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Optimizing Morpho-Physiological Traits of Black Gram (*Vigna mungo* L.) in the Summer Season Mediated by Putrescine and Cadaverine

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ABSTRACT

Background: Black gram (*Vigna mungo* L.), a vital legume crop, frequently encounters growth challenges due to environmental stress, leading to reduced yield and productivity. This study aimed to determine the impact of putrescine and cadaverine on the morpho-physiological growth of the black gram variety MASH 1008.

Methods: The present piece of work was executed on the Research Farm of Lovely Professional University in the summer season of 2022-2023 in a randomized block design with a total of 8 possible combinations of polyamines *i.e.* Putrescine and Cadaverine with 3 replications.

Result: Among the treatments, the most effective treatment was T_3, *i.e.* putrescine (1.5 mM) +cadaverine (1 mM), which was observed as a statistically highly significant at p<0.05% for most of the parameters, resulting in maximum plant height, number of leaves, fresh weight, dry weight, leaf area, leaf area index (LAI), crop growth rate (CGR), leaf area duration (LAD), chlorophyll index and total chlorophyll content, Chlorophyll a and b (66.74 cm, 20.47, 71.52, 12.27 g plant^{-1}, 433.90 cm^2 plant^{-1}, 1.91, 54.91 mg g^{-1} day^{-1}, 0.938, 54.13, 2.71, 1.13 and 1.17 mg g^{-1} at 90 DAS respectively. These results strongly suggest that applying polyamines, particularly the combination of putrescine and cadaverine, can significantly enhance morpho-physiological growth and yield of black gram, especially under adverse environmental conditions.

Key words: Black gram, Cadaverine, Growth parameter, Polyamine, Putrescine.

INTRODUCTION

Black gram (*Vigna mungo* L.), usually cultivated in Southern Asia, is also named urad, urd bean, urad dal, mash or black matte bean, *etc*. (Swaminathan *et al.,* 2023). It is a popularly grown legume crop belonging to the family Fabaceae. It has a high nutritional value from the point of food and energy, constituting 24% protein, 1.3% fat, 3.2% minerals, 60% carbohydrate, 0.9% fiber, 385 mg phosphorus, 154 mg calcium, 9.1 mg ironand a trace amount of vitamin B-complex in compared to cereals that are grown in undeveloped and under-developed countries around the globe (Reddy *et al.,* 2023). It is a short-duration (80-85 days) pulse crop favorable to all seasons as a sole, fallow inter, or inter-mixed crop. India alone has production and productivity of 4.49 m ha, 2.92 m tonsand 651 kg ha^{-1} of black gram, respectively (Directorate of Economics and Statistics, 2016-17). Highest leading states that produce black gram in India are Madhya Pradesh (43.59%), Uttar Pradesh (20.76 %), Rajasthan (10.00%), Maharashtra (8.06%) and Chhattisgarh (3.79%) (Thriveni *et al.*, 2024). In the southern states of India like Karnataka, black gram alone occupies 71 thousand ha with a production of 20 thousand tons and productivity is 282 kg ha^{-1} (Thriveni *et al.,* 2024). Despite all those records, black gram's productivity is insufficient to feed the growing Indian population. Consequently, productivity improvement is urgently needed. Various disciplines are working to increase the productivity of black gram and develop methods like foliar application of organic and inorganic sources of nutrients for maximizing the crop's genetic potential (Shao *et al.,* 2022). This method is very efficient and economically feasible in providing nutrient requirements during critical growth stages (Shao *et al.,* 2022). To obtain higher seed yield, diverting food from the sink to the source and enhancing vegetative growth in black gram is essential (Tyagi *et al.,* 2023). Growth stimulating substances like growth regulators increase and improve broad physiological parameters *viz.*, alteration of plant formation, integrated partitioning, increase of photosynthesis, translocation of nutrients, improving nitrogen metabolism, initiation of flowering, pod formation, delaying the senescence of leaves and improved seed quality (Bhargav *et al.,* 2023).

