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Boron Supplementation On Brassica Napus: Unraveling The Best Growth Stages For Paramount Yield

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ABSTRACT

Agriculture has a multifaceted dependency of crops and their production strategies on the nutrients. Habitually, the crops are dependent upon twain nutrients i.e, macro and micro. Boron, being a vital micro-nutrient, plays an important role in many metabolic mechanisms in plants. Low pH and low availability of boron may hinder the crops from thriving. Rapeseed, being a nutrient sensitive, oil-seed crop, gets immensely

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effected by the boron deficiency at its particular growth stages. For the seizure of boron deficiency effects at varied stages and different levels of boron supplementation, a two-year experiment was executed during the years 2022-23 and 2023-24 in Rabi season in the region of Islamabad, Pakistan. The soil conditions were calcareous and loamy with a pH of 7.40-8.73. NARC-Sarsoon was used as experimental material. Seven treatments were used in two categories of Split doses (T2, T3, T4/ 0.075g/40kg) and single full doses (T1, T5, T6, T7/0.15g/40kg) with a control, on seedling, budding and, flowering stages. Analyses performed via ANOVA, Correlation heatmap and, Principal Component Analysis characterized promising outcomes for the full foliar spray of boron at flowering stage and treatment at 0.15grams per 40 kilograms of soil (T-07).

Keywords: Rapeseed, Micro-Nutrients, Boron Supplementation, PCA, Heat Map

Introduction

The crop development and production has a time-honored base for dependency upon nutrients, emphasizing the fact of realization of nutrient dynamic base, mechanisms for uptake from the soil by the crop plants and their biological reciprocation for unlocking the crop betterment blockades (Wawrzyńska & Sirko, 2014). Last two decades hold prominent importance in realm of research work on morpho-physiological mechanisms of crop plants apropos of nutrients uptake (Krouk & Kiba, 2020). The twain plant nutrients are macro and micro, contemporary in varied quantities. Not only macronutrients i.e. carbon dioxide (CO2), water (H2O) and oxygen (O) which are needed in massive amount, there are other fourteen (14) micronutrients which are required in a very specific little quantity; N, O, P, C, K, Mg, Fe, Cu, H, Ca, Mn, S, Cl, Co, Ni, Zn and, B; whose absence creates havoc in plants and growth is affected adversely (Mengel et al., 2001).

Among micronutrients, one of the important and prominent one is Boron, needed for proper growth and development accompanied by fecundity and many physiological processes in crop plants (Brown et al., 2002). Reported facts state that, cell wall structural coherence is also due to boron. In the cell wall of tobacco and squash plants, near about 95% boron is reported by Hu & Brown (1994). The soils with high pH, fewer organic matter contents, soaring carbon dioxide concentrations and alkalinity are deficient in boron (Hua et al., 2020; Zaib et al., 2023a). Importance of boron in plants growth and development established its rule about a century ago but still its prominence detection is in highlights of the researchers (Lewis, 2019). Lignification, synthesis and, composition of cell wall (Goldbach et al., 2000), carbohydrates, nucleic acid, auxins and sugar metabolism, enzymatic activities and photosynthesis all are beholden by boron (Princi et al., 2016).

Near about 96% boron (H3BO3) is absorbed by the plants from soil in the guise of the boron while 4% is up took in the configuration of borate anions (Bolaños at el., 2004; Abbas et al., 2023). Simplifying the facts, the monocotyledonous plants' cell wall are consisted of fewer pectin as compared to dicotyledonous plants thus requiring less boron and face minimum boron toxicity (Chormova et al., 2016).

