with their respective controls.

4.1.8 Net photosynthetic rate

The values for net photosynthetic rate (P_N) increased as the growth advanced from day 30 to 60, in both the varieties (Table 6). However, the plants raised in the soil amended with different levels of NaCl showed a decrease in net photosynthetic rate and the loss was concentration dependent of the salt. Lowest values were recorded in the plants raised with 5.6 dsm^{-1} salt that decreased P_N by 32% and 27% in Varuna and 53% and 46% in RH-30 at 30 and 60 DAS respectively, when compared to their control plants.

4.1.9 Stomatal conductance

The presence of NaCl in the soil decreased the values of stomatal conductance (g_s) in the plants of both the varieties (Table 6). Out of the three tested concentrations of NaCl, the highest concentration (5.6 dsm^{-1}) was most toxic that lowered the values by 40% and 52% in Varuna and RH-30 respectively at 60 DAS, compared with the respective control.

4.1.10 Internal CO$_2$ concentration

Internal CO$_2$ concentration (C_i) showed a pattern similar to that of stomatal conductance (g_s) (Table 7). The decrease in internal CO$_2$ level was relative to the increase in NaCl in the soil. Therefore, even at the lowest level of NaCl (2.8 dsm^{-1}) the loss was 9% and 16% in Varuna and RH-30 respectively at 60 DAS, as compared to their control plants.

4.1.11 Transpiration rate

The data presented in table 7 shows that the presence of NaCl in the soil caused a significant decrease in transpiration rate (E) both in Varuna and RH-30. The loss increased with the increasing concentration of NaCl. In terms of percentage 5.6 dsm^{-1} of NaCl lowered the transpiration rate by 27% and 19% in Varuna and 39% and 29% in RH-30 at 30 and 60 DAS

Table 6: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm⁻¹) on net photosynthetic rate (μmol CO_2 m⁻² s⁻¹) and stomatal conductance (mol H_2O m⁻² s⁻¹) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Net photosynthetic rate (P_N)						Stomatal conductance (g_s)					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	20.00	17.29	18.65	26.10	22.26	24.18	0.062	0.059	0.061	0.088	0.085	0.087
NaCl (2.8 dsm⁻¹)	15.60	10.54	13.07	22.40	15.58	18.99	0.040	0.028	0.034	0.065	0.051	0.058
NaCl (4.2 dsm⁻¹)	14.40	9.33	11.87	20.60	13.80	17.20	0.033	0.025	0.029	0.057	0.045	0.051
NaCl (5.6 dsm⁻¹)	13.60	8.12	10.86	19.10	12.02	15.56	0.030	0.020	0.025	0.053	0.041	0.047
Mean	15.90	11.32		22.05	15.92		0.041	0.033		0.066	0.056	

LSD at 5%	V	=	0.44 (Sig)	V	=	0.49 (Sig)	V	=	0.001 (Sig)	V	=	0.002 (Sig)
	T	=	0.63 (Sig)	T	=	0.70 (Sig)	T	=	0.002(Sig)	T	=	0.003 (Sig)
	V x T	=	0.89 (Sig)	V x T	=	0.99 (Sig)	V x T	=	0.003(Sig)	V x T	=	0.005 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 7: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on internal CO_2 concentration (ppm) and transpiration rate (mmol $H_2O m^{-2} s^{-1}$) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Internal CO_2 concentration (C_i) | | | | | | Transpiration rate (E) | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	327	309	318	374	350	362	3.19	3.00	3.10	4.71	4.44	4.58
NaCl (2.8 dsm^{-1})	277	228	252	340	294	317	2.82	2.19	2.51	4.42	3.59	4.01
NaCl (4.2 dsm^{-1})	261	216	238	325	262	293	2.58	1.98	2.28	4.23	3.24	3.74
NaCl (5.6 dsm^{-1})	249	200	224	310	241	275	2.32	1.83	2.08	3.81	3.15	3.48
Mean	278	238		337	286		2.73	2.25		4.29	3.61	

LSD at 5%	V = 11.94 (Sig)	V = 1.10 (Sig)	V = 0.12 (Sig)	V = 0.04 (Sig)
	T = 17.13 (Sig)	T = 1.56 (Sig)	T = 0.17 (Sig)	T = 0.05 (Sig)
	V x T = 27.37 (Sig)	V x T = 2.21 (Sig)	V x T = 0.24 (Sig)	V x T = 0.07 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

respectively, compared to their controls. The observation revealed that the decrease was more prominent in RH-30 than Varuna.

4.1.12 Maximum quantum yield of PSII (Fv/Fm)

The maximum quantum yield of PSII increased when the growth progressed from 30 to 60 days in both the varieties (Table 8). The variety Varuna possessed higher values for Fv/Fm than RH-30. However, the yield showed a linear decrease to the increase in NaCl concentration in both the varieties. The maximum loss was recorded at the highest NaCl concentration (5.6 dsm^{-1}) which was 29% and 21% in Varuna and 35% and 28% in RH-30 at 30 and 60 DAS respectively, compared to their controls.

4.1.13 Nitrate reductase (NR) activity

With the advancement of the growth, the NR activity increased in both the varieties (Table 8). The decrease in NR activity was proportional to the level of NaCl in the soil and therefore, the highest concentration (5.6 dsm^{-1}) was most toxic and caused maximum inhibition which was 53% less in variety RH-30 at 30 DAS, compared with the control. The decrease in NR activity was more prominent at 30d stage than 60 DAS.

4.1.14 Carbonic anhydrase (CA)activity

The activity of CA increased with the advancement of plant age (30 to 60 DAS). However, the values decreased significantly with the increasing level of NaCl in the soil (Table 9). Out of the three concentrations of salt, the highest concentration (5.6 dsm^{-1}) was most damaging that decreased the activity by 34% and 26% in Varuna and 42% and 41% in RH-30 at 30 and 60 DAS, over their controls.

4.1.15 Catalase (CAT) activity

The level of CAT showed a completely reverse trend (Table 9), whose activity increased with an increase in the level of the stress (salt) and the plant age of both the varieties. In terms of percentage, salt (5.6 dsm^{-1}) increased the CAT activity by 40% and 27% in Varuna and 23% and 16% in RH-30 at 30 and 60 DAS respectively, compared to their respective control plants.

4.1.16 Peroxidase (POX) activity

It is evident from the table 10 that the activity of peroxidase, in both the varieties, increased with an increase in the level of NaCl stress in the soil. A maximum increase of 57% and

Table 8: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on maximum quantum yield of PSII and nitrate reductase (nmole NO_2 g^{-1} FM s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Maximum quantum yield of PSII (Fv/Fm)						Nitrate reductase activity					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	0.771	0.649	0.710	0.979	0.811	0.895	429	419	424	570	540	555
NaCl (2.8 dsm^{-1})	0.683	0.547	0.615	0.904	0.710	0.807	356	284	320	517	388	452
NaCl (4.2 dsm^{-1})	0.611	0.478	0.545	0.826	0.654	0.740	334	222	278	491	356	423
NaCl (5.6 dsm^{-1})	0.548	0.423	0.486	0.770	0.587	0.679	300	196	248	469	324	396
Mean	0.653	0.524		0.870	0.691		354	280		511	402	
LSD at 5%	V = 0.023 (Sig)			V = 0.076 (Sig)			V = 11.44 (Sig)			V = 9.10 (Sig)		
	T = 0.019 (Sig)			T = 0.021 (Sig)			T = 16.18 (Sig)			T = 12.87 (Sig)		
	V x T = 0.047 (Sig)			V x T = 0.099 (Sig)			V x T = 22.44 (Sig)			V x T = 18.20 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 9: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and catalase (mM H_2O_2 decomposed g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Carbonic anhydrase activity						Catalase activity					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	2.06	1.98	2.02	2.42	2.26	2.34	419	374	396	442	390	416
NaCl (2.8 dsm^{-1})	1.66	1.40	1.53	2.17	1.71	1.94	532	433	482	503	421	462
NaCl (4.2 dsm^{-1})	1.56	1.16	1.36	1.95	1.51	1.73	557	448	502	530	436	483
NaCl (5.6 dsm^{-1})	1.35	0.93	1.14	1.76	1.30	1.53	586	460	523	561	452	506
Mean	1.65	1.36		2.07	1.69		523	428		509	424	
LSD at 5%	V	=	0.06 (Sig)	V	=	0.14 (Sig)	V	=	9.05 (Sig)	V	=	13.59 (Sig)
	T	=	0.11 (Sig)	T	=	0.17 (Sig)	T	=	12.81 (Sig)	T	=	19.22 (Sig)
	V x T	=	0.15 (Sig)	V x T	=	0.26 (Sig)	V x T	=	18.11 (Sig)	V x T =	NS	

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 10: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on peroxidase (units g^{-1} FM) and superoxide dismutase (units g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Peroxidase activity | | | | | | Superoxide dismutase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	12.00	7.09	9.55	16.08	9.21	12.65	125	119	122	156	145	150
NaCl (2.8 dsm^{-1})	16.44	8.36	12.40	20.26	10.32	15.29	184	159	171	209	172	190
NaCl (4.2 dsm^{-1})	17.04	9.15	13.10	22.03	10.68	16.36	202	175	188	234	191	212
NaCl (5.6 dsm^{-1})	18.84	9.57	14.21	22.83	11.51	17.17	246	215	230	285	239	262
Mean	16.08	8.54		20.30	10.43		189	167		221	186	

LSD at 5%												
	V	=	0.22 (Sig)	V	=	0.16 (Sig)	V	=	12.75 (Sig)	V	=	6.98 (Sig)
	T	=	0.31 (Sig)	T	=	0.23 (Sig)	T	=	18.04 (Sig)	T	=	9.88 (Sig)
	V x T	=	0.44 (Sig)	V x T	=	0.32 (Sig)	V x T	=	26.11 (Sig)	V x T = NS		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

42% in Varuna and 35% and 25% in RH-30, over their respective controls, at 30 and 60 DAS were recorded with the highest level of NaCl (5.6 dsm^{-1}). Varuna expressed higher values than RH-30, at both the stages of sampling.

4.1.17 Superoxide dismutase (SOD) activity

The activity of SOD also followed a trend of response similar to that of CAT and POX (Table 10). Leaves of the plants that were raised with 5.6 dsm^{-1} of soil amended NaCl possessed maximum values of SOD activity that were 96% and 82% more in Varuna and 80% and 64% more in RH-30 than their respective controls at 30 and 60 DAS respectively. The variety RH-30 possessed lower values for the SOD activity than the Varuna at both the sampling stages.

4.1.18 Proline content

It is evident from table 11 that the proline content of the leaves was comparatively higher in the plants that were raised in the soil added with different concentrations of NaCl. The values increased with an increase in the concentration of salt (salt level) as well as age of the plants. In terms of percentage, the maximum increase was 71% and 47 % in Varuna and RH-30 respectively, as compared to their respective controls, at 60 day stage of the plant growth. RH-30 had lower values than Varuna at both the stages of sampling.

4.1.19 Yield characteristics per plant

All the observed yield characteristics (number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant) exhibited a significant linear decrease in their values in response to an increase in NaCl concentration in both the varieties (Table 12). The maximum loss in the values of all the above yield characteristics was noticed in RH-30 at the highest stress level of NaCl (5.6 dsm^{-1}). In terms of per cent the maximum decrease in the number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant was 25%, 18%, 10% and 34% in Varuna and 30%, 33%, 10% and 47% in RH-30 at 5.6 dsm^{-1} of NaCl respectively, as compared to the control plants.

4.2 Experiment 2

This experiment was set up according to simple randomized block design to study the effect of foliar application of HBL (10^{-8} M) or EBL (10^{-8} M) on *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30 during the winter season of 2009-10. All the agronomic and

Table 11: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on proline content (μmol g^{-1} FM) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Proline Content					
	30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	13.84	13.50	13.67	19.37	17.55	18.46
NaCl (2.8 dsm^{-1})	21.31	19.17	20.24	28.86	21.58	25.22
NaCl (4.2 dsm^{-1})	23.80	20.65	22.23	31.96	22.81	27.39
NaCl (5.6 dsm^{-1})	25.60	22.81	24.21	33.31	25.79	29.55
Mean	21.14	19.03		28.38	21.93	
LSD at 5%	V	=	0.20 (Sig)	V	=	0.27 (Sig)
	T	=	0.28 (Sig)	T	=	0.39 (Sig)
	V x T	=	0.40 (Sig)	V x T	=	0.55 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 12: Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on pods plant^{-1}, seeds pod^{-1}, 100 seed mass (mg) and seed yield plant^{-1} (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Pods plant^{-1}			Seeds pod^{-1}			100 seeds mass			Seed yield plant^{-1}		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	220	211	215	13.20	11.42	12.31	311	304	307	7.23	6.50	6.87
NaCl (2.8 dsm^{-1})	187	170	178	11.72	9.25	10.49	298	279	288	5.86	4.94	5.40
NaCl (4.2 dsm^{-1})	176	162	169	11.16	8.68	9.92	287	265	276	5.27	4.03	4.65
NaCl (5.6 dsm^{-1})	165	147	156	10.83	7.65	9.24	275	252	263	4.77	3.45	4.11
Mean	187	172		11.73	9.25		294	277		5.78	4.73	
LSD at 5%	V = 4.96 (Sig)			V = 0.13 (Sig)			V = 5.86 (Sig)			V = 0.16 (Sig)		
	T = 7.02 (Sig)			T = 0.18 (Sig)			T = 9.01 (Sig)			T = 0.23 (Sig)		
	V × T = NS			V × T = 0.26 (Sig)			V × T = NS			V × T = 0.33 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

cultural practices remained the same as described in Experiment 1. The foliage of 29 days old seedlings was sprayed either with DDW (control), Tween-20 (0.5%), ethanol (5%), HBL (10^{-8} M) or EBL (10^{-8} M). The plants were sampled at 30 and 60 DAS.

4.2.1 Shoot and root length

The shoot and root length increased as growth progressed from 30 to 60 DAS in both Varuna and RH-30 (Table 13). The plants sampled at 60 day stage exhibited a significant increase in both shoot and root length, provided their foliage was sprayed with HBL or EBL, compared with the control that was at par with Tween-20 and ethanol. Varuna excelled in its response to the treatment than RH-30. The spray of HBL increased the shoot length by 66% and 55% and root length by 43% and 38% in Varuna and RH-30 respectively whereas EBL increased shoot length by 70% and 62% and root length 56% and 41% respectively in the two varieties, at 60 DAS, compared to their control plants.

4.2.2 Shoot and root fresh mass

As the growth advanced from 30 to 60 DAS, the fresh mass of shoot and root increased (Table 14). Foliar spray of Tween-20 and ethanol did not influence the fresh mass. However, HBL and EBL significantly increased the shoot and root fresh mass, noted at 60 DAS in both the varieties. In terms of the percentage, the EBL increased the shoot and root fresh mass by 65% and 66% in Varuna and 62% and 31% in RH-30 at 60 DAS, compared to their respective control plants.

4.2.3 Shoot and root dry mass

The dry mass of shoot and root showed a pattern similar to that of fresh mass in both the varieties at the both sampling stages (Table 15). The foliar spray of EBL excelled over that of HBL in increasing the dry mass of shoot and root at 60 DAS. The foliar spray of EBL increased the shoot dry mass by 84% and 78% and root dry mass by 77 and 66% in Varuna and RH-30 respectively as compared to their respective controls.

4.2.4 Leaf area

Leaf area increased as growth progressed from 30 to 60 DAS in both the varieties (Table 16). The foliar spray of HBL or EBL significantly increased leaf area at 60 day stage, in both the varieties. The increase in leaf area by HBL treatment was 28% and 23% and by

Table 13: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-8} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on shoot and root length (cm) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot length | | | | | | Root length | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	18.74	15.39	17.07	74.12	68.62	71.37	16.39	15.17	15.78	24.58	22.57	23.58
Tween-20	18.80	15.40	17.10	74.14	68.65	71.39	16.40	15.17	15.78	24.63	22.56	23.59
Ethanol	18.81	15.42	17.12	74.14	68.64	71.39	16.29	15.13	15.71	24.67	22.59	23.63
HBL	18.85	15.45	17.15	123.33	106.84	115.09	16.43	15.21	15.82	35.39	31.37	33.38
EBL	18.89	15.49	17.19	126.15	111.71	118.93	16.46	15.26	15.86	38.59	32.04	35.32
Mean	18.81	15.43		94.38	84.89		16.39	15.19		29.57	26.23	

LSD at 5%	V	=	0.48 (Sig)	V	=	4.09 (Sig)	V	=	0.22 (Sig)	V	=	0.81 (Sig)
	T	=	NS	T	=	6.46 (Sig)	T	=	NS	T	=	1.29 (Sig)
	V × T	=	NS	V × T	=	NS	V × T	=	NS	V × T	=	1.83 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 14: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-8} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on shoot and root fresh mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot fresh mass | | | | | | Root fresh mass | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	6.34	5.50	5.92	11.91	10.50	11.21	2.80	2.42	2.61	5.42	4.59	5.01
Tween-20	6.28	5.42	5.85	11.88	10.47	11.18	2.76	2.35	2.56	5.40	4.55	4.98
Ethanol	6.37	5.52	5.94	11.94	10.52	11.23	2.79	2.38	2.58	5.43	4.58	5.01
HBL	6.42	5.57	5.99	18.93	16.17	17.55	2.83	2.43	2.63	8.07	6.42	7.25
EBL	6.46	5.61	6.03	19.65	17.01	18.33	2.90	2.48	2.69	8.99	7.20	8.10
Mean	6.37	5.52		14.86	12.94		2.82	2.41		6.66	5.47	
LSD at 5%	V = 0.10 (Sig)			V = 0.86 (Sig)			V = 0.08 (Sig)			V = 0.44 (Sig)		
	T = NS			T = 1.36 (Sig)			T = NS			T = 0.70 (Sig)		
	V x T = NS			V x T = NS			V x T = NS			V x T = 0.99 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 15: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-8} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on shoot and root dry mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot dry mass | | | | | | Root dry mass | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	1.77	1.56	1.67	3.46	2.99	3.23	0.781	0.592	0.687	1.52	1.13	1.33
Tween-20	1.74	1.52	1.63	3.50	2.96	3.23	0.781	0.590	0.686	1.58	1.14	1.36
Ethanol	1.75	1.52	1.64	3.52	2.95	3.24	0.780	0.588	0.684	1.56	1.13	1.35
HBL	1.78	1.56	1.67	6.08	4.96	5.52	0.789	0.595	0.692	2.46	1.79	2.13
EBL	1.81	1.57	1.69	6.36	5.32	5.84	0.792	0.597	0.695	2.69	1.88	2.29
Mean	1.77	1.55		4.58	3.84		0.785	0.592		1.96	1.41	
LSD at 5%	V	=	0.07 (Sig)	V	=	0.08 (Sig)	V	=	0.02 (Sig)	V	=	0.04 (Sig)
	T	=	NS	T	=	0.13 (Sig)	T	=	NS	T	=	0.07 (Sig)
	V x T	=	NS	V x T =		0.19 (Sig)	V x T =		NS	V x T =		0.10 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 16: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-8} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on leaf area (cm^2) and SPAD Chlorophyll values in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Leaf area | | | | | | SPAD Chlorophyll value | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	24.89	23.20	24.05	44.23	33.40	38.82	44.50	37.22	40.86	58.74	48.38	53.56
Tween-20	24.92	23.25	24.09	44.26	33.42	38.84	44.90	37.43	41.17	58.80	48.41	53.61
Ethanol	24.84	23.12	23.98	44.20	33.35	38.78	44.60	37.13	40.87	58.82	48.36	53.59
HBL	24.95	23.29	24.12	56.61	41.08	48.85	59.18	45.78	52.48	73.42	56.12	64.77
EBL	24.99	23.36	24.18	61.92	44.75	53.34	61.85	47.64	54.75	75.77	58.53	67.15
Mean	24.92	23.24		50.24	37.20		51.01	41.04		65.11	51.96	

LSD at 5%	V	=	0.92 (Sig)	V	=	0.96 (Sig)	V	=	0.42(Sig)	V	=	0.47 (Sig)
	T	=	NS	T	=	1.51 (Sig)	T	=	0.67(Sig)	T	=	0.74 (Sig)
	V x T	=	NS	V x T	=	2.14 (Sig)	V x T	=	0.95 (Sig)	V x T = 1.05 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

EBL 40% and 34% in Varuna and RH-30 respectively at 60 DAS, compared to their controls. Out of the two BR analogues EBL excelled over HBL in both the varieties.

4.2.5 SPAD chlorophyll values

As evident from the table 16, the SPAD chlorophyll values increased as growth progressed from 30 to 60 DAS in both the varieties. Tween-20/ethanol were ineffective, however application of either of the BR analogues (HBL or EBL; 10^{-8} M) generated the most promising response and significantly increased the chlorophyll content in both the varieties. EBL increased the SPAD values of chlorophyll by 39% and 29% in Varuna and 28% and 21% in RH-30 at 30 and 60 DAS respectively when compared to their respective controls. Out of the two BR analogues, EBL was more effective than HBL.

4.2.6 Electrolyte leakage

Cnmpared to 30 day stage, the leakage of ions from the leaves was less at 60 DAS in both the varieties (Table 17). Foliar spray of BR analogues (HBL or EBL), tween-20 or ethanol had not significant influence on the electrolyte leakage in the leaves at 30 DAS. However, at 60 day stage the values were significantly lower in the leaves that received the treatment of BRs in both varieties, being more prominent in Varuna than in RH-30. In terms of percentage, EBL excelled over HBL and decreased electrolyte leakage by 30% and 24% in Varuna and RH-30 respectively at 60 DAS, compared to their respective control plants.

4.2.7 Leaf water potential

Leaf water potential in the leaves which were sprayed with either of the BR analogue (HBL or EBL) increased significantly, being more prominent at 30 day stage than at 60 day, in both the varieties (Table17). Tween-20 and ethanol spray had no impact on leaf water potential. EBL increased the leaf water potential by 37% and 30% in Varuna and 31% and 24% in RH-30 at 30 DAS and 60 DAS respectively, compared to the respective controls. The values of leaf water potential were higher in Varuna than in RH-30 at both the sampling stages. EBL excelled in its effect over HBL.

4.2.8 Net photosynthetic rate

The net photosynthetic rate increased substantially as the age of the plants progressed from 30 to 60 DAS in both the varieties (Table18). Moreover, the leaves of the plants sprayed with HBL/EBL phntosynthesized at higher rate than the control plants at both the stages of

Table 17: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-9} M) and 24-epibrassinolide (EBL; 10^{-9} M) as foliar spray (29 d stage) on electrolyte leakage (%) and leaf water potential (MPa) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Electrolyte leakage						Leaf Water Potential					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	7.30	8.33	7.82	5.72	6.82	6.27	-0.63	-0.73	-0.73	-0.46	-0.53	-0.50
Tween-20	7.21	8.27	7.74	5.74	6.80	6.27	-0.63	-0.73	-0.68	-0.45	-0.52	-0.49
Ethanol	7.30	8.31	7.81	5.74	6.81	6.28	-0.62	-0.73	-0.68	-0.46	-0.52	-0.49
HBL	7.28	8.31	7.80	4.34	5.44	4.89	-0.45	-0.54	-0.50	-0.35	-0.43	-0.39
EBL	7.26	8.30	7.78	4.03	5.19	4.61	-0.40	-0.50	-0.45	-0.32	-0.40	-0.36
Mean	7.27	8.30		5.11	6.21		-0.53	-0.65		-0.41	-0.48	
LSD at 5%	V = 0.09 (Sig)			V = 0.14 (Sig)			V = 0.016 (Sig)			V = 0.02 (Sig)		
	T = NS			T = 0.22 (Sig)			T = 0.025 (Sig)			T = 0.03 (Sig)		
	V x T = NS			V x T = NS			V x T = 0.036 (Sig)			V x T = 0.05 (Sig)		

V – Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 18: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-8} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Net photosynthetic rate (P_N)						Stomatal Conductance (g_s)					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	21.66	19.13	20.40	27.50	23.91	25.71	0.066	0.054	0.060	0.095	0.074	0.085
Tween-20	21.55	19.01	20.28	27.48	23.87	25.68	0.067	0.054	0.061	0.097	0.075	0.086
Ethanol	21.47	18.98	20.23	27.47	23.84	25.66	0.065	0.052	0.059	0.095	0.073	0.084
HBL	29.67	25.63	27.65	34.37	28.66	32.59	0.111	0.085	0.098	0.148	0.105	0.127
EBL	30.75	26.30	28.53	36.85	30.12	34.88	0.121	0.092	0.107	0.168	0.111	0.139
Mean	25.02	21.81		31.21	26.59		0.086	0.067		0.121	0.088	
LSD at 5%	V	=	0.36 (Sig)	V	=	0.51 (Sig)	V	=	0.0001(Sig)	V	=	0.0003 (Sig)
	T	=	0.57 (Sig)	T	=	0.81 (Sig)	T	=	0.0002(Sig)	T	=	0.0005 (Sig)
	V × T	=	0.80 (Sig)	V × T	=	1.15 (Sig)	V × T	=	0.0003(Sig)	V × T	=	0.0008 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

growth (30 and 60 DAS). A maximum increase of about 42% and 37% was recorded in the leaves of Varuna and RH-30 respectively, sprayed with EBL (10^{-8} M), whereas with HBL the increase was 37% and 34% at 30 DAS, compared to their respective control plants. Out of the two varieties, Varuna performed better than RH-30.

4.2.9 Stomatal conductance

The values of stomatal conductance in the leaves of both Varuna and RH-30 exhibited an increase as the growth advanced from 30 to 60 DAS (Table 18). The values increased significantly in the leaves of both the varieties that received either of the brassinosteroid analogues (HBL/EBL) at 30 and 60 DAS. EBL was more effective than HBL. EBL increased the stomatal conductance by 83% and 70% in Varuna and RH-30 respectively at 30 DAS compared to their respective control plants.

4.2.10 Internal CO_2 concentration

The internal CO_2 concentration followed a pattern similar to that of stomatal conductance (Table 19). Foliar spray of either of the brassinosteroid analogues (HBL/EBL) significantly increased the internal CO_2 concentration in both the varieties at 30 and 60 DAS. Maximum increase in internal CO_2 concentration was recorded at 30 DAS in the leaves sprayed with EBL (10^{-8} M) which was 27% and 13% higher in Varuna and RH-30, over their control plants.

4.2.11 Transpiration rate

The transpiration rate in the plants of both varieties (Varuna and RH-30) expressed a response similar to that of g_s (Table 19). A significant increase in the transpiration rate was recorded in the plants sprayed with either of the hormone (HBL/EBL). EBL (10^{-8} M) sprayed leaves showed a maximum increase in the values at 30 DAS which was 49% and 30% more in Varuna and RH-30 respectively, over the control plants. Plants exhibited a similar trend at 60 DAS.

4.2.12 Maximum quantum yield of PSII (Fv/Fm)

The values for Fv/Fm increased as the growth progressed from 30 to 60 d stage (Table 20). The leaves of plants treated with either of the brassinosteroid analogues (HBL/EBL) had higher values for Fv/Fm, over the control plants. EBL excelled over HBL in enhancing the values that were 36% and 24% in Varuna and 29% and 18% higher in

Table 19: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-8} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O m^{-2} s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Internal CO_2 concentration (C_i) | | | | | | Transpiration rate (E) | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	**Mean**	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	318	280	299	365	315	340	3.27	2.90	3.09	4.30	3.90	4.10
Tween-20	320	281	300	366	315	340	3.24	2.83	3.04	4.31	3.94	4.13
Ethanol	318	278	298	363	312	337	3.20	2.79	2.99	4.28	3.98	4.13
HBL	388	322	355	422	350	386	4.44	3.59	4.02	5.46	4.62	5.04
EBL	404	316	360	419	345	382	4.87	3.77	4.32	5.85	4.78	5.32
Mean	349	295		387	327		3.80	3.18		4.84	4.24	
LSD at 5%	V	=	17.46 (Sig)	V	=	2.74 (Sig)	V	=	0.10 (Sig)	V	=	0.05 (Sig)
	T	=	27.61 (Sig)	T	=	4.33 (Sig)	T	=	0.17 (Sig)	T	=	0.08 (Sig)
	V × T	=	NS	V × T	=	6.13 (Sig)	V × T	=	0.24 (Sig)	V × T	=	0.12 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 20: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-8} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on maximum quantum yield of PSII (Fv/Fm) and nitrate reductase activity (nmole NO_2 g^{-1} FM s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Maximum quantum yield of PSII (Fv/Fm) | | | | | | Nitrate reductase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	0.779	0.654	0.717	0.972	0.819	0.896	423	390	406	522	468	495
Tween-20	0.775	0.649	0.712	0.975	0.816	0.896	426	392	409	523	468	495
Ethanol	0.773	0.646	0.709	0.979	0.822	0.900	428	393	410	522	467	494
HBL	1.000	0.807	0.904	1.140	0.921	1.031	626	527	576	679	571	625
EBL	1.060	0.846	0.953	1.210	0.970	1.090	647	562	604	715	608	661
Mean	0.877	0.720		1.055	0.869		510	452		592	516	
LSD at 5%	V	=	0.010 (Sig)	V	=	0.014 (Sig)	V	=	13.26 (Sig)	V	=	13.93 (Sig)
	T	=	0.015 (Sig)	T	=	0.023 (Sig)	T	=	20.97 (Sig)	T	=	22.03 (Sig)
	V × T	=	0.022 (Sig)	V × T	=	0.032 (Sig)	V × T	=	NS	V × T	=	NS

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

RH-30 at 30 and 60 DAS respectively, compared to the control plants. The variety Varuna was more responsive to the treatment than RH-30.

4.2.13 Nitrate reductase (NR) activity

As evident from table 20, the leaves of both varieties that received foliar spray of HBL or EBL (10^{-8} M) possessed significantly higher activity of nitrate reductase, at both the stages of growth (30 and 60 DAS), as compared to their respective controls. Moreover, the enzyme activity increased with the advancement of age (30 to 60 DAS). EBL proved better than HBL that enhanced the activity by 53% and 37% in Varuna and 44% and 30% in RH-30, over their respective controls at 30 and 60 DAS, respectively.

4.2.14 Carbonic anhydrase (CA) activity

The activity of carbonic anhydrase followed the trend similar to that of NR (4.2.13). The activity of CA increased with the age of the plant (Table 21). Brassinosteroids (HBL/EBL) applied to the foliage of the plants, improved the CA activity up to a significant level. Maximum values for enzyme activity were recorded in the leaves sprayed with EBL (10^{-8}M) and were 41% and 30% in Varuna and 31% and 19% in RH-30 more than the control plants at 30 and 60 DAS respectively. The other analogue HBL (10^{-8}M) increased the values of CA activity by 32% and 21% in Varuna and 25% and 17% in RH-30 at 30 and 60 DAS, over the control, respectively.

4.2.15 Catalase (CAT) activity

The activity of catalase enzyme increased as the growth progressed from 30 to 60 DAS (Table 21) which improved further with the application of BRs (10^{-8} M). Maximum values were recorded with EBL where CAT activity improved by 28% and 20% in Varuna and 19% and 13% in RH-30 at 30 and 60 DAS respectively compared to their water sprayed controls.

4.2.16 Peroxidase (POX) activity

The data depicted in table 22 clearly revealed an increase in the activity of POX, as growth progressed from 30 to 60 d stage and also in response to the two analogues of brassinosteroids (HBL/EBL) in both the varieties (Varuna and RH-30). Out of them, EBL proved better than HBL and enhanced the activity to a maximum level. The response of POX activity to the brassinosteroids analogues was similar to that of CAT activity (4.2.15).

Table 21: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-8} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and catalase (mM H_2O_2 decomposed g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Carbonic anhydrase activity | | | | | | Catalase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	2.10	1.99	2.04	2.46	2.25	2.35	423	372	397	443	390	416
Tween-20	2.12	2.00	2.06	2.51	2.23	2.37	424	372	398	443	389	416
Ethanol	2.10	2.02	2.06	2.43	2.20	2.31	425	373	399	443	388	415
HBL	2.77	2.49	2.63	2.98	2.52	2.75	508	424	466	514	425	469
EBL	2.98	2.61	2.79	3.20	2.69	2.94	541	443	492	532	440	486
Mean	2.41	2.22		2.71	2.38		464	396		475	406	
LSD at 5%	V	=	0.10 (Sig)	V	=	0.14 (Sig)	V	=	10.25 (Sig)	V	=	12.84 (Sig)
	T	=	0.18 (Sig)	T	=	0.20 (Sig)	T	=	15.79 (Sig)	T	=	18.37 (Sig)
	V x T	=	NS	V x T =		0.33 (Sig)	V x T	=	NS	V x T =		NS

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 22: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-8} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on peroxidase (units g^{-1} FM) and superoxide dismutase (units g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Peroxidase activity | | | | | | Superoxide dismutase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	12.79	7.30	10.05	17.13	9.52	13.33	137	118	127	171	142	156
Tween-20	12.83	7.31	10.07	17.18	9.54	13.36	135	114	124	169	140	154
Ethanol	12.85	7.32	10.08	17.13	9.49	13.31	137	119	128	172	142	157
HBL	18.42	9.93	14.17	22.78	11.58	17.18	167	138	152	200	160	180
EBL	20.08	10.80	15.44	24.32	12.38	18.35	178	141	159	215	165	190
Mean	15.39	8.53		19.71	10.50		150	126		185	149	

LSD at 5%	V = 0.16 (Sig)	V = 0.13 (Sig)	V = 6.84 (Sig)	V = 7.43 (Sig)
	T = 0.25 (Sig)	T = 0.21 (Sig)	T = 10.82 (Sig)	T = 11.76 (Sig)
	V x T = 0.36 (Sig)	V x T = 0.30 (Sig)	V x T = NS	V x T = NS

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.2.17 Superoxide dismutase (SOD) activity

It is evident from table 22 that the leaves of the plants of Varuna and RH-30 which received HBL or EBL (10^{-8} M), possessed higher SOD activity, over their respective controls at both the stages of growth (30 and 60 DAS). The HBL improved the SOD activity by 22% and 17% in Varuna and 17 and 13% in RH-30, over the control, at 30 and 60 DAS. However, EBL induced a prominent impact where the activity increased by 30% and 26% in Varuna and 19% and 16 % in RH-30, over the water sprayed control plants at 30 and 60 DAS, respectively.

4.2.18 Proline content

The leaf proline content in both the varieties (Varuna and RH-30) increased with age and the spray of either HBL or EBL (10^{-8} M). The plants that received EBL possessed highest quantities of proline as compared to their water sprayed controls. The values increased by 47% and 22 % in Varuna and 21% and 14% in RH-30 at 30 and 60 DAS respectively, compared to their respective control plants (Table 23).

4.2.19 Yield characteristics per plant

At harvest, number of pods and seed yield per plant were significantly increased by the foliar application of either HBL or EBL where EBL (10^{-8} M) generated maximum response which was more pronounced in Varuna than RH-30 (Table 24). A maximum increase of 41% and 34%, and 35% and 31% in number of pods and seed yield per plant was recorded in Varuna and RH-30 respectively, over the control, by the treatment with EBL (10^{-8} M). However, the other characteristics (number of seeds per pod and 100 seed mass) were not increased significantly.

4.3 Experiment 3

This experiment was carried out with an aim to study the effect of various concentrations (0, 10, 20 or 30 mM) of proline in the two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss. All the agricultural practices were same as in Experiment 1. At 29 DAS, the plants were sprayed with DDW (control), 10 mM, 20 mM or 30 mM of proline. The plant samples were collected at 30 and 60 DAS to assess various parameters same as in Experiment 1. The rest of the plants were allowed to grow to maturity and harvested (120 DAS) to study yield characteristics.

Table 23: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-9} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on proline content (μmol g^{-1} FM) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Proline content					
	30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	13.00	12.29	12.65	19.37	17.82	18.59
Tween-20	13.08	12.32	12.70	19.41	17.85	18.63
Ethanol	13.13	12.35	12.74	19.44	17.82	18.63
HBL	18.07	16.59	17.33	23.05	20.14	21.59
EBL	19.11	14.87	16.99	23.63	20.31	21.97
Mean	15.28	13.68		20.98	18.79	
LSD at 5%	V = 0.51 (Sig)			V = 0.27 (Sig)		
	T = 0.81 (Sig)			T = 0.44 (Sig)		
	V x T = 1.15 (Sig)			V x T = 0.62 (Sig)		

V = Varieties; T = Treatments; Sig - Significant; NS - Non-significant

Table 24: Effect of tween-20 (0.5%), ethanol (5%), 28-homobrassinolide (HBL; 10^{-8} M) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) on pods plant^{-1}, seeds pod^{-1}, 100 seed mass (mg) and seed yield plant^{-1} (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Pods plant^{-1}			Seeds pod^{-1}			100 seed mass			Seed yield plant^{-1}		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	231	211	221	12.640	12.00	12.32	319	307	313	7.13	6.84	6.98
Tween-20	234	212	223	12.690	12.04	12.37	323	309	316	7.19	6.89	7.04
Ethanol	236	214	225	12.730	12.08	12.41	321	311	316	7.24	6.93	7.09
HBL	316	278	297	12.750	12.18	12.47	328	313	320	9.41	8.69	9.05
EBL	325	286	305	12.780	12.23	12.51	333	316	324	9.55	8.96	9.26
Mean	268	240		12.72	12.11		324	311		8.10	7.66	
LSD at 5%	V	=	3.03 (Sig)	V	=	0.36 (Sig)	V	=	7.68(Sig)	V	=	0.11 (Sig)
	T	=	4.80 (Sig)	T	=	NS	T	=	NS	T	=	0.17 (Sig)
	V x T	=	6.79 (Sig)	V x T =		NS	V x T	=	NS	V x T	=	0.24 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.3.1 Shoot and root length

Length of shoot and root increased as the growth progressed from 30 to 60 DAS (Table 25). Foliar spray of different concentrations of proline (10, 20 or 30 mM) significantly increased the values for shoot and root length of both the varieties at 60 DAS. Out of the three concentrations of proline used, 20 mM generated the maximum response and increased the length of shoot and root by 47% and 35% in Varuna and 39% and 28% in RH-30 at 60 DAS, as compared to their respective control plants. Varuna proved better than RH-30 in its response.

4.3.2 Shoot and root fresh mass

As the age of the plants of the two varieties progressed from 30 to 60 DAS, fresh mass of shoot and root increased (Table 26). Among the three concentrations (10, 20 or 30 mM) of proline, 20 mM generated a maximum response as compared to the control plants both in Varuna and RH-30 at 60 DAS. The per cent increase was higher in Varuna than in RH-30 at 60 DAS. Spray of proline (20 mM) increased the fresh mass of shoot and root by 45% and 33% in Varuna and 37% and 28% in RH-30 respectively at 60 DAS, as compared to their respective control plants.

