

Research Article

Biostimulants and calcium-mediated optimization in morpho-phenological growth of mung bean (*Vigna radiata* L.) in the summer season**Poonam Kumari**

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Abstract

Mung bean, botanically referred to as *Vigna radiata* is valued for its superb quality of nutrition, which includes a large amount of protein, minerals, and vitamins, making it a staple worldwide. Morpho-phenological traits are critical indicators of plant growth and development, which influence the overall yield and quality of the mung bean; thus, optimizing these traits can improve the yield barriers. Therefore, this study aimed to analyze the impact of biostimulants and calcium-mediated response on the morpho-phenological growth of mung bean while assessing the growth, plant height (cm), number of leaves, leaf area plant⁻¹ (cm²), leaf area index (LAI), crop growth rate (CGR), leaf area duration (LAD), SPAD reading, and total chlorophyll content (mg g⁻¹) were used. Among the treatment combinations, T₇ (Calcium, 10mM + Bio-priming with Rhizobium + Putrescine, 3mM) showed statistically significant improvements (p < 0.05%) in all measured parameters. This treatment yielded the highest values across the board. The second-best results were observed with the T₅ (Calcium, 10mM + Putrescine, 3mM), which also showed significant improvements for most of the parameters at both intervals, i.e. 30 and 60 days after sowing (DAS). Moreover, the analysis of the data in terms of percent increase/decrease over control produced a clear view of the morpho-physiological growth in the T₇ and T₅ treatments compared to the control, highlighting their effectiveness. Overall, this study revealed the significance of a synergistic approach of calcium, putrescine, and biopriming with rhizobium to optimize the morpho-phenological traits and enhance the yield of mung bean in the summer season.

Keywords: Calcium, Leaf area duration, Leaf area index, Mung bean, Putrescine, *Rhizobium***INTRODUCTION**

Mung bean (*Vigna radiata* L.) is well-known for its ability to thrive in all climates and soil types, making it a desirable crop in traditional and modern agricultural systems (Megu *et al.*, 2024). In addition to its use in agriculture, mung bean is known for its outstanding nutritional composition, characterized by elevated quantities of protein, fiber, vitamins, and minerals. Shattering loss is one of the main problems caused by yield loss in mung bean plants, and this causes a huge loss in mung bean production (Ranjan *et al.*, 2024). Biopriming is a sustainable agriculture practice that involves

treating seeds with beneficial bacteria to improve the process of seed germination and promote root growth. Rhizobia species are particularly notable in bio-priming techniques because of their capacity to establish symbiotic associations with leguminous plants, including soybeans, peas, mung beans, and chickpeas (Lohitha and Dutta, 2022). These microorganisms also produce growth-promoting substances like phytohormones or enzymes that coordinate nutrient uptake and its utilization by the plant (Suhail and Hussein, 2024; Chaurasia *et al.*, 2024). Putrescine, a chemical belonging to the class of polyamines, garners significant interest across the disciplines of biology, medicine, and agriculture due

to its diverse range of physiological functions and potential applications. It plays crucial roles in cellular functions such as cell growth, differentiation, and programmed cell death, underscoring its importance in maintaining optimal health (Kumar *et al.*, 2024). This highlights its significance in agricultural productivity and food quality (Kolesnikov *et al.*, 2024).

The application of calcium to plant leaves has gained widespread recognition as a beneficial agricultural practice for enhancing crop yield, quality, and resilience to environmental challenges (Liu *et al.*, 2024). Calcium is a crucial nutrient for the growth and development of plants, as it plays important roles in maintaining the formation of cell walls, the integrity of membranes, the activation of enzymes, and the functioning of signalling pathways (Kour *et al.*, 2023). Moreover, using calcium as a foliar treatment is a highly effective method of improving the efficiency of nutrient absorption. It also coordinates in mitigating abiotic and biotic stress (Domnariu *et al.*, 2024). Optimizing morpho-phenological traits of mung bean in the summer season to enhance the yield potential is most needed. Thus, this study aimed to analyze the impact of biostimulants and calcium-mediated response on the morpho-phenological growth of mung bean (*Vigna radiata* L.)

