

EFFECTS OF BENZYL AMINO PURINE ON GROWTH, ANTIOXIDANTS, AND CHLOROPHYLL CONTENTS OF *PHASEOLUS VULGARIS* L. CULTIVATED UNDER HEAT STRESS

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Abstract

Heat stress can inhibit plant growth, delaying photosynthesis and nutrient uptake. Prolonged exposure can lead to wilting, reduced yields, and even plant death. Using benzyl amino purine (BAP) can be an effective technique to overcome this problem. Benzyl aminopurine (BAP) is a synthetic cytokinin crucial for plant growth, stimulating cell division and proliferation, promoting protein synthesis, and aiding in tissue culture techniques. The current study used BAP to amend *Phaseolus vulgaris* L. growth under heat stress. Four treatments, i.e., control, 0.001% BAP, 0.003% BAP, 0.005% BAP, were applied in 4 replications following a completely randomized design. Results showed that 0.005% BAP caused significant enhancement in *Phaseolus vulgaris* L., shoot length (~43%), root length (~62%), shoot fresh weight (~71%), root fresh weight (~90%), shoot dry weight (~29%), and root dry weight (~44%) over the control under heat stress. Compared to the control, a significant improvement in *Phaseolus vulgaris* L., chlorophyll a (~54%), chlorophyll b (~38%), and total chlorophyll (~70%) validates the potential of 0.005% BAP under heat stress. Furthermore, improvement in N, P, K, and Ca concentration in leaves and roots showed the effective functioning of 0.005% BAP compared to the control in heat stress. In conclusion, 0.005% BAP is recommended as the best solution for mitigating heat stress in *Phaseolus vulgaris* L.

Key words: Antioxidant, Benzyl aminopurine, Chlorophyll content, Growth attributes, *Phaseolus vulgaris* L.

Introduction

Climate change poses significant challenges, such as heat stress and drought, lowering agricultural productivity (Cotrina Cabello *et al.*, 2023). Heat stress, a key concern globally, is estimated to impact food security due to climate change increasingly (Farhad *et al.*, 2023). Research indicates a notable rise in average global temperatures, a significant portion of which has increased in recent decades. The elevated temperatures will likely adversely affect crop growth and yield, especially in tropical and subtropical regions. Heat stress occurs when plants are subjected to prolonged high temperatures, which is detrimental to their growth, development, and productivity (Stone, 2023). Heat stress affects plant morphology by causing stunted growth, wilting, and changes in leaf structure to cope with water loss and heat absorption (Bechtaoui *et al.*, 2021). Biochemically, it triggers the production of harmful reactive oxygen species, leading to oxidative damage to cell components. This stress also alters the activity of enzymes, hormones, and other biochemical compounds involved in photosynthesis and stress response pathways.

Benzyl aminopurine (BAP), known as N6-benzyladenine, is a synthetic cytokinin regulating plant growth. It plays a crucial role in cell division, differentiation, and growth (Rehman *et al.*, 2023). Its chemical structure resembles naturally occurring cytokinins, particularly adenine, allowing it to interact with plant cytokinin receptors and trigger growth-promoting responses. BAP stimulates cell division and proliferation by promoting protein and nucleic acid synthesis, shoots and root tissue growth, leaf expansion, and plant vigor. It is also used in tissue culture techniques for shoot proliferation or micropropagation (Selim *et al.*, 2023).

Phaseolus vulgaris L., is commonly consumed for its pods and seeds (Khaleeq *et al.*, 2023). Nutritionally, *Phaseolus vulgaris* L., is rich in various essential nutrients. They contain dietary fiber, protein, carbohydrates, minerals, and phytochemicals, including phenolic compounds (Aydar *et al.*, 2023). Dietary fiber and resistant starch contribute to weight management, blood sugar regulation, heart health, and improved gut function. Heat stress negatively affects *Phaseolus vulgaris* L. growth by hindering germination, stunting plant growth, reducing photosynthesis, exacerbating water stress, disrupting

nutrient uptake, impeding reproductive development, and increasing susceptibility to diseases and pests (Devi *et al.*, 2023). Overall, it significantly reduces yield and quality.

The objective of the present study was to investigate the potential of BAP in alleviating heat stress in *Phaseolus vulgaris* L., cultivation. This research aims to select the best application rate of BAP on the growth of *Phaseolus vulgaris* L. under heat stress. By addressing this knowledge gap and proposing an environmentally friendly strategy to mitigate the adverse impacts of heat stress on *Phaseolus vulgaris* L., cultivation, this study contributes to the broader goal of ecosystem and crop preservation.

Material and Methods

Experimental site: In 2022, a pot experiment was conducted in the experimental area of ResearchSolution (30°09'41.6"N 71°36'38.0" E). Random soil sampling was done from the research area to characterize soil physicochemical properties. The soil and irrigation water parameters before experimentation were given in Table 1.

Table 1. Soil and irrigation parameters before experimentation

Soil	Values	References
pH	8.13	(Page <i>et al.</i> , 1983)
ECe (dS/m)	5.37	(Rhoades, 1996)
Soil organic matter (%)	0.30	(Nelson & Sommers, 1982)
Available phosphorus (µg/g)	4.81	(Kuo, 2018)
Total nitrogen (%)	0.003	(Bremner, 1996)
Extractable sodium (µg/g)	245	(Donald & Hanson, 1998)
Extractable potassium (µg/g)	111	(Pratt, 2016)
Texture	Clay loam	(Gee & Bauder, 2018)
Irrigation	Values	References
pH	7.61	
EC (µS/cm)	884	
Bicarbonates (meq./L)	6.67	
Chloride (meq./L)	0.01	(Estefan <i>et al.</i> , 2013)
Ca+Mg (meq./L)	2.68	
Carbonates (meq./L)	0.05	
Sodium (mg/L)	186	

Collecting, sterilization, and sowing of seeds: This study acquired *Phaseolus vulgaris* L., seeds from a licensed seed dealer. Each pot was filled with 10kg of soil. After that, ten seeds were sown per pot. Two seedlings in each pot were maintained by thinning after germination.

Treatments plan: Treatments include control without any treatment and three benzyl aminopurine (BAP) levels (0.001%, 0.003%, 0.005% BAP). Two foliar applications were made after one week of germination, with a 15-day gap in each foliar. To introduce heat stress, plants were shifted in the greenhouse, where temperature was raised to 40°C for 24 hours before applying the first and second foliar of BAP. However, no-stress plants were subjected to natural optimal growth temperature. All the treatments were applied under no stress and heat stress in 4 replications following a completely randomized design.

