



RESEARCH ARTICLE

6-benzylaminopurine treatment delays senescence and enhances antioxidant defenses in ivy gourd (*Coccinia grandis* (L.) Voigt)

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Abstract

Ivy gourd faces several postharvest challenges that limit its marketability and shelf life. These include rapid yellowing, water loss and tissue softening that accelerate deterioration. This study investigates the impact of 6-benzylaminopurine (BAP) treatments on the quality and shelf-life of ivy gourd fruits during ambient storage, given its reported role in delaying senescence in horticultural produce. Fruits were dipped with BAP at 1 mM, 2 mM and 3 mM concentrations, parameters including weight loss, chlorophyll, carotenoid, ascorbic acid content, antioxidant capacity and enzymatic activities were measured over a 10-day period. Treated fruits showed significantly reduced weight loss, with the 3 mM showing the lowest loss of 23.16 % at 10 days after storage (DAS). Chlorophyll and carotenoid retention were also higher in treated fruits, with the 3 mM treatment maintaining 11.15 mg/g fresh weight (FW) chlorophyll and 0.88 mg/g carotenoids at 10 DAS. Additionally, treatments reduced oxidative stress, as shown by lower malondialdehyde content (54.94 % compared to control) at 10 DAS. Enzymatic activities, such as catalase and peroxidase, were enhanced under 3 mM treatment, with catalase at 116.28 EU/mg protein/min and peroxidase at 140.25 EU/mg protein/min at 10 DAS. These results demonstrate that 6-benzylaminopurine, especially at 3 mM, is effective in delaying senescence, enhancing antioxidant defences and preserving ivy gourd quality during storage up to 10 days.

Keywords: 6-benzylaminopurine; antioxidant capacity; ivy gourd; postharvest treatment; shelf life

Introduction

Ivy gourd (*Coccinia grandis* L. Voigt) is a tropical plant species belonging to the family Cucurbitaceae. Known by names like tindora, little gourd, kundru, kovakkai, kowai fruit and scarlet gourd (1), it is native to Africa and Asia, including India, Philippines, China, Indonesia, Malaysia, Thailand, Vietnam, Eastern Papua New Guinea and the Northern Territories, Australia (2).

Ivy gourd fruits are a valuable source of natural therapeutic agents, with no reported adverse effects or toxicity for human consumption (3). Nutritionally, they are rich in antioxidants and bioactive compounds such as flavonoids, polyphenols, carotenoids, α -tocopherol, ascorbic acid and β -carotene (4), along with high levels of potassium (K) and calcium (Ca), which are beneficial for human health (5). Pharmacologically, ivy gourd shows promise in treating conditions such as leprosy, asthma and jaundice (6). Its bioactivities, particularly relevant to human health, include antioxidant, anti-inflammatory, antidiabetic, analgesic and anticancer effects (7, 8). Additionally, it has demonstrated hepatoprotective and antifungal properties. These therapeutic claims are supported by *in vitro* and *in vivo* studies, though further

clinical research is needed to confirm their efficacy in human populations.

Ivy gourd faces several critical postharvest challenges that limit its marketability and shelf life. A primary concern is its elevated respiration and transpiration rates in warm ambient conditions, which accelerate deterioration (9). In general, ivy gourd is sold without packaging or temperature control, leading to rapid moisture loss and a shelf life of merely 4-5 days at room temperature (10). As the fruit matures, it transitions from green to pale green and eventually to pink, a stage marked by increased mass loss, shriveling and wilting. Fully ripened pink fruits are considered unsuitable for culinary use due to their poor texture and flavor (11). Continuous respiration during storage accelerates ripening and quality deterioration (12).

One of the most noticeable symptoms of postharvest stress is shriveling, which results from excessive moisture loss, particularly in the absence of proper storage practices. In ivy gourd, this leads to the toughening of the exterior pericarp and a significant loss of tenderness, diminishing both visual appeal and consumer acceptability (13). The resulting textural degradation,

combined with rapid ripening and the lack of postharvest interventions, significantly contributes to postharvest losses. Therefore, addressing these issues through effective pre- and post-harvest management practices is crucial to enhance shelf life, maintain quality and reduce market losses.

BAP is a plant growth regulator that has demonstrated considerable efficacy in enhancing the postharvest storage quality of fruits and vegetables. It has been shown to mitigate chilling injury and enhance stress resistance across various crops (14, 15). The compound effectively reduces chilling injury by maintaining elevated levels of chlorophyll, ascorbic acid, total phenolics and total antioxidant capacity. Furthermore, it enhances the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT). Additionally, treatment with BAP increases adenosine triphosphate (ATP) content and energy charge, thereby contributing to improved fruit quality during storage (14). Several preservation methods in ivy gourd have been explored, including edible coatings like guar gum and alginate (13), external applications of mannitol and sorbitol (10) and a gum acacia/pectin/pullulan (GPPL) polysaccharide composite film (16). Additionally, modified atmosphere packaging has been studied for its effectiveness in maintaining fruit quality (9). However, research on BAP for ivy gourd is limited.

This study investigates the effects of BAP on the postharvest quality and shelf life of ivy gourd, with particular focus on antioxidant enzyme activity. The study addresses the need for sustainable and consumer-safe preservation strategies for this underutilized vegetable crop.

Materials and Methods

Fruit selection and treatment

The ivy gourd cultivar Indira Kundru-35 was harvested in August 2022 at commercial maturity (6-7 cm long and have a bright green colour) from a field in Ramana village, Varanasi, Uttar Pradesh. The fruits were sorted and only those that were healthy and undamaged, devoid of blemishes, diseases, pests and mechanical injuries, were selected based on uniformity in size, shape, colour and maturity. A total of 100 fruits per treatment were chosen. The selected fruits were surface sterilized in a 2 % sodium hypochlorite solution for 5 min, after which they were dried to remove surface moisture. Control fruits were subjected to treatment with distilled water. Ivy gourd fruits underwent treatment with aqueous solutions of 6-benzylaminopurine (Sisco Research Laboratories Pvt. Ltd., Mumbai, India, extra pure AR, 99 %) at concentrations of 1.0 mM, 2.0 mM and 3.0 mM, achieved by immersing them in the solution at 25 °C for a duration of 5 min. The treatment duration was standardized across all samples and excess moisture was eliminated through air drying in ambient condition. Following air drying under sterile conditions, the fruits were placed in corrugated fiber board containers and stored under ambient conditions, maintaining a temperature of 25 ± 1 °C and a relative humidity of 85 %. Weight loss was measured using 10 fruits per treatment and for biochemical analysis, 4 replicates per treatment were collected at 2-day intervals throughout the storage period. Observations on physico-chemical and functional quality attributes, as well as antioxidant enzyme activity, were recorded at 2-day intervals.

Fresh weight loss

The weight of treated and control fruits was recorded at regular intervals using an electronic balance to measure weight loss during storage. Final weight loss was calculated using the following formula, with results expressed in percentage (%). The results were averaged across replicates.

Total chlorophyll and total carotenoid content

The total chlorophyll content was determined following earlier described methods (17). For this, 1.0 g of fruit skin was crushed with 80 % acetone to extract the chlorophyll pigment. Later, the crushed sample was strained and the volume was adjusted with 80 % acetone up to 10 mL. Afterwards, the sample was put through Remi CPR 24 plus Refrigerated Centrifuge, centrifugation at 10000 rpm for 10 min. Subsequently, absorbance was recorded in spectrophotometer at 645 and 663 nm with reference to the blank prepared with 80 % acetone. Lastly, the total chlorophyll content was calculated using the following formula. The results were expressed as mg/g FW.

Total chlorophyll content (mg/g fw)=

$$\frac{20.2 \times A_{645} + 8.02 \times \text{Volume of sample}}{1000 \times \text{Weight of sample}}$$

Total carotenoid content was estimated following procedure described previously (18). A 1.0 g fruit sample was crushed in 80 % acetone to extract carotenoids. The extract was transferred to a separating funnel and 20 mL petroleum ether and 5 % sodium sulphate were added. After carotenoid pigments migrated to the petroleum ether layer, it was transferred to a 50 mL volumetric flask and the volume was adjusted with petroleum ether. Absorbance was measured at 470 nm, with petroleum ether as the blank. Results were expressed as mg/g FW.

