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Higher Boron Accumulation is Associated with Low-Boron Tolerance in Mustard

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Abstract

Boron (B) is an essential micronutrient required for optimal plant growth and development. Seed yield and quality are often compromised in plants grown under limited soil B. Indian mustard (*Brassica juncea* L.) is one of the most susceptible plants to B limitation. In this study, twenty-seven Indian mustard genotypes were grown in hydroponic culture with different B concentrations, and growth performances were evaluated based on their root and shoot growth. Geeta, RH406, and Maya were found to be low-B tolerant, moderate, and sensitive genotypes, respectively. In Geeta, root meristem cells showed higher viability than RH406 and Maya under low B conditions. Geeta had a significantly higher B concentration in the root and youngest opened leaf (YOL) than Maya and RH406. The pectin levels in the cell wall of the root and the YOL varied across different genotypes under different B conditions. The pectin levels in the cell wall of YOL were lowest in Geeta and highest in Maya under sufficient B conditions. Based on these results, we suggest that a higher B uptake and translocation and possibly lower pectin levels are the key factors responsible for low B tolerance in *B. juncea* genotypes.

Discipline: Agricultural Environment
Additional keywords: boron accumulation, boron nutrition, *Brassica juncea*

Introduction

Boron (B) is an essential micronutrient primarily required for the cross-linking of pectic polysaccharide rhamnogalacturonan II (RG-II) to confer stability and elasticity of cell walls in vascular plants (Funakawa & Miwa 2015). Therefore, the continuous supply of B is vital for optimal plant growth and development. B deficiency is the second most important micronutrient constraint after Zn for optimal crop production worldwide (Goyal et al. 2012). More than 130 field crops across 80 different countries were reported to be affected by B deficiency (Brdar-Jokanović 2020). B has a narrow window between deficiency and toxicity in most plants; thus, maintaining optimal tissue B concentration is important for plant growth (Jothi & Takano 2023).

The rapeseed-mustard group of crops ranked as the third most important oilseed crops in the world in terms of area and production. In India, rapeseed-mustard crops contribute to 1/3rd of the total oilseed production. In 2021-2022, its production reached a record 11.7 million tonnes (Annual Report 2021-22 of the Ministry of Agricultural and Farmers Welfare, Government of India). Rapeseed-mustard crops are mainly cultivated for oil and condiment purposes. Oil content in these crops varies from 33 to 46%, and the average oil recovery is around 32 to 38%. The group rapeseed-mustard crops consist of various Brassica species, including *Brassica rapa* (yellow sarson), *B. napus* (gobhi sarson), *B. carnita* (karan rai), and *B. juncea* (Indian mustard). These groups of crops are widely cultivated in the northern plains and eastern belts of India. Among others, *B. juncea* is India's most important and largely cultivated winter season (rabi) oilseed crop, which thrives best in light to heavy loam soils having 24 cm-40 cm of rainfall (Shekhawat et al. 2012).

Similar to other rapeseed-mustard crops, *B. juncea* is very sensitive to B deficiency (Dhaliwal et al. 2022).

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Soil B content in major mustard-producing states of India, especially in West Bengal and Assam, is alarmingly low (Prasad et al. 2014, Das et al. 2017). B deficiency (≤ 0.5 mg B/kg soil of hot-water extraction) ranges from 2% in alluvial soils of Gujarat to 68% in red soils in Bihar, with a mean of 23.2% for the whole country (Singh 2008, Shukla & Behera 2019). A maximum occurrence of B deficiency (54%-86%) was recorded in Alfisol soils of Assam and West Bengal, due to readily leaching of B under high rainfall conditions (Shukla et al. 2021).

Many field studies in India revealed that *B. juncea* is very responsive to soil- and foliar-B application. The application of 1.73 kg B/ha on B-deficient calcareous soil in Rajasthan increases the *B. juncea* yield by 39% (Kumararaja et al. 2015). The highest numbers of siliquae/plant, seeds/siliquae, and increase in seed yield (36%) and oil content (52%) were observed on application of 1.5 kg B/ha in inceptisol soils in Uttar Pradesh (Yadav et al. 2016). The application of 3 kg B/ha in sandy loam soils with neutral pH (7.2) in Uttar Pradesh and 2 kg B/ha in sandy loam soils with slightly alkaline pH (7.9) in Rajasthan showed the highest biomass, number of seeds/siliquae, seed yield increment in *B. juncea* (Choudhary & Bhogal 2017, Mosam & Umesha 2022). A foliar spray of 0.2% and 1% borax during the flowering stage resulted in 10.6% and 37.7% increase in seed yield in rainfed Indian mustard cultivated in sandy loam soils of Delhi and Punjab, respectively (Rana et al. 2005, Dhaliwal et al. 2022). These results indicate that B fertilization is necessary to improve the yield and oil quality of *B. juncea*.

Currently, the application of B fertilizers is the only means to alleviate B deficiency in India (Kumararaja et al. 2015). B application not only improves the yield but also the quality of the products. Unlike other mineral nutrient deficiencies, visual symptoms of B deficiency in *B. juncea* leaves are not very prominent (Kumararaja et al. 2015). Therefore, diagnosing B deficiency and following appropriate B fertilization regimes is very difficult. Identification and adoption of low B-use-efficient *B. juncea* genotypes is therefore crucial for low-B-input sustainable oil seed production (Stangoulis et al. 2000, Kumararaja et al. 2015).