Fertilizers and irrigation: To fulfill the nutritional requirements of *Phaseolus vulgaris* L., nitrogen (N) and phosphorus (P) were applied at rates of 25 (0.31 g/pot) and 50 kg/acre (0.62g/pot), respectively. Urea was applied in two splits, while Single Super Phosphate (SSP) was also utilized. The potassium was applied at the rate of 20

kg/acre (0.25g/pot) using potassium sulfate. Pot moisture levels were carefully monitored and regulated throughout the experiment using a moisture meter (YIERYI 4 in 1; Shenzhen, Guangdong Province, China). Control groups were maintained at normal moisture levels and consistently maintained at 70% field capacity (Boutraa *et al.*, 2010).

Harvesting and data collection: After 65 days, the plant's morphological attributes data were calculated using an analytical grade balance. Samples were subjected to 70 °C heating for 48 hours to determine dry weight in an oven.

Chlorophyll and carotenoid content: To assess the chlorophyll (a, b, and total) in freshly harvested leaf samples, we ground the samples with 5 ml of 80% acetone by following the method Arnon, (1949). The absorbance was subsequently measured at 645, 663 nm, and 480nm wavelength for chlorophyll contents and carotenoids (Kirk & Allen, 1965).

Antioxidants: Catalase (CAT) activity was measured by observing H₂O₂ decomposition and reducing absorbance at 240 nm (Aebi, 1984). Ascorbate peroxidase (APX) activity was assessed by monitoring ascorbate oxidation in the presence of H₂O₂ at 290 nm (Nakano & Asada, 1981).

Determination of GSH: To evaluate the glutathione (GSH) level, we added an equal volume of 5% sulfosalicylic acid (w/v) to the homogenate, then centrifuged it for 10 minutes at 12,000×g. The obtained supernatant was used for analysis by mixing it with a 100 mM phosphate buffer (pH 7.0) and 5,5-dithiobis (2-nitrobenzoic acid), and the absorbance was determined spectrophotometrically at 412 nm (Anderson, 1985).

Total soluble proteins: Fresh leaf samples were standardized with 10mL of 50 mM potassium phosphate buffer, centrifuged at 10,000 × g for 15 minutes, and protein concentration in the extract was measured using the standard protocol Bradford, (1976).

Relative water content: We followed the method of Weatherley, (1950) to assess leaf water content. We recorded initial fresh weight (FW), soaked leaves until fully turgid, noting turgid weight (TW), then dried leaves for the final dry weight (DW). RWC was calculated as follows:

$$\text{RWC (\%)} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100$$

Shoot and root nutrients: Initially, sulfuric acid (Mills & Jones, 1991) was used to digest samples for nitrogen analysis. However, for analysis of phosphorus and potassium di-acid mixture (HNO₃:HClO₄) was used (Miller, 1997). Nitrogen content was determined using a modified micro-Kjeldahl method (Mills & Jones, 1991). Conversely, the phosphorus content was assessed at 420 nm using the yellow color method with the aid of a spectrophotometer (Mills & Jones, 1991). Flame photometer was used to quantify potassium content.

Statistical analysis

The data was examined using conventional statistical methods (Steel *et al.*, 1997). Two-way ANOVA was conducted using OriginPro software. Convex hull, hierarchical cluster analysis, and Pearson correlation analysis were performed by OriginPro software (OriginLab Corporation, 2021).

Results

Shoot length, shoot fresh and shoot dry weight: In no stress, adding 0.001% BAP led to an ~8% increase in shoot length, 0.003% BAP exhibited ~17%, and 0.005% BAP resulted in a ~31% increase compared to the control. Under heat stress, applying 0.001% BAP led to a ~9% increase, 0.003% BAP, and 0.005% BAP resulted in ~23% and ~43% increases in shoot length than the control (Fig. 1A).

Under no stress, a significant ~24%, ~11%, and ~38% increase in shoot fresh weight was observed with 0.001% BAP, 0.003% BAP, 0.005% BAP over the control. Under heat stress, applying 0.001% BAP, 0.003% BAP, and 0.005% BAP treatments resulted in ~49%, ~20%, and ~71% increase in shoot fresh weight compared to the control (Fig. 1B).

The shoot dry weight increased by ~6% with 0.001% BAP, ~12% with 0.003% BAP, and ~19% with 0.005% BAP under no stress, and under heat stress, caused ~8%, ~16%, and ~29% rise than the control (Fig. 1C).

Root length, root fresh and root dry weight: Applying 0.001% BAP, 0.003% BAP, and 0.005% BAP in no stress showed ~12%, ~22%, and ~29% increases in root length compared to the control. Under heat stress, the root length increased by ~13% with 0.001% BAP, ~35% with 0.003% BAP, and ~62% with 0.005% BAP in comparison to the control (Fig. 2A).