In the thick of oilseed crops in the world, Rapeseed (Brassica napus) accounts significance as a potential oilseed crop along with a bio-energy crop; making it a fresh

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intensive growing crop worldwide. Due to optimum nutrient needs, the excess or deficiency of any nutrient i.e. macro or micro (Brown et al., 2002), can catastrophically effect its' growth. Optimum level of micronutrients is very essential to be maintained in the soils where rapeseed has to be cultivated (White & Hammond, 2008). As for boron is in the argument, its' deficiency can lead to boron stress in rapeseed, spawning a condition of 'flowering without seed setting' (Liu, 1999). Rapeseed yield could be affected by the boron deficiency; globally the second common nutrient deficiency, which can onward be cured only via supplementation of boron rich fertilizers (Ahmad et al., 2012). Astound results according to the reported researches, state that optimum boron in rapeseed could lead to improved seed yield and quality via enhancing oil content and oleic acid content, and minimizing Glucosinolates and Erucic acid content (Yang et al., 1993; Malhi et al., 2003; Chen et al., 2004). Inspections of boron deficiency in Brassica crops start from leaves, stems and proceed to reproductive parts. Results of deficiency move ahead to, poor pod and seed-setting, ultimately total yield reduction.

Soil fertility is the greatest affecting factor to boron up take by the Brassica crops (Zhang, Zhao, Shi & Xu, 2014). Boron transportation starts from roots and proceeds to the leaves and stems, where it starts accumulation and its' translocation halts there. Even the same piece of soil has different boron levels and boron supplementation is varied at different stages of growth, negatively impacting the plants feedback (Xu, Yang, Wang, & Wu, 2002), emphasizing the accurate boron supplementation at precise growth stages for maximized and positive results. Bearing in mind, the present experiments were designed with the aim to check the boron deficiency effects in Islamabad, Pakistan soils at different growth stages of Brassica napus L. to access the most sensitive stage of growth and to conclude the right time and stage of boron supplementation to Rapeseed.

Material and Methods

To investigate boron deficiency effects on Brassica napus, two experiments were conducted in consecutive sequence during the years of 2021-22 and 2023-24 at National Agriculture Research Center, Islamabad.

Brassica napus variety used in both years was NARC Sarsoon. Seven doses and one control were used at Seedling, budding and flowering stages to analyze boron effects. The experiment was laid out in Completely Randomized Design with two factor factorial and three replications. Texture of soil of Islamabad is loamy sandy, calcium enriched and with an alkaline pH of 7.40-8.73 and electrical conductivity (EC) of this area is 0.74 ds/m (CABI Digital Library/ https://www.cabidigitallibrary.org). Chemical analysis of the soil used in this experiment was performed in collaboration with Land Resources Research Institute, NARC.

According to the obtained results of soil analysis, the boron content in soil was measured as 0.85mg/Kg, for the first year while for the second year, the boron contents in soil were 0.51 mg/Kg. The experimental soil was subjected to supplemental boron application. Boric acid application was kept direct in soil for full doses while it was dissolved in distilled water for foliar treatments in dilutions as, 0.1% Boron solution was prepared by dissolving 9g of boric acid in 1 liter of water and 0.05% Boron solution was prepared by dissolving 4.5g boric acid in 1 liter of

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water. Doses were applied in two categories; Full dose and one-time split dose (Table.1).

Table.1 Single & Split boron doses

Type of Doses	% of Doses	Time of application
Single dose	0.15g/40kg soil	At the time of sowing
Single dose	@0.1%	At time of seedling stage in form of foliar spray
Single dose	@0.1%	At time of budding stage in form of foliar spray
Single dose	@0.1%	At time of flowering stage in form of foliar spray
½ doses	0.075g/40Kg soil Foliar spray@0.05%	At time of sowing and spray atseedling stage
½ doses	0.075g/40Kg soil Foliar spray@0.05%	At time of sowing and spray budding stage
½ doses	0.075g/40Kg soil Foliar spray@0.05%	At time of sowing and spray flowering stage

Data recorded

Parameters recorded were, days to flowering, days to maturity, plant height (cm), number of branches per plant, number of pods per plant, Pod length (cm), number of seeds per pod and, total yield (gm).