MATERIALS AND METHODS

Experimental site

An investigation was strategized and implemented to know the impact of biostimulants and calcium-mediated optimization on the morpho-physiological growth to improve the yield and yield contributing characters in mung bean (*Vigna radiata* L.) during the summer season (March to May) of 2022 and 2023 at the research farm of Lovely Professional University.

Treatment details

The experiment was executed with eight possible combinations of the treatments in Randomised Block Design (RBD) in triplicate with SML-668 variety of green gram. The combinations of the treatments are composed of Rhizobium, Putrescine (3 mM), and Calcium (10 mM), wherein Rhizobium was used as a bioprimer while calcium and putrescine were used as a foliar application through knapsack hand sprayer. Standard agronomical practices were employed during the experiment in both consecutive seasons (Kumar *et al.*, 2018; Paradva, 2018).

Growth analysis

To analyze the morpho-phenological growth of the green gram, plant height, number of leaves, leaf area, leaf area index (LAI), crop growth rate (CGR), leaf area duration (LAD), SPAD reading, and total chlorophyll content were used to conclude. To measure the leaf

area an instrument leaf area meter (Model No.211) was used to compute the LAI, CGR, and LAD, the following formulas were used as proposed by (Watson, 1947; Watson, 1952 and Power *et al.*, 1967).

$$\text{Leaf Area Index} = \frac{\text{Total leaf area (cm}^2\text{)}}{\text{Total ground area (cm}^2\text{)}} \quad \text{Eq. 1}$$

$$\text{Crop Growth Rate} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{1}{A} \quad \text{Eq. 2}$$

Whereas,

W_2 and W_1 is the dry weight at second and first time

T_2 and T_1 is the time intervals

A is the land area

$$\text{Leaf Area Duration} = \frac{L_1 + L_2}{2} \times T_2 - T_1 \quad \text{Eq. 3}$$

Whereas,

L_1 and L_2 are the LAI of the second and first intervals

T_2 and T_1 is the time intervals

SPAD reading

An instrument was used to record the SPAD reading over the field, i.e. SPAD meter (Model no. SPAD-502 Plus), which represents the greenness of the plant in terms of relative chlorophyll content. Five randomly selected plants from each plot were used to record the SPAD reading, and averages of 25 readings were presented to conclude the results.

Total chlorophyll content

A well-established procedure proposed by (Arnon, 1949) was used to estimate the amount of total chlorophyll. The extraction of pigments from the leaf samples was mediated by the 10ml of 80% acetone till the complete maceration. Whatman's filter paper was used to separate the fiber and filtrates in 25ml of graduated volumetric flasks while rinsing the pestle and mortar till the complete transfer of pigment and, in the end, maintained final volume up to 25 ml by adding the 80% acetone. However, 80% acetone was used to calibrate the Spectrophotometer during the optical density measurement at 663 and 645 nm, while a given formula was used to calculate the amount of total chlorophyll.

$$\text{Total chlorophyll} = (20.2 (D_{645}) + 8.02 (D_{663})) \times \frac{V}{1000 \times W} \text{ mg g}^{-1} \quad \text{Eq. 4}$$

Whereas,

V = Final volume

W = weight of sample

Statistical analysis

The present experiment was repeated twice in random-

ized block design while pooled for statistical analysis using SPSS software to determine the significance of variance ($p < 0.05\%$) while DMRT was used to compare the difference among the mean.

RESULTS AND DISCUSSION

Plant height, number of leaves, and leaf area plant⁻¹

The treatments evaluated over two consecutive summer seasons revealed plant height statistically significant at ($p < 0.05\%$). Among the entire combinations of the treatments, T₇ (Calcium, 10mM) + Bio-priming with Rhizobium + Putrescine, 3mM) measured the highest value of plant height, reaching 46.68 ± 1.29 and 66.19 ± 0.47 cm during the summer season at both 30 and 60 days after sowing (DAS). Data presented in (Table 1) illustrates the treatment impacts, with T₅ (Calcium, 10mM) + Putrescine, 3mM) exhibiting comparable results to T₇, recording 46.02 and 43.02 cm in both seasons at 30 DAS, while T₅ and T₆ were on par with T₇, measuring 64.28 and 65.67 cm during the summer.