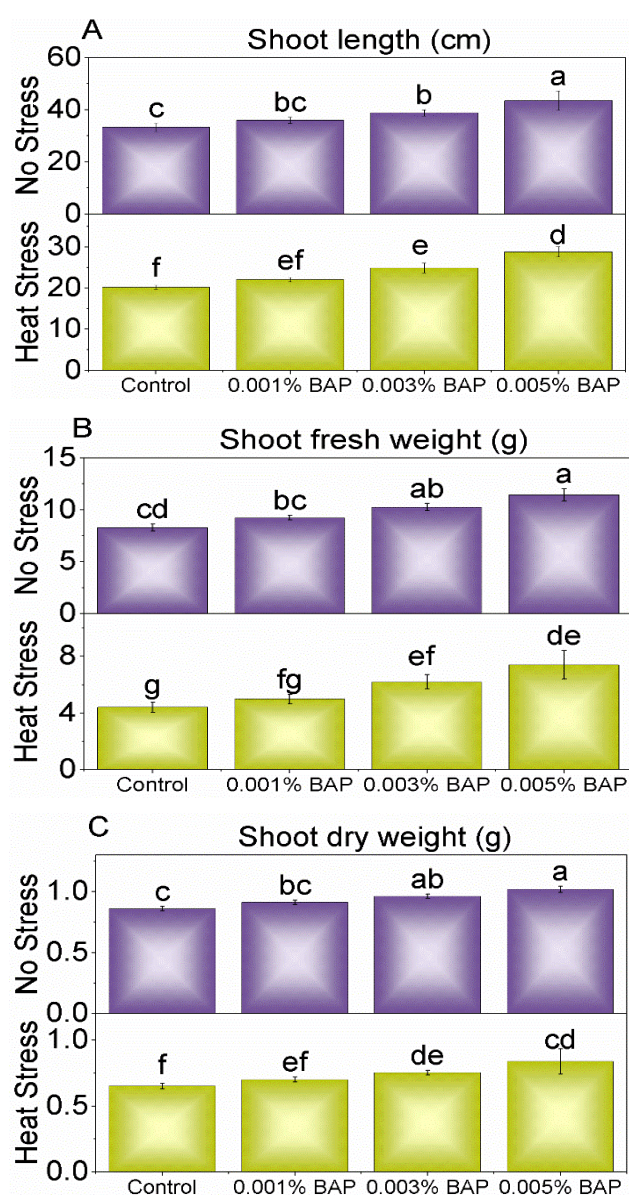


Fig. 1. The effect of different concentrations of BAP on shoot length (A), shoot fresh weight (B), and shoot dry weight (C) of *Phaseolus vulgaris* L., grown under no stress and heat stress. The Tukey test measured significant differences at (p<0.05); distinct letters on the bars are the mean of four replicates.

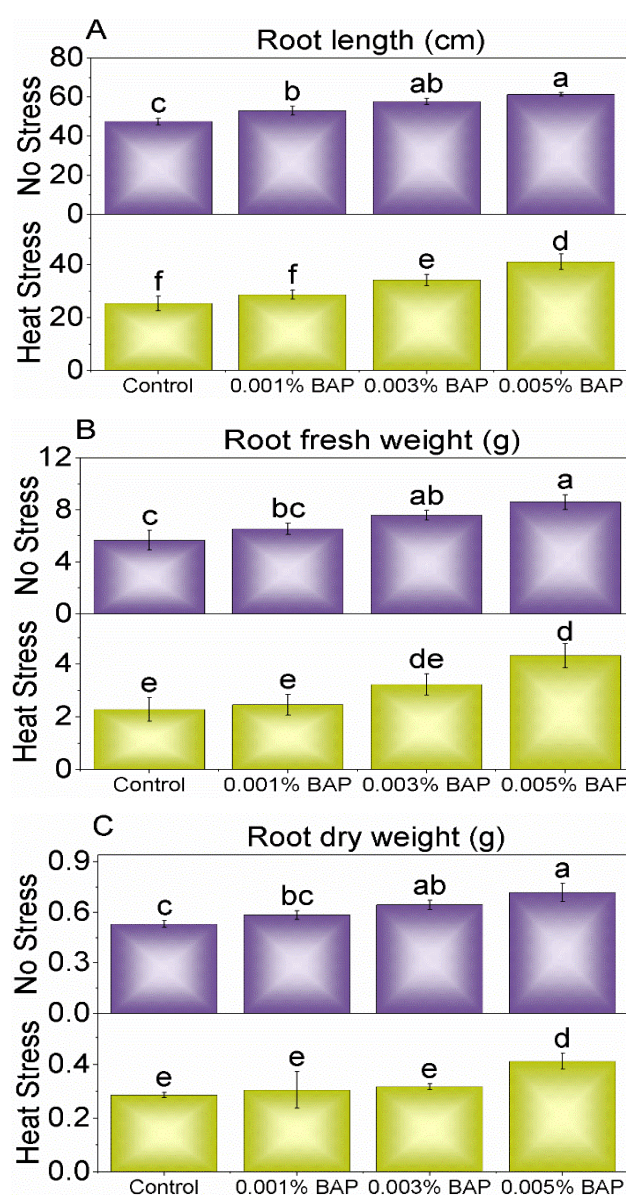


Fig. 2. The effect of different concentrations of BAP on root length (A), root fresh weight (B), and root dry weight (C) of *Phaseolus vulgaris* L., grown with and without heat stress. The Tukey test measured significant differences at (p<0.05); distinct letters on the bars are the mean of four replicates.