Statistical Analysis

Statistical analysis performed included Analysis of variance (ANOVA), for description statistics along with least significant differences (LSD) using Statistix 8.1. Principle component analysis (PCA) biplot along with scree plot and correlation analysis to study impact of different parameters on seed yield was performed by R studio.

Results

Treatments portrayed significant variations for all measured parameters (Table.2). Next factor year is also highly significant for all parameters. Thus, indicating the difference in years cause substantial variations among all recorded parameters. The interaction among these two factors is also highly significant, indicating that effect of each treatment on measured variables depends on the year. The error accounts for the random variation in the experiment and it did not include those variations which are present due to treatment or years or by their interactions instead it accounts for errors in measurement or inherent biological variability.

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Sources of variation i.e. replications, treatments, years and their interactions were all significant, indicating that all sources have meaningful impact on measured variables.
Figure.1 and Figure.2 indicated the data for the parameters as, days to flowering, days to maturity, plant height, number of branches per plant, number of pods per plant, Pod length, number of seeds per pod and, total yield across different treatments (T-00 to T-07) during the years 2021-2022 and 2023-2024, represented by patterned and solid bars, respectively. Over both the years, variation is perceived in the number of days to flowering among the treatments. Control consistently had the highest number of days to flowering in both years, portraying difference from the other applied treatments.
The days to flowering generally remained similar between the first and second years across all treatments, though there were slight variations. The reduction in the number of days to flowering in the treatments compared to the control (T-00) depicted that the applied treatments, positively influenced the flowering process. The consistency across years implies that the treatments have a stable effect over time. The statistical analysis supports the conclusion that treatments, particularly T-03 to T-07, significantly reduce the time to flowering compared to the control, with some variations in the magnitude of the effect.
Figure 1 and 2, illustrates the number of days to maturity among treatments over two years. Comprehensible differences were observed in the number of days to maturity, while the control took the longest time for maturity. This parameter delineated fluctuations among control and treatments.
Significant differences were observed for plant height as shown in the Figure.1 & 2. Difference of the plant height among the years, for the same treatments indicated that the treatments and their potential to affect any trait are also attributed to the environmental fluctuations.
Both the Figures of all the treatments for number of pods indicated varied figures and significant differences, over both the years. Treatment-3 produced highest number of pods per plant during the first year. Treatment-1 and Treatment-6 outlined relatively consistent performance over both the years. Fluctuations were observed in said parameter over both the years which may be attributed due to environmental factors.
The trend of the number of branches for the consecutive years is portrayed in Figure (1) and (2) indicating the Treatment-5 as loftier than other applied treatments in first year, followed by Treatment-4 and Treatment-6.
Significant differences were observed for pod length over both the years with peculiarity to Treatment-7. Though, slight fluctuations in this trend were observed between the years that may be due to some abiotic factors.
Number of seeds per pod highlighted significant difference (Figure.1 & 2) over both the years. Maximum seed setting per pod was observed tor the treatment-7, thus an increase in the overall yield.
Yield per plant portrayed significant differences over both the years. Treatment-7 accorded best results in both the experiments.

Table 2. Analysis of Variance for studied parameters

Source of	Df	DF	DM	PH	NBP^{-1}	NPP^{-1}	NSP^{-1}	PL	TY

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variation									
Replication	2	25.33	5.58	13.226	0.152	0.016	1.02	1.00	19.54
Treatment	7	68.85**	17.90**	142.72*	1.419**	128.46**	10.63**	3*	61.01*
Year (Y)	1	50.02**	102.08**	355.61**	12.251**	15211.9**	3.93*	360.10*	8600.13*
T X Y	7	33.63**	39.08**	87.59*	0.953**	157.27**	5.07*	20.36*	23.07*
Error	30	0.911	2.45	28.83	0.044	1.74	0.87	0.002	1.17
Total	47								

Treatment-07 vouchsafed for both the years in yield per plant. Treatment-1 and Treatment-2 remained parallel consecutively. Treatment-3 and Treatment-4 did not show a significant difference in yield between the two years, suggesting that these treatments may offer more stable performance over time.

