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RESEARCH ARTICLE

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

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Abstract

Boron toxicity is an environmental constraint that limits crop productivity worldwide. The aim of the present study was to explore the boron-induced modulation in growth, photosynthesis, antioxidant system under varied levels of boron in two varieties of *Brassica juncea* L. Czern & Coss var. Varuna and Chapka rohini. The surface-sterilized seeds of these varieties were sown in soil amended with different levels (0, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) of boron and were sampled at 45 d stage of growth. The boron treatments (20, 30, 40, 50 or 60 mg kg^{-1}) significantly decreased growth, net photosynthetic rate and its related attributes, chlorophyll fluorescence, SPAD value of chlorophyll, and leaf carbonic anhydrase and nitrate reductase activities whereas, the proline content and the level of various antioxidant enzymes (catalase, peroxidase and superoxide dismutase) increased in both the varieties. Out of the graded concentrations of boron, 20 mg kg^{-1} was least toxic and 60 mg kg^{-1} B generated maximum toxicity. However, the application of 10 mg kg^{-1} B did not generate any significant effect in almost all the parameters. Furthermore, the variety Varuna was found more tolerant than Chapka rohini to the boron stress and possessed higher values for growth, photosynthetic attributes and antioxidant enzymes. The variation in the responses of these two varieties to boron toxicity is attributed to their differential photosynthetic traits, SPAD chlorophyll value and antioxidant capacity, which could be used as potential markers for screening mustard plants for boron tolerance.

INTRODUCTION

Boron (B) is an essential micronutrient required for the normal growth of higher plants and is absorbed by plants from soil solution in the form of boric acid (Dordas et al., 2000). There is a narrow window between deficiency and toxicity in soil-plant systems however, the risk of inducing toxicity should not be ignored (Herrera-Rodriguez et al., 2010; Siddiqui et al., 2013). The content of B varies in the soil as some soils contain insufficient B to support normal plant growth, while others contain excess of B, which causes toxicity in plants (Esim et al., 2012). It has long been known that the optimum B level for one species could be either toxic or insufficient for other species (Blevins and Lukaszewski, 1998). In soils deficiency may take place below 0.5 mg B kg^{-1} while soil containing more than 5 mg B kg^{-1} could cause toxicity symptoms in most of the crops (Gupta, 2007).

Boron toxicity is an important micronutrient disorder affecting the productivity of many cultivated crops across the world, including oilseed crops i.e., Brassicas (Campbell et al., 1998). The most obvious symptoms of toxicity are leaf burns and chlorotic and necrotic patches, often visible at the margins and tips of older leaves. These symptoms reflect the tissue distribution of boron in most species, with accumulation at the ends of the transpiration stream (Nable et al., 1990). Plant growth and seed yield are typically reduced when boron toxicity is present (Yau and Saxena, 1997). In addition, B toxicity causes physiological and morphological defects in plants such as

decreased shoot and root growth (Lovatt and Bates, 1984; Nable et al., 1997), inhibition of photosynthesis and lower stomatal conductance (Lovatt and Bates, 1984), decreased proton extrusion from roots (Roldan et al., 1992), decreased root cell division (Liu et al., 2000; Choi et al., 2007), deposition of lignin and suberin in the roots (Ghanati et al., 2002), increased membrane permeability, lipids peroxidation and hydrogen peroxide (H_2O_2) content, and changed the activities of antioxidant enzymes (Herrera-Rodriguez et al., 2010; Esim et al., 2012).

Like other abiotic stresses (drought, salinity, cold, heat and heavy metals), excess B lead to the production of reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$) radicals, hydrogen peroxide, and free singlet oxygen (Siddiqui et al., 2013; Archana and Pandey 2015; Landi et al. 2014). These over-accumulated ROS trigger detrimental effects on cellular processes, including oxidative damage to nucleic acids, proteins, lipids, and resulting in the alteration in antioxidative enzymes activities (Molassiotis et al., 2006; Tombuloglu et al., 2012). To prevent themselves from the harmful effects of these reactive molecules, plant have evolved an effective scavenging system of antioxidant enzymes such as superoxide dismutase, peroxidase and catalase (Apel and Hirt, 2004). Among them, superoxide dismutase as a major scavenger decomposes superoxide ion ($O_2^{\bullet-}$) to H_2O_2 and molecular oxygen that are further detoxified to O_2 and H_2O by catalase and/or peroxidase via ascorbate-glutathione pathway (Karabal et al., 2003). It was observed that high supply of B invoked the formation of ROS which induced oxidative damage by lipid peroxidation and accumulation of hydrogen peroxide in leaves (Karabal et al., 2003; Molassiotis et al., 2006). Cervilla et al. (2012) also reported oxidative damage and altered antioxidative enzyme activity in tomato leaves under B toxicity, indicating a crucial role of antioxidant system in conferring tolerance to B stress in plants.

The present study was carried out to examine the changes in growth, photosynthetic characteristics, chlorophyll pigments, chlorophyll fluorescence, and level of non-enzymatic and enzymatic antioxidants in two varieties i.e., Varuna and Chapka rohini of mustard (*Brassica juncea* L.) to the different concentrations of B.