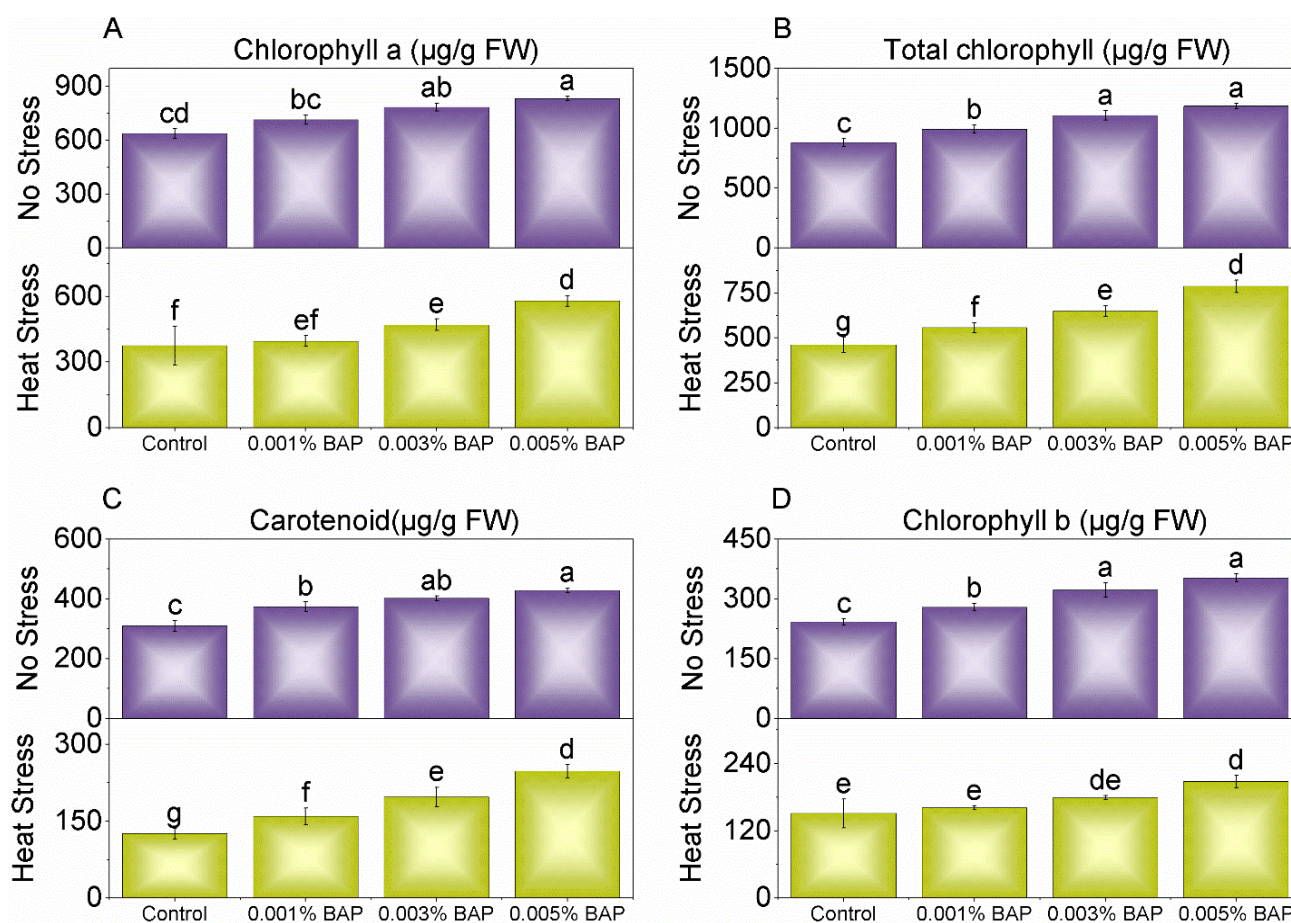


Fig. 3. The effect of different concentrations of BAP on chlorophyll a (A), total chlorophyll (B), carotenoid (C), and chlorophyll b (D) of *Phaseolus vulgaris* L., grown with and without heat stress. The Tukey test measured significant differences at ($p < 0.05$); distinct letters on the bars are the mean of four replicates.

Compared to the control, the treatment of 0.001% BAP resulted in a ~16% increase in no stress, and 0.003% and 0.005% BAP resulted in a ~34% and ~52% increase in root fresh weight, respectively. In heat stress, the 0.001% BAP showed an ~8%, and 0.003% and 0.005% BAP treatment exhibited a ~42% and ~90% increase in root fresh weight over the control (Fig. 2B).

Application of 0.001% BAP, 0.003% BAP, and 0.005% BAP under no stress showed ~10%, ~22%, and ~35% increase, and under heat stress, caused ~6%, ~11%, and ~44% increase, respectively in root dry weight in comparison to the control (Fig. 2C).

Chlorophyll and carotenoid content: Chlorophyll a content increases by ~12% with 0.001% BAP, ~23% with 0.003% BAP, and ~31% with 0.005% BAP without stress than the control. With heat stress, 0.001% BAP showed a ~5% increase in chlorophyll a content, 0.003% BAP led to a ~25%, and 0.005% BAP resulted in a ~54% increase over the control (Fig. 3A).

Compared to the control, the application of 0.001% BAP exhibited a ~15% increase, 0.003% BAP and 0.005% BAP exhibited a ~33% and ~46% increase in chlorophyll b content. Under heat stress, adding 0.001% BAP, 0.003% BAP, and 0.005% BAP treatments resulted in a ~7%, ~18%, and ~38% increase in chlorophyll b content from the control (Fig. 3B).

A significant ~13%, ~26%, and ~35% increase in total chlorophyll content was recorded with 0.001% BAP, 0.003% BAP, and 0.005% BAP in no stress over the control. Adding 0.001% BAP, 0.003% BAP, and 0.005% BAP with heat stress showed ~21%, ~41%, and ~70% increase in total chlorophyll from the control (Fig. 3C).

Adding 0.001% BAP, 0.003% BAP, and 0.005% BAP under no stress resulted in ~21%, ~30%, and ~38% rise in carotenoid and under heat stress showed ~27%, ~57%, and ~97% rise in comparison to the control (Fig. 3D).

CAT, APX, and GSH: Without stress, the CAT activity decreased by ~27% with 0.001%, ~15% with 0.003%, and ~49% with 0.005% BAP, respectively, over the control. Under heat stress, the 0.001% BAP, 0.003% BAP, and 0.005% BAP treatments showed ~18%, ~11%, and ~28% decreases in CAT activity compared to the control (Fig. 4A).

In comparison to the control, adding 0.001% BAP showed a ~39% decrease under stress, and 0.003% BAP led to an ~17%, and 0.005% BAP exhibited a ~60% decrease, respectively, in APx and under heat stress, caused ~23%, ~9%, and ~44% decrease over the control (Fig. 4B).

GSH level decreased by ~28%, ~70%, and ~99% with 0.001%, 0.003%, and 0.005% BAP under no stress, and heat stress caused ~10%, ~21%, and ~33% decreases, respectively in comparison to the control (Fig. 4C).