4.3.3 Shoot and root dry mass

Like fresh mass (4.3.2), dry mass of shoot and root also increased as the age of plants progressed from 30 to 60 DAS both in Varuna and RH-30 (Table 27). Out of the levels (10, 20 or 30 mM) of proline, a medium concentration (20 mM) proved best in increasing the dry mass of shoot and root in both the varieties at 60 DAS. The shoot and root dry mass improved by 48% and 42% in Varuna and 40% and 34% in RH-30 respectively at 60 DAS, compared to their respective control plants. Varuna responded more effectively to proline than RH-30 at 60 DAS.

4.3.4 Leaf area

Like other growth parameters (4.3.1, 4.3.2 and 4.3.3) leaf area increased as the growth advanced from 30 to 60 DAS in the plants of Varuna and RH-30 (Table 28). Proline (20 mM) generated maximum increase in the leaf area which was 23% and 18% in Varuna and RH-30 respectively at 60 DAS, compared to their respective control plants. Out of the two varieties, Varuna responded better than RH-30.

Table 25: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on shoot and root length (cm) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot length | | | | | | Root length | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	19.00	15.63	17.32	73.72	59.23	66.48	16.000	14.920	15.46	24.48	22.23	23.36
Proline (10 mM)	19.04	15.65	17.34	102.47	77.13	89.80	16.007	14.950	15.48	31.09	26.68	28.89
Proline (20 mM)	19.10	15.68	17.39	108.37	82.47	95.42	16.180	14.990	15.59	33.05	28.68	30.87
Proline (30 mM)	19.07	15.67	17.37	104.68	80.09	92.39	16.110	14.970	15.54	31.82	28.00	29.91
Mean	19.05	15.66		97.31	74.73		16.07	14.96		30.11	26.39	
LSD at 5%	V = 0.96 (Sig)			V = 4.56 (Sig)			V = 0.18(Sig)			V = 0.58 (Sig)		
	T = NS			T = 6.45 (Sig)			T = NS			T = 0.82 (Sig)		
	V x T = NS			V x T = NS			V x T = NS			V x T = 1.17 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 26: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on shoot and root fresh mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Shoot fresh mass						Root fresh mass					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	6.20	5.39	5.79	11.54	9.97	10.76	2.74	2.39	2.57	5.31	4.54	4.93
Proline (10 mM)	6.21	5.39	5.80	15.46	12.96	14.21	2.77	2.41	2.59	6.48	5.40	5.94
Proline (20 mM)	6.25	5.41	5.83	16.73	13.71	15.22	2.80	2.42	2.61	7.06	5.81	6.44
Proline (30 mM)	6.24	5.40	5.82	16.04	13.38	14.71	2.79	2.42	2.61	6.69	5.54	6.12
Mean	6.23	5.39		14.94	12.51		2.78	2.41		6.39	5.32	
LSD at 5%	V	=	0.11 (Sig)	V	=	0.64 (Sig)	V	=	0.15 (Sig)	V	=	0.28 (Sig)
	T	=	NS	T	=	0.91 (Sig)	T	=	NS	T	=	0.39 (Sig)
	V x T	=	NS	V x T	=	1.23 (Sig)	V x T	=	NS	V x T	=	0.56 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 27: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on shoot and root dry mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot dry mass | | | | | | Root dry mass | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	1.82	1.60	1.71	3.54	3.09	3.32	0.769	0.532	0.651	1.48	1.02	1.25
Proline (10 mM)	1.82	1.61	1.72	4.81	4.02	4.42	0.770	0.530	0.650	1.85	1.22	1.54
Proline (20 mM)	1.86	1.64	1.75	5.24	4.33	4.79	0.775	0.533	0.654	2.10	1.37	1.74
Proline (30 mM)	1.84	1.64	1.74	5.03	4.23	4.63	0.773	0.532	0.653	1.92	1.32	1.62
Mean	1.84	1.62		4.66	3.92		0.772	0.532		1.84	1.23	
LSD at 5%	V = 0.04 (Sig)			V = 0.07 (Sig)			V = 0.031(Sig)			V = 0.04 (Sig)		
	T = NS			T = 0.10 (Sig)			T = NS			T = 0.06 (Sig)		
	V x T = NS			V x T = 0.14 (Sig)			V x T = NS			V x T = 0.09 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.3.5   SPAD chlorophyll values

Chlorophyll content (SPAD values) increased with the plant age (Table 28). Out of the two varieties, Varuna possessed higher chlorophyll content than RH-30 at both the growth stages. Proline (10, 20 or 30 mM) spray increased the chlorophyll content in both the varieties at both the stages of growth being more at 30 DAS than at 60 DAS. 20 mM proved the best concentration that increased the chlorophyll content by 24% and 20% in Varuna and RH-30 at 30 DAS respectively, when compared to their respective control plants.

4.3.6   Electrolyte leakage

As the growth advances from 30 to 60 DAS, the leakage of ions from leaves decreased in both Varuna and RH-30 (Table 29). Foliar spray of graded concentrations (10, 20 or 30 mM) of proline had no significant impact on the leakage of ions at 30 day stage. However, at 60 DAS the electrolyte leakage decreased significantly in both the varieties. Out of the different concentrations of proline, 20 mM proved best that checked the loss by 18% and 15% in Varuna and RH-30 respectively at 60 DAS, compared to their respective control plants.

4.3.7   Leaf water potential

Proline (10, 20 or 30 mM) applied to the foliage increased the leaf water potential in Varuna and RH-30 at both the stages of growth, the per cent increase was more at 30 DAS than at 60 DAS (Table 29). A moderate concentration (20 mM) improved the leaf water potential by 21% and 18% in Varuna and 16% and 11% in RH-30 at 30 and 60 DAS respectively, when compared to their respective control plants.

4.3.8   Net photosynthetic rate

As depicted in table 30, the photosynthetic rate increased from day 30 to 60 both in Varuna and RH-30. Exogenous application of proline significantly enhanced the net photosynthetic rate (P_N), more in Varuna than RH-30, at both the sampling stages. Among various concentrations (10, 20 or 30 mM) of proline tested, foliar spray of 20 mM generated maximum response and enhanced P_N by 27% and 25% in Varuna and 25% and 17% in RH-30 at 30 and 60 DAS, respectively compared to their control plants. Varuna photosynthesized at a higher rate than RH-30.

Table 28: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on leaf area (cm^2) and SPAD chlorophyll values in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Leaf area						SPAD Chlorophyll value					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	25.17	23.36	24.27	41.53	37.96	39.75	45.17	38.00	41.59	60.97	49.36	55.17
Proline (10 mM)	25.20	23.38	24.29	48.17	42.89	45.53	54.16	43.02	48.59	68.59	53.11	60.85
Proline (20 mM)	25.28	23.44	24.36	51.08	44.79	47.94	56.24	45.52	50.88	73.10	56.12	64.61
Proline (30 mM)	25.24	23.40	24.32	49.42	43.65	46.54	54.97	44.49	49.73	70.66	55.14	62.90
Mean	25.22	23.39		47.55	42.32		52.64	42.76		68.33	53.43	

LSD at 5%												
	V	= 1.04 (Sig)		V	= 1.05 (Sig)		V	= 0.45(Sig)		V	= 1.27 (Sig)	
	T	= NS		T	= 1.49 (Sig)		T	= 0.63(Sig)		T	= 1.81 (Sig)	
	V x T	= NS		V x T	= 1.71 (Sig)		V x T	= 0.90 (Sig)		V x T	= 2.55 (Sig)	

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 29: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on electrolyte leakage (%) and leaf water potential (MPa) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Electrolyte leakage						Leaf water potential					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	7.68	8.72	8.20	5.79	6.78	6.29	-0.65	-0.72	-0.69	-0.45	-0.53	-0.49
Proline (10 mM)	7.72	8.70	8.21	5.22	6.27	5.75	-0.57	-0.66	-0.62	-0.41	-0.50	-0.46
Proline (20 mM)	7.78	8.84	8.31	4.71	5.74	5.23	-0.51	-0.60	-0.56	-0.37	-0.47	-0.42
Proline (30 mM)	7.79	8.84	8.32	5.01	6.02	5.52	-0.54	-0.63	-0.59	-0.39	-0.48	-0.44
Mean	7.74	8.76		5.18	6.20		-0.57	-0.65		-0.41	-0.49	
LSD at 5%	V	=	0.16 (Sig)	V	=	0.24 (Sig)	V	=	0.0073 (Sig)	V	=	0.0100 (Sig)
	T	=	NS	T	=	0.33 (Sig)	T	=	0.0104 (Sig)	T	=	0.0141 (Sig)
	V x T	=	NS	V x T	=	NS	V x T	=	0.0147 (Sig)	V x T	=	0.0199 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 30: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Net photosynthetic rate (P_N)						Stomatal conductance (g_s)					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	21.09	19.48	20.29	28.00	25.73	26.87	0.059	0.050	0.055	0.079	0.067	0.073
Proline (10 mM)	25.20	22.75	23.98	31.44	28.12	29.78	0.082	0.065	0.074	0.106	0.086	0.096
Proline (20 mM)	26.78	24.25	25.51	34.94	30.28	32.61	0.089	0.073	0.081	0.112	0.092	0.102
Proline (30 mM)	26.15	23.57	24.86	33.60	29.17	31.39	0.085	0.065	0.075	0.109	0.088	0.099
Mean	24.81	22.51		31.99	28.33		0.079	0.063		0.102	0.083	

LSD at 5%												
	V	=	0.29 (Sig)	V	=	0.37 (Sig)	V	=	0.0012 (Sig)	V	=	0.009 (Sig)
	T	=	0.41 (Sig)	T	=	0.53 (Sig)	T	=	0.0017 (Sig)	T	=	0.003 (Sig)
	V x T	=	0.58 (Sig)	V x T	=	0.75 (Sig)	V x T	=	0.0024 (Sig)	V x T	=	0.015 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.3.9 Stomatal conductance

The stomatal conductance (g_s) followed a pattern similar to that of P_N (4.3.8) and the values increased in the plants, sprayed with different concentrations of proline (Table 30). Proline (20 mM) induced maximum response and increased the g_s by 50% and 41% in Varuna and 46% and 37% in RH-30 at 30 and 60 DAS respectively, compared to the respective control plants.

4.3.10 Internal CO_2 concentration

Foliar spray of proline increased internal CO_2 concentration (C_i) at both stages of the growth (30 and 60 DAS) (Table 31). Proline (20 mM) enhanced the values by 18% and 16% in Varuna and 16% and 11% in RH-30 at 30 and 60 DAS respectively over their control plants.

4.3.11 Transpiration rate

The transpiration rate (E) of the plants expressed a response similar to that of C_i (4.3.10) where proline had a positive effect at the two samplings (30 and 60 days) in both Varuna and RH-30 (Table 31). A maximum increase was recorded in the plants sprayed with 20 mM proline where the values increased by 29% and 25% in Varuna and 20% and 15% in RH-30 at 30 and 60 DAS respectively, over their respective control plants.

4.3.12 Maximum quantum yield of PSII (Fv/Fm)

The values for Fv/Fm were slightly higher at 60 d stage than at 30 d stage in both Varuna and RH-30 and were significantly improved by proline treatment (Table 32). The plants that received proline (20 mM) exhibited maximum value which was higher by 22% and 17% in Varuna and RH-30 respectively, at 30 DAS over their control plants. Varuna showed better response than RH-30, to proline treatment, assessed at the two stages of growth (30 and 60 DAS).

4.3.13 Nitrate reductase (NR) activity

It is evident from table 32 that the plants that were sprayed with different proline concentrations (10, 20 or 30 mM) possessed significantly higher NR activity at the two stages of growth (30 and 60 DAS) in comparison to the control. Maximum values for the enzyme activity (32% and 25% at 30 and 60 DAS, respectively, over their control plants) were noted in the plants sprayed 20 mM proline in Varuna. Proline was more effective in Varuna than RH-30.

Table 31: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O m^{-2} s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Internal CO_2 concentration (C_i)						Transpiration rate (E)					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	320	298	309	364	317	340	3.49	2.77	3.13	4.73	3.82	4.28
Proline (10 mM)	358	326	342	393	335	364	4.29	3.21	3.75	5.63	4.13	4.88
Proline (20 mM)	378	345	361	424	351	387	4.53	3.32	3.93	5.91	4.39	5.15
Proline (30 mM)	374	333	353	415	340	377	4.42	3.29	3.86	5.72	4.24	4.98
Mean	357	325		399	335		4.18	3.15		5.49	4.14	

LSD at 5%	V = 15.26 (Sig)	V = 1.09 (Sig)	V = 0.08 (Sig)	V = 0.04 (Sig)
	T = 21.59 (Sig)	T = 1.54 (Sig)	T = 0.11 (Sig)	T = 0.06 (Sig)
	V x T = NS	V x T = 2.18 (Sig)	V x T = 0.16 (Sig)	V x T = 0.09 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 32: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on maximum quantum yield of PSII (Fv/Fm) and nitrate reductase activity (nmole NO_2 g^{-1} FM s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Maximum quantum yield of PSII (Fv/Fm) | | | | | | Nitrate reductase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	0.783	0.663	0.723	0.975	0.815	0.895	435	380	407	552	476	514
Proline (10 mM)	0.871	0.720	0.796	1.050	0.863	0.957	522	437	479	655	529	592
Proline (20 mM)	0.954	0.775	0.865	1.080	0.883	0.982	574	471	522	690	571	630
Proline (30 mM)	0.902	0.749	0.826	1.060	0.867	0.964	552	456	504	676	551	613
Mean	0.877	0.727		1.041	0.857		520	436		643	531	

LSD at 5%	V	= 0.08 (Sig)	V	= 0.04 (Sig)	V	= 12.98 (Sig)	V	= 10.13 (Sig)
	T	= 0.11 (Sig)	T	= 0.06 (Sig)	T	= 15.68 (Sig)	T	= 14.32 (Sig)
	V × T	= 0.16 (Sig)	V × T	= 0.09 (Sig)	V × T	= NS	V × T	= NS

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.3.14   Carbonic anhydrase (CA) activity

The activity of CA increased with the age of the plants (Table 33). The foliar spray of proline (10, 20 or 30 mM) increased the values by 18%, 24% and 20% in Varuna and 14%, 20% and 17% in RH-30 respectively, at 30 DAS. The 20 mM proline proved best at the two stages of the growth (30 and 60 DAS) in both the varieties (Varuna and RH-30).

4.3.15   Catalase (CAT) activity

The data shown in table 33 clearly revealed that activity of catalase enzyme increased as the growth progressed from 30 to 60 DAS and also in response to the proline (10, 20 or 30 mM) both in Varuna and RH-30. Out of the proline concentrations, 20 mM proved best and enhanced the CAT activity by 19% and 13% in Varuna and 13% and 8% in RH-30 at 30 and 60 DAS respectively, over their control plants. Varuna possessed higher activity of CAT enzyme than RH-30.

4.3.16   Peroxidase (POX) activity

The activity of POX exhibited a similar trend as that of CAT (4.3.15) in both the varieties (Table 34). The leaves of the plants that were sprayed with different concentrations (10, 20 or 30 mM) of proline possessed higher POX activity as compared to water sprayed plants in both the varieties. Proline (20 mM) induced maximum increases that were 35% and 29% in Varuna and 29% and 20% in RH-30 at 30 and 60 DAS respectively, more than their control plants.

4.3.17   Superoxide dismutase (SOD) activity

As evident from table 34, the leaves of plants sprayed with proline, possessed higher SOD activity, compared to those sprayed with water only. Foliar spray of 20 mM of proline proved best that enhanced the activity by 20% and 15% in Varuna and 16% and 10% in RH-30 at 30 and 60 DAS respectively, over their control plants. Moreover, plants of Varuna possessed higher activity of SOD than RH-30.

4.3.18   Proline content

The proline content in fresh leaves increased as the age progressed from 30 to 60 DAS in Varuna and RH-30 (Table 35). Like other parameters, exogenous application of 20 mM of

Table 33: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and catalase (mM H_2O_2 decomposed g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Carbonic anhydrase activity | | | | | | Catalase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	2.07	1.97	2.02	2.49	2.28	2.38	417	380	398	449	404	426
Proline (10 mM)	2.44	2.24	2.34	2.82	2.40	2.61	467	402	434	481	416	448
Proline (20 mM)	2.56	2.36	2.46	2.98	2.52	2.75	497	431	464	509	437	473
Proline (30 mM)	2.48	2.30	2.39	2.91	2.46	2.68	489	414	451	493	427	460
Mean	2.38	2.21		2.80	2.41		467	406		483	421	

LSD at 5%												
V	=	0.07 (Sig)	V	=	0.13 (Sig)		V	=	12.38 (Sig)	V	=	13.34 (Sig)
T	=	0.11 (Sig)	T	=	0.10 (Sig)		T	=	17.51 (Sig)	T	=	18.86 (Sig)
V x T	=	0.16 (Sig)	V x T	=	0.20 (Sig)		V x T	=	NS	V x T	=	NS

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 34: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on peroxidase (units g^{-1} FM) and superoxide dismutase (units g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Peroxidase activity | | | | | | Superoxide dismutase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	13.00	6.92	9.96	16.34	8.60	12.47	133	121	127	166	147	156
Proline (10 mM)	16.64	8.44	12.54	20.10	9.82	14.96	153	135	144	183	155	169
Proline (20 mM)	17.55	8.97	13.26	21.05	10.31	15.68	160	141	150	191	163	177
Proline (30 mM)	16.90	8.75	12.83	20.41	10.02	15.22	157	136	146	186	153	169
Mean	16.02	8.27		19.48	9.69		150	133		181	154	
LSD at 5%	V = 0.18 (Sig)			V = 0.25 (Sig)			V = 9.91(Sig)			V = 6.56 (Sig)		
	T = 0.26 (Sig)			T = 0.35 (Sig)			T = 5.01(Sig)			T = 7.00 (Sig)		
	V × T = 0.37 (Sig)			V × T = 0.50 (Sig)			V × T = NS			V × T = NS		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 35: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on proline content (μmol g^{-1} FM) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Proline content | | | | | |
| | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	14.00	12.62	13.31	20.16	17.73	18.95
Proline (10 mM)	16.94	14.77	15.86	22.68	19.36	21.02
Proline (20 mM)	17.92	15.68	16.80	23.58	20.30	21.94
Proline (30 mM)	17.64	15.14	16.39	23.14	19.99	21.56
Mean	16.63	14.55		22.39	19.35	
LSD at 5%	V	$\approx$	0.14 (Sig)	V	=	0.21 (Sig)
	T	$\approx$	0.20 (Sig)	T	=	0.30 (Sig)
	V x T	$\approx$	0.29 (Sig)	V x T	=	0.42 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

proline generated maximum response and increased the values by 28% and 17% in Varuna and 24% and 14% in RH-30 at 30 and 60 DAS, respectively, compared to the control. Moreover, Varuna had higher content of proline than RH-30.

4.3.19 Yield characteristics per plant

Foliar application of proline (10, 20 or 30 mM) significantly increased number of pods and seed yield per plant where 20 mM generated maximum response in both the varieties (Table 36). Here the respective increase in the number of pods and seed yield per plant was 25% and 30% in Varuna and 22% and 25% in RH-30, over their control plants.

4.4 Experiment 4

This experiment was performed to explicate the effect of BR application on the two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss under salt stress. All the agricultural practices remained same as in Experiment 1. Three different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl was applied through soil at the time of sowing. At 29 DAS, the foliage of the plants was sprayed with DDW or aqueous solution of BRs (HBL/EBL). The plants were sampled in a same way as mentioned in experiment 1.

4.4.1 Shoot and root length

The length of shoot and root increased as the growth progressed from 30 to 60 DAS (Table 37). The plants raised in the soil supplemented with different levels of NaCl (2.8, 4.2, or 5.6 dsm^{-1}) at the time of sowing generated a linear decrease in the values of shoot and root length in a proportion to the NaCl more drastically in RH-30 than Varuna. However, sprayed with brassinosteroid analogues (HBL/EBL) to the leaves at 29 d old plants significantly increased the shoot and root length at 60 d stage of growth. The application of EBL as foliar spray proved better than HBL where shoot length improved by 70% and 60% and root length by 57% and 43% in Varuna and RH-30 respectively, at 60 DAS, compared to the control plants. Moreover, the ill effect generated on the shoot length by the lower concentrations (2.8 or 4.2 dsm^{-1}) of NaCl was completely neutralized by BRs (10^{-8} M), the values were comparable with that of the control. However, in case of root the BRs could neutralize the damage of only the lowest NaCl (2.8 dsm^{-1}). Varuna was more responsive than RH-30.

Table 36: Effect of proline (10, 20, or 30 mM) as foliar spray (29 d stage) on pods plant^{-1}, seeds pod^{-1}, 100 seed mass (mg) and seed yield plant^{-1} (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Pods plant^{-1}			Seeds pod^{-1}			100 seed mass			Seed yield plant^{-1}		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	220	208	214	12.90	12.16	12.53	312	303	307	7.62	7.14	7.38
Proline (10 mM)	257	237	247	12.91	12.18	12.54	316	305	310	8.73	7.99	8.36
Proline (20 mM)	275	254	264	12.98	12.21	12.59	319	308	313	9.90	8.93	9.41
Proline (30 mM)	260	241	250	12.96	12.19	12.57	318	306	312	9.29	8.50	8.89
Mean	253	235		12.93	12.18		316	305		8.88	8.14	

LSD at 5%												
	V	=	4.71 (Sig)	V	=	0.38 (Sig)	V	=	5.34 (Sig)	V	=	0.44 (Sig)
	T	=	6.67 (Sig)	T	=	NS	T	=	NS	T	=	0.62 (Sig)
	V × T	=	NS	V × T	=	NS	V × T	=	NS	V × T	=	NS

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 37: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on shoot and root length (cm) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Shoot length						Root length					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	17.00	15.00	16.00	72.00	68.00	70.50	16.80	14.51	15.65	26.29	23.72	25.00
HBL	17.20	15.28	16.24	118.80	108.30	113.55	16.84	14.42	15.63	37.33	32.49	34.91
EBL	17.31	15.13	16.22	122.40	110.40	116.40	16.77	14.53	15.65	41.28	33.92	37.60
NaCl (2.8 dsm^{-1})	13.37	11.21	12.29	62.78	55.00	58.89	12.00	9.23	10.61	21.34	18.01	19.67
NaCl (4.2 dsm^{-1})	11.23	9.05	10.14	55.21	48.45	51.83	9.54	6.81	8.17	17.56	13.78	15.67
NaCl (5.6 dsm^{-1})	9.86	7.67	8.76	46.89	38.33	42.61	7.21	5.36	6.28	15.71	10.37	13.04
NaCl (2.8 dsm^{-1}) +HBL	13.43	11.03	12.23	82.09	64.86	73.48	11.93	9.29	10.61	25.09	21.34	23.21
NaCl (4.2 dsm^{-1})+HBL	10.88	7.80	9.34	74.88	60.03	67.45	9.91	6.22	8.06	22.61	17.55	20.08
NaCl (5.6 dsm^{-1})+HBL	9.15	7.20	8.18	62.80	51.82	57.31	7.73	4.93	6.33	21.03	15.65	18.34
NaCl (2.8 dsm^{-1}) +EBL	13.74	10.95	12.35	86.40	66.93	76.66	12.43	9.58	11.00	25.02	22.29	23.65
NaCl (4.2 dsm^{-1})+EBL	11.22	7.65	9.44	78.48	64.17	71.32	9.59	5.95	7.77	23.92	18.97	21.44
NaCl (5.6 dsm^{-1})+EBL	9.35	7.50	8.43	65.68	55.96	60.82	7.39	4.79	6.09	21.56	16.84	19.20
Mean	12.81	10.46		77.36	66.10		11.51	8.80		24.90	20.41	

LSD at 5%
V = 0.47 (Sig) V = 2.60 (Sig) V = 0.51(Sig) V = 0.87 (Sig)
T = 1.01 (Sig) T = 5.52 (Sig) T = 1.08(Sig) T = 1.85 (NS)
V x T = NS V x T = NS V x T = NS V x T = NS

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.4.2   Shoot and root fresh mass

The data depicted in the table 38 indicate that fresh mass of shoot and root exhibited an increase as the growth progressed from 30 to 60 DAS. The foliar application of brassinosteroid analogues (HBL/EBL) increased the shoot and root fresh mass at 60 DAS where EBL was more effective that gave 62% and 65% increase, over the control plants in Varuna. However, the plants raised in the soil amended with varying levels of NaCl (2.8, 4.2, or 5.6 dsm^{-1}) showed significant reduction in the shoot and root fresh mass but this loss was more prominent at higher concentration of NaCl in both the varieties. The damage caused by the lower concentrations (2.8 or 4.2 dsm^{-1}) of NaCl was completely nullified by BRs (10^{-8} M). EBL was more effective than HBL. Among the varieties, Varuna was more responsive than RH-30.

4.4.3   Shoot and root dry mass

As evident from table 39 dry mass of shoot and root followed a pattern similar to that of their fresh mass (4.4.2) where stress caused a decrease but BRs improved the values and overcome the impact of stress, particularly that of lower concentrations of NaCl. The values here were comparable with that of the control. Varuna generated a better response than RH-30.

4.4.4   Leaf area

The leaf area of both the varieties (Varuna and RH-30) increased from 30 to 60 DAS (Table 40). The values decreased as the NaCl level (2.8, 4.2 or 5.6 dsm^{-1}) increased in the soil. However, BRs counteracted the impact of the stress where both the analogues (EBL and HBL) completely overcome the damage caused by the NaCl (2.8 dsm^{-1}) at day 60. Varuna had higher values and better response than RH-30.

4.4.5   SPAD chlorophyll values

At both the stages of the growth (30 and 60 DAS) the values were significantly affected by the treatment (Table 40). NaCl generated stress decreased the chlorophyll content but the impact was overcome by the BRs. The plants that received lower levels of NaCl (2.8 or 4.2 dsm^{-1}) and given spray of BRs to their foliage did not show any ill effect of the salt. The values were significantly higher than the control. EBL was better in its effect than HBL that increased the chlorophyll content by 39% and 30% in Varuna and 27% and 22% in RH-30 at

Table 38: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on shoot and root fresh mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot fresh mass | | | | | | Root fresh mass | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	6.78	5.51	6.14	12.62	10.16	11.39	2.89	2.43	2.66	5.47	4.88	5.17
HBL	6.84	5.56	6.20	19.69	14.83	17.26	2.86	2.39	2.62	8.75	7.46	8.10
EBL	6.79	5.63	6.21	20.44	15.24	17.84	2.94	2.43	2.68	9.03	7.80	8.41
NaCl (2.8 dsm^{-1})	5.21	3.78	4.49	10.75	6.95	8.84	1.90	1.31	1.60	4.01	3.06	3.53
NaCl (4.2 dsm^{-1})	4.41	2.96	3.68	9.98	6.37	8.17	1.46	1.07	1.26	3.71	2.81	3.26
NaCl (5.6 dsm^{-1})	3.66	2.10	2.88	9.12	5.43	7.27	1.21	0.84	1.02	3.08	1.97	2.52
NaCl (2.8 dsm^{-1})+HBL	5.49	3.31	4.40	15.39	10.36	12.87	1.82	1.28	1.55	5.96	5.12	5.54
NaCl (4.2 dsm^{-1})+HBL	4.95	2.81	3.88	14.39	8.84	11.61	1.42	1.11	1.26	4.75	3.81	4.28
NaCl (5.6 dsm^{-1})+HBL	4.34	2.59	3.46	11.36	8.03	9.69	1.09	0.68	0.88	4.16	3.46	3.81
NaCl (2.8 dsm^{-1})+EBL	5.42	3.36	4.39	16.02	10.67	13.34	1.76	1.31	1.53	6.29	5.37	5.83
NaCl (4.2 dsm^{-1})+EBL	5.02	2.87	3.94	15.02	9.45	12.23	1.45	1.11	1.28	5.13	4.09	4.61
NaCl (5.6 dsm^{-1})+EBL	4.27	2.64	3.45	12.12	7.52	9.82	1.12	0.73	0.92	4.92	3.95	4.43
Mean	5.27	3.59		13.91	9.49		1.83	1.39		5.44	4.48	
LSD at 5%	V = 0.35 (Sig)			V = 0.10 (Sig)			V = 0.07(Sig)			V = 0.05 (Sig)		
	T = 0.70 (Sig)			T = 0.22 (Sig)			T = 0.16(Sig)			T = 0.12 (Sig)		
	V x T = NS			V x T = 0.32 (Sig)			V x T = 0.23 (Sig)			V x T = 0.17 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 39: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on shoot and root dry mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Soot dry mass | | | | | | Root dry mass | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	1.73	1.51	1.62	3.20	2.74	2.97	0.790	0.600	0.695	1.46	1.09	1.27
HBL	1.76	1.52	1.64	5.60	4.52	5.06	0.792	0.604	0.698	2.51	1.81	2.16
EBL	1.78	1.49	1.63	5.86	4.85	5.35	0.788	0.609	0.698	2.69	1.95	2.32
NaCl (2.8 dsm^{-1})	1.33	0.97	1.15	2.67	1.85	2.26	0.561	0.310	0.435	1.20	0.745	0.97
NaCl (4.2 dsm^{-1})	1.16	0.82	0.99	2.51	1.67	2.09	0.491	0.245	0.368	1.10	0.661	0.88
NaCl (5.6 dsm^{-1})	1.02	0.56	0.79	2.23	1.20	1.71	0.410	0.229	0.319	1.01	0.571	0.79
NaCl (2.8 dsm^{-1})+HBL	1.21	0.72	0.96	4.00	2.82	3.41	0.608	0.374	0.491	1.56	0.97	1.26
NaCl (4.2 dsm^{-1})+HBL	1.00	0.62	0.81	3.26	2.46	2.86	0.545	0.311	0.428	1.51	0.93	1.22
NaCl (5.6 dsm^{-1})+HBL	0.90	0.56	0.73	2.82	2.19	2.50	0.498	0.264	0.381	1.27	0.79	1.03
NaCl (2.8 dsm^{-1})+EBL	1.23	0.74	0.98	4.22	2.93	3.57	0.614	0.366	0.490	1.60	1.05	1.32
NaCl (4.2 dsm^{-1})+EBL	0.99	0.59	0.79	3.36	2.57	2.96	0.547	0.306	0.426	1.54	1.00	1.27
NaCl (5.6 dsm^{-1})+EBL	0.88	0.60	0.74	3.04	2.26	2.65	0.508	0.252	0.380	1.33	0.87	1.10
Mean	1.25	0.89		3.56	2.67		0.596	0.373		1.57	1.04	
LSD at 5%	V = 0.055 (Sig)			V = 0.05 (Sig)			V = 0.0057 (Sig)			V = 0.03 (Sig)		
	T = 0.117 (Sig)			T = 0.12 (Sig)			T = 0.0120 (Sig)			T = 0.07 (Sig)		
	V × T = 0.166 (Sig)			V × T = 0.17 (Sig)			V × T = 0.0170 (Sig)			V × T = 0.10 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 40: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on leaf area (cm^2) and SPAD Chlorophyll values in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Leaf area | | | | | | SPAD Chlorophyll value | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	25.17	24.21	24.69	38.80	36.31	37.55	43.50	38.40	40.95	58.72	51.07	54.89
HBL	25.20	24.40	24.80	50.05	44.37	47.21	57.42	47.62	52.52	73.93	59.24	66.58
EBL	25.31	24.32	24.81	54.71	48.66	51.68	60.47	48.77	54.62	76.34	62.31	69.32
NaCl (2.8 dsm^{-1})	20.18	15.87	18.02	33.40	26.76	30.08	35.20	27.23	31.21	51.56	41.98	46.77
NaCl (4.2 dsm^{-1})	15.87	13.43	14.65	28.45	22.51	25.48	30.65	22.69	26.67	47.45	35.67	41.56
NaCl (5.6 dsm^{-1})	13.26	10.03	11.64	25.98	19.34	22.66	26.34	19.34	22.84	40.67	32.45	36.56
NaCl (2.8 dsm^{-1}) +HBL	19.88	15.49	17.68	43.14	37.58	40.36	47.41	39.17	43.29	66.94	54.13	60.53
NaCl (4.2 dsm^{-1})+HBL	15.86	13.56	14.71	39.73	31.22	35.47	44.81	33.79	39.30	64.00	47.49	55.74
NaCl (5.6 dsm^{-1})+HBL	13.59	9.68	11.63	32.20	27.95	30.07	36.14	30.72	33.43	54.02	44.94	49.48
NaCl (2.8 dsm^{-1}) +EBL	20.14	15.73	17.93	44.62	38.48	41.55	48.72	40.70	44.71	68.12	56.17	62.14
NaCl (4.2 dsm^{-1})+EBL	16.11	13.31	14.71	42.25	33.11	37.68	46.55	34.94	40.74	65.18	49.53	57.35
NaCl (5.6 dsm^{-1})+EBL	13.34	9.44	11.39	33.36	29.33	31.34	37.32	32.25	34.785	54.60	46.88	50.74
Mean	18.66	15.79		38.89	32.96		42.87	34.63		60.13	48.48	

LSD at 5%												
	V = 0.59 (Sig)			V = 1.46 (Sig)			V = 0.55(Sig)			V = 0.08 (Sig)		
	T = 1.25 (Sig)			T = 3.09 (Sig)			T = 1.18(Sig)			T = 0.18 (Sig)		
	V x T = 1.77 (Sig)			V x T = NS			V x T = 1.66 (Sig)			V x T = 0.26 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

30 and 60 DAS respectively over the water sprayed control plants. Varuna was highly responsive than RH-30.

4.4.6 Electrolyte leakage

The two BR analogues (HBL/EBL) significantly reduced the leakage of ions under stress free or NaCl stressed conditions at 60 DAS (Table 41). The plants raised in the soil, amended with different levels of NaCl (2.8, 4.2 or 5.6 dsm^{-1}) showed marked increase in the leaf electrolyte leakage. The maximum increase was reported in RH-30 under highest concentration of NaCl (5.6 dsm^{-1}) which was 31% and 26% at 30 and 60 DAS respectively, over the control plants. Moreover, application of EBL/HBL as a follow up treatment to NaCl (2.8 dsm^{-1}) stressed plants completely neutralized the ill effect of the salt at 60 DAS. Varuna was more responsive than RH-30.

4.4.7 Leaf water potential

Foliar spray of brassinosteroids analogues (HBL/EBL) increased the leaf water potential compared with the control plants (Table 41) at both the stages of growth (30 and 60 DAS). Out of the two analogues, EBL proved best and significantly enhanced the values of leaf water potential in Varuna by 30.9% and in RH-30 by 23.2% at 60 DAS, over their respective control plants. However, the highest level of NaCl (5.6 dsm^{-1}) showed most inhibitory action and reduced the leaf water potential by 39% and 26% in Varuna than the control at 30 and 60 DAS respectively. Moreover, BRs, as a follow up treatment to NaCl (2.8 or 4.2 dsm^{-1}) stressed plants completely restored the leaf water potential, where EBL was more effective than HBL. Among the two varieties, RH-30 was more sensitive to NaCl than Varuna.

