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Research Article
Effects of Cytokinin on Physiological and Biochemical Indicators of Some Tomato Varieties (*Solanum lycopersicum* L.) Cultivated in Vietnam

[1]Le Van Trong, [2]Bui Bao Thinh, [3]Nguyen Hung, [1]Le Thi Huyen and [1]Ha Thi Phuong

[1]Faculty of Natural Sciences, Hong Duc University, Thanh Hoa, Vietnam
[2]School of Natural Sciences, Far Eastern Federal University, Vladivostok, Russia
[3]Vietnam-Hungary Industrial University, Hanoi, Vietnam

Abstract

Background and Objective: Tomato (*Solanum lycopersicum* L.) is widely cultivated and consumed in many countries around the world, including Vietnam. Cytokinin (CK) is a plant hormone that plays a crucial role in numerous plant growth and developmental processes. A study was carried out to analyze the impacts of 6-benzyl adenine (6-BA, a type of CK) on some physiological and biochemical parameters of tomato varieties at the 5-leaf stage and the flowering stage cultivated in Thanh Hoa province, Vietnam. **Materials and Methods:** The experiments consisted of two factors (6-BA concentration and tomato variety) arranged in a Split Plot design with three replications, in which the concentration of 6-BA (0 (control), 6 and 12 mg L^{-1}) was the whole-plot factor and the tomato variety ($P_{375,}$ CS_1, P_n and F) was the split-plot factor. **Results:** The results showed that indicators including plant height, dry matter content, chlorophyll a content, chlorophyll b content, total chlorophyll content, carotenoid content, vitamin C content, total organic acid content, catalase enzyme activity and peroxidase enzyme activity of tomato varieties treated with 6-BA at a concentration of 6 and 12 mg L^{-1} were higher than those of the control group. The study also showed that impacts of 6-BA on tomato physiological and biochemical parameters depend not only on the concentration of 6-BA but also on the developmental stages and plant varieties. **Conclusion:** In general, CK has markedly influenced the physiological and biochemical parameters of tomato varieties. These results provide some insights into the role of CK in tomato growth and development.

Key words: Tomato, *Solanum lycopersicum*, 6-benzyl adenine, cytokinin, physiology, biochemistry, growth

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Corresponding Author: Le Van Trong, Faculty of Natural Sciences, Hong Duc University, Thanh Hoa, Vietnam

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a family Solanaceae species, is native to South America. This species is widely cultivated and consumed all over the world[1]. According to FAOSTAT[2], tomato is cultivated in 176 countries. It is a vegetable with high economic value and an essential export item for many countries in both fresh and processed product forms. The US ranks first in terms of tomato consumption, followed by European countries. Tomatoes are high in nutrition and a good source of lycopene, beta-carotene, phenols, folate, potassium, vitamin C, flavonoids and vitamin E, which are essential for the human body[3,4]. It is of high medicinal value thanks to its sweet taste, cooling action, antibacterial and detoxification properties. Tomatoes can reduce the risk of cardiovascular disease, prevent the formation of cancer-causing free radicals, especially prostate cancer[3,5].

Cytokinin (CK) is an important plant hormone that stimulates strong cell division and has a pronounced effect on the plant organ formation and morphogenesis, mainly shoot morphogenesis[6-8]. Studies have demonstrated that CK has a direct inducing effect on shoot formation in plants, increasing the number of new shoots compared to untreated plants. Therefore, CK is applied as a stimulant to the plants cultivated mainly for leaves such as tea, tobacco[9,10] and plants with flower buds such as carnation and chrysanthemum poinsettia, petunia, fuchsia and lily[11,12]. Currently, for many types of seedlings, after successful germination process in pots, foliar spray with CK at suitable concentrations is applied to effectively induce branching and shoot formation[7,13]. Treatments with CK during the period from fruit set to harvesting stage also bring about the dramatic increase in fruit tree yield[14]. This plant hormone also affects metabolic processes such as the synthesis of nucleic acids, proteins and chlorophyll, thus regulating plants' physiological processes[6,15-18]. It regulates plants' senescence and the retardation of plant senescence by increasing the content of chlorophyll[15,16]. High CK content makes the leaves stay green longer; thus, more nutrients are transported and transduced to cellular targets for leaves growth[16].