The uptake and requirement of B greatly differ among the rapeseed-mustard genotypes under B deficiency. Among the rapeseed-mustard crops, the genotypic variations in B use efficiency have been well documented in *B. napus* (Xue et al. 1998; Stangoulis et al. 2001; Xu et al. 2001, 2002; Yang et al. 2013; Zhang et al. 2014). B deficiency resulted in floral abortion and a significant drop in the number of siliquae, seed setting, and seed yield in *B. napus* cultivars during the field trials in China and Bangladesh (Zaman et al. 1998, Xue et al. 1998, Shi et al. 2009). Our current knowledge of genotypic variation among the *B. juncea* genotypes on their B-deficiency responses is very limited. Stangoulis et al. (2000) have conducted a field and a glass house-level screening for the B-efficient *B. juncea* genotypes. They selected five mustard genotypes for studying their response under two B conditions: B deficiency (0 mg B kg^{-1}) and B sufficiency (2 kg B/ha in the field; 0.25 mg B/kg in the glass house) in Myponga sand (pH 5.4), Australia. Among the five *B. juncea* genotypes screened for low B tolerance, two were from Australia (397-23-2-3-2, CSIRO 6), and three were from India (TM 18, Pusa Bold, and CPI 81792). Based on the shoot dry weight, B content, and seed yield, they identified that all three Indian mustard genotypes performed better than the Australian genotypes under low B conditions (Stangoulis et al. 2000). Choudhary & Bhogal (2017) have conducted a field study to identify the genotypic variation among the three Indian mustard varieties (Aravali, Laxmi, Vardan) under five levels of B concentrations (0, 0.5, 1.0, 1.5 and 2.0 kg B/ha) in sandy loam soil (pH 7.9) in Rajasthan, India. Among these cultivars, Laxmi was the most promising in yield and quality under low B conditions (Choudhary & Bhogal 2017).

In the present study, we screened twenty-seven *B. juncea* genotypes for their physiological responses to low B supply using a hydroponic culture system. The phenotypic alterations in responses to low B conditions were monitored at the early vegetative stages in representative low-B tolerant, moderately tolerant, and sensitive genotypes. Biomass, root and shoot length, cell viability, tissue B concentration, and the levels of pectin in cell wall fractions were studied to identify the physiological basis for low B tolerance in mustard genotypes.

Materials and methods

1. Plant material and growth conditions

Twenty-seven genotypes of Indian mustard received from the Directorate of Rapeseed-Mustard Research (DRMR), Rajasthan, India, were used in this study. Seeds were surface sterilized using 70% ethanol for 10 mins, followed by 0.5% (w/v) sodium hypochlorite for 10 mins, and washed 3 times in sterile water before drying onto sterile filter paper. The seeds were germinated on moistened filter paper for five days in the dark. Germinated seedlings with similar root lengths were selected and grown in Hoagland's solution (Hoagland & Arnon 1950) at two varying B concentrations: 0.46 μM B (B deficient) and 46 μM B (B sufficient; control

treatment). In a pilot experiment, we determined that 1/100th of the B sufficient concentration (0.46 µM B) induced B deficiency symptoms in *B. juncea* genotypes. The seedlings were first transplanted into plant tissue culture containers made of autoclavable polycarbonate and polypropylene (planton boxes L7.5 × B7.5 × H10 cm; Tarson, Kolkata, India) with 1/4th strength Hoagland's solution with respective B concentration as described in previous literature (Pan et al. 2012). The plants were grown for 5 days in a culture room at 22^{o}C under a 16/8 h day/night photoperiod with a photon flux density of 300-360 µmol/m^2/s. Then, the roots were carefully rinsed with deionized water, and the plants were transferred to half-strength Hoagland's solution with respective B concentrations for 5 days. Finally, the plants were shifted to full-strength Hoagland's solution with respective B concentrations, and the nutrient solution was replaced every five days. The main root length and the shoot length were recorded, and roots and shoots were harvested separately after the plants had grown with 3-4 leaves for about 21 days. The experiment was designed in a completely randomized block with two treatments. The pH was maintained at 5.8 using 0.5 M H_2SO_4 or 1 M NaOH.

The plant samples were divided into two groups: the first to determine the dry weight of the biomass and B concentration, and the second to be immediately frozen in liquid nitrogen for cell wall extraction.

2. Cell viability staining in mustard root tips

Germinated mustard seedlings were grown on Hoagland's solution for 3 weeks at two varying B concentrations: 0.46 µM B (B deficient) and 46 µM B (B sufficient; control treatment). Cell viability of mustard root tips was estimated by staining with fluorescein diacetate (FDA) according to the method described by Jones & Senft (1985). The FDA (Sigma, USA) primary stock solution was prepared at a 5 mg/mL concentration in acetone. The secondary stock solution was prepared by adding 4 µl of primary stock solution to 1 mL of ultrapure water (Merck Millipore, Germany). Roots were excised from the respective mustard genotypes, washed well in ultrapure water for one minute, and stained for 10 mins in FDA (20 µg/mL) solution under dark conditions. The stained roots were then rinsed with ultrapure water for one minute, and images were acquired by confocal microscopy (LSM 880 equipped with filter set and 40× lens, Carl Zeiss, Germany) with an excitation wavelength; 488 nm emission wavelength; 520 nm - 530 nm. The fluorescence intensity was quantified using ImageJ software (Schindelin et al. 2012).

3. Analysis of B

Individually separated root and youngest opened leaves (YOL) samples were dried in an oven at 65°C until they reached a constant weight. Dried plant samples were then dry-ashed at 550°C for 4 h and dissolved in 0.1 M HCl. The suspension was centrifuged to avoid any insoluble matter, and the supernatant was used to determine the B concentration spectrophotometrically using the azomethine-H method at 420 nm (Wolf 2008). The statistical analysis was performed using Two-way ANOVA with Tukey's multiple comparison test.

4. Preparation of the cell wall materials

The roots were separated from the remaining plant parts and washed for 2-3 mins with ultrapure water (Merck Millipore, Germany). Samples from each treatment were pooled, frozen in liquid nitrogen, and ground into a fine powder with a mortar and pestle. The cell walls were extracted from the homogenized samples using the previously described method (Hu & Brown 1994). Briefly, 1 g of homogenized samples were washed with 10 mL ice-cold HEPES buffer (pH 7.0) containing 2 mM potassium metabisulfite. The suspension was then ultrasonicated at 0.5 cycles with 50% amplitude in a Hielscher ultrasonic device (model UP200S, Hielscher Ultrasound Technology, Brandenburg, Germany) by immersion in an ice bath. After ten cycles of sonication for 5 min each, the suspensions were centrifuged at 5,000 ×g for 10 mins. The insoluble pellet was washed twice with 10 ml of ultrapure water and centrifuged at 5,000 ×g for 10 mins. The residue was sequentially washed with ice-cold 80%, 95%, 100% (v/v) ethanol, chloroform: methanol (1:1, v/v), and acetone (80% v/v). All procedures were carried out in polyethylene vessels. The air-dried pellets, designated as an alcohol-insoluble residue (AIR), were used to quantify the amount of pectin.

