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Research Article

Impact of *rhizobium* inoculation and boron application on morphological alterations and biochemical triggers in pea (*Pisum sativum* L.)

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Abstract
Rhizobium is a bacterial culture that enhances the speed of biological nitrogen fixation in association with root nodules in pulse crops while boron act as a catalyst for cell division hence the size of nodules and sugar translocation in the plant. An experiment was conducted by considering *rhizobium* as a seed inoculant and boron as a soil applicant in two peas (*Pisum sativum* L.) varieties Arkel and Azad Pea-3 to know the morphological alterations in plant height (cm), fresh and dry weight g plant^{-1}), number of leaves (plant^{-1}), and days required for the first flowering and picking. However, the biochemical triggers were also observed for soil plant analysis development reading (SPAD), total chlorophyll (mg g^{-1}) and derivative (chlorophyll a and b), protein (mg g^{-1}), and proline content (µg g^{-1}) in both varieties of pea (Arkel and Azad Pea-3). The performance of V_2 was superior to V_1 for most of the morphological and biochemical parameters. The combination of *rhizobium* + 2.0 kg boron ha^{-1} (T_5) was observed as the best treatment compared to the rest of the treatments in V_1, while the *rhizobium* + 1.5 kg boron ha^{-1} (T_4) in V_2. Among the treatment combinations, T_5 and T_4 were recorded as minimum days for the first flowering (39.3 and 43.0), while the maximum days for the first picking (75 and 84) in V_1 and V_2. The highest SPAD reading, total chlorophyll, and protein were also noticed in T_5 and T_4, while chlorophyll a was recorded at maximum value in T_4 for both varieties. However, the value of chlorophyll b was recorded as maximum in T_4 and T_3 at 50 DAS, while T_5 and T_4 were at 75 DAS. A trigger of proline content was also noticed in T_5 and T_4 of both varieties compared to the control set. The positive alteration in morphology and biochemical changes in pea plants due to the application of *rhizobium* in combination with boron interlink well for the betterment of yield via increasing the rate of photosynthesis.

Keywords: Boron, Chlorophyll, Proline, Protein, *Rhizobium*

INTRODUCTION

The pea (*Pisum sativum* L.) is one of the magistral crops grown worldwide as a multi-use in which green vegetables and pulse are one of them. It is a rich source of protein, carbohydrates, and minerals like Fe, B, and Mo; moreover, it is a crucial element in symbiotic nitrogen fixation in pulse crops (Kelly *et al.,* 2021). The symbiotic association between the root nodules and *rhizobium* culture is significant in enabling legumes to fix nitrogen from the atmosphere into the soil. At the same time, deviation negatively impacts the rate of biological nitrogen fixation (Pulido-Suárez *et al.,* 2021). *Rhizobium* influences not only the growth of nodules but also the growth of roots and shoots in pulse crops (Laguerre *et al.,* 2007). Symbiosomes with endosymbiotic rhizobia bacteria in nodules serve as a temporary plant organelle for facilitating nitrogen fixation. In contrast, bacteria metabolically depend on the host legume (Coba de la Pena *et al.,* 2018). Biological nitrogen fixation maintains soil health if symbiosis is effective in which glycoconjugate from *rhizobium*'s cell surface plays a crucial role in establishing the symbiosis between legumes and rhizobia during the development of root nodules in legumes (Wheatley *et al.,* 2020). Integrated approaches of boron and the rest of the essential elements help maintain soil fertility (Quddus *et al.,* 2018). The scarcity of boron increases enzymatic and nonenzymatic oxidation, leading to increased polyphenol oxidase (Shireen *et al.,* 2018). However, with changes in the leaf area and its composition, boron poisoning may impair the rate of photosynthesis (Antonopoulou *et al.,* 2017). The availability of boron in soil accelerates photosynthesis and boosts the synthesis of carbohydrates and its translocation from source to sink; consequently, entire morphological growth, including vegetative and reproductive, both benefited (Sharma and Sharma, 2016 and Qamar *et al.,* 2016). However, the quantity of boron is needed more for the development of nodules than for other plant parts (Redondo□Nieto *et al.,* 2003). The main objective of the current study was to know the collective effort of *Rhizobium* and boron on the morphological alteration and biochemical changes in favor of the yield of two pea plant varieties of Arkel and Azad Pea-3.