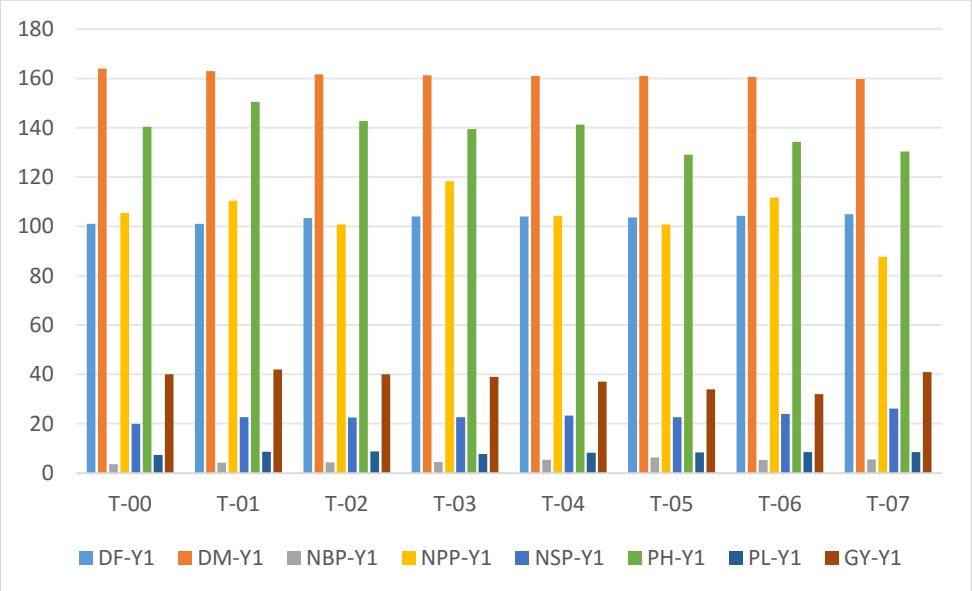


Figure 1 Comparative performance of different treatments (T-00 to T-07) on growth and yield-related traits of the crop during Year 1.

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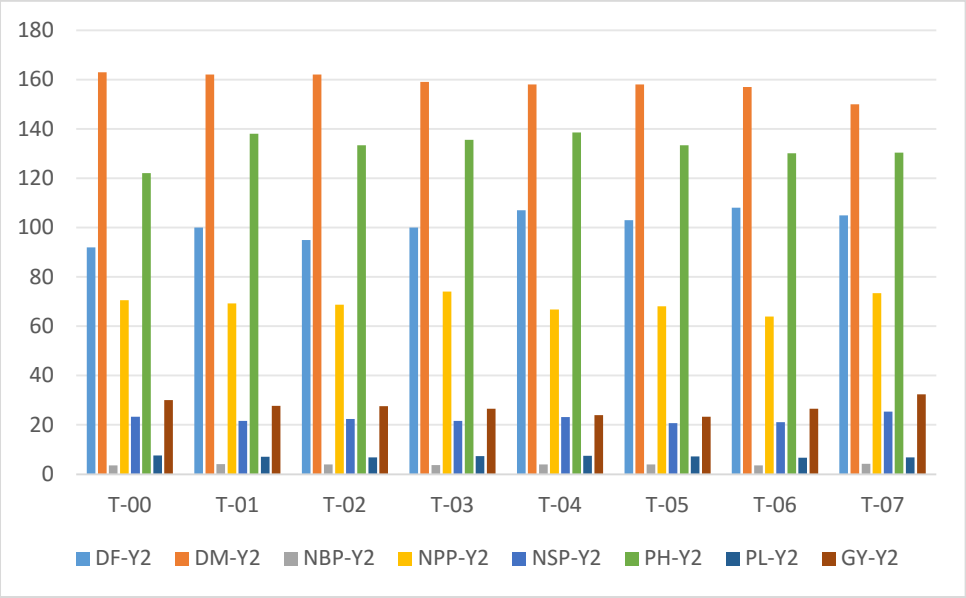


Figure 2 Comparative performance of different treatments (T-00 to T-07) on growth and yield-related traits of the crop during Year 2.

