

28-Homobrassinolide Modulation of Osmolytes in *Brassica Juncea L.* Under Salt Stress



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KEYWORDS : Brassica juncea, salt stress, 28-homobrassinolide, sugars, glycine betaine

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ABSTRACT

The aim of present study was to explore the ameliorative impact of 28-homobrassinolide on morpho-physiological attributes and osmolytes of Brassica juncea L. exposed to oxidative stress caused by salt stress (180 mM NaCl). For this, experiments were carried out at the Plant Conservatory, Department of Botany, Punjabi University, Patiala. 28-homoBL (10-6, 10-9 and 10-12M) primed and unprimed seeds of B. Juncea L. were examined for total carbohydrates, total soluble sugars and accumulation of proline and glycine betaine. In conclusion 28-homoBL showed great potential in protecting the Brassica juncea L. plants from oxidative stress caused by (NaCl) salt stress but in very dose dependent manner

1. Introduction

Salt stress is one of the most important environmental stresses that adversely affect plant growth and development thereby limiting plant productivity. It causes reduction in crop yield and alteration in plant metabolism including reduced water potential, ion imbalance and toxicity. Several hormones are implicated in modulating the plant responses to oxidative stress, including ethylene [1], abscisic acid [2], salicylic acid (SA) [3] and brassinosteroids (BRs) [4]. Brassinosteroids are a class of plant polyhydroxysteroids that are ubiquitously distributed in the plant kingdom. These compounds, when applied exogenously, improve plant quality and yield. They have been further explored for stress-protective properties in plants against a number of stresses like salt [5] and heat [6]. The studies conducted with brassinosteroid in biosynthetic mutants of pea (*Pisum sativum*) [7] and faba bean (*Vicia faba*) [8] provided compelling evidence for the vital role of this group of growth regulators for normal growth and development. However, it is unclear whether BRs are involved in the accumulation of osmolytes under oxidative stresses. *Brassica juncea L.* is an important oilseed crop known for its oil content, edible and medicinal uses. The present study was undertaken to observe the growth and accumulation of osmolytes under the influence of 28-homobrassinolide (28-homoBL).

2. Materials and methods

2.1 Seed Treatments and Growth Conditions

Seeds of *B. juncea L.* cultivar. (RLC-1) were procured from the Department of Plant Breeding, Punjab Agriculture University, Ludhiana, India. Seeds were surface sterilized with 0.01% HgCl₂ and rinsed 5-6 times with double distilled water. The sterilized seeds were soaked for 8h in different concentrations of 28-homoBL (Sigma-Aldrich. USA) (10-6, 10-9 and 10-12M). The treated seeds were propagated in triplicate in cemented pots under natural field conditions. 3 kg soil was added to each pot and 1 litre solution of 180 mM NaCl was added to each pot at the time of sowing. Plants were sampled on the 30th day after sowing for measuring morphological and biochemical parameters.

2.2 Morphological parameters

Shoot length of *B. juncea L.* plants was taken on 30th day after sowing.

2.3 Biochemical parameters

2.4 Total proline content

Total proline content was determined by the method of Bates, [9]. Fresh plant material was homogenized in 3 % aqueous sulphosalicylic acid and the homogenate was centrifuged to 10,000

rpm. Supernatant was taken for estimation of proline content. The reaction mixture consisting of 2 ml supernatant, 2 ml acid ninhydrin and 2 ml of glacial acetic acid was boiled at 100°C for 1 hr. After termination of the reaction in ice bath, the reaction mixture was extracted with 4 ml toluene and the absorbance was read at 520 nm.

2.5 Glycine betaine content

Glycine betaine content was estimated according to the method of Grieve and Grattan, [10]. 0.5 g tissue was crushed and mechanically shaken in 20 ml deionized water for 24 h at 25°C. After filtration 0.5 ml extract was mixed with 1 ml of 2N H₂SO₄ solution. Add 0.2 ml of potassium tri-iodide solution was added and reactants were gently stirred with a vortex mixture. Tubes were stored at 4 °C for 16 h and then 3 ml supernatant and 9 ml of 1,2 dichloroethane was pour in it. By passing continuous stream of air for 1-2 minutes two layers were separated, upper aqueous layer was discarded and optical density of organic layer was recorded at 365 nm. The concentrations of glycinebetaine were estimated by using standard curve developed with different concentration of glycine betaine.