MATERIALS AND METHODS

Preliminary information

The present study was conducted over the Research Farm of Lovely Professional University in 2019-20 during the *rabi* season. Two popular varieties of pea Arkel and Azad Pea-3 (V_1 and V_2) were used to analyze the impact of *rhizobium* as a seed inoculant and boron as a soil applicant in pea crops. The seeds of pea varieties were procured from the Punjab Agriculture University, Ludhiana, Punjab. Both varieties were employed with three replications and six treatments and executed in factorial randomized block Design (FRBD) whereas T_0= (without any treatments), T_1= *rhizobium*, T_2= *Rhizobium* + 0.5 kg Boron ha^{-1}, T_3= *rhizobium* + 1 kg Boron ha^{-1}, T_4= *rhizobium* + 1.5 kg Boron ha$^{—1}$, T_5= *rhizobium* + 2 kg Boron ha^{-1} were used for each variety. A recommended dose of fertilizers @ 20 N and 25 kg P_2O_5 per acre was applied in the research field at the time of sowing while K_2O is not recommended in Punjab.

Morphological attributes

The morphological observations like plant height (cm), fresh and dry weight of (g plant^{-1}), number of leaf plant^{-1}, days required for first flowering, and days required for first picking were measured by following standard protocol, whereas the five randomly selected plants from each plot were considered to measure the morphological attributes and the average value was used interpret the data with treatments.

SPAD reading and estimation of total chlorophyll

Soil plant analysis development reading (SPAD) was measured by SPAD meter (Model No- SPAD-502 Plus), which measures the greenness in the leaf in terms of chlorophyll while the analysis of total chlorophyll content through a biochemical process was also estimated using a method (Arnon, 1949).

Computation of total chlorophyll, a, and b

The computation of total chlorophyll, chlorophyll 'a', and 'b' were carried out as per the formula given below.

Total chlorophyll = 20.2 (D645) + 8.02 (D663) x V / 1000 x W …..Eq. 1

Chlorophyll a = 12.7 (D663) + 2.69 (D645) x V / 1000 x W …..Eq. 2

Chlorophyll b = (22.9 X D646) – (4.68 X D 663) x V / 1000 x W …..Eq. 3

Whereas

V= Final volume of sample

W= Weight of a sample taken for the extraction of pigment

Protein estimation

The measurement of protein was carried out as per the protocol of (Lowry *et al.,* 1951) in which Folin Phenol was used as a reagent and bovine serum albumin (BSA) as a standard to quantify the amount of protein present in the sample.

Proline estimation

Proline content was carried out as per the protocol of (Bates *et al*., 1973), in which toluene was used to separate the proline from the sample while pure proline was used as a standard to quantify the presence of proline in the sample.

Statistical analysis

Replicated data of each character studied in the present part of the research work was analyzed through SPSS software 23rd version while the least significant differences (LSD) and Duncan's multiple range test (DMRT) were used to examine the significance among the treatments at a probability (P≤0.05).