The number of leaves plant⁻¹ measured at two consecutive intervals during the entire life span, i.e. 30 and 60 DAS are presented in Table 1, revealing that the recorded parameter was statistically significant at ($p < 0.05\%$). Among the treatment combinations, T₇ (Calcium, 10mM)+Bio-priming with Rhizobium + Putrescine, 3mM) measured the highest number of leaves plant⁻¹ 40.80 ± 0.60 and 57.43 ± 0.44 in the summer season, at the intervals of 30 and 60 DAS which was followed by treatments T₅, T₆, T₄, T₃, T₁ T₂ respectively. Out of all the sets of treatments, T₅ (Calcium, 10mM) + Putrescine, 3mM) was recorded at par with T₇ 56.47 at 60DAS in the summer season of mung bean.

Leaf area is one of the most important parameters that is directly linked with the leaf area index and leaf area duration of the crop. Moreover, it is equally responsible

for synthesizing carbohydrates through photosynthesis as chlorophyll. The influence of treatments is presented in Table 1 wherein the leaf area was recorded as statistically significant at ($p < 0.05\%$). The highest extension in leaf area was measured in T₇ (Calcium, 10mM + Bio priming with Rhizobium + Putrescine, 3mM) 331.60 ± 5.2 and 760.27 ± 3.7 cm² in the summer season as compared to the control set of treatment 287.60 ± 3.7 and 618.87 ± 2.6 cm² at both the intervals 30 and 60 DAS. Out of all the sets of treatments, T₅ (Calcium, 10mM) + Putrescine, 3mM) was recorded at par with T₇ 748.40 at 60DAS in the summer season (Table 1). Data presented in (Table 2) showed an increase of the highest % increase over control in T₇ 13.3.

Leaf area index, crop growth rate and leaf area duration

The experimental findings indicated that the treatments applied to the mung bean also positively influenced the leaf area index (LAI). The influence of treatments is presented in Fig. 1, wherein the LAI was recorded as statistically significant in both seasons at ($p < 0.05\%$), i.e. Summer seasons. Among the sets of treatments, the maximum leaf area index was measured in T₇ (Calcium, 10mM)+Bio priming with Rhizobium + Putrescine, 3mM) 1.47 to 3.38 for the Summer season at 30 and 60 DAS followed by the treatment T₅ (Calcium, 10mM) + Putrescine, 3mM) which is at par with T₇ 3.32 at 60DAS in summer season as shown in Fig. 1 while the control set of treatment recorded the lowest LAI corresponding with all other treatments. Moreover, data depicted in (Table 2) also revealed the highest % increase over control of LAI in T₇ 13.1 and 11.7 in their respective seasons.

The gain in crop growth rate presented in (Fig. 2) reveals the impact of individual and combinations of bio-priming, putrescine, and calcium in green gram during

Table 1. Treatments impact on plant height (cm), number of leaves, and leaf area plant⁻¹, in the mung bean of the summer season (2022-23)

Treatments	Plant height		Number of leaves		Leaf area	
	30DAS	60DAS	30DAS	60DAS	30DAS	60DAS
T ₀	41.87±0.81	62.01±0.73	31.17±0.31	48.07±0.85	287.60±3.7	618.87±2.6
T ₁	42.93±1.19	63.19±1.00	33.90±0.54	51.07±0.31	294.67±2.8	639.30±0.5
T ₂	42.55±0.61	62.78±0.21	32.83±0.25	50.13±0.94	300.93±1.8	633.27±1.6
T ₃	43.45±0.35	63.32±0.12	35.47±0.29	52.67±0.29	305.47±0.6	641.67±1.3
T ₄	44.25±0.26	64.28±0.25	36.23±0.19	53.87±0.60	310.10±2.0	679.13±11.9
T ₅	46.02±0.89	65.67±0.30	39.03±0.65	56.47±0.40	323.63±1.6	748.80±9.1
T ₆	45.06±1.23	65.39±0.94	37.47±1.16	55.17±0.19	314.03±0.9	716.77±3.5
T ₇	46.68±1.29	66.19±0.47	40.80±0.60	57.43±0.44	331.60±5.2	760.27±3.7
C.D. at ($p < 0.05\%$)	1.593	0.934	1.069	1.007	4.969	13.205