Correlation Analysis

This Pearson's correlation Figure depicts the correlation coefficient (r) among different variables such as days to Maturity, days to flowering, plant height, pod length, number of seeds per pod, number of branches per plant, number of pods per plant and, yield per plant. The correlation coefficients are color coded ranging from -1 (Strongly negative) to +1 (Strongly positive).

Correlation coefficient for days to maturity and days to flowering stood at 0.7. A strongly positive and significant correlation was observed for said parameters. For days to maturity and plant height value of correlation coefficient stood at 0.76, hence a significant positive correlation was presented between said parameters. Lofty plant heights were observed for a greater number of days to maturity. A strong positive and significant correlation was observed for number of branches per plant and total yield. Higher yield is associated with greater number of branches per plant. For number of branches per plant and yield, values of correlation coefficient were 0.67. While, for the second year, number of branches per plant portrayed a strong positive correlation with number of seeds per pod The value correlation coefficient (r) for these variables stood at 0.77, suggesting that the increase in the number of branches per plant is parallel to number of seeds per pod. A strong positive correlation was portrayed between number of branches per plant and pod length with a correlation coefficient at 0.65. Moreover, number of branches per plant depicted a strong positive correlation with total seed yield in parallel, and correlation coefficient stood at 0.69.

A moderately positive correlation was observed for parameters such as between yield per plant and days to maturity, between number of seeds per pod and yield per plant, between plant height and days to flowering, whereas, in the second year, a moderate positive correlation was observed between plant height and days to maturity.

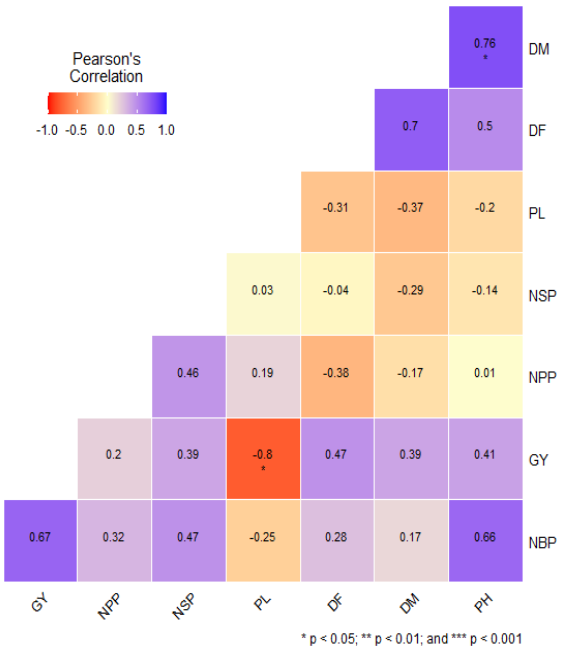
In first year, a weak negative correlation was observed between pod length and days