Malondialdehyde content

The malondialdehyde (MDA) content in ivy gourd fruit during postharvest storage was quantified using previously reported methods (19). A sample weighing 1.0 g was homogenized in 5 mL of 5 % (w/v) trichloroacetic acid (TCA) and subsequently centrifuged at 10000 rpm for 15 min. A 2 mL aliquot of the resulting supernatant was combined with 2 mL of 5 % TCA containing 0.6 % (w/v) thiobarbituric acid (TBA), heated at 90 °C for 20 min and then rapidly cooled. Absorbance measurements were taken at 450, 532 and 600 nm. The MDA content was expressed as nmol/g FW.

Ascorbic acid content

The ascorbic acid content in ivy gourd fruit was quantified following the methodology described earlier (20). A 1.0 g sample was homogenized in 3 % metaphosphoric acid and subsequently diluted to 100 mL with the same solution. A 10 mL aliquot was titrated with 2,6-dichlorophenol indophenol dye until a pink endpoint was sustained for 15 sec. The titre value obtained was utilized to calculate the ascorbic acid content, which was expressed as mg/100 g FW.

Total phenol content

The total phenol content in ivy gourd fruit was assessed using the method outlined in previous studies. A 1.0 g sample was extracted with 10 mL of 80 % ethanol, then centrifuged at 10000 rpm for 10 min and the supernatant was collected for analysis. A 200 µL portion of the extract was combined with 2.8 mL of

distilled water and 0.5 mL of 1 N Folin-Ciocalteu reagent. After 3 min, 2 ml of 20 % sodium carbonate was added to the mixture. The solution was incubated in dark condition for 90 min and its absorbance was measured at 760 nm. Gallic acid served as the standard and the results were reported as microgram gallic acid equivalent per gram of fresh weight ($\mu\text{g GAE/g FW}$).

Total flavonoids content

The total flavonoid content of ivy gourd fruit was determined using the aluminum chloride method (22). A 1.0 g sample was mixed with 10 mL of methanol and centrifuged at 10000 rpm for 10 min. A 1 mL aliquot of the supernatant was added to a test tube containing 4 mL of distilled water and 0.3 mL of 5 % sodium nitrite solution. After 5 min, 0.3 mL of 10 % aluminum chloride solution was added and the mixture was stored at room temperature in dark condition for 6 min. Subsequently, 2 mL of 1 N sodium hydroxide solution was added and the final volume was adjusted to 10 mL with distilled water. The absorbance was measured at 510 nm using a spectrophotometer, alongside a reagent blank. A standard calibration curve was prepared using rutin and the total flavonoid content was expressed as microgram rutin equivalents/g FW ($\mu\text{g RE/g FW}$).

Total antioxidant capacity

The total antioxidant capacity was evaluated using the CUPRAC assay (Cupric Reducing Antioxidant Capacity) (23). In this method, the sample extract was mixed with a solution containing 80 % ethanol, copper (II) chloride (10^{-2} M), neocuproine ($7.5 \times 10^{-3}\text{ M}$) and ammonium acetate buffer (pH 7.0). Absorbance of the mixture was measured at 450 nm using a spectrophotometer. The total antioxidant capacity was calculated from a standard curve using Trolox as standard. The results were quantified and expressed as $\mu\text{mol Trolox equivalents/g FW}$ ($\mu\text{mol TE/g FW}$).

Radical scavenging activity (DPPH assay)

The radical scavenging activity of ivy gourd fruit was evaluated utilizing the DPPH assay (24). A solution comprising 0.2 mL of the sample extract and 3.8 mL of 0.0634 mM DPPH in 95 % methanol was incubated for 30 min at room temperature in the dark condition, after which the absorbance was recorded at 515 nm. Radical scavenging activity was calculated according to the formula: $100 \times (\text{Abs}_0 - \text{Abs}_1) / \text{Abs}_0$ where, Abs_0 is the beginning absorbance at 515 nm against a reagent blank, obtained by measuring the same volume of solvent and Abs_1 is the final absorbance of the sample extract after 30 min. The activity was quantified as percentage inhibition.

Estimation of total soluble proteins

A 1.0 g fruit sample was homogenized in 10 mL of phosphate buffer, then centrifuged at 20000 rpm for 15 min (25). The supernatant was collected for protein estimation. To 0.1 mL of the extract, 1 mL of alkaline copper reagent was added, mixed and allowed to stand for 15 min. Next, 0.2 mL of 1:1 (v:v) diluted Folin-Ciocalteu phenol reagent was added and the solution was incubated in the dark at room temperature for 30 min, developing a blue color. The volume was adjusted to 5 mL with double-distilled water and absorbance was measured at 750 nm. The protein content was calculated from a standard curve using bovine serum albumin (BSA). Protein content was expressed as $\mu\text{g/g FW}$.

Catalase (CAT) (EU/mg protein/min)

Catalase activity in ivy gourd fruit was measured at 2-day intervals, following (26). A 100 mg fruit sample was homogenized in 5 mL of 0.1 M phosphate buffer, then the crude extract was centrifuged at 10000 rpm for 20 min at 4 °C and the extract stored at low temperature. The enzyme assay involved mixing 2.6 mL buffer, 0.1 mL enzyme extract and 0.1 mL 1 % H_2O_2 , with changes in absorbance at 240 nm recorded every 15 sec for 2 min. A blank was prepared with buffer instead of enzyme extract. Catalase activity was calculated using the extinction coefficient ($39.4\text{ mM}^{-1}\text{ cm}^{-1}$) for H_2O_2 decomposition and expressed per EU/mg protein/min.

Peroxidase (POX) (EU/mg protein/min)

Peroxidase (POX) activity in ivy gourd fruit was measured at 2-day intervals following method in previous reports (27). Enzyme extraction was done by homogenizing 100 mg of fruit pulp in 5 mL of 0.1 M phosphate buffer, followed by centrifugation at 10000 rpm for 20 minutes at 4 °C. The reaction mixture, consisting of phosphate buffer, pyrogallol, 50 μM H_2O_2 and enzyme extract, was incubated at 25 °C for 5 min and terminated with 0.5 mL of 5 % sulfuric acid. Absorbance was measured at 420 nm using a spectrophotometer (Elico, SL196) and POX activity was calculated as change in absorbance expressed per EU/mg protein/min.

Polyphenol oxidase (PPO) (EU/mg protein/min)

Polyphenol oxidase (PPO) activity in ivy gourd fruit was measured at 2-day intervals (27). Enzyme extraction was performed by homogenizing 100 mg of fruit pulp in 5 mL of 0.1 M phosphate buffer, followed by centrifugation at 10000 rpm for 20 min at 4 °C. The reaction mixture, containing phosphate buffer, pyrogallol and enzyme extract, was incubated at 25 °C for 5 min and terminated with 0.5 mL of 5 % sulfuric acid. Absorbance was measured at 420 nm and PPO activity was calculated and expressed as EU/mg protein/min.

Statistical analysis

The experiment was conducted using a factorial completely randomized design (FCRD) with 4 replications per treatment. Data collected from different treatments and storage durations were analyzed using analysis of variance (ANOVA), with treatment and storage duration as the sources of variation. Results are expressed as mean \pm standard error. Mean comparisons between treatments were made using the HSD Tukey's test, with a significance level set at $p \leq 0.05$. All statistical analyses were performed using SAS version 14.3 (SAS Institute, Cary, NC, USA).

Results

Fresh weight loss

Table 1 presents the effects of postharvest BAP treatments on the weight loss of ivy gourd fruits during storage under ambient conditions over a 10-day period. The results demonstrate that all treatments, including the control group, show a progressive increase in weight loss (%) as the storage duration extends from 2 to 10 DAS. Weight loss was lowest in 3 mM BAP (23.16 % at 10 DAS) compared to 43.13 % in control. In contrast, fruits treated with BAP, particularly at a concentration of 3mM, exhibited significantly lower weight loss across all time points, with values ranging from 3.17 % at 2 DAS to 23.16 % at 10 DAS. The BAP treatments at 1mM and 2 mM also showed reduced weight loss, with 1mM BAP

Table 1. Effect of postharvest 6-benzylaminopurine treatments on weight loss (%) of ivy gourd fruits during storage at ambient condition.

Treatments	Weight loss (%)				
	Days after storage (DAS)				
	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS
BAP 1mM	3.89 ± 0.16b	8.29 ± 0.05b	11.25 ± 0.22b	19.89 ± 0.18b	28.25 ± 0.11b
BAP 2mM	3.4 ± 0.1bc	7.17 ± 0.09c	10.02 ± 0.12c	18.27 ± 0.15c	25.25 ± 0.06c
BAP 3mM	3.17 ± 0.14c	6.66 ± 0.11c	9.68 ± 0.13c	17.12 ± 0.27d	23.16 ± 0.21d
Control	7.09 ± 0.29a	15.47 ± 0.46a	22.99 ± 0.29a	36.6 ± 0.3a	43.13 ± 0.61a

Values are mean ± standard error of four replicate determinations (n=4). Treatment values followed by the same letters are not significantly different ($p \leq 0.05$).

starting at 3.89 % and reaching 28.25 % at 10 DAS and 2 mM BAP displaying similar trends from 3.40 % at 2 DAS to 25.25 % at 10 DAS.