RESULTS AND DISCUSSION

Morphological growth of pea plant

Results obtained from the present piece of work represented the influence of the seed and soil application of *rhizobium* and Boron on morphological alteration and biochemical triggers in pea plants. The plant height, fresh weight, dry weight, and the number of leaves were the most fundamental traits of growth that were used to evaluate the impact of treatments on morphological alteration (Table 1). As per the results, T_5 (*rhizobium* + 2 kg Boron ha^{-1}) recorded the significantly highest value (p≤0.05%) irrespective of the rest of the treatments for plant height, fresh weight, and dry weight, and the number of leaves plant-$^{-1}$ (69.7cm, 85.7g, 35.4g, 64.7 respectively) in V_1 (Arkel). However, T_4 (*rhizobium* + 1.5 kg Boron ha^{-1}) was recorded as one of the significantly best treatments (72.5cm, 94.3g, 39.3g, 65.3 $plant^{-1}$) in V_2 for the same parameters. The close data analysis also reflected the difference among the varieties while finding that V_2 performed comparatively better than V_1. The performance of T_3 (*rhizobium* + 1 kg Boron ha^{-1}) was found second most (67.6cm, 82.4g, 35.1g, 60.7 and 70.7cm, 88.2g, 36.7g, 63.3 plant $^{-1}$) in both the varieties (V_1 and V_2). The days required for first flowering were observed significantly least days in T_5 and T_4 (39.3 and 43.0), while the minimum days for first picking out were recorded in T_2 and T_1 (71 and 79.7) as compared to the rest of the treatments in V_1 and V_2 (Table 1).

SPAD reading, total chlorophyll, a and b

The impact of given treatments was also reflected in biochemical triggers of the SPAD reading, total chlorophyll, chlorophyll a, chlorophyll b, protein, and proline content in both varieties (V_1 and V_2) in which SPAD reading delineated the greenness of the leaf in terms of chlorophyll. Data depicted in (Fig. 1) showed the impact of seed and soil application of *rhizobium* and boron on the SPAD reading was recorded as a significant difference at (p≤0.05%). Out of all the treatment combinations, the highest value of SPAD reading was detected in T_5 and T_4 (32.8 and 35.4) in the respective varieties (V_1 and V_2) whereas V_2 was comparatively better than V_1 most of the time at all the times of observations. In addition to this, T_5 and T_4 were also recorded to have significantly the highest amount of total chlorophyll content in V_1 and V_2 at all the intervals of observation (0.62, 0.86, 1.81, and 0.64, 0.87, 1.85mg g^{-1}) while it also showed V_2 is marginally better than V_1 (Fig. 2). Moreover, both the parameters i.e. SPAD reading and total chlorophyll content showed an almost similar trend regarding the treatments in both the varieties.

Data regarding chlorophyll a and b are depicted in Fig. 3 showing the impact of treatments on both traits. The significantly highest quantity of chlorophyll a was derived from T_5 and T_4 (0.666, 0.713 and 1.30, 1.28 mg g^{-1}) at both the time of observation 50 and 75 DAS while the chlorophyll b was derived from T_4 and T_3 (0.54 and 0.59 mg g^{-1}) in their respective varieties at 50 DAS. In contrast, it declined sharply in the entire set of treatments at 75 DAS and recorded a significantly highest value of chlorophyll b in T_5 and T_4 (0.19 and 0.16 mg g^{-1}) as compared to rest of the treatments, including the control set in their respective varieties (Fig. 4).

Protein and proline content in pea plant

The data depicted in Fig. 5 showed an increment of quality parameter, i.e. protein content at both the intervals in the entire set of treatments, while the significantly highest quantity of protein was recorded in T_5 and T_4 (57.38 and 55.44 mg g^{-1}) in respective varieties of V_1 and V_2 at 75 DAS. The varietal response due to the impact of treatments showed that V_1 was found superior over V_2 for protein content. A trigger of osmoregulatory compound proline content was detected in the leaf of pea plants due to the impact of *rhizobium* inoculation and boron application in both varieties (Fig. 6). However, the highest trigger was recorded in T_5 and

Table 1 Effect of *rhizobium* and boron application on morphological growth at harvest