In Vietnam, numerous imported tomato varieties with high quality and productivity are widely cultivated in many provinces. Currently, thanks to its dominant economic value compared to other produce, tomato production and cultivation area tend to increase. It has encouraged farmers and gardeners to make a substantial investment in this plant as well as in the application of growth stimulants for yield improvement[19]. In other words, the application of growth stimulants in general and CK, in particular, is increasingly popular in Vietnam. In this study, the impacts of CK at different concentrations on different tomato varieties during plant developmental stages are analyzed to indicate how this hormone regulates the physiological and biochemical parameters of plants, thus examining the effects of CK on the growth and development of tomatoes.

MATERIALS AND METHODS

Plant material and experimental design: In this research, four tomato varieties ($P_{375,}$ CS_1, P_n and F) were provided by Thanh Hoa Seed Center (Vietnam) and 6-benzyl adenine (6-BA, a type of CK) was collected from Nhat Tan Chemical Company (Vietnam).

The experiments were conducted in a net house at the Faculty of Agriculture-Forestry-Fishery, Hong Duc University (Thanh Hoa, Vietnam) from February-May 2018. 6-BA was applied on four tomato varieties with three concentrations: 0 (control), 6 and 12 mg L^{-1}. The experiments were arranged in a Split Plot design with two factors: the concentration of 6-BA was the whole-plot factor and the tomato variety was the split-plot factor. The experiments were repeated three times, with a total of 36 plots. Each plot area was 10 m^2 (2.5×4 m) and the total area was 360 m^2.

Treatments and agronomic practices: When seedlings reached 20 cm, they were transplanted into the plots with 50% of their stems deep underground because the root development would soon improve, making the tomato plants much more robust and more resistant. Nitrogen, phosphorus, potassium fertilizers (2: 1.5: 2) were applied at developmental stages. Bed preparation consisted of total organic substrate amount, total phosphorus amount, 1/3 of nitrogen amount and 1/3 of potassium amount. The rest of nitrogen and potassium was later used in supplementary fertilizations.

The supplementary application of 6-BA was carried out based on different treatments to ensure the concentration and dosage of 5 mL per plant. Foliar spray with 6-BA was conducted in the morning (from 5:30-6:30 am) twice a week on a periodical basis.

For the first ten days after transplanting, each plant was watered regularly every day with about 500 mL of warm water (at 25-30°C) in the afternoon (from 4:00-5:00 pm), for leaves to dry before the sunset. Plants were carefully cared for and

monitored for timely and scientific prevention and detection of pests and diseases.

Sampling method: Sample collection was conducted using a mixed sampling method. Across the experimental area, samples were collected at many points, on many plants of normal growth and development, without pests or diseases, of the same senescence and care conditions. The sample collection was carried out in the morning, at the 5-leaf and flowering stages for each experimental formula. Then samples were transferred to the laboratory for analysis.

Plant height: Plant heights were measured at the 5-leaf and flowering stages by a measuring instrument with an accuracy of 0.1 mm. At the stage of 5-leaf, plant heights were measured from the root collar to the tip of terminal buds. Those positions were then marked using paint. At the flowering stage, the plant heights were measured from the marked position to the tip of terminal buds.

Dry matter content: With treatments for each variety, three plants were collected randomly and placed into plastic bags to avoid dehydration. They were then brought to the laboratory where their initial fresh weights (B) were measured. Next, weighed plants were put into a drying oven at the temperature of 105°C until they reached unchangeable weights, which were the dry plant weights after drying (b). Dry matter content was determined using the formula:

$$X = \frac{b}{B} \times 100$$

where, X is dry matter content (%), B is initial fresh weight of plants (g) and b is dry plant weight after drying (g).

Leaf pigment content: Fresh tomato leaf samples were frozen in liquid nitrogen and then ground into powder. Total 5 mg of the powder and 100 µL of distilled water were put into a test tube, let still for 10 min. Total 8 mL of acetone, 80% was added for chlorophyll extraction. Centrifugation was conducted to extract the filtrate (10 mL) and the optical density was measured at corresponding wavelengths. The pigment content was determined using the formula[4,8]:

$$C_a\ (mg\ L^{-1}) = 10.3 \times E_{663} - 0.918 \times E_{644}$$

$$C_b\ (mg\ L^{-1}) = 19.7 \times E_{644} - 3.87 \times E_{663}$$

$$C_{(a+b)}\ (mg\ L^{-1}) = 6.4 \times E_{663} + 18.8 \times E_{644}$$

$$C_{carotenoid}\ (mg\ L^{-1}) = 4.695 \times E_{440.5} - 0.268 \times C_{(a+b)}$$