Total chlorophyll and total carotenoid content

Total chlorophyll content data is presented in Table 2. The initial (0 DAS) chlorophyll content of ivy gourd fruits was measured at 15.80 ± 0.42 mg/g FW. Throughout the storage period, a gradual decline in chlorophyll content was observed across all treatments. The BAP 3 mM treatment proved most effective in preserving chlorophyll, with levels decreasing from 15.96 ± 0.39 mg/g FW at 2 DAS to 11.15 ± 0.28 mg/g FW at 10 DAS. Conversely, the control group exhibited the most pronounced decline, with chlorophyll content dropping from 13.64 ± 0.23 mg/g FW at 2 DAS to 4.01 ± 0.11 mg/g FW at 10 DAS. The BAP 1mM and 2 mM treatments resulted in moderate reductions, with final chlorophyll contents of 9.42 ± 0.22 mg/g FW and 10.12 ± 0.35 mg/g FW at 10 DAS, respectively.

In the storage of ivy gourd fruits, the increase in the storage period led to higher accumulation of total carotenoids, irrespective of treatments, while chlorophyll degradation was observed, indicating delayed senescence and the rise in carotenoids acted as a ripening indicator. The initial carotenoid content in ivy gourd fruits was recorded at 0.29 ± 0.003 mg/g FW. Over the 10-day storage period (Table 3), the control group demonstrated the most significant increase in carotenoid content, rising from 0.51 ± 0.05 mg/g FW at 2 DAS to 2.23 ± 0.08 mg/g FW at 10 DAS. Among the BAP treatments, the 1mM concentration resulted in the most notable increase, from 0.38 ± 0.03 mg/g FW at 2 DAS to 1.14 ± 0.01 mg/g FW at 10 DAS. The BAP 2 mM and 3 mM treatments also exhibited increases in carotenoid content. Specifically, BAP 2 mM

increased from 0.36 ± 0.03 mg/g FW at 2 DAS to 0.93 ± 0.01 mg/g FW at 10 DAS, while BAP 3 mM rose from 0.34 ± 0.02 mg/g FW at 2 DAS to 0.88 ± 0.02 mg/g FW at 10 DAS.

Malondialdehyde content (MDA)

MDA, a marker of lipid peroxidation and oxidative stress, accumulates in plant tissues during post-harvest storage, leading to cellular damage and reduced quality. BAP treatments significantly reduced MDA accumulation in ivy gourd fruits during storage, indicating lower lipid peroxidation and improved membrane integrity compared to the control (Table 4). While MDA content increased in all samples over time, the rate of increase was markedly lower in BAP-treated fruits. At 10 DAS, the control exhibited the highest MDA level (1.62 nmol/g FW), whereas BAP 1 mM, 2 mM and 3 mM treatments maintained significantly lower levels of 0.94, 0.79 and 0.73 nmol/g, respectively. BAP 3 mM consistently recorded the lowest MDA content across all time points, remaining relatively stable compared to other treatments, particularly in the early days of storage, though a noticeable increase was observed at 10 DAS (0.73 nmol/g FW).

Ascorbic acid content

Ascorbic acid levels declined gradually in all treatments over time, except in BAP-treated fruits, which retained significantly higher levels compared to the control (Table 5), highlighting BAP's role in preserving nutritional value. Ascorbic acid, a major antioxidant, contributes to both the shelf life and nutritional quality of the fruit by slowing oxidative degradation. BAP 3 mM was most effective, maintaining ascorbic acid content closest to the initial value (26.84 mg/100 g FW), with 16.68 mg/100 g FW remaining at 10 DAS,

Table 2. Effect of postharvest 6-benzylaminopurine treatments on total chlorophyll content (mg/g FW) of ivy gourd fruits during storage at ambient condition.

Treatments	Total chlorophyll content (mg/g FW)				
	Days after storage (DAS)				
	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS
BAP 1mM	15.2 ± 0.39a	13.86 ± 0.49b	12.4 ± 0.58b	11.03 ± 0.46b	9.42 ± 0.22b
BAP 2mM	15.23 ± 0.27a	14.2 ± 0.34ab	12.7 ± 0.26ab	11.62 ± 0.26ab	10.12 ± 0.35b
BAP 3mM	15.96 ± 0.39a	15.09 ± 0.29a	13.73 ± 0.24a	12.19 ± 0.24a	11.15 ± 0.28a
Control	13.64 ± 0.23b	10 ± 0.21c	8.61 ± 0.11c	5.3 ± 0.13c	4.01 ± 0.11c
Initial value (0 DAS): 15.80 ± 0.42 (mg/g)					

Values are mean ± standard error of four replicate determinations (n=4). Treatment values followed by the same letters are not significantly different ($p \leq 0.05$).

Table 3. Effect of postharvest 6-benzylaminopurine treatments on total carotenoids content (mg/g FW) of ivy gourd fruits during storage at ambient condition.

Treatments	Total carotenoids content (mg/g FW)				
	Days after storage (DAS)				
	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS
BAP 1mM	0.38 ± 0.03b	0.54 ± 0.01b	0.67 ± 0.02b	0.85 ± 0.01b	1.14 ± 0.01b
BAP 2mM	0.36 ± 0.03b	0.5 ± 0.03b	0.61 ± 0.03b	0.79 ± 0.01b	0.93 ± 0.01c
BAP 3mM	0.34 ± 0.02b	0.45 ± 0.02b	0.56 ± 0.02c	0.72 ± 0.02b	0.88 ± 0.02c
Control	0.51 ± 0.05a	0.79 ± 0.03a	1.05 ± 0.03a	1.48 ± 0.11a	2.23 ± 0.08a
Initial value (0 DAS): 0.29 ± 0.003 (mg/g)					

Values are mean ± standard error of four replicate determinations (n=4). Treatment values followed by the same letters are not significantly different ($p \leq 0.05$).

Table 4. Effect of postharvest 6-benzylaminopurine treatments on malondialdehyde content (nmol/g FW) of ivy gourd fruits during storage at ambient condition.

Treatments	Malondialdehyde content (nmol/g FW)				
	Days after storage (DAS)				
	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS
BAP 1mM	0.29 ± 0.008d	0.41 ± 0.004b	0.55 ± 0.003d	0.69 ± 0.005d	0.94 ± 0.016c
BAP 2mM	0.26 ± 0.007e	0.39 ± 0.081b	0.5 ± 0.002f	0.61 ± 0.003f	0.79 ± 0.005e
BAP 3mM	0.24 ± 0.003f	0.36 ± 0.006b	0.48 ± 0.003g	0.58 ± 0.006g	0.73 ± 0.008f
Control	0.39 ± 0.003a	0.6 ± 0.002a	0.88 ± 0.023a	1.17 ± 0.031a	1.62 ± 0.011a
Initial value (0 DAS): 0.24 ± 0.01 (nmol/g)					

Values are mean ± standard error of four replicate determinations (n=4). Treatment values followed by the same letters are not significantly different ($p \leq 0.05$).

Table 5. Effect of postharvest 6-benzylaminopurine treatments on ascorbic acid content (mg/100 g FW) of ivy gourd fruits during storage at ambient condition.

Treatments	Ascorbic acid content (mg/ 100 g FW)				
	Days after storage (DAS)				
	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS
BAP 1mM	24.26 ± 0.03c	22.98 ± 0.19b	20.31 ± 0.02c	18.34 ± 0.04c	15.67 ± 0.03c
BAP 2mM	24.99 ± 0.03b	23.91 ± 0.23a	20.95 ± 0.02b	18.74 ± 0.03b	16.07 ± 0.04b
BAP 3mM	26.27 ± 0.03a	24.47 ± 0.23a	21.43 ± 0.09a	19.14 ± 0.03a	16.68 ± 0.02a
Control	23.1 ± 0.17d	20.31 ± 0.37c	16.68 ± 0.02d	14.22 ± 0.03d	9.83 ± 0.24d
Initial value (0 DAS): 26.84 ± 0.23 (mg/100 g)					

Values are mean ± standard error of four replicate determinations (n=4). Treatment values followed by the same letters are not significantly different ($p \leq 0.05$).

compared to only 9.83 mg/100 g FW in the control. BAP 2 mM and 1 mM treatments also slowed the decline, though to a lesser extent.