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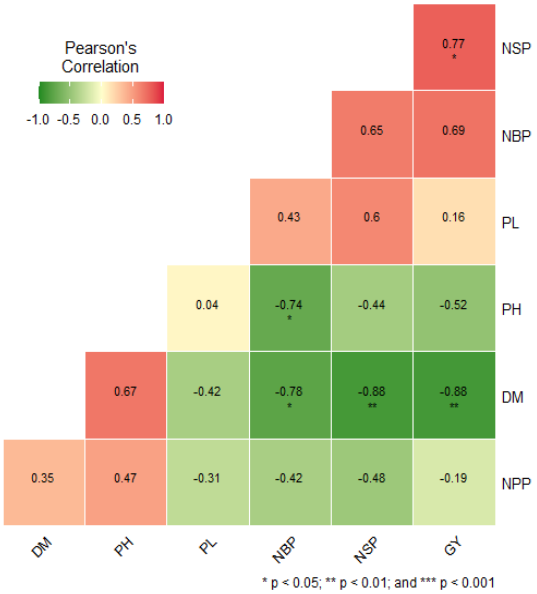
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to flowering, suggesting a slight tendency for plants with longer pod length to flower earlier. For pod length and grain yield a significant but negative correlation was depicted. This could be due to the trade-off between pod length and grain yield, as more nutrients are utilized in increasing pod length hence resulting in less seed setting. The value of correlation coefficient observed for these parameters stood at -0.8, whereas, in the second-year experiment, a strong negative correlation was observed among days to maturity, plant height, number of seeds per pod and, number of branches per plant. The significant correlations suggest reliable relationships that are unlikely to occur by random chance.



Correlation Heat Map-Year.2022-23



Correlation Heat Map-Year.2023-24

Figure 3. Correlation heatmaps illustrating the relationships among agronomic and yield-related traits during 2022–23 and 2023–24. The color scale represents the direction and magnitude of correlation coefficients (r), ranging from –1 (strong negative, blue/green) to +1 (strong positive, red/orange).

Principle Component Analysis (Biplot Analysis)

In Principle components analysis, vectors that point in same direction are positively correlated while those pointing in opposite direction are negatively correlated.

Biplot Figures for the first- and second-year experiments represented Principal components, portraying the relationship of different variables recorded. Along with detection of variance in the parameters and genotypes, PCA also insight in to correlation among variables and treatments and also highlighted the distribution and grouping of samples based on principal components in both the years.

Biplot Figure for the year 2022-23, illustrated two principal components i.e. PC1 and PC2: accounting 63.8% and 14.9% of total variance in the data set respectively

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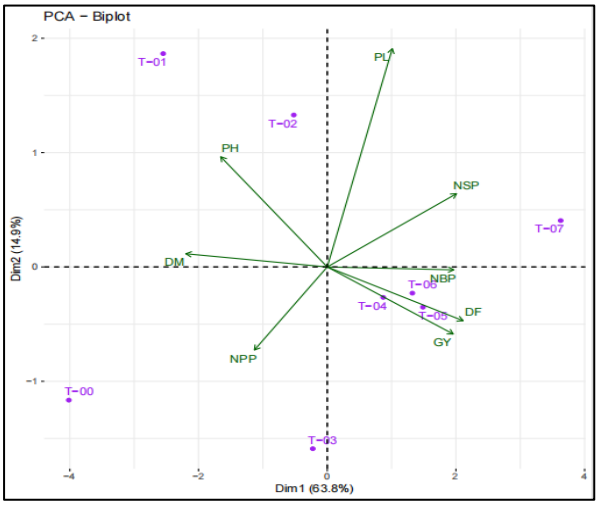
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accounting for 78% total variation while Biplot Figure for the year 2023-24 illustrated a total variation of 68.2% with PC1 at 41.8% and PC2 at 26.4% of total variance. Hence it provided comprehensive view of data in two dimensions. Direction and length of each vector indicated the direction and magnitude of variable influence on principal components.

