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28-Homobrassinolide ameliorates the saline stress in chickpea (*Cicer arietinum* L.)

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Abstract

The response of chickpea (*Cicer arietinum* L.) cv. KPG-59 to pre-sowing seed treatment with 28-homobrassinolide (HBR) and/or sodium chloride (NaCl) was investigated. The seeds imbibed in aqueous solution of 10^{-10} or 10^{-8} M of HBR for 8 h, resulted in an increase in the values for most of the characteristics of shoot and root at 90-day stage and seed yield, at harvest. The plants resulting from the seeds soaked in HBR (10^{-8} M) possessed 23% and 31% higher leaf nitrate reductase (E.C. 1.6.6.1) and carbonic anhydrase (E.C. 4.2.2.1) activities, 34% more dry mass, 30% higher nodule number, 31% and 18% more nodule fresh and dry mass, compared with water soaked, control. Leghaemoglobin content and nitrogenase activity (E.C. 1.7.99.2) were 28% and 30% higher while nodule nitrogen and carbohydrate contents decreased by 5% and 6%, compared with the control. Moreover, seed yield increased by 26% over the control, at harvest. The values for all the above characteristics declined significantly, in the plants raised from the seeds soaked in NaCl. However, this ill effect was overcome, if NaCl treatment was given before or after HBR treatment.

Keywords: *Cicer arietinum*; Chlorophyll; Carbonic anhydrase; 28-Homobrassinolide; Nitrate reductase; Nitrogenase; Proline

1. Introduction

Soil salinity is one of the major environmental problems that poses a severe threat to the growth of plants and their productivity. It 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). The salinity stress, particularly in legumes, adversely effects the nodulation; the activity of nitrogenase (Sheokand et al., 1995). Glutamin synthetase and NADH-glutamate synthase (Soussi et al., 1998). The stress generated by NaCl, also decreases the level of nitrate reductase (Aslam et al., 1984), the primary enzyme in the assimilation of nitrate (Solomonson and Barber, 1990). All these and other related events lead to poor plant growth and productivity.

Out of the various compounds exploited to alleviate the plant stress (Chakrabarti and Mukherjee, 2003), the brassinosteroids (BRs) are recognized as a novel group to regulate the plant growth and their productivity (Rao et al., 2002). Moreover, BRs are also recognized as regulators of transcription and translation (Kalinich et al., 1985; Bajguz, 2000) thereby improving the level of total proteins (Bajguz, 2000), enzymes (Hayat and Ahmad, 2003; Hayat et al., 2003; Fariduddin et al., 2004), the rate of nitrogen fixation (Vardhini and Rao, 1999) and finally the seed yield, at harvest (Hayat and Ahmad, 2003; Hayat et al., 2003; Fariduddin et al., 2004, 2005).

Besides this, BRs have an ameliorative role in plants, under biotic and abiotic stresses (Rao et al., 2002). However, it will be pre-mature to assign specificity in its action, under these conditions, unless additional work is undertaken in this direction. Chickpea is considered to be sensitive to salt stress (Dua, 1992). This work was, therefore, undertaken to explore the possible remedial measures by using 28-homobrassinolide, a highly active and stable steroidal hormone (Khripach et al., 2000). We tested the hypothesis that the application of 28-homobrassinolide will ameliorate the ill effect of salinity on the growth of chickpea plants and finally improve the yield potential.

2. Materials and methods

The seeds of chickpea (*Cicer arietinum* L.) cv. KPG-59 were obtained from National Seed Corporation Ltd., New Delhi. The

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healthy seeds were surface sterilized with 5% sodium hypochlorite solution followed by repeated washing with double distilled water (DDW). These seeds of the first set were soaked, for 8 h in: (i) DDW, (ii) 10^{-10} M HBR, (iii) 10^{-8} M HBR, (iv) 1 mM NaCl, or (v) 10 mM NaCl. In the second set, the seeds were soaked for 4 h, in 1 or 10 mM of NaCl and then transferred to 10^{-10} or 10^{-8} M of HBR for additional 4 h. In the third set, the seeds were soaked, for 4 h in 10^{-10} or 10^{-8} M of HBR and resoaked in 1 or 10 mM of NaCl, for another 4 h.