Total phenol content

Total phenols declined across all treatments over time, BAP-treated fruits retained significantly higher phenolic content than the control throughout the storage period (Fig. 1A). BAP at 3 mM was most effective, maintaining phenol levels relatively higher than other treatments, with 91.13 µg GAE/g FW remaining at 10 DAS, compared to the initial value of 144.75 µg GAE/g FW, reflecting a 40 % reduction. This was substantially higher than the control, which decreased sharply to 50.03 µg GAE/g. The BAP 2 mM and 1 mM treatments showed intermediate values but remained significantly higher than the control.

Total flavonoids content

BAP treatments significantly influenced the retention of total flavonoid content in ivy gourd fruits during ambient storage (Fig. 1B). Although flavonoid levels decreased over time in all treatments, BAP-treated fruits retained significantly higher levels compared to the control, which may contribute to enhanced antioxidant and anti-browning potential, helping to maintain fruit quality during storage. Among treatments, BAP 3 mM was the most effective in slowing flavonoid degradation, with a content of 3.64 µg RE/g FW at 10 DAS, compared to only 1.23 µg RE/g FW in the control. BAP 1 mM and 2 mM also showed better retention than the control, though slightly lower than 3 mM. Notably, no significant difference was observed among BAP treatments at 8 DAS, but by 10 DAS, clear distinctions re-emerged.

Total antioxidant capacity

The findings indicate that application of BAP significantly influenced the total antioxidant capacity of ivy gourd fruits during storage under ambient conditions (Fig. 2A). Initially, the total antioxidant capacity was recorded at 3.36 µmol TE/g FW. Throughout the storage period, a decline was observed across all treatments; however, the 3 mM BAP treatment proved most effective in preserving antioxidant capacity. This treatment

commenced with 3.14 µmol TE/g FW and gradually decreased to 1.01 µmol TE/g FW by 10 DAS. The 2 mM BAP treatment began at 3.09 µmol TE/g FW, declining to 0.97 µmol TE/g FW by 10 DAS, while the 1mM BAP treatment exhibited a more rapid decrease from 3.01 to 0.89 µmol TE/g FW. In contrast, the control group, which did not receive BAP treatment, started at 2.77 µmol TE/g FW and experienced the most pronounced decline, reaching only 0.24 µmol TE/g FW by 10 DAS. 3 mM BAP preserved ~4-fold higher antioxidant capacity than control at 10 DAS.

Radical scavenging activity (DPPH assay)

Fig. 2B presents the effect of BAP treatments on the DPPH radical scavenging activity of ivy gourd fruits during storage at ambient conditions. Initially, the DPPH radical scavenging activity was 46.21 % and over the course of storage, all treatments showed a decline in this activity. However, the BAP treatments at 1mM, 2 mM and 3 mM concentrations maintained higher scavenging activity compared to the control group. At 2 DAS, the 1mM, 2 mM and 3 mM treatments had scavenging activities of 43.93 %, 44.42 % and 44.51 %, respectively, while the control group had 40.83 %. At 10 DAS, the 3 mM BAP treatment retained 25 % activity, nearly double that of the control (13.54 %).

Total soluble proteins

The total protein content in ivy gourd fruits was preserved more effectively with BAP treatments compared to the control group (Table 6). The 3 mM BAP treatment maintained the highest protein content, starting with 16.74 µg/g FW at 2 DAS and gradually decreasing to 10.5 µg/g FW by 10 DAS. Similarly, the 2 mM BAP treatment exhibited relatively high protein levels, starting with 15.82 µg/g FW and decreasing to 9.92 µg/g FW by 10 DAS. In contrast, the control group showed a significant decline in total protein content, dropping from 12.01 µg/g FW at 2 DAS to only 3.54 µg/g FW by 10 DAS.

Catalase (CAT)

The effect of BAP treatments on CAT activity in ivy gourd fruits during storage at ambient conditions was significant (Fig. 3A).

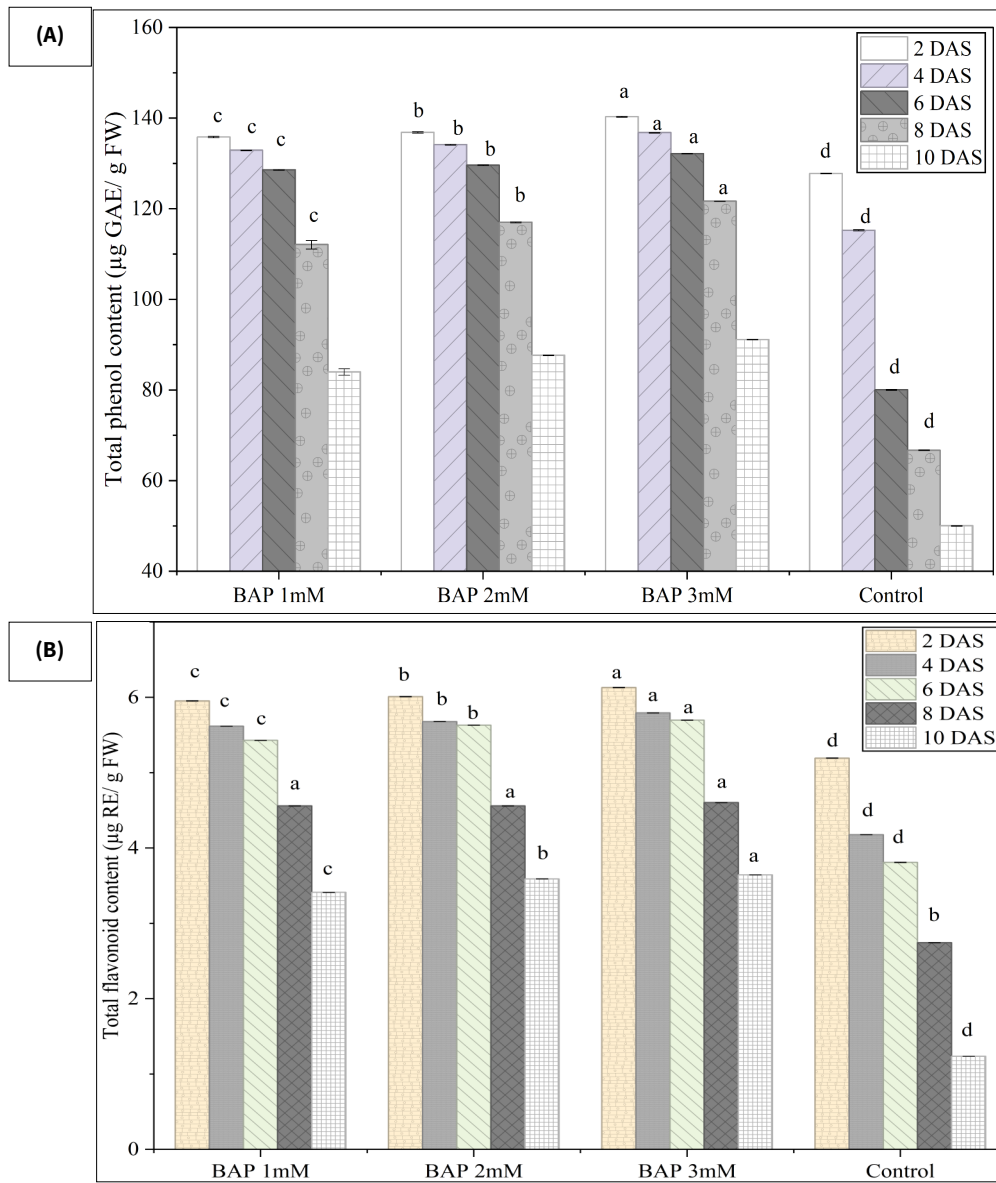
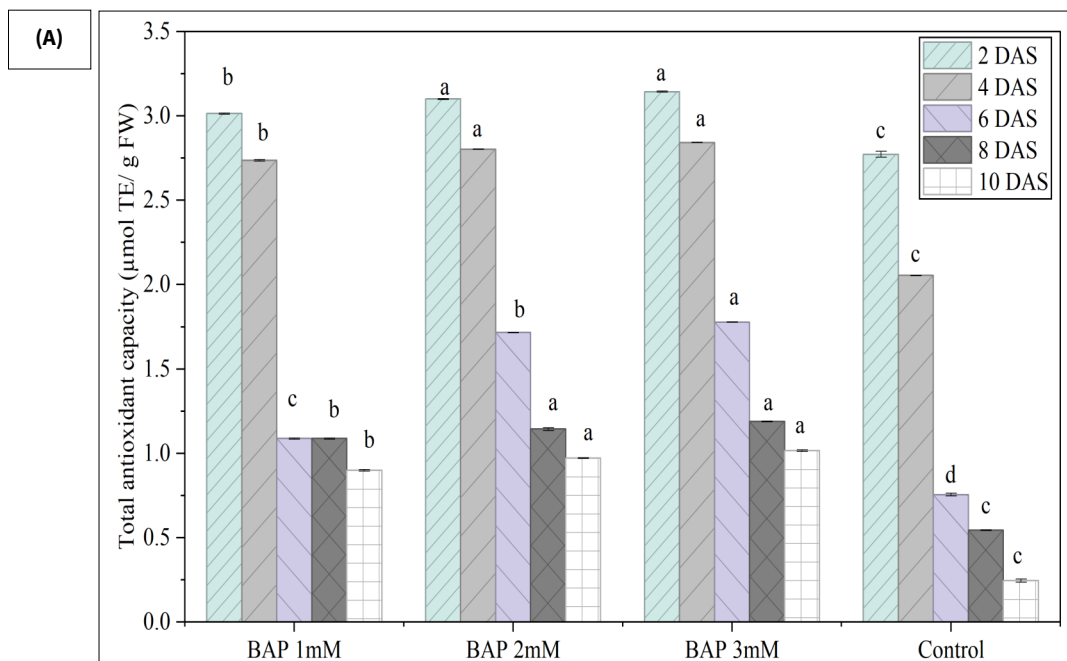