4.4.8 Net photosynthetic rate and related attributes

The net photosynthetic rate (P_N) and its related attributes i.e. stomatal conductance (g_s), internal CO_2 concentrations (C_i), and transpiration rate (E) increased as the age of plants progressed from 30 to 60 DAS (Tables 42 and 43). The leaves supplied with either of the BR analogues (HBL/EBL) to the stress free and stressed plants had added efficiency to photosynthesize and also had higher photosynthetic attributes in both the varieties at the two stages of growth (30 and 60 DAS). Furthermore, EBL established itself better than HBL in increasing P_N, g_s, C_i, and E in Varuna by 37%, 73%, 13%, and 36% respectively, over the control at 60 DAS. In contrast to this, plants raised in the soil fed with NaCl (2.8, 4.2 or 5.6

Table 41: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on electrolyte leakage (%) and leaf water potential (Mpa) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Electrolyte leakage | | | | | | Leaf water potential | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	7.16	8.12	7.64	5.80	6.78	6.29	-0.66	-0.75	-0.70	-0.42	-0.56	-0.49
HBL	7.20	8.14	7.67	4.35	5.42	4.88	-0.46	-0.55	-0.50	-0.32	-0.46	-0.39
EBL	7.24	8.17	7.71	4.00	5.08	4.54	-0.40	-0.51	-0.45	-0.29	-0.43	-0.36
NaCl (2.8 dsm^{-1})	7.90	9.40	8.65	6.20	7.57	6.88	-0.77	-0.89	-0.83	-0.46	-0.63	-0.54
NaCl (4.2 dsm^{-1})	8.67	9.87	9.27	6.78	7.90	7.34	-0.81	-0.94	-0.87	-0.49	-0.69	-0.59
NaCl (5.6 dsm^{-1})	9.13	10.67	9.90	6.91	8.59	7.75	-0.92	-1.02	-0.97	-0.53	-0.74	-0.63
NaCl (2.8 dsm^{-1})+HBL	7.42	8.52	7.97	5.22	6.37	5.79	-0.59	-0.72	-0.65	-0.36	-0.50	-0.43
NaCl (4.2 dsm^{-1})+HBL	7.73	9.20	8.46	5.74	7.18	6.46	-0.67	-0.82	-0.74	-0.40	-0.58	-0.49
NaCl (5.6 dsm^{-1})+HBL	8.59	10.26	9.42	6.72	8.13	7.42	-0.81	-0.97	-0.89	-0.48	-0.67	-0.57
NaCl (2.8 dsm^{-1})+EBL	7.23	8.39	7.81	5.04	6.23	5.63	-0.57	-0.69	-0.63	-0.34	-0.48	-0.41
NaCl (4.2 dsm^{-1})+EBL	7.51	8.88	8.19	5.51	6.64	6.07	-0.65	-0.79	-0.72	-0.39	-0.55	-0.47
NaCl (5.6 dsm^{-1})+EBL	8.27	9.62	8.94	6.43	7.93	7.18	-0.77	-0.93	-0.85	-0.47	-0.65	-0.56
Mean	7.84	9.10		5.73	6.99		-0.67	-0.79		-0.41	-0.58	
LSD at 5%	V = 0.15 (Sig)			V = 0.15 (Sig)			V = 0.008(Sig)			V = 0.008 (Sig)		
	T = 0.33 (Sig)			T = 0.33 (Sig)			T = 0.017 (Sig)			T = 0.016 (Sig)		
	V x T = 0.47 (Sig)			V x T = 0.47 (Sig)			V x T = 0.024 (Sig)			V x T = 0.023 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 42: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Net photosynthetic rate (P_N) | | | | | | Stomatal conductance (g_s) | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	20.19	17.53	18.86	26.65	22.77	24.71	0.062	0.051	0.056	0.087	0.071	0.079
HBL	27.46	23.49	25.47	34.38	27.78	31.08	0.104	0.081	0.092	0.137	0.099	0.118
EBL	28.67	24.19	26.43	36.51	29.60	33.05	0.113	0.087	0.100	0.151	0.107	0.129
NaCl (2.8 dsm^{-1})	15.71	10.56	13.13	22.92	15.93	19.42	0.041	0.024	0.032	0.063	0.043	0.053
NaCl (4.2 dsm^{-1})	14.34	9.32	11.83	21.00	14.10	17.55	0.034	0.021	0.027	0.056	0.037	0.046
NaCl (5.6 dsm^{-1})	13.65	8.18	10.91	19.40	12.27	15.83	0.030	0.017	0.023	0.052	0.034	0.043
NaCl (2.8 dsm^{-1}) +HBL	22.41	17.88	20.14	30.70	23.56	27.13	0.064	0.047	0.055	0.097	0.072	0.084
NaCl (4.2 dsm^{-1})+HBL	20.59	14.33	17.46	27.71	19.92	23.81	0.064	0.037	0.0505	0.094	0.059	0.076
NaCl (5.6 dsm^{-1})+HBL	15.22	10.83	13.02	23.18	16.84	20.01	0.047	0.035	0.041	0.070	0.055	0.062
NaCl (2.8 dsm^{-1}) +EBL	23.01	18.44	20.72	31.93	24.59	28.26	0.067	0.049	0.058	0.101	0.074	0.087
NaCl (4.2 dsm^{-1})+EBL	21.40	15.21	18.30	28.51	21.17	24.84	0.064	0.039	0.051	0.095	0.061	0.078
NaCl (5.6 dsm^{-1})+EBL	15.99	11.53	13.76	23.97	18.21	21.09	0.051	0.037	0.044	0.076	0.058	0.067
Mean	19.88	15.12		27.23	20.56		0.062	0.044		0.089	0.064	
LSD at 5%	V = 0.14 (Sig)			V = 0.09 (Sig)			V = 0.010 (Sig)			V = 0.014 (Sig)		
	T = 0.30 (Sig)			T = 0.20 (Sig)			T = 0.007 (Sig)			T = 0.015 (Sig)		
	V x T = 0.42 (Sig)			V x T = 0.28 (Sig)			V x T = 0.015 (Sig)			V x T = 0.001 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 43: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O m^{-2} s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Internal CO_2 concentration (C_i)						Transpiration rate (E)					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	319	297	308	370	337	353	3.29	3.12	3.20	4.77	4.46	4.61
HBL	386	338	362	429	374	401	4.41	3.93	4.17	6.12	5.22	5.67
EBL	402	356	379	418	387	402	4.84	4.12	4.48	6.49	5.44	5.96
NaCl (2.8 dsm^{-1})	271	219	245	335	286	310	2.94	2.25	2.59	4.42	3.62	4.02
NaCl (4.2 dsm^{-1})	253	204	228.5	320	252	286	2.66	2.03	2.34	4.32	3.24	3.78
NaCl (5.6 dsm^{-1})	240	190	215	304	227	265	2.38	1.91	2.14	3.89	3.13	3.51
NaCl (2.8 dsm^{-1}) +HBL	335	267	301	392	316	354	3.68	3.17	3.42	5.46	4.54	5.00
NaCl (4.2 dsm^{-1})+HBL	299	261	280	381	310	345	3.35	2.80	3.07	4.91	4.23	4.57
NaCl (5.6 dsm^{-1})+HBL	272	228	250	347	272	309	2.89	2.49	2.69	4.48	3.96	4.22
NaCl (2.8 dsm^{-1}) +EBL	341	278	309	407	339	373	3.84	3.20	3.52	5.77	4.68	5.22
NaCl (4.2 dsm^{-1})+EBL	315	273	294	412	323	367	3.41	2.87	3.14	5.00	4.32	4.66
NaCl (5.6 dsm^{-1})+EBL	287	243	265	352	303	327	2.99	2.43	2.71	4.57	3.74	4.15
Mean	310	262		372	310		3.39	2.86		5.02	4.23	

LSD at 5%	V = 2.71 (Sig)	V = 0.83 (Sig)	V = 0.015(Sig)	V = 0.014 (Sig)
	T = 5.76 (Sig)	T = 1.76 (Sig)	T = 0.033(Sig)	T = 0.031 (Sig)
	V × T = 8.14 (Sig)	V × T = 2.50 (Sig)	V × T = 0.047(Sig)	V × T = 0.043 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

dsm^{-1}) showed a significant reduction in all the photosynthetic attributes. The highest NaCl concentration decreased the values of P_N, g_s, C_i, and E in Varuna by 32%, 51%, 24%, and 27% respectively, than the non-stressed control plants at 30 DAS whereas the same concentration of NaCl in RH-30 at 30 DAS decreased the values by 53%, 66%, 36% and 38% respectively, compared with the control. Beside this, 10^{-8} M EBL/HBL, as a follow up treatment to NaCl (2.8 and 4.2 dsm^{-1}) stressed, Varuna plants completely neutralized the damaging effect on net photosynthetic rate and its related attributes and partially that of 5.6 dsm^{-1} NaCl at 60 DAS. Out of two varieties, RH-30 was vulnerable to stress than Varuna.

4.4.9 Maximum quantum yield of PSII (Fv/Fm)

The values of Fv/Fm increased as the age of plants advanced from 30 and 60 DAS (Table 44). However, application of NaCl as soil amendment caused significant reduction in Fv/Fm in both the varieties at the stages of growth (30 and 60 DAS). The degree of damage was more prominent at early stage of growth than the latter stage. The leaves of the plants sprayed with either of the brassinosteroid analogues (HBL/EBL) had higher values for Fv/Fm, over the control plants. Moreover, as a follow up treatment with 10^{-8} M of EBL/HBL improved Fv/Fm values in both the varieties (Varuna and RH-30) and also completely neutralized the damage caused by the lowest concentration of NaCl (2.8 dsm^{-1}). Varuna was more responsive to BRs than RH-30.

4.4.10 Nitrate reductase (NR) activity

The activity of NR decreased significantly with increase in the soil generated salt stress (Table 44). The degree of damage was more pronounced in RH-30 than Varuna. Application of 10^{-8} M of either of the brassinosteroid analogues (HBL/EBL) significantly increased the NR activity in both the varieties at both the stages of growth. Moreover, EBL spray proved better and increased the NR activity by 36% and 28% in Varuna and RH-30 respectively over their control plants at 60 DAS. In addition to this, exogenous application of EBL (10^{-8} M), as a follow up treatment to the foliage of stressed plants completely neutralized the toxic effects generated by the low concentration of NaCl (2.8 dsm^{-1}) and partially that of other concentrations.

Table 44: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on maximum quantum yield of PSII (Fv/Fm) and nitrate reductase (nmole NO_2 g^{-1} FM s^{-1}) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Maximum quantum yield of PSII (Fv/Fm) | | | | | | Nitrate reductase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	0.776	0.651	0.713	0.978	0.831	0.904	430	417	423	571	550	560
HBL	0.986	0.800	0.893	1.150	0.922	1.036	635	562	598	742	660	701
EBL	1.063	0.846	0.954	1.220	0.980	1.100	653	583	618	776	709	742
NaCl (2.8 dsm^{-1})	0.684	0.544	0.614	0.901	0.724	0.812	354	280	317	515	398	456
NaCl (4.2 dsm^{-1})	0.612	0.475	0.543	0.825	0.673	0.749	333	221	277	490	364	427
NaCl (5.6 dsm^{-1})	0.550	0.423	0.486	0.769	0.600	0.684	300	194	247	468	330	399
NaCl (2.8 dsm^{-1}) +HBL	0.838	0.677	0.757	1.110	0.897	1.003	516	433	474	708	606	657
NaCl (4.2 dsm^{-1})+HBL	0.752	0.574	0.663	1.010	0.822	0.916	442	350	396	588	495	541
NaCl (5.6 dsm^{-1})+HBL	0.651	0.468	0.559	0.889	0.681	0.785	369	304	336	536	456	496
NaCl (2.8 dsm^{-1}) +EBL	0.869	0.696	0.782	1.150	0.930	1.040	528	450	489	736	629	682
NaCl (4.2 dsm^{-1})+EBL	0.791	0.598	0.694	1.040	0.847	0.943	451	362	406	610	517	563
NaCl (5.6 dsm^{-1})+EBL	0.675	0.518	0.596	0.919	0.731	0.825	382	316	349	559	445	502
Mean	0.771	0.606		0.997	0.803		449	372		608	513	
LSD at 5%	V = 0.033 (Sig)			V = 0.024 (Sig)			V = 2.40(Sig)			V = 6.06 (Sig)		
	T = 0.087 (Sig)			T = 0.045 (Sig)			T = 5.10 (Sig)			T = 10.87(Sig)		
	V x T = 0.110 (Sig)			V x T = 0.063 (Sig)			V x T = 7.21 (Sig)			V x T = NS		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.4.11 Carbonic anhydrase (CA) activity

The activity of CA increased with the advancement of the growth (Table 45). BRs (HBL/EBL) improved the values both in the stress-free and stressed plants where EBL generated values that were comparatively more than the HBL. However, the plants raised in NaCl amended soil possessed significantly lower CA activity at the two stages of sampling (30 and 60 DAS) in both the varieties than their control plants. Moreover, the degree of damage caused by NaCl was in proportion to its concentrations (2.8, 4.2 or 5.6 dsm^{-1}). The application of BRs to the foliage of the stressed plants, completely nullified the effect of NaCl (2.8 dsm^{-1}), and also partially neutralized the ill effect of higher levels of the salt at both the stages of growth. Varuna was more resistant than RH-30.

4.4.12 Activities of antioxidant enzymes

It is evident from the tables 45 and 46 that activity of all the three antioxidant enzymes (CAT, POX and SOD) significantly increased in response to NaCl and/or BRs at 30 and 60 DAS, in both the varieties (Varuna and RH-30). Control plants possessed minimum activity of the antioxidant enzymes. The level of the enzymes increased with an increase in the level of the stress and increased further with the application of either of the brassinosteroid analogues (HBL/EBL). Maximum increase in CAT, POX and SOD was recorded in the plants administered with NaCl (5.6 dsm^{-1}) with foliar application of 10^{-8} M of EBL that was 71%, 124% and 118%, more in Varuna over the control, at 30 DAS. The variety RH-30 possessed lesser antioxidant enzymes activity than Varuna.

4.4.13 Proline content

As depicted in table 47, the proline content in the leaves increased with the advancement of age in both the varieties (Varuna and RH-30). Moreover, the leaf proline content got an additive effect of the stress and the BRs treatments. Out of the two varieties, Varuna possessed high proline content than RH-30. The maximum proline content was recorded in the plants that were raised in the soil amended with 5.6 dsm^{-1} and sprayed with EBL, as a follow up treatment in both the varieties at 60 DAS. In terms of percentage, the increase was 107% and 73% in Varuna and RH-30 respectively, over the control plants at 60 DAS.

Table 45: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and catalase (mM H_2O_2 decomposed g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Carbonic anhydrase activity						Catalase activity					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	2.05	1.96	2.00	2.40	2.29	2.34	419	372	395	440	386	413
HBL	2.72	2.45	2.58	2.90	2.56	2.73	511	427	469	506	416	461
EBL	2.89	2.59	2.74	3.12	2.72	2.92	540	442	491	532	432	482
NaCl (2.8 dsm^{-1})	1.66	1.35	1.50	2.15	1.73	1.94	530	432	481	500	418	459
NaCl (4.2 dsm^{-1})	1.54	1.14	1.34	1.93	1.52	1.72	552	447	499	528	434	481
NaCl (5.6 dsm^{-1})	1.32	1.01	1.16	1.76	1.33	1.54	588	459	523	563	450	506
NaCl (2.8 dsm^{-1}) +HBL	2.29	2.01	2.15	2.73	2.37	2.55	569	468	518	572	463	517
NaCl (4.2 dsm^{-1})+HBL	2.06	1.58	1.82	2.44	2.03	2.23	666	509	587	624	501	562
NaCl (5.6 dsm^{-1})+HBL	1.80	1.46	1.63	2.20	1.78	1.99	708	528	618	664	524	594
NaCl (2.8 dsm^{-1}) +EBL	2.37	2.04	2.20	2.83	2.46	2.64	586	483	534	589	482	535
NaCl (4.2 dsm^{-1})+EBL	2.07	1.72	1.89	2.48	2.30	2.39	678	520	599	633	505	569
NaCl (5.6 dsm^{-1})+EBL	1.87	1.54	1.70	2.56	1.85	2.20	720	550	635	691	540	615
Mean	2.05	1.74		2.45	2.08		588	469		570	462	

LSD at 5%						
	V = 0.02 (Sig)		V = 0.05 (Sig)		V = 4.33(Sig)	V = 4.09 (Sig)
	T = 0.06 (Sig)		T = 0.11 (Sig)		T = 9.18(Sig)	T = 8.68 (Sig)
	V × T = 0.08 (Sig)		V × T = 0.15 (Sig)		V × T = 12.99 (Sig)	V × T = 12.27(Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 46: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on peroxidase (units g^{-1} FM) and superoxide dismutase (units g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Peroxidase activity						Superoxide dismutase activity					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	12.69	7.15	9.92	17.13	9.41	13.27	131	112	121	163	137	150
HBL	18.40	9.71	14.05	22.61	11.39	17.00	162	133	147	190	156	173
EBL	19.79	10.57	15.18	24.32	12.14	18.23	178	137	157	204	163	183
NaCl (2.8 dsm^{-1})	17.35	8.49	12.92	21.56	10.58	16.07	190	145	167	215	160	187
NaCl (4.2 dsm^{-1})	18.00	9.27	13.63	23.40	10.96	17.18	211	162	186	242	181	211
NaCl (5.6 dsm^{-1})	19.86	9.68	14.77	24.38	11.83	18.10	250	200	225	293	221	257
NaCl (2.8 dsm^{-1}) +HBL	20.05	10.71	15.38	25.18	12.79	18.98	234	179	206	267	191	229
NaCl (4.2 dsm^{-1})+HBL	23.98	12.58	18.28	30.32	14.67	22.49	252	194	223	290	201	245
NaCl (5.6 dsm^{-1})+HBL	26.52	13.73	20.12	32.54	15.99	24.26	275	212	243	307	220	263
NaCl (2.8 dsm^{-1}) +EBL	20.68	10.92	15.80	25.86	13.45	19.65	243	184	213	275	195	235
NaCl (4.2 dsm^{-1})+EBL	26.01	13.01	19.51	31.40	15.24	23.32	259	205	232	296	210	253
NaCl (5.6 dsm^{-1})+EBL	28.55	14.16	21.35	34.26	16.46	25.36	286	221	253.5	312	230	271
Mean	20.99	10.83		26.08	12.91		222	173		254	188	

LSD at 5%	V = 0.06 (Sig)	V = 0.07 (Sig)	V = 1.05(Sig)	V = 1.97 (Sig)
	T = 0.14 (Sig)	T = 0.16 (Sig)	T = 2.24(Sig)	T = 4.18 (Sig)
	V × T = 0.20 (Sig)	V × T = 0.22 (Sig)	V × T = 3.17 (Sig)	V × T = 5.92 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 47: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on proline content (μmol g^{-1} FM) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Proline content					
	30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	13.17	12.09	12.63	18.95	16.98	17.96
HBL	18.17	15.96	17.06	22.55	19.36	20.95
EBL	19.49	15.11	17.30	23.12	19.70	21.41
NaCl (2.8 dsm^{-1})	20.10	17.00	18.55	27.23	20.56	23.89
NaCl (4.2 dsm^{-1})	22.65	18.27	20.46	30.24	22.00	26.12
NaCl (5.6 dsm^{-1})	24.30	20.29	22.29	32.57	24.67	28.62
NaCl (2.8 dsm^{-1}) +HBL	21.86	18.49	20.17	30.13	22.05	26.09
NaCl (4.2 dsm^{-1})+HBL	24.33	20.19	22.26	32.59	26.52	29.55
NaCl (5.6 dsm^{-1})+HBL	28.57	22.36	25.46	37.14	29.12	33.13
NaCl (2.8 dsm^{-1}) +EBL	22.52	20.42	21.47	31.64	22.75	27.19
NaCl (4.2 dsm^{-1})+EBL	25.28	21.21	23.24	32.78	25.47	29.12
NaCl (5.6 dsm^{-1})+EBL	29.50	23.69	26.59	39.41	29.54	34.47
Mean	22.49	18.76		29.86	23.22	

LSD at 5%	V	= 0.41(Sig)	V	= 0.24 (Sig)
	T	= 0.88(Sig)	T	= 0.52 (Sig)
	V x T	= 1.25 (Sig)	V x T	= 0.74 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.4.14 Yield characteristics per plant

The plants raised in the soil amended with different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl showed significant reduction in seed yield and its characteristics (number of pods per plant, number of seeds per pod and 100 seed mass) in the two cultivars (Varuna and RH-30) at harvest (Table 48) in a concentration dependent manner. However, stress-free plants sprayed with either of the BRs generated significantly higher values for all the above characteristics. Moreover, BRs also overcame the adverse impact of the stress and could cause complete recovery against the lowest level (2.8 dsm^{-1}) of NaCl and a partial one against higher concentrations of the salt. Varuna was more responsive than RH-30.

4.5 Experiment 5

This experiment was laid down to elucidate the effect of exogenous proline application on the salinity induced changes in *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. All the agricultural practices remained same as in Experiment 1. The NaCl (2.8, 4.2 or 5.6 dsm^{-1}) was applied through soil. The plants were sprayed with DDW/proline (20 mM) and then sampled as mentioned in the Experiment 1.

4.5.1 Shoot and root length

The length of shoot and root increased as the age of plants progressed from day 30 to 60, in both the varieties (Table 49). Exogenous foliar application of 20 mM proline significantly increased the length of shoot and root both in Varuna and RH-30 at 60 DAS. Here in terms of percentage the shoot length increased by 45% and 38% and root length 33% and 28% in Varuna and RH-30 respectively, over the control plants. However, plants raised in the soil amended with different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl exhibited a decrease in shoot and root length where variety RH-30 was more vulnerable than Varuna. The application of proline as a follow up treatment to the stressed plants partially neutralized the damaging effects of the salt.

4.5.2 Shoot and root fresh mass

As depicted in the table 50, the fresh mass of shoot and root increased with the advancement of age in both the varieties (Varuna and RH-30). However, the soil applied NaCl (2.8, 4.2 or 5.6 dsm^{-1}) caused a significant reduction in fresh mass of shoot and root at 30 and 60 DAS. The highest concentration (5.6 dsm^{-}) caused maximum reduction in fresh mass of shoot by

Table 48: Effect of 28-homobrassinolide (HBL; 10^{-8} M) or 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on pods plant^{-1}, seeds pod^{-1}, 100 seed mass (mg) and seed yield plant^{-1} (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Pods plant^{-1}			Seeds pod^{-1}			100 seed mass			Seed yield plant^{-1}		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	225	213	219	13.00	11.58	12.29	317	309	313	7.77	6.27	7.02
HBL	307	281	294	13.08	11.62	12.35	321	311	316	10.33	8.03	9.18
BL	318	290	304	13.15	11.66	12.40	325	313	319	10.49	8.25	9.37
NaCl (2.8 dsm^{-1})	192	173	182	11.46	9.30	10.38	301	276	288	6.34	4.72	5.53
NaCl (4.2 dsm^{-1})	180	164	172	10.86	8.61	9.73	289	267	278	5.64	3.85	4.74
NaCl (5.6 dsm^{-1})	168	147	157	10.56	7.65	9.10	282	250	266	5.04	3.30	4.17
NaCl (2.8 dsm^{-1})+HBL	220	200	210	11.50	9.36	10.43	303	280	291	8.38	5.76	7.07
NaCl (4.2 dsm^{-1})+HBL	211	181	196	10.92	8.75	9.83	292	271	281	7.38	5.45	6.41
NaCl (5.6 dsm^{-1})+HBL	195	176	185	10.68	7.70	9.19	284	260	272	6.99	5.20	6.09
NaCl (2.8 dsm^{-1})+EBL	231	208	219	13.21	9.42	11.31	305	285	295	8.62	6.52	7.57
NaCl (4.2 dsm^{-1})-EBL	218	193	205	11.54	8.79	10.16	293	271	282	8.00	5.95	6.97
NaCl (5.6 dsm^{-1})+EBL	202	185	193	10.94	9.93	10.43	284	259	271	7.45	5.51	6.48
Mean	222	200		11.74	9.53		299	279		7.70	5.73	
LSD at 5%	V = 3.46 (Sig)			V = 0.03 (Sig)			V = 3.05 (Sig)			V = 0.09 (Sig)		
	T = 7.35 (Sig)			T = 0.06 (Sig)			T = 6.48 (Sig)			T = 0.19 (Sig)		
	V x T = NS			V x T = 0.07 (Sig)			V x T – NS			V x T = 0.27 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 49: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on shoot and root length (cm) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot length | | | | | | Root length | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	19.70	15.58	17.64	76.81	59.04	67.92	15.54	14.31	14.92	23.51	21.45	22.48
Proline (20 mM)	19.65	15.38	17.51	112.14	81.43	97.40	15.56	14.35	14.95	31.50	27.46	29.48
NaCl (2.8 dsm^{-1})	15.30	11.58	13.44	66.67	47.11	56.89	11.16	9.34	10.25	19.27	16.30	17.78
NaCl (4.2 dsm^{-1})	12.59	9.68	11.13	58.45	41.67	50.06	9.03	6.06	7.54	15.88	12.34	14.11
NaCl (5.6 dsm^{-1})	12.01	8.06	10.03	50.69	33.65	42.17	6.99	5.37	6.18	14.14	9.40	11.77
NaCl (2.8 dsm^{-1})+Proline	15.37	11.68	13.52	72.97	53.13	63.05	11.25	9.44	10.34	20.68	17.80	19.24
NaCl (4.2 dsm^{-1})+Proline	12.61	9.81	11.21	69.13	47.82	58.47	9.09	6.15	7.62	19.27	14.59	16.93
NaCl (5.6 dsm^{-1})+Proline	12.02	8.10	10.06	64.52	42.51	53.51	7.24	5.44	6.34	17.16	12.01	14.58
Mean	14.91	11.23		71.42	50.95		10.73	8.81		20.18	16.42	
LSD at 5%	V = 0.09 (Sig)			V = 1.50 (Sig)			V = 0.12 (Sig)			V = 0.73 (Sig)		
	T = 0.15 (Sig)			T = 2.27 (Sig)			T = 0.19 (Sig)			T = 1.16 (Sig)		
	V x T = 0.21 (Sig)			V x T = 3.35 (Sig)			V x T = 0.28 (Sig)			V x T = 1.64 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 50: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on shoot and root fresh mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Shoot fresh mass						Root fresh mass					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	6.19	5.23	5.71	11.63	9.57	10.6	2.49	2.00	2.24	4.73	3.79	4.26
Proline (20 mM)	6.25	5.18	5.71	16.72	12.93	14.82	2.38	1.96	2.17	6.36	4.85	5.60
NaCl (2.8 dsm^{-1})	4.76	3.20	3.98	9.89	6.55	8.22	1.57	1.05	1.31	3.50	2.40	2.95
NaCl (4.2 dsm^{-1})	4.15	2.65	3.40	9.15	6.00	7.57	1.24	0.86	1.05	3.12	2.17	2.64
NaCl (5.6 dsm^{-1})	3.65	2.14	2.89	8.43	5.10	6.76	1.02	0.75	0.88	2.78	1.59	2.18
NaCl (2.8 dsm^{-1}) +Proline	4.83	3.24	4.03	11.04	8.32	9.68	1.62	1.10	1.36	3.97	2.88	3.42
NaCl (4.2 dsm^{-1})+Proline	4.27	2.75	3.51	10.11	6.98	8.54	1.27	0.90	1.08	3.69	2.65	3.17
NaCl (5.6 dsm^{-1})+Proline	3.71	2.38	3.04	9.30	6.63	7.96	1.09	0.79	0.94	3.16	2.27	2.71
Mean	4.72	3.35		10.78	7.76		1.59	1.18		3.91	2.83	
LSD at 5%	V = 0.06 (Sig)			V = 0.38 (Sig)			V = 0.03(Sig)			V = 0.05 (Sig)		
	T = 0.10 (Sig)			T = 0.61 (Sig)			T = 0.04(Sig)			T = 0.08 (Sig)		
	V x T = 0.15 (Sig)			V x T = 0.86 (Sig)			V x T = 0.06 (Sig)			V x T = 0.12 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

41% and 59% and that of root by 59% and 62% in Varuna and RH-30 respectively, compared with their control plants as early as 30 DAS. The application of proline on the stress free plants improved the values that were 43% and 34% in Varuna and 35% and 27% in RH-30 more for shoot and root, over the control at 60 DAS. However, as a remedial treatment, proline did not generate significant impact on the stressed plants.

4.5.3   Shoot and root dry mass

The dry mass of shoot and root followed the pattern similar to that of their fresh mass (4.5.2). The exogenous application of 20 mM proline increased the shoot and root dry mass in both Varuna and RH-30 at 60 DAS (Table 51). The increase was more pronounced in variety Varuna than RH-30. In terms of percentage, the increase in shoot and root dry mass was 49% and 41% respectively in Varuna, over the control plants at 60 DAS. However, the plants raised in the soil amended with different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl, the dry mass of the shoot and root decreased which was in proportion to the salinity level in the soil. The decrease was prominent at early stage (30 DAS) than at latter stage (60 DAS) of the growth. Proline (20 mM) as a follow up treatment to stressed plants partially neutralized the toxic effects of the lowest concentration (2.8 dsm^{-1}) of NaCl only in variety Varuna at 60 DAS.

4.5.4   Leaf area

Like other growth parameters (4.5.1, 4.5.2 and 4.5.3), leaf area also increased as the growth progressed from 30 to 60 DAS in the plants of Varuna and RH-30 (Table 52). Moreover, exogenous application of 20 mM proline had an additive effect and increased the leaf area by 23% and 18% in Varuna and RH-30 respectively, over the control plants, at 60 DAS. However, the leaf area of the plants raised in the soil amended with different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl decreased with an increase in the level of NaCl. However, proline spray to the foliage of stressed plants partially neutralized the harmful effects at 60 DAS. Out of the two varieties, Varuna was more responsive to proline than RH-30.

4.5.5   SPAD chlorophyll values

The exogenous application of 20 mM proline to the foliage enhanced the SPAD chlorophyll values in both varieties at 30 and 60 DAS (Table 52). The response was prominent after 24h (i.e. at 30 day stage) of the treatment. However, presence of NaCl in the soil

Table 51: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on shoot and root dry mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot dry mass | | | | | | Root dry mass | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	1.38	1.20	1.29	2.30	2.14	2.22	0.766	0.608	0.687	1.41	1.10	1.25
Proline (20 mM)	1.35	1.15	1.25	3.43	3.01	3.22	0.760	0.600	0.680	1.99	1.46	1.72
NaCl (2.8 dsm^{-1})	1.04	0.69	0.86	1.95	1.47	1.71	0.542	0.298	0.420	1.16	0.76	0.96
NaCl (4.2 dsm^{-1})	0.90	0.58	0.74	1.84	1.29	1.56	0.472	0.255	0.363	1.07	0.68	0.87
NaCl (5.6 dsm^{-1})	0.77	0.37	0.57	1.60	0.97	1.28	0.402	0.250	0.326	1.00	0.58	0.79
NaCl (2.8 dsm^{-1}) +Proline	1.08	0.74	0.91	2.16	1.86	2.01	0.543	0.297	0.420	1.28	0.90	1.09
NaCl (4.2 dsm^{-1})+Proline	0.94	0.61	0.77	2.07	1.71	1.89	0.474	0.257	0.365	1.22	0.83	1.02
NaCl (5.6 dsm^{-1})+Proline	0.84	0.43	0.63	1.91	1.49	1.70	0.405	0.255	0.330	1.11	0.71	0.91
Mean	1.03	0.72		2.15	1.74		0.545	0.352		1.28	0.88	
LSD at 5%	V = 0.02 (Sig)			V = 0.03 (Sig)			V = 0.01(Sig)			V = 0.02 (Sig)		
	T = 0.03 (Sig)			T = 0.04 (Sig)			T = 0.02(Sig)			T = 0.03 (Sig)		
	V x T = 0.05 (Sig)			V x T = 0.06 (Sig)			V x T = 0.02 (Sig)			V x T = 0.05 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 52: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm⁻¹) on leaf area (cm²) and SPAD chlorophyll values in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Leaf area						SPAD Chlorophyll value					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	24.82	22.39	23.60	39.21	34.03	36.62	45.29	36.08	40.68	67.02	55.80	61.41
Proline (20 mM)	24.76	22.31	23.53	48.39	40.22	44.30	56.83	43.36	50.09	80.35	63.16	71.75
NaCl (2.8 dsm⁻¹)	19.98	14.55	17.26	33.72	25.18	29.45	36.78	25.95	31.36	58.88	54.87	56.87
NaCl (4.2 dsm⁻¹)	15.49	12.30	13.89	29.00	20.74	24.87	31.67	21.20	26.43	53.68	46.35	50.01
NaCl (5.6 dsm⁻¹)	13.14	9.50	11.32	26.21	18.32	22.26	26.26	18.00	22.13	46.14	42.35	44.24
NaCl (2.8 dsm⁻¹)+Proline	20.10	14.77	17.43	36.46	30.96	33.71	43.47	33.19	38.33	68.36	53.01	60.68
NaCl (4.2 dsm⁻¹)-Proline	15.88	12.31	14.09	32.93	26.88	29.90	38.49	28.86	33.67	59.64	48.54	54.09
NaCl (5.6 dsm⁻¹)-Proline	13.15	9.63	11.39	29.79	24.50	27.14	35.77	25.97	30.87	58.30	45.76	52.03
Mean	18.41	14.77		34.46	27.60		39.32	29.08		61.55	51.23	
LSD at 5%	V = 0.50 (Sig)			V = 0.98 (Sig)			V = 0.12 (Sig)			V = 0.84 (Sig)		
	T = 0.80 (Sig)			T = 1.56 (Sig)			T = 0.19 (Sig)			T = 1.34 (Sig)		
	V x T = 1.13 (Sig)			V x T = NS			V x T = 0.27 (Sig)			V x T = 1.89 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

significantly decreased SPAD chlorophyll values, in a concentration dependent manner. NaCl (5.6 dsm^{-1}) decreased the SPAD chlorophyll by 42% and 50% in Varuna and RH-30 respectively, compared with the control, at 30 DAS. However, 20 mM proline as a follow up treatment to NaCl stressed plants neutralized the toxic effects of the lowest concentration (2.8 dsm^{-1}) of NaCl and restored the value at par to the control in the Varuna at 60 DAS.

4.5.6   Electrolyte leakage

As the growth progressed from 30 to 60 DAS, the electrolyte leakage decreased in both Varuna and RH-30 (Table 53). The soil applied NaCl (2.8, 4.2 or 5.6 dsm^{-1}) significantly increased the electrolyte leakage in both the varieties, where 5.6 dsm^{-1} was most damaging that increased the electrolyte leakage by 26% and 33% in Varuna and RH-30 respectively, over the control plants at 30 DAS. However, the exogenous spray of proline significantly decreased the electrolyte leakage at 60 DAS as compared to the unsprayed control plants. Moreover, the follow-up treatment with proline spray to stressed plants completely neutralized the impact of lowest concentration (2.8 dsm^{-1}) of NaCl on the leakage at 60 DAS more in Varuna than RH-30.

4.5.7   Leaf water potential

As depicted in table 53, foliar application of 20 mM proline increased the leaf water potential (LWP) in both the varieties (Varuna and RH-30) at the two stages of growth (30 and 60 DAS). In terms of percentage the proline increased the LWP by 23% and 16% in Varuna and RH-30 respectively, over the control plants, at 30 DAS. The soil applied NaCl (2.8, 4.2 or 5.6 dsm^{-1}) caused significant reduction in LWP in the varieties at both the stages of growth. The decrease was more pronounced in RH-30 than Varuna at 30 DAS. However 20mM proline, applied as a follow-up treatment, completely neutralized the loss because of the lowest concentration (2.8 dsm^{-1}) of NaCl at 60 DAS.

4.5.8   Net photosynthetic rate and related attributes

As the age progressed from 30 to 60 DAS, the net photosynthetic rate (P_N) and its related attributes i.e. stomatal conductance (g_s), internal CO_2 concentration (C_i), and transpiration rate (E) increased in both the varieties (Tables 54 and 55). Moreover, spray of 20 ml proline to the foliage of plants significantly increased the net photosynthetic rate and related attributes in both the varieties at 30 and 60 DAS. In terms of percentage the P_N increased by

Table 53: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on electrolyte leakage (%) and leaf water potential (MPa) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Electrolyte leakage | | | | | | Leaf water potential | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	7.24	8.18	7.71	5.78	6.70	6.24	-0.64	-0.72	-0.68	-0.47	-0.52	-0.49
Proline (20 mM)	7.28	8.18	7.73	4.79	5.89	5.34	-0.49	-0.60	-0.54	-0.39	-0.46	-0.42
NaCl (2.8 dsm^{-1})	8.02	9.40	8.71	6.20	7.40	6.80	-0.72	-0.86	-0.79	-0.52	-0.59	-0.55
NaCl (4.2 dsm^{-1})	8.60	9.98	9.29	6.67	7.85	7.26	-0.81	-0.91	-0.86	-0.57	-0.65	-0.61
NaCl (5.6 dsm^{-1})	9.17	10.87	10.02	6.99	8.42	7.70	-0.87	-1.03	-0.95	-0.61	-0.71	-0.66
NaCl (2.8 dsm^{-1}) +Proline	7.52	8.91	8.21	5.66	6.76	6.21	-0.69	-0.80	-0.74	-0.45	-0.51	-0.48
NaCl (4.2 dsm^{-1})+Proline	8.03	9.48	8.75	6.06	7.23	6.64	-0.73	-0.87	-0.80	-0.51	-0.59	-0.55
NaCl (5.6 dsm^{-1})+Proline	8.68	10.30	9.49	6.47	7.90	7.18	-0.79	-0.94	-0.86	-0.54	-0.63	-0.58
Mean	8.07	9.41		6.07	7.27		-0.72	-0.84		-0.51	-0.58	
LSD at 5%	V = 0.08 (Sig)			V = 0.05 (Sig)			V = 0.004 (Sig)			V = 0.008 (Sig)		
	T = 0.13 (Sig)			T = 0.09 (Sig)			T = 0.006 (Sig)			T = 0.013 (Sig)		
	V x T = 0.19 (Sig)			V x T = 0.13 (Sig)			V x T = 0.009 (Sig)			V x T = 0.018 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 54: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Net photosynthetic rate (P_N) | | | | | | Stomatal conductance (g_s) | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	20.73	18.00	19.36	27.25	23.40	25.32	0.064	0.053	0.058	0.092	0.075	0.083
Proline (20 mM)	26.53	22.35	24.44	34.06	27.50	30.78	0.096	0.077	0.086	0.129	0.102	0.115
NaCl (2.8 dsm^{-1})	16.19	10.96	13.57	23.34	16.35	19.84	0.042	0.025	0.033	0.069	0.045	0.057
NaCl (4.2 dsm^{-1})	14.90	9.78	12.34	21.45	14.51	17.98	0.032	0.022	0.027	0.059	0.038	0.048
NaCl (5.6 dsm^{-1})	14.04	8.45	11.24	19.78	12.60	16.19	0.030	0.018	0.024	0.055	0.035	0.045
NaCl (2.8 dsm^{-1})+Proline	20.10	16.38	18.24	28.61	23.86	26.23	0.052	0.039	0.045	0.085	0.066	0.075
NaCl (4.2 dsm^{-1})+Proline	17.20	12.42	14.81	25.34	20.00	22.67	0.049	0.036	0.042	0.076	0.058	0.067
NaCl (5.6 dsm^{-1})+Proline	16.16	10.44	13.30	23.43	16.15	19.79	0.044	0.033	0.038	0.072	0.054	0.063
Mean	18.23	13.59		25.41	19.29		0.051	0.037		0.079	0.059	
LSD at 5%	V = 0.035 (Sig)			V = 0.037 (Sig)			V = 0.0004(Sig)			V = 0.0004 (Sig)		
	T = 0.056 (Sig)			T = 0.059 (Sig)			T = 0.0007(Sig)			T = 0.0007 (Sig)		
	V x T = 0.080 (Sig)			V x T = 0.084 (Sig)			V x T = 0.0009(Sig)			V x T = 0.0010 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 55: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O m^{-2} s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Internal CO_2 concentration (C_i) | | | | | | Transpiration rate (E) | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	330	311	320	374	349	361	3.30	2.87	3.08	4.81	4.07	4.44
Proline (20 mM)	393	361	377	437	383	410	4.30	3.45	3.87	5.97	4.63	5.30
NaCl (2.8 dsm^{-1})	280	229	254	339	293	316	2.89	2.06	2.47	4.56	3.30	3.93
NaCl (4.2 dsm^{-1})	263	216	239	325	260	292	2.67	1.83	2.25	4.37	2.96	3.66
NaCl (5.6 dsm^{-1})	243	201	222	307	240	273	2.35	1.71	2.03	3.87	2.76	3.31
NaCl (2.8 dsm^{-1})+Proline	323	283	303	381	335	358	3.36	2.35	2.85	5.05	3.58	4.31
NaCl (4.2 dsm^{-1})+Proline	293	258	275	347	310	328	2.97	2.29	2.63	4.71	3.33	4.02
NaCl (5.6 dsm^{-1})+Proline	270	227	248	329	275	302	2.77	1.98	2.37	4.42	3.17	3.79
Mean	299	260		354	305		3.08	2.32		4.72	3.47	
LSD at 5%	V = 1.44 (Sig)			V = 2.09 (Sig)			V = 0.02(Sig)			V = 0.017 (Sig)		
	T = 2.28 (Sig)			T = 3.31 (Sig)			T = 0.03(Sig)			T = 0.027 (Sig)		
	V × T = 3.23 (Sig)			V × T = 4.68 (Sig)			V × T = 0.04(Sig)			V × T = 0.038 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

28% and 24%; g_s: 50% and 45%; C_i: 19% and 16% and E: 30% and 20% in Varuna and RH-30 respectively, over their control plants, at 30 DAS. However, the soil applied NaCl (2.8, 4.2 or 5.6 dsm^{-1}) caused significant decrease in P_N and its related attributes in both the varieties, being more prominent in RH-30 than Varuna. The application of 20 mM proline to the stressed plants completely neutralized the damaging effects of the lowest concentration (2.8 dsm^{-1}) of NaCl in Varuna at 60 DAS.