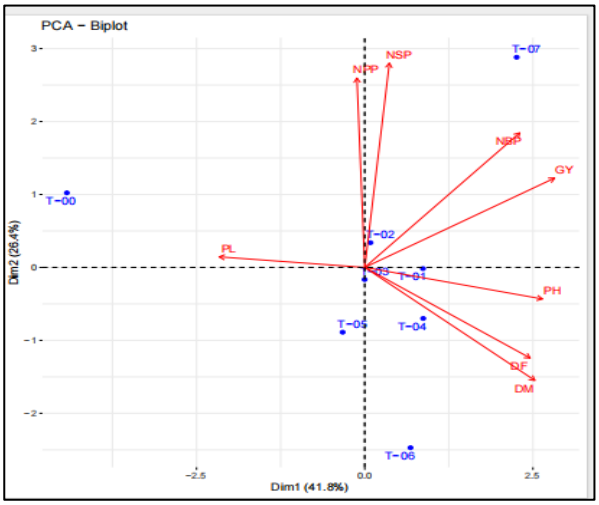
In the Figure (A), Plant height and pod length vectors pointed in similar direction indicating a strong positive correlation, number of seeds per pod and number of branches per plant depicted a positive correlation as vectors of both variables had same direction. Vector direction for number of pods per plant was opposite to number of seeds per pod and pod length indicated a negative correlation among said variables. While the purple dots represented different applied treatments. Treatment-1 was positioned positively along PC2, indicating higher values of variables for days to maturity and plant height. Said description portrayed that plants with treatment-1 showed elevated plant height and days to maturity. Treatment-2 was also positioned parallel to treatment-1 hence, also depicting same trends for mentioned variables. Control was positioned negatively along both PCs, indicating lower values for plant height and days to maturity. While treatment-7 was positioned as to indicate higher values for number of seeds per pod and number of branches per plant, whereas in Figure (B) for the year 2023-24, the strong positive correlation was indicated by total yield, plant height, days to flowering and days to maturity. Number of seeds per pod and number of pods per plant were in strong positive alignment in PC2. Pod length was negatively correlated to mentioned variables, as the vector was pointed in opposite direction. Treatments were projected on the vectors to understand their variable contribution, in which Treatment-7 indicated high effect on variables like yield, plant height, days to flowering and days to maturity.

A



BIPLOT, YEAR 2022-23

B



BIPLOT, YEAR 2023-24

Figure 4. Principal Component Analysis (PCA) biplots illustrating relationships

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among agronomic and yield related traits and treatment distributions during 2022–23 (A) and 2023–24 (B).

Discussion

Combined ANOVA shows that each factor i.e., treatment and years and their interaction had a significant effect on parameters studied (dependent variables). This implies that not only such factors i.e. treatment or year, individually influence the outcome but the interaction between treatment and year also plays critical role in influencing each parameter. The parameter days to flowering indicated prolonged duration for control whereas all the boron treatments applied positively affected the said parameter and reduced days to flowering, particularly T-03 to T-07, significantly reduced the time to flowering compared to the control, with some variations in the magnitude of the effect, which could be beneficial in contexts where earlier flowering is desired. Similar results were reported by Dinh et al., (2021), indicating that low levels of boron in the soil could impact flowering process due to drop in cell elongation and pollen formation process ultimately prolonging the process of flower formation.

Comprehensible differences were observed in the number of days to maturity, while the control took the longest time for maturity as longest number of days to flowering were also observed by control hence resulting in late maturity. This parameter delineated fluctuations among control and treatments (T-03 to T-07). Similar results were reported by Malhi et al., 2003; Safdar et al., (2023), showing that boron deficiency slow down the process of cell elongation, flower initiation and completion consequently causing late maturity in Brassica napus.

Potential differences i.e. increase in plant height, were posed by various treatments (T-01, T-04 followed by T-07) as compared to the control, between years. Difference of the plant height between the years, for the same treatments may be attributed to the environmental fluctuations. Increase in number of pods per plant was posed by Treatment-3 during the first year and Treatment-1 and 6 outlined relatively consistent performance over both the years, depicting the improvement of pod production and yield onward via boron applications. Total yield per plant portrayed notable behaviour for all the treatments, but filtered treatment (T-7) as a more promising dosage level for mentioned trait' improvement. Yang et al., (2009); Safdar et al., (2023), reported similar results in their two year studies on micro-nutrients i .e. boron and zinc.