Fig. 1. Effect of postharvest 6-benzylaminopurine treatments on **(A)** Total phenol content (µg GAE/ g FW) and **(B)** Total flavonoid content (µg RE/g FW) of ivy gourd fruits during storage at ambient condition. DAS-days after storage. Different letters across similar pattern of bar graph indicates significantly different at $p \leq 0.05$ as per Tukey's HSD post-hoc analysis.



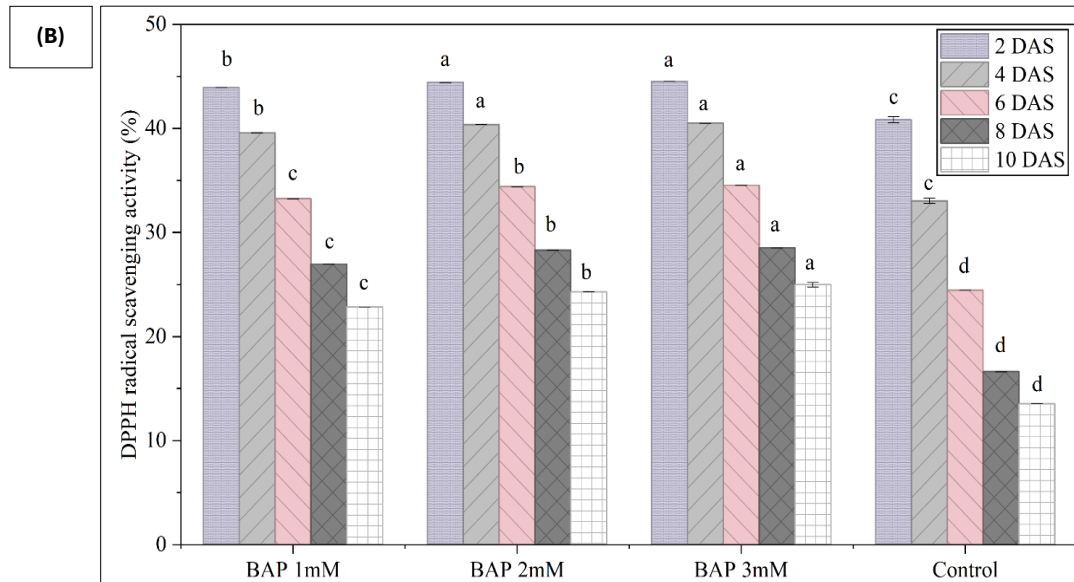


Fig. 2. Effect of postharvest 6-benzylaminopurine treatments on (A) Total antioxidant capacity ($\mu\text{mol TE/g FW}$) and (B) DPPH radical scavenging activity (%) of ivy gourd fruits during storage at ambient condition. DAS- days after storage. Different letters across similar pattern of bar graph indicates significantly different at $p \leq 0.05$ as per Tukey's HSD post-hoc analysis.

Table 6. Effect of postharvest 6-benzylaminopurine treatments on total protein content ($\mu\text{g/g FW}$) of ivy gourd fruits during storage at ambient condition.

Treatments	Total protein content ($\mu\text{g/g FW}$)				
	Days after storage (DAS)				
	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS
BAP 1mM	14.1 \pm 0.24b	12.55 \pm 0.21b	11.17 \pm 0.19b	9.94 \pm 0.17b	8.85 \pm 0.15b
BAP 2mM	15.82 \pm 0.53a	14.08 \pm 0.47a	12.53 \pm 0.42a	11.15 \pm 0.37a	9.92 \pm 0.33a
BAP 3mM	16.74 \pm 0.29a	14.89 \pm 0.26a	13.26 \pm 0.23a	11.8 \pm 0.2a	10.5 \pm 0.18a
Control	12.01 \pm 0.2c	10.69 \pm 0.18c	9.51 \pm 0.16c	5.8 \pm 0.1c	3.54 \pm 0.06c
Initial value (0 DAS): 17.19 \pm 0.31 ($\mu\text{g/g FW}$)					

Values are mean \pm standard error of four replicate determinations ($n=4$). Treatment values followed by the same letters are not significantly different ($p \leq 0.05$)

Enhanced CAT activity, which breaks down hydrogen peroxide (H_2O_2), indicates a strengthened ROS defense in BAP-treated fruits. The 3 mM BAP treatment consistently showed the highest catalase activity, peaking at 220.91 EU/mg protein/min at 6 DAS and although it declined to 116.28 EU/mg protein/min by 10 DAS, it remained higher than the control. The 2 mM BAP treatment also showed a notable increase in catalase activity, reaching 193.61 EU/mg protein/min at 6 DAS. In contrast, the control group exhibited significantly lower catalase activity, with a dramatic decline over the storage period, dropping to 19.01 EU/mg protein/min by 10 DAS.

Peroxidase (POD)

POD activity in ivy gourd fruits was significantly influenced by BAP treatments (Fig. 3B), as POD is involved in the oxidation of phenolic compounds, contributing to lignification and enhancing the plant's response to stress. The 3 mM BAP treatment showed the highest peroxidase activity, peaking at 191.97 EU/mg protein/min at 4 DAS and maintaining relatively high levels of activity, with a value of 140.25 EU/mg protein/min at 10 DAS. The 2 mM BAP treatment also maintained high POD activity levels, with a peak of 188.37 EU/mg protein/min at 4 DAS. However, the control group displayed a sharp decline in peroxidase activity, which decreased to 14.69 EU/mg protein/min by 10 DAS.

Polyphenol oxidase (PPO)

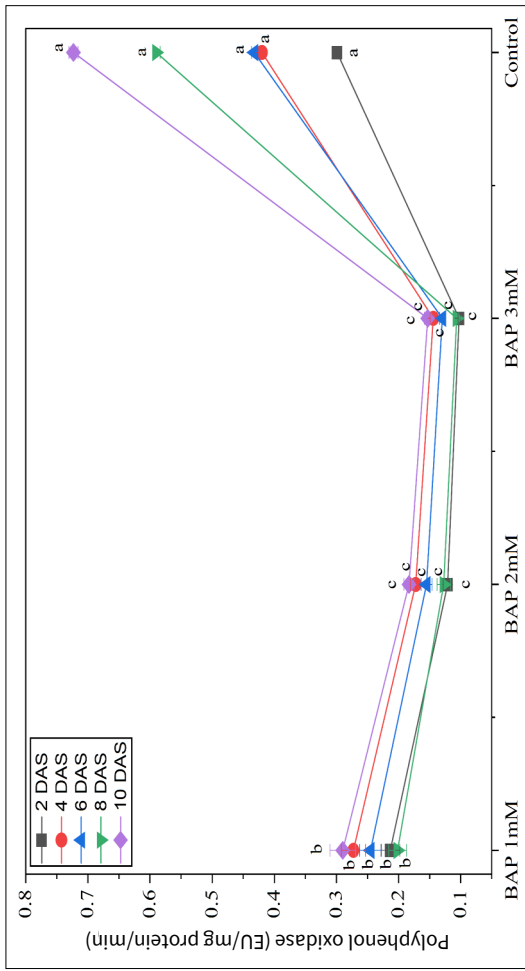
PPO activity was lower in the BAP-treated fruits compared to the control (Fig. 3C). The control group exhibited the highest PPO

activity, increasing from 0.29 EU/mg protein/min at 2 DAS to 0.72 EU/mg protein/min by 10 DAS. In contrast, all BAP treatments maintained significantly lower PPO activity levels, with the 3 mM BAP treatment showing values ranging from 0.1 to 0.15 EU/mg protein/min throughout the storage period.