5. Determination of cell wall uronic acids

Chemical fractionation of cell wall uronic acids (pectin, hereafter) from AIR was performed by using trans-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid (CDTA), and Na_2CO_3, as described by Wu et al. (2017). The AIR was sequentially extracted with sodium acetate buffer (50 mM, pH 6.5) containing 0.05 M CDTA for 12 h at 24°C in a horizontal shaker. The suspension was then centrifuged (5,000 ×g, 5 min), and the collected supernatant was designated as a CDTA-soluble or chelator-soluble pectin fraction. The CDTA-insoluble residue was washed twice with an extraction solution (sodium acetate buffer, 50 mM, pH 6.5) and transferred into a new tube. The CDTA-insoluble pellets were then resuspended in 50 mM Na_2CO_3 (alkali-soluble pectin)

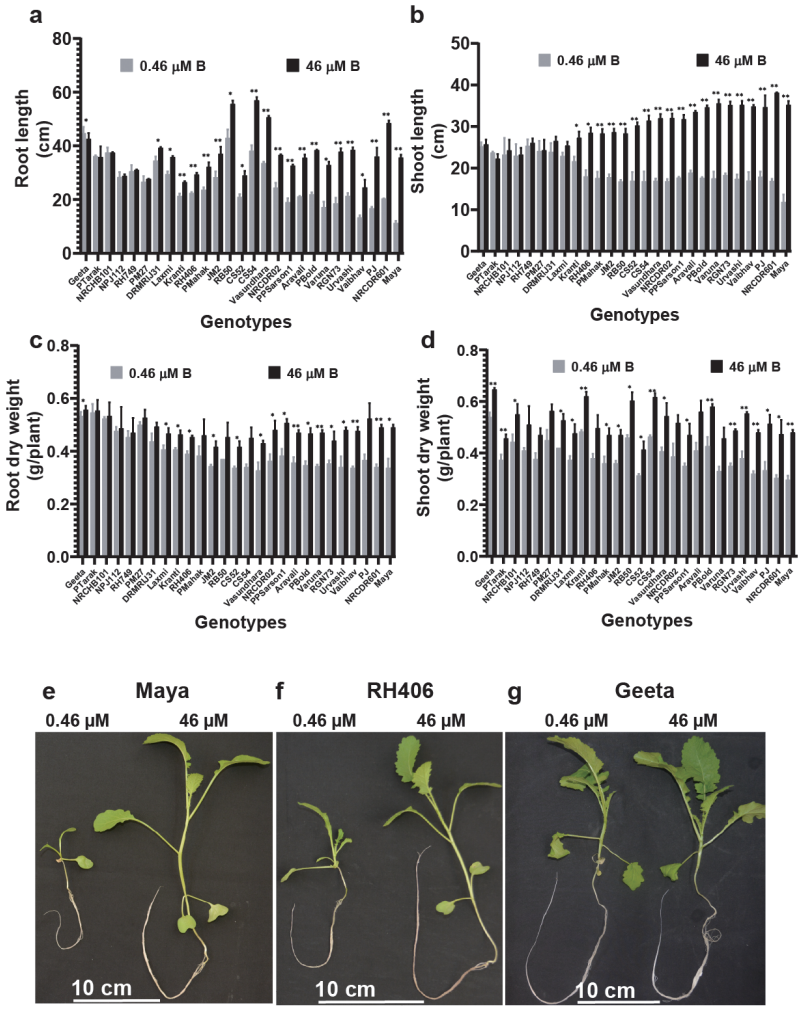


Fig. 1. Growth variations of 27 mustard genotypes under low-B and B-sufficient conditions

The germinated seedlings with similar root lengths were selected and grown in Hoagland's solution at two different B concentrations: 0.46 µM (low B) and 46 µM (sufficient B). The seedlings were first transplanted into 1/4th strength Hoagland's solution and subsequently transferred to half-strength and full-strength solution every five days. (a-d) The shoots and roots were harvested after 21 days, and length and dry weights were measured. The means ± SDs from three plants are shown. Scale bar: 10 cm. Multiple *t* test analyses were performed to verify the statistical significance (*: $P \leq 0.05$; **: $P \leq 0.01$). (e-g) Pictures of the low-B tolerant genotype Geeta, moderately sensitive genotype RH406, and sensitive genotype Maya are shown.

and incubated for 12 h at 24°C. The alkali-soluble pectin fraction was collected after centrifugation at 5,000 ×g for 20 min at 4°C. The amount of pectin in chelator-soluble and alkali-soluble pectin fractions was determined using the m-hydroxy diphenyl colorimetric method as per the procedure described by Blumenkrantz and Asboe-Hansen (1973). A standard curve was plotted with galacturonic acid, and the amount of pectin was expressed in galacturonic acid equivalents. A statistical analysis was performed using Two-way ANOVA with Tukey's multiple comparison test.

Results

1. Geeta showed a better growth rate compared to Maya and RH406 under low-B conditions

We used a hydroponic-based growth system to evaluate the growth of Indian mustard (*B. juncea*) genotypes under low B conditions. Based on the preliminary screening of the effect of varying B concentrations on plant growth in Hoagland's medium, we chose 0.46 µM B to serve as the low-B condition and 46 µM B (standard concentration in Hoagland's medium) as the B-sufficient condition. Out of the 27 mustard genotypes, 21 and 19 genotypes showed significantly