Pod length and seeds per pod showed consistent behavior for control and treatments in first year while second year filtered the treatment (T-7) as a more promising dosage level for mentioned traits' improvement. Another yield contributing parameter, number of branches per plant remained consistent for control in both the years while Treatment-5 stood loftier than other applied treatments in first year, followed by Treatment-4 and Treatment-6 implying that the betterment of said trait could be achieved by boron supplementation. Malhi et al., (2003) reported similar results, when conducted an experiment of boron application in soils of china, where addition of said micro-nutrient resulted in improvement of plant height, pod bearing branches, pods per plant and seed setting per pod ultimately, increasing the total yield of crop.

Significant variations were obtained within replications, in the same year in both the experiments among all studied parameters while some fluctuations were observed

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between years which may be due to the environmental variations or management practices. Though, variations with in replications and in experimental setup are not reliable only on treatments and years. Thus, boron supplementation resulted in improvement of most of the yield contributing parameters. Similar results were reported by Mortvedt, (1994), indicating that the boron supplementation via soil and foliar application, is the most effective way to complement boron content to the plants. Significant relationship among key agronomic parameters and their influence on the total yield in Brassica napus was highlighted by the correlation analysis.The results indicated a strong positive correlation between days to flowering and days to maturity ($r = 0.7$), besides plant height and days to maturity ($r = 0.76$), intimating the fact, longer growth span may contribute to elevated plant height. Another positive correlation was also indicated among total plant yield ($r = 0.67$) and seeds per pod ($r = 0.77$) with number of branches. Thus demonstrating the vital role of mentioned yield contributing parameter. Moreover, moderately positive correlations were observed for yield per plant and days to maturity, along with seed count per pod and total yield, reinforcing the fact that reproductive efficiency contribute to the yield potential of the crop. However, parameters such as pod length ($r = -0.8$) and total yield are significantly and negatively correlated to each other. Suggesting, a tradeoff between pod length and grain yield. These findings delineate that the optimization of number of branches and seed count per pod could be effective in yield enhancement, while excessive pod elongation may prove to curtail the yield. These insights clarify a foundation for targeted breeding strategies to improve Brassica napus productivity.

Among two experimental years, the association among varied parameters was depicted via Principal Component Analysis (PCA). The trait distribution on biplot Figures for 2022-23 and 2023-24 evoked 78% and 68.2% of total variance, respectively. In first year i.e., 2022-2023, the parameters: number of branches per plant and number of seeds per pod depicted positive alignment contrarily, a negative correlation was observed for pod length and number of seeds per pod. Distribution of treatment variation was also illustrated in the biplot, where an increased plant height and days to maturity were observed for Treatment-1 and Treatment-2 as compared to control. Higher number of branches and soared seed count was observed for Treatrment-7.

In second year i.e., 2023-2024, key findings indicated a negative correlation for pod length and total yield, days to flowering and days to maturity. Mentioned parameters were also influenced by Treatment-7. Principle Component Analysis unfolded the impact of different boron treatments on plant yield and its components suggesting a recuperated production of Brassica napus could be achieved by standard management practices.

Conclusions

Present experiments signify the broad variation among the boron treatments applied to rapeseed and, also the significant variation among the stages of application of supplementation. Sources of variation i.e. replications, treatments, years and their interactions were all significant, indicating that all sources have meaningful impact on measured variables. The parameters which depicted prominent outcomes under applied treatments included days to flowering, plant height, number of pods per plant,

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seeds per pod and total yield. The treatment at which all the parameters stood distinguished, filtered to be the Treatment-07 and, the stage of treatment application which was better influenced by treatments was full dose foliar application at flowering stage i.e. at 0.15 grams per 40 kilograms of soil. Correlation and principle component analysis also portrayed the significance among studied parameters and supported the outstanding results of treatment-07 at flowering stage of foliar application. Thus treatment-07 could be used in future initiatives at flowering stage via foliar application for studying the boron effects and its supplementation sequels.

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Declaration
Authors declare no competing interest.

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