4.5.9   Maximum quantum yield of PSII (Fv/Fm)

As evident from table 56, the exogenous spray of 20 mM proline to the foliage of plants increased the Fv/Fm values in the varieties (Varuna and RH-30) at both the stages of growth (30 and 60 DAS). The per cent increase in Fv/Fm value was 20% and 8% in Varuna and 14% and 6% in RH-30, over their control plants, at 30 and 60 DAS respectively. However, soil applied NaCl (2.8, 4.2 or 5.6 dsm^{-1}) significantly decreased the values. Moreover, the proline application to the salt-stressed plants completely overcomes the toxic effects generated by the lowest concentration (2.8 dsm^{-1}) of NaCl in Varuna at 60 DAS. The variety RH-30 was more prone to the stress than the Varuna.

4.5.10   Nitrate reductase (NR) activity

As depicted in table 56, the activity of NR increased as the growth advanced from 30 to 60 DAS in both the varieties (Varuna and RH-30). However, the activity significantly decreased with the increase in the NaCl concentration in the soil. Application of 20 mM proline significantly increased the NR activity in both the varieties at both the stages of growth. In terms of percentage the NR activity increased by 33% and 25% in Varuna and 24% and 19% in RH-30, over their control plants, at 30 and 60 DAS respectively. In addition, exogenous application of proline (20 mM) to the foliage of stressed plants completely neutralized the toxic effects generated by lowest concentration (2.8 dsm^{-1}) of NaCl.

4.5.11   Carbonic anhydrase (CA) activity

The application of different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl significantly decreased CA activity in the leaves of plants of Varuna and RH-30 in a concentration dependent manner both at 30 and 60 DAS (Table 57). However, the application of 20 mM proline to the foliage of the plants enhanced the activity significantly by 23% and 17% in Varuna and 18% and

Table 56: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on maximum quantum yield of PSII (Fv/Fm) and nitrate reductase activity (nmole NO$_2$ g^{-1} FM s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Maximum quantum yield of PSII (Fv/Fm) | | | | | | Nitrate reductase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	0.780	0.663	0.721	0.971	0.815	0.893	425	397	411	565	518	541
Proline (20 mM)	0.936	0.755	0.845	1.060	0.872	0.966	565	492	528	709	621	665
NaCl (2.8 dsm^{-1})	0.691	0.559	0.625	0.892	0.713	0.802	358	268	313	512	388	450
NaCl (4.2 dsm^{-1})	0.618	0.487	0.552	0.815	0.663	0.739	330	210	270	482	341	411
NaCl (5.6 dsm^{-1})	0.555	0.431	0.493	0.768	0.595	0.681	293	186	239	461	310	385
NaCl (2.8 dsm^{-1}) +Proline	0.741	0.596	0.668	1.000	0.806	0.903	463	404	433	632	533	582
NaCl (4.2 dsm^{-1})+Proline	0.670	0.483	0.576	0.893	0.717	0.805	369	277	323	525	424	474
NaCl (5.6 dsm^{-1})+Proline	0.624	0.424	0.524	0.825	0.643	0.734	344	234	289	497	362	429
Mean	0.702	0.549		0.903	0.728		393	308		547	437	
LSD at 5%	V = 0.09 (Sig)			V = 0.14 (Sig)			V = 5.93 (Sig)			V = 7.72 (Sig)		
	T = 0.12 (Sig)			T = 0.20 (Sig)			T = 9.38 (Sig)			T = 9.11 (Sig)		
	V x T = 0.20 (Sig)			V x T = 0.32 (Sig)			V x T = 13.27 (Sig)			V x T = 15.24 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 57: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and catalase (mM H_2O_2 decomposed g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Carbonic anhydrase activity						Catalase activity					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	2.08	1.97	2.03	2.45	2.28	2.36	428	377	402	453	393	423
Proline (20 mM)	2.54	2.32	2.43	2.88	2.53	2.70	513	424	468	515	424	469
NaCl (2.8 dsm^{-1})	1.69	1.34	1.52	2.21	1.77	1.99	535	436	485	521	425	473
NaCl (4.2 dsm^{-1})	1.59	1.16	1.37	1.97	1.56	1.76	570	450	510	543	440	491
NaCl (5.6 dsm^{-1})	1.38	0.99	1.18	1.80	1.38	1.59	600	461	530	578	455	516
NaCl (2.8 dsm^{-1}) +Proline	2.16	1.82	1.99	2.61	2.21	2.41	547	452	499	552	459	505
NaCl (4.2 dsm^{-1})+Proline	1.70	1.41	1.55	2.30	1.82	2.06	637	478	557	588	471	529
NaCl (5.6 dsm^{-1})+Proline	1.51	1.23	1.37	2.10	1.59	1.84	684	512	598	643	487	565
Mean	1.83	1.53		2.29	1.89		564	448		549	444	
LSD at 5%	V = 0.08 (Sig)			V = 0.13 (Sig)			V = 3.69 (Sig)			V = 6.04 (Sig)		
	T = 0.14 (Sig)			T = 0.21 (Sig)			T = 5.85 (Sig)			T = 9.55 (Sig)		
	V x T = 0.19 (Sig)			V x T = 0.30 (Sig)			V x T = 8.27 (Sig)			V x T = 13.50(Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

10% in RH-30, over their control plants, at 30 and 60 DAS respectively. Moreover, the damaging effects of the lowest concentration (2.8 dsm^{-1}) of NaCl were completely neutralized by the follow-up action of proline (20 mM) in Varuna and partially in RH-30.

4.5.12 Activities of antioxidant enzymes

The activity of antioxidant enzymes (CAT, POX and SOD) increased as the growth progressed from 30 to 60 DAS in both the varieties (Tables 57 and 58). The application of 20 mM proline as a foliar spray and/or different concentrations (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl through soil resulted in the increase of the activity of these enzymes significantly over their control plants. The highest concentration (5.6 dsm^{-1}) of NaCl with 20 mM proline generated maximum increase in CAT (59.8%), POX (92.9%) and SOD (88.3%) in Varuna, over their control plants, at 30 DAS. Out of the two varieties RH-30 possessed lower activity of antioxidant enzymes than Varuna, at both 30 and 60 DAS.

4.5.13 Proline content

The foliar application of 20 mM proline significantly increased the endogenous proline content maximum values being 28% and 23% in Varuna and RH-30, compared with the control plants at 30 DAS (Table 59). Moreover, soil applied NaCl also increased the proline content and the increase was in proportion to the concentration of NaCl. Furthermore, proline application to the stressed plants had an additive effect. The maximum increase was recorded in the plants treated with highest concentration of NaCl and sprayed with 20 mM proline in both the varieties.

4.5.14 Yield characteristics per plant

The plants raised in the soil amended with different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl possessed a sharp decrease in the yield characteristics (number of pods per plant, number of seeds per pod, 100 seed mass and seed yield) in both Varuna and RH-30, at harvest (Table 60). The decrease was in proportion to the degree of NaCl stress. However, the spray of 20 mM proline to the foliage of stress free plants significantly increased the number of pods per plant (24% and 21%) and seed yield (30 and 25%) in Varuna and RH-30 respectively, over their control plants. Moreover, the spray of proline to stressed plants partially neutralized the harmful effects of NaCl (2.8 dsm^{-1}) in Varuna whereas, the other NaCl concentrations (4.2 or 5.6 dsm^{-1}) were not neutralized at all by 20 mM proline spray.

Table 58: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on peroxidase (units g^{-1} FM) and superoxide dismutase (units g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Peroxidase activity | | | | | | Superoxide dismutase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	12.22	7.34	9.78	16.60	9.68	13.14	120	110	115	150	134	142
Proline (20 mM)	16.55	9.46	13.00	21.74	11.61	16.67	146	127	136	174	147	160
NaCl (2.8 dsm^{-1})	16.64	8.54	12.59	20.87	10.76	15.81	170	142	156	199	154	176
NaCl (4.2 dsm^{-1})	17.49	9.50	13.49	22.71	11.29	17.00	187	156	171	220	170	195
NaCl (5.6 dsm^{-1})	19.09	9.93	14.51	23.45	12.13	17.79	214	167	190	256	189	222
NaCl (2.8 dsm^{-1}) +Proline	17.35	9.83	13.59	22.74	12.39	17.56	193	168	180	235	164	199
NaCl (4.2 dsm^{-1})+Proline	21.62	11.01	16.31	25.56	13.74	19.65	213	185	199	243	182	212
NaCl (5.6 dsm^{-1})+Proline	23.58	12.25	17.91	27.88	15.00	21.44	226	188	207	261	202	231
Mean	18.07	9.73		22.69	12.08		183	155		217	167	
LSD at 5%	V = 0.10 (Sig)			V = 0.14 (Sig)			V = 1.52 (Sig)			V = 2.78 (Sig)		
	T = 0.15 (Sig)			T = 0.22 (Sig)			T = 2.41 (Sig)			T = 4.39 (Sig)		
	V x T = 0.22 (Sig)			V x T = 0.31 (Sig)			V x T = 3.41 (Sig)			V x T = 6.21 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 59: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on proline content (μmol g^{-1} FM) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Proline content | | | | | |
| | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	14.00	12.53	13.26	19.95	17.41	18.68
Proline (20 mM)	17.99	15.43	16.71	23.38	19.84	21.61
NaCl (2.8 dsm^{-1})	20.51	16.11	18.31	27.78	20.08	23.93
NaCl (4.2 dsm^{-1})	23.11	17.45	20.28	29.56	21.56	25.56
NaCl (5.6 dsm^{-1})	25.23	19.76	22.49	33.78	25.12	29.45
NaCl (2.8 dsm^{-1}) +Proline	22.40	17.79	20.09	29.32	21.76	25.54
NaCl (4.2 dsm^{-1})+Proline	24.08	18.79	21.43	31.92	22.98	27.45
NaCl (5.6 dsm^{-1})+Proline	26.70	21.80	24.25	35.51	27.33	31.42
Mean	21.75	17.45		28.90	22.01	

LSD at 5%						
	V	=	0.08(Sig)	V	=	0.16 (Sig)
	T	=	0.13(Sig)	T	=	0.26 (Sig)
	V × T	=	0.18(Sig)	V × T	=	0.38 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 60: Effect of proline (20 mM) as foliar spray (29 d stage) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm⁻¹) on pods plant⁻¹, seeds pod⁻¹, 100 seed mass (mg) and seed yield plant⁻¹ (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Pods plant⁻¹			Seeds pod⁻¹			100 seed mass			Seed yield plant⁻¹		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	232	200	216	12.83	11.72	12.27	318	300	309	7.00	6.29	6.64
Proline (20 mM)	288	242	265	13.01	11.75	12.38	319	298	308	9.11	7.88	8.49
NaCl (2.8 dsm⁻¹)	197	159	178	11.49	9.49	10.49	298	267	282	5.67	4.81	5.24
NaCl (4.2 dsm⁻¹)	184	146	165	10.88	8.90	9.89	280	243	261	5.03	3.87	4.45
NaCl (5.6 dsm⁻¹)	173	131	152	10.52	7.83	9.17	268	231	249	4.46	3.33	3.89
NaCl (2.8 dsm⁻¹) +Proline	213	178	195	11.65	9.61	10.63	300	270	285	6.49	5.28	5.88
NaCl (4.2 dsm⁻¹)+Proline	204	161	182	10.99	8.90	9.94	287	257	272	6.19	4.90	5.54
NaCl (5.6 dsm⁻¹)+Proline	187	152	169	10.60	8.00	9.30	274	243	258	5.60	4.46	5.03
Mean	209	171		11.49	9.53		293	263		6.19	5.10	
LSD at 5%	V = 6.55 (Sig)			V = 0.57 (Sig)			V = 7.56 (Sig)			V = 0.11 (Sig)		
	T = 10.35 (Sig)			T = 0.31 (Sig)			T = 11.01 (Sig)			T – 0.17 (Sig)		
	V x T = NS			V x T – 0.74 (Sig)			V x T = NS			V x T = 0.25 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.6 Experiment 6

This experiment was designed with an aim to elucidate the cumulative effect of the foliar spray of EBL and proline on the salinity induced changes in *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. All the agricultural practices were same as in the Experiment 1. Different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl were applied through soil at the time of sowing. The subsequent plants were sprayed by DDW or proline and/or EBL at 28 and 29 DAS respectively. The plant samples were then collected at 30 and 60 DAS to assess all the parameters mentioned in the Experiment 1. The rest of the plants were allowed to grow up to maturity and were harvested at 120 DAS to study the yield characteristics.

4.6.1   Shoot and root length

As the growth progressed from 30 to 60 DAS, the length of shoot and root increased (Table 61). Application of 10^{-8} M EBL and/or 20 mM proline to the foliage of mustard plants increased the shoot and root length at 60 DAS. However, the plants raised in the soil fed with NaCl (2.8, 4.2 or 5.6 dsm^{-1}) had significantly lesser values for shoot and root length in both varieties (Varuna and RH-30) at 30 and 60 DAS, compared with the control. The damage was more pronounced in RH-30 than Varuna. Moreover, follow up treatment of EBL (10^{-8} M) and proline (20 mM) spray to NaCl stressed plants, completely neutralized the ill effects generated by NaCl (2.8 and 4.2 dsm^{-1}) at 60 DAS.

4.6.2   Shoot and root fresh mass

The data in table 62 depicted that the spray of a combination of proline and EBL proved best and increased the shoot and root fresh mass significantly in both Varuna and RH-30, over their respective control plants, at 60 DAS. However, the NaCl (2.8, 4.2 or 5.6 dsm^{-1}) applied through soil brought a sharp decline in the fresh mass of shoot and root and the loss was concentration dependent. Moreover, the application of EBL and proline combination to the salt stressed plants not only completely neutralized the harmful effects generated by 2.8 and 4.2 dsm^{-1} concentrations of NaCl in both varieties at 60 DAS but the values were significantly higher than the control plants. The variety Varuna was more responsive to the treatments than RH-30.

Table 61: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on shoot and root length (cm) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Shoot length						Root length					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	15.97	14.82	15.39	73.40	67.87	70.63	17.00	15.81	16.40	26.09	23.87	24.98
Proline (20 mM)	15.94	14.83	15.38	105.23	90.34	97.78	17.00	15.79	16.39	35.00	32.56	33.78
EBL (10^{-8} M)	15.96	14.82	15.39	123.45	107.12	115.28	17.02	15.82	16.42	42.54	34.00	38.27
Proline (20 mM)+ EBL (10^{-8} M)	16.00	14.92	15.46	126.98	111.31	119.14	17.10	15.83	16.46	40.96	34.61	37.78
NaCl (2.8 dsm^{-1})	12.47	10.75	11.61	63.85	54.45	59.15	12.34	10.01	11.17	21.56	18.14	19.85
NaCl (4.2 dsm^{-1})	10.22	9.11	9.66	57.00	48.10	52.55	9.55	6.43	7.99	17.74	13.93	15.83
NaCl (5.6 dsm^{-1})	9.50	7.49	8.49	48.23	38.45	43.34	7.87	5.55	6.71	15.78	10.50	13.14
NaCl (2.8 dsm^{-1}) + Proline +EBL	12.50	10.82	11.66	90.28	78.73	84.50	12.41	10.08	11.24	29.35	25.82	27.58
NaCl (4.2 dsm^{-1})+ Proline +EBL	10.28	9.19	9.73	85.51	75.34	80.42	9.69	6.48	8.08	28.43	24.82	26.62
NaCl (5.6 dsm^{-1}) +Proline +EBL	9.58	7.56	8.57	74.87	59.73	67.30	7.91	5.69	6.80	23.74	18.61	21.17
Mean	12.84	11.43		84.88	73.14		12.78	10.74		28.11	23.68	
LSD at 5%	V = 0.17 (Sig)			V = 3.17 (Sig)			V = 0.11(Sig)			V = 1.12 (Sig)		
	T = 0.27 (Sig)			T = 5.02 (Sig)			T = 0.18(Sig)			T = 1.77 (Sig)		
	V x T= 0.39 (Sig)			V x T= NS			V x T= 0.26 (Sig)			V x T = NS		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 62: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on shoot and root fresh mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot fresh mass | | | | | | Root fresh mass | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	6.69	5.17	5.93	12.34	9.61	10.97	2.93	2.40	2.66	5.65	4.53	5.09
Proline (20 mM)	6.73	5.19	5.96	17.34	13.00	15.17	2.90	2.38	2.64	7.12	5.64	6.38
EBL (10^{-8} M)	6.70	5.17	5.93	19.99	14.01	17.00	2.95	2.41	2.68	9.27	7.19	8.23
Proline (20 mM)+ EBL (10^{-8} M)	6.73	5.29	6.01	21.96	14.79	18.37	3.00	2.49	2.74	9.46	7.38	8.42
NaCl (2.8 dsm^{-1})	4.99	3.10	4.04	10.56	6.69	8.62	1.89	1.29	1.59	4.24	2.89	3.56
NaCl (4.2 dsm^{-1})	4.45	2.55	3.50	9.81	6.10	7.95	1.40	1.02	1.21	3.78	2.56	3.17
NaCl (5.6 dsm^{-1})	3.99	2.04	3.01	9.00	5.12	7.06	1.15	0.69	0.92	3.15	1.70	2.42
NaCl (2.8 dsm^{-1}) + Proline +EBL	5.02	3.15	4.08	15.79	10.76	13.27	1.94	1.32	1.63	7.06	4.89	5.97
NaCl (4.2 dsm^{-1})+ Proline +EBL	4.55	2.57	3.56	14.87	10.19	12.53	1.49	1.08	1.28	6.49	4.03	5.26
NaCl (5.6 dsm^{-1}) +Proline +EBL	4.07	2.27	3.17	11.93	8.65	10.29	1.26	0.65	0.95	4.75	3.62	4.18
Mean	5.39	3.65		14.35	9.89		2.09	1.57		6.09	4.44	
LSD at 5%	V = 0.09 (Sig)			V = 0.09 (Sig)			V = 0.10(Sig)			V = 0.08 (Sig)		
	T = 0.14 (Sig)			T = 0.14 (Sig)			T = 0.15(Sig)			T = 0.13 (Sig)		
	V × T= 0.20 (Sig)			V × T = 0.20 (Sig)			V × T = 0.22 (Sig)			V × T = 0.19 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.6.3   Shoot and root dry mass

· The dry mass of shoot and root exhibited a pattern similar to that of the fresh mass (4.6.2). The plants raised in the soil amended with different levels (2.8, 4.2 or 5.6 dsm⁻¹) of NaCl showed a marked decrease in the shoot and root dry mass (Table 63). The variety RH-30 was more prone to stress than Varuna. However, the spray of EBL and proline combination significantly increased the dry mass of shoot and root at 60 DAS, maximum being 93% and 88% more in Varuna, over the control plants. Moreover, the spray of EBL and proline combination as a follow up treatment to NaCl stressed plants completely neutralized the ill effects of NaCl (2.8 and 4.2 dsm⁻¹) at 60 DAS.

4.6.4   Leaf area

The data in table 64 showed that the leaf area increased as the growth progressed from 30 to 60 DAS in both the varieties (Varuna and RH-30). Furthermore, the spray of proline, EBL or their combination to the foliage of stress free plants enhanced the leaf area and the per cent increase by EBL+ proline was 41% and 35% in Varuna and RH-30 respectively, over their control plants at 60 DAS. However, the leaf area had a sharp decrease as the level of NaCl (2.8, 4.2 or 5.6 dsm⁻¹) increased in the soil in both varieties being more prominent in RH-30 than Varuna. Moreover, the spray of EBL and proline combination together to the stressed plants completely neutralized the damaging effects of NaCl (2.8 or 4.2 dsm⁻¹) and partly that of 5.6 dsm⁻¹, in Varuna and RH-30 at 60 DAS.

4.6.5   SPAD chlorophyll values

The plants at 60 DAS had higher SPAD chlorophyll than at 30 DAS (Table 64). Moreover, the application of proline and/or EBL to the foliage further increased the SPAD chlorophyll values and the combination of these two was most effective in both the varieties. However, the plants grown in the soil amended with NaCl (2.8, 4.2, or 5.6 dsm⁻¹) showed a negative response where 5.6 dsm⁻¹ of NaCl triggered maximum decrease which was 42% and 31% in Varuna and 49%, 37% in RH-30 at 30 and 60 DAS, compared with non-stressed control plants, respectively. The loss generated by NaCl (2.8 or 4.2 dsm⁻¹) was completely overcome by the follow-up treatment with EBL plus proline and the values were significantly higher than those of the control. The impact of higher level (5.6 dsm⁻¹) was partially overcome by the combination.

Table 63: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on shoot and root dry mass (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Shoot dry mass | | | | | | Root dry mass | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	1.84	1.39	1.61	3.40	2.53	2.96	0.795	0.613	0.704	1.49	1.13	1.31
Proline (20 mM)	1.84	1.37	1.60	5.01	3.50	4.25	0.795	0.611	0.703	2.04	1.46	1.75
EBL (10^{-8} M)	1.86	1.40	1.63	6.12	4.32	5.22	0.798	0.614	0.706	2.58	1.85	2.21
Proline (20 mM)+ EBL (10^{-8} M)	1.92	1.41	1.66	6.57	4.35	5.46	0.799	0.624	0.711	2.81	1.89	2.35
NaCl (2.8 dsm^{-1})	1.41	0.83	1.12	2.90	1.72	2.31	0.564	0.300	0.432	1.28	0.834	1.05
NaCl (4.2 dsm^{-1})	1.20	0.68	0.94	2.73	1.50	2.11	0.494	0.249	0.371	1.10	0.699	0.89
NaCl (5.6 dsm^{-1})	1.05	0.51	0.78	2.34	1.10	1.72	0.400	0.220	0.310	0.97	0.598	0.78
NaCl (2.8 dsm^{-1}) + Proline +EBL	1.39	0.85	1.12	4.42	2.76	3.59	0.573	0.301	0.437	1.80	1.20	1.50
NaCl (4.2 dsm^{-1})+ Proline +EBL	1.23	0.71	0.97	3.67	2.58	3.12	0.492	0.257	0.374	1.68	1.16	1.42
NaCl (5.6 dsm^{-1}) +Proline +EBL	1.12	0.50	0.81	3.26	2.28	2.77	0.405	0.239	0.322	1.42	0.97	1.19
Mean	1.48	0.96		4.04	2.66		0.611	0.402		1.71	1.17	

LSD at 5%												
	V	= 0.03 (Sig)		V	= 0.04 (Sig)		V	= 0.003(Sig)		V	= 0.08 (Sig)	
	T	= 0.05 (Sig)		T	= 0.07 (Sig)		T	= 0.006(Sig)		T	= 0.12 (Sig)	
	V × T	= 0.08 (Sig)		V × T	= 0.10 (Sig)		V × T	= 0.008(Sig)		V × T	= 0.18 (Sig)	

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 64: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on leaf area (cm^2) and SPAD Chlorophyll values in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Leaf area | | | | | | SPAD chlorophyll value | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	24.93	23.26	24.09	38.64	35.35	36.99	44.81	37.62	41.21	60.94	50.59	55.76
Proline (20 mM)	25.03	23.28	24.15	47.51	40.45	43.98	54.34	45.00	49.67	73.02	57.52	65.27
EBL (10^{-8} M)	25.12	23.34	24.23	53.12	45.89	49.50	62.21	47.01	54.61	79.28	61.23	70.25
Proline (20 mM)+ EBL (10^{-8} M)	25.00	23.40	24.20	54.48	47.72	51.10	64.53	48.91	56.72	81.05	63.24	72.14
NaCl (2.8 dsm^{-1})	19.87	14.75	17.31	33.34	26.08	29.71	36.21	27.10	31.65	53.52	41.44	47.48
NaCl (4.2 dsm^{-1})	15.30	13.10	14.20	28.45	21.78	25.11	31.21	22.20	26.70	48.34	34.83	41.58
NaCl (5.6 dsm^{-1})	12.87	9.62	11.24	25.76	19.23	22.49	25.89	18.90	22.39	42.00	31.75	36.87
NaCl (2.8 dsm^{-1}) + Proline +EBL	19.69	14.89	17.29	45.21	38.89	42.05	51.97	41.75	46.86	75.56	58.17	66.86
NaCl (4.2 dsm^{-1})+ Proline +EBL	15.23	13.26	14.24	42.08	36.06	39.07	48.84	38.74	43.79	72.51	55.14	63.82
NaCl (5.6 dsm^{-1}) +Proline +EBL	12.96	9.54	11.25	37.02	31.11	34.06	41.67	32.73	37.20	59.72	47.45	53.58
Mean	19.60	16.84		40.56	34.25		46.16	35.99		64.59	50.13	
LSD at 5%	V = 0.51 (Sig)			V = 0.89 (Sig)			V = 0.06 (Sig)			V = 0.09 (Sig)		
	T = 0.81 (Sig)			T = 1.41 (Sig)			T = 0.09 (Sig)			T = 0.15 (Sig)		
	V x T = 1.15 (Sig)			V x T = 1.99 (Sig)			V x T = 0.14 (Sig)			V x T = 0.21 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.6.6 Electrolyte leakage

The electrolyte leakage from the leaves increased with an increasing level of NaCl in the soil. Out of the three concentrations (2.8, 4.2 or 5.6 dsm^{-1}), 5.6 dsm^{-1} of NaCl proved highly toxic that increased the leakage by 27% and 33% in Varuna and RH-30, over the control, respectively at 30 DAS (Table 65). Proline and/or EBL checked the loss of electrolytes significantly and the combination of the two proved best at 60 DAS. Moreover, the follow-up application of the salt stresses plants with proline plus EBL minimized the impact of the stress and brought the values of electrolyte leakage below to that of the control plants.

4.6.7 Leaf water potential

The data in the table 65 revealed that the leaves of both varieties (Varuna and RH-30) under NaCl stress possessed significantly lower leaf water potential than their control plants in a concentration dependent manner. However, leaves exposed to proline and/or EBL, to stress free plants as foliar spray, had significantly higher values at both the stages of growth (30 and 60 DAS). The combined effect of proline and EBL proved best. The loss of leaf water potential under stress was totally nullified by the follow-up treatment with EBL plus proline. Moreover, at 60 d stage all the salt-stressed plants had LWP at par with the control or more than that, provided they were given a follow-up treatment with proline plus EBL.

4.6.8 Net photosynthetic rate and related attributes

The values for net photosynthetic rate (P_N) and its related attributes i.e., stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) increased with the advancement of the plant growth (Tables 66 and 67). The foliar application of proline and/or EBL to the plants significantly improved the values, both at 30 and 60 DAS. Proline plus EBL spray proved best and increased the P_N by 53% and 44%; g_s: 96% and 76%; C_i: 33% and 24% and E: 54% and 33% in Varuna and RH-30 respectively, over their control plants at 30 DAS. NaCl at three levels (2.8, 4.2 or 5.6 dsm^{-1}) administered through the soil generated stress and decreased P_N and all its related attributes significantly at both the stages of the growth (30 and 60 DAS) in a concentration dependent manner in both the varieties. The NaCl-induced decrease was more pronounced in variety RH-30 than Varuna. However, the toxicity generated by the two lower concentrations (2.8 or 4.2 dsm^{-1}) of NaCl was completely overcome by the follow up application of proline plus and EBL and the values

Table 65: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on electrolyte leakage (%) and leaf water potential (MPa) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Electrolyte leakage						Leaf water potential					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	7.15	8.19	7.67	5.82	6.78	6.30	-0.64	-0.73	-0.68	-0.48	-0.58	-0.53
Proline (20 mM)	7.19	8.24	7.71	4.63	5.82	5.22	-0.52	-0.63	-0.57	-0.39	-0.52	-0.45
EBL (10^{-8} M)	7.10	8.16	7.63	4.10	5.26	4.68	-0.41	-0.50	-0.45	-0.33	-0.44	-0.38
Proline (20 mM)+ EBL (10^{-8} M)	7.26	8.21	7.73	3.49	4.67	4.08	-0.30	-0.43	-0.36	-0.29	-0.40	-0.34
NaCl (2.8 dsm^{-1})	7.95	9.43	8.69	6.23	7.51	6.87	-0.73	-0.85	-0.79	-0.52	-0.65	-0.58
NaCl (4.2 dsm^{-1})	8.53	9.98	9.25	6.73	7.94	7.33	-0.81	-0.93	-0.87	-0.57	-0.72	-0.64
NaCl (5.6 dsm^{-1})	9.09	10.93	9.98	7.02	8.57	7.79	-0.87	-1.01	-0.94	-0.61	-0.78	-0.69
NaCl (2.8 dsm^{-1}) + Proline +EBL	6.29	7.61	6.95	4.48	5.89	5.18	-0.48	-0.61	-0.54	-0.34	-0.46	-0.40
NaCl (4.2 dsm^{-1})+ Proline +EBL	6.93	8.27	7.60	5.23	6.44	5.83	-0.55	-0.70	-0.62	-0.38	-0.53	-0.45
NaCl (5.6 dsm^{-1}) +Proline +EBL	7.72	9.33	8.52	5.99	7.39	6.69	-0.69	-0.83	-0.76	-0.46	-0.61	-0.53
Mean	7.52	8.82		5.37	6.62		-0.60	-0.72		-0.43	-0.56	
LSD at 5%	V = 0.07 (Sig)			V = 0.05 (Sig)			V = 0.013 (Sig)			V = 0.004 (Sig)		
	T = 0.12 (Sig)			T = 0.09 (Sig)			T = 0.021 (Sig)			T = 0.006 (Sig)		
	V x T = 0.17 (Sig)			V x T = 0.12 (Sig)			V x T = 0.030 (Sig)			V x T = 0.009 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 66: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Net photosynthetic rate (P_N)						Stomatal conductance (g_s)					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	20.79	18.20	19.49	27.33	23.29	25.31	0.063	0.056	0.059	0.089	0.078	0.083
Proline (20 mM)	26.31	22.31	24.31	33.60	27.16	30.38	0.093	0.078	0.085	0.124	0.101	0.112
EBL (10^{-8} M)	29.36	24.61	26.98	37.86	30.64	34.25	0.110	0.091	0.100	0.152	0.116	0.134
Proline (20 mM)+ HBL (10^{-8} M)	31.81	26.30	29.05	40.23	32.26	36.24	0.124	0.099	0.111	0.163	0.120	0.141
NaCl (2.8 dsm^{-1})	16.16	11.10	13.63	23.67	16.54	20.10	0.041	0.027	0.034	0.065	0.046	0.055
NaCl (4.2 dsm^{-1})	14.76	9.78	12.27	21.23	14.43	17.83	0.034	0.024	0.029	0.056	0.038	0.047
NaCl (5.6 dsm^{-1})	14.00	8.43	11.21	19.86	12.67	16.26	0.030	0.019	0.024	0.051	0.033	0.042
NaCl (2.8 dsm^{-1}) + Proline +EBL	24.78	20.38	22.58	34.16	26.59	30.37	0.074	0.061	0.067	0.107	0.088	0.097
NaCl (4.2 dsm^{-1})+ Proline +EBL	22.76	19.01	20.88	30.88	24.52	27.70	0.069	0.058	0.063	0.099	0.085	0.092
NaCl (5.6 dsm^{-1}) +Proline +EBL	17.57	14.38	15.97	25.77	19.33	22.55	0.056	0.047	0.051	0.084	0.068	0.076
Mean	21.83	17.45		24.25	22.74		0.069	0.056		0.099	0.077	

LSD at 5%	V = 0.12 (Sig)			V = 0.09 (Sig)			V = 0.0004(Sig)			V = 0.0003 (Sig)		
	T = 0.20 (Sig)			T = 0.14 (Sig)			T = 0.0007(Sig)			T = 0.0005 (Sig)		
	V x T = 0.28 (Sig)			V x T = 0.20 (Sig)			V x T = 0.0009(Sig)			V x T = 0.0008 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 67: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on internal CO_2 concentration (ppm) and transpiration rate (mmol H_2O m^{-2} s^{-1}) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

	Internal CO_2 concentration (C_i)						Transpiration rate (E)					
	30 DAS			60 DAS			30 DAS			60 DAS		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	322	290	306	371	326	348	3.32	2.84	3.08	4.92	4.15	4.53
Proline (20 mM)	376	333	354	432	360	396	4.26	3.32	3.79	6.08	4.78	5.43
EBL (10^{-8} M)	408	346	377	442	375	408	4.86	3.62	4.24	6.64	5.00	5.82
Proline (20 mM)+ EBL (10^{-8} M)	431	361	396	471	391	431	5.13	3.78	4.45	7.28	5.35	6.31
NaCl (2.8 dsm^{-1})	274	213	243	333	272	302	2.92	2.03	2.47	4.54	3.31	3.92
NaCl (4.2 dsm^{-1})	253	200	226	316	241	278	2.63	1.80	2.21	4.36	3.00	3.68
NaCl (5.6 dsm^{-1})	237	184	210	303	217	260	2.38	1.65	2.01	3.93	2.81	3.37
NaCl (2.8 dsm^{-1}) – Proline +EBL	347	301	324	415	345	380	4.18	3.03	3.60	6.54	4.64	5.59
NaCl (4.2 dsm^{-1})+ Proline +EBL	333	298	315	389	338	363	3.85	2.95	3.40	5.90	4.27	5.08
NaCl (5.6 dsm^{-1}) +Proline +EBL	296	240	268	356	290	323	3.12	2.30	2.71	4.69	3.58	4.13
Mean	327	276		382	315		3.66	2.73		5.48	4.08	
LSD at 5%	V = 1.21 (Sig)			V = 1.30 (Sig)			V = 0.06 (Sig)			V = 0.13 (Sig)		
	T = 1.91 (Sig)			T = 2.06 (Sig)			T = 0.10 (Sig)			T = 0.20 (Sig)		
	V x T = 2.71 (Sig)			V x T = 2.92 (Sig)			V x T = 0.14 (Sig)			V x T = 0.29 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

were more than that of the control. Moreover, the treatment partially overcome the impact of the NaCl (5.6 dsm^{-1}).

4.6.9 Maximum quantum yield of PSII (Fv/Fm)

The values of Fv/Fm were inversely proportional to the concentration of NaCl (2.8, 4.2 or 5.6 dsm^{-1}) availability to the plants of Varuna and RH-30 (Table 68). Moreover, 5.6 dsm^{-1} of NaCl was most toxic and reduced the values in Varuna and RH-30 by 30% and 35%, respectively at 30 DAS and by 22% and 28% at 60 DAS, compared to the corresponding control plants. However, proline and/or EBL given as a follow-up treatment to the leaves of stress free or NaCl-stressed plants, improved the values significantly. Moreover, the combination of proline and EBL completely overcome the impact of the two lower concentrations (2.8 or 4.2 dsm^{-1}) of NaCl and partially that of 5.6 dsm^{-1}.

4.6.10 Nitrate reductase (NR) activity

With the advancement of plant age, the NR activity increased in both Varuna and RH-30 (Table 68). The values of NR were higher in the leaves of both varieties which received proline and/or EBL as foliar application. Moreover, the combination of proline and EBL together induced highest increase that was 60% in Varuna and 50% in RH-30, over their control plants at 30 DAS. Salt-stress lowered the values but like the other parameters, proline plus EBL completely overcome the damaging effect of 2.8 and 4.2 dsm^{-1} of salt and the values were significantly higher than the control.

4.6.11 Carbonic anhydrase (CA) activity

The foliage of plants exposed to proline and/or EBL had higher activity of CA (Table 69) compared with the control. The maximum increase was recorded in the plants which received the combination of proline and EBL which was 51% more in Varuna at 30 DAS, over the control plants. However, the plants grown in the soil amended with varied levels of NaCl (2.8, 4.2 or 5.6 dsm^{-1}) showed a decrease in the activity of CA and maximum loss (50%) was induced by 5.6 dsm^{-1} in RH-30 at 30 DAS, compared with the stress free control plants. However, the application of proline plus EBL as a follow-up treatment to the NaCl stressed plants completely neutralizes the salt toxicity where Varuna was more responsive than RH-30.