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Polyamines play a vital role in influencing yield potential in pulses. Foliar application of these compounds offers several advantages, including enhanced nutrient utilization, reduced losses through leaching and fixationand more efficient regulation of nutrient uptake by plants (Jadhav *et al.*, 2019). Acting as growth regulators, polyamines promote cell division and differentiation, facilitate DNA and protein synthesis, modulate ion channelsand help regulate plant metabolism in response to both biotic and abiotic stresses (Rai *et al.,* 2019). Diamines like putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane) have been shown to affect several plant processes. Despite being less studied, Cadaverine has demonstrated the potential to boost development under specific conditions (Deotale *et al.,* 2019). Putrescine is well-known for stabilizing membrane integrity and enhancing stress tolerance. It has been observed that the exogenous application of putrescine and cadaverine increases plant growth and results in better yield (Rai *et al.,* 2019). Hence, the present experiment was conducted to observe the effect of foliar application of polyamines (putrescine and cadaverine) on black gram to enhance the morpho-physiological quality.

MATERIALS AND METHODS

Experimental Site

The field experiment was conducted at the Agricultural Research Farm of Lovely Professional University, Phagwara, Punjab, India, to investigate and strategize the effect of polyamines on optimizing the morpho-physiological growth of black gram (*Vigna mungo* L.) mediated by putrescine and cadaverine to improve yield and yield-attributing characteristics in black gram during the summer season of years of 2022 and 2023.

Treatment details

The experiment was carried out in a randomized block design (RBD) with 8 treatments and 3 replications. The total experimental area was divided into 24 and the variety used for this experiment was (Mash 1008) variety of black gram. To perform this experiment, polyamines are used in both combined and individual forms, *i.e.,* Putrescine (0.75 mM, 1.5 mM, 3 mM) and Cadaverine (0.5 mM, 1 mM, 2 mM) applied in the field by foliar application through knapsack sprayer. In both years seasons, standard agronomical practices were used during the entire growth period.

Morphological traits

To analyze the morphological traits of black gram, plant height, number of leaves, fresh weight, dry weight and leaf area were used. In order to measure it, measuring scale, weighing balance, hot air oven and leaf area meter (Model no.211) was used.

Phenological traits

To analyze the influence of phenological traits, leaf area index (LAI), crop growth rate (CGR) and leaf area duration (LAD) were used while in order to calculate it, the following formulas proposed by (Watson, 1947; Watson 1952 and Power *et al.,* 1967) were used.

$$\text{Leaf area index} = \frac{\text{Total leaf area (cm}^2\text{)}}{\text{Total ground area (cm}^2\text{)}}$$

Whereas,
Total leaf area is the sum of the area of all leaves in a given region.
Ground area is the ground covered by the plant canopy:

$$\text{Crop growth rate} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{1}{A}$$

Whereas,
W_1 and W_2 = Dry weight at second and first time.
T_2 and T_1 = Time interval.
A = Land area.

$$\text{Leaf area duration} = \frac{L_1 + L_2}{2} \times T_2 - T_1$$

Whereas,
L_1 and L_2 = LAI of the second and first intervals.
T_2 and T_1 = Time interval.

Biochemical analysis

Two different methods are used to calculate the chlorophyll index and chlorophyll content. For chlorophyll index, an instrument is known as SPAD METER (Model no. SPAD-502 Plus), while for chlorophyll content, a well-known procedure is used by Arnon (1942). The main reagent for this procedure is 80% acetone. The plant sample is extracted from the field and crushed in 10 ml of 80% acetone till it completely converts from solid to liquid. Whatman filter paper no. 42 is used to filtrate the fiber from the solution in 25 ml of graduated volumetric flasks and the final volume up to 25 ml by adding 80% acetone. However, 80% acetone calibrates the spectrophotometer during the 663 and 645nm optical density measurements. In contrast, a given formula was used to calculate the amount of chlorophyll a, chlorophyll band total chlorophyll.

$$\text{Chl a} = 12.7 \times (D663) - 2.69 \times (D645) \times \frac{V}{1000 \times W} \text{ mg g}^{-1}$$

$$\text{Chl b} = 22.9 \times (D663) - 4.68 \times (D645) \times \frac{V}{1000 \times W} \text{ mg g}^{-1}$$

Total chlorophyll =

$$20.2 + (D663) + 8.02 + (D645) \times \frac{V}{1000 \times W} \text{ mg g}^{-1}$$

Where as,
W= Weight of sample
V= Volume of sample

Statistical analysis

The collected data was analyzed using particular software OPSTAT (Operational statistics) and Duncan's multiple range test compared the mean of data at a 5% significance level.