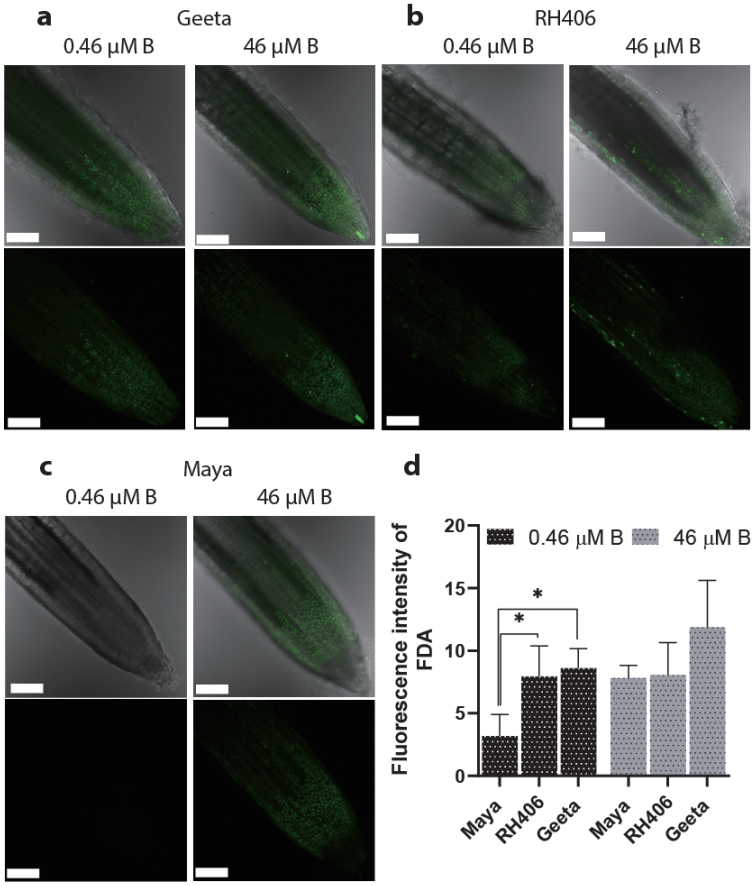


Fig. 2. Cell viability of mustard roots under a low B condition by fluorescein diacetate (FDA)
(a-c) The germinated mustard seedlings were transferred to Hoagland's solution containing 46 µM and 0.46 µM boric acid and cultivated for 3 weeks. The roots were washed in ultrapure water and then incubated in FDA (20 µg/mL) solution for 10 mins in dark conditions. The imaging was acquired from confocal microscopy (Carl Zeiss LSM880; 40× lens) with an excitation filter; 450-490 nm and a barrier filter; 520 nm. Scale bar 100 µm. (d) The fluorescence intensity of the FDA was quantified using ImageJ software. The means ± SDs from 3-7 plants are shown. Two-way ANOVA analysis with Tukey's multiple comparison test was performed to verify the statistical significance (α= 0.05; *: $P < 0.05$).

inhibited root and shoot growth, respectively, under low-B conditions (Fig. 1a and b). We observed that the low-B conditions negatively influenced the root length in 21 mustard genotypes but not in the genotypes Geeta, Pusa Tarak, NRCHB101, NPJ112, RH749, and PM27. Similarly, the shoot length was reduced significantly in 19 mustard genotypes but not in the genotypes Geeta, Pusa Tarak, NRCHB101, NPJ112, RH749, PM27, DRMRIJ31, and Laxmi (Fig. 1a, b; SI Fig. 1). Compared to other genotypes, Maya showed the highest reduction in root (47%) and shoot (60%) growth under low B conditions (Fig. 1a, b). The root and shoot dry weight responses showed relatively similar trends to that of root and shoot length under low-B conditions (Fig. 1c, d). For further analysis, we chose Geeta, RH406, and Maya as representative genotypes for the low-B tolerant, moderate low-B tolerant, and low-B sensitive genotypes, respectively, to study their growth variations under B sufficient and low-B conditions (Fig. 1e-g).

2. B-deficiency induces higher cell death in low-B sensitive mustard genotype Maya

To compare the B-deficient symptoms in the three representative genotypes, root cell viability was evaluated using FDA staining. The live cells can be visualized by green patches. Exposure to low B conditions reduced live cell intensity in the roots of all three mustard genotypes compared to B-sufficient conditions (Fig. 2a-c). Under low-B conditions, the root consisted of more live cells in the genotypes RH406 and Geeta compared to Maya (Fig. 2d). The above results indicate that the root tip of Maya is most sensitive, that of RH406 is moderately sensitive, and that of Geeta is most tolerant to the low-B condition.

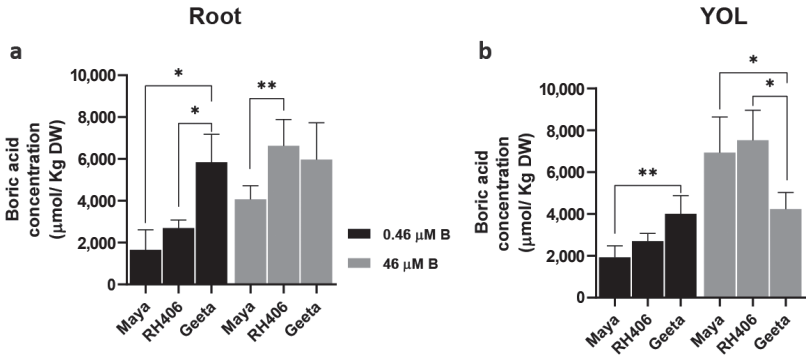


Fig. 3. B concentrations in root and youngest opened leaf (YOL) of the three contrasting mustard varieties

(a, b) The plants were cultivated under deficient (0.46 µM) and sufficient (46 µM) B conditions for 21 days, and then roots (a) and youngest opened leaves (b) were dried in an oven at 65°C until they reached a constant weight. Dried samples were then dry-ashed at 550°C for 4 h and dissolved in 0.1 M HCl. The supernatant was used to determine the B concentration spectrophotometrically by the azomethine-H method at 420 nm (Wolf, 1971). The means ± SDs from three plants are shown. Two-way ANOVA analysis with Tukey's multiple comparison test was performed to verify the statistical significance (α= 0.05; *: $P < 0.05$; **: $P < 0.01$).

