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

ABSTRACT
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ABSTRACT

Establishment of dose dependent responses of brassinosteroids and proline against salinity stress in Brassica juncea

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Six pot experiments were conducted, during 2009-2012 to elucidate the effect of brassinosteroids and/or proline on salinity induced changes in Brassica juncea (L.) Czem & Coss cv. Varuna and RH-30. Salt stress was developed by soil-applied NaCl, whereas brassinosteroid analogues (HBL/EBL) and proline were applied to the plant foliage. The salient features of each of the six experiments are mentioned below.

Experiment 1

This experiment was conducted to evaluate the effects of sodium chloride (NaCl) on Brassica juncea (L.) Czem & Coss cv. Varuna and RH-30. The healthy seeds, surface sterilized with 0.01% mercuric chloride, were sown in the earthen pots (25x25 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⁻³) of NaCl were mixed with the soil. Three plants per pot and five pots per treatment were maintained. The pots were arranged in a simple randomized block design, in the net house of Department of Botany, Aligarh Muslim University, Aligarh. After 30 and 60 DAS, a selected number of plants were assessed for various morphological and physio-biochemical parameters. The rest of the plants were allowed to attain maturity and were harvested to study the yield characteristics (120 DAS). The decrease in the values, caused by NaCl was more pronounced in RH-30 than Varuna. The highest concentration (5.6 ds m⁻³) of the salt was most toxic. All the parameters [growth, chlorophyll content (SPAD level), leaf water potential, photosynthetic attributes, maximum quantum yield of PSII (Fv/Fm), activity of nitrate reductase and carbonic anhydrase enzyme], except antioxidant enzymes, proline content and electrolyte leakage showed a linear decrease as the salt content of the soil increased. 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 laid down 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 were the same as in Experiment 1. At 29 DAS, the foliage of the plants was sprayed 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 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 attributes. The foliar spray of BR analogues increased plant growth, SPAD chlorophyll level, leaf water potential, net photosynthetic rate and its related attributes, Fv/Fm, activities of various enzymes (nitrate reductase, carbonic anhydrase and antioxidant enzymes), leaf proline content, number of pods per plant and seed yield per plant in both the varieties. 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 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 the same as in Experiment 1. At 29 DAS, the foliage of the plants was sprayed with DDW (control), 10 mM, 20 mM or 30 mM of proline. The parameters and the pattern of sampling 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, activity 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 most effective. Varuna showed better response than RH-30.

Experiment 4
This experiment was laid down to elucidate the ameliorative effects of BR analogues on the salinity induced changes in *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. All the agricultural practices and the mode of application of NaCl and BR analogues remained the same as in Experiment 1 and 2, respectively. The characteristics studied and their assessment pattern were the same as mentioned in experiment 1. The presence of NaCl in the soil 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 neutralized the impact of the salt by improving the values of the parameters, studied. The loss in Varuna, under lower NaCl concentrations (2.8 and 4.2 dsm⁻¹) was completely recovered by the follow-up treatment with EBL (10⁻⁸ M) and partially in RH-30. The activity of antioxidant enzymes and total proline content in both the varieties increased with the level of NaCl 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 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 mode of application of NaCl and that of proline remained the same as in Experiment 1 and 3, respectively. The pattern of sampling and the parameters studied were the same as in Experiment 1. The salt stress 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 more prominent in Varuna. The proline application had an additive effect in increasing its own level and the activity of antioxidant enzymes in stressed plants. Proline application improved the yield of the plants under stress free conditions and also in plants, exposed to lower concentration (2.8 dsm⁻¹) of NaCl, particularly in Varuna.

Experiment 6

This experiment was carried out to explicate the cumulative effect of EBL (BR analogue) and proline as foliar spray on the stress-free and stressed plants of *Brassica juncea* (L.) Czern & Coss cv. Varuna and RH-30. All the agricultural practices, mode of treatment of NaCl and the parameters studied remained the same as in Experiment 1. The plants were sprayed with DDW or proline and/or EBL at 28 and 29 DAS, respectively. The mode of sampling and analysis was same as mentioned in
Experiment 1. 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 various enzymes (nitrate reductase and carbonic anhydrase) 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 in *Brassica juncea.*