2.6 Total Soluble sugars

Total soluble sugars content was estimated by following Loewus, [11]. Known weight of dried plant material was homogenized in 80% of ethanol then centrifuged at 3000x g for 15 minutes and the extract was collected for sugars estimation. For total sugars 0.05ml of extract was diluted to 2 ml by distilled water and add 3ml cold anthrone reagent and mixed thoroughly. Then mixture was heated for 10 min in boiling water bath and cooled rapidly at room temperature. O.D. was recorded at 630 nm. Amount of total sugars was calculated and expressed as mg/g¹ DW tissue.

2.7 Total Carbohydrates

Total carbohydrates content was determined according to Dubois, [12]. 0.05ml of extract was diluted to 2 ml by distilled water and 0.05 ml of phenol reagent was added to it and mixed thoroughly. Then 5 ml of H₂SO₄ was added rapidly. Blank was prepared by taking distilled water instead of extract. The samples were allowed to stand at room temperature for 30 minutes and optical density was taken at 485 nm. Standard curve was prepared by using glucose (20-100 mg). The amount of carbohydrates was calculated and expressed as mg/g¹ dry weight. The data obtained were presented as means ± SE.

3. Results and Discussion

In the present study, *B. juncea L.* plants showed susceptibility to salt stress as shoot length was 28% inhibited in 180 mM

NaCl treatment but on application of different concentrations of 28-homoBL (10⁻⁶M, 10⁻⁹M and 10⁻¹²M) showed 11%, 59% and 32% increase (Figure 1A). Salt stress decreased shoot length of barley and radish seeds by Cavusoglu and Kabar, [13]. Our previous studies also illustrated that 28-homoBL protect plants starting from their germination till maturity by up and down regulation of various non-enzymatic and enzymatic activities at the cellular level Sirhindi, [14].

In case of osmolytes, the present results showed that the contents of total proline content was significantly induced under salt stress at higher levels (Figure. 1 B) in *B. juncea* L. plants compared with those of unstressed plants. Total proline content (119 %) was induced significantly in 180 mM salt as compared to distilled water control. Applications of different concentrations of 28-homoBL (10⁻⁶M, 10⁻⁹M, 10⁻¹²M) under salt stress enhanced 142% 179%, 129% proline content to significant high level. These results are consistent with Fedina, [15]. Accordingly, Proline has been considered as a carbon and nitrogen source for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules and also, a free radical scavenger [16] Proline is accumulated in many plants that are exposed to water, salinity or cold stress, and the accumulation of proline was positively correlated with stress tolerance [17]. In maize plants that were exposed to water stress proline content increased significantly [18]. In this study, proline content increased remarkably in the *Brassica juncea* L. plants under salt stress and 28-homoBL treatment caused a significant increase in the proline content of plants under salt stress; conditions were compared with those plants which were under salt stress alone (Figure 1B). Proline accumulation was found to be an early response to salt stress, which acts as an osmotic protectant and increased accumulation shows greater tolerance to salt and drought stress. Increased levels of proline contribute to the turgor maintenance of cells and its accumulation is considered as a stress indicator in several plant species under salt stress conditions [19, 20].

Similarly, in case of glycine betaine (Figure 1C) salt stress showed its positive effect. Maximum (142 %, 179%, 129 %) increase in glycine betaine content was found in 180 mM NaCl, supplemented with (10⁻⁶M, 10⁻⁹M and 10⁻¹²M) concentrations of 28-homoBL. Exogenous application of 28-homoBL to plants before exposing to salt stress in our present work showed ameliorative potential in mitigating toxic effect of salt particularly on osmotic relations at cellular level by modulating the level of glycine betaine. Our results are in accordance to that of Rattan *et al.*, [21].