Note: T₀= Control, T₁= Calcium (10mM), T₂= Bio priming (Rhizobium), T₃= Putrescine (3mM), T₄= Calcium (10mM) + Bio priming (rhizobium), T₅=Calcium (10mM) + Putrescine (3mM), T₆= Bio priming (rhizobium) + Putrescine (3mM), T₇=Calcium (10mM) + Bio priming (rhizobium) + Putrescine (3mM)

Table 2. Treatments impact on percent increased /decreased over the control at 30 DAS in the mung bean of the summer season (2022-23)

Treatments	LAI at 30DAS	CGR 30-60 DAS	LAD 30-60 DAS	SPAD reading 30DAS	Total chlorophyll at 30 DAS
T ₀	1.28	5.57	51.35	30.8	0.65
T ₁	1.31 [+22.3]	5.69 [+1.9]	52.85 [+2.8]	34.4 [+10.5]	0.76 [+14.5]
T ₂	1.34 [+4.3]	5.57 [+0.0]	52.90 [+2.9]	33.1 [+6.9]	0.71 [+8.5]
T ₃	1.36 [+5.7]	5.63 [+1.0]	53.64 [+4.3]	36.0 [+14.4]	0.80 [+18.8]
T ₄	1.38 [+7.1]	5.68 [+1.7]	56.30 [+8.8]	37.1 [+17.0]	0.85 [+23.5]
T ₅	1.44 [+11.0]	5.76 [+3.1]	61.56 [+16.6]	39.2 [+21.4]	0.95 [+31.6]
T ₆	1.40 [+8.3]	5.76 [+3.1]	58.99 [+13.0]	38.0 [+18.9]	0.88 [+26.1]
T ₇	1.47 [+13.1]	5.87 [+4.9]	62.74 [+18.2]	40.0 [+23.0]	1.05 [+38.1]

Note: T₀= Control, T₁= Calcium (10mM), T₂= Bio priming (Rhizobium), T₃= Putrescine (3mM), T₄= Calcium (10mM) + Bio priming (rhizobium), T₅=Calcium (10mM) + Putrescine (3mM), T₆= Bio priming (rhizobium) + Putrescine (3mM), T₇=Calcium (10mM) + Bio priming (rhizobium) + Putrescine (3mM); Data presented in parenthesis showed percent increase over control

two consecutive years of summer seasons. The crop growth rate (CGR) was recorded as statistically significant at ($p < 0.05\%$) between the intervals of 30 to 60 days. Out of all the combinations of the treatments, T₇ (Calcium, 10mM) + Bio priming with Rhizobium + Putrescine, 3mM) measured the highest value of CGR $5.87 \text{ mg cm}^2 \text{ day}^{-1}$ in the summer season while the second highest was recorded in T₅ (Calcium, 10mM) + Putrescine, 3mM) and T₆ (Bio-priming with Rhizobium + Putrescine, 3mM) $5.76 \text{ mg cm}^2 \text{ day}^{-1}$. The data presented in (Table 2) revealed the highest % increase over control was depicted in T₇ 4.9% in their respective seasons.

The impact of applied treatments was studied with leaf area duration (LAD) in green gram during two consecutive summer seasons. Data depicted in (Fig. 2) revealed the impact of treatments wherein the leaf area duration (LAD) was recorded as statistically significant at ($p < 0.05\%$). Among the treatments, T₇ showed the highest value of LAD at 62.74 days in the summer season. The treatment T₅ recorded the second highest value of LAD 61.56 days as compared to the control set of treatments 51.35 days. A close analysis of the LAD concerning % increase over control appeared in T₇ 18.2 days (Table 2).

SPAD reading and total chlorophyll

The impact of treatments was also tested on SPAD reading to correlate the amount of chlorophyll synthesis in two consecutive years of the summer season. Data depicted in Fig. 3 reveals that the SPAD reading was

recorded as statistically significant at ($p < 0.05\%$). Wherein the T₇ (Calcium, 10mM) + Bio priming with Rhizobium + Putrescine, 3mM) showed its highest values of 40.0 and 39.3 in the summer season at both intervals, i.e. 30 and 60 DAS. Out of all combinations of the treatment, T₅ was recorded at par with T₇, i.e., 39.2 and 61.4, respectively, in the summer season at 30 and 60 DAS. Data presented in Table 2 showed an increase over control of around 23% in their respective season at 30 DAS.