RESULTS AND DISCUSSION

Morphological traits

The response of different levels of polyamine (putrescine and cadaverine) was recorded at various stages of black gram (MASH 1008). Growth parameters like plant height (cm), fresh weight (g plant^{-1}), dry weight (g plant^{-1}), number of leaves, leaf area, leaf area index, crop growth rate (g m^{-2} day^{-1}) and leaf area duration (m^{-2} m^{-2}day^{-1}) were taken for observation are given in Table 1, 2 and 3.

Plant height is one of the most important characteristics of plants, as it provides space for branches, leaves, flowersand pods. As observed in Table 1, polyamine application significantly increased plant height in black gram. The highest plant height was recorded in treatment T_3 at both 50 and at harvest, measuring 63.74 cm and 66.74 cm, respectively, with an application of putrescine (1.5 mM) combined with cadaverine (1 mM). The significant increase in plant height is due to the application of putrescine and cadaverine, known as growth regulators. Due to their rapid cell division, polyamines tend to modulate the metabolism of plants (Ahmed *et al.* 2013). The same responses were observed by (Jadhav *et al.* 2019) and (Roy and Roy, 2022). Significantly, the highest fresh weight was observed in T_3 at both 50 and harvest, measuring 37.24 g and 71.52 g, respectively, with the same formulation of putrescine (1.5 mM) combined with cadaverine (1 mM). Baroowa and Gogoi (2015) reported that polyamines stimulate cell enlargement, leading to tissue growth, directly affecting the plant biomassand increasing plants' fresh and dry weight. Similar results were obtained in dry-weight; the highest observation was recorded in T_3 in both 50 and at harvest, *i.e.* 6.99 g and 12.27 g, respectively, with putrescine (1.5 mM) combined with cadaverine (1 mM). Ahmed (2013) supports this result as it was reported that polyamines' enhanced activity of photosynthate leads to the production of carbohydrates, which are known as the energy source for growth, resulting in increased dry biomass. According to Table 1, it is clearly shown that foliar application of putrescine (1.5mM) combined with cadaverine (1mM) increased plant height, fresh weightand dry weight at 50 DAS by 23.58% 22.20% and 43.40%, at harvest 24.41%, 17.57% and 38.07% respectively.

One essential morphological characteristic that significantly impacts photosynthesis, plant health, growth stages, resource allocationand agricultural productivity is the number of leaves. It is a crucial indicator of a plant's physiological state and ability to generate yield and biomass. Monitoring and optimizing the number of leaves is vital for ensuring plant health in research and practical agriculture. Observations taken at 60 and 90 days after sowing (DAS) showed that the highest number of leaves was recorded in treatment T_3, *i.e.* 18.39 and 20.47, respectively, with foliar application of a combined dose of putrescine (1.5 mM) and cadaverine (1 mM). This result is supported by Ahmed *et al.* (2013), as it was reported that putrescine interacts with endogenous hormones like auxin, gibberellinand cytokinin, which leads to an increase in leaf number and growth too. In contrast, the cadaverine role is somewhat similar to putrescine as cadaverine balances the hormones of plants and reduces oxidative damage, which helps plants cope with any damage internally. This statement was also relevant to Rajpal and Tomar (2020) and Tomar *et al.* (2017). The study of leaf area is critical to understanding the dynamics of plant growth and their interaction with the environment because all plant metabolic processes are linked to the leaf and its shape

Table 1: Effect of polyamines [putrescine and cadaverine] on growth parameters in black gram.