Treatment details		Plant height (cm)	Fresh weight $plant^{-1}$ (g)	Dry weight $plant^{-1}$ (g)	Number of leaves $Plant^{-1}$	Days required for the first flowering	Days required for first picking
V_1	T_0	60.6^{a}	71.2^{a}	28.7^{a}	49.7^{a}	43.0^{bcd}	70.0^{a}
	T_1	63.5^{ab}	76.4^{ab}	31.7^{bc}	55.3^{abcd}	41.4^{ab}	71.3^{a}
	T_2	62.9^{ab}	76.2^{ab}	30.1^{ab}	53.3^{abc}	42.3^{abc}	71.0^{a}
	T_3	67.6^{bcd}	82.4^{bc}	35.1^{de}	60.7^{de}	41.0^{ab}	71.3^{a}
	T_4	64.8^{abc}	79.3^{abc}	32.0^{bc}	58.7^{bcde}	41.0^{a}	72.0^{a}
	T_5	69.7^{bcd}	85.7^{c}	35.4^{de}	64.7^{e}	39.3^{ab}	74.0^{a}
V_2	T_0	62.9^{ab}	75.6^{ab}	31.8^{bc}	52.0^{ab}	48.0^{e}	79.7^{b}
	T_1	63.7^{ab}	80.5^{bc}	33.6^{cd}	55.3^{abcde}	47.7^{e}	79.7^{b}
	T_2	68.7^{bcd}	84.8^{bc}	35.7^{de}	62.7^{e}	46.0^{de}	81.3^{bc}
	T_3	70.7^{cd}	88.2^{cd}	36.7^{e}	63.3^{e}	45.7^{cde}	83.0^{bc}
	T_4	72.5^{d}	94.3^{d}	39.3^{f}	65.3^{e}	43.0^{bcd}	85.0^{c}
	T_5	64.2^{abc}	84.6^{bc}	35.2^{de}	59.7^{cde}	46.0^{ab}	80.7^{b}

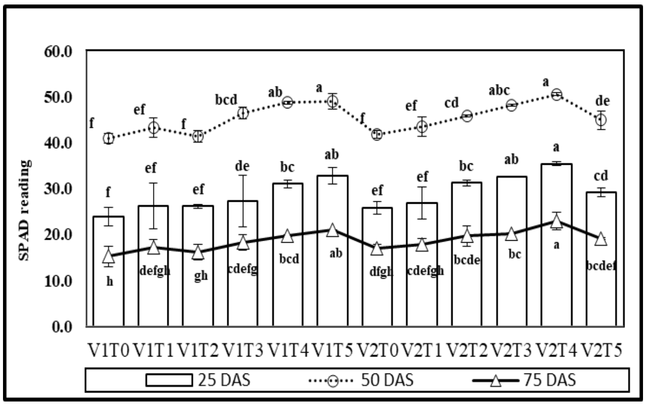

Fig. 1. *Effect of rhizobium and boron application on SPAD reading*

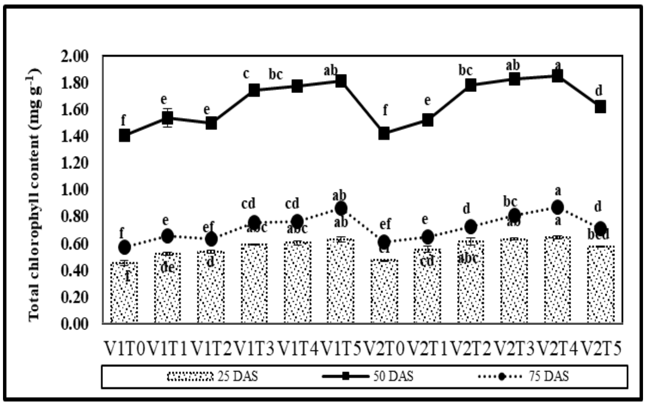

Fig. 2. *Effect of rhizobium and boron application on total chlorophyll content (mg g*$^{-1}$*)*

T_4 (123.17 and 107.58 µg g^{-1}) as compared to the rest of the treatments including the control one (91.72 and 86.68 µg g^{-1}). A close analysis of the data showed that the trigger of proline content in V_1 is high irrespective of V_2 (Fig. 6).