MATERIALS AND METHODS

Plant material and experimental design

The authentic and healthy seeds of *Brassica juncea* L. var. Varuna and Chapka rohini obtained from National Seed Corporation Ltd. (New Delhi, India) were surface sterilized with 1% sodium hypochlorite solution, followed by rinsing with deionized water at least thrice. These surface-sterilized seeds of two varieties were sown in earthen pots (20 cm in diameter, 20 cm in depth) filled with sandy loam soil and farmyard manure (mixed to the ratio of 6:1) and lined in a net house, where the average day/night temperature, relative humidity and photoperiod were 25°C/20, 6±3% and 12 h, respectively. Before sowing seeds, the different levels of B (0, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) were given through the soil in the form of boric acid (H_3BO_3). 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 45 DAS to assess the growth, photosynthetic and biochemical parameters. The remaining plants were allowed to grow up to maturity and were harvested at 120 DAS to study the yield characteristics.

Plant growth analysis

The plants were uprooted and washed under running tap water to dislodge the soil particles to analyze the several growth parameters. The length of the shoot and root was measured on a meter scale. The shoots and roots were weighed separately to record their fresh mass and placed in an oven run at 80°C for 72 h. The samples were weighed again to obtain their respective dry mass. The leaf area was measured by leaf area meter (ADC Bioscientific, Hoddesdon, Herts, UK).

Determination of SPAD value of chlorophyll and photosynthetic parameters

The chlorophyll content in the leaves was measured with the help of a SPAD chlorophyll meter (Konica Minolta Sensing Inc., Japan). The rate of photosynthesis and its related parameters (photosynthetic rate, stomatal conductance, internal CO_2 concentration, and transpiration rate, were measured by using a portable photosynthesis system (LI-COR 6400; LI-COR Lincoln, NE, USA). Where, the air temperature, relative humidity, CO_2 concentration and photosynthetic photon flux density (PPFD) were maintained at 25°C, 85%, 600 µmol mol^{-1} and 800 µmol mol^{-2} s^{-1}, respectively. The measurements were made in the uppermost fully expanded leaves between, 11:00 and 13:00 hours.

Analysis of chlorophyll fluorescence i.e. maximum quantum yield of photosystem II (Fv/Fm)

Chlorophyll fluorescence i.e., maximum quantum yield of photosystem II (Fv/Fm) was measured by using a leaf chamber fluorometer (LI-COR 6400-40, LI-COR Lincoln, NE, USA). All the measurements were carried out at a PPFD of 1,500 µmol m^{-2} s^{-1} with a constant airflow rate of 500 µmol s^{-1}. The sampled leaf was dark adapted for 30 min, prior to the measurement of Fv/Fm.

Determination of nitrate reductase and carbonic anhydrase activity

Nitrate reductase activity was measured following the method laid down by Jaworski (1971). The fresh leaf samples were cut into small pieces and transferred to plastic vials, containing phosphate buffer (pH 7.5), KNO_3 and

isopropanol which were incubated at 30°C for 2 h. After incubation, sulfanilamide and N-1-naphthylethylenediamine hydrochlorides solutions were added. The absorbance was read at 540 nm on a spectrophotometer (Spectronic 20D; Milton Roy, USA). Carbonic anhydrase activity was determined using the procedure described by Dwivedi and Randhawa (1974). The leaf samples were cut into small pieces in cystein hydrochloride solution. These samples were blotted and transferred to the test tubes followed by the addition of phosphate buffer (pH 6.8), 0.2 M $NaHCO_3$, bromothymol blue, and the methyl red indicator, at the last. This reaction mixture was titrated against 0.05 N HCl. The activity of the enzyme was expressed on a fresh mass basis.

Antioxidant enzyme activity

For the assay of antioxidant enzymes, the leaf tissue (0.5 g) was homogenized in 50 mM phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone. The homogenate was centrifuged at 15,000 g for 10 min at 4°C and the supernatant was used as source of enzymes catalase, peroxidase and superoxide dismutase. Catalase and peroxidase were assayed following the method described by Chance and Maehly (1956). Catalase was estimated by titrating the reaction mixture consisting of phosphate buffer (pH 6.8), 0.1 M H_2O_2, enzyme extract and 2% H_2SO_4 against 0.1N $KMnO_4$ solution. The reaction mixture for peroxidase consisted of pyragallol, phosphate buffer (pH 6.8), 1% H_2O_2 and enzyme extract. Change in absorbance due to catalytic conversion of pyragallol to perpurogallin was noted at an interval of 20 s for 2 min, at 420 nm on a spectrophotometer. A control set was prepared by using deionized water instead of enzyme extract. The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Beauchamp and Fridovich (1971). The reaction mixture consisted 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA and 0-50 µL enzyme extract and were placed under 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 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.

Determination of proline content

The proline content in fresh leaf was determined by adopting the method of Bates et al., (1973). The samples were extracted in sulphosalicylic acid. To the extract, an equal volume 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 of toluene layer was read at 528 nm on a spectrophotometer (Spectronic-20D, Milton Roy, USA).

Statistical analysis

Data were statistically analyzed using SPSS, 17.0 for windows (SPSS, Chicago, IL, USA). Standard error was calculated and analysis of variance (ANOVA) was performed on the data to determine the least significance difference (LSD) between treatment means with the level of significance at $P \leq 0.05$.