After that, pigment content contained in 1 g of fresh leaves was determined using the formula:

$$A = \frac{C \times V}{P \times 1000}$$

where, E_{663}, E_{644} and $E_{440.5}$ are extinction coefficients of chlorophyll for wavelengths of 663, 644 and 440.5 nm, C_a, C_b, C_{a+b} are chlorophyll a, b and total chlorophyll content, A is pigment content expressed in mg g^{-1} Fresh Weight (FW), C is pigment content in the extracted filtrate (mg L^{-1}), V is volume of extracted filtrate (10 mL) and P is sample weight (g).

The total organic acid content: About 5 g of fresh tomato leaves were finely ground using a porcelain mortar and then transferred to a 50 mL volumetric flask. Distilled water was added to the scale. Mixed well. A 10 mL of the extracted filtrate was transferred into a 100 mL conical flask; a few drops of reagent phenolphthalein were added to the solution. Titration of the solution with 0.1 N NaOH solution was carried out until a persistent pink appeared. Total organic acid content was determined using the formula[4]:

$$X = \frac{a \times V_1 \times 100}{V_2 \times P}$$

where, X is total organic acid content in extracted filtrate (mg/100 g FW), P is sample weight (g), V_1 is total volume of extracted filtrate (mL), V_2 is volume of the analyte (mL), a: Volume of titrant 0.1 N NaOH (mL).

Vitamin C content: Vitamin C content was determined using the titration method. A 5 g of fresh tomato leaves were finely ground using porcelain mortar together with 5 mL of HCl, 5%, then transferred to a volumetric flask. Distilled water was added to the scale of 50 mL and mixed well. Then 20 mL of the extracted filtrate was transferred into a 100 mL conical flask. Titration of the solution with I_2 liquid (starch was used as a color indicator) was carried out until the blue color appeared. Vitamin C content was determined using the formula[4]:

$$X = \frac{V \times V_1 \times 0.00088 \times 100}{V_2 \times b}$$

where, X is vitamin C content (mg/100 g FW), V is volume of diluted sample solution (mL), V_1 is volume of titrant I_2 0.01 N

(mL), V_2 is volume of analyte solution (mL), V_2 is weight of analyte material (g), 0.00088 is weight of Vitamin C amount (g) equimolar to 1 mL of I_2 0.01 N.

Peroxidase enzyme activity: A 5 g of fresh tomato leaves were finely ground using a porcelain mortar and a veronal buffer was gradually added to the volume of 10 mL. Centrifugation was carried out using a refrigerated centrifuge at the speed of 20000 rpm for 20 min. The extracted filtrate was used to determine enzyme activity. About 0.1 mL of 0.2 M pyrogallol solution; 1 mL of veronal buffer; 1.2 mL of water; 0.5 mL of H_2O_2 solution and 0.2 mL of extracted enzyme filtrate was added into a test tube. The reaction mixture was incubated at 30°C for 10 min and then stopped by the addition of 1 mL of H_2SO_4, 5%. The optical density (E) was measured using the absorbance of purpurogallin from the calibration curve at 430 nm wavelength. Peroxidase enzyme activity was determined using the formula[4]:

$$A = \frac{E \times (a \times b)}{p \times d \times t}$$

where, A is peroxidase enzyme activity (UI/g/sec); E is measured optical density, a is total volume of enzyme extract (mL), b is degree of dilution of the extract, p is weight of plant sample (g) is d is tube thickness (cm), t is Time (ses).