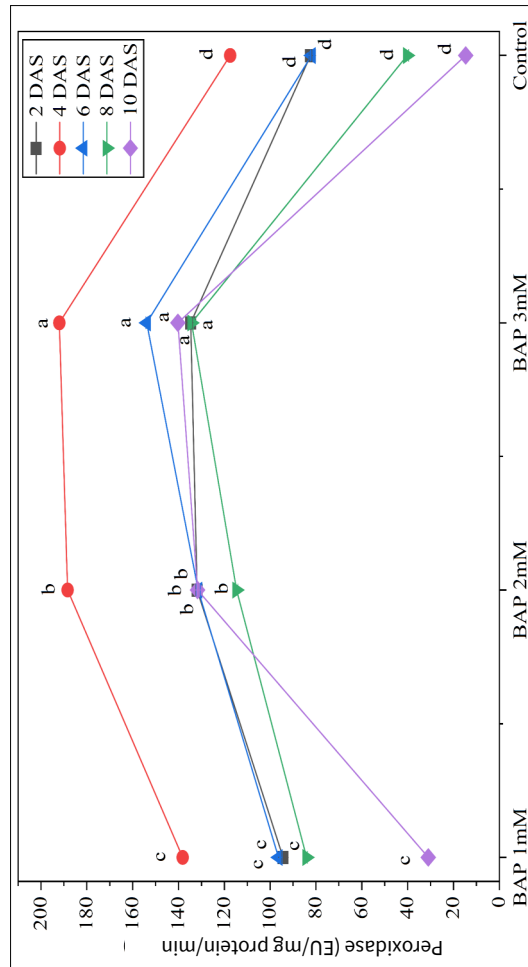
Hierarchical Clustering Dendrogram and Heatmap

Analysis of hierarchical clustering presented in Fig. 4 indicates that, at 2 DAS, a significant divergence was observed between the control and BAP-treated groups. The control group exhibited higher values for weight loss, carotenoid content, MDA and lowest retention of chlorophyll content. In contrast, BAP treatment, particularly at higher concentrations (3 mM), resulted in notable reductions in protein, phenol and ascorbic acid levels. The 3 mM BAP treatment also promoted elevated levels of DPPH radical scavenging activity and CAT, indicating enhanced antioxidant activity. Clustering analysis revealed that the responses of the ivy gourd were strongly influenced by BAP concentration, with distinct groupings for each treatment, suggesting a clear dose-dependent effect.

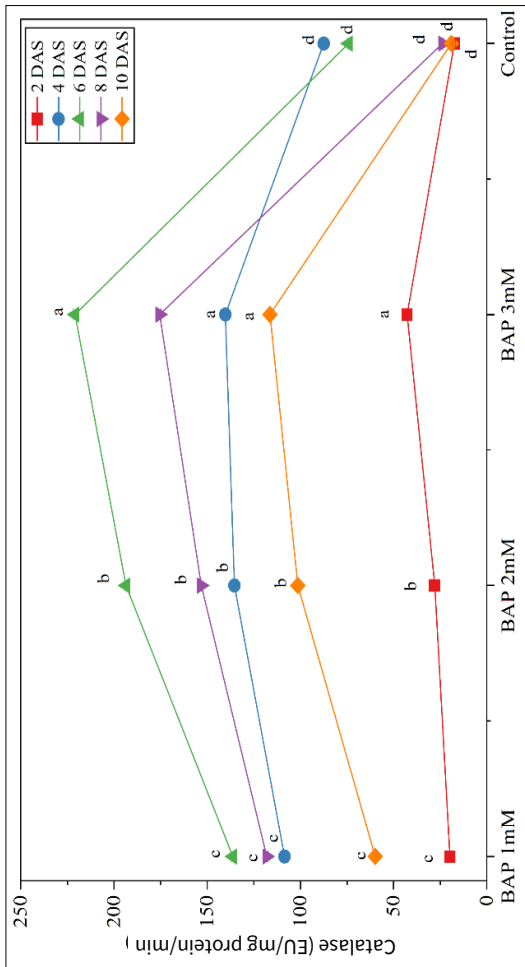
By 10 DAS, the effects of BAP treatments on the fruit had moderated, with fewer pronounced differences between the control and BAP-treated groups (Fig. 5). Although the control still exhibited higher values for weight loss and carotenoid content, the differences were less marked compared to 2 DAS. BAP treatments at 1 mM and 2 mM resulted in milder effects on PPO and chlorophyll content, while flavonoids and antioxidants remained



(A)



(B)



(C)

Fig. 3. Effect of postharvest 6-benzylaminopurine treatments on **(A)** Catalase (EU/mg protein/min), **(B)** Peroxidase (EU/mg protein/min) and **(C)** Polyphenol oxidase (EU/mg protein/min) of Ivy gourd fruits during storage at ambient condition. DAS- days after storage. Different letters across similar pattern of bar graph indicates significantly different at $p \leq 0.05$ as per Tukey's HSD post-hoc analysis.

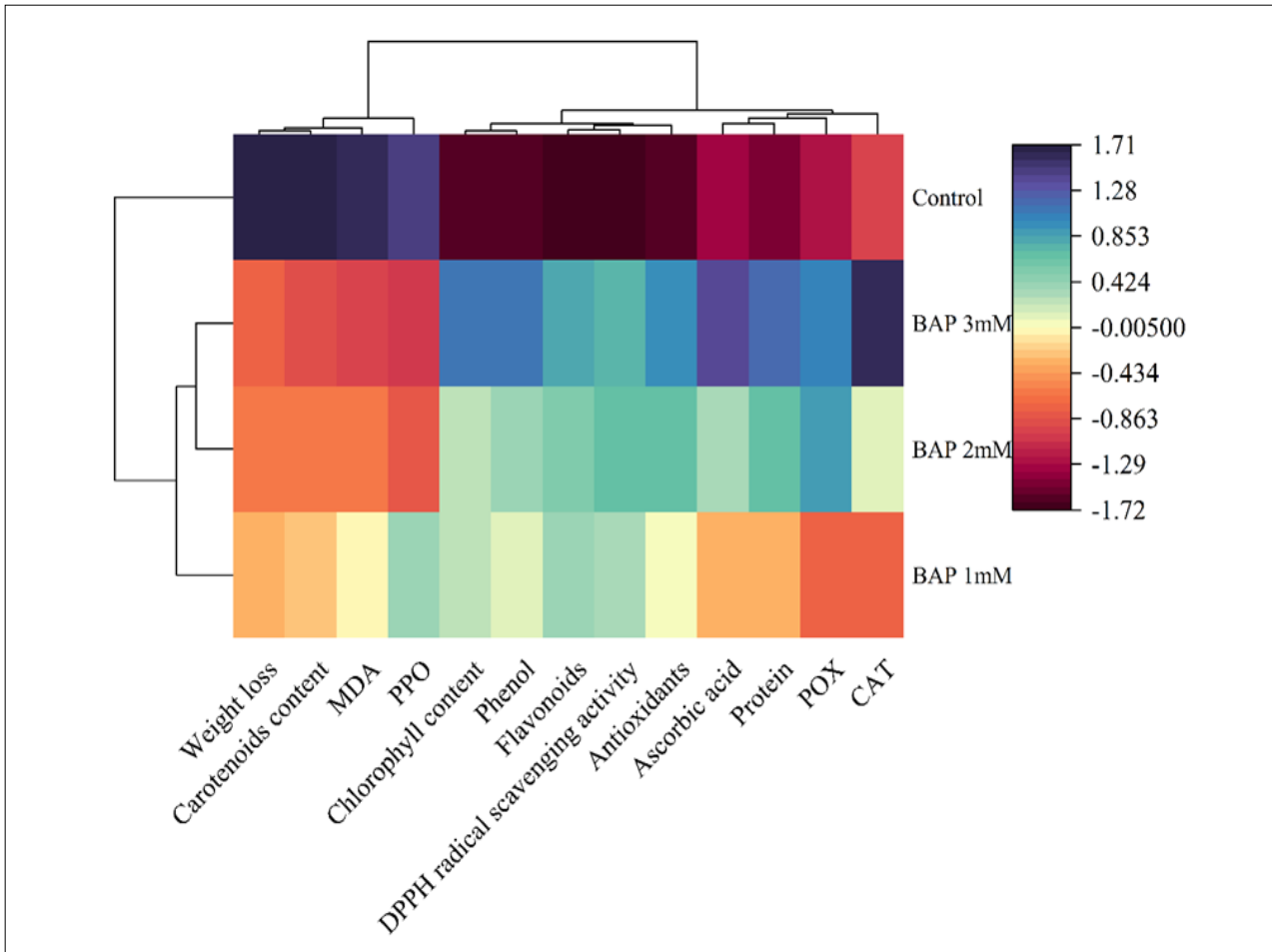


Fig. 4. Hierarchical clustering heatmap depicting the relationships among BAP treatments and standardized physiological and enzymatic characteristics of ivy gourd at 2 DAS.