RESULTS

Growth biomarkers

The length, fresh and dry mass of the shoot and root, and leaf area of both the varieties showed a marked decrease on being subjected to different levels of B (Fig. 1 and 2A). Out of the different levels (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of B, 10 mg kg^{-1} did not generate significant effect whereas, 20 mg kg^{-1} proved least toxic. However, the highest concentration of B (60 mg kg^{-1}) generated severe damage and caused maximum per cent decrease in the shoot length by 24% and 39%, root length by 31% and 44%, shoot fresh mass by 26% and 39%, root fresh mass by 37% and 51%, shoot dry mass by 30% and 39%, root dry mass by 36% and 43% and leaf area by 28% and 41% in Varuna and Chapka rohini respectively, compared with their respective controls. The damage was more prominent in Chapka rohini than in Varuna.

SPAD value of chlorophyll

Maximum chlorophyll content was recorded in unstressed (control) plants where the variety Varuna possessed higher values for chlorophyll content than Chapka rohini (Fig. 2B). Out of different levels (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of B, 10 mg kg^{-1} did not generate significant decline but above this there was observed significant decline in chlorophyll content. Moreover, the highest concentration (60 mg kg^{-1}) of B caused maximum decrease by 26% and 38% in Varuna and Chapka rohini respectively, compared to their controls.

Photosynthetic parameters

The plants raised from the seeds sown in the soil fed with different levels (10, 20, 30, 40, 50 or 60 mg kg^{-1}) of B except 10 mg kg^{-1} showed significant decrease in the net photosynthetic rate (P_N) and its related attributes [stomatal conductance (g_s), internal CO_2 concentration (Ci) and transpiration rate (E)] in Varuna and Chapka rohini (Fig. 2C-F). The decrease was proportionate to the concentrations of B. The highest concentration (60 mg kg^{-1}) of B decreased P_N, g_s, Ci and E by 31%, 35%, 25%, and 22% in Varuna and 44%, 50%, 41% and 36% in Chapka rohini

respectively, when compared to their control plants. Moreover, the Chapka rohini was more sensitive to B stress than Varuna.

Maximum quantum yield of photosystem II (Fv/Fm)

Fig. 3A shows that the presence of different concentrations (20, 30 40, 50 or 60 mg kg^{-1}) of B in the soil decreased the maximum quantum yield of photosystem II (Fv/Fm) in both the varieties. However, the highest concentration of B (60 mg kg^{-1}) proved most deleterious and decreased the Fv/Fm by 26% and 38% in Varuna and in Chapka rohini respectively, compared to their controls. Moreover, 10 mg kg^{-1} B did not generate significant impact in Fv/Fm. The order of toxicity of B was 20<30<40<50<60 mg kg^{-1}. The variety Varuna possessed higher values for Fv/Fm than Chapka rohini.

Carbonic anhydrase and nitrate reductase activity

The plants raised in the presence of different levels (20, 30, 40, 50 or 60 mg kg^{-1}) of B had lower activity of carbonic anhydrase and nitrate reductase enzymes compared with unstressed (control) plants in concentration dependent manner (Fig. 3B and C). Therefore, 60 mg kg^{-1} generated maximum toxicity in both carbonic anhydrase (18% and 30% lower than control) and nitrate reductase (28% and 36% lower than control) in Varuna and Chapka rohini respectively, compared to the respective controls. Moreover, 10 mg kg^{-1} B did not induce significant effect in carbonic anhydrase and nitrate reductase activity. The loss in the activity of these enzymes was more prominent in Chapka rohini than Varuna.

Antioxidant enzymes

Unlike the other parameters, the activity of antioxidant enzymes catalase, peroxidase and superoxide dismutase showed completely different response (Fig. 3D-F). The data revealed that the antioxidant enzyme activity increased in response to the concentrations (20, 30, 40, 50 or 60 mg kg^{-1}) of B in the soil in both the varieties. The plants raised in the soil amended with the highest B level (60 mg kg^{-1}) possessed maximum values for antioxidant enzymes in both the varieties. The values for catalase, peroxidase and superoxide dismutase activity increased by 40%, 44% and 54% in Varuna and 25%, 30% and 39% in Chapka rohini respectively, compared to their respective control plants.

Proline content

As evident from the Fig. 4A, the leaf proline content was higher in the plants that were raised in the presence of excess B in the soil. The values increased with an increase in the concentration of the B, whereas 10 mg kg^{-1} did not generate any significant increase in proline content. Maximum values were found in the plants which were fed with 60 mg kg^{-1} of B through the soil in both the varieties and the increase was 69% and 51% in Varuna and Chapka rohini respectively, over the respective controls.

Yield attributes

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 values in response to the different concentrations of B (20, 30 40, 50 or 60 mg kg^{-1}) present in the soil in both the varieties, at harvest (Fig. 5). The maximum reduction in the values of all yield attributes was noticed at 60 mg kg^{-1} of B and decreased the number of pods per plant, number of seeds per pod, 100 seed mass and seed yield by 28%, 19%, 15% and 25% in Varuna and 34%, 27%, 24% and 31% in Chapka rohini at respectively, compared to their control plants. Furthermore, there was no significant reduction in all yield parameters at10 mg kg^{-1}. The variety Chapka rohini was more prone to the stress than the Varuna.