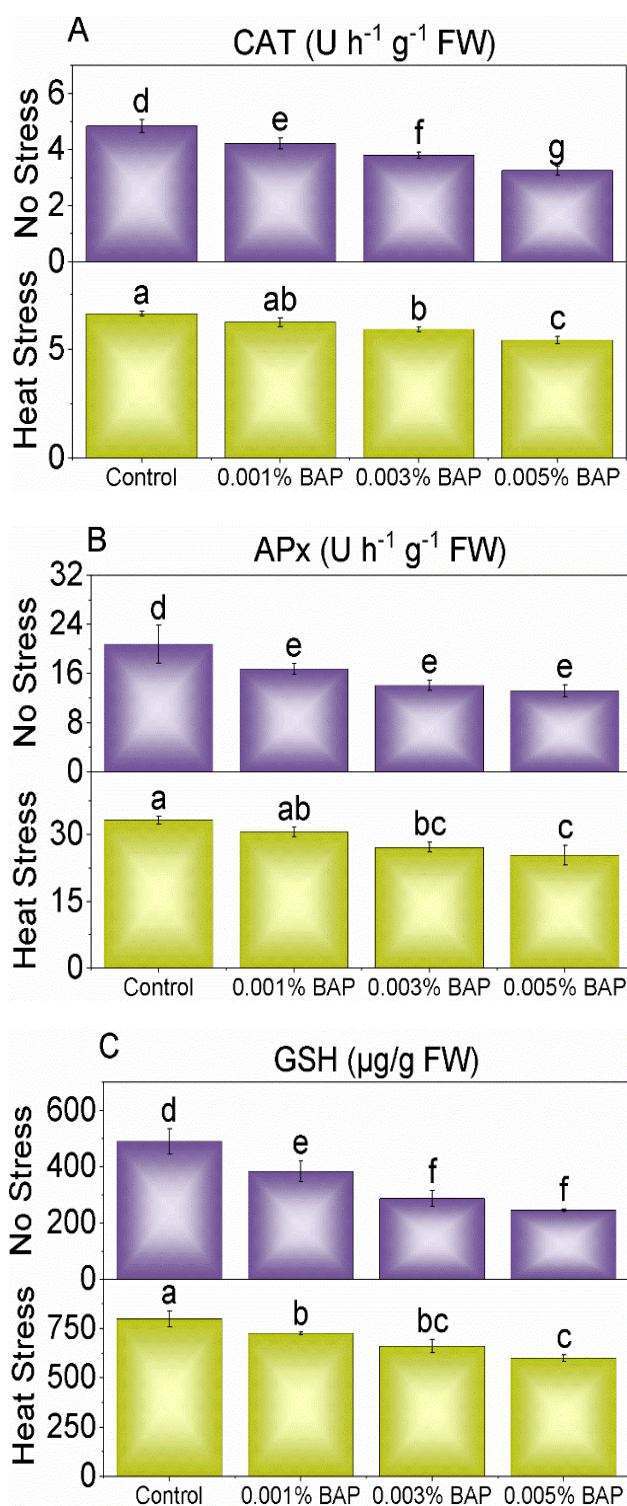


Fig. 4. The effect of different concentrations of BAP on CAT (A), APx (B), and GSH (C) of *Phaseolus vulgaris* L., grown with and without heat stress. The Tukey test measured significant differences at ($p < 0.05$); distinct letters on the bars are the mean of four replicates.

RWC and Total soluble protein: In no stress, applying 0.001% BAP, 0.003% BAP, and 0.005% BAP treatments resulted in a ~11%, ~19%, and ~27% increase, respectively, in RWC from the control. Under heat stress, the treatments of 0.001% BAP led to a ~12% increase, and 0.003% BAP and 0.005% BAP exhibited ~26% and ~42% increases in RWC over the control (Fig. 5A).

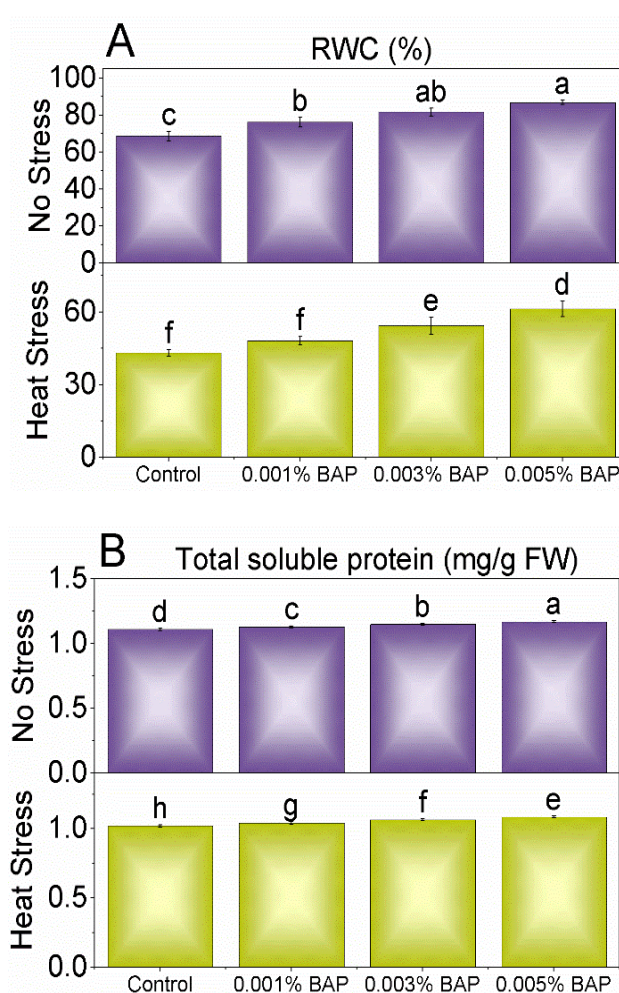


Fig. 5. The effect of different concentrations of BAP on RWC (A) and total soluble protein (B) of *Phaseolus vulgaris* L., grown with and without heat stress. The Tukey test measured significant differences at ($p < 0.05$); distinct letters on the bars are the mean of four replicates.

Adding 0.001% BAP, 0.003% BAP, and 0.005% BAP under no stress showed a ~2, ~4%, and ~5% increase in total soluble protein compared to the control. Applying 0.001% BAP, 0.003% BAP, and 0.005% BAP under heat stress showed ~2%, ~4%, and ~6% rise in total soluble protein over the control (Fig. 5B).

Shoot nutrients: Under no stress, 0.001% BAP treatment demonstrated a ~36% increase, 0.003% BAP showed a ~69% increase, and 0.005% BAP exhibited a ~97% increase, respectively, in shoot N compared to the control. Shoot N level is increased by ~23% with 0.001% BAP, ~61%, and ~93% with 0.003% BAP and 0.005% BAP under heat stress from the control (Table 2).

The shoot P in no stress increases by ~13% with 0.001% BAP, ~27% with 0.003% BAP, and ~42% with 0.005% BAP than the control. Under heat stress, shoot P is increased by ~4% with 0.001% BAP, ~48% with 0.003% BAP, and ~90% with 0.005% BAP from the control (Table 2).

Under no stress, the treatments of 0.001% BAP resulted in an ~11% increase, and 0.003% BAP and 0.005% BAP led to ~25% and ~38% increases, respectively, in shoot K from the control. Under heat stress,

shoot K is increased by ~15% with 0.001% BAP, ~44% with 0.003% BAP, and ~84% with 0.005% BAP than the control (Table 2).

Under no stress, adding 0.001% BAP, 0.003% BAP, and 0.005% BAP increases the shoot Ca level by ~15%, ~26%, and ~40% over the control, while under heat stress increases by ~32%, ~56%, and ~96%, respectively (Table 2).

Without stress, adding 0.001% BAP showed a ~12% increase, 0.003% BAP led to an ~18%, and 0.005% BAP resulted in a ~29% increase, respectively, in shoot Fe over the control. Related to the control, in heat stress, the shoot Fe is increased by ~17% with 0.001% BAP, ~51% with 0.003% BAP, and ~99% with 0.005% BAP, respectively (Table 2).