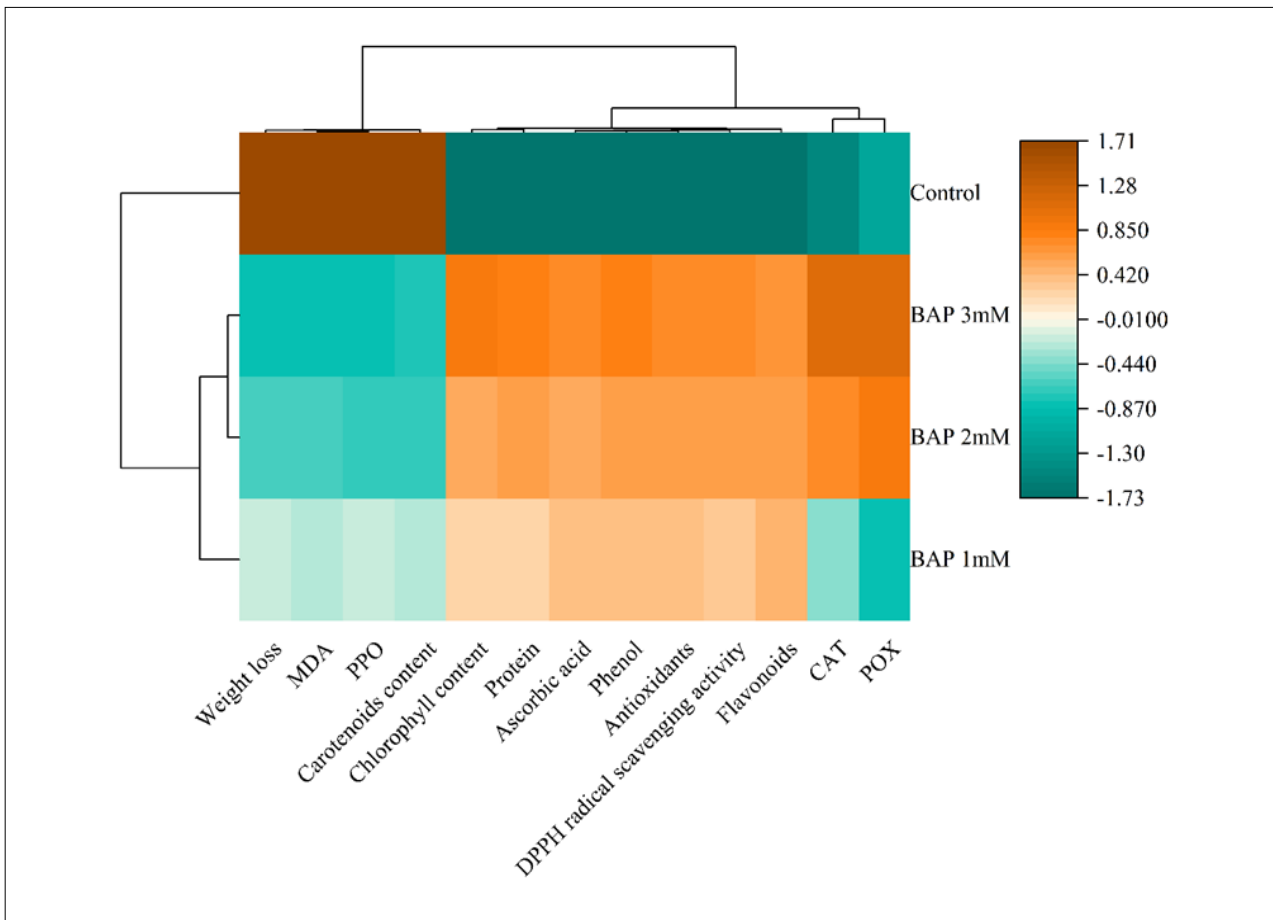


Fig. 5. Hierarchical clustering heatmap depicting the relationships among BAP treatments and standardized physiological and enzymatic characteristics of ivy gourd at 10 DAS.

relatively stable, with less variability than observed earlier. Hierarchical clustering indicated a shift towards a more uniform response across treatments over time, suggesting that the plants had either adapted to the BAP treatment or experienced a reduction in sensitivity as they matured.

Discussion

BAP-treated fruits effectively reduce weight loss in ivy gourd throughout the storage period (Fig. 6). This may be due to delayed senescence and preserving chloroplast function, thereby slowing metabolic processes that contribute to weight reduction (28). It also enhances antioxidant enzyme activity, reducing oxidative damage and maintaining cellular integrity (29). Similar results are also reported on BAP-treated crops, such as pak choi (30), Chinese flowering cabbage (31) and pointed gourd (32), demonstrated comparable minimal weight loss during storage.

A reduction in total chlorophyll was observed in BAP-treated fruits during storage. BAP plays a key role in delaying aging by slowing chlorophyll degradation, which is typically triggered by aging and environmental stress. It enhances photosynthesis by promoting mesophyll cell growth, increasing leaf size and activating chlorophyll-related genes like non-yellow colouring 1 (NYC1) and NYC-1 like (NOL), thus maintaining chlorophyll levels and improving overall photosynthetic efficiency during storage (33, 34). Furthermore, BAP likely mitigated oxidative stress by enhancing antioxidant activity, which helped the preserve chlorophyll and plant tissues post-harvest. Similar results of BAP on chlorophyll content are also reported on pak choi (30), Chinese flowering cabbage (31, 35) and pointed gourd (32), where decrease in chlorophyll breakdown was observed during storage.

A slight increase in total carotenoid content was observed in BAP-treated fruits, whereas a sharp rise was noted in untreated fruits during storage. BAP regulates reactive oxygen species (ROS) and enhances antioxidant enzyme activity, mitigating oxidative stress and stabilizing carotenoids, particularly under storage conditions where ROS production typically increases. By boosting enzymes like SOD, CAT and POD, BAP reduces carotenoid

degradation. Additionally, BAP's effect on photosynthetic efficiency, gene expression and metabolic processes further contributes to reduced carotenoid loss (36, 37). However, studies on crops such as pointed gourd (32), Chinese flowering cabbage (31) and carrot (38) showed a decrease in carotenoid content following BAP treatment.

Malondialdehyde, a marker of lipid peroxidation and oxidative stress, accumulates in plant tissues during post-harvest storage, leading to cellular damage and reduced quality (39). The application of BAP treatment demonstrated varying degrees of effectiveness in reducing MDA content in ivy gourd by enhancing the plant's antioxidant defence mechanisms. BAP mitigates this by enhancing antioxidant enzyme activities, such as catalase and ascorbate peroxidase, which reduce ROS levels and stabilize membrane integrity (31). Additionally, BAP influences stress-responsive gene expression, further lowering MDA accumulation and improving post-harvest resilience (39). BAP treatment effectively lowers MDA levels in Chinese flowering cabbage (31) and pointed gourd (32).

During storage, treated fruits showed lesser decline in ascorbic acid compared to untreated control. BAP preserved ascorbic acid levels in plants by augmenting the activities of antioxidant enzymes such as SOD and POD, which alleviate oxidative stress and prevent the degradation of ascorbic acid during storage. By delaying senescence and stabilizing cell membranes, BAP aids in preserving ascorbic acid and other nutrients, thereby maintaining the nutritional quality of ivy gourd (15). The application of BAP has been shown to sustain higher ascorbic acid levels in various crops, including pointed gourd (32), Chinese flowering cabbage (31) and cauliflower (40).