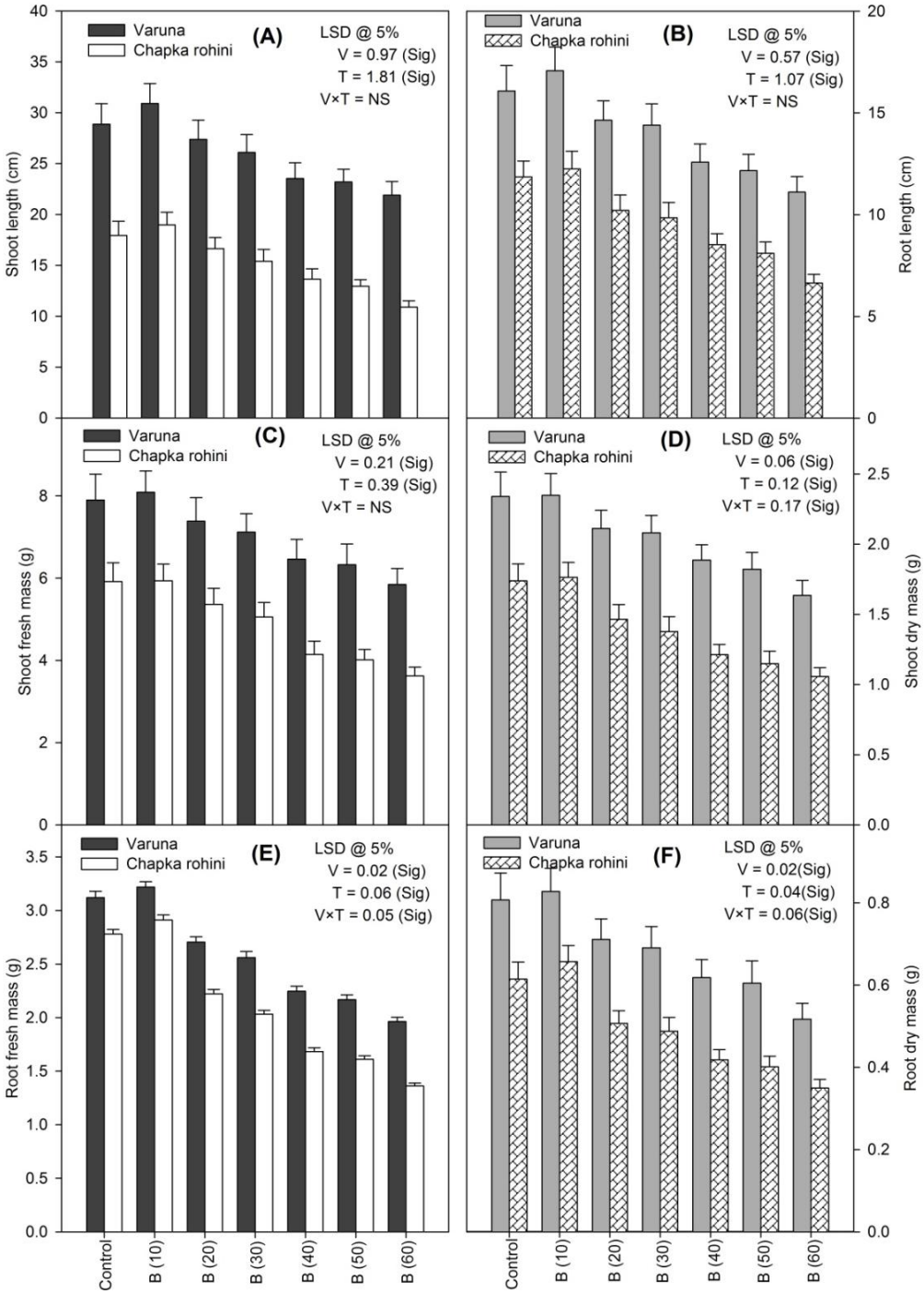

Fig. 1 Effect of different levels of soil applied B (Control, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) on (A) shoot length, (B) root length, (C) shoot fresh mass, (D) shoot dry mass, (E) root fresh mass, (F) root dry mass, in two varieties (Varuna and Chapka rohini) of *Brassica juncea* at 45 DAS. Bar represents the standard error.