Root nutrients

Applying 0.001% BAP, 0.001% BAP, and 0.005% BAP in no stress exhibits a ~12%, ~25%, and ~38% increase in root N compared to the control. Under heat stress, the application of 0.001% BAP resulted in a ~22% increase, 0.003% BAP showed a ~50% increase, and 0.005% BAP demonstrated a ~89% increase in root N from the control (Table 3).

In the absence of stress, from the control, the treatment 0.001% BAP showed a ~9% increase, 0.003% BAP exhibited a ~19% increase, and 0.005% resulted in an ~29% increase, and under heat stress, caused a ~18%, ~40%, and ~65% increases, respectively in root P (Table 3).

Without stress, a significant ~11%, ~25%, and ~36% was observed with 0.001% BAP, 0.001% BAP, and

0.005% BAP in root K, and in heat stress, showed ~36%, ~61%, and ~99% increases, respectively in comparison to the control (Table 3).

Compared to the control, without stress, the root Ca content is increased by ~21% with 0.001% BAP, ~34%, and ~41% with 0.003% BAP and 0.005% BAP, respectively. In heat stress, ~25%, ~58%, and ~96% increases were observed with 0.001% BAP, 0.003% BAP, and 0.005% in root Ca from the control (Table 3).

Under no stress, adding 0.001% BAP led to a ~19% increase, 0.003% BAP resulted in a ~14% increase, and 0.005% BAP showed a ~25% increase in root Fe than the control. Under heat stress, 0.001% BAP increased Fe content by ~51%, 0.003% by ~24%, and 0.005% by ~91% compared to the control (Table 3).

Convex hull and hierarchical cluster analysis: The convex hull analysis was conducted on the dataset, categorizing the data points into four distinct treatment groups: Control, 0.001% BAP, 0.003% BAP, and 0.005% BAP. For the Control group, the convex hull was determined by points representing the treatment conditions, spanning from (97.46%, 1.23%) to (-6.54021, -0.41913). In the 0.001% BAP treatment group, the convex hull covered points ranging from (-6.0506, -0.55529) to (-4.37418, 0.26387). The convex hull for the 0.003% BAP treatment contained points extending from (-4.37418, 0.26387) to (-2.05551, 0.36308). Finally, the convex hull for the 0.005% BAP treatment was defined by points covering from (-2.05551, 0.36308) to (0.79528, 2.49345) (Fig. 6A).

Table 2. The effect of different concentrations of BAP on shoot N, P, K, Ca, and Fe concentration of *Phaseolus vulgaris* L., grown with and without heat stress.

Treatment	Shoot N (%)	Shoot P (%)	Shoot K (%)	Shoot Ca (%)	Shoot Fe (%)
No stress					
Control	11.09d	0.65d	5.50cd	2.43c	0.08bc
0.001% BAP	15.04c	0.74c	6.12bc	2.79bc	0.09ab
0.003% BAP	18.74b	0.83b	6.86ab	3.05ab	0.10a
0.005% BAP	21.80a	0.92a	7.58a	3.39a	0.11a
Heat stress					
Control	2.96g	0.27g	2.66f	1.19e	0.04e
0.001% BAP	3.63fg	0.28g	3.06ef	1.60de	0.05de
0.003% BAP	4.76ef	0.40f	3.83e	1.90d	0.06d
0.005% BAP	5.70e	0.51e	4.89d	2.38c	0.08c

The Tukey test measured significant differences at ($p < 0.05$); distinct letters on the bars are the mean of four replicates

Table 3. The effect of different concentrations of BAP on root N, P, K, Ca, and Fe concentration of *Phaseolus vulgaris* L., grown with and without heat stress.

Treatment	Root N (%)	Root P (%)	Root K (%)	Root Ca (%)	Root Fe (%)
No Stress					
Control	0.65cd	2.01c	2.17cd	3.73c	0.26bc
0.001% BAP	0.71bc	2.24c	2.41bc	4.51b	0.29ab
0.003% BAP	0.77ab	2.50b	2.71ab	4.99a	0.30a
0.005% BAP	0.84a	2.76a	2.95a	5.28a	0.32a
Heat Stress					
Control	0.38f	0.92f	1.10f	1.63g	0.12e
0.001% BAP	0.44f	1.12f	1.50ef	2.03f	0.15de
0.003% BAP	0.53e	1.38e	1.78de	2.57e	0.18d
0.005% BAP	0.62d	1.74d	2.20c	3.19d	0.24c

The Tukey test measured significant differences ($p < 0.05$); distinct letters on the bars are the mean of four replicates

