Chapter-6

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 ds m⁻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 ds^{-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.) Czem & 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.) Czem & 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.) Czem & 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.