Soluble sugars in plants found to be accumulated at higher level in salinity and temperature stress stress and act as osmoprotectants [22, 23]. Treatment of (10⁻⁶M, 10⁻⁹M and 10⁻¹²M) 28-homoBL to salt stressed plants in present case (Figure 1D), increased level of total soluble sugars (51 %, 69 %, 36 %) which may protect the cellular metabolic machinery by maintaining homeostasis of cells under salinity. Verma *et al.*, [24] reported increased sugar level with the treatment of BRs in *Arachis hypogaea* and Vardhini *et al.*, [25] reported the increased carbohydrate fractions like reducing sugars and starch in the radish roots with the treatment of BRs.

In this work, the contribution of total soluble sugars accumulation to osmotic adjustment was significant, since the total soluble sugars content increased with an increase in salinity (Figure 1D). Similar results were obtained by Morant-Manceau, [26] who reported that the concentrations of sugars change in response to salt stress in *Triticum dicoccum*. Carbohydrates such as soluble sugars (glucose, fructose, sucrose, fructans) accumulate under salt stress to accommodate the ionic balance in the vacuoles [27]. Their major functions are osmoprotection, osmotic adjustment, carbon storage, radical scavenging and stabilization of the

structure of proteins [28]. In our results total carbohydrates content increased in 28-homobrassinolide treated *B. juncea* L. plants (Figure 1E), which are supplemented with 180 mM concentration of salt (105 %, 107 %, and 48 %).

4. Conclusion

The present study shows that, although salt is essential for normal plant growth and physiological processes, above threshold value salt is toxic and may result in growth inhibition and altered metabolic processes. The observations of the present study clearly indicate salt stress-protective properties of 28-homoBL in *Brassica juncea* plants. Stress ameliorative properties of 28-homoBL are clearly demonstrated by better growth, increased level of osmolytes in plants to which various 180 mM and 28-homobrassinolides were applied. It points to the possibility of BR-regulated stress protection in plants but extensive studies are still needed on various aspects related to stress.

5. Future Prospects

Plants often experience various abiotic and biotic stresses like drought, high or low temperature, flooding, salinity, metal toxicity, UV-radiations, herbicides and pathogen stress which adversely affected the crop production and yield. Plants adopted various strategies to adopt the stress conditions and osmolytes accumulation is one of among defensive strategies. Application of BRs at appropriate dose further develops the stress tolerance by enhancing the accumulation of osmolytes production which helps the plants to overcome the stress conditions. Thus our study will further helpful to study the complex and fine mechanisms of osmolytes participation in the creation of resistant plants and help to explore the fundamental signaling mechanism of BRs induced plant stress protection in abiotic stressed plants.

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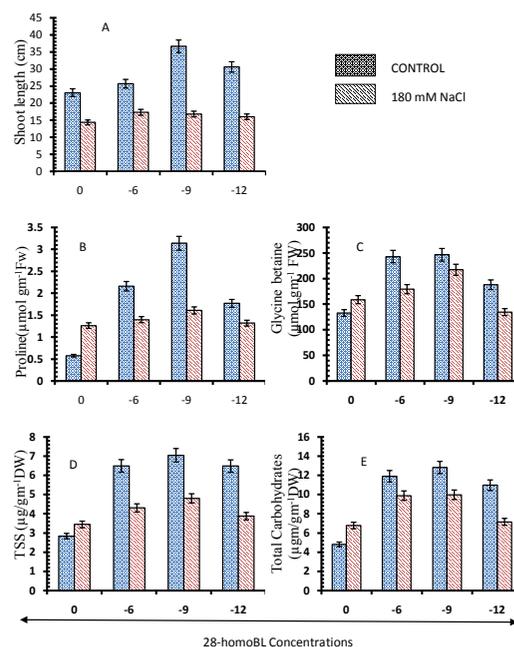


Figure 1A-E- Effect of 28-homoBL on Shoot length (A), Total Proline (B), Total Glycine betaine (C), Total soluble Sugars content (D), Total carbohydrates (E), on 30 days old *B. juncea* L. plants under salt stress [Bars represent the SE (n=3)]

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