3. Maya and RH406 had significantly reduced B concentration in root and YOL under low B conditions

To investigate whether the growth difference under low-B conditions depends on the differential accumulation of B in tissues, we measured the B concentration in the root and youngest open leaf (YOL) of three mustard genotypes (Fig. 3, SI Fig. 3). Under B-sufficient (46 µM) conditions, there was no significant difference in root B concentration between Geeta and RH406. However, root B concentration in Maya was 39% lower than in RH406. Under low-B (0.46 µM) conditions, Geeta had a significantly higher root B concentration compared to RH406 (54% reduction) and Maya (72% reduction) (Fig. 3a). In YOL, RH406 displayed a 78% higher B than Geeta under sufficient B conditions. Whereas, genotype Geeta showed a 108% higher YOL B than Maya under low B conditions (Fig. 3b). Intriguingly, the concentration of B in roots and YOL remained similar (not significantly different) under the low- and B-sufficient conditions in Geeta but not in RH406 and Maya in three independent experiments (Fig. 3, SI Fig. 3). These results suggest that the uptake and translocation of B are the main factors influencing low-B tolerance in the selected genotypes.

4. Three genotypes had differential levels of pectin in the cell wall

Plant species with more cell wall pectin require more tissue B to maintain cell wall integrity (Hu & Brown 1994). Genetic variations in the concentration of cell wall pectin were observed in different *B. napus* genotypes with varying B efficiency (Zhang et al. 2014). Here, we studied the potential differences in the amount and structure of pectin among the *B. juncea* genotypes by measuring the levels of pectin in the chelator (CDTA) -soluble and alkali (Na_2CO_3) -soluble fractions of cell walls. Although there is a potential risk of losing water-soluble polysaccharides such as pectin with our method using an aqueous buffer (HEPES buffer), we have obtained consistent results with slight variations in the amounts of pectin (Fig. 4; SI Fig. 4).

The sum of pectin amounts in the chelator-soluble and alkali-soluble cell wall fractions in the roots of the three genotypes were not largely different under both low- and B-sufficient conditions (Fig. 4a, SI Fig. 4a). However, in the case of YOL, the sum of pectin amounts in these fractions was higher in Maya compared to RH406 and Geeta under B-sufficient conditions ($P < 0.01$, Fig. 4b, SI Fig. 4b). The presence of more B binding sites (pectin) in YOL may be one of the reasons for the low-B sensitivity of Maya.

In Maya, the proportion of chelator-soluble pectin in the sum of pectin was relatively higher under low-B conditions compared to B-sufficient conditions in the roots ($P < 0.01$ in two independent experiments, as shown in Fig. 4c, SI Fig. 4c) and in the YOL ($P < 0.01$ in one experiment in Fig. 4d but not significant in SI Fig. 4d). There were no significant changes in the proportion of chelator-soluble pectin in both the root and YOL of RH406 and Geeta. It is likely that the low-B accumulation in Maya's tissues (Fig. 3a, b, SI Fig. 3a, b) reduced the crosslinking of RG-II and affected the structure of pectin.

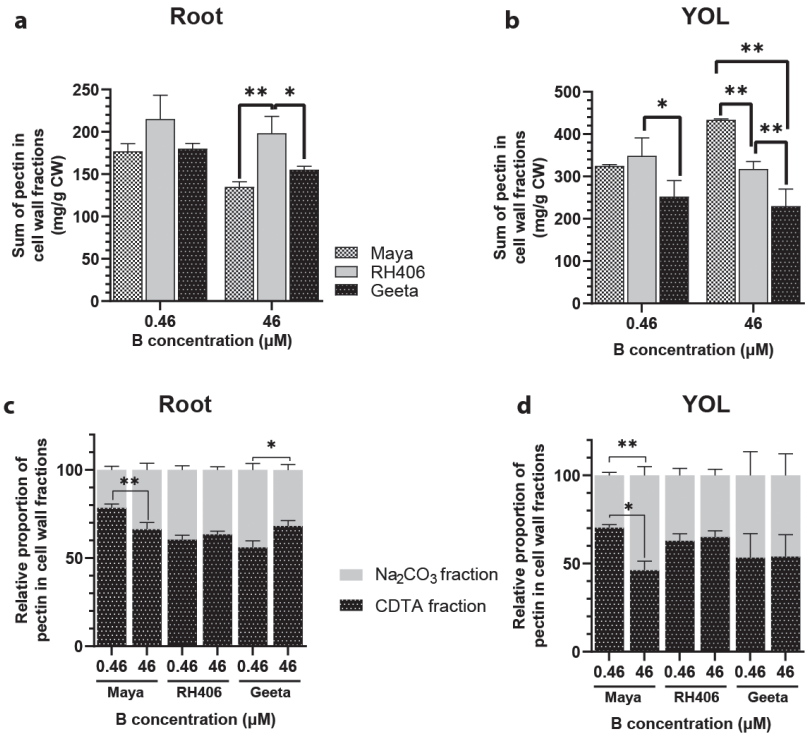


Fig. 4. Amount of pectin in root and youngest opened leaf (YOL) cell wall fractions
Pectin in plant tissues was determined using the m-hydroxy diphenyl colorimetric method as per the procedure described by Blumenkrantz and Asboe-Hansen (1973). Pectin was estimated by comparison with the standard curve of galacturonic acid. The sum of pectin was calculated by summing pectin amounts in CDTA and Na_2CO_3 fractions present in the respective tissues. The relative pectin proportion in CDTA and Na_2CO_3 fractions are represented in (c, d). The means ± SDs from three plants are shown. Two-way ANOVA with Tukey's multiple comparison test was performed to verify the statistical significance (α= 0.05; *: $P < 0.05$; **: $P < 0.01$).

Discussion

The present study revealed a substantial variation in response to low-B conditions in 27 *B. juncea* genotypes. The low-B sensitive genotypes showed a significant reduction in root length, shoot length, and lower biomass production (dry weight). However, the genotypes Geeta, Pusa Tarak, NRCHB101, NPJ112, RH749, PM27, DRMRIJ31, and Laxmi showed similar root and shoot growth under low-B conditions compared to B-sufficient conditions (Fig. 1, SI Fig. 1).