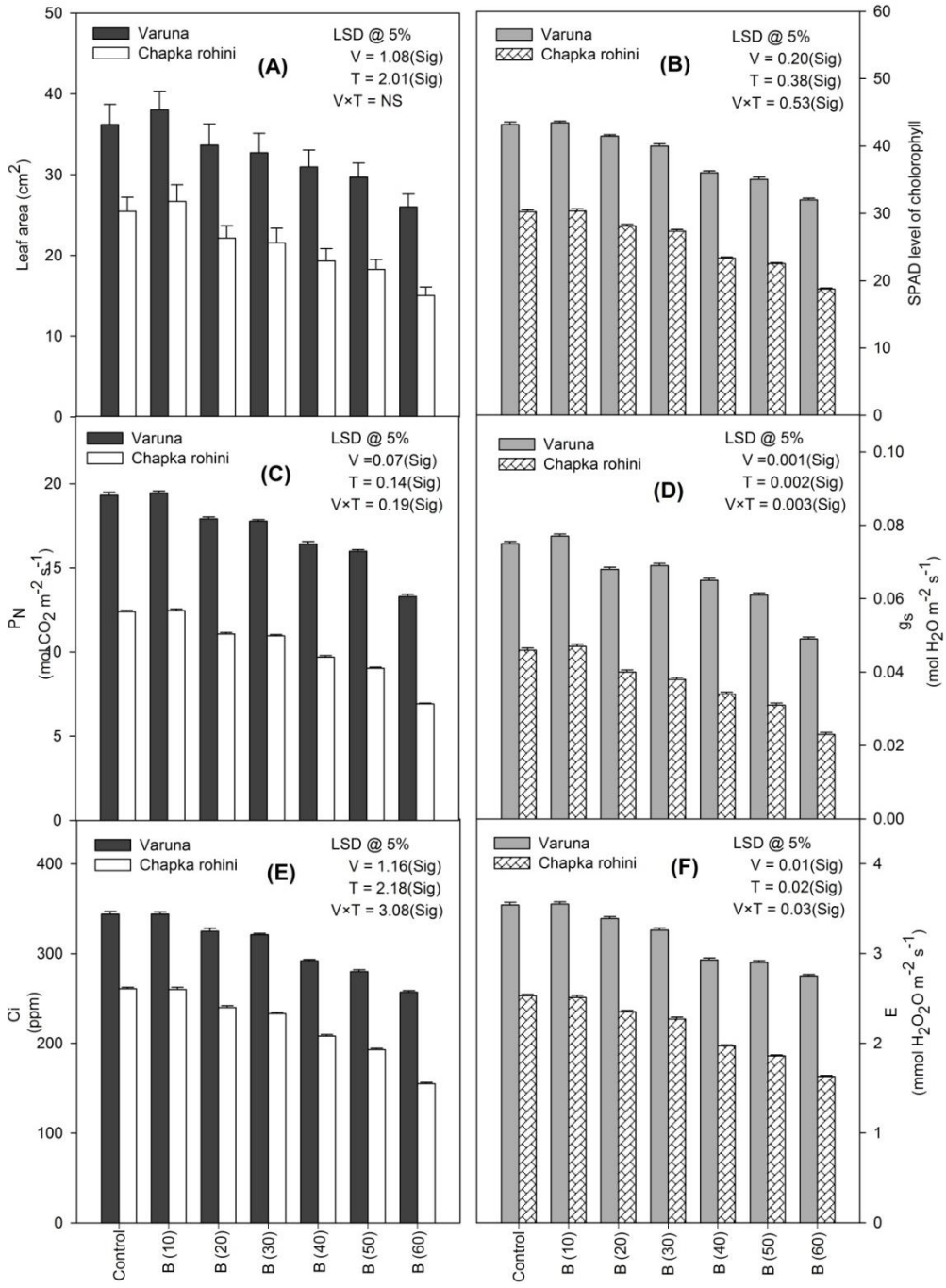

Fig. 2 Effect of different levels of soil applied B (Control, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) on (A) leaf area, (B) SPAD value of chlorophyll, (C) net photosynthetic rate (P_N), (D stomatal conductance (g_s), (E)) internal CO_2 concentration (Ci), (F) transpiration rate (E), in two varieties (Varuna and Chapka rohini) of *Brassica juncea* at 45 DAS. Bar represents the standard error.