To optimize the morph-phenological growth, total chlorophyll content was estimated at two intervals: 30 DAS and 60 DAS in green grams during two consecutive years of summer seasons. Data presented in Fig. 4 revealed that the parameters across the seasons were noticed as statistically significant at 30 DAS ($p < 0.05\%$) in the summer season, while it was recorded as statistically non-significant at 60 DAS. Results indicated that out of all the combinations of the treatments, T₇ recorded the maximum value of total chlorophyll of 1.07 and 2.33 in the Summer season. Of all the treatments, T₆ and T₇ were recorded at par with T₇ in Summer. A scrutiny of the data showed an increment over control was 39.2 in their respective seasons at 30 DAS (Table 2).

Morpho-physiological parameters are key components for improving yield potential; therefore, it is essential to optimize their growth, and thus, they could contribute positively. Plant height, number of leaves, leaf area, leaf area index, crop growth rate, and leaf area duration are some of the most important parameters of crop growth, which directly correlate with the life span, ma-

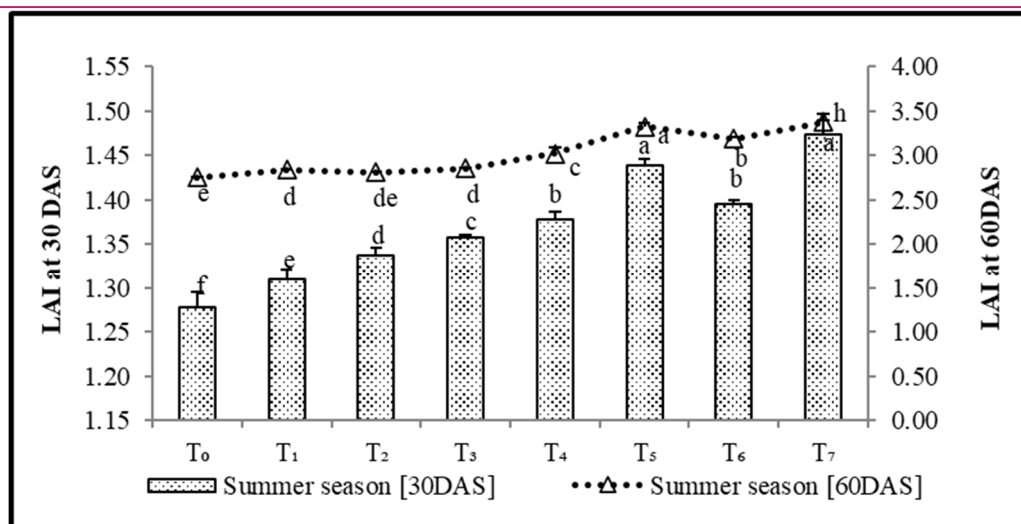


Fig. 1. Treatment impact on leaf area index in the summer season

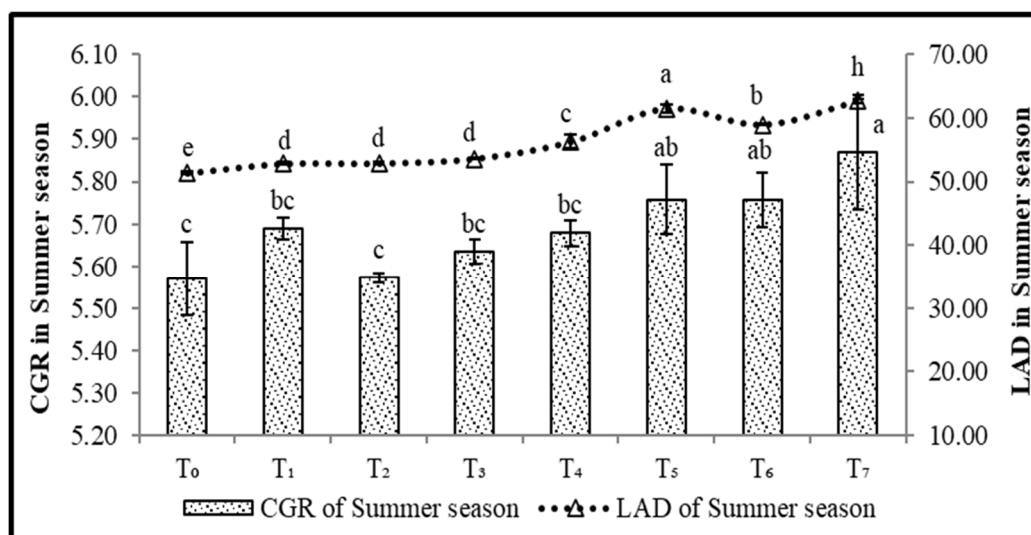


Fig. 2. Treatment impact on crop growth rate ($\text{mg cm}^2 \text{ day}^{-1}$) and leaf area duration (days) between 30-60 DAS in the summer season

turity, and ability to compete for light. In the present investigation, the plant height, number of leaves, leaf area (Table 1), leaf area index, crop growth rate, and leaf area duration (Fig. 1 and 2) were recorded significantly highest ($P < 0.05\%$) in T₇, which was a combination of Calcium, Putrescine and bio priming with rhizobium which