Table 68: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on maximum quantum yield of PSII (Fv/Fm) and nitrate reductase (nmole NO_2 g^{-1} FM s^{-1}) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Maximum quantum yield of PSII (Fv/Fm) | | | | | | Nitrate reductase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	0.773	0.651	0.712	0.975	0.808	0.891	432	378	405	570	492	531
Proline (20 mM)	0.930	0.754	0.842	1.090	0.876	0.983	566	465	515	708	584	646
EBL (10^{-8} M)	1.020	0.826	0.923	1.210	0.950	1.080	658	536	597	780	624	702
Proline (20 mM)+ EBL (10^{-8} M)	1.150	0.904	1.027	1.360	1.030	1.195	695	567	631	820	669	744
NaCl (2.8 dsm^{-1})	0.685	0.543	0.614	0.900	0.703	0.801	354	257	305	513	350	431
NaCl (4.2 dsm^{-1})	0.611	0.473	0.542	0.820	0.645	0.732	328	200	264	483	315	399
NaCl (5.6 dsm^{-1})	0.538	0.419	0.478	0.756	0.581	0.668	299	172	235	436	284	360
NaCl (2.8 dsm^{-1}) + Proline +EBL	0.927	0.729	0.828	1.210	0.945	1.077	548	438	493	763	590	676
NaCl (4.2 dsm^{-1})+ Proline +EBL	0.858	0.670	0.764	1.130	0.880	1.005	514	419	466	695	553	624
NaCl (5.6 dsm^{-1}) +Proline +EBL	0.703	0.559	0.631	0.965	0.743	0.854	395	306	350	591	419	488
Mean	0.819	0.652		1.041	0.816		478	373		631	489	
LSD at 5%	V = 0.07 (Sig)			V = 0.13 (Sig)			V = 3.48 (Sig)			V = 6.07 (Sig)		
	T = 0.13 (Sig)			T = 0.21 (Sig)			T = 5.50 (Sig)			T = 9.60 (Sig)		
	V x T = 0.18 (Sig)			V x T = 0.32 (Sig)			V x T = 7.79 (Sig)			V x T = 13.58 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 69: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on carbonic anhydrase (mol CO_2 g^{-1} FM s^{-1}) and catalase (mM H_2O_2 decomposed g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Carbonic anhydrase activity | | | | | | Catalase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	2.12	2.01	2.07	2.40	2.33	2.37	420	382	401	445	398	421
Proline (20 mM)	2.65	2.33	2.49	2.83	2.63	2.73	497	431	464	504	420	462
EBL (10^{-8} M)	3.02	2.63	2.83	3.12	2.77	2.95	537	455	496	534	435	484
Proline (20 mM)+ EBL (10^{-8} M)	3.24	2.82	3.03	3.44	3.07	3.25	600	515	557	560	453	506
NaCl (2.8 dsm^{-1})	1.71	1.37	1.54	2.14	1.78	1.96	524	440	482	506	428	467
NaCl (4.2 dsm^{-1})	1.59	1.18	1.38	1.91	1.57	1.74	556	458	507	533	442	487
NaCl (5.6 dsm^{-1})	1.39	0.97	1.18	1.73	1.36	1.55	592	469	530	567	467	517
NaCl (2.8 dsm^{-1}) + Proline +EBL	2.56	2.15	2.35	3.08	2.64	2.86	655	557	606	654	525	589
NaCl (4.2 dsm^{-1})+ Proline +EBL	2.33	2.03	2.18	2.77	2.44	2.61	730	607	668	734	581	657
NaCl (5.6 dsm^{-1}) +Proline +EBL	2.00	1.65	1.83	2.46	2.15	2.31	831	683	757	805	636	720
Mean	2.26	1.91		2.59	2.28		594	499		584	478	

LSD at 5%												
	V = 0.08 (Sig)			V = 0.05 (Sig)			V = 4.38 (Sig)			V = 5.33 (Sig)		
	T = 0.13 (Sig)			T = 0.14 (Sig)			T = 6.94 (Sig)			T = 8.44 (Sig)		
	V x T = 0.19 (Sig)			V x T = 0.18 (Sig)			V x T = 9.81 (Sig)			V x T = 11.93(Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

4.6.12 Activities of antioxidant enzymes

The activity of antioxidant enzymes (CAT, POX and SOD) exhibited an increase with the progress of the growth from 30 to 60 DAS (Tables 69 and 70). Furthermore, the activity of these enzymes increased in the presence of NaCl, proline and/or EBL. The maximum activity of these enzymes was recorded in Varuna, grown in the soil amended with 5.6 dsm^{-1} of NaCl and sprayed with the combination of proline and EBL. In terms of percentage, the activity of CAT enzyme increased by 97% and 80%, POX by 164% and 130%, SOD by 144% and 118% in Varuna at 30 and 60 DAS respectively, over the control plants. Minimum antioxidant enzyme activity was recorded in control plants of both varieties.

4.6.13 Proline content

Leaf proline content increased in both the varieties fed either with NaCl (2.8, 4.2 or 5.6 dsm^{-1}), proline and/or EBL over the control plants (Table 71). Out of the two varieties, Varuna possessed higher proline content than R11-30. Moreover, the maximum values for proline content in both the varieties were recorded in the plants fed with highest level of NaCl (5.6 dsm^{-1}) and given a follow up treatment of combination of proline and EBL. A maximum increase of 116% at 30 DAS and 101% at 60 DAS, over the control plants, was noted in Varuna, where EBL was given as a follow up treatment with NaCl (5.6 dsm^{-1}) as a soil amendment.

4.6.14 Yield characteristics per plant

With the increase of NaCl concentration from 2.8 to 5.6 dsm^{-1} number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant significantly decreased in both the varieties, at harvest (Table 72). The toxic response of NaCl was more pronounced in variety RH-30 than Varuna. However, the foliar application of proline and/or EBL on plants under stress-free conditions significantly increased the number of pods per plant and seed yield per plant, over the control. The maximum values for number of pods per plant, and seed yield per plant were observed in Varuna under stress free conditions which received the combination of proline and EBL that were 50% and 44% more, over the control plants. The foliar spray of proline plus EBL proved most effective in nullifying the toxicity caused by 2.8 and 4.2 dsm^{-1} of NaCl particularly in Varuna where these values were significantly higher than the control.

Table 70: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on peroxidase (units g^{-1} FM) and superoxide dismutase (units g^{-1} FM) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Peroxidase activity | | | | | | Superoxide dismutase activity | | | | | |
| | 30 DAS | | | 60 DAS | | | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	12.37	7.28	9.82	16.72	9.68	13.20	135	120	127	170	147	158
Proline (20 mM)	16.42	9.29	12.85	21.21	11.42	16.31	160	139	149	191	164	177
EBL (10^{-8} M)	19.26	10.64	14.95	23.61	12.52	18.06	176	145	160	214	171	192
Proline (20 mM)+ EBL (10^{-8} M)	20.91	11.50	16.20	24.74	13.26	19.00	187	152	169	221	183	202
NaCl (2.8 dsm^{-1})	16.40	8.59	12.49	21.08	10.89	15.98	195	154	174	220	174	197
NaCl (4.2 dsm^{-1})	17.52	9.57	13.54	22.90	11.22	17.06	210	170	190	249	193	221
NaCl (5.6 dsm^{-1})	19.55	9.90	14.72	23.72	12.15	17.93	249	208	228	301	237	269
NaCl (2.8 dsm^{-1}) + Proline +EBL	23.75	13.10	18.42	30.43	15.48	22.95	274	228	251	333	260	296
NaCl (4.2 dsm^{-1})+ Proline +EBL	29.06	14.34	21.70	35.44	17.52	26.48	307	249	278	353	271	312
NaCl (5.6 dsm^{-1}) +Proline +EBL	32.78	16.23	24.50	38.62	18.87	28.74	330	261	295	372	288	330
Mean	20.80	11.04		25.84	13.30		222	182		262	208	
LSD at 5%	V = 0.05 (Sig)			V = 0.11 (Sig)			V = 4.73 (Sig)			V = 5.27 (Sig)		
	T = 0.08 (Sig)			T = 0.18 (Sig)			T = 7.48 (Sig)			T = 8.33 (Sig)		
	V × T = 0.12 (Sig)			V × T = 0.25 (Sig)			V × T = 10.57 (Sig)			V × T = 11.78(Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 71: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on proline content (μmol g^{-1} FM) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at 30 and 60 DAS.

| | Proline content | | | | | |
| | 30 DAS | | | 60 DAS | | |
	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	13.60	12.43	13.01	19.04	17.15	18.09
Proline (20 mM)	17.21	15.08	16.14	22.17	19.52	20.84
EBL (10^{-8} M)	19.86	16.48	18.17	23.22	20.51	21.86
Proline (20 mM)+ EBL (10^{-8} M)	22.57	17.65	20.11	29.13	22.46	25.79
NaCl (2.8 dsm^{-1})	20.63	17.43	19.03	28.00	20.99	24.49
NaCl (4.2 dsm^{-1})	23.12	18.78	20.95	31.12	22.10	26.61
NaCl (5.6 dsm^{-1})	25.00	20.65	22.82	32.45	25.08	28.76
NaCl (2.8 dsm^{-1}) + Proline +EBL	25.43	21.63	23.53	33.51	26.07	29.79
NaCl (4.2 dsm^{-1})+ Proline +EBL	26.93	22.37	24.65	35.98	27.44	31.71
NaCl (5.6 dsm^{-1}) +Proline +EBL	29.51	24.23	26.87	38.46	30.52	34.49
Mean	22.38	18.67		29.30	23.18	

LSD at 5%
V = 0.09 (Sig)   V = 0.09 (Sig)
T = 0.15 (Sig)   T = 0.15 (Sig)
V x T = 0.21 (Sig)   V x T = 0.22 (Sig)

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Table 72: Effect of proline (20 mM) and 24-epibrassinolide (EBL; 10^{-8} M) as foliar spray (28 and 29 d stages) and/or soil applied sodium chloride (NaCl; 2.8, 4.2, or 5.6 dsm^{-1}) on pods plant^{-1}, seeds pod^{-1}, 100 seed mass (mg) and seed yield plant^{-1} (g) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern & Coss at harvest (120 DAS).

	Pods plant^{-1}			Seeds pod^{-1}			100 seed mass			Seed yield plant^{-1}		
	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean	Varuna	RH-30	Mean
Control	228	215	221.	13.00	11.81	12.40	316	301	308	7.40	6.77	7.08
Proline (20 mM)	281	254	267	13.03	11.80	12.41	316	299	307	9.58	8.40	8.99
EBL (10^{-8} M)	320	289	304	13.00	11.84	12.42	314	300	307	9.98	8.89	9.43
Proline (20 mM)+ EBL (10^{-8} M)	342	313	327	13.04	11.82	12.43	319	303	311	10.66	9.41	10.03
NaCl (2.8 dsm^{-1})	190	173	181	11.67	9.84	10.75	296	265	280	6.02	5.10	5.56
NaCl (4.2 dsm^{-1})	172	154	163	11.03	9.00	10.01	276	252	264	5.34	4.09	4.71
NaCl (5.6 dsm^{-1})	159	140	149	10.36	7.95	9.15	271	230	250	4.82	3.62	4.22
NaCl (2.8 dsm^{-1}) + Proline +EBL	257	232	244	11.82	9.92	10.87	297	269	283	8.36	7.18	7.77
NaCl (4.2 dsm^{-1})+ Proline +EBL	246	225	235	11.10	8.98	10.04	283	254	268	7.92	6.57	7.24
NaCl (5.6 dsm^{-1}) +Proline +EBL	216	195	205	10.44	8.03	9.23	267	238	252	7.25	6.02	6.63
Mean	241	219		11.84	10.09		295	271		7.73	6.60	
LSD at 5%	V = 5.73 (Sig)			V = 0.61 (Sig)			V = 8.69 (Sig)			V = 0.29 (Sig)		
	T = 9.07 (Sig)			T = 0.24 (Sig)			T = 11.34 (Sig)			T = 0.46 (Sig)		
	V x T = NS			V x T = NS			V x T = NS			V x T = 0.65 (Sig)		

V = Varieties; T = Treatments; Sig = Significant; NS = Non-significant

Discussion

DISCUSSION

In the first instance plants maintain a homeostatic balance with the environment therefore, even a slight deviation from normalcy may impose plants under stress. Thus under a narrow range of environmental fluctuations some plants sustain growth and reproduce successfully whereas others may show growth flexibility under a wide range of fluctuations, like variations in light, temperature, water and/or nutrients. The crop species either show a diminished capacity to adapt to sub-optimal conditions or may perform better because of slight molecular changes incorporated due to long term selection and cultivation. There are varied external factors that regulate the repression and/or de-repression of genes out of which light (Thompson and White, 1991), pollutants/elicitors/phytotoxins (Royals *et al.*, 1992), phytohormones (Cleland, 1999) and metabolites such as proline (Ashraf and Foolad, 2007) are noteworthy. Out of these major factors, salinity, brassinosteroids (BRs) and proline were selected for the present study with an aim to elucidate whether exogenous brassinosteroids and/or proline application could confer tolerance against salt stress? And if so which BR analogue and proline concentration would be most effective in overcoming the stress.

The enzyme carbonic anhydrase (CA) catalyzes the reversible inter-conversion of CO_2 and HCO_3, whose activity is largely determined by photon flux density, concentration of CO_2, Zn availability (Tiwari *et al.*, 2005) and/or genetic expression (Kim *et al.*, 1994). The enzyme ensures a constant supply of CO_2 to Rubisco, at the level of grana of the chloroplast (Majeau and Coleman, 1994; Price *et al.*, 1994), otherwise Rubisco activity seizes at the ambient concentration of inorganic carbon (Majeau and Coleman, 1994). In the present study, the NaCl-induced stress could have caused a decrease in the activity of CA (Tables 9, 45, 57 and 69) a reason to inactivate Rubisco (Soussi *et al.*, 1998) which could have sequentially reduced photosynthetic carbon metabolism, leaf chlorophyll content and photosynthetic efficiency (Seeman and Critihley, 1985). Moreover, the negative impact of the salinity on gene expression of CA (Liu *et al.*, 2012) could be another reason for the observed decrease in its activity. Similarly Ali *et al.* (2007a), Hayat *et al.* (2010c, 2011a), Idrees *et al.* (2012), Liu *et al.* (2012) have reported the loss in CA activity, under stress. Proline is a well-known enzyme protectant (Krall *et al.*, 1989) which is due to the fact that 3-D structure of proteins (enzymes) is governed by hydrophobic/hydrophilic interactions between side chains of the constituent amino

acids. Proline interferes with these side chains and thus plays a protective role (Paleg *et al.*, 1981) thereby increases the activity of enzymes. A similar type of interaction might be occurring between CA and proline to enhance the activity of the enzyme. Moreover, foliar application of BRs (HBL or EBL) increases the activity of CA by elevating the rate of CO_2 assimilation (Yu *et al.*, 2004) may be because of the enhanced expression of genes that encode other enzymes of the calvin cycle which also play an important role in the regeneration of RUBP, thereby maximizing the carboxylation rate of Rubisco (Xia *et al.*, 2009). A similar concept has also been floated by Hayat *et al.* (2010b) in tomato and Swamy and Rao (2009) in *Pelargonium graveoleus*. Therefore, application of proline and EBL to NaCl-stressed or stress-free plants might have imparted additive effects on CA (Table 69).

The nitrate assimilation that represents a very small pool of total leaf protein (Larcher, 1995) involves the enzyme, nitrate reductase (NR) whose activity declines significantly with increasing level of NaCl in the soil (Tables 8, 44, 56 and 68). The reason could be the stress-induced enzyme inhibition and/or its metabolic dysfunction (Hopkins, 1995). The enzyme catalyzes the conversion of nitrate to nitrite which is a rate limiting step in the process of nitrate reduction (Salisbury and Ross, 1992). Soil-salinity is known to retard the nitrate uptake by the plants (Aslam *et al.*, 1984) which is substrate cum the inducer of NR (Solomonson and Barber, 1990), therefore causes a decline in the level of NR. However, proline application improved the NR activity both in stressed and stress free plants possibly because of proline induced increase in the total phenolic contents (Kwok and Shetty, 1998) which in turn prevent auxin degradation (Schneider and Whitman, 1974). Higher auxin levels could have increased the NR activity as proposed by Ahmad and Hayat (1999) and Hayat *et al.* (2009). Moreover, BRs alone or as a follow-up treatment to NaCl-stressed plants, improved their NR activity that could be an expression of BRs impact on translation and/or transcription machinery (Khripach *et al.*, 2003). The additional possible reason could be the involvement of BRs in increasing the substrate (NO_3^-) level by acting at the level of cell membrane (Mai *et al.*, 1989) as BRI 1 peptide has basic residues at P-3, P-4, P-6 and a hydrophobic residue at P-5, related to phosphorylated Ser which is similar to regulatory phosphorylation sites of sucrose-phosphate synthase (SPS), NR and HMGCoA reductase (HMR) in their sequence of amino acids (Man-Ho *et al.*, 2000). A decrease in NR activity under salt stress and also the anti-stress effects of

BRs is in conformity with other studies (Anuradha and Rao, 2003; Shahid *et al.*, 2011; Hayat *et al.*, 2010e). Moreover, both proline (Schneider and Whitman, 1974) and BRs (Nemhauser *et al.*, 2004) prevent auxin degradation under stress therefore, its elevated level increases the activity of NR (Ahmad and Hayat, 1999; Hayat *et al.*, 2009). Therefore, it looked quite obvious that a cumulative effect of proline and brassinosteroids, in our studies (Table 68) could have enhanced the activity of NR.

The plants, under salt-stress loose a significant level of leaf chlorophyll (SPAD value) (Zhao *et al.*, 2007; Hayat *et al.*, 2010e; Hayat *et al.*, 2011a; Ahmad *et al.*, 2012; Akbari ghogdi *et al.*, 2012; Heidari, 2012 and Tables 4, 40, 52 and 64), in a concentration and variety dependent manner, possibly salinity either inhibits its synthesis or accelerates the degradation of chlorophyll molecules (Iyengar and Reddy, 1996). However, this harmful effect of NaCl was overcome in the plants sprayed with BRs (HBL or EBL) and/or proline (Tables 40, 52 and 64). Being membrane bound, the stability of chlorophyll molecules highly depends on the membrane integrity which has been possibly maintained, in our study by proline application as it acts as a membrane stabilizer (Ashraf and Foolad, 2007). These studies are in conformity with other crops (Wani *et al.*, 2012; Ahmed *et al.*, 2010; Ahmed *et al.*, 2011b; Aggarwal *et al.*, 2011). Moreover, BRs also elevate the level of chlorophyll in various crops (Bhatia and Kaur, 1997; Hayat *et al.*, 2000, 2001, 2011b; Yu *er al.*, 2004, Ali *et al.*, 2006, 2007a; Yusuf *et al.*, 2011) because of their involvement in improving the related transcription and/or translation machinery (Bajguz, 2000 and Bajguz and Asami, 2005). Brassinosteroids also retard the rate of degradation of chlorophyll molecules and that of the proteins associated with them, in particular the proteins of light-harvesting complexes located in thylakoid membranes (Hola, 2011).

The NaCl-stress causes closure of stomata due to salt-induced ABA accumulation (Yang and Lu, 2005), thereby decreases partial pressure of CO_2 in the stroma (Iyengar and Reddy, 1996) that becomes the main reason for the observed loss of stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) in the present study (Tables 6, 7, 42, 43, 54, 55, 66 and 67). Cumulative response of all these ill effects could have led to the observed decrease in net photosynthetic rate (P_N) (Tables 6, 42, 54 and 66). The net photosynthetic rate has already positively correlated with g_s and C_i (Lu *et al.*, 2009). Moreover, stress-induced activation of the process of senescence and a shift in the activities of related enzymes as a result of the

changes in cytoplasmic structure and negative feedback of reduced sink activity (Iyengar and Reddy, 1996) and slow pace of transport of photosynthates, under potassium deficiency (Cakmark, 2005) cause a significant loss in the rate of photosynthesis. The decrease in SPAD chlorophyll values (Tables 4, 40, 52 and 64) and CA activity (Tables 9, 45, 57 and 69) are the other reasons to justify the lowering of P_N in NaCl-stressed plants. A positive correlation between P_N and chlorophyll content (SPAD value) (Figs. 1, 2 and 3) as well as between P_N and CA (Figs. 4, 5 and 6) further corroborate the present observations. The support is also gained from others (Noreen *et al.*, 2010; Akram and Ashraf, 2011; Saleem *et al.*, 2011; Wu *et al.*, 2012; Wang *et al.*, 2012; Eisa *et al.*, 2012; Ahmad *et al.*, 2012). The recovery in P_N and the related attributes (g_s, C_i and E) in the salt-stressed plants could be attained by exposing them to BRs and/or proline as a follow-up treatment (Tables 18, 19, 30, 31, 42, 43, 54, 55, 66 and 67). Since photosynthesis is mainly dependent on the stomatal movement and the metabolism of mesophyll cells (proteins associated with PSI, PSII and chlorophyll) (Lawlor and Cornic, 2002; Athar and Ashraf, 2005), therefore it can be inferred from the present study that the exogenous application of proline to stressed plants causes an increase in stomatal conductance by maintaining appropriate cellular turgor (Kamran *et al.*, 2009) thereby facilitating sub-stomatal accumulation and assimilation of CO_2 at a higher pace. The observations suggest that the photosynthetic enhancement primarily corresponds to the increased stomatal conductance with higher CO_2 diffusion rate within the leaves to activate P_N. Ahmed *et al.* (2010) in young *Olea europaea* plants proposed similar inferences. Moreover, higher chlorophyll contents (Tables 28, 52 and 64) and CA activity (Tables 33, 57 and 69) under exogenous proline application would also expectedly result in higher P_N. The two important enzymes that initiate the process of photosynthesis i.e., CA and Rubisco are activated by BRs (Yu *et al.*, 2004; Anuradha and Rao, 2009; Hayat *et al.*, 2011b, 2012b; Yusuf *et al.*, 2011). The higher CA activity increases the carboxylation state of Rubisco (Bajguz and Asami, 2005), thereby improves P_N. These observations are further corroborated by the observed positive correlation between CA and P_N (Figs. 4, 5 and 6). It may be derived from the present observations that BRs improved the CO_2 concentration (Tables 19, 43 and 67) by increasing g_s (Tables 18, 42 and 66) and also the efficiency of light harvesting system by elevating the level of chlorophyll (Tables 16, 40 and 64). These in a cumulative action speeded up the net
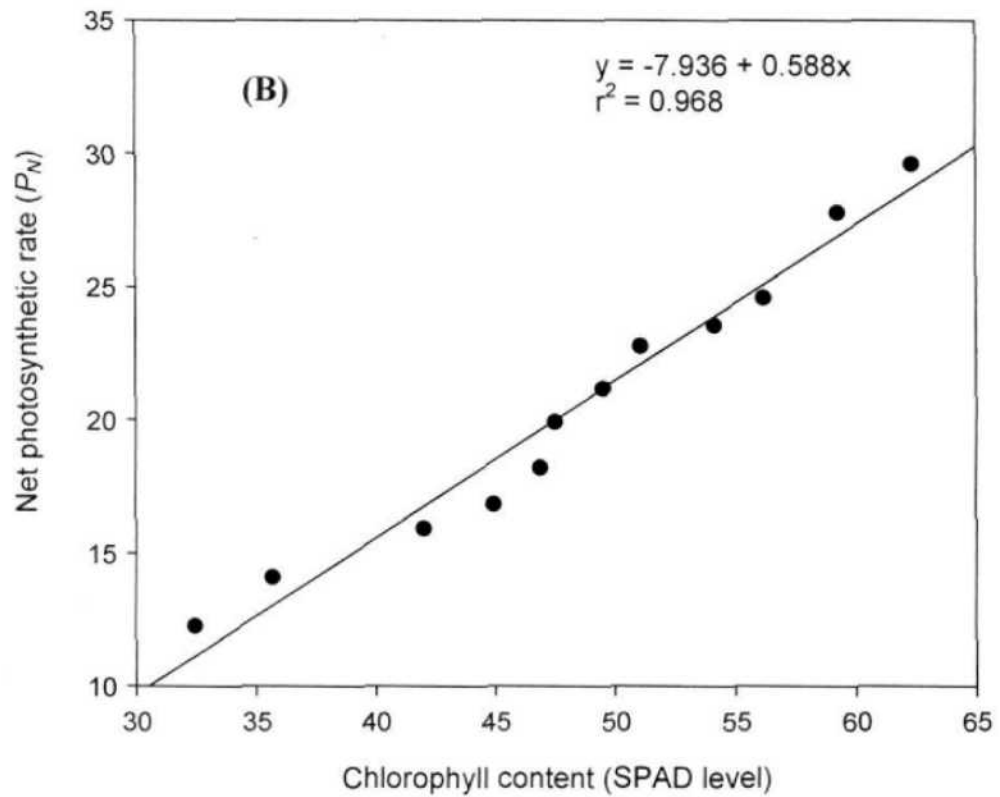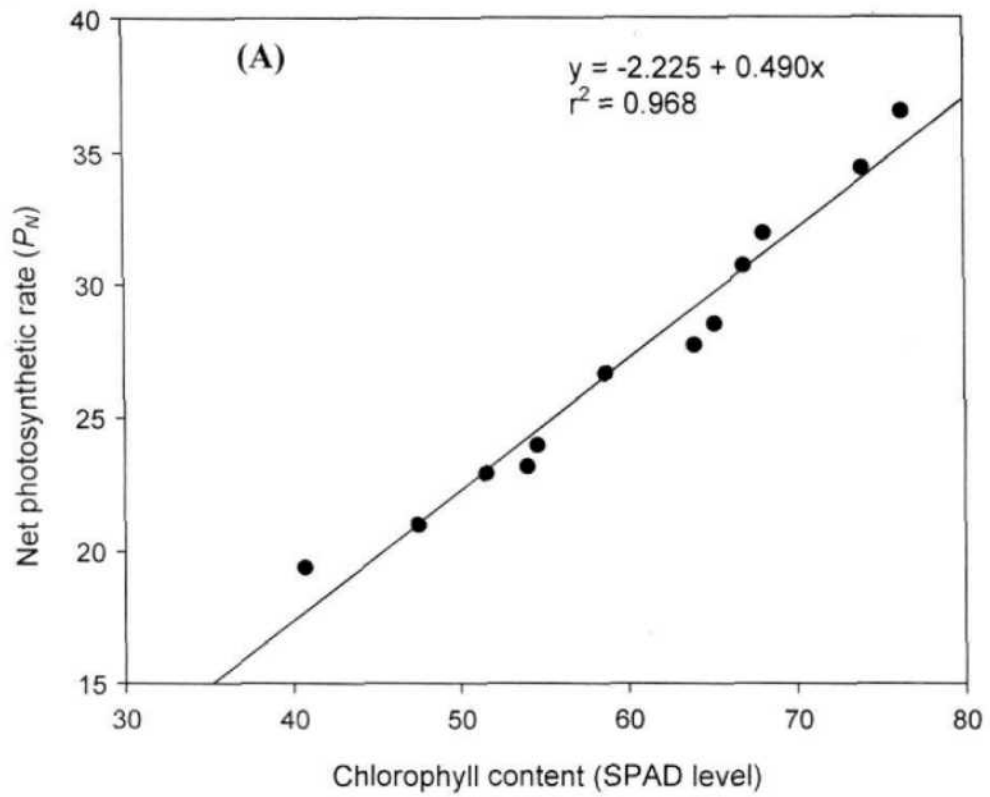
Figure 1 Correlation coefficient values between net photosynthetic rate and chlorophyll content (SPAD level) in (A) Varuna and (B) RH-30 (Experiment 4).
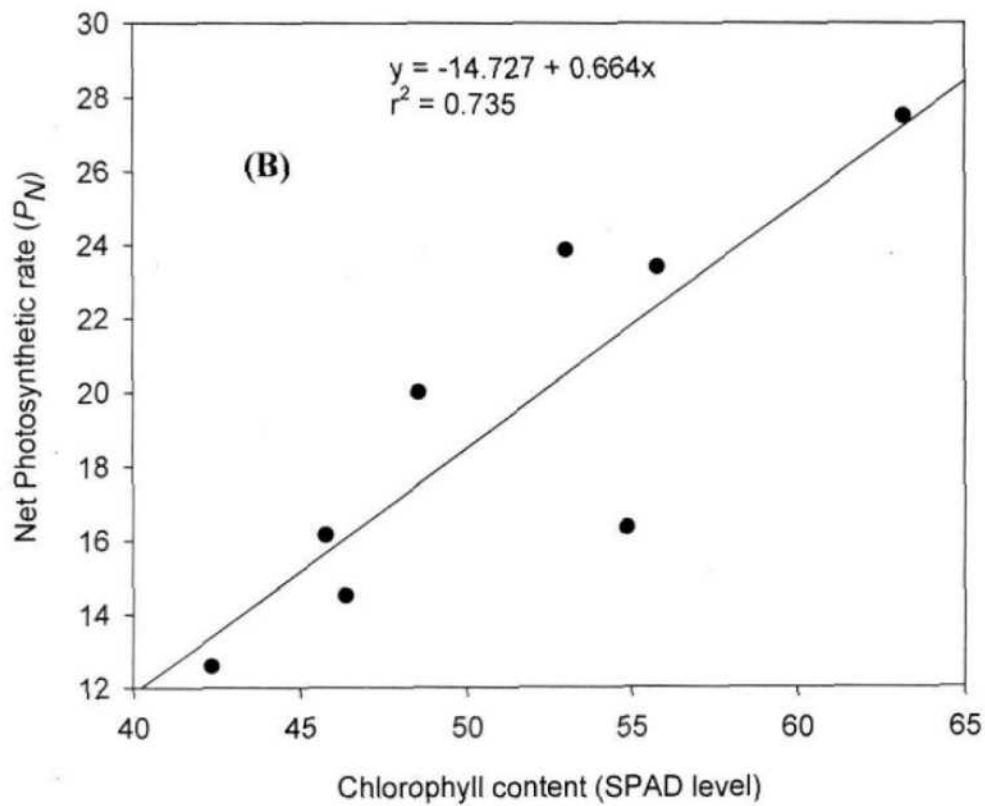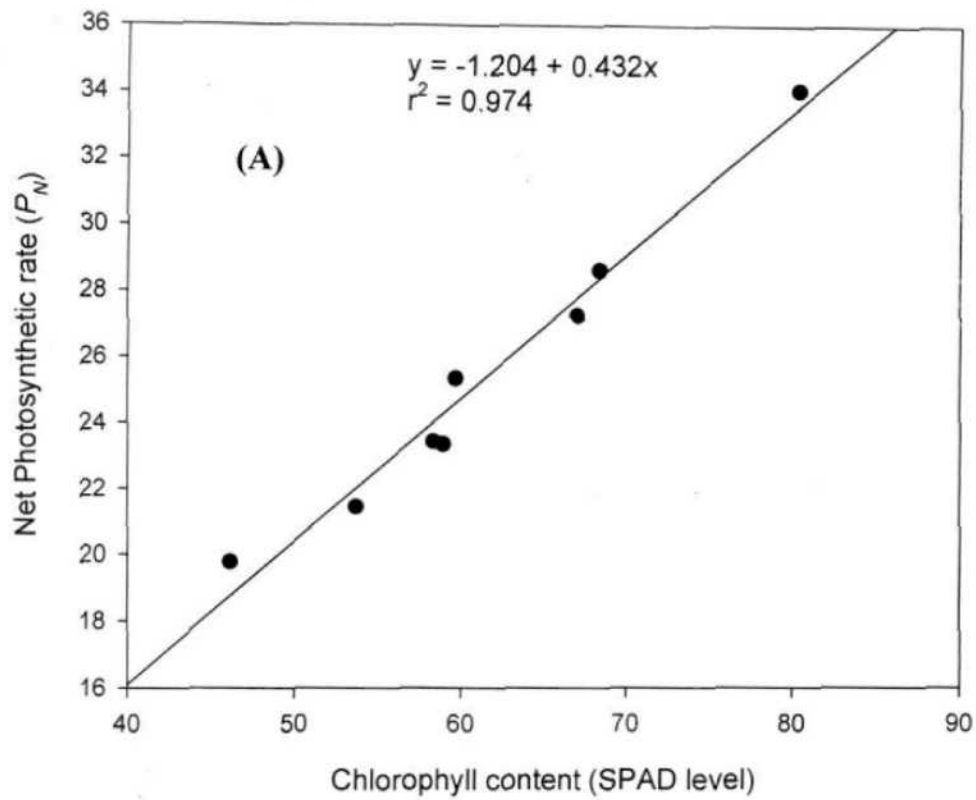
Figure 2 Correlation coefficient values between net photosynthetic rate and chlorophyll content (SPAD level) in (A) Varuna and (B) RH-30 (Experiment 5).
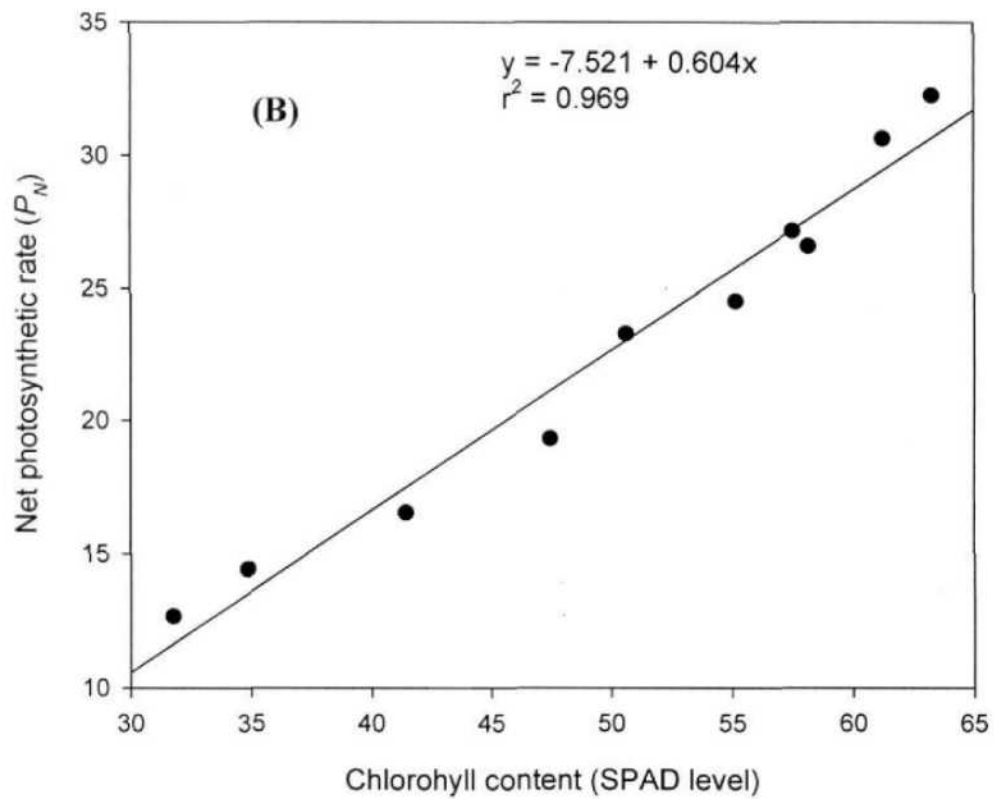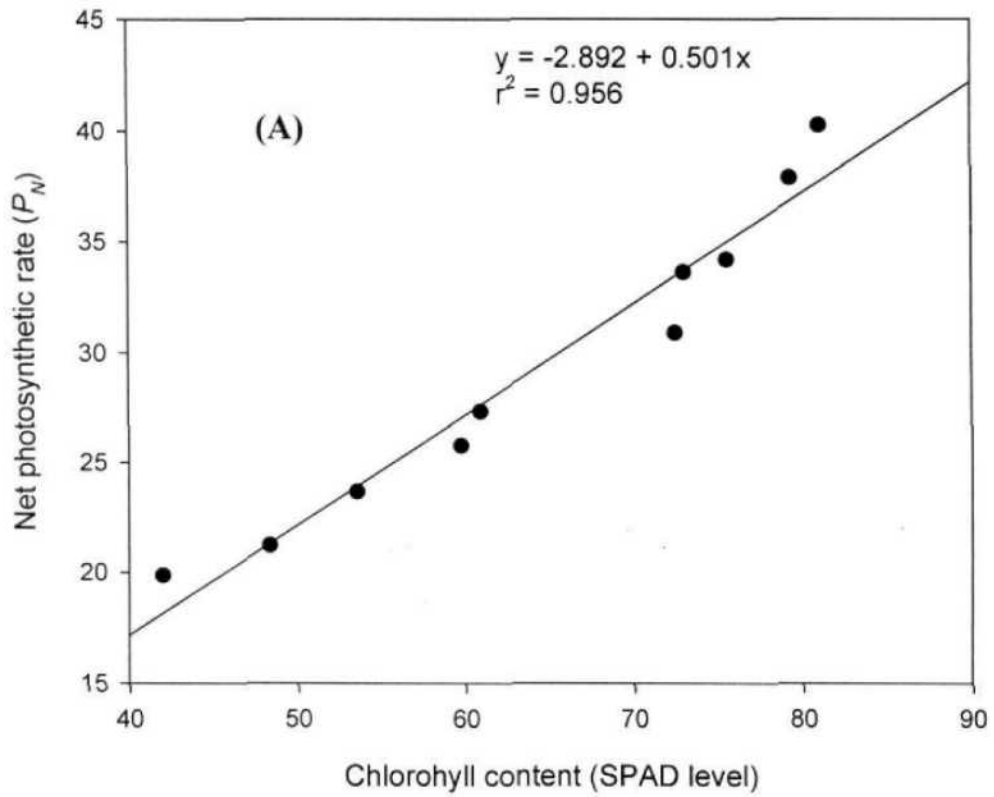
Figure 3 Correlation coefficient values between net photosynthetic rate and chlorophyll content (SPAD level) in (A) Varuna and (B) RH-30 (Experiment 6).
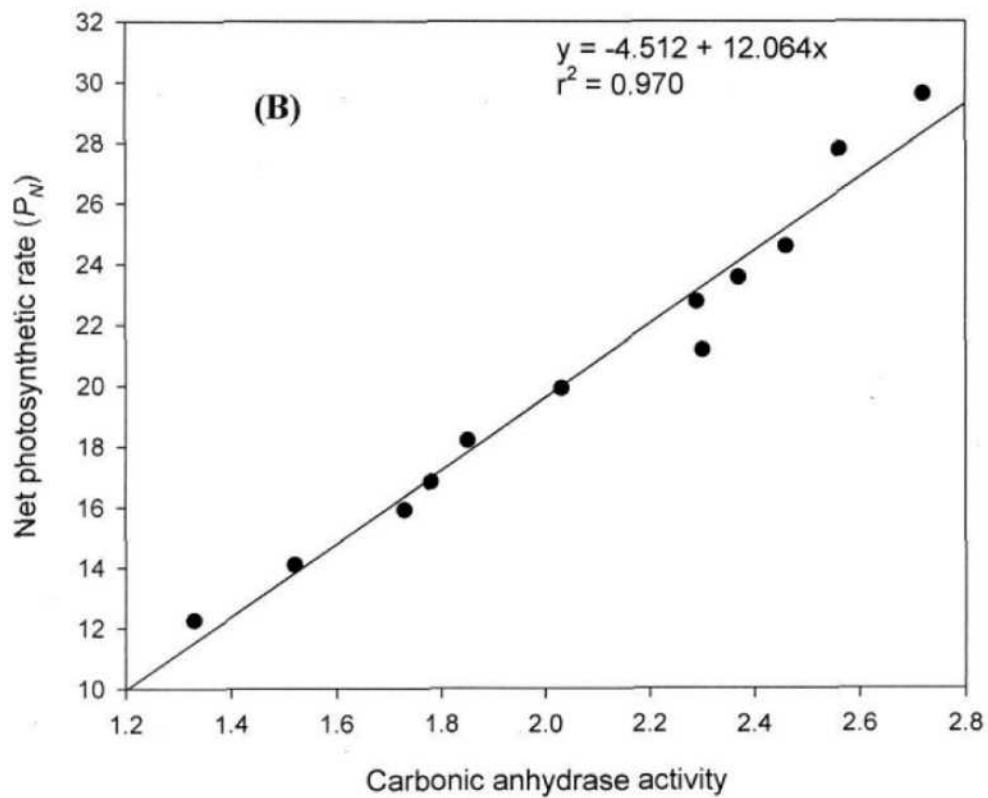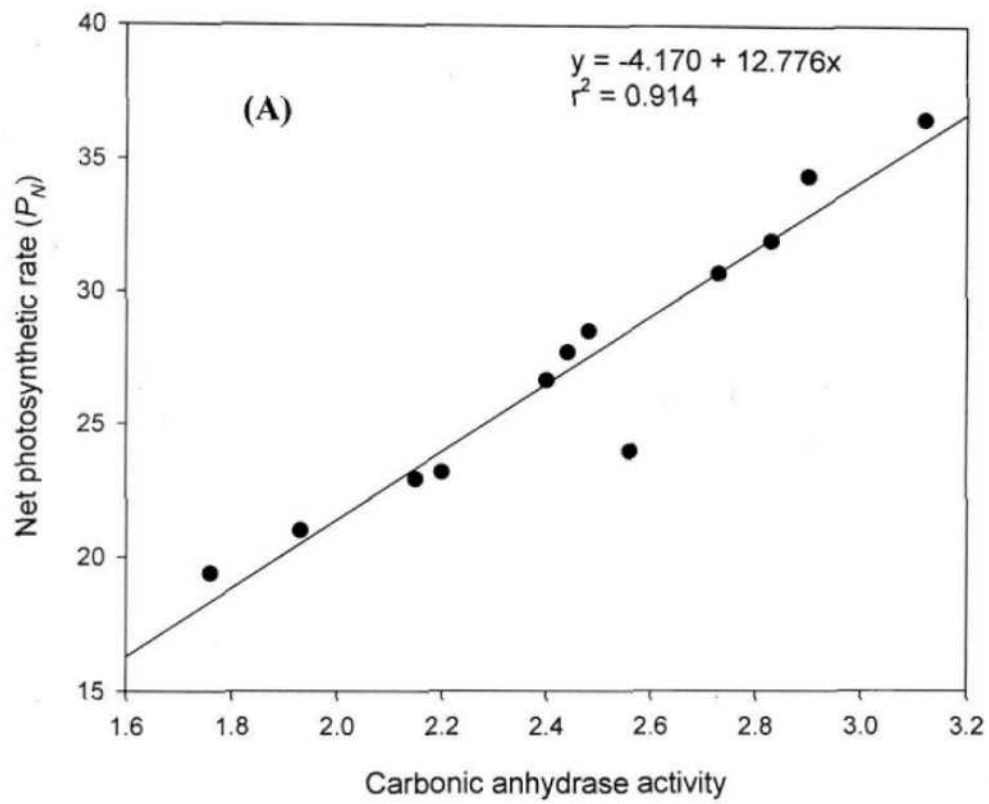
Figure 4 Correlation coefficient values between net photosynthetic rate and carbonic anhydrase activity in (A) Varuna and (B) RH-30 (Experiment 4).