Catalase enzyme activity: Total 5 g of fresh tomato leaves were finely ground using a porcelain mortar together with glass powder and 0.3 g of $CaCO_3$. Added 20 mL of distilled water, carefully ground into a homogenous solution. The solution was then transferred into a 50 mL volumetric flask. Distilled water was added to the scale. The solution was mixed well. After 30 min, centrifugation was conducted. Two 100 mL conical flasks were used. About 10 mL of filtrate was put into each flask. The control flask was heated for boiling for 3 min (to inactivate the enzyme), then cooled. Total 20 mL of distilled water and 3 mL of H_2O_2, 1% solution were added to each flask. Let the flasks stay at 25°C for 30 min. Then 5 mL of H_2SO_4, 10% solution was added. The solutions were titrated with 0.1 N $KMnO_4$ solutions until a light pink color appeared for 1 min. Catalase enzyme activity was determined using the formula[4]:

$$X = \frac{(V_1 - V_2) \times 1.7 \times V_x}{V_c \times 30 \times 0.034 \times a}$$

Where, X is catalase enzyme activity (mM H_2O_2 g^{-1} min^{-1}), V_1 is volume of 0.1 N $KMnO_4$ used in the titration of H_2O_2 in the control flask (mL), V_2 is Volume of 0.1 N $KMnO_4$ used in the titration of H_2O_2 in the experimental flask (mL), V_x is total volume of enzyme extract (mL), V_c is volume of extracted filtrate analyte (mL), a is weight of ground sample, 1.7 is conversion factor for the number of mL of 0.1 N $KMnO_4$ titrant to weight (mg) of titrated H_2O_2, 30 is enzyme activity time (min), 0.034 is conversion factor for mg to micromol H_2O_2.

Statistical analysis: Statistical data analysis was performed using IRRISTAT software (version 5.0) for Analysis of Variance (ANOVA). All experiments were conducted three times independently. The results are expressed as mean values and Standard Deviation (SD). Different letters in tables represent significant differences for p-value$\leq$0.05.

RESULTS AND DISCUSSION

Impacts of 6-BA on plant height and dry matter content: Plant height is an important indicator as it relates to the yield and stable development of tomato plants[19]. It is also a crucial criterion for assessing the effects of growth regulators on plants[19]. The impacts of 6-BA on plant height of tomato varieties at the 5-leaf and the flowering stages were illustrated in Table 1. Regarding plant height, 6-BA exerted the most significant effects on P_{375} variety. At the 5-leaf stage, 6-BA at a concentration of 6 mg L^{-1} induced the plant height from 8.52-8.67 cm (1.76% higher than the control). With 6-BA at a concentration of 12 mg L^{-1}, there was an increase in plant height to 8.94 cm (4.93% higher than the control). At the flowering stage, the impacts of 6-BA on plant height were even more profound. At a concentration of 6 mg L^{-1}, 6-BA promoted the plant height from 34.25-35.18 cm (2.72% higher than the control), while 6-BA at a concentration of 12 mg L^{-1} brought about a rise in plant height to 37.05 cm (8.18% higher than the control). The effects of 6-BA on plant heights of CS_1, P_n and F varieties were more noticeable at the 5-leaf stage than those at the flowering stage (Table 1). It can be interpreted that 6-BA does impact the plant height of different tomato varieties and the impacts vary considerably among different varieties. Also, different treatment concentrations might exert impacts on different levels. Treatments with a concentration of 12 mg L^{-1} promoted plant height increase better than those of 6 mg L^{-1}. The impacts of 6-BA on plant height of tomato varieties can be arranged in descending order of P_{375}>P_n>F>CS_1. These results agree with those of other research, proving that 6-BA does promote plant height[12,20].

Dry matter content is an indicator that directly links to crop yield[21]. High yield first requires high biological