was followed by T₅, with the combination of Calcium and Putrescine. This means that the additional growth among the T₇ and T₅ was somehow due to the bioprimering treatment of rhizobium while the differences in growth among the combinations of treatment and control (T₀) are due to metabolic event caused by the use of calcium and putrescine.

The contribution of calcium, putrescine, and bio priming with rhizobium in favor of morphological growth of green gram is well documented by (Sajid *et al.*, 2020; Hussein *et al.* 2023; Khiangte *et al.*, 2023) who reported that Ca, putrescine, and bioprimering with rhizobium have their entity in favor of morphological growth in plants. Wherein, Ca provides structural strength in the

form of calcium magnesium pectate, which acts as a cementing agent thus, it helps in modulating the morphological growth such as plant height, dry weight, and the number of leaves in cucumber (Zhang *et al.*, 2024 and Kazemi, 2013) and putrescine is a kind of polyamine which known for the regulation of growth and development which is a part of morphological growth (Shu *et al.*, 2015) while the establishment of seedling, gain in dry weight and the number of leaves were reported in mung bean (Nawaz *et al.*, 2021).

Crop growth rate (CGR) refers to the increase in the dry matter of plants on a specific area of land during a specific period. It is often used in studies to determine the impact of treatments on growth. Results depicted in (Fig. 2) revealed that the CGR was statistically significant in both seasons, which is similar to the finding of Jadhav *et al.* (2018), who reported that foliar application of putrescine had the highest leaf area and CGR while the improvement in CGR and NAR in green gram by the use of rhizobium was also reported by