To our knowledge, few studies have been conducted on the B efficiency in *B. juncea* (Stangoulis et al. 2000, Choudhary & Bhogal 2017). Stangoulis et al. (2000) have included in their study three genotypes of Indian mustard, TM-18, CPI81792, and Pusa Bold, of which the former two genotypes have become obsolete, and their seeds are currently unavailable in India. However, Pusa Bold is still a widely cultivated and popular variety for the mustard breeders in India, which we have included in our current study. The Pusa Bold has shown high B efficiency in an earlier study conducted at the pot and field levels (Stangoulis et al. 2000). Nevertheless, in our hydroponic study, Pusa Bold was found moderately low-B tolerant. Pusa Bold is an old mustard variety released in 1985, with the high-yielding mustard varieties (RH406, RB50, Pusa Mahak, Laxmi, JM2, RH749, Pusa Tarak, PM27, NRCHB101, NPJ112, & Geeta) having been released very recently. Since there was no available information on mustard B efficiency, one possible explanation is that the breeders may have accidentally introduced low-B tolerant genotypes in the mustard gene pool over the years. Hence, we assume that B-efficiency is an important selection pressure in Indian soils. Choudhary & Bhogal (2017) have studied the growth variation of three mustard genotypes, Aravali, Laxmi, and Vardan, under different B concentrations (0, 0.5, 1, 1.5, & 2 kg B/ha) at the field level. Among the other two genotypes, Laxmi was identified as high-yielding, followed by Aravali and Vardan under different B conditions. Among the three genotypes, we have included genotypes Laxmi and Aravali in our study. Based on the relative root length

(RRL%) and shoot and root dry weight biomass, we identified Laxmi as a low-B tolerant and Aravali as a moderate low-B tolerant genotype (Fig. 1, SI Fig. 1).

Previous studies have demonstrated that low B decreases the cell viability in BY-2 cells, rose cells, and trifoliate orange roots (Dordas & Brown 2005, Koshiba et al. 2009, Wu et al. 2020). In our current study, we observed that cell viability decreased in root tips of mustard genotypes under low B conditions. In addition, we also observed significant differences in cell viability among mustard genotypes (Fig. 2, SI Fig. 2). The genotype Geeta showed relatively higher rates of viable cells under low B conditions compared to RH406 and Maya. We also observed that only the genotype Maya exhibited a relatively higher proportion of CDTA-soluble pectin under low-B conditions compared to the sufficient B condition (Fig. 4c, d, SI Fig. 4c, d). The increase in CDTA-soluble pectin implies that the pectin is loosely bound to the cell wall. We hypothesize that the impairment of cell wall integrity in Maya under low-B conditions may be due to lower crosslinking rates by borate.

In our study, we observed a clear correlation between the B concentrations in the root and YOLs and the growth of the roots and shoots across the three genotypes under low B conditions (Figs. 1, 3). This finding suggests that the uptake and translocation of B is the major factor controlling B efficiency among the three genotypes. Previous studies in *B. napus* have shown the importance of the uptake and translocation of B for the genetic diversity of B efficiency (Zhang et al. 2014, Jothi & Takano 2023). Importantly, genetic variation in a boric acid channel *BnaA3.NIP5;1* expressed in the lateral root cap cells was shown to determine the differential rates of B uptake and growth under low B conditions (He et al. 2021). The field trials showed the correlation between the relative expression levels of *BnaA3.NIP5;1* and seed yield among 29 *B. napus* varieties under low B conditions. In future studies, it is important to analyze the expression and function of B transport proteins in *B. juncea*.

In *B. napus*, pectin levels are also linked to B efficiency. The genotype Wester 10, which is B-inefficient, was found to have a higher amount of cell wall pectin in the leaves and roots compared to the B-efficient genotype Qingyou 10 (Pan et al. 2012). The authors concluded that the low B sensitivity of Wester 10 is due to the presence of higher B-binding sites (pectin) and limited accumulation and allocation of B to the cell walls (Pan et al. 2012). In our study, we observed a negative correlation between pectin levels under a B-sufficient condition and B-efficiency in the YOL but not in the roots. Although further studies are needed, the presence of higher B binding sites in leaves may be one of the reasons for the low B sensitivity of Maya. It is important to study how pectin accumulates differently and the physiological significance of this variation in various genotypes of the *Brassica* family.

In conclusion, we propose that the mechanism responsible for the low-B tolerance of the mustard genotype Geeta is higher uptake and translocation of B. The lower levels of pectin in YOL may also contribute to the low-B tolerance. In the low-B sensitive genotype Maya, low B accumulation and potentially high pectin amount decrease the rate of pectin crosslinking by borate. As a result, symptoms of B deficiency, such as root cell death, are exaggerated. The existence of diversity in plant responses to low B is fundamental for breeding to improve B-efficiency in *B. juncea* genotypes to meet higher yields. Further analysis of B uptake, RGII-B dimerization, and functional characterization of key B transport proteins will help us understand the genetic differences in B-efficiency among the mustard genotypes.

References

Brdar-Jokanović, M. (2020) Boron toxicity and deficiency in agricultural plants. *International Journal of Molecular Sciences*, **21**, 1424.

Camacho-Cristóbal, J. J. et al. (2004) Boron deficiency causes accumulation of chlorogenic acid and caffeoyl polyamine conjugates in tobacco leaves. *Journal of Plant Physiology*, **161**, 879-881.

Choudhary, S. & Bhogal, N. S. (2017) Effect of boron on yield, quality and its uptake in Indian mustard (*Brassica juncea* L) genotypes. *Annals of Plant and Soil Research*, **19**, 394-397.

Das, S. K. et al. (2017) Secondary and micronutrients: Deficiency symptoms and management in organic farming. *Inno. Farm.*, **2**, 209-211.

Dhaliwal, S. S. et al. (2022) Biofortification of oil quality, yield, and nutrient uptake in Indian mustard (*Brassica juncea* L.) by foliar application of boron and nitrogen. *Frontiers in Plant Science*, **13**, 976391.

Dong, X. et al. (2022) The regulation of plant lignin biosynthesis under boron deficiency conditions. *Physiologia Plantarum*, **174**, e13815.