The treated seeds were washed with DDW, to remove adhering solutions and inoculated with *Rhizobium* sp. The seeds were sown in earthen pots (25 cm diameter), filled with sandy loam soil and farmyard manure, in a ratio of 9:1. Three plants per pot were maintained. The plants were sampled at 60, 90 and 120 days, after sowing to assess their dry mass and number, fresh and dry mass of nodules. The level of nitrogenase, nitrate reductase and carbonic anhydrase and the contents of leghaemoglobin, nitrogen, protein, proline, carbohydrate and chlorophyll were also determined. The remaining plants were harvested 160 days after sowing (DAS) for assessing the yield characteristics. The experiment was conducted in 2002–2003 and 2003–2004. The results presented in the tables are pooled data of both the years.

2.1. Measurement of growth characteristics

Three plants per treatment were uprooted at 60, 90 and 120 DAS. The samples were washed under tap water and dried in a hot air oven, run at 80 °C for 24 h. The samples were weighed to obtain dry mass per plant. To obtain nodule number per plant the whole mass of soil with the plant, from each treatment, was taken out of the pot and placed in a bucket, filled with water. The roots were then washed under running tap water and the nodules were counted. The nodules from each pot were weighed to get their fresh mass. The nodules were transferred to petriplates for overnight drying in an oven at 80 °C. The dried material was weighed to obtain dry mass of nodules per plant.

2.2. Determination of enzyme activities

The activity of nitrogenase was determined, in the fresh samples by the method of Hardy et al. (1968). Nodules were incubated with acetylene for 30 min at 27 ± 2 °C. The air (5 cm^3), from each incubation chamber, was removed with a syringe and ethylene content was measured on a gas chromatograph (GC 5700, Nucon, New Delhi) equipped with 1.8 m Porapack N (80/100 mesh) column, a flame ionization detector and an integrator. Nitrogen was used as carrier gas. The flow rate of nitrogen, hydrogen and oxygen were 0.5, 0.5 and 5 mL s^{-1}. The oven temperature was 100 °C and that of the detector was 150 °C. Ethylene identification was based on the retention time and was quantified by comparing with the standard curve drawn with pure ethylene.

The activity of nitrate reductase was determined, in fresh leaf samples, by the procedure explained by Jaworski (1971). This method is based on the reduction of nitrate to nitrite, whose quantity was estimated calorimetrically at 540 nm. Leaf carbonic anhydrase activity was assayed by the method of Dwivedi and Randhava (1974) where 200 mg of fresh sample was cut into small pieces in 0.2 M cystein hydrochloride, at 4 °C. These pieces were transferred to test tube containing phosphate buffer (pH 6.8) and 0.2 M sodium bicarbonate. The CO_2 released, during catalytic action of enzyme on $NaHCO_3$, was estimated by titrating the reaction mixture against HCl, using methyl red as an indicator.

2.3. Leghaemoglobin content

The leghaemoglobin content, in fresh nodules, was quantified as described by Sadasivam and Manickam (1992). It was extracted with sodium phosphate buffer (pH 7.4). The extract was divided equally into two test tubes, each one added with same volume of alkaline pyridine reagent. The hemochrome formed, was read at 539 and 556 nm on a spectrophotometer (Spectronic 20D, Milton Roy, USA) after adding a few crystals of potassium hexacyanoferrate and sodium dithionite, respectively.

2.4. Nitrogen content

The nodule nitrogen content was estimated by employing the method used by Lindner (1944). Oven dried samples were digested with sulphuric acid and H_2O_2 to get a clear solution. To the digested material, 2.5N NaOH, 1% sodium silicate and Nessler's reagent were added. The absorbance of solution was read at 525 nm, on a spectrophotometer.

2.5. Total carbohydrate content

The carbohydrate was extracted following the method of Yih and Clark (1965) and estimated by adopting the procedure of Dubois et al. (1956). Dried powder of nodules was transferred to a glass centrifuge tube, containing 1.5N H_2SO_4. The sample was centrifuged at 4000 rpm, for 10 min. One cubic centimetre of the supernatant was taken in a test tube to which 1 cm^3 of 5% distilled phenol was added. The absorbance was read at 490 nm, using spectrophotometer.

2.6. Total protein content

The protein content, in leaves and seeds, was determined by the method of Lowry et al. (1951). The samples were homogenized in DDW and 5% trichloroacetic acid was added to precipitate the proteins. The precipitate was dissolved in 1% NaOH solution. Blue colour developed by using Folin phenol reagent and the absorbance was read at 660 nm, using spectrophotometer.

2.7. Proline content

The proline content in fresh leaf sample was determined by adopting the method of Bates et al. (1973). Sample was extracted in sulphosalicyclic acid. In the extract, an equal quantity of glacial acetic acid and ninhydrin were added. The sample was