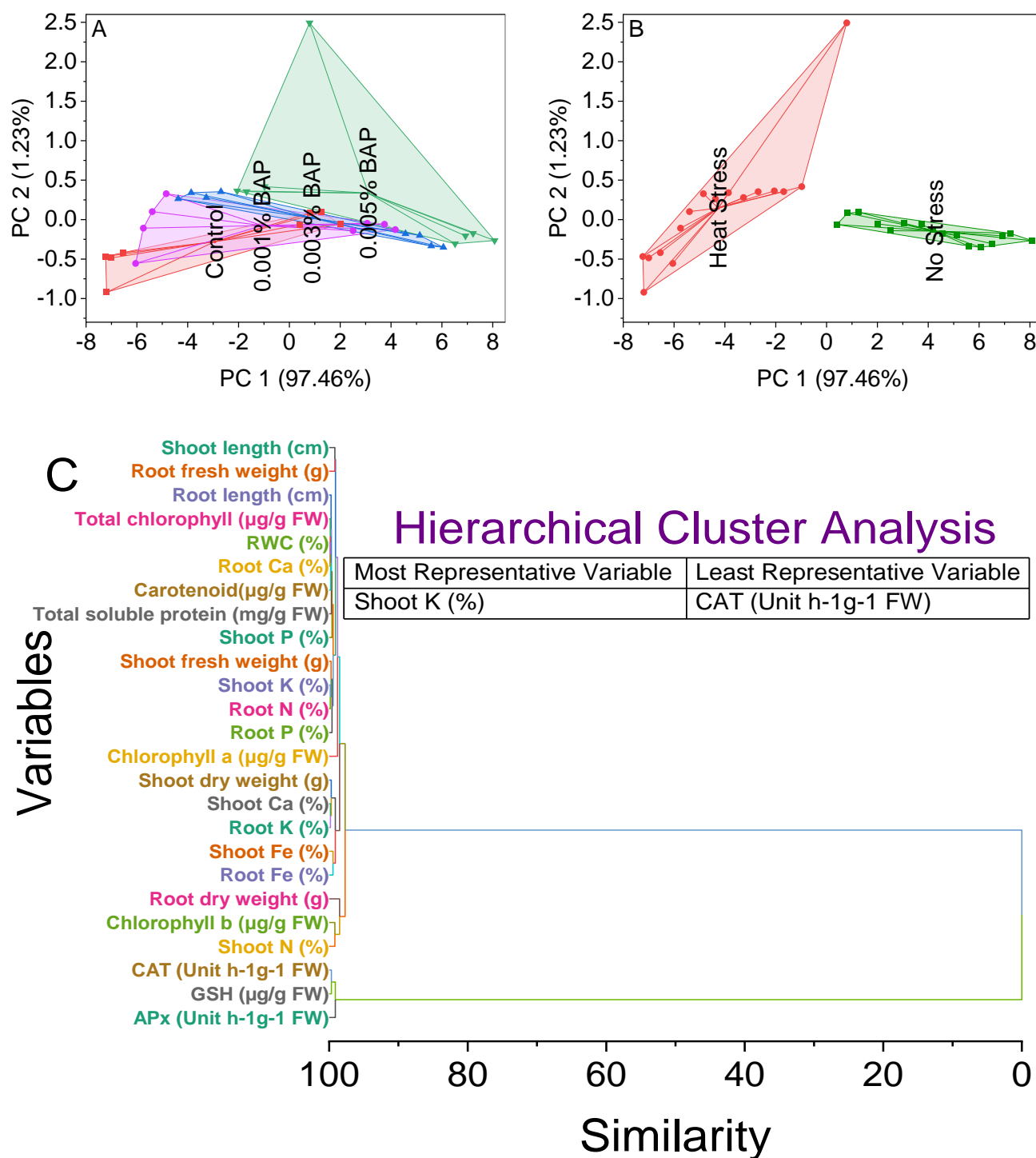


Fig. 6. Cluster plot convex hull for treatments (A), stress levels (B), and Hierarchical cluster plot (C) for studied attributes.

The first principal component (PC 1) captures a substantial 97.46% of the overall variance, while PC 2 contributes 1.23%. Examining the stress distribution across the samples delineates two main categories: no stress and heat stress. Samples categorized under no stress exhibit scores clustered within a relatively narrow range, characterized by values such as (0.40125, -0.06241), (2.50848, -0.14082), and (8.07598, -0.26547). Conversely, samples experiencing heat stress are marked by notably higher PC 1 scores alongside varying PC 2 values, as evident in coordinates such as (-7.19074, -0.9208), (-5.39443, 0.10076), and (0.79528, 2.49345) (Fig. 6B).

Notable associations emerge, such as the similarity between RWC and root Ca with a score of 0.11463, indicating a potential relationship in their behavior. Similarly, variables like total chlorophyll and variable (26) exhibit a lower but discernible similarity score of 0.03849, suggesting a modest connection. Associations with higher similarity scores include pairs like shoot P and an unnamed variable (34) with a score of 0.42032, highlighting a strong correspondence in their patterns. Remarkably, variables such as shoot length and root fresh weight display an exceptionally high similarity score of 0.82531, indicating a very close relationship in their characteristics (Fig. 6C).

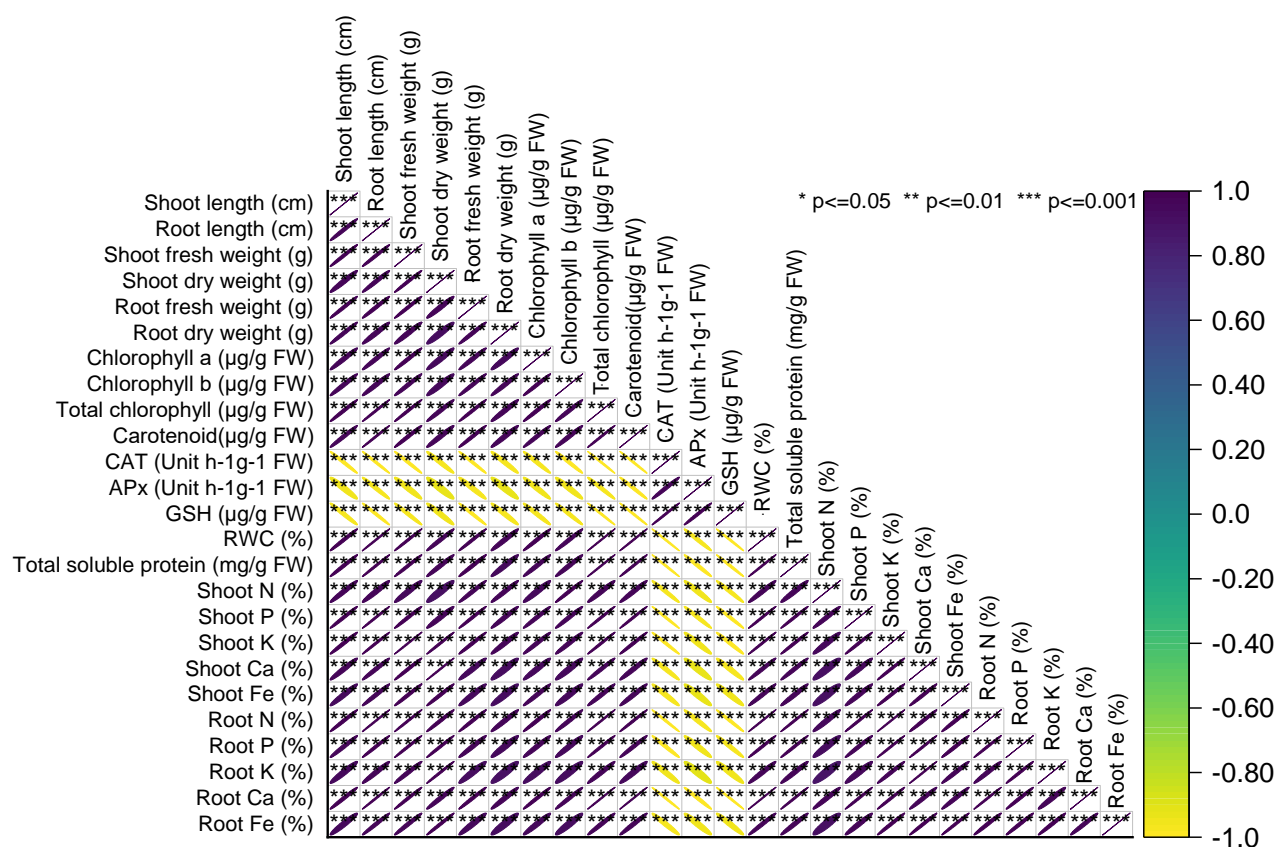


Fig. 7. Pearson correlation analysis for the studied attributes.