Dordas, C. & Brown, P. H. (2005) Boron deficiency affects cell viability, phenolic leakage and oxidative burst in rose cell cultures. *Plant and Soil*, **268**, 293-301.

Funakawa, H. & Miwa, K. (2015) Synthesis of borate cross-linked rhamnogalacturonan II. *Frontiers in Plant Science*, **6**, 138445.

Gigli-Bisceglia, N. et al. (2019) Plant cell wall integrity maintenance in model plants and crop species-relevant cell wall components and underlying guiding principles. *Cellular and Molecular Life Sciences*, **77**, 2049-2077.

Goyal, A. (2012) *Crop Plant*. IntechOpen, London, UK.

He, M. et al. (2021). Genetic variation of *BnaA3. NIP5; 1* expressing in the lateral root cap contributes to boron

deficiency tolerance in *Brassica napus*. *PLoS Genetics*, **17**, e1009661.

Hoagland, D. R. & Arnon, D. I. (1950) The water-culture method for growing plants without soil. *Circular* **347**. California Agricultural Experiment Station, The College of Agriculture, University of California, Barkley, USA.

Hu, H. & Brown, P. H. (1994) Localization of boron in cell walls of squash and tobacco and its association with pectin (evidence for a structural role of boron in the cell wall). *Plant Physiology*, **105**, 681-689.

Jones, K. H. & Senft, J. A. (1985) An improved method to determine cell viability by simultaneous staining with fluorescein diacetate-propidium iodide. *Journal of Histochemistry & Cytochemistry*, **33**, 77-79.

Jothi, M. & Takano, J. (2023) Understanding the regulatory mechanisms of B transport to develop crop plants with B efficiency and excess B tolerance. *Plant and Soil*, **487**, 1-20.

Koshiba, T. et al. (2009) Boron nutrition of tobacco BY-2 cells. V. Oxidative damage is the major cause of cell death induced by boron deprivation. *Plant and Cell Physiology*, **50**, 26-36.

Kumararaja, P. et al. (2015) Application of boron enhances Indian mustard (*Brassica juncea*) productivity and quality under boron deficient calcareous soil in semi-arid environment. *Ecology, Environment and Conservation*, **21**, S249-S254.

Li, M. et al. (2017) Effect of boron deficiency on anatomical structure and chemical composition of petioles and photosynthesis of leaves in cotton (*Gossypium hirsutum* L.). *Scientific Reports*, **7**, 4420.

Liu, G. et al. (2014) Boron deficiency is correlated with changes in cell wall structure that lead to growth defects in the leaves of navel orange plants. *Scientia Horticulturae*, **176**, 54-62.

Mosam, P. K. & Umesha, C. (2022) Effect of boron levels and row spacing on growth and yield of mustard (*Brassica juncea* L.). *International Journal of Plant & Soil Science*, **34**, 789-794.

Ogden, M. et al. (2018) Feeding the walls: how does nutrient availability regulate cell wall composition? *International Journal of Molecular Sciences*, **19**, 2691.

O'Neill, M. A. et al. (2001) Requirement of borate cross-linking of cell wall rhamnogalacturonan II for Arabidopsis growth. *Science*, **294**, 846-849.

Pan, Y. et al. (2012) Differences in cell wall components and allocation of boron to cell walls confer variations in sensitivities of Brassica napus cultivars to boron deficiency. *Plant and Soil*, **354**, 383-394.

Prasad, R. et al. (2014). Boron in Indian agriculture–A review. *Indian Journal of Agronomy*, **59**, 511-517.

Rana, K. et al. (2005) Influence of phosphorus, sulphur and boron on growth, yield, nutrient uptake and economics of Indian mustard (*Brassica juncea*) under rainfed conditions. *Indian Journal of Agronomy*, **50**, 314-316.

Schindelin, J. et al. (2012) Fiji: an open-source platform for biological-image analysis. *Nature Methods*, **9**, 676-682.

Shekhawat, K. et al. (2012) Advances in agronomic management of Indian mustard (*Brassica juncea* (L.) Czernj. Cosson): an overview. *International Journal of Agronomy*, **2012**, 1-14.

Shi, L. et al. (2009) Inheritance of boron efficiency in oilseed rape. *Pedosphere*, **19**, 403-408.

Shukla, A. K. & Behera, S. K. (2019) All India coordinated research project on micro- and secondary nutrients and pollutant elements in soils and plants: research achievements and future thrusts. *Indian Journal of Fertilisers*, **15**, 522-543.

Shukla, A. K. et al. (2021) Deficiency of phyto-available sulphur, zinc, boron, iron, copper and manganese in soils of India. *Scientific Reports*, **11**, 1-13.

Singh, M. V. (2008) Micronutrient deficiencies in crops and soils in India. *In* Micronutrient Deficiencies in Global Crop Production, ed. Alloway B. J., Springer Dordrecht, Netherlands, pp. 93-125.

Stangoulis, J. C. R. et al. (2000) Boron efficiency in oilseed rape: I. Genotypic variation demonstrated in field and pot grown *Brassica napus* L. and *Brassica juncea* L. *Plant and Soil*, **225**, 243-251.

Stangoulis, J. C. R. et al. (2001) The efficiency of boron utilisation in canola. *Functional Plant Biology*, **28**, 1109-1114.

Wiercigroch, E. et al. (2017) Raman and infrared spectroscopy of carbohydrates: A review. *Spectrochimica Acta Part A: Molecular and Biomolecular*, **185**, 317-335.

Wolf, B. (1971) The determination of boron in soil extracts, plant materials, composts, manures, water and nutrient solutions. *Communications in Soil Science and Plant Analysis*, **2**, 363-374.

Wu, X. et al. (2017) Boron deficiency in trifoliate orange induces changes in pectin composition and architecture of components in root cell walls. *Frontiers in Plant Science*, **8**, 1882.

Wu, X. et al. (2020) How the cells were injured and the secondary metabolites in the shikimate pathway were changed by boron deficiency in trifoliate orange root. *Plant Physiology and Biochemistry*, **151**, 630-639.