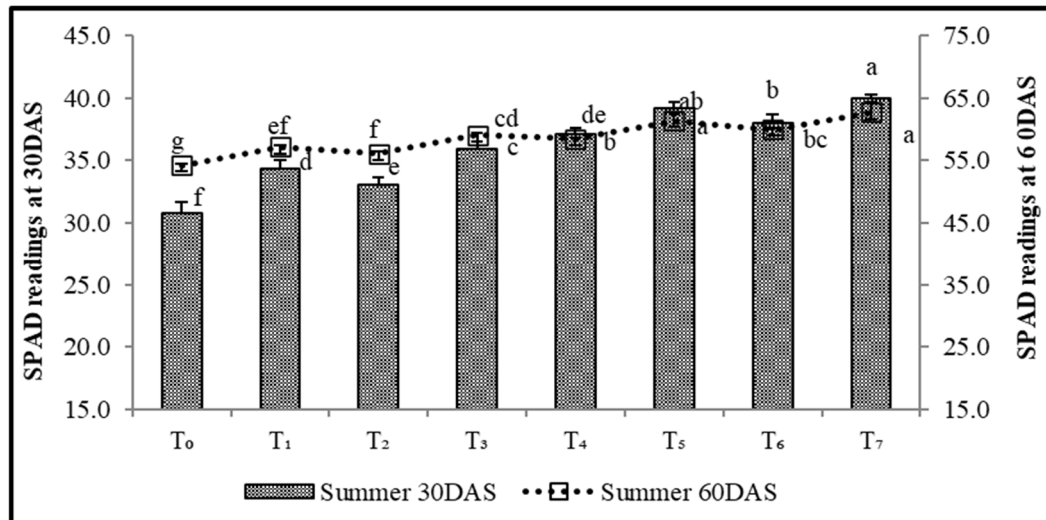


Fig. 3. Treatment impact on SPAD reading in the summer season

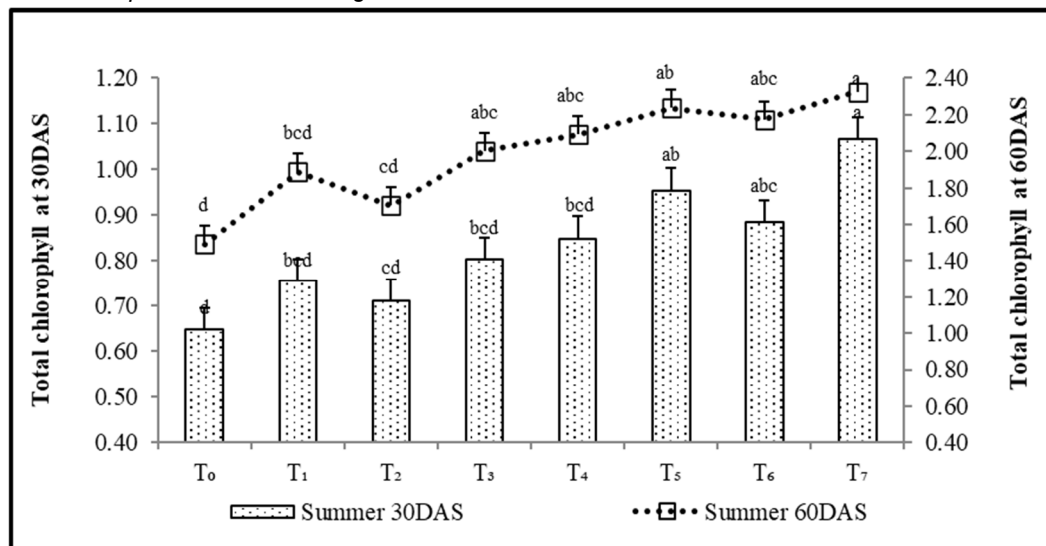


Fig. 4. Treatment impact on chlorophyll content (mg g^{-1}) in the summer seasons

(Naragund *et al.*, 2020). The morpho-physiological growth of plants is directly dependent upon the rate of photosynthesis, which is directly linked to the amount of chlorophyll content. The results depicted in Figs. 3 and 4 showed a significantly higher SPAD reading and total chlorophyll content in T₇. Increase of chlorophyll due to the use of calcium, putrescine, and rhizobium are well reported in cucumber, black gram, green gram, and cowpea (Kazemi, 2013; Jadhav *et al.*, 2019; Suchak and Pandya, 2020; Arumugam *et al.*, 2010) with different treatment combinations. The use of calcium, putrescine, and rhizobium not only improves the leaf area, LAI, CGR, LAD but also improves the SPAD reading and total chlorophyll content, which indicates the betterment of yield and yield attributes in green gram.

Conclusion

The present study concluded that out of all the combinations of the treatment, T₇ (Calcium, 10mM) + Bi-

oprining with Rhizobium) + Putrescine, 3mM) emerged as one of the most effective combinations to optimize the morpho-phenological traits, which included plant height, number of leaves, leaf area, leaf area index (LAI), crop growth rate (CGR), and leaf area duration (LAD) studied in mung bean wherein calcium and putrescine were applied as a foliar application and bio-priming with *rhizobium* as a seed treatment. Additionally, the synergistic effect of these treatments was reflected in improving SPAD readings and total chlorophyll content, indicating enhanced photosynthetic potential in mung bean. Among the various treatments, T₇ was the most effective combination for the summer season. These findings provide valuable insights into using bio-stimulants to improve growth and productivity, which may help enhance mung bean production.

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Conflict of interest

The authors declare that they have no conflict of interest.

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