Pearson correlation analysis: Strong positive correlations are observed among several parameters. For instance, shoot length exhibits a high positive correlation with root length (0.97948), shoot fresh weight (0.98461), shoot dry weight (0.96104), root fresh weight (0.98372), and root dry weight (0.97911). Similarly, total chlorophyll demonstrates strong positive correlations with most variables, such as chlorophyll a (0.98357) and chlorophyll b (0.99296). Conversely, variables like CAT and APx exhibit strong negative correlations with most parameters, indicating an inverse relationship. Notably, shoot N displays positive correlations with various parameters, including shoot P (0.97202) and shoot K (0.98738). Conversely, CAT and APx demonstrate strong negative correlations with several variables, suggesting an inverse relationship. Additionally, RWC is highly positively correlated with total soluble protein (0.99502), indicating a strong association between these parameters (Fig. 7).

Discussion

Heat stress affects plant morphology by causing stunted growth, wilting, and changes in leaf structure to cope with water loss and heat absorption (Bechtaoui *et al.*, 2021). This study explores the influence of benzyl amino purine (BAP) on the growth of *Phaseolus vulgaris L.* cultivation. Our investigation aims to select the best application rate of BAP on the growth of *Phaseolus vulgaris L.* plants. BAP is known to stimulate cell division and elongation, particularly in meristematic tissues, which are regions of active growth in

plants (Sosnowski *et al.*, 2023). By representing the action of natural cytokinin's, BAP activates cell cycle-related genes and promotes the synthesis of proteins involved in cell division (Sytar *et al.*, 2019). This leads to increased shoot length, as observed in the results (Fig. 1A). The higher concentrations of BAP likely result in greater activation of cell division processes, leading to more pronounced increases in shoot length. BAP not only influences shoot growth but also affects root development (Ramadan *et al.*, 2023). BAP has been shown to promote lateral root formation elongation and enhance root branching (Kurepin *et al.*, 2011). Additionally, cytokinins regulate the balance between shoot and root growth, promoting overall root biomass (Sosnowski *et al.*, 2023). Under heat stress conditions, where root growth is inhibited, applying BAP could mitigate these effects by stimulating root growth through its hormone-like activity. The observed increases in chlorophyll and carotenoid content can be attributed to the role of cytokinin's in regulating photosynthetic processes. Cytokinin's have been shown to enhance chlorophyll biosynthesis by upregulating the expression of genes involved in chlorophyll synthesis pathways (Janečková, 2021). Additionally, cytokinin's influence chlorophyll degradation rates, leading to higher chlorophyll content (Sosnowski *et al.*, 2023). Conversely, carotenoids are accessory pigments in photosynthesis and play a crucial role in scattering excess light energy and protecting chlorophyll from photodamage (Maoka, 2020). The increase in carotenoid content may reflect a response to the enhanced photosynthetic activity stimulated by BAP. Cytokinin's like

BAP are also involved in plant stress responses, including heat stress (Hudeček *et al.*, 2023). They interact with other hormonal pathways, such as abscisic acid (ABA) and ethylene, to regulate stress signaling and tolerance mechanisms. Catalase (CAT) serves as a vital enzyme in the breakdown of hydrogen peroxide (H₂O₂) into water and oxygen, which is crucial for scavenging reactive oxygen species (ROS) and protecting plant cells from oxidative harm (Sharma *et al.*, 2021). The observed decline in CAT activity with increasing BAP concentration may result from the modulation of ROS levels, as BAP could potentially influence ROS production or signaling pathways, consequently downregulating CAT expression or activity. Ascorbate Peroxidase (APx) also holds significance in the plant antioxidant defense system, responsible for reducing hydrogen peroxide using ascorbate as an electron donor (Bouremani *et al.*, 2023). The diminishing APx activity with escalating BAP concentration hints at a complex interaction between BAP and the antioxidant system, possibly through interference with the ascorbate-glutathione cycle or other ROS detoxification pathways. Glutathione (GSH), a major non-enzymatic antioxidant, is critical in maintaining cellular redox homeostasis (Jiang *et al.*, 2016). The notable decrease in GSH levels observed alongside increasing BAP concentration may stem from either direct ROS scavenging by BAP or alterations in the expression of genes involved in GSH synthesis or recycling. Regarding relative water content (RWC) and total soluble protein, the rising RWC with increasing BAP concentration suggests BAP's involvement in boosting water uptake or retention in plant tissues. BAP might influence factors like stomatal conductance, root hydraulic conductivity, or aquaporin activity, thus enhancing water availability and mitigating water loss during stress conditions (Kirkby *et al.*, 2023). The slight elevation in total soluble protein with BAP concentration hints at a potential role for BAP in fostering protein synthesis or stability, possibly through the activation of signaling pathways associated with protein biosynthesis or protection against denaturation under stressful circumstances. Regarding shoot and root nutrient levels (N, P, K, Ca, Fe), the notable enhancements with increasing BAP concentration indicate BAP's potential to enhance nutrient uptake or assimilation. BAP could stimulate root growth, enhance the activity of nutrient transporters, or modulate ion channels, thereby improving nutrient uptake efficiency (Kirkby *et al.*, 2023). Particularly under heat stress conditions, the amplified increases in nutrient levels with BAP treatment suggest a potential for BAP in alleviating heat-induced nutrient stress by possibly enhancing the expression of stress-responsive genes involved in nutrient acquisition or remobilization, thus enriching plant nutrient status under stressful environments.

Conclusion

In conclusion, 0.005% BAP is an effective amendment to increase *Phaseolus vulgaris* L., growth under heat stress. It can effectively improve morphological attributes and chlorophyll contents while regulating the antioxidants to mitigate heat stress in *Phaseolus vulgaris* L. Treatment 0.005% BAP also improves nutrient absorption in shoots and roots which are essential for plant growth. It is

recommended that 0.005% BAP can be used to increase the *Phaseolus vulgaris* L., growth. More investigations are suggested at the field level to declare 0.005% BAP as the best amendment dose for alleviating heat stress effect in different crops.

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