Treatments	Plant height (cm)		Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})	
	50 DAS	At harvest	50 DAS	At harvest	50 DAS	At harvest
T_0	48.71^{e}±0.23	50.45^{d}±1.26	30.70^{f}±0.41	63.48^{e}±0.76	3.96^{e}±0.41	7.60^{e}±0.72
T_1	59.62^{bc}±1.42	61.43^{b}±1.66	32.40^{d}±0.26	66.30^{c}±0.58	6.15^{bc}±0.26	10.45^{bcd}±0.58
	(18.30%)	(17.89%)	(11.07%)	(5.27%)	(33.60%)	(27.29%)
T_2	54.92^{d}±0.92	57.29^{c}±1.55	31.91^{de}±0.26	65.66^{cd}±0.43	5.09^{d}±0.26	8.99^{de}±0.43
	(11.31%)	(11.94%)	(4.50%)	(3.81%)	(22.30%)	(15.46%)
T_3	63.74^{a}±1.11	66.74^{a}±2.26	37.24^{a}±0.19	71.52^{a}±0.44	6.99^{a}±0.1	12.27^{a}±0.44
	(23.58%)	(24.41%)	(22.20%)	(17.57%)	(43.40%)	(38.07%)
T_4	57.34^{cd}±2.40	60.43^{bc}±4.02	32.22^{d}±0.37	66.02^{cd}±0.33	5.82^{c}±0.37	9.77^{cd}±0.33
	(15.05%)	(16.57%)	(9.60%)	(4.74%)	(32.01%)	(29.62%)
T_5	62.15^{ab}±1.79	64.32^{ab}±1.32	35.43^{b}±0.44	69.48^{b}±0.42	6.69^{ab}±0.44	11.32^{ab}±0.42
	(21.52%)	(21.57%)	(15.55%)	(13.35%)	(40.83%)	(32.88%)
T_6	61.95^{ab}±1.30	64.00^{ab}±1.06	34.68^{c}±0. 68	68.82^{b}±0.52	6.36^{bc}±0.68	10.80^{abc}±0.52
	(21.28%)	(21.18%)	(12.73%)	(11.49%)	(37.82%)	(29.62%)
T_7	50.93^{e}±1.66	52.89^{d}±1.46	31.33^{ef}±0.33	65.29^{d}±0.73	4.41^{e}±0.33	8.22^{e}±0.73
	(4.36%)	(4.62%)	(1.73%)	(2.03%)	(10.39%)	(7.58%)
C.D. at (p<0.05%)	2.799	3.752	0.726	0.845	0.601	1.417

Note: Data presented in parenthesis shows a percent increase over control.

and size. Similar results were obtained in the leaf area; the data was recorded at 50 and harvest, in which the highest was recorded in T_3, *i.e.* 558.24 and 433.90, respectively, with combined application of putrescine (1.5 mM) and cadaverine (1mM). This result was supported by Mutlu and Bozcuk (2013) and Yousefi *et al.* (2021). According to Table 2, it is clearly shown that foliar application of putrescine (1.5 mM) combined with cadaverine (1 mM) increased the number of leaves and leaf area at 60 DAS by 44.07% and 32.36% and at harvest by 37.45% and 33.22%.

Phenological traits

Data presented in Table 3 indicates the effect of putrescine and cadaverine on leaf area index (LAI), crop growth rate (CGR) and leaf area duration (LAD). These parameters are the main factors observing plant growth, as the leaf area index indicates the capacity of the plant to absorb the total sunlight for the photosynthesis process. Eventually, LAI will be directly proportional to increased biomass and productivity. CGR indicates the growth of plants or accumulation of biomass over time and LAD is the time plants remain active in photosynthesis; all the above parameters help in assisting plant growth. Here, the data is taken at 50 and harvest; the highest LAI was observed in T_3, *i.e.* 2.46 and 1.91, respectively, with the application of a combined dose of putrescine (1.5 mM) and cadaverine (1mM). This result was supported by Ahmed *et al.* (2013) also, it was reported by Gupta *et al.* (2012) application of

Table 2: Effect of polyamines [putrescine and cadaverine] on the number of leaves and leaf area in black gram.

Treatment	Number of leaves plant^{-1}		Leaf area cm^2 plant^{-1}	
	50 DAS	At harvest	50 DAS	At harvest
T_0	10.29^{f}±0.37	12.80^{e}±0.55	377.58^{f}±12.14	289.78^{e}±6.48
T_1	14.72^{cd}±0.88	16.89^{b}±1.11	455.36^{cd}±24.12	336.28^{c}±5.44
	(30.11%)	(24.21%)	(17.08%)	(13.83%)
T_2	13.36^{de}±0.83	15.11^{cd}±0.86	426.89^{de}±13.45	317.21^{d}±5.08
	(23.03%)	(15.25%)	(11.55%)	(8.65%)
T_3	18.39^{a}±1.07	20.47^{a}±0.46	558.24^{a}±22.17	433.90^{a}±9.58
	(44.07%)	(37.45%)	(32.36%)	(33.22%)
T_4	13.41^{de}±0.46	15.46^{c}±0.17	449.18^{cde}±16.49	328.93^{cd}±15.09
	(23.31%)	(17.17%)	(15.94%)	(11.90%)
T_5	16.26^{b}±0.69	19.23^{a}±0.25	494.62^{b}±6.95	370.53^{b}±7.26
	(36.73%)	(33.43%)	(23.66%)	(21.79%)
T_6	14.92^{bc}±0.74	17.30^{b}±0.84	481.19^{bc}±32.96	359.66^{b}±11.58
	(31.07%)	(25.99%)	(21.53%)	(19.43%)
T_7	12.19^{e}±1.22	14.00^{de}±1.12	414.89^{ef}±17.14	311.76^{d}±9.95
	(15.64%)	(8.57%)	(8.99%)	(7.05%)
C.D. at (p<0.05%)	1.44	1.28	35.50	17.62