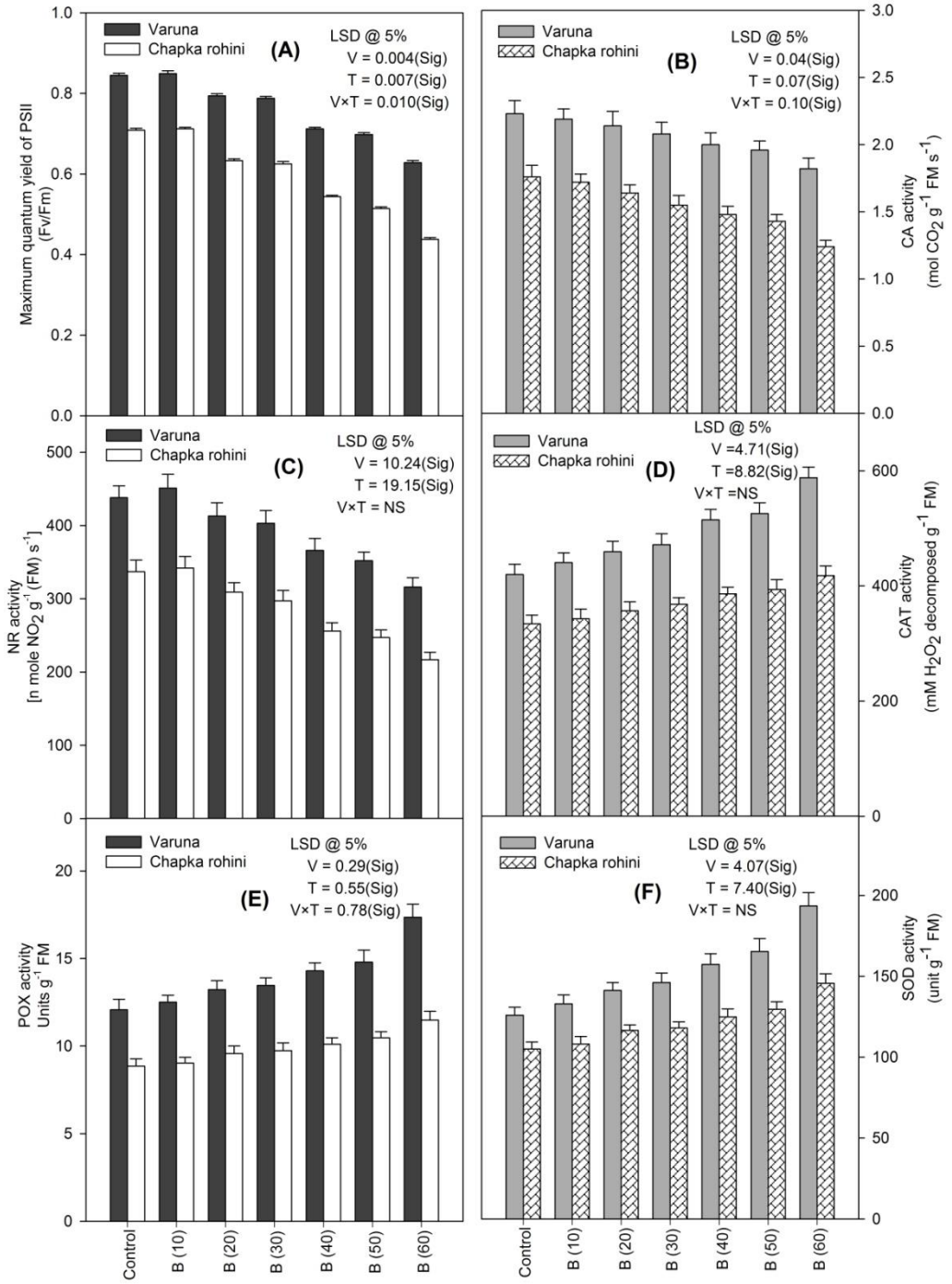

Fig. 3 Effect of different levels of soil applied B (Control, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) on (A) maximum quantum yield of photosynthesis (Fv/Fm), (B) carbonic anhydrase (CA) activity, (C) nitrate reductase (NR) activity, (D) catalase (CAT) activity, (E) peroxidase (POX) activity, (F) superoxide dismutase (SOD) activity, in two varieties (Varuna and Chapka rohini) of *Brassica juncea* at 45 DAS. Bar represents the standard error.

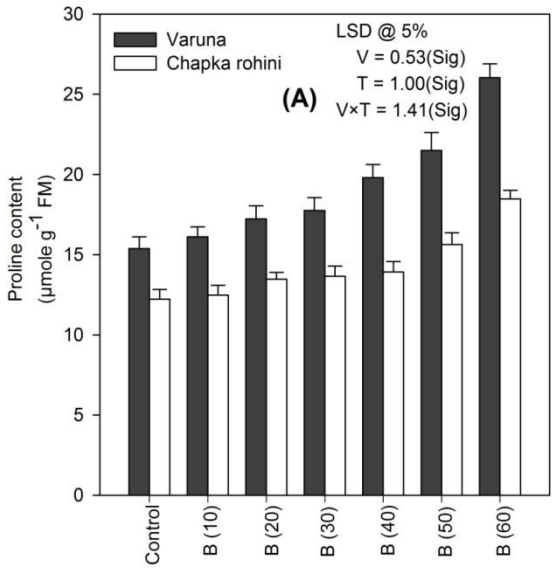

Fig. 4 Effect of different levels of soil applied B (Control, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) on (A) leaf proline content, in two varieties (Varuna and Chapka rohini) of *Brassica juncea* at 45 DAS. Bar represents the standard error.