$$y = -3.813 + 12.760x$$
$$r^2 = 0.973$$

$$y = -4.815 + 12.740x$$
$$r^2 = 0.967$$
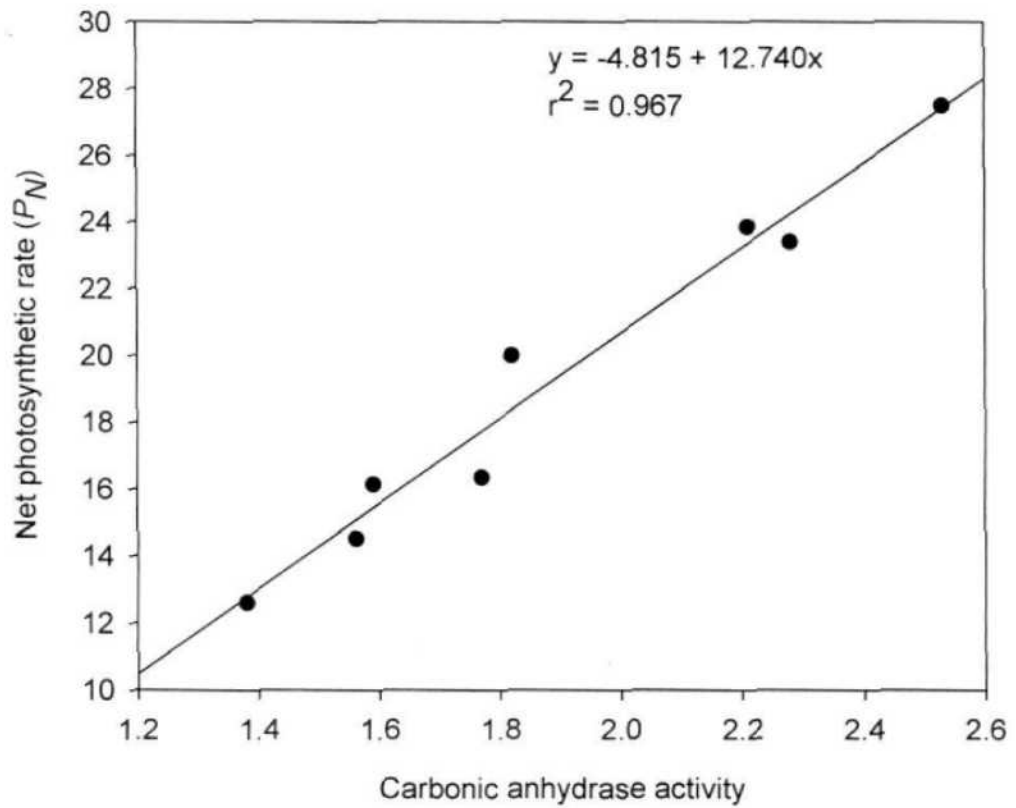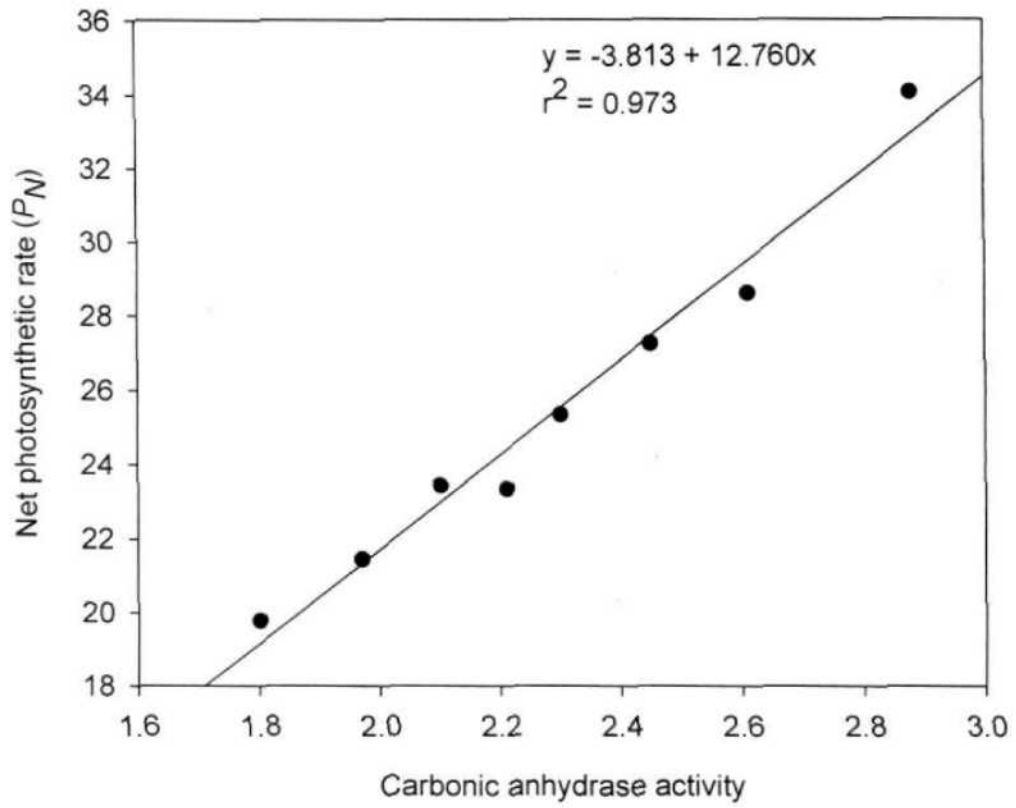
Figure 5 Correlation coefficient values between net photosynthetic rate and carbonic anhydrase activity in (A) Varuna and (B) RH-30 (Experiment 5).
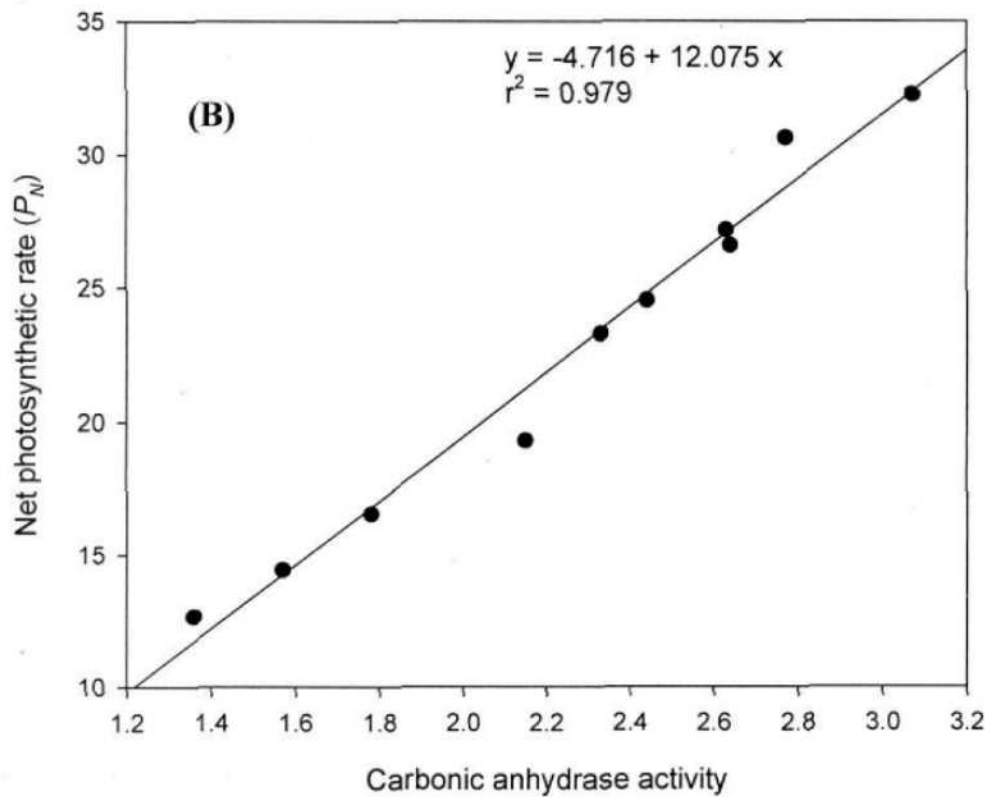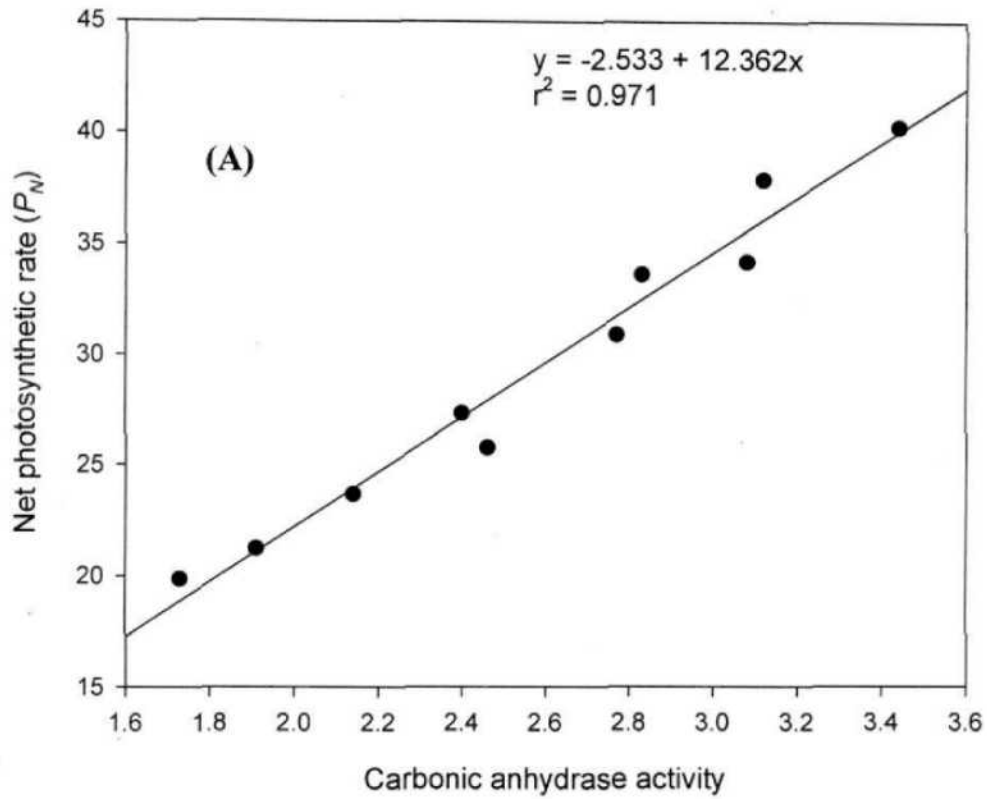
Figure 6 Correlation coefficient values between net photosynthetic rate and carbonic anhydrase activity in (A) Varuna and (B) RH-30 (Experiment 6).

photosynthetic rate of the plants (Hola, 2011 and Tables 18, 42 and 66). Similar reasons have also been given by others to explain the increase in P_N values in the crops treated with BRs under various abiotic stresses (Alam *et al.*, 2007; Ali *et al.*, 2008a,b; Hasan *et al.*, 2008; Hayat *et al.*, 2010a,b, 2012b; Fariduddin *et al.*, 2009a, 2011, Yusuf *et al.*, 2011). Besides this, BRs and/or proline also improved the cell water relations i.e. leaf water potential (Tables 17, 29, 41, 53, 65) and membrane structure and its stability (Wang and Zeng, 1993; Slathia *et al.*, 2012; Yan *et al.*, 2011) so as to decrease the electrolyte leakage (Tables 17, 29, 41, 53, 65) which could have been helpful in maintaining normal cellular metabolism.

The NaCl decreased the photochemical efficiency which has been ascribed with the suppression of PSII activity (Mehta *et al.*, 2010 and Tables 8, 44, 56 and 68). This suggests that the salt stress caused damage to PSII electron transport (Megdiche *et al.*, 2008) where it blocks the electron transfer from the primary acceptor plastoquinone (QA) to the secondary acceptor plastoquinone (QB) at the acceptor side of PSII which lead to the decrease in Fv/Fm values (Mehta *et al.*, 2010; Shu *et al.*, 2012). Similar observations have been reported in *Triticum aestivum* (Shahbaz *et al.*, 2008; Kanwal *et al.*, 2011), *Vigna radiata* (Hayat *et al.*, 2010e), *Brassica napus* (Naeem *et al.*, 2010), *Solanum melongena* (Wu *et al.*, 2012), *Cucumis sativus* (Shu *et al.*, 2012), *Brassica juncea* (Ahmad *et al.*, 2012) under salt stress. However, the spray of BRs and/or proline to the stressed/stress-free plants improved the values of Fv/Fm (Tables 20, 32, 44, 56 and 68). Similarly observations have also been reported in *Triticum aestivum* (Shahbaz *et al.*, 2008), *Brassica juncea* (Hayat *et al.*, 2012a) and *Solanum melongena* (Wu *et al.*, 2012). Brassinosteroids protect PSII against over-excitation, under salt stress that otherwise could have caused the loss of integrity of thylakoid membranes (Ogweno *et al.*, 2008). Moreover, PSII machinery gets similar type of protection from applied proline (Tables 56 and 68) which may be supported by Oukarroum *et al.* (2012), Moustakas *et al.* (2011) and Yan *et al.* (2011) who cultured the plants under various types of stresses.

Plants possess complex antioxidative defense system comprising of non-enzymatic (such as proline) and enzymatic components (such as CAT, POX, SOD) to scavenge reactive oxygen species (ROS), produced during their exposure to stress. Various cell organelles (chloroplasts, mitochondria, and peroxisomes) are the seat for the synthesis and scavenging of ROS, the pathways are recognized and well-

coordinated (Pang and Wang, 2008). Under normal conditions, ROS are generated at a very slow pace and an appropriate balance is maintained between their productions and quenching. However, various environmental stresses disturb this balance as they give rise to rapid increases in the intra and inter-cellular ROS levels (Noctor *et al.*, 2002; Sharma *et al.*, 2010) which may induce oxidative damage to lipids, proteins and nucleic acids (Sharma *et al.*, 2012). In order to avoid this oxidative damage, plants raise the level of endogenous enzymatic and non-enzymatic scavenging components (Sharma *et al.*, 2010 and Tables 9-11, 45-47, 57-59 and 69-71). Different other crop plants such as *Helianthus annuus* (Noreen *et al.*, 2009), *Panicum miliaceum* (Sabir *et al.*, 2011), *Triticum aestivum* (Ashraf *et al.*, 2010a), and *Carthamus tinctorius* (Siddiqi, 2010), *Solanum lycopersicum* (Hayat *et al.*, 2010c), *Brassica juncea* (Hayat *et al.*, 2011a; Ahmad *et al.*, 2012) are also reported to behave similarly. Salt-induced increase in endogenous proline content (Tables 11, 47, 59 and 71) could have been due to the increased rate of hydrolysis of proteins (Irigoyen *et al.*, 1992) as protein synthetic machinery is diverted towards the proline accumulation (Claussen, 2005). Secondly, an enhanced level of proline could be due to its slower rate of degradation (Kiyosue *et al.*, 1996). Similar observations have also been reported earlier in *Brassica juncea* (Ahmad *et al.*, 2012; Hayat *et al.*, 2007b, 2011a; Yusuf *et al.*, 2008), *Vigna radiata* (Hayat *et al.*, 2010e), *Solanum lycopersicum* (Hayat *et al.*, 2010c) and *Beta vulgaris* (Farkhondeh *et al.*, 2012) grown under salt stress. Out of the two cultivars tested, Varuna possessed higher proline content and the activity of CAT, POX and SOD enzymes than RH-30. Such types of responses differing in salt tolerance have been reported earlier in which salt tolerant varieties possessed better antioxidative defense system (both enzymatic and non-enzymatic components) than the salt sensitive varieties (Sabir *et al.*, 2011; Hayat *et al.*, 2011a). In the present study, we noted that the treatment of stress-free and stressed plants with BRs and/or proline improved their antioxidant enzymes activity and the proline content (Tables 21-23, 33-35, 45-47, 57-59 and 69-71). Being a membrane stabilizer, proline application results in its rapid uptake coupled with its *de novo* synthesis (Zhu *et al.*, 1990; Santos *et al.*, 1996), thereby increasing the endogenous level of proline (Tables 35, 59 and 71). Proline action is carried over through its involvement at transcription and/or translation level (Cuin and Shabala, 2007; Ashraf and Foolad, 2007). Furthermore, higher endogenous proline content improves water uptake (Jain *et al.*,

2001) thereby maintains leaf water potential (Tables 29, 53 and 65). However, application of higher proline concentrations impose a check on its biosynthesis through feedback inhibition thereby decreasing the endogenous proline content (Zhang *et al.,* 1995; Garcia-Rios *et al.,* 1997 and Table 35).

BRs regulate the antioxidant enzymes activity in the tissues where free radical accumulation occurs (Ashraf *et al.,* 2010b). This peculiarity of BRs of managing cells in dual state i.e. to provide defense and to promote growth, places them in the list of novel regulators in plant growth (Sun *et al.,* 2010). BRs confer the tolerance through the increased activity of NADPH oxidase enzyme and elevate the level of H_2O_2 in the apoplast (Xia *et al.,* 2009). Brassinosteroid perception by receptors activates the plasma membrane-bound NADPH oxidase (RBOH) which results in the elevation of the level of H_2O_2 to initiate protein phosphorylation cascade (Xia *et al.,* 2009). The H_2O_2 mediates the transcriptional induction of defense or antioxidant genes. Transcription factors may be activated via a phosphorylation cascade by MAPKs (Mitogen-activated protein kinases). Finally, the products of target genes participate directly in cellular protection against the stress (Xia *et al.,* 2009). In addition to this, on the basis of molecular, physiological and genetic studies it is reported that the enhanced expression of *det-2* genes results in an increase in the activity of antioxidant enzymes that provides resistance to oxidative stress in *Arabidopsis* (Cao *et al.,* 2005). Moreover, BRs induce the expression of proline biosynthetic genes (Ozdemir *et al.,* 2004) which results in the accumulation of proline in the stressed plants (Tables 47 and 71). Similarly proline accumulation has been reported in *Sorghum bicolor,* under osmotic stress (Vardhini and Rao, 2003), *Cicer arietinum,* under cadmium stress (Hasan *et al.,* 2008), *Brassica juncea,* under NaCl stress (Hayat *et al.,* 2012a), Zn stress (Arora *et al.,* 2010) and copper stress (Fariduddin *et al.,* 2009a), *Raphanus sativus,* under copper stress (Choudhary *et al.,* 2012), *Arachis hypogaea,* under *in vitro* conditions (Verma *et al.,* 2012), *Pisum sativum,* under salt stress (Shahid *et al.,* 2011). Proline is designated as a natural cytosolic osmoticum and scavenges free radicals, interacts with macromolecules of the cells such as enzymes, DNA and membranes to stabilize their structure and function (Anjum *et al.,* 2000; Kavi Kishor *et al.,* 2005). Among various compatible solutes, only proline has the property to protect plants from singlet oxygen and free radical damages, that results from the stress (Alia *et al.,* 1997). Therefore, a combination of BRs and proline elevated the

plant proline content and the level of antioxidant enzymes (CAT, POX, SOD) possibly through its action at transcription and/or translation, as discussed (pp. 87-88) earlier, thereby prevented the mustard plants against the damage caused by the salinity stress.

In the present study mustard plants, exposed to three levels of soil amended NaCl, had reduction in growth traits, reflected in the form of loss in length, fresh and dry mass of shoot and root and leaf area (Tables 1-4, 37-40, 49-52 and 61-64). The salt stress is known to cause reduction in cell division and elongation (Yasseen *et al.*, 1987; Pitann *et al.*, 2009) which is mainly due to salt induced alterations in the nutrient uptake, reactive oxygen species accumulation (Ashraf, 2009), inhibition of the activity of cytoplasmic enzymes, turgor loss (Pitann *et al.*, 2009) and hormonal imbalance (Ashraf *et al.*, 2010b; Iqbal and Ashraf, 2010) which in turn impair plant growth and biomass production. Moreover, the growth inhibition, under salinity stress could be partly due to the shortage of energy (observed slower rate of photosynthesis) as the processes involved in the transport of salts and repair of salt damage on membranes or proteins is energy consuming (Kleinkopf and Wallace, 1974). Similar impact of salt stress on the growth of *Beta vulgaris* (Ghoulam *et al.*, 2002), *Brassica juncea* (Hayat *et al.*, 2006, Hayat *et al.*, 2011a), *Cicer arientinum* (Ali *et al.*, 2007a), *Solanum lycopersicum* (Zribi *et al.*, 2009; Hayat *et al.*, 2010c), *Helianthus annuus* (Akram and Ashraf, 2011), *Capsicum annuum* (Chartzoulakis and Klapaki, 2000), *Populous alba* (Imada and Tamai, 2009), *Fragaria ananassa* (Keutgen and Pawelzik, 2009), *Morus alba* (Ahmad and Sharma, 2010), *Abelmoschus esculentus* (Saleem *et al.*, 2011) and *Panicum miliaceum* (Sabir *et al.*, 2011), has been reported earlier. The ill effects generated by the salt stress can, however, be overcome by the application of BRs and/or proline alone or as a follow-up treatment to salt-stressed plants. The increase in the endogenous proline content (Table 35, 59 and 71) by its application to the plant foliage protects the enzymes (Khedr *et al.*, 2003) and 3-D structure of proteins (Paleg *et al.*, 1981), cell organelles and membranes by checking lipid peroxidation (Okuma *et al.*, 2004) and facilitates the supplies of energy for plant growth and survival, thereby helps to overcome stress (Hoque *et al.*, 2007; Ashraf and Foolad, 2007). Therefore, higher proline content acts as an osmoregulator to overcome the impact of salt stress and improves plant growth (Csonka and Hanson,

1991 and Yancey, 1994 and Tables 49-52 and 61-64). Similarly, Deivani *et al.* (2011) reported higher proline content in rice plants associated with improved growth.

The foliar spray of BRs (HBL or EBL) to plants under stress or non-stress conditions enhanced all the growth traits in both the mustard varieties (Tables 13-16, 37-40 and 61-64). BRs are involved to modulate a number of metabolic phenomena leading to the plant tolerance against the stress (Ashraf *et al.*, 2010b). The amelioration of salt stress by BR application is well documented in plants of *Oryza sativa* (Anuradha and Rao, 2003; Ozdemir *et al.*, 2004), *Phaseolus vulgaris* and *Hordeum vulgare* (Akram and Abdel-Fattah, 2006), *Triticum aestivum* (Qayyum *et al.*, 2007; Eleiwa *et al.*, 2011), *Cicer arietinum* (Ali *et al.*, 2007a), *Capsicum annuum* (Houimili *et al.*, 2008), *Cucumis sativus* (Xia *et al.*, 2009), *Pisum sativum* (Shahid *et al.*, 2011), *Fragaria ananassa* (Karlidag *et al.*, 2011), *Cajanus cajan* (Dalio *et al.*, 2011), *Phaseolus vulgaris* (Rady, 2011), *Brassica juncea* (Hayat *et al.*, 2012a), *Solanum melongena* (Wu *et al.*, 2012), *Vigna sinensis* (El-Mashad and Mohamed, 2012). BRs have a positive impact on cell division and cell elongation (Catterou *et al.*, 2001), regulation of genes encoding XTHs (xyloglucan endo-transglyucosylase/ hydrolase) i.e., the enzymes responsible for the modification of cell wall activity and cell enlargement, cellulose synthase and sucrose synthase (Ashraf *et al.*, 2010b) that play a vital role in growth and development of plants. Besides this, BRs also act along with auxins to stimulate cell elongation (Katsumi, 1991). Exogenous application of BRs accelerates plant growth and development, however, the extent of their effects may vary with plant species and the concentration applied (Ashraf *et al.*, 2010b). *Brassica* plants sprayed with BRs possessed larger leaves (Table 16, 40 and 64) which could have been an expression of activated cell division and cellular enlargement (Clouse and Sasse, 1998; Bajguz and Tretyn, 2003). Similarly BRs have improved the leaf area in *Vigna radiata*, under aluminum stress (Ali *et al.*, 2008a), *Brassica juncea*, under copper stress (Fariduddin *et al.*, 2009a), and *Triticum aestivum*, under stress-free conditions (Shahbaz *et al.*, 2008). Moreover, a combination of proline and BRs had an additive effect on growth and development (Tables 61-64). It looked quite obvious in the light of the above discussion as both of them individually generated beneficial effects.

It is quite evident (Aldesuquy and Ibrahim, 2001; Afroz *et al.*, 2005; Ali *et al.*, 2007a and Asgari *et al.*, 2012 and Tables 12, 48, 60 and 72) that the observed yield

characteristics, at harvest, decreased significantly under salt-stress in a concentration dependent manner. The cultivar, Varuna expressed slight resistance, compared with RH-30. The most prominent reason that could have contributed most to this loss is the poor vegetative plant growth (Tables 1-4, 37-40, 49-52 and 61-64). The other related phenomenon may be limited supply of photosynthates under lower pace of CO_2-reduction (Tables 6, 42, 54 and 66 and Chen *et al.*, 2009) and unfavorable nature of conducting pathway (Aldesuquy and Ibrahim, 2001) where the leaves start behaving as sinks rather than source (Arbona *et al.*, 2005). This causes inhibition of assimilate movement towards the developing reproductive organs, to make them weak and less productive (Tables 12, 48, 60 and 42). However, BRs and/or proline applied to the foliage improved the values for yield characteristics both in stressed and stress-free plants (Tables 24, 36, 48, 60, and 72). The extended life span of vegetative and reproductive organs under the impact of proline (Balestrasse *et al.*, 2004) and/or BRs (Iwahori *et al.*, 1990) could be the reason other than referred earlier where an improvement in any one of the parameters could have a favorable impact on the other. Therefore, increased seed yield, under BRs (Tables 24, 48 and 72) may be an expression of higher rate of photosynthesis (Tables 18, 42 and 66) that facilitated the availability of more carbohydrates for metabolism and export to the sink (Bajguz and Asami, 2005) for healthy growth. Similarly, higher biological yield in passion fruit, correlating with higher photosynthetic CO_2 assimilation, under BRs (Gomes *et al.*, 2003) was reported. To strengthen the above statement a positive correlation was observed between P_N and seed yield (Figs. 7, 8 and 9). BRs are also reported to favor the yield characteristics in *Vigna radiata* and *Brassica juncea* (Fariduddin, 2002) and also in *Cicer arientinum*, under saline stress (Ali *et al.*, 2007a) and cadmium stress (Hasan *et al.*, 2008) and *Lycopersicon esculentum* under cadmium stress (Hayat *et al.*, 2010a). From the above discussion it is quite evident that both proline and BRs individually have a positive (additive) effect on most of the characteristics determining growth and yield. Therefore, it may not be a surprise if the combination of these two increased the values further (Table 72) to give better yield both under stress and stress-free conditions.

In the light of the presentations, it can be inferred that the concentration (NaCl) dependent variation appeared almost in all the parameters investigated. Moreover, out of the two BR analogues (HBL or EBL), EBL proved more effective in
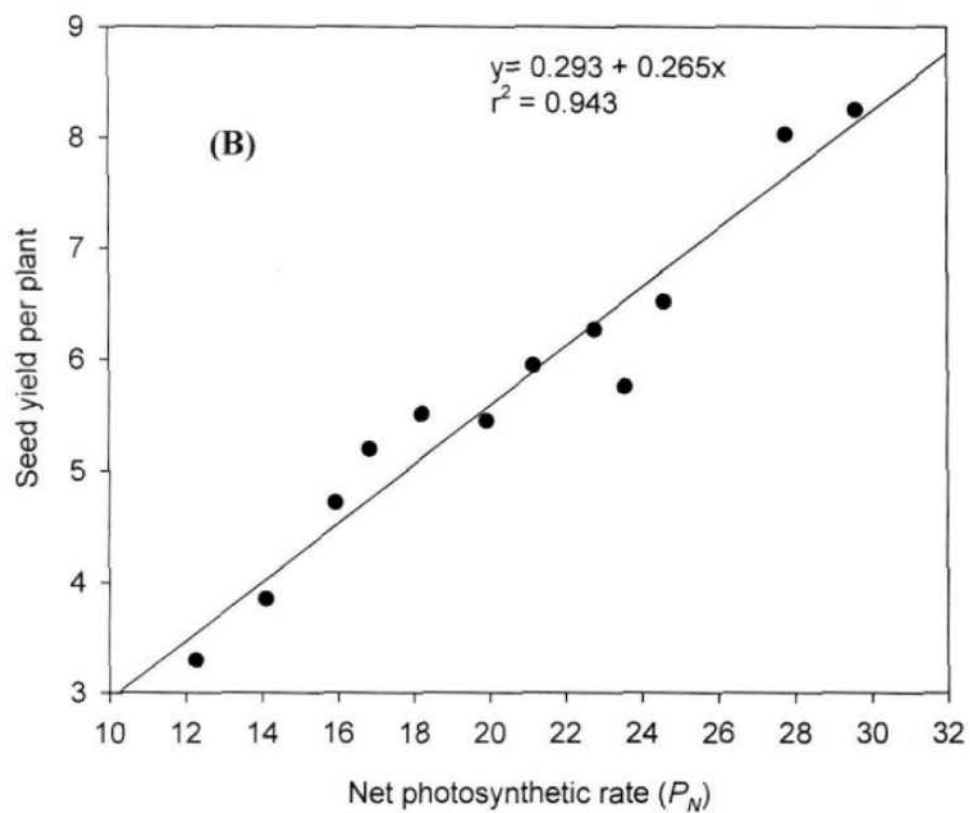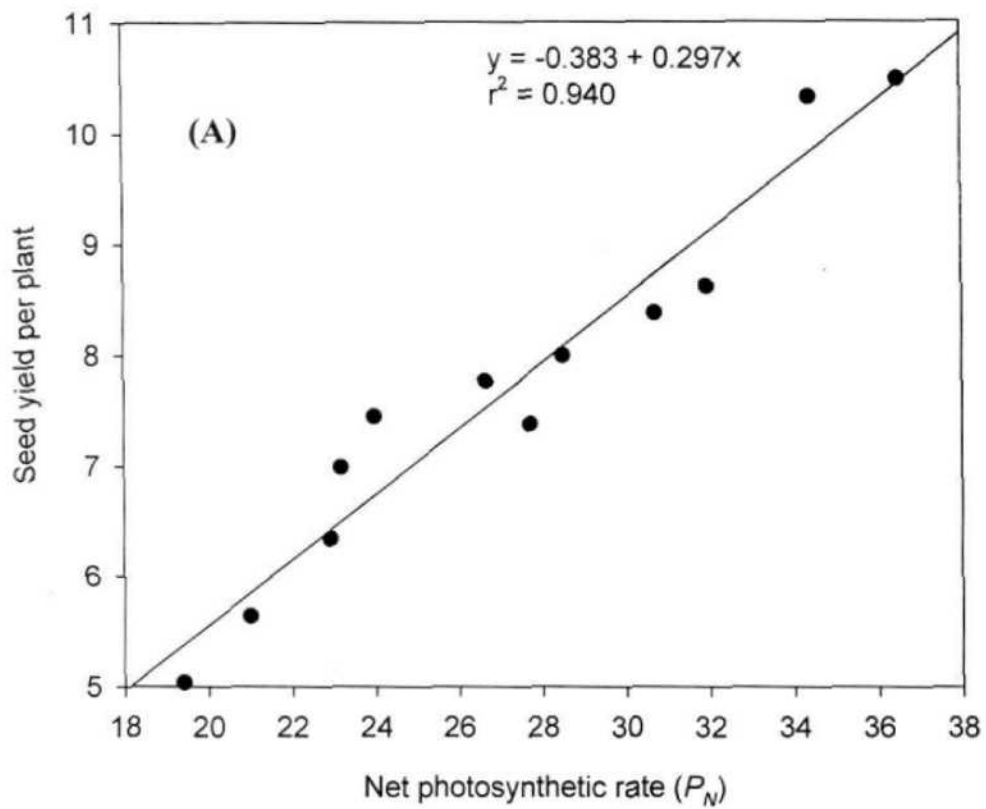
Figure 7 Correlation coefficient values between seed yield per plant and net photosynthetic rate in (A) Varuna and (B) RH-30 (Experiment 4).
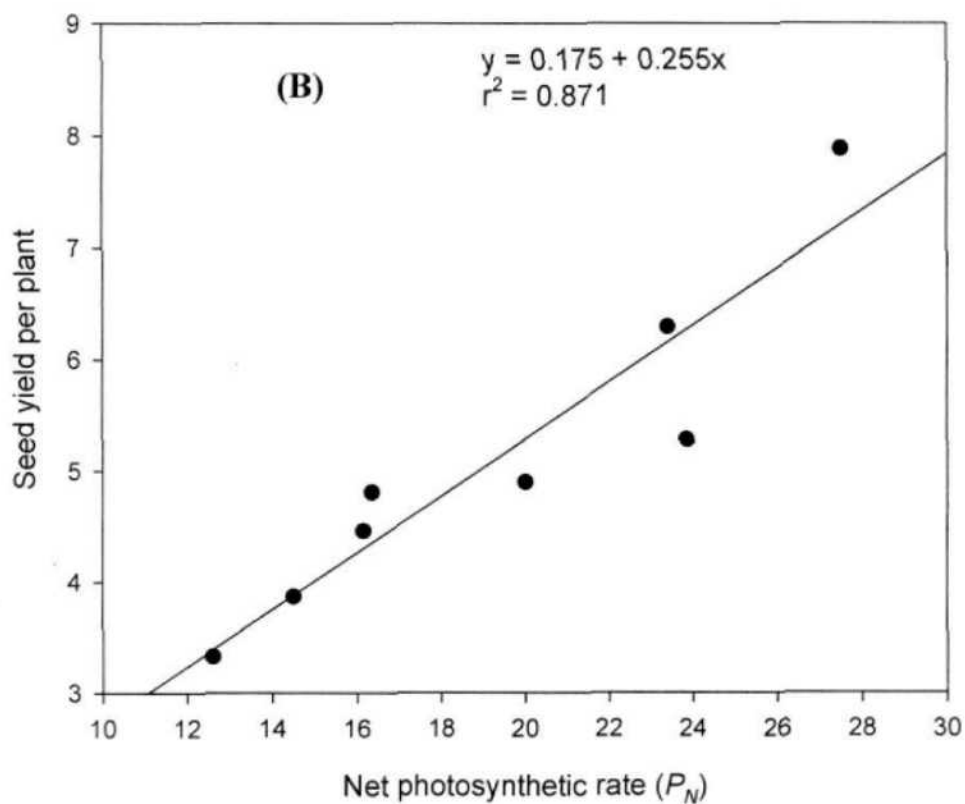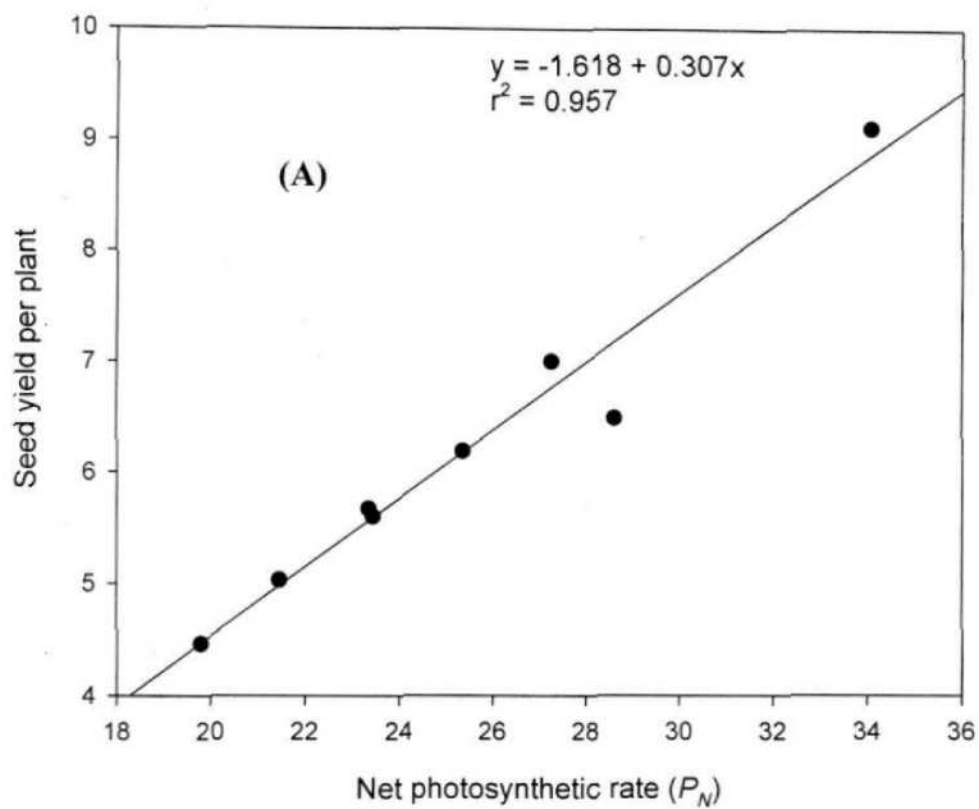
Figure 8 Correlation coefficient values between seed yield and net photosynthetic rate in (A) Varuna and (B) RH-30 (Experiment 5).
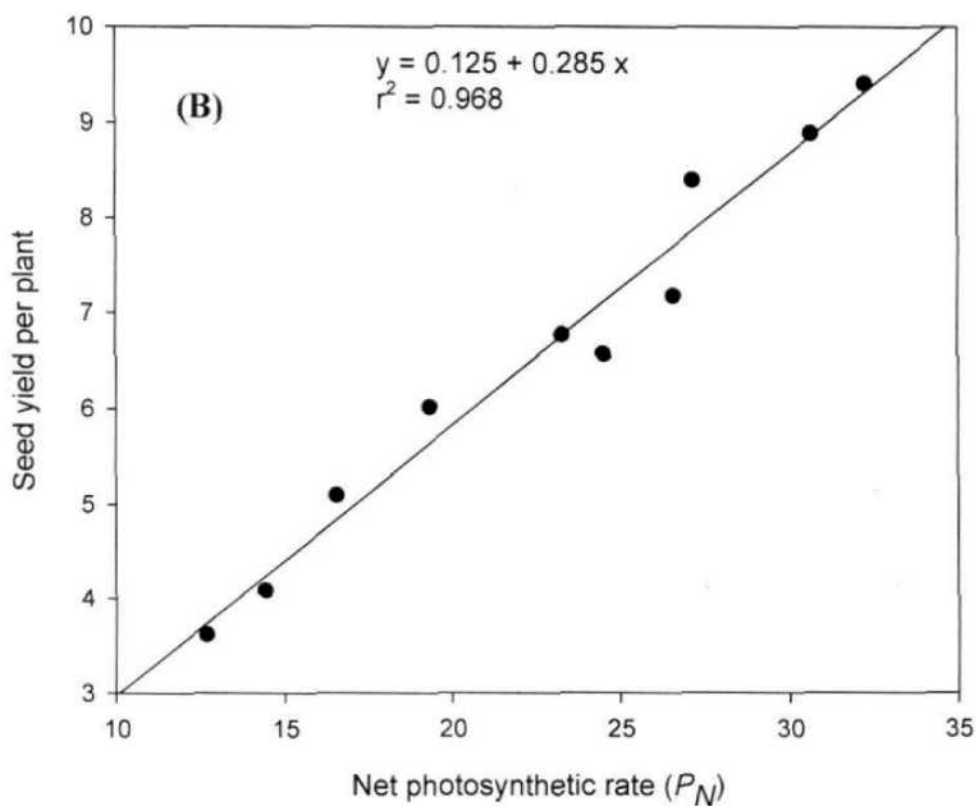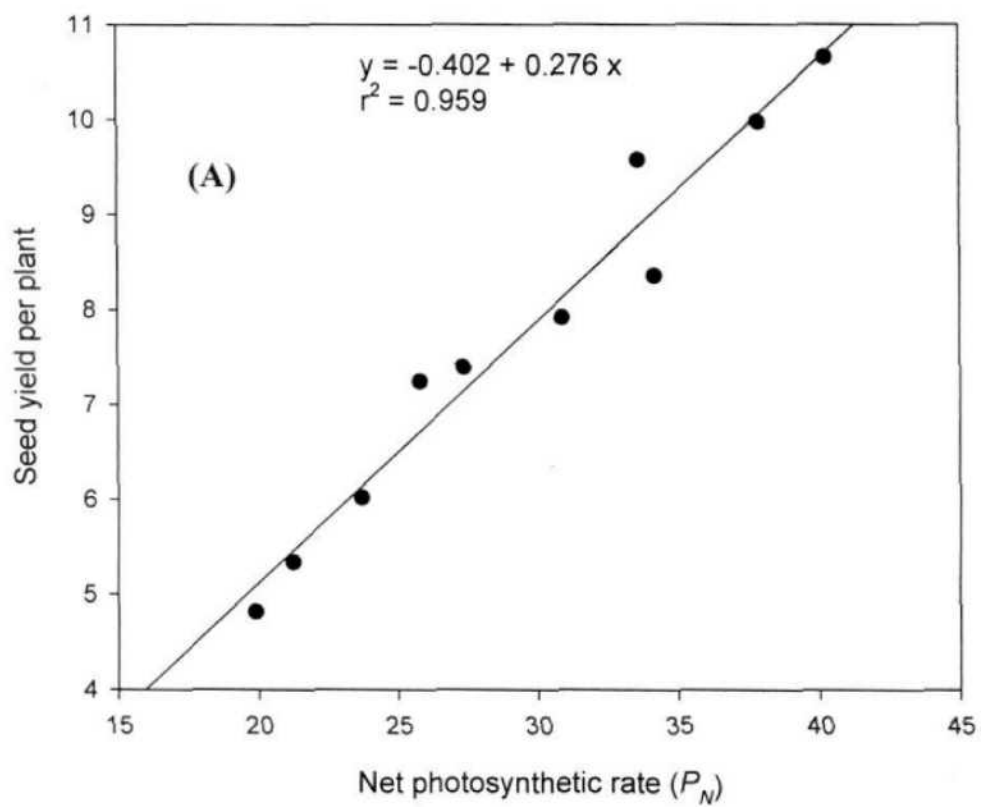
Figure 9 Correlation coefficient values between seed yield and net photosynthetic rate in (A) Varuna and (B) RH-30 (Experiment 6).