Data depicted in Table 1 shows morphological alterations in the entire set of treatments while T5 and T4 proved a potential source of treatments that had a high impact on morphological traits like plant height, fresh and dry weight of plants, and the number of leaves in their respective varieties. The results are well related to the findings of Ragaa and Safinaz (2013), who affirmed that the use of *rhizobium* promotes the morphological growth of pulse crops by enhancing the speed of nitrogen fixation. It is already well established that *rhizobium* culture establishes a symbiotic association with root nodules in Leguminosae crop resulting in an increase in nitrogen fixation and availability of nitrogen in the soil which in turn supports the morphological growth of plants (Mabrouk *et al.,* 2018; Lindström and Mousavi, 2020 and Mus *et al.,* 2016). In addition to this, the amount of nitrogen fixation process also depends upon the number and size of root nodules.

Boron is one of the efficient sources that not only enhance the number and size of root nodules but also enhances the availability of nitrate by interacting in the nitrogen metabolic process (Bellaloui *et al.,* 2014). Qamar *et al.,* (2016) reported that the presence of boron in an appropriate amount in the soil boosts the synthesis of carbohydrates and its translocation from source to sink mediated by encouraging the rate of photosynthesis; consequently, entire morphological growth and yield and yield attributes benefited (Sharma and Sharma, 2016 and EL-Mahmoudy *et al.,* 2019) while in contrast, the scarcity of boron leading to increase the amount of polyphenol oxidase in the plant (Shireen *et al.,* 2017). The biochemical triggers like total chlorophyll, chlorophyll a, b, protein content, and osmoregulatory amino acid were increased due to the impact of given treatments in pea plants (Fig. 2 to 6). Moreover, the SPAD reading was also increased as total chlorophyll in the same treatment (Fig. 1). The results of this study were positively correlated with the finding of Bejandi *et al.,* (2012) who reported that seed inoculation with *rhizobium* and the application of micronutrients increase the content of chlorophyll and protein in the pea plant. *rhizobium* is well known for the mechanism of nitrogen fixation (Wang *et al.,* 2018) while nitrogen is

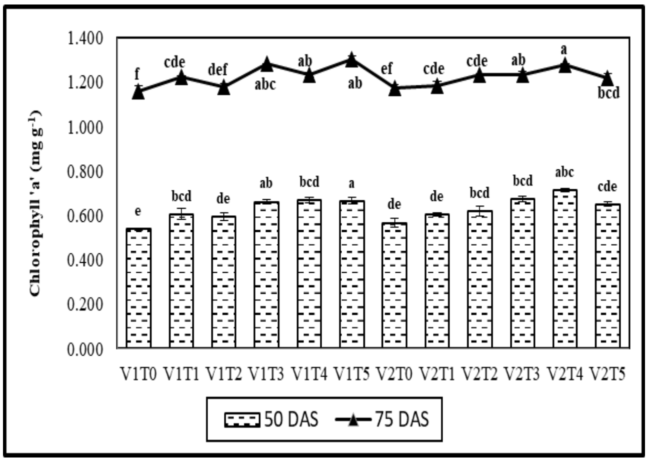

Fig. 3. *Effect of rhizobium and boron applications on chlorophyll 'a' (mg g*$^{-1}$*)*

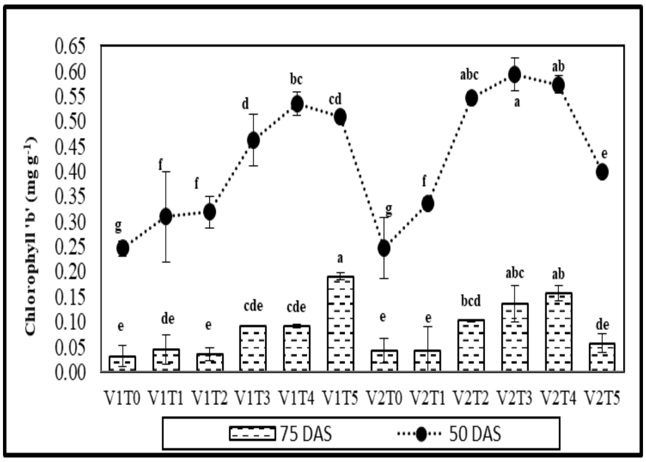

Fig. 4. *Effect of rhizobium and boron application on chlorophyll 'b' (mg g*$^{-1}$*)*