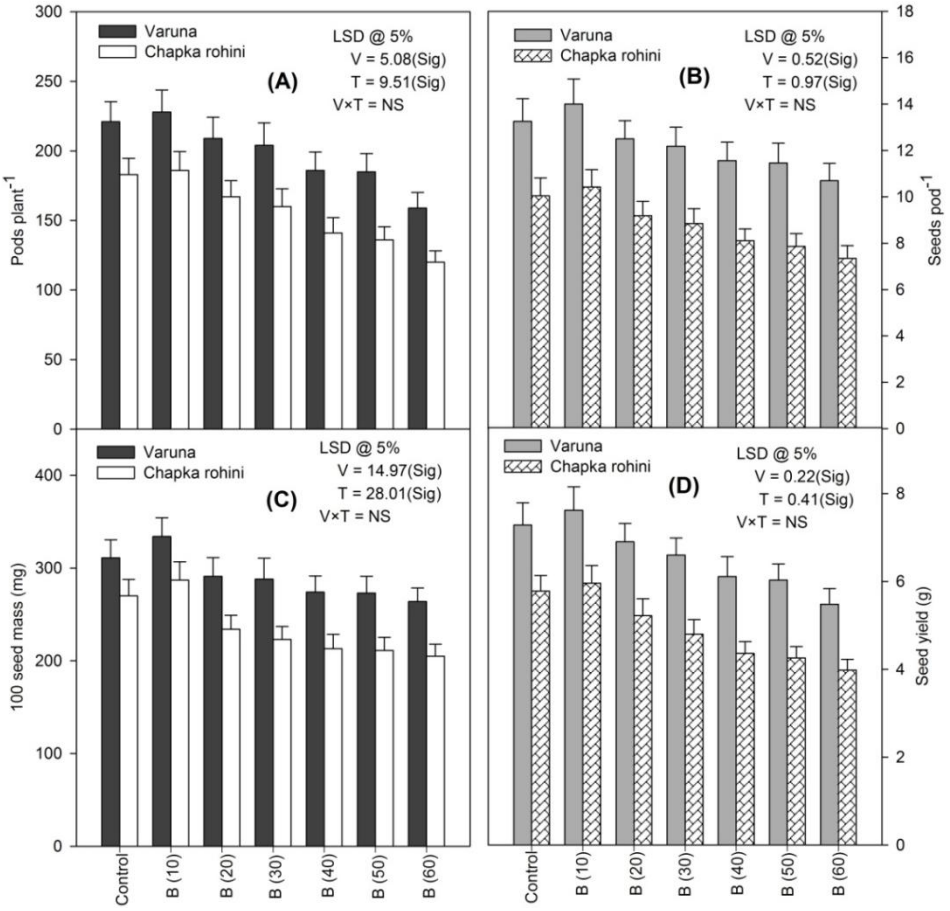

Fig. 5 Effect of different levels of soil applied B (Control, 10, 20, 30, 40, 50 or 60 mg kg^{-1}) on (A) pods $plant^{-1}$, (B) seeds pod^{-1}, (C) 100 seed mass, (D) seed yield, in two varieties (Varuna and Chapka rohini) of *Brassica juncea* at 45 DAS. Bar represents the standard error.

DISCUSSION

All the growth parameters (fresh and dry mass of roots and shoots, their lengths and the leaf area) was not significantly reduced in mustard plants grown in the soil amended with 10 mg kg^{-1} of B but was significantly reduced with 20, 30, 40, 50 or 60 mg kg^{-1} of B on both varieties i.e. Varuna and Chapka rohini (Fig. 1 and 2A). High levels of B suppressed plant growth which might be due to the reduction in cell division and elongation (Lovatt and Bates, 1984; Nable et al., 1997) because these processes are responsible in the retardation of normal biosynthetic activities or energy transduction, protein synthesis, and inhibition of many cellular processes (Reid et al., 2004) and altered activities of antioxidant enzymes (Karabal et al., 2003; Keles et al., 2004) which will naturally impair plant growth and finally the yield (Fig. 5). These results are in conformity with tomato (Cervilla et al., 2009), apple rootstocks (Mouhtaridou et al., 2004), mustard (Javid et al., 2014), wheat (Coskun et al., 2014) and watermelon (Hamurcu et al., 2015). Present study also revealed that the root growth (root length, fresh weight and dry weight) was more affected than the growth of shoots due to B toxicity in both the varieties i.e., Varuna and Chapka rohini (Fig. 1). This might be due to the more accumulation of B in roots that resulted in reduced elongation and lateral root development (Kaur et al., 2006). The inhibition in root elongation may be correlated with a decrease in either cell numbers or cell width (Choi et al., 2007; Ardic et al., 2009). Furthermore, out of the two varieties, Varuna is likely more tolerant as reflected by minimum loss in plant growth. This relative tolerance of genotype of a species to B could possibly be due to differences in their abilities to passively transport of B, probably due to differences in membrane composition affecting transmembrane movement of B. Lipid composition of the plasma membrane could affect the total uptake of B in mutants of *Arabidopsis* (Dordas et al., 2000). Therefore, the improved tolerance of Varuna to B could be related to higher exclusion of B in the roots mediated by reduced permeability of membrane lipids and/or presence of carriers (BOR and NIP) essential for exclusion (Miwa et al., 2007).

The SPAD value of chlorophyll decreased significantly in the B stressed (20, 30, 40, 50 or 60 mg kg^{-1}) leaves of both varieties (Fig. 2B). This inhibition of chlorophyll value might be due to the B induced production of reactive oxygen species (ROS) (Reid et al., 2004; Camacho-Cristobal et al., 2008; Han et al., 2009) which lead to photo-oxidative damages in organic molecules (Papadakis et al., 2004). The observations are further corroborated by the findings in *Vigna radiata* (Hasnain et al., 2011) and *Phaseolus vulgaris* (Nagesh et al., 2012). In other studies, oxidative damage in apple and grape (Gunes et al., 2006) and photooxidation damage to organic molecules in orange plants were induced by B toxicity (Cervilla et al., 2007).Besides this, fig. 2C-F revealed that B induced decrease in net photosynthetic rate (P_N) and related attributes (g_s, Ci, and E) along with chlorophyll fluorescence i.e., maximum quantum yield of photosystem II (Fv/Fm) (Fig. 3A) in a concentration dependent manner. One of the probable reason for the reduction of P_N, g_s, Ci, and E (Fig. 2C-F) is the structural damage of thylakoids, which affects the photosynthetic transport of electrons, as indicated by the reduction of the ratio between variable fluorescence and initial fluorescence (Fv/F_0) (Pereira et al., 2000). The decrease in net photosynthetic rate could be attributed to oxidation of chlorophyll and chloroplastic membranes, which might be exacerbated by the excess B in the soil (Lee, 2006; Ardic et al., 2009). Furthermore, increased levels of B reduced the internal CO_2 concentration, so less of the absorbed photon-energy, captured by the light harvesting system, is expected to be used in the electron transport system, thus decreasing photosynthesis (Aftab et al., 2011; Landi et al., 2012). A significant reduction in CO_2 assimilation due to B toxicity has also been reported in various species such as summer squash (Lovatt and Bates, 1984), kiwi fruits (Sotiropoulos et al., 2002), citrus (Han et al., 2009; Sheng et al., 2010) and pear (Wang et al., 2011). The diminution of maximum quantum yield of photosystem II i.e., chlorophyll fluorescence (Fv/Fm) (Fig. 3F) under B stress due to the molecular O_2 operates as an alternative acceptor for non-utilized electrons and light energy (Velez-Ramirez et al., 2011), resulting thus in the generation of ROS. The ability of ROS to cause photooxidative damages in organic molecules could probably explain the structural damages in the chloroplasts, and the reductions of leaf chlorophyll (Fig. 2B; Han et al., 2009; Chen et al., 2012).