improving the values of most of the morphological, physiological and biochemical parameters in the presence as well as in the absence of NaCl-induced stress. This superiority of EBL over HBL may be because of differences in the structure and stability of these two analogues (Khripach *et al.,* 2000; 2003). S-oriented alkyl (methyl or ethyl) group at C-24 of side chain is present in almost all BRs while EBL and CS (another BR analogue) being exceptions carry R-oriented alkyl group on the side chain of the steroid nucleus (Plate IV). It is, therefore, inferred that the attachment of EBL at its receptor on the plasma membrane leads to more distorted three dimensional structure conformational state as compared to HBL. This thermodynamically acquired new stable state of EBL which seems to be more actively involved in triggering wide array of cascades, more efficiently involved than HBL. However, further study is warranted to know about the transcription factors that are involved in BKI-1 dissociation with BAK-1 to avail the binding in BRI-1 at membrane (Swaczynova *et al.,* 2007; Hategan *et al.,* 2010; Codreanu and Russinova, 2010).

Out of the three concentrations of proline used, the medium concentration (20 mM) proved most effective. The possible reason for this impact is attributed to the fact that proline activates a cycle of cytosolic proline synthesis from glutamate and mitochondrial proline degradation which simultaneously provided $NADP^+$ to drive cytosolic purine biosynthesis (Hare, 1998). An induction of *Arabidopsis* gene encoding proline dehydrogenase (PDH), a mitochondrial enzyme, by exogenous proline (Kiyosue *et al.,* 1996; Nakashima *et al.,* 1998) is consistent with this hypothesis. However, at higher proline levels, feedback inhibition of δ-1-pyroline-5-carboxylate synthetase (P5CS) (Garcia-Rios *et al.,* 1997; Zhang *et al.,* 1995) blocks the biosynthetic portion of this cycle and thereby inhibits organogenesis, as in *Arabidopsis* (Hare *et al.,* 2001).

The foliar spray of a combination of EBL and proline improved almost all the growth parameters both in the presence or absence of the salt stress. A diagrammatic summary of the effect of proline and/or BRs on the salinity induced changes in plants is shown in Plate V. However, more study is needed at molecular level to disclose the crosstalk between BRs and proline and with other phytohormones in providing tolerance against the stress. Out of the two cultivars, Varuna was found more tolerant to salt stress. This varied growth response of the two varieties of mustard could
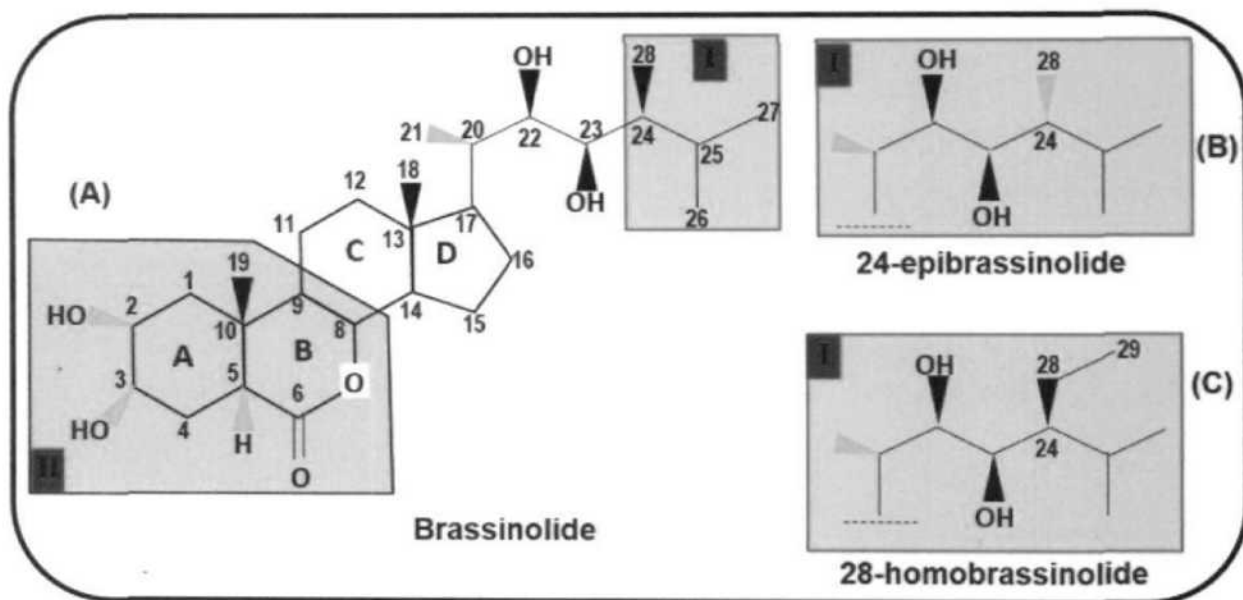
Plate IV: Structural difference between (A) Brassinolide, (B) 24-epibrassinolide and (C) 28-homobrassinolide

✓ The structures of other BRs differ from brassinolide (A) within the boxed areas I and II.

✓ The two important BRs, 24-EBL (B) and 28-HBL (C), differ from BL by the substituent in the side chain at C-24 or by its configuration (R or S orientation) at C-24, respectively.
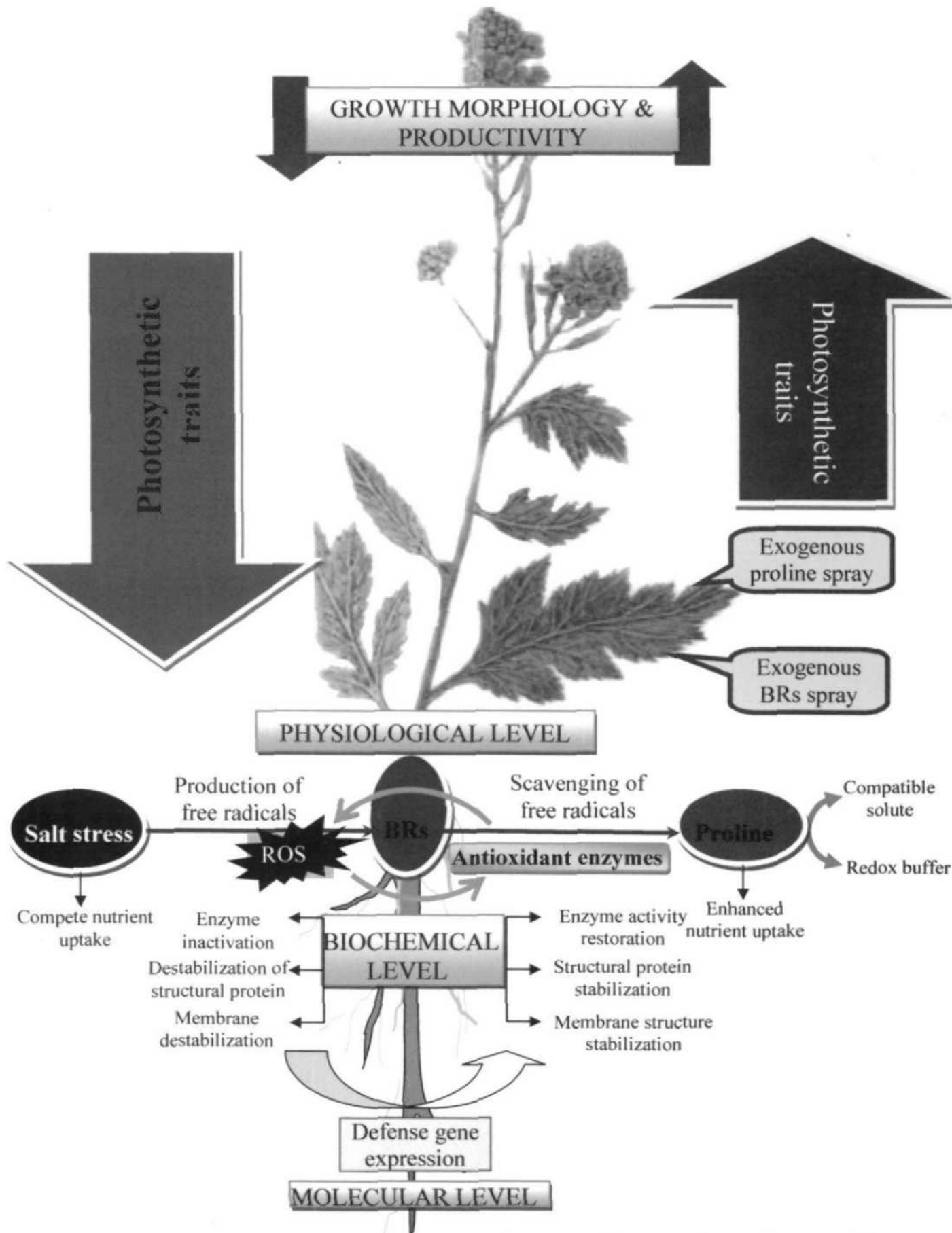
Plate V: Diagrammatic representation of the effects of proline and/or brassinosteroids on the salinity induced changes in plants.

possibly be due to differential regulation of the processes related to growth at their genetic, biochemical and physiological levels.

Conclusions

The present study revealed:

1. Out of the three levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl applied through the soil, 5.6 dsm^{-1} generated maximum toxicity and damage in the plants.

2. Out of the two varieties (Varuna and RH-30), the variety RH-30 was more susceptible to the stress than Varuna.

3. Out of the two BR analogues (HBL/EBL) used in this study, EBL excelled over HBL to generate favorable responses both in stressed and stress-free plants.

4. Out of the various concentrations (10, 20 or 30 mM) of proline, 20 mM proved the best in inducing resistance to the salt stress.

5. All the morphological, photosynthetic and various biochemical parameters decreased significantly with the increasing level of NaCl, amended into the soil.

6. All the morphological biomarkers and photosynthetic traits along with various biochemical parameters increased significantly in the plants treated with either of the BR analogues (HBL/EBL), over the control (water sprayed) plants.

7. Toxic effects generated by the lower concentration of NaCl were completely neutralized by the follow up treatment with either of the BR analogues where EBL excelled in its effect over HBL.

8. Activity of the enzymes (nitrate reductase and carbonic anhydrase) and the values for all the photosynthetic attributes increased by the application of either of the BR analogues, alone or as a follow up treatment to plants, exposed to NaCl.

9. Antioxidant enzymes (catalase, peroxidase and superoxide dismutase) and proline accumulation in the plants increased in response to BRs and/or proline alone or as a follow-up treatment to NaCl stressed plants.

10. The foliar application of proline (20 mM) and EBL (10^{-8} M) in combination or alone as a follow-up treatment to stressed plants showed an additive effect thereby maintained healthy growth and productivity.

11. The yield of the plants decreased significantly in the plants grown in the soil supplemented with different levels of NaCl in a concentration dependent manner.

12. The seed yield of the plants was significantly increased by the application of BRs (HBL/EBL) and/or proline application. Combination of EBL with proline was most effective.

13. The foliar spray of EBL (10^{-8} M) with proline (20 mM) as a follow-up treatment proved most potent salt stress alleviator by enhancing the level of antioxidant system and osmolyte (proline), manifested as rich growth, higher rate of photosynthesis, and biological yield of plants.

Summary

SUMMARY

This thesis is based on the following five chapters:

☛ **Chapter 1** includes the significance of the problem entitled, "Establishment of dose dependent responses of brassinosteroids and proline against salinity stress in *Brassica juncea*".

☛ **Chapter 2** represents a comprehensive review of the available literature, related with the above problem, pertaining to growth, metabolism, and yield characteristics of the plants.

☛ **Chapter 3** explains the details of the materials and methods employed in conducting the experiments and assessing the physical and chemical characteristics of the biological material.

☛ **Chapter 4** is comprised of the tabulated data recorded during this study and its brief description.

☛ **Chapter 5** deals with the possible explanations for the observations, in the light of the earlier findings.

The salient features of the observations recorded in each of the six experiments are summarized below:

Experiment 1

This experiment was carried out to study the effect of soil amended doses of sodium chloride (NaCl) on *Brassica juncea* (L.) Czern & Coss, cv. Varuna and RH-30. The healthy looking seeds were surface sterilized with 0.01% mercuric chloride solution for 5 min, followed by washing with double distilled water (DDW), at least thrice, to remove the traces of mercuric chloride adhered to the seed surface. These surface sterilized seeds were then sown in the earthen pots (25×25 cm) filled with sandy loam soil and farmyard manure, mixed in the ratio of 6:1. The three concentrations (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl were mixed with the soil. Thinning was done 7 d after sowing (DAS), leaving three plants per pot where five pots were maintained per treatment. The pots were arranged in a simple randomized block design, in the net house of Department of Botany, Aligarh Muslim University, Aligarh. The plants after 30 and 60 DAS were assessed for growth, chlorophyll content (SPAD level), leaf water potential, electrolyte leakage, photosynthetic attributes, maximum quantum yield of PSII (Fv/Fm), activities of nitrate reductase, carbonic anhydrase and various

antioxidant enzymes (catalase, peroxidase and superoxide dismutase) and proline content before/after being removed from the soil. The rest of the plants were allowed to attain maturity and were harvested to study the yield characteristics (120 DAS). The plants of the two cultivars showed significantly different response to graded concentrations of NaCl. The decrease caused by NaCl was more pronounced in RH-30 than Varuna. The highest concentration (5.6 dsm^{-1}) of the salt was most toxic. All the parameters, except antioxidant enzymes, proline content and electrolyte leakage showed a linear decrease as the level of salt increased in the soil. Varuna showed higher antioxidant enzymes activity at all the levels of salt, compared to RH-30. At harvest, all the yield attributes i.e. number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant exhibited a marked reduction in response to NaCl.

Experiment 2

This experiment was carried out to study the impact of two BR analogues (HBL/EBL) on *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. All the agricultural practices, including the dose of organic fertilizers were same as in Experiment 1. Twenty nine days old plants foliage were sprayed with DDW (control), tween-20 (0.5%), ethanol (5%), HBL (10^{-8} M) or EBL (10^{-8} M). The plant were assessed at 30 and 60 DAS for the parameters, as in Experiment 1. A set of plants was allowed to grow to maturity and were harvested (120 DAS) to study the yield characteristics. The foliar spray of BR analogues generated a favorable response in the plants by increasing growth, chlorophyll content (SPAD level), leaf water potential, photosynthetic attributes, Fv/Fm, activities of nitrate reductase, carbonic anhydrase and antioxidant enzymes and total proline content. However, this treatment decreased the leaf electrolyte leakage in the plants. Moreover, the spray of BRs significantly increased the number of pods per plant and seed yield per plant. Out of the two BR analogues, EBL excelled in its effects, over HBL. Varuna showed better response than RH-30.

Experiment 3

This experiment was carried out with an aim to study the effect of three concentrations (10, 20 or 30 mM) of proline on *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. All the agricultural practices were same as in Experiment 1. At 29 DAS, the foliage of the resulting plants was sprayed with DDW (control), 10

mM, 20 mM or 30 mM of proline. The parameters and the pattern of assessment was same as in Experiment 1. The foliar spray of proline improved plant growth, SPAD chlorophyll level, leaf water potential, net photosynthetic rate and related attributes, Fv/Fm, activities of various enzymes (nitrate reductase, carbonic anhydrase and antioxidant enzymes) and total proline content and the number of pods per plant and seed yield per plant in both the varieties. Out of the three concentrations of proline tested, medium concentration (20 mM) proved to be most effective. Varuna showed better response than RH-30.

Experiment 4

This experiment was laid down with an aim to elucidate the remedial effects of BR analogues on the salinity induced changes in *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. All the agricultural practices remained the same as in Experiment 1. The NaCl (2.8, 4.2 or 5.6 dsm^{-1}) was applied to the soil before sowing. Foliage of 29 days old plants was sprayed with DDW/aqueous solution (10^{-8} M) of BRs (HBL/EBL). The characteristic studies and their assessment pattern was the same as mentioned in experiment 1. The presence of NaCl caused a significant decline in the values of most of the parameters, in a concentration dependent manner, except those of electrolyte leakage, total proline content and activity of antioxidant enzymes in the leaves that increased. However, foliar spray of BRs (HBL/EBL) alone or as a follow-up treatment to the NaCl stressed plants improved the values of the parameters studied and also overcome the salinity-induced damages. The damages to the Varuna plants under lower NaCl concentrations (2.8 and 4.2 dsm^{-1}) were completely overcome by the follow-up treatment with EBL (10^{-8} M) Varuna and partially in RH-30. The activity of antioxidant enzymes and total proline content in both the varieties increased with the level of NaCl in the soil and BRs had an additive effect. Varuna possessed higher values for all the attributes than RH-30.

Experiment 5

This experiment was carried out to elucidate the effect of exogenous proline application to the foliage of *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30 in the presence or absence of NaCl-induced stress. All the agricultural practices and the parameters studied remained the same as in Experiment 1. The NaCl (2.8, 4.2 or 5.6 dsm^{-1}) was applied to the soil before sowing. At 29th day of the sowing, the foliage of the stressed/stress-free plants was sprayed with DDW/proline (20 mM). The

plants were assessed at 30 and 60 DAS for the parameters mentioned in Experiment 1. The remaining plants were allowed to grow to maturity and were harvested (120 DAS) to study the yield characteristics. The presence of NaCl decreased the values for almost all the parameters in a concentration dependent manner but total proline content, activity of various antioxidant enzymes and electrolyte leakage increased with the level of stress in both the cultivars. However, the exogenous application of proline alone or as a follow-up treatment to stressed plants improved the values of most of the parameters and completely alleviated the adverse effects of lower concentration (2.8 dsm^{-1}) of NaCl which was prominent in Varuna. The proline application had an additive effect in increasing the proline content and antioxidant enzymes activity in the stressed plants. Proline application improved the yield of the plants under stress free conditions and also on being exposed to lower concentration (2.8 dsm^{-1}) of NaCl particularly in Varuna.

Experiment 6

This experiment was designed to explicate the cumulative effect of EBL (BR analogue) and proline as foliar spray on the stress-free and salt-induced changes in *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. All the agricultural practices and the parameters studied remained same as in Experiment 1. Three levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl was applied to the soil. The plants were sprayed with DDW or proline and/or EBL at 28 and 29 DAS, respectively. The plants were subjected to analysis at 30 and 60 DAS for the parameters, mentioned in Experiment 1. The rest of the plants were allowed to grow up to maturity and were harvested at 120 DAS to study the yield characteristics. As the level of NaCl increased in the soil, the values for growth, SPAD chlorophyll level, leaf water potential, photosynthetic attributes, Fv/Fm and activities of nitrate reductase and carbonic anhydrase enzymes decreased significantly in both the varieties. The electrolyte leakage, proline content and activity of antioxidant enzymes improved as the salt stress increased. The variety RH-30 was more susceptible to NaCl stress than Varuna. However, foliar application of proline and/or EBL improved the values of these attributes, that too more effectively in Varuna than in RH-30. The damage caused to most of the parameters by the lower two concentrations of NaCl (2.8 and 4.2 dsm^{-1}) was completely neutralized by proline and EBL combination in both the varieties. The activity of antioxidant enzymes (catalase, peroxidase, and superoxide dismutase) and total proline content in

both the varieties increased with an increase in the level of stress and the follow-up treatment with proline and EBL had an additive effect. Varuna possessed higher values for these attributes in response to all the treatments than RH-30. The spray of proline and EBL combination was established as the most suited treatment in the alleviation of NaCl-induced stress.

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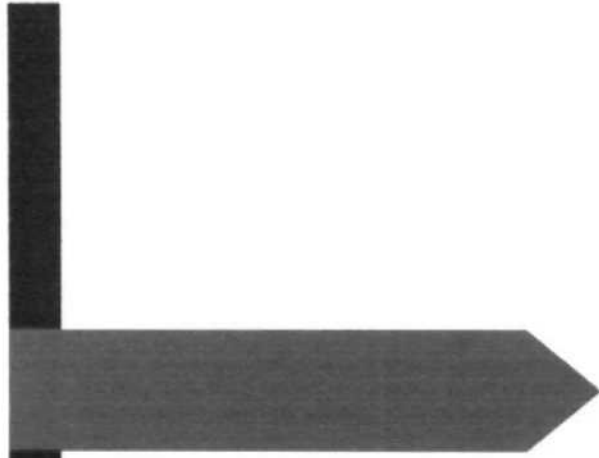
Appendix

APPENDIX

1   Preparation of reagents for the estimation of nitrate reductase activity

1.1   0.1M Phosphate buffer (pH 7.4)

27.20 g of KH_2PO_4 and 45.63 g of $K_2HPO_4.7H_2O$ were dissolved separately in 1000 cm^3 of DDW. The above solutions of KH_2PO_4 and $K_2HPO_4.7H_2O$ were then mixed in the ratio of 16:84 to get the solution of required concentration.

1.2   0.2M KNO_3

20.20 g of KNO_3 was dissolved in sufficient DDW and final volume was made up to 1000 cm^3, using DDW.

1.3   5% Isopropanol

5 cm^3 of isopropanol was pipetted into sufficient DDW and final volume was made up to 100 cm^3, using DDW.

1.4   1% Sulphanilamide

1 g of sulphanilamide was dissolved in 100 cm^3 of 3N HCl which was prepared by dissolving 25.86 cm^3 of HCl in sufficient DDW and final volume was maintained to 100 cm^3, by using DDW.

1.5   0.02% N-1-Naphthylethylenediamine dihydrochloride (NED-HCl)

20 mg of NED-HCl was dissolved in sufficient DDW and final volume was made up to 100 cm^3, by using DDW.

2   Preparation of reagents for the estimation of carbonic anhydrase activity

2.1   Cysteine hydrochloride solution (0.2M)

48 g of cysteine hydrochloride was dissolved in sufficient DDW and final volume was made up to 1000 cm^3, by using DDW.

2.2   Sodium Phosphate buffer (pH 6.8)

27.80 g of NaH_2PO_4 and 53.65 g of Na_2HPO_4 were dissolved separately in sufficient DDW and final volume was made up to 1000 cm^3. 51 cm^3 of NaH_2PO_4 and 49 cm^3 of Na_2HPO_4 were then mixed to get the required solution.

2.3   Alkaline sodium bicarbonate solution

16.80 g of sodium bicarbonate ($NaHCO_3$) was dissolved in aqueous 0.2M NaOH solution [0.8 g NaOH $(1000 cm^3)^{-1}$] and final volume was made up to 1000 cm^3, by using DDW.

2.4   *0.002% bromothymol blue*

0.002 g of bromothymol blue was dissolved in sufficient DDW and final volume was made up to 1000 cm^3 by using DDW.

2.5   *0.5N HCl*

4.3 cm^3 of pure HCl was pipetted in sufficient DDW and final volume was made up to 1000 cm^3, by using DDW.

2.6   *Methyl red indicator*

5 mg of methyl red was dissolved in sufficient ethanol and final volume was made to 100 cm^3, using ethanol.

3.   **Preparation of reagents for catalase estimation**

3.1   *Phospahte buffer (0.1M) for pH 6.8*

3.54 g of Na_2HPO_4 was dissolved in 100 cm^3 of DDW and 3.72 g of NaH_2PO_4 was added to 100 cm^3 of DDW. Then 12.3 cm^3 of Na_2HPO_4 was added to 87.7 cm^3 of NaH_2PO_4 to get the solution of required concentration and pH.

3.2   *H_2O_2 (0.1M)*

0.34 cm^3 of H_2O_2 was added to 100 cm^3 of distilled water.

3.3   *Sulphuric acid (2%)*

2 cm^3 of H_2SO_4 was added to 98 cm^3 of DDW.

3.4   *0.1N Potassium permanganate*

This was made by dissolving 0.162 g of $KMnO_4$ in 500 cm^3 of DDW.

4   **Preparation of reagents for peroxidase estimation**

4.1   *Pyrogallol phosphate buffer*

It was prepared by mixing 25 cm^3 of pyrogallol in 75 cm^3 phosphate buffer (pH 6).

5   **Preparation of reagents for superoxide dismutase**

5.1   *Phosphate buffer (50mM) for pH 7.8*

It was prepared by mixing 1.78 g Na_2HPO_4 and 1.56 g of NaH_2PO_4 in 100 cm^3 of DDW separately. 91.5 cm^3 of Na_2HPO_4 with 8.5 cm^3 of NaH_2PO_4 were mixed to get phosphate buffer of pH 7.8.

5.2   *Methionine (13mM)*

It was prepared by dissolving 0.193 g of methionine in 100 cm^3 of DDW.

5.3   *Nitrobluetetrazolium (NBT) (75μM)*

6.13 mg of NBT was dissolved in 100 cm^3 of DDW.

5.4   *Riboflavin (2µM)*

0.0753 mg of riboflavin was dissolved in 100 cm³ of DDW.

5.5   *EDTA (0.1M)*

2.92 g EDTA was dissolved in 100 cm³ of DDW.

6   **Preparation of reagents for proline estimation**

6.1   *Sulfosalicylic acid (3%)*

3 g of sulfosalicylic acid was dissolved in sufficient DDW and final volume was maintained to100 cm³, by using DDW.

6.2   *Acid ninhydrin solution*

1.25 g of ninhydrin was dissolved in a mixture of warm, 30 cm³ of glacial acetic acid and 6 M phosphoric acid (pH 1.0) with agitation till it got dissolved. It was stored at 4°C and used within 24 h.

The 6M phosphoric acid was prepared by mixing 11.8 cm³ of phosphoric acid with 8.2 cm³ of DDW.
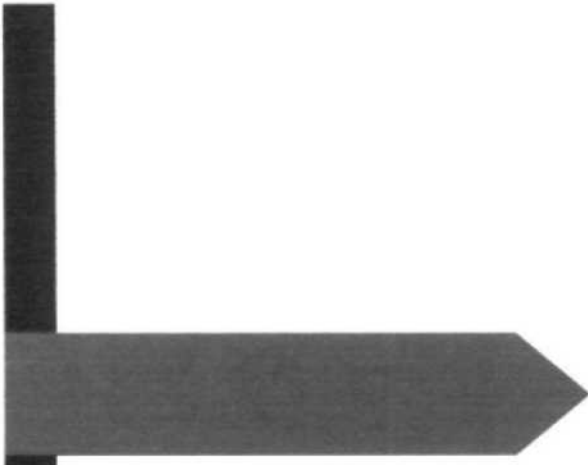
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ORIGINAL ARTICLE

Salt-induced modulation in growth, photosynthesis and antioxidant system in two varieties of *Brassica juncea*

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KEYWORDS

Abstract The present study was carried out to examine salt-induced modulation in growth, photosynthetic characteristics and antioxidant system in two cultivars of *Brassica juncea* Czern and Coss varieties (Varuna and RH-30). The surface sterilized seeds of these varieties were sown in the soil amended with different levels (2.8, 4.2 or 5.6 dsm^{-1}) of sodium chloride under a simple randomized block design. The salt treatment significantly decreased growth, net photosynthetic rate and its related attributes, chlorophyll fluorescence, SPAD value of chlorophyll, leaf carbonic anhydrase activity and leaf water potential, whereas electrolyte leakage, proline content, and activity of catalase, peroxidase and superoxide dismutase enzymes increased in both the varieties at 30 d stage of growth. The variety Varuna was found more resistant than RH-30 to the salt stress and possessed higher values for growth, photosynthetic attributes and antioxidant enzymes. Out of the graded concentrations (2.8, 4.2 or 5.6 dsm^{-1}) of sodium chloride, 2.8 sm^{-1} was least toxic and 5.6 dsm^{-1} was most harmful. The variation in the responses of these two varieties to salt stress is attributed to their differential photosynthetic traits, SPAD chlorophyll value and antioxidant capacity, which can be used as potential markers for screening mustard plants for salt tolerance.

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1. Introduction

Plants throughout their life cycle experience various types of environmental stresses (such as drought, salinity, high temperature, cold, heavy metal and other similar stresses) due to their sessile nature. Among these stresses, salinity stress has become the limiting factor for the productivity of agricultural crops by affecting germination, plant vigor and finally crop yield (Munns and Tester, 2008; Zhang et al., 2011). There are various effects of salinity stress on plants such as ion toxicity, water stress, oxidative stress, nutritional imbalances, alterations in metabolic processes, disorganization of membranes, reduction in division and expansion of cells, and genotoxicity (Hasegawa et al., 2000; Zhu, 2007). These adverse effects collectively lead to the reduction in plant growth, development and finally biological yield.

During the onset and development of salt stress within the plant, all major processes such as photosynthesis, protein syn-

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thesis and lipid metabolism are affected (Parida and Das, 2005; Hasanuzzaman et al., 2012; Rahdari et al., 2012). Photosynthesis is severely affected during salinity stress which is mediated through a decrease in stomatal conductance (Parida et al., 2004; Yan et al., 2012), internal CO_2 partial pressure and gaseous exchange through stomata (Iyengar and Reddy, 1996). The decrease in photosynthesis under saline conditions is considered as one of the most important factors restricting plant growth and productivity (Manikandam and Desingh, 2009). Salinity reduces plant productivity first by reducing plant growth during the phase of osmotic stress and subsequently by inducing leaf senescence during the phase of toxicity when excessive salt is accumulated in transpiring leaves (Munns, 2002). Many plants have evolved various mechanisms either to exclude salt from their cells or to tolerate its presence within the cells. They counteract the toxic effects of the stress through the production of osmolytes or by an increasing activity of antioxidant enzymes (Ashraf and Foolad, 2007; Ahmad et al., 2010). Antioxidant enzymes such as superoxide dismutase, peroxidase and catalase etc. help plants to withstand harmful effects of the environmental stress. In plants, superoxide dismutase scavenges superoxide anions (O_2^-) and converts them to hydrogen peroxides (Alscher et al., 2003). Catalase, the second line of defense, converts these lethal hydrogen peroxides to water and molecular oxygen. The effectiveness of oxidative defense system in plants can be measured by the activity of antioxidant enzymes and level of non-enzymatic antioxidants such as proline (Geebelen et al., 2002; Ahmad et al., 2012).

Brassica juncea (L.) Czern and Coss is an important oil-seed crop, which often experiences saline stress as it is grown extensively in the arid and semi-arid regions of the world (Singh et al., 2001). India ranks second in the world with regard to the production of Brassicas (Afroz et al., 2005) and supplies nearly 7% of the world's edible oil (Khan et al., 2002). A number of biotic and abiotic stresses contribute to yield losses and this low economic yield is related to the crop's susceptibility. There is a greater need to improve crop plants for salinity tolerance, however Brassica has a considerable potential to grow in salt-affected areas. One of the approaches is the improvement of salt tolerance of the cultivated species. The identification of tolerant genotype provides an initial germplasm base for breeding salt-tolerant crops. The present study was therefore carried out to examine the salt-induced modulation in growth, photosynthetic characteristics, chlorophyll pigments, leaf fluorescence, antioxidant enzymes and levels of non-enzymatic antioxidants in two varieties of Brassica i.e. Varuna and RH-30. Such studies will facilitate the evaluation of the relative performance of varieties and characterization of mechanism of salt tolerance which in turn will be helpful in effective breeding for salt tolerance.

2. Material and methods

2.1. Plant material and treatment

The authentic and healthy seeds of B. juncea (L.) Czern and Coss cv. Varuna and RH-30 were procured from National Seed Corporation Ltd. New Delhi, India. Before sowing the seeds were surface sterilized with 0.01% mercuric chloride solution followed by rinsing with sterilized, double distilled water (DDW),

at least thrice, to remove the traces of adhered mercuric chloride to the seed surface. The surface sterilized seeds of these two cultivars were sown in earthen pots (25×25 cm) containing soil amended with different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl. Pots were amended with the recommended dose of fertilizers (nitrogen from urea, single superphosphate, and muriate of potash) added at rates of 40, 138 and 26 mgkg^{-1} of soil, respectively at the time of sowing. Thinning was done on the 7th day after sowing (DAS), leaving three plants per pot. Each treatment was represented by five pots. Irrigation was done with tap water as and when required. The plants were up-rooted at 30 DAS to assess the following parameters. The remaining plants were allowed to grow up to maturity and were harvested at 120 DAS to study the yield characteristics.

2.2. Growth parameters

The plants were removed along with soil at 30 DAS and dipped in water to dislodge the adhering soil particles without injuring the roots. The length of the root and shoot was measured on a meter scale. The roots were then separated from the shoot and blotted. The roots and shoot were weighed separately to record their fresh mass and placed in an oven (80 °C for 72 h). The samples were weighed again to record the respective dry mass. Leaf area was ascertained by gravimetric method by tracing the outline of the leaf on graph sheet and counting the squares covered by it on graph paper.