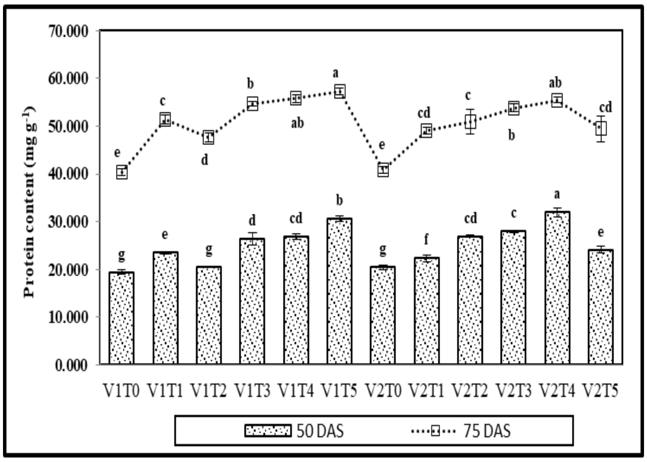

Fig. 5. *Effect of rhizobium and boron application on protein content (mg g^{-1})*

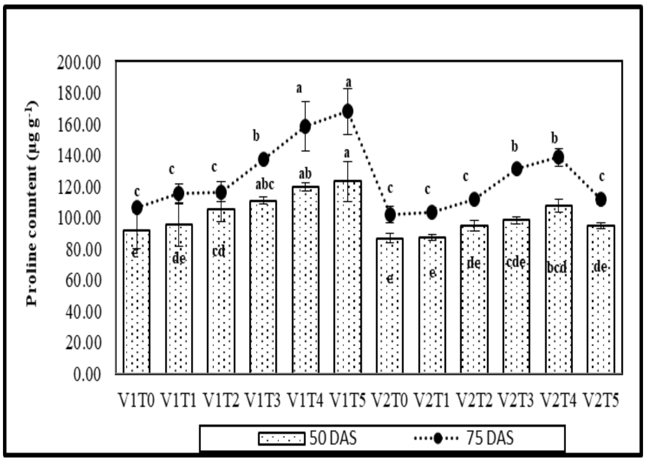

Fig. 6. *Effect of rhizobium and boron application on proline content (µg g^{-1})*

a base compound of both the molecules chlorophyll and protein (Sara *et al.,* 2013). Therefore, the inoculation of *rhizobium* is beneficial for enhancing the content of chlorophyll, protein, and subsequently the rate of photosynthesis, and carbohydrates in the plant. In addition to this, boron is also useful for increasing the root, shoot growth, structural integrity, cell division, number and size of root nodules, and sugar translocation from source to sink, while it also reduces flower drop in pulse crop (Bariya *et al.,* 2014; Raj *et al.,* 2019; Vitry *et al.,* 2010 and Shireen *et al.,* 2018). Moreover, the synthesis of osmolyte proline may be increased due to the variable environment during the growth period (Siddique *et al.,* 2018). Therefore, the integrated approach of *rhizobium* inoculation and soil application of boron may be coordinated well to modify the morphological structure by triggering the biochemical synthesis in the pea plant even under adverse environmental conditions.

Conclusion

The present study affirmed that the given treatments positively impacted morphological alteration and biochemical triggers in both the varieties of pea, i.e. Arkel and Azad Pea-3 with a bit of variation. Among the treatment combinations, T_5 (*rhizobium* + 2 kg Boron) and T_4 (*rhizobium* + 1.5 kg Boron ha^{-1}) were detected as the best combination for most of the morphological and biochemical traits studied. The increase in total chlorophyll content and their derived a and b confirmed the accelerated rate of photosynthesis and, subsequently, synthesis of carbohydrates which may have helped in the better morphological growth of pea plants. The additional amount of protein synthesis in respective treatments showed the efficiency of treatments for better grain quality, while the synthesis of proline in T_5 and T_4 treatments may help against the collision in variable environments.

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Conflict of interest

The authors declare that they have no conflict of interest.

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