The excess B (10, 20, 30, 40, 50 or 60 mg kg^{-1}), except 10 mg kg^{-1} B caused the inhibition of carbonic anhydrase and nitrate reductase e activity (Fig. 3B-C). The variety, Varuna expressed slight tolerance, compared with Chapka rohini. The possible reason behind this may be that B has ability of metabolic disruption by binding to the ribose moieties of molecules such as ATP, NADH or NADPH (Reid et al., 2004) restricting the uptake of nitrate (Harnandez et al., 1996) and also an inhibition and/or metabolic dysfunction of the enzyme protein (Hopkins, 1995). Boron toxicity caused the inhibition of protein synthesis through the formation of borate esters with ribose (Reid 2007) and also altered the activities of several enzymes, and consequently the plant metabolism (Herrera-Rodrigues et al., 2010).

As a natural course plants exposed to stress produce large quantities of ROS (Schutzendubell and Polle, 2002) that may oxidize proteins, lipids and nucleic acids resulting in abnormalities at the level of cell (Sharma et al., 2010). Boron toxicity also causes an oxidative stress because of the formation of ROS such as superoxides and hydroxy and peroxy radicals as induced in many other ionic stress which can damage metabolic processes, altering membranes through lipid per oxidation, and provoking cell death in the plant (Molassiotis et al., 2006). In order to counteract

these ROS, plants induce the synthesis of antioxidant metabolites (proline, ascorbate, glutathione etc.) and enzymes (peroxidase, superoxide dismutase, catalase etc.) that neutralize the toxic effects of ROS generated through stress. We have found that the mustard plants raised the level of endogenous enzymes such as catalase, peroxidase and superoxide dismutase and the non-enzymatic component such as proline in the presence of B stress (Fig. 3D-F and 4A). Our result corroborate previous reports indicating an increase in catalase and superoxide dismutase activity in response to excess B in barley (Karabal et al., 2003), in tomato (Cervilla et al., 2007) in apple rootstocks (Sotiropoulos et al., 2006; Molassiotis et al., 2006), in chickpea (Ardic et al., 2009) while an increase in peroxidase activity has been reported in chickpea (Ardic et al., 2009). Increased accumulation of hydrogen peroxide in leaves and roots of *Brassica juncea* was accompanied by enhanced activities of catalase, peroxidase, and superoxide dismutase also strengthen our findings (Archana and Pandey, 2015). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater tolerance to oxidative damage (Sudhakar et al., 2001). Moreover, the induction of these antioxidant metabolisms coincided with an elevated rate of proline (Fig. 4A) at highest dose (60 mg kg^{-1}) of B, indicating that excess B induced oxidative damage. Increased proline levels are another common response of plants upon oxidative stress (Karabal et al., 2003). The stimulated proline accumulation in the mustard under the influence of higher levels of the applied B is in agreement with the result obtained in tomato and pepper (Eraslan et al., 2007), in wheat (Metwally et al., 2012). Proline also protects enzymes and membranes against oxidative stress (Agarwal and Pandey, 2004). Therefore, in the present study, Varuna possessed enhanced activities of antioxidants like, catalase, peroxidase, superoxide dismutase and higher proline content than in Chapka rohini, which suggest that antioxidative defense system could be one of the effective components of mechanism of tolerance of mustard plants to B toxicity.

Plants exposed to the varying levels of B (10, 20, 30, 40, 50 or 60 mg kg^{-1}) in the soil showed a reduction in yield characteristics (number of pods per plant, number of seeds per pod, 100 seed mass and seed yield per plant) soil (Fig. 5). However, variety Varuna performed better and showed a lesser loss in yield than Chapka rohini could be attributed to improved plant growth (Fig. 1 and 2A). Similar findings were reported by Mirshekari (2012) and Cokkizgin (2013), who observed a restricted, seedling vigour index of *Anethum graveolens* and *Phaseolus vulgaris* at high level of B concentrations, respectively. Under conditions of excess B supply, its concentration in the cytosol may rise and cause metabolic disturbances by formation of complexes with NAD^+ of the ribonucleotide units that form a key part of the RNA structure (Loomis and Durst, 1992). The adverse effects of B on plant metabolic activities are more probably related to chlorosis and necrosis, loss of photosynthetic capacity, leading to their poor growth and seed setting and eventually reduction in plant productivity (Reid, 2007) (Fig. 5). Reduction in yield could be attributed to decrease in assimilates under limited water and nutrient supply to the photosynthetic organs in the presence of excessive trace elements (Hasnain et al., 2011).

CONCLUSION

From present study, it concluded that the presence of boron (20, 30, 40, 50 or 60 mg kg^{-1} B added through soil) significantly retarded plant growth, pace of photosynthesis, and ultimately the seed yield in both the varieties viz., Varuna and Chapka rohini of the *Brassica juncea* L. even though the plants exhibited a higher antioxidant enzyme activity and an accumulation of proline content (the protective mechanism). The variety Chapka rohini was more sensitive to the boron toxicity than Varuna.

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