Xu, F. et al. (2002) Boron uptake and retranslocation in cultivars of *Brassica napus* differing in boron efficiency. *In* Boron in Plant and Animal Nutrition, eds. Goldbach et al, Springer New York, USA, pp. 127-135.

Xu, F. S. et al. (2001) Mapping boron efficiency gene(s) in Brassica napus using RFLP and AFLP markers. *Plant Breeding*, **120**, 319-324.

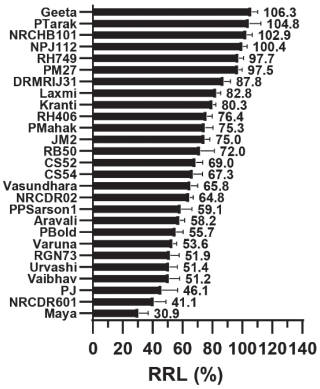
Xue, J. et al. (1998) Differential response of oilseed rape (*Brassica napus* L.) cultivars to low boron supply. *Plant and Soil*, **204**, 155-163.

Yadav, S. N. et al. (2016) Effect of boron on yield attributes, seed yield and oil content of mustard (*Brassica juncea* L.) on an inceptisol. *Journal of the Indian Society of Soil Science*, **64**, 291-296.

Yang, L. et al. (2013) Characteristics of root boron nutrition confer high boron efficiency in Brassica napus cultivars. *Plant and Soil*, **371**, 95-104.

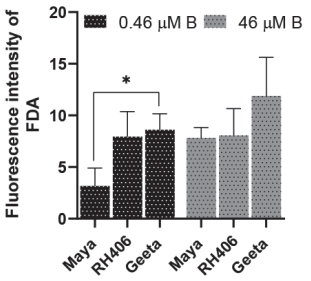
Zaman, N. W. et al. (1998) Yield and fertility of *Brassica napus* as affected by boron deficiency in soil. *Thai Journal of Agricultural Science*, **31**, 92-97.

Zhang, D. et al. (2014) Physiological and genetic responses to boron deficiency in *Brassica napus*: A review. *Soil Science and Plant Nutrition*, **60**, 304-313.



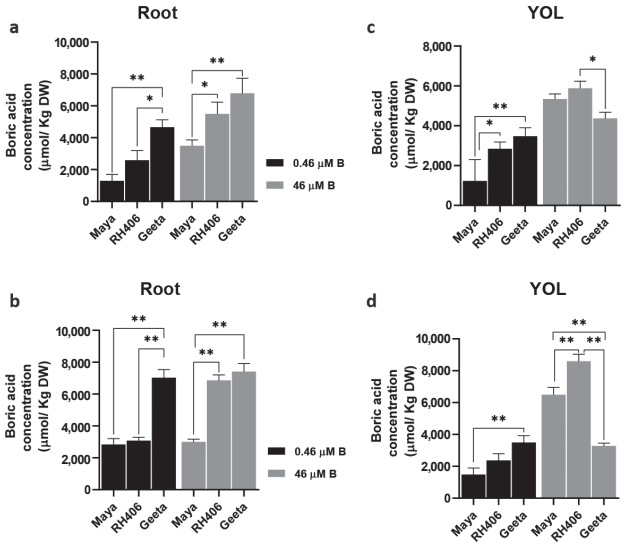
SI Fig. 1. Relative root length (RRL) of 27 mustard genotypes under low B condition

RRL represents the root length at 0.46 µM B divided by that at 46 µM B. The data in Figure 1 were used.



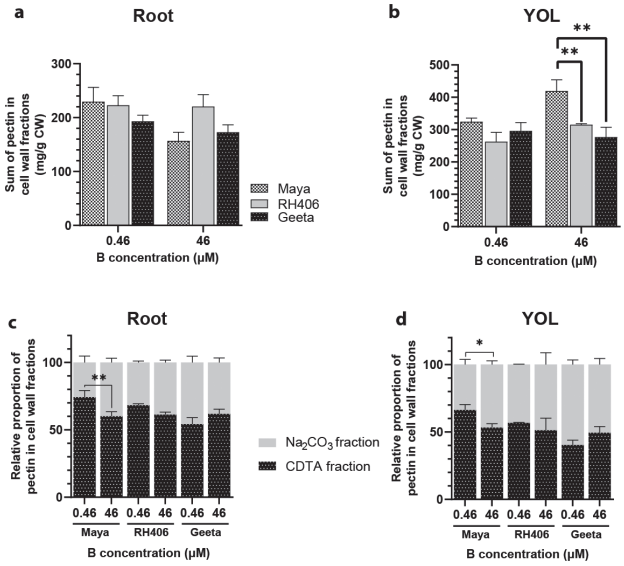
SI Fig. 2. Additional datasets of the fluorescence intensities of the FDA

The means ± SDs from 3-7 plants are shown. Two-way ANOVA analysis with Tukey's multiple comparison test was performed to verify the statistical significance (α= 0.05; *: $P < 0.05$).



SI Fig. 3. Second (a, c) and third (b, d) additional datasets of B concentrations in root (a, b) and youngest opened leaf (YOL) (c, d) of the three contrasting mustard varieties

The method described in the legend of Figure 3 was followed to estimate the B concentration in root and YOL tissues in two independent experiments. The means ± SDs from three plants are shown. Two-way ANOVA analysis with Tukey's multiple comparison test was performed to verify the statistical significance (α= 0.05; *: $P < 0.05$; **: $P < 0.01$).



SI Fig. 4. Additional datasets: Amount of pectin in root and youngest opened leaf (YOL) cell wall fractions

Pectin in plant tissues was determined using the m-hydroxy diphenyl colorimetric method as per the procedure described by Blumenkrantz and Asboe-Hansen (1973). Pectin was estimated by comparison with the standard curve of galacturonic acid. The sum of pectin was calculated by summing pectin amounts in CDTA and Na_2CO_3 fractions present in the respective tissues. The relative pectin proportion in CDTA and Na_2CO_3 fractions are represented in (c, d). The means ± SDs from three plants are shown. Two-way ANOVA with Tukey's multiple comparison test was performed to verify the statistical significance (α= 0.05; *: $P < 0.05$; **: $P < 0.01$).