2.3. Leaf water potential and electrolyte leakage

The leaf water potential (LWP) was measured by Psypro water potential system (Wescor Inc. USA). Total inorganic ions leaked out of the leaf were quantified by the method described by Sullivan and Ross (1979). Twenty leaf discs were taken in a boiling test tube containing 10 mL of DDW, and electrical conductivity was measured (EC_a). The tubes were heated at 45 °C and 55 °C for 30 min in water bath, and electrical conductivity was measured (EC_b). The contents were again boiled at 100 °C for 10 min, and electrical conductivity was recorded (EC_c). The electrolyte leakage was calculated using the formula:

$$\text{Electrolyte leakage} \quad (\%) = \frac{EC_b - EC_a}{EC_c} \times 100$$

2.4. Carbonic anhydrase (CA) activity

The activity of CA was determined following the procedure described by (Dwivedi and Randhawav, 1974). The leaf samples were cut into small pieces and suspended in cysteine hydrochloride solution. The samples were incubated at 4 °C for 20 min and then filtered. The filtrate was transferred to the test tubes, containing phosphate buffer (pH 6.8) followed by the addition of alkaline bicarbonate solution and bromothymol blue. The samples were incubated at 4 °C for 20 min. The reaction mixture was titrated against 0.05 N HCl after the addition of 0.2 mL of methyl red indicator.

2.5. SPAD value of chlorophyll and photosynthetic attributes

SPAD chlorophyll meter (Minolta 502) was used to assess the SPAD value of chlorophyll in the intact leaves. The photosyn-

thetic attributes [net photosynthetic rate (P_N), stomatal conductance (g_s), internal CO_2 concentration (C_i), and transpiration rate (E)] were measured by using portable photosynthetic system (LICOR-6400, Lincoln, NE, USA). These measurements were recorded on the uppermost fully expanded leaf of the main branch between 11:00 and 13:00 h, under bright sunlight. The atmospheric conditions during measurements were: photosynthetically active radiation 1016 ± 6 μmol m^{-2} s^{-1}, relative air humidity $60 \pm 3\%$, atmospheric temperature 22 ± 1 °C, and atmospheric CO_2 360 μmol mol^{-1}. The ratio of atmospheric CO_2 to intercellular CO_2 concentration was constant.

2.6. Maximum quantum yield of PSII

The maximum quantum yield of PSII (Fv/Fm) was measured on the adaxial surface of the intact leaf using portable photosynthesis system (LICOR-6400, Lincoln NE, USA). Prior to measurements, plants were left for 30 min in dark at room temperature. The chlorophyll molecules were excited for 10 s by actinic light with a photon flux density of 40 μmol m^{-2} s^{-1}.

2.7. Antioxidant enzymes

Fresh leaves (0.5 g) were homogenized with 5 mL of 50 mM phosphate buffer (pH 7.0) containing 1% PVP (polyvinylpyrrolidone). The homogenate was centrifuged at 10080g for 10 min. The supernatant was collected and used as a source for enzyme assay. This whole extraction process was carried out at 4 °C. The assay of peroxidase (POX) and catalase (CAT) was done by adapting the method of Chance and Maehly (1956). Activity of CAT was measured by titrating the reaction mixture [phosphate buffer (pH 6.8), 0.1 M H_2O_2, enzyme extract and 2% H_2SO_4] against 0.1 N KMnO$_4$. The activity of POX was measured by observing the change in the absorbance of reaction mixture [pyragallol phosphate buffer (pH 6.8), 1% H_2O_2 and enzyme extract], due to catalytic conversion of pyragallol to purpurogallin at an interval of 20 s for 2 min at 420 nm. A control set was prepared by using DDW instead of enzyme extract.

The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of NBT by using the method of Beauchamp and Fridovich (1971). The reaction mixture [50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM nitroblue tetrazolium (NBT), 2 μM riboflavin, 0.1 mM EDTA and 0–50 μl enzyme extract] in tubes was placed under 15 W fluorescent lamps for starting the reaction. After 10 min, the reaction was stopped by switching off the light. Non-illuminated reaction mixture was used as a blank. The absorbance was measured at 560 nm and the SOD activity was expressed as unit g^{-1} fresh mass. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photo-reduction.

2.8. Leaf proline content

The proline content in fresh leaf was determined by the method given by Bates et al. (1973). The samples were extracted in sulphosalicylic acid. To the extract, an equal volume (2 mL) of glacial acetic acid and ninhydrin solutions was added. The sample was heated at 100 °C, to which 5 mL of toluene was

added after cooling in ice bath. The absorbance by toluene layer was read at 528 nm, on a spectrophotometer (Spectronic-20D, Milton Roy, USA).

2.9. Statistical analysis

Treatment means were compared by the analysis of variance using SPSS (SPSS ver. 17, Chicago, United States). Least Significant Difference (LSD) was calculated at the 5% level of probability. Standard error between the replicates was also calculated.

3. Results

3.1. Growth characteristics

The length, fresh and dry mass of the shoot and root of both the varieties showed a marked decrease on being subjected to different levels of NaCl (2.8, 4.2 or 5.6 dsm^{-1}), applied through soil (Figs. 1a–f). Out of the different levels of NaCl, lowest concentration (2.8 dsm^{-1}) proved least toxic. However, the highest concentration of NaCl (5.6 dsm^{-1}) generated severe damage and caused maximum per cent decrease in the shoot length of Varuna and RH-30 (39.3% and 47.1%) and that of root length by 55.0% and 61.2%, compared with their respective controls. Moreover, NaCl (5.6 dsm^{-1}) also caused a maximum decrease (47.0% and 58.0%, compared with the controls) in the leaf area of Varuna and RH-30 (Fig. 2a). The damage was more prominent in RH-30 than in Varuna.

3.2. Leaf water potential and electrolyte leakage

The variety RH-30 had lower values for leaf water potential than Varuna (Fig. 2b). The presence of NaCl in the soil decreased these values where the maximum loss was observed at 5.6 dsm^{-1} which was 34.3% and 37.8% less than the controls in Varuna and RH-30, respectively. However, the presence of NaCl in the soil caused a significant increase in the electrolyte leakage in both the varieties (Fig. 2c). The leakage increased as the concentration of NaCl was increased. The highest level (5.6 dsm^{-1}) of NaCl increased the electrolyte leakage by 26.7% and 32.3% in Varuna and RH-30, respectively, as compared to their controls.

3.3. Carbonic anhydrase (CA) activity

Plants of both the varieties raised in the soil amended with different levels of NaCl (2.8, 4.2 or 5.6 dsm^{-1}) possessed a lower activity of CA enzyme in comparison to their controls (Fig. 2d). The decrease was proportional to the soil NaCl level and therefore the highest salt concentration was most toxic and caused maximum inhibition (42.0% less than the control) in variety RH-30. The loss in the activity of the enzyme was more prominent in variety RH-30 than Varuna, at all the concentrations of the NaCl.

3.4. SPAD value of chlorophyll

The plants grown in the soil amended with varied concentrations of NaCl possessed significantly lower SPAD values of chlorophyll than unstressed control plants (Fig. 3a). Out of the NaCl concentrations 5.6 dsm^{-1} was most toxic and de-
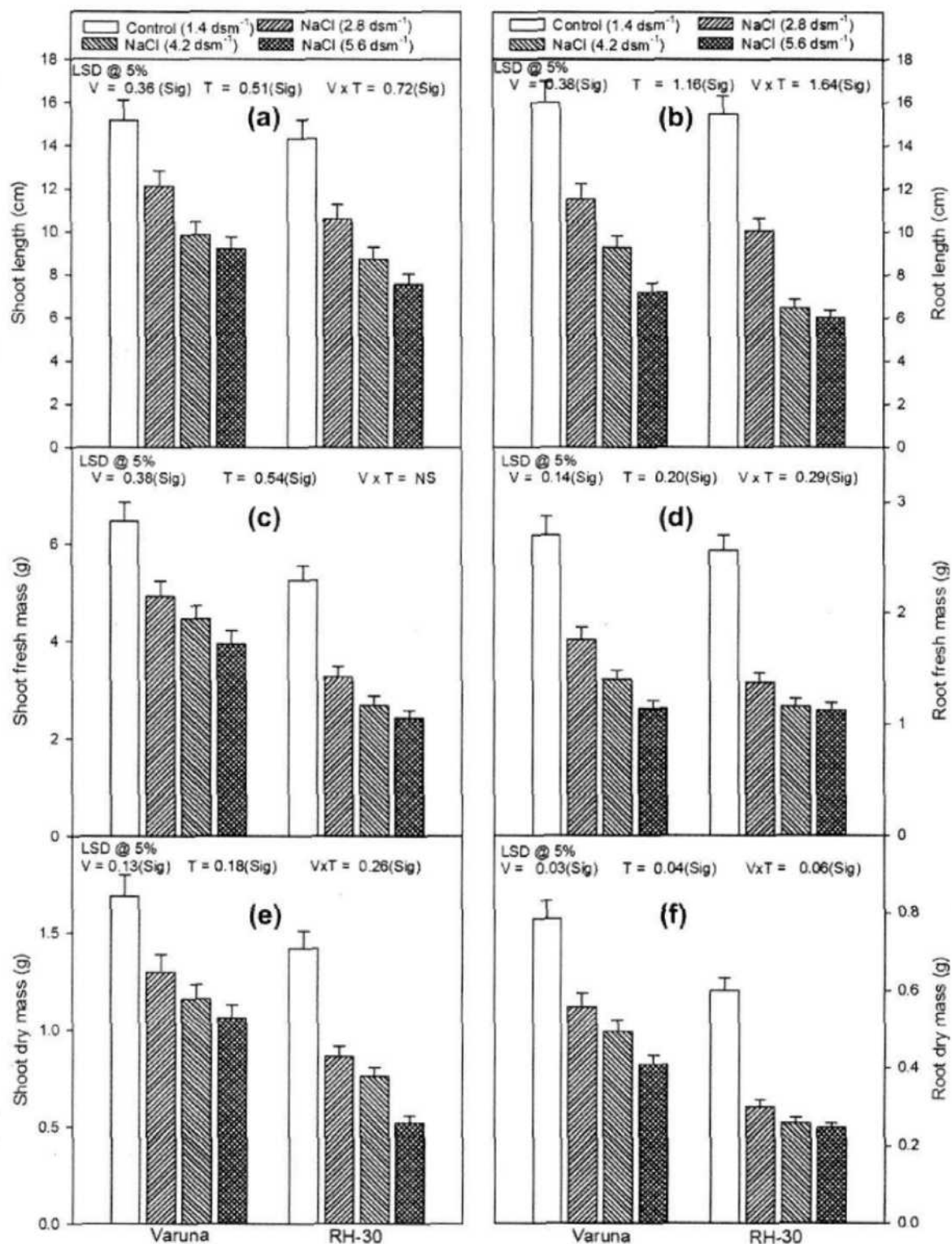
Figure 1 Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on length (cm), fresh mass (g) and dry mass (g) of shoot and root in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern and Coss at 30 DAS.

creased the values by 41.9% and 50.0% in Varuna and RH-30 respectively, compared to the respective controls. RH-30 was more prone than Varuna to salt stress.

3.5. Photosynthetic attributes

The plants raised from the seeds sown in the soil fed with different levels (2.8, 4.2 or 5.6 dsm^{-1}) of NaCl showed signifi-

cant decrease in the net photosynthetic rate (P_N) and its related attributes [stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E)] in Varuna and RH-30 (Figs. 3b–e). The decrease was proportionate to the concentrations of the NaCl. The 5.6 dsm^{-1} of NaCl decreased P_N, g_s, C_i and E by 32.0%, 51.6%, 23.9% and 27.2% in Varuna and 53.0%, 66.1%, 35.3% and 39.0% in RH-30, respectively when compared to their control plants.
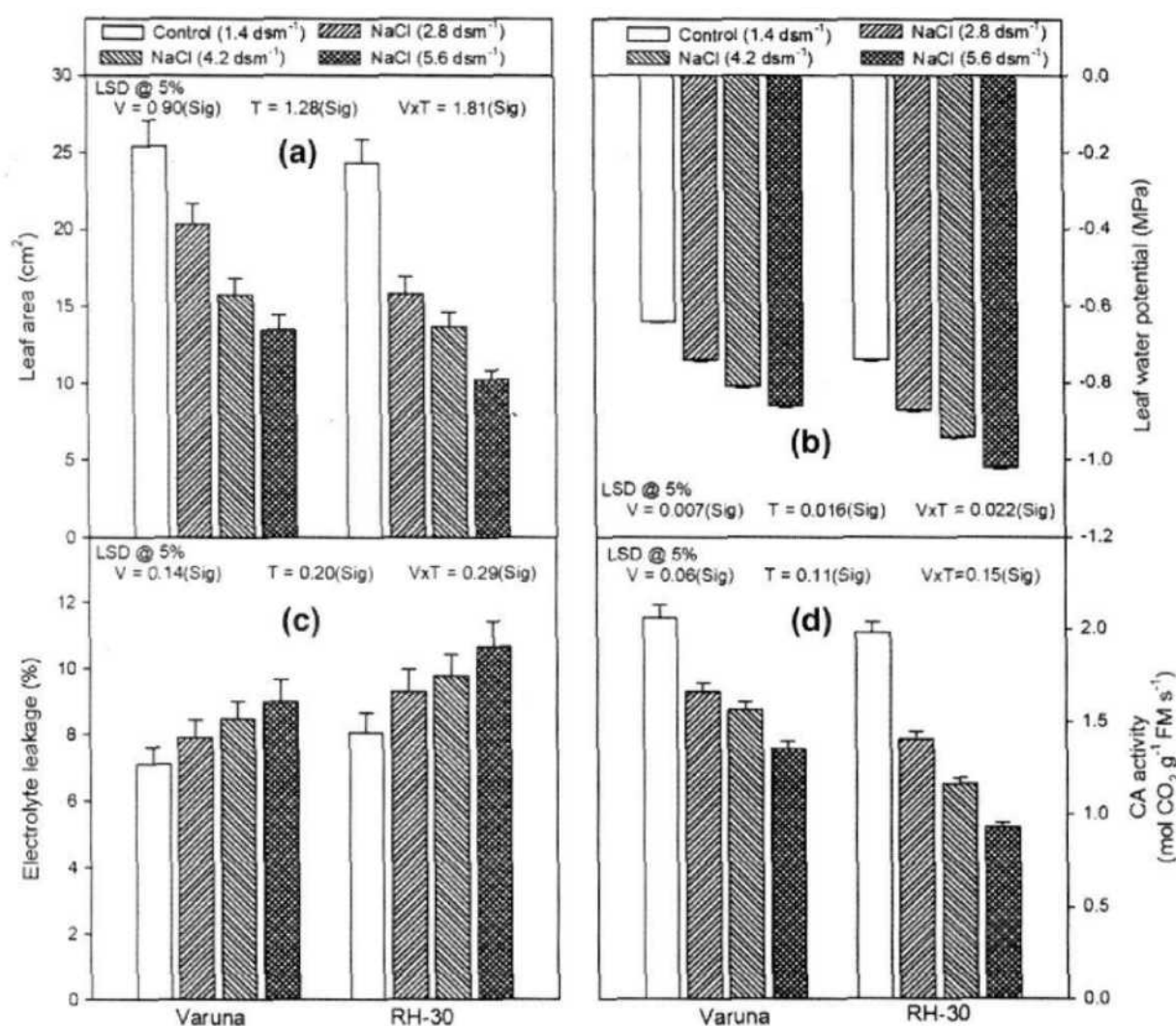
Figure 2 Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on (a) leaf area, (b) Leaf water potential, (c) electrolyte leakage, and (d) carbonic anhydrase (CA) activity in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern and Coss at 30 DAS.

Moreover, the variety RH-30 was more sensitive to salt stress than Varuna.

3.6. Maximum quantum yield of PSII

As depicted in Fig. 3f, the maximum quantum yield of PSII (Fv/Fm) showed a linear decrease with the increase in the concentration of NaCl in both the varieties (Varuna and RH-30). The maximum loss was recorded at the highest concentration (5.6 dsm^{-1}) which was 28.9% and 34.8% in Varuna in RH-30 respectively, compared to their controls. The variety Varuna possessed higher values for Fv/Fm than RH-30.

3.7. Antioxidant enzyme activities

Unlike the other parameters, the activity of antioxidant enzymes [catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD)] showed completely different response (Figs. 4a–c). The data revealed that the antioxidant enzyme activity increased in response to the concentrations of NaCl in the soil in both the varieties (Varuna and RH-30). The

plants raised in the soil amended with the highest NaCl level (5.6 dsm^{-1}) possessed maximum values for antioxidant enzymes in both the varieties. The values for CAT, POX and SOD activity increased by 39.8%, 57.0% and 96.8% in Varuna and 22.9%, 34.9% and 80.6% in RH-30, respectively compared to their respective control plants.

3.8. Leaf proline content

The leaf proline content was higher in the plants that were fed with NaCl (Fig. 4d). The values increased with an increase in the concentration of the salt. Maximum values were found in the plants which were fed with 5.6 dsm^{-1} of NaCl through the soil in both the varieties and the increase was 84.9% and 68.9% in Varuna and RH-30, respectively, over the respective controls.

3.9. Yield characteristics

Yield characteristics (number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant) were significantly affected and exhibited a linear decrease in their
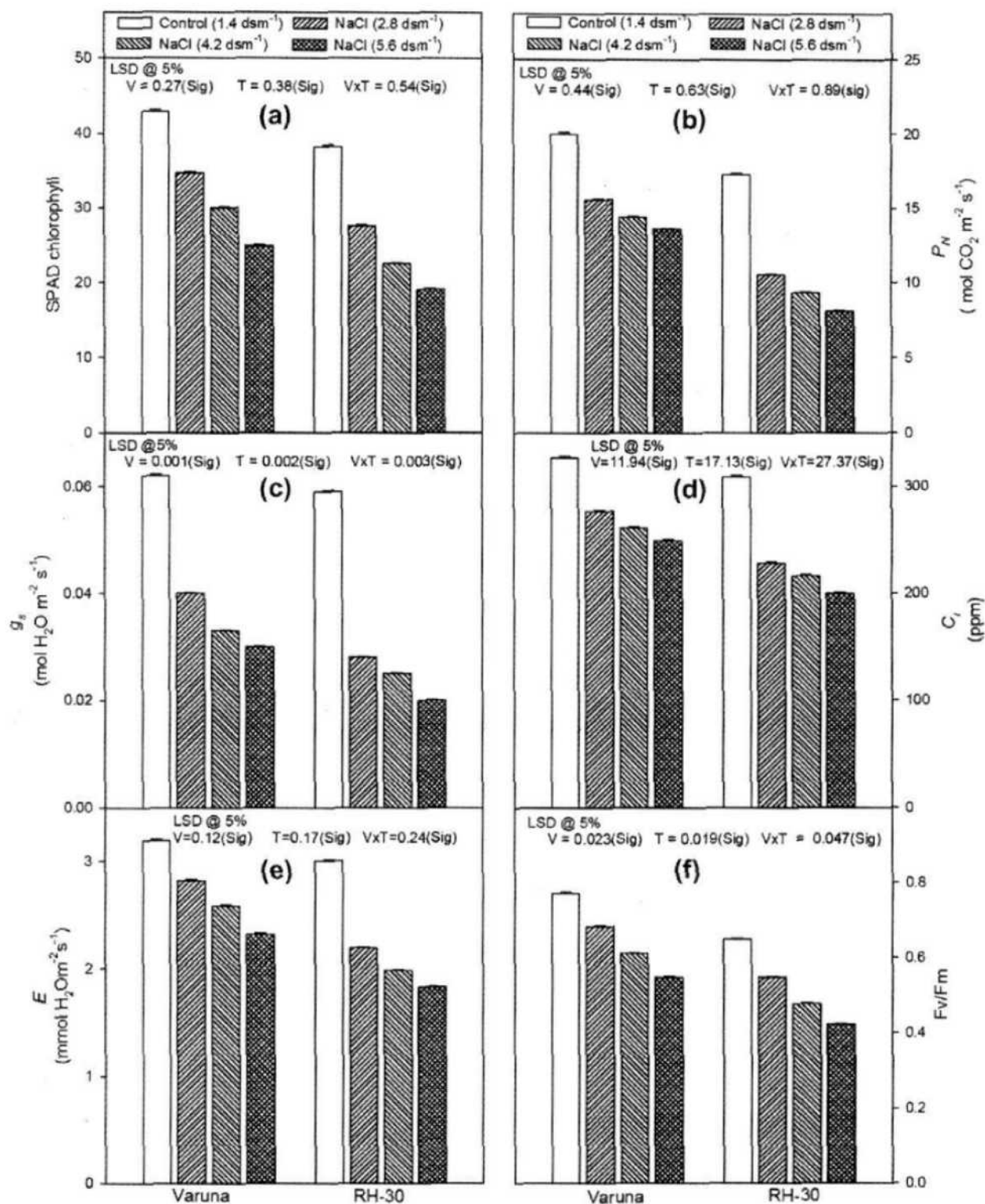
Figure 3 Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on (a) SPAD chlorophyll, (b) Net photosynthetic rate (P_N), (c) stomatal conductance (g_s), (d) internal CO_2 concentration (C_i), (e) transpiration rate (E), and (f) maximum quantum yield of PSII (Fv/Fm) in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern and Coss at 30 DAS.

values in response to the NaCl present in the soil in both the varieties (Figs. 5a–d). The maximum reduction in the values of all the above yield characteristics was noticed in RH-30 at the highest level of NaCl (5.6 dsm^{-1}). The number of pods per plant, number of seeds per pod, 100 seed mass and seed yield decreased by 25.0%, 17.9%, 10.3% and 34.0% in Varuna and 30.3%, 33.0%, 10.2% and 46.9% in RH-30 at

5.6 dsm^{-1} of NaCl respectively, as compared to their control plants.

4. Discussion

Salt stress (2.8, 4.2 or 5.6 dsm^{-1} of NaCl added to the soil) considerably decreased growth of the plants in both the varie-
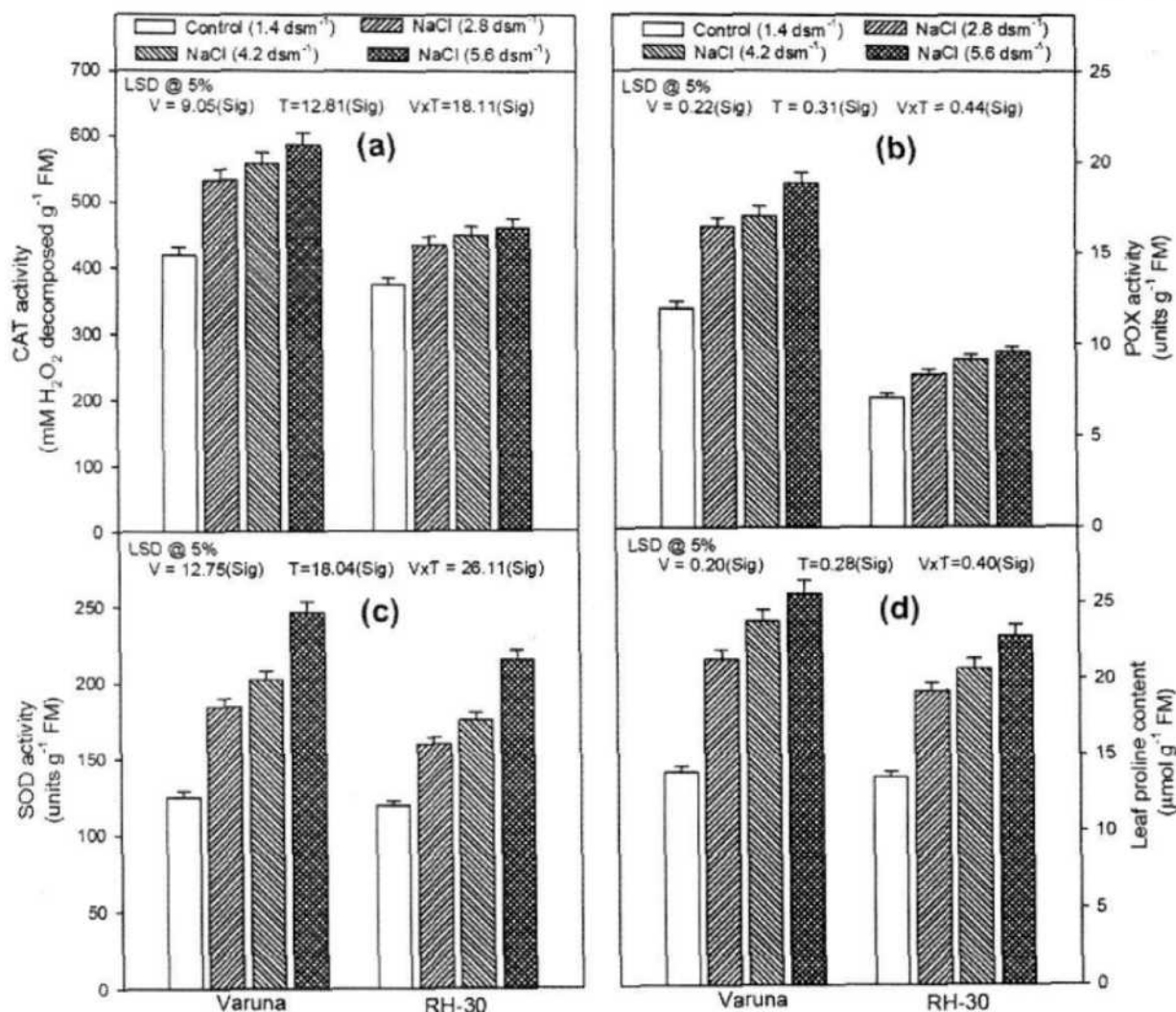
Figure 4 Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on (a) catalase (CAT) activity, (b) peroxidase (POX) activity, (c) superoxide dismutase (SOD) activity, and (d) leaf proline content in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern and Coss at 30 DAS.

ties (Varuna and RH-30) as reflected by reduced length, fresh mass and dry mass of roots and shoot and leaf area (Figs. 1a–f and 2a). These observations are in conformity with tomato (Hayat et al., 2010a), sunflower (Akram and Ashraf, 2011), mulberry (Ahmad and Sharma, 2010), okra (Saleem et al., 2011), mustard (Hayat et al., 2011) and proso millet (Sabir et al., 2011). Out of the two cultivars, as also reported by Hayat et al. (2011), Varuna is likely more salt tolerant. This varied growth response of the two varieties of mustard could possibly be due to differential regulation of the processes related to growth at their genetical, biochemical and physiological levels. The salt stress is known to cause reduction in cell division and elongation (Pitann et al., 2009) which is mainly due to salt induced alterations in the nutrient uptake, induced formation of reactive oxygen species (Ashraf, 2009), inhibition of cytoplasmic enzymes, turgor loss (Pitann et al., 2009) and hormonal imbalance (Ashraf et al., 2010) which will naturally impair plant growth and finally the yield (Fig. 5a–d).

The SPAD value of chlorophyll decreased significantly in the stressed leaves of plants of both the varieties (Fig. 3a). The reason behind this loss is that the salinity either inhibits synthesis and/or accelerates the degradation of existing chloro-

phyll molecules (Iyengar and Reddy, 1996). These results are in conformity with Hayat et al. (2011), Ahmad et al. (2012), Ghogdi et al. (2012) and Heidari (2012).

Leaf carbonic anhydrase (CA) enzyme catalyzes the reversible hydration of CO_2 and maintains its constant supply to Rubisco, at the level of the grana of the chloroplast (Price et al., 1994). In the present study the CA activity decreased as the NaCl concentration increased (Fig 2d). Since NaCl inhibits the activity of the key enzymes (Rubisco and PEP carboxylase) of photosynthesis (Soussi et al., 1998), decrease in the activity of CA could be due to similar reasons. Moreover, NaCl induced regulation of genes for CA synthesis (Liu et al., 2012) could be another reason for the observed decrease in the activity of enzyme. The decrease in CA activity is further corroborated by the findings of Hayat et al. (2011), Idrees et al. (2012) and Liu et al. (2012).

Plants exposed to the increasing levels of NaCl in the soil showed a diminished net photosynthetic rate (P_N) accomplished by a significant decrease in the stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) (Figs. 3b–e). The reduction in the level of photosynthetic capacity under salinity might be largely due to the stomatal
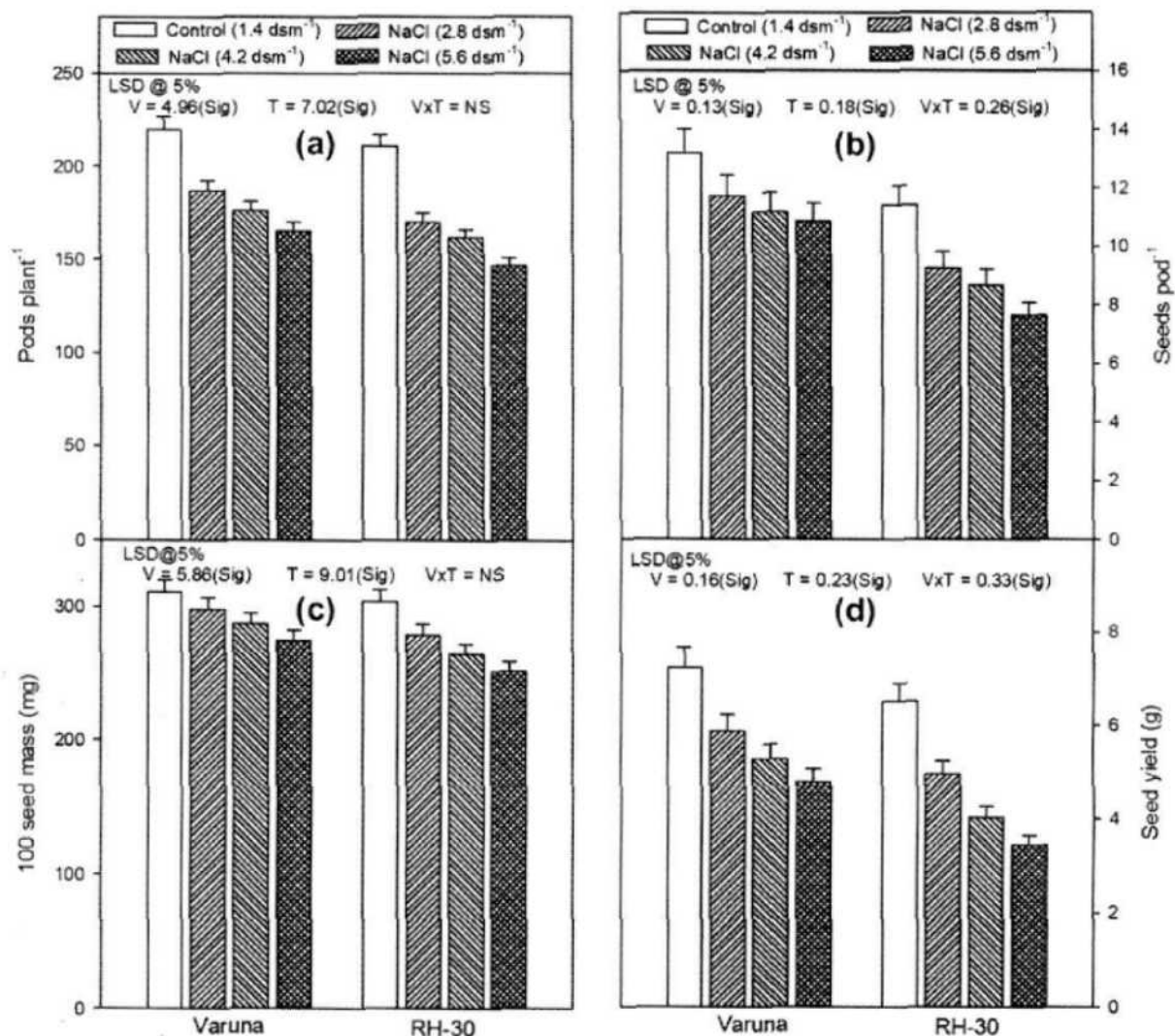
Figure 5 Effect of soil applied sodium chloride (NaCl; 2.8, 4.2 or 5.6 dsm^{-1}) on (a) pods plant^{-1}, (b) seeds pod^{-1}, (c) 100 seed mass and (d) seed yield in two varieties (Varuna and RH-30) of *Brassica juncea* (L.) Czern and Coss at harvest (120 DAS).

closure, brought about by salt-induced ABA accumulation, which will limit automatically the photosynthetic CO_2 assimilation (Saleem et al., 2011). The poor P_N values, under salt stress, were noted to be positively related to the observed decrease in g_s and C_i (Lu et al., 2009). Speeding up of the senescence of plant organs and shift in the activity of enzymes induced by the modifications in cytoplasmic structures and negative feedback of diminished sink activity associated with slow transport of photosynthates are other possible reasons for the salinity induced decrease in P_N and its related attributes (Iyengar and Reddy, 1996). The observed decrease in SPAD value of chlorophyll (Fig. 3a) and CA activity (Fig. 2d) are also the other reasons for low P_N in salt stressed plants. Similar observations have also been made by others (Ahmad et al., 2012; Eisa et al., 2012; Wang et al., 2012; Wu et al., 2012).

The NaCl decreases the photochemical efficiency which has been ascribed with the suppression of PSII activity (Mehta et al., 2010). In the present study, it has been observed that NaCl applied through the soil caused a significant reduction in the values of quantum yield of PSII (Fv/Fm) (Fig. 3f) suggesting the salt stress induced perturbations in electron transport of PSII (Megdiche et al., 2008). Salinity blocks the electron transfer from the

primary acceptor, plastoquinone (Q_A) to the secondary acceptor, plastoquinone (Q_B) at the acceptor side of PSII which leads to the decrease in Fv/Fm (Shu et al., 2012) These results are in conformity with the studies in *Triticum aestivum* (Kanwal et al., 2011), *Vigna radiata* (Hayat et al., 2010b), *Brassica napus* (Naeem et al., 2010), *Solanum melongena* (Wu et al., 2012), *Cucumis sativus* (Shu et al., 2012), *B. juncea* (Ahmad et al., 2012), exposed to salt stress.

Under stress conditions (such as drought and salinity) plant cells accumulate osmolytes in order to reduce the osmotic potential which increases the water absorption capacity, maintain turgor pressure at a certain extent and protects the cell growth. This whole phenomenon is called osmotic adjustment (Wang et al., 2012). In the present study the water potential decreased with an increase in the salt concentration (Fig. 2b) indicating that the leaves of *B. juncea* have evolved certain mechanisms to adjust its survival, under salt stress. Moreover, the varied range of increase in the ion concentration in the leaves caused varied degree of reduction in water potential under salt stress (Liu, 2004). These results are in conformity with Eisa et al. (2012), Wang et al. (2012) and Naeem et al. (2010). The cell membranes under various environmental stress are subjected

to changes, like the loss of integrity and increase in permeability (Blokhina et al. 2003) which cause an increase in the electrolyte leakage as observed in the present study (Fig. 2c).

Plants possess complex antioxidative defense system comprising of non-enzymatic (such as proline) and enzymatic components (such as CAT, POX, SOD) to scavenge reactive oxygen species (ROS) produced during stress. The production and scavenging of ROS occurs in different cell organelles such as chloroplasts, mitochondria and peroxisomes, however, pathways are well coordinated (Pang and Wang, 2008). Under normal conditions, ROS are generated at very low levels and a homeostasis is maintained between production and quenching of these molecules. This balance could be disturbed by the environmental stress, giving rise to a rapid increase in intercellular ROS levels which induce oxidative damage to lipids, proteins, and/or nucleic acids (Sharma et al., 2010). In order to cope with the oxidative damages under stress, plants raise the level of endogenous enzymes (CAT, POX and SOD) and the non-enzymatic component such as proline (Sharma et al., 2010 and Figs. 4a–d). Similar observations have been reported earlier in different crops such as *Kochia scoparia* (Nabati et al., 2011), pro-so millet (Sabir et al., 2011), wheat (Ashraf et al., 2010), safflower (Siddiqi, 2010), tomato (Hayat et al., 2010a) and *B. juncea* (Hayat et al., 2011; Ahmad et al., 2012). Under water or salinity stress the increase in proline content (Fig. 4d) may be due to the surpassing of the rate of protein hydrolysis over that of its synthesis (Irigoyen et al., 1992). Moreover, a higher proline content may be the result of its slower rate of breakdown or the diversion of protein synthesis so as to accumulate more proline. Similar observations have also been reported earlier in response to salt stress in *B. juncea* (Hayat et al., 2011; Ahmad et al., 2012), *V. radiata* (Hayat et al., 2010b) and sugar beet (Farkhondeh et al., 2012). It seems that the increased level of proline has a protective role in plants, exposed to stress (Hayat et al., 2010b). Therefore, in the present study, variety Varuna, a salt tolerant, possessed a higher proline content and the activity of CAT, POX and SOD enzymes than RH-30, a salt-sensitive. Such plant responses, differing in salt tolerance have been studied earlier in which salt tolerant varieties possessed better antioxidative defense system than the salt sensitive varieties (Sabir et al., 2011; Hayat et al., 2011). The yield characteristics (number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant) decreased significantly with an increase in NaCl level of the soil (Figs. 5a–d). However, variety Varuna performed better and showed a lesser loss than RH-30. This reduction in seed yield and its other related parameters, with an increase in the level of NaCl could be attributed to poor plant growth (Figs. 1a–f), resulting from the reduced rate of photosynthesis (Fig. 3b; Chen et al., 2009). These results are in conformity with those of Ali et al. (2007) and Asgari et al. (2012). Under salt stress, the thickness of the pathway elements conducting assimilates gets reduced (Aldesuquy and Ibrahim, 2001), at the same time the leaves start behaving as sink rather than source (Arbona et al., 2005) the cumulative effect being the inhibition of assimilate movement toward the developing reproductive organs leading to their poor growth and seed setting (Figs. 5a–d).

5. Conclusion

From the present study, it can be concluded that sodium chloride in a concentration (2.8, 4.2 or 5.6 dsm^{-1}) dependent man-

ner through soil significantly retarded plant growth, pace of photosynthesis and ultimately the seed yield in the *B. juncea* (L.) Czern and Coss cv. Varuna and RH-30, even though the plants exhibited a higher antioxidant enzyme activity and an accumulation of proline (the protective mechansims). The variety RH-30 was more sensitive to the salt stress than Varuna.

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