Table 1: Impacts of cytokinin on plant height and dry matter content

Variety	Stage	Concentration (mg L^{-1})	Plant height (cm)	Compared to the control (%)	Dry matter (%)	Compared to the control (%)
P_{375}	5-leaf	Control	8.52±0.257^{b}	100.00	10.93±0.762^{c}	100.00
		6	8.67±0.712^{b}	101.76	11.88±0.587^{b}	108.69
		12	8.94±0.423^{a}	104.93	12.58±0.430^{a}	115.10
	Flowering	Control	34.25±0.726^{c}	100.00	13.85±0.424^{c}	100.00
		6	35.18±0.469^{b}	102.72	14.90±0.505^{b}	107.58
		12	37.05±0.651^{a}	108.18	15.42±0.182^{a}	111.33
CS_1	5-leaf	Control	6.81±0.462^{c}	100.00	12.97±0.426^{b}	100.00
		6	6.92±0.427^{b}	101.62	13.58±0.323^{b}	104.70
		12	7.09±0.753^{a}	104.11	19.22±0.353^{a}	118.89
	Flowering	Control	31.82±0.468^{b}	100.00	15.42±0.386^{c}	100.00
		6	31.93±0.492^{b}	100.35	17.01±0.603^{b}	106.31
		12	32.57±0.751^{a}	102.36	19.22±0.323^{a}	120.12
P_n	5-leaf	Control	7.25±0.497^{c}	100.00	9.38±0.105^{b}	100.00
		6	7.54±0.463^{b}	104.00	10.55±0.431^{b}	112.47
		12	7.83±0.824^{a}	108.00	12.25±0.387^{a}	130.60
	Flowering	Control	33.21±0.491^{b}	100.00	13.38±0.228^{b}	100.00
		6	33.27±0.153^{b}	100.18	13.42±0.344^{b}	100.30
		12	33.85±0.825^{a}	101.93	14.99±0.373^{a}	112.03
F	5-leaf	Control	6.58±0.457^{b}	100.00	9.31±0.630^{b}	100.00
		6	6.84±0.255^{a}	103.95	10.19±0.423^{b}	109.45
		12	6.95±0.250^{a}	105.62	12.01±0.247^{a}	129.00
	Flowering	Control	29.23±0.459^{b}	100.00	11.54±0.356^{b}	100.00
		6	29.45±0.421^{a}	100.75	12.62±0.523^{b}	109.36
		12	29.58±0.256^{a}	101.20	14.53±0.355^{a}	125.91

The values represent the Mean±SD of three independent measurements, For data of the same variety, at the same growth stage and data column, values with the same letter represent non-significant differences; values with different letters represent a significant difference at the significance level $p \leq 0.05$

productivity (the total amount of dry matter accumulated) and a high economic coefficient[21]. Apart from plant height, analysis of dry matter content of tomato varieties at the 5-leaf and flowering stages was also illustrated in Table 1. 6-BA had more significant impacts on dry matter accumulation than on plant height. At the 5-leaf stage, the most significant impacts of 6-BA on dry matter accumulation were recorded in P_n variety. Dry matter accumulation of P_n variety treated with 6-BA at a concentration of 6 mg L^{-1} increased by 12.47% and P_n variety treated with 6-BA at a concentration of 12 mg L^{-1} increased by 30.60% compared to the control. At the flowering stage, the impacts of 6-BA on dry matter accumulation of tomato plants were less remarkable than those at the 5-leaf stage. The most significant effects were recorded in F variety, with an increase of 9.36% in the treatment of 6 mg L^{-1} concentration and 25.91% in the treatment of 12 mg L^{-1} concentration. The lowest data were collected in P_n variety with an increase of only 0.30% in the treatment of 6 mg L^{-1} concentration and 12.03% in the treatment of 12 mg L^{-1} concentration compared to the control. The reason for more remarkable impacts of 6-BA on dry matter accumulation of tomato plants at the 5-leaf stage than those at the flowering stage is that at the stage of 5-leaf, plants tend to accumulate organic matter, which promotes faster plant growth, as a necessary preparation for the upcoming flowering stage. The impacts of 6-BA on dry matter content of tomato varieties can be arranged in descending order of $F>P_n>CS_1>P_{375}$. Research results by Ghani *et al.*[22] and Gadallah[17] also state that CK supports the accumulation of dry matter content in plants.

Impacts of 6-BA on leaf pigment content: Chlorophyll refers to a group of photosynthetic pigments found in plants. Leaf pigment content acts as a crucial criterion for assessing plant photosynthetic capacity, thus playing an essential role in crop yield[23]. Impacts of 6-BA on chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents are presented in Table 2. 6-BA exerted effects on chlorophyll content at both 5-leaf and flowering stages and at different levels in different varieties. The most significant effect was recorded in a CS_1 variety. At the 5-leaf stage, in treatment with a concentration of 6 mg L^{-1}, the content of chlorophyll a increased from 0.265-0.273 mg g^{-1} FW, that of chlorophyll b rose from 0.222-0.253 mg g^{-1} FW and there was also an increase in total chlorophyll content from 0.499-0.545 mg g^{-1} FW. At the same time, in treatment with a concentration of 12 mg L^{-1}, the total chlorophyll content increased from 0.499-0.531 mg g^{-1} FW. Meanwhile, at the flowering stage, total chlorophyll content increased from 1.230-1.337 mg g^{-1} FW in treatment with a concentration of 6 mg L^{-1} and 1.529 mg g^{-1} FW in treatment