Table 3: Effect of polyamines [putrescine and cadaverine] on phenological parameters, LAI, CGR and LAD in black gram.

Treatment	LAI		CGR g m^{2}day^{-1}		LAD	
	50 DAS	At harvest	25-50 DAS	50 DAS-harvest	25-50 DAS	50-harvest
T_0	1.66±0.03	1.28±0.01	29.48^{f}±0.58	36.98^{f}±0.20	0.421^{d}±0.02	0.647^{a}±0.09
T_1	2.00±0.07	1.49±0.01	34.51^{cd}±0.72	42.94^{cd}±0.63	0.724^{ab}±0.04	0.764^{a}±0.18
	(17.16%)	(13.55%)	(14.58%)	(13.89%)	(41.89%)	(15.32%)
T_2	1.88±0.04	1.40±0.01	32.83^{de}±0.63	41.32^{de}±0.60	0.596^{bc}±0.08	0.692±0.09
	(11.72%)	(8.32%)	(10.19%)	(10.51%)	(29.47%)	(6.51%)
T_3	2.46±0.07	1.91±0.02	45.87^{a}±1.57	54.91^{a}±1.56	0.790^{a}±0.06	0.938^{a}±0.17
	(32.49%)	(32.88%)	(35.71%)	(32.67%)	(46.78%)	(31.00%)
T_4	1.98±0.04	1.45±0.04	34.74^{c}±0.58	43.52^{c}±0.80	0.692^{ab}±0.12	0.702^{a}±0.21
	(16.15%)	(11.72%)	(15.13%)	(15.03%)	(39.20%)	(7.81%)
T_5	2.18±0.02	1.64±0.02	39.03^{b}±0.08	47.76^{b}±0.17	0.746^{a}±0.05	0.823^{a}±0.20
	(23.99%)	(21.59%)	(24.47%)	(22.57%)	(43.64%)	(21.41%)
T_6	2.13±0.08	1.59±0.03	37.79^{b}±1.67	47.14^{b}±1.83	0.733^{a}±0.05	0.788^{a}±0.08
	(22.24%)	(19.20%)	(21.98%)	(21.56%)	(42.62%)	(17.86%)
T_7	1.82±0.04	1.38±0.03	31.27^{ef}±0.92	39.70^{e}±1.20	0.486^{cd}±0.10	0.676^{a}±0.11
	(9.12%)	(6.71%)	(5.71%)	(6.88%)	(13.52%)	(4.31%)
C.D. at (p<0.05%)	0.16	0.074	1.83	1.95	0.13	NS