The total phenolic content in BAP-treated fruits exhibited a significant decline throughout the storage period. BAP enhances the total phenolic content in ivy gourd by activating pathways involved in phenolic biosynthesis. It stimulates phenylalanine ammonia-lyase (PAL), a crucial enzyme in the phenylpropanoid pathway, which is essential for the synthesis of phenolic compounds. Furthermore, BAP influences the plant's antioxidant defence mechanisms, aiding in the mitigation of oxidative stress

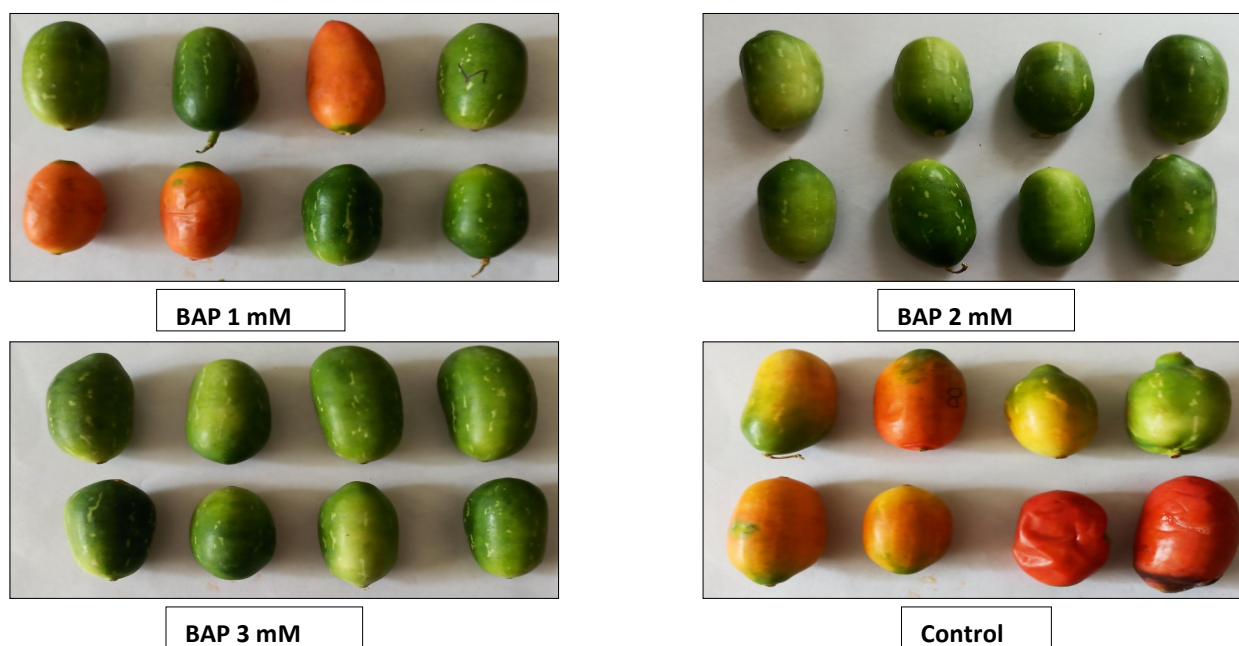


Fig. 6. Effect of 6-benzylaminopurine treatments on ivy gourd fruit after 10 days of storage.

and stabilization of phenolic compounds during storage. These effects, in conjunction with delaying the senescence process, contribute to maintaining elevated phenolic levels, thereby enhancing post-harvest quality (41). The application of BAP has also been observed to sustain high phenolic content in various crops, including pointed gourd (32), cauliflower (40) and broccoli florets (42).

The total flavonoid content in BAP-treated fruits decreased as the storage period advanced; however, this reduction was significantly less pronounced compared to untreated fruits. The application of BAP may affect flavonoid concentrations in ivy gourd by activating cellular mechanisms that enhance the production of secondary metabolites, although specific studies on ivy gourd are limited. Cytokinins, such as BAP, activate enzymes within the phenylpropanoid pathway, including PAL, which is essential for flavonoid biosynthesis (43). By promoting cell growth and division, BAP may indirectly increase flavonoid production during post-harvest storage, although its effects can vary depending on growth conditions and the presence of other growth regulators (44-46). Similar findings have been reported in pointed gourd (32).

During storage, fruits treated with BAP demonstrated a reduction in total antioxidant capacity; however, this decline was notably less pronounced compared to untreated fruits. BAP facilitates the synthesis of key antioxidant enzymes which are essential for neutralizing ROS and mitigating oxidative stress during post-harvest storage. Furthermore, BAP enhances phenolic content in plants, thereby improving radical scavenging by neutralizing free radicals. By bolstering antioxidant defences and preserving cell membrane integrity, BAP significantly enhances the radical scavenging activity of ivy gourd during storage (47). BAP treatment has been shown to augment radical scavenging activity in various crops, including pointed gourd (32), Chinese flowering cabbage (31), cauliflower (40) and broccoli florets (42).

The total protein content in BAP-treated fruits exhibited a decrease with prolonged storage time; however, this decline was less significant compared to untreated fruits. BAP influences several physiological processes, including protein biosynthesis, primarily by modulating antioxidation pathways. By alleviating oxidative stress, BAP aids in maintaining cellular integrity and prevents protein degradation (48). BAP also regulates enzymatic activities, augmenting the activity of antioxidant enzymes such as superoxide dismutase, catalase and ascorbate peroxidase, which protect proteins from oxidative damage and preserve their stability during storage (47). Additionally, BAP suppresses protease activity, reducing protein degradation and ensuring the preservation of proteins synthesized during the growth phase. Similar observations have been reported previously in Chinese flowering cabbage (31) and broccoli (40).

BAP plays a pivotal role in modulating the activities of antioxidant enzymes. The activity of CAT showed a significant increase in all fruits treated with BAP compared to the control group, peaking at 6 DAS before gradually declining. Among the various treatments, the application of 3 mM BAP resulted in the highest CAT activity throughout the storage period. BAP treatment enhances the activity of CAT, a crucial enzyme that aids in neutralizing H₂O₂. This reduction in ROS mitigates oxidative damage, thereby preserving plant tissue quality and extending

shelf life (36). Increased catalase activity has been observed in various crops, including cucumber (49), green asparagus (47), broccoli florets (42) and Chinese flowering cabbage (31), demonstrating the broad applicability of BAP in enhancing antioxidant defense mechanisms. In fruits treated with BAP, peroxidase activity exhibited a significant increase during the initial days of storage, followed by a gradual decline. In contrast, the control group demonstrated considerably lower activity, which decreased rapidly. BAP also enhances the activity of POD, another important antioxidant enzyme that helps neutralize ROS, particularly under stress conditions such as high temperature. Studies have shown that BAP-treated plants, including tomatoes, exhibit increased peroxidase activity, suggesting BAP's role in enhancing a plant's ability to scavenge ROS (15). This boost in POD activity further supports plant defense systems against oxidative damage. Throughout the storage period, the activity of PPO was consistently lower in fruits treated with BAP compared to the control group. BAP's influence extends to PPO, an enzyme responsible for enzymatic browning, as it reduces the availability of its substrates. BAP enhances antioxidant activities, including SOD and peroxidases, which decrease ROS levels. Lower ROS levels result in the preservation of polyphenolic compounds, reducing the substrates available for PPO and limiting browning during post-harvest storage (15). These effects underscore the crucial role of BAP in enhancing antioxidant enzyme activities, thereby improving plant quality and shelf life during storage.

Conclusion

The present study demonstrates that BAP treatments significantly enhance postharvest quality on ivy gourd by reducing weight loss, preserving chlorophyll and carotenoid content and sustaining antioxidant capacity during storage. Among the treatments, 3 mM BAP, was most effective in slowing oxidative degradation, enhanced enzymatic activities of CAT and POD and suppressing PPO—the key enzyme associated with enzymatic browning. These combined effects contributed to improved fruit quality and a practical strategy to extend the shelf life during postharvest handling and marketing of ivy gourd. Future research should focus on optimizing BAP concentrations and evaluating its interactions with other postharvest treatments such as modified atmosphere packaging or edible coatings. Additionally, exploring synergistic combinations with other growth regulators or storage techniques may further enhance storability and nutritional quality across a wider range of fruits and vegetables. Safety and regulatory considerations should also be addressed to ensure practical adoption.

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Authors' contributions

AKP conceptualized and overseen the programme. NY carried out the experimentation. AKS, KB and BKD provided the scientific guidance. NY and NAB made the data analysis. NY, NAB

and DRS prepared the manuscript. AKP, AKS, KB and BKD carried out critical revision and data interpretation.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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