Table 2: Impacts of cytokinin on tomato leaf pigment content

Variety	Stage	Concentration mg L^{-1}	Chlorophyll a (mg g^{-1} FW)	Compared to the control (%)	Chlorophyll b (mg g^{-1} FW)	Compared to the control (%)	Total chlorophyll (mg g^{-1} FW)	Compared to the control (%)	Carotenoid content (mg g^{-1} FW)	Compared to the control (%)
P_{375}	5-leaf	Control	0.262±0.009^{b}	100.00	0.242±0.003^{b}	100.00	0.501±0.002^{b}	100.00	0.072±0.010^{b}	100.00
		6	0.275±0.001^{b}	104.96	0.249±0.003^{b}	102.89	0.512±0.008^{b}	102.20	0.078±0.009^{b}	100.33
		12	0.292±0.008^{a}	111.45	0.262±0.003^{a}	108.26	0.552±0.007^{a}	110.18	0.081±0.003^{a}	112.50
	Flowering	Control	0.513±0.002^{c}	100.00	0.636±0.003^{c}	100.00	1.378±0.003^{c}	100.00	0.093±0.008^{c}	100.00
		6	0.535±0.002^{b}	104.29	0.713±0.004^{b}	112.10	1.397±0.003^{b}	101.38	0.095±0.001^{b}	102.15
		12	0.548±0.009^{a}	106.82	0.752±0.004^{a}	118.24	1.409±0.007^{a}	102.25	0.099±0.007^{a}	106.45
CS_1	5-leaf	Control	0.265±0.003^{c}	100.00	0.222±0.005^{c}	100.00	0.499±0.004^{c}	100.00	0.064±0.003^{c}	100.00
		6	0.273±0.003^{b}	103.01	0.253±0.005^{b}	113.96	0.545±0.006^{a}	109.22	0.066±0.002^{b}	103.13
		12	0.281±0.005^{a}	106.03	0.260±0.007^{a}	117.12	0.531±0.008^{b}	106.41	0.068±0.005^{a}	106.25
	Flowering	Control	0.524±0.005^{b}	100.00	0.706±0.004^{c}	100.00	1.230±0.005^{b}	100.00	0.083±0.004^{c}	100.00
		6	0.542±0.003^{b}	103.43	0.795±0.005^{b}	112.63	1.337±0.005^{b}	108.66	0.086±0.006^{b}	103.61
		12	0.590±0.006^{a}	112.60	0.939±0.003^{a}	133.00	1.529±0.003^{a}	124.70	0.089±0.006^{a}	107.23
P_n	5-leaf	Control	0.253±0.006^{b}	100.00	0.222±0.008^{c}	100.00	0.462±0.001^{b}	100.00	0.063±0.007^{c}	100.00
		6	0.255±0.002^{b}	100.79	0.253±0.004^{b}	113.96	0.505±0.004^{a}	109.31	0.065±0.009^{b}	103.17
		12	0.264±0.004^{a}	104.35	0.257±0.002^{a}	115.77	0.509±0.004^{a}	110.17	0.069±0.006^{a}	109.52
	Flowering	Control	0.545±0.007^{b}	100.00	0.799±0.008^{c}	100.00	1.342±0.004^{c}	100.00	0.084±0.002^{b}	100.00
		6	0.563±0.007^{b}	103.30	0.898±0.004^{b}	112.39	1.365±0.004^{b}	101.71	0.085±0.002^{b}	101.19
		12	0.615±0.003^{a}	112.84	1.108±0.005^{a}	138.67	1.392±0.004^{a}	103.73	0.088±0.005^{a}	104.76
F	5-leaf	Control	0.275±0.005^{b}	100.00	0.266±0.004^{a}	100.00	0.526±0.003^{c}	100.00	0.055±0.002^{c}	100.00
		6	0.280±0.004^{b}	101.82	0.206±0.001^{b}	77.44	0.539±0.005^{b}	102.47	0.062±0.008^{b}	112.73
		12	0.291±0.002^{a}	105.82	0.268±0.004^{a}	100.75	0.553±0.007^{a}	105.13	0.064±0.005^{a}	116.36
	Flowering	Control	0.536±0.004^{b}	100.00	0.869±0.005^{b}	100.00	1.405±0.007^{b}	100.00	0.078±0.003^{c}	100.00
		6	0.538±0.008^{b}	100.37	0.875±0.007^{b}	100.69	1.412±0.003^{b}	100.50	0.080±0.003^{b}	102.56
		12	0.594±0.004^{a}	110.82	0.946±0.004^{a}	108.86	1.492±0.003^{a}	101.71	0.082±0.004^{a}	105.13