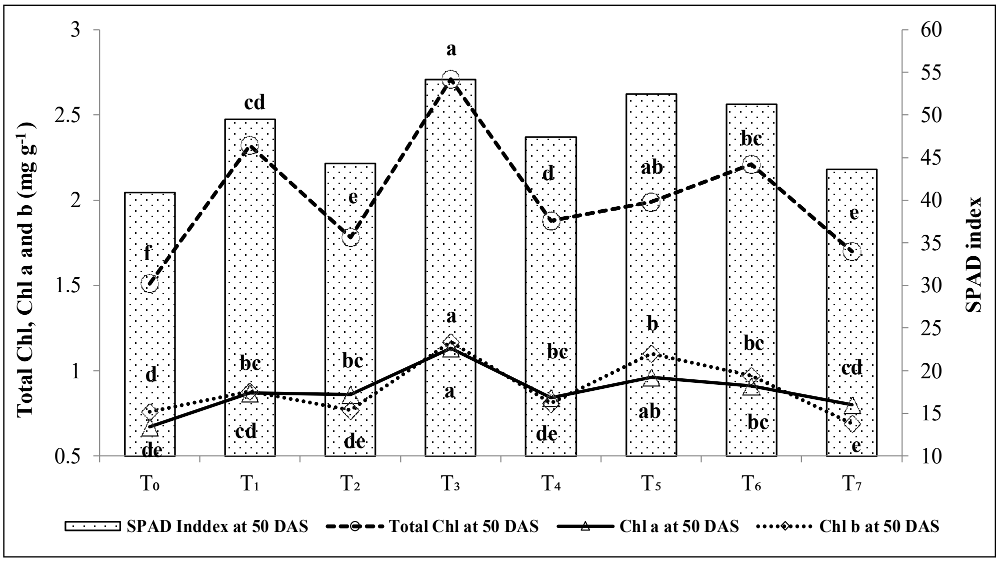

Fig 1: Effect of putrescine and cadaverine on total Chl, Chl a, Chl b and Chlorophyll index (SPAD) at 50 DAS in black gram.

exogenous putrescine increased the leaf area of chickpeas by 23% and 16% under abiotic stress conditions in maize leaf area increased by 45% and 91% reported by Agbodjato *et al.* (2016) and Gholami *et al.* (2009). Similar findings were observed for the crop growth rate (CGR), measured in g m^{-2} day^{-1} with data collected at the 25-50 day and 50-harvest intervals. The highest CGR values were noted in treatment T_3, registering 45.87 and 54.91, respectively. Additionally, the leaf area duration (LAD) measured in m^2 m^{-2} day^{-1}, also peaked in T_3, with values of 0.790 and 0.938. These results align with the research of Lee *et al.* (1997) and Pandey *et al.* (2000), which demonstrated that exogenous application of putrescine at 1 mM significantly enhances plant biomass production, thereby directly boosting the crop growth rate.

According to Table 3, it is clearly shown that foliar application of putrescine (1.5mM) combined with cadaverine (1mM) increased the LAI at 50 and harvest by 32.49% and 32.88%, CGR and LAD at 25-50das by 35.71% and 46.78% at 50-harvest by 32.67% and 31.00% respectively.

Chlorophyll content and chlorophyll index (SPAD)

Fig 1 represents the chlorophyll index and chlorophyll content at 50 DAS and harvest. Determination of chlorophyll is one of the critical factors in observing the growth of plants, as the chlorophyll index offers a method to evaluate the chlorophyll levels in leaves, which are closely linked to a plant's capacity for photosynthesis and overall well-being. Fig 1 presents the chlorophyll index at both 50 days and at harvest, with the highest values observed in treatment T3, measuring 54.13 and 32.21, respectively. Additionally, Fig 1 provides detailed data on chlorophyll content, including Chl a, Chl band total chlorophyll for these two-time points. The peak values for Chl a was recorded at 0.88 mg g^{-1} at 50 days and 0.97 mg g^{-1} at harvest. For Chl b, the maximum values were 2.71 mg g^{-1} and 1.85 mg g^{-1}, respectively, while total chlorophyll reached its highest at 1.13 mg g^{-1} and 1.17 mg g^{-1}. This data underscores the significant impact of treatment T_3 on chlorophyll levels throughout the growth period. This result was supported by Jahan *et al.* (2022), who found that putrescine can affect the activity of enzymes crucial for chlorophyll production. By supporting or stabilizing these enzymes, putrescine helps sustain or increase chlorophyll levels, particularly during stress or nutrient shortage. According to Fig 1, it is clearly shown that foliar application of putrescine (1.5 mM) combined with cadaverine (1 mM) increased the chlorophyll index at 50 and at harvest by 24.42% and 35.93%, Chl a, b and total at 25DASby 44.18%, 44.20% and 41.06% at harvest by 48.05%, 46.21% and 35.48% respectively.

CONCLUSION

Based on experiment results, the combined application of putrescine (1.5 mM) + cadaverine (1 mM), was most effective at both 50 DAS and harvest clearly showing that polyamines (putrescine and cadaverine) significantly enhanced the plant height, fresh weight, dry weight, number of leaves, leaf area, LAI, CGR, LAD, chlorophyll index and chlorophyll content (Chl a, b and total) due to its ability of cell division, nutrient uptake, balancing the hormones and its efficacy. Hence, the foliar application of polyamines is a promising technique to enhance the growth of crops in black gram.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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