The values represent the Mean±SD of three independent measurements, For data of the same variety, at the same growth stage and data column, values with the same letter represent non-significant differences, values with different letters represent a significant difference at the significance level $p \leq 0.05$

with a concentration of 12 mg L^{-1}. It can be shown that under the application of 6-BA at concentrations of 6 and 12 mg L^{-1}, the leaf chlorophyll content increased, especially at the stage of 5-leaf and at a concentration of 6 mg L^{-1}. Besides, the rates varied in different varieties. The impacts of 6-BA on chlorophyll content of tomato varieties can be arranged in descending order of CS_1>P_n>P_{375}>F. In this research, leaf chlorophyll content of tomatoes increased when plants were treated with 6-BA and this result was in agreement with those of previous research[15,17].

Together with chlorophyll, carotenoid is an important photosynthetic pigment. It does not only transfer photon energy to chlorophyll but protects chlorophyll pigments from photo-oxidation in the presence of light and oxygen[24]. Impacts of 6-BA on carotenoid content at the 5-leaf and flowering stages were shown in Table 2. The most significant effects were recorded in F variety. Under the application of 6-BA, the carotenoid content at the stage of 5-leaf increased from 0.055-0.062 mg g^{-1} FW when treated with a concentration of 6 mg L^{-1} and increased to 0.064 mg g^{-1} FW when treated with a concentration of 12 mg L^{-1}. Whereas, at the flowering stage, the carotenoid content witnessed a rise from 0.078-0.080 mg g^{-1} FW when applied with 6-BA at 6 mg L^{-1} and an increase to 0.082 mg g^{-1} FW when applied with 6-BA at 12 mg L^{-1}. It can be seen that carotenoid content increased in treatments with both 6 and 12 mg L^{-1} 6-BA applications and impacts of 6-BA on carotenoid content in these tomato varieties might be arranged in descending order of F>P_{375}>CS_1>P_n.

The results showed that the application of CK at a reasonable concentration promoted carotenoid and synthetic chlorophyll capacities in tomato plants. These results are in agreement with those of previous research, proving the fact that CK might increase leaf chlorophyll content, making leaves stay green longer and at the same time, CK contributes to avoiding leaf senescence[15,16].

Impacts of 6-BA on Vitamin C and total organic acid contents: Despite low content in plants, Vitamin C is predominantly essential for the healthy growth and development of plants, thanks to its contribution to the enhancement of plant resistance against the living environment[25]. The application of 6-BA has some effects on leaf vitamin C content in tomato plants (Table 3). Regarding vitamin C content, 6-BA exerted the most significant effect on F variety. For plants of F variety, at the 5-leaf stage, the application of 6 mg L^{-1} 6-BA helped increase vitamin C content by 2.42% compared to the control and in treatment

Table 3: Impacts of cytokinin on Vitamin C and total organic acid contents

Variety	Stage	Concentration (mg L^{-1})	Vitamin C (mg/100 g FW)	Compared to the control (%)	Total organic acid (mg/100 g FW)	Compared to the control (%)
P_{375}	5-leaf	Control	13.70±0.008^{b}	100.00	75.18±0.415^{b}	100.00
		6	13.70±0.009^{b}	100.00	81.30±0.250^{b}	108.14
		12	13.90±0.008^{a}	101.46	85.42±0.331^{a}	113.62
	Flowering	Control	27.10±0.002^{c}	100.00	50.17±0.384^{b}	100.00
		6	43.21±0.002^{b}	159.45	54.28±0.334^{b}	108.19
		12	59.40±0.002^{a}	219.19	60.62±0.428^{a}	120.83
CS_1	5-leaf	Control	11.80±0.004^{b}	100.00	90.37±0.441^{c}	100.00
		6	11.90±0.003^{a}	100.85	92.49±0.337^{b}	102.35
		12	11.90±0.003^{a}	100.85	95.80±0.405^{a}	106.01
	Flowering	Control	26.30±0.002^{c}	100.00	60.65±0.352^{b}	100.00
		6	40.02±0.007^{b}	152.18	66.12±0.408^{b}	109.19
		12	50.16±0.004^{a}	190.72	75.54±0.375^{a}	124.55
P_n	5-leaf	Control	18.30±0.016^{b}	100.00	110.15±0.537^{b}	100.00
		6	18.50±0.005^{a}	101.09	110.28±0.513^{b}	100.12
		12	18.60±0.001^{a}	101.64	112.72±0.832^{a}	102.33
	Flowering	Control	46.00±0.002^{c}	100.00	60.08±0.425^{c}	100.00
		6	60.56±0.005^{b}	131.65	78.34±0.450^{b}	130.39
		12	70.32±0.021^{a}	152.87	95.47±0.333^{a}	158.91
F	5-leaf	Control	16.50±0.027^{c}	100.00	80.87±0.530^{c}	100.00
		6	16.90±0.021^{b}	102.42	87.36±0.591^{b}	108.03
		12	17.80±0.004^{a}	107.88	99.41±0.252^{a}	122.93
	Flowering	Control	27.50±0.002^{b}	100.00	55.78±0.378^{c}	100.00
		6	60.12±0.004^{a}	218.62	64.45±0.333^{b}	115.54
		12	60.72±0.003^{a}	220.80	70.28±0.337^{a}	125.99

The values represent the Mean±SD of three independent measurements, For data of the same variety, at the same growth stage and data column, values with the same letter represent non-significant differences, values with different letters represent a significant difference at the significance level $p \leq 0.05$

with a concentration of 12 mg L^{-1}, vitamin C content raised by 7.88% compared to the control. At the flowering stage, the impacts of 6-BA were more remarkable, with an increase of 118.62% in treatment with 6 mg L^{-1} concentration and 120.80% in treatment with 12 mg L^{-1} concentration compared to the control. When treated with 6-BA, tomato plants of P_{375} variety also witnessed an increase in leaf vitamin C content. At the 5-leaf stage, while 6-BA at 6 mg L^{-1} made no changes in vitamin C content, the application of 6-BA at 12 mg L^{-1} led to rising by 1.46%. At the flowering stage, 6-BA at 6 mg L^{-1} brought about growth by 59.45% and 6-BA at 12 mg L^{-1} led to rising by 119.19% in vitamin C content compared to the control. The application of 6-BA also exerted some impacts on CS_1 và P_n plants regarding leaf vitamin C content. At the flowering stage and in treatment with a concentration of 12 mg L^{-1}, an increase by 90.72% was recorded in CS_1 variety, while a rise by 52.87% was collected in P_n variety in comparison with the control. It can be interpreted that 6-BA does impact the leaf vitamin C content of different tomato varieties and the impacts vary considerably among different varieties. Besides, different treatment concentrations might exert impacts on different levels. Treatment with a concentration of 12 mg L^{-1} promotes leaf vitamin C content more remarkably than those of 6 mg L^{-1}. Study results by Shams *et al.*[26] also show that CK application causes an increase in vitamin C content in pepper fruit. The impacts of 6-BA on vitamin C content of tomato varieties can be arranged in descending order of $F>P_{375}>CS_1>P_n$.

Besides, total organic acid acts as a dominant bridge linking the metabolism processes in plants directly related to the energy metabolism of plants[27]. 6-BA contributed to an increase in total organic acid content at both 5-leaf and flowering stages of tomato varieties (Table 3). For F variety, at the stage of 5-leaf, total organic acid content increased by 8.03% in treatment with a concentration of 6 mg L^{-1} and by 22.93% in treatment with a concentration of 12 mg L^{-1} compared to the control. At the flowering stage, total organic acid content increased by 15.54% in treatment with a concentration of 6 mg L^{-1} and by 25.99% in treatment with a concentration of 12 mg L^{-1} compared to the control. Regarding CS_1 variety, at the stage of 5-leaf, total organic acid content increased by 2.35% in treatment with a concentration of 6 mg L^{-1} and by 6.01% in treatment with a concentration of 12 mg L^{-1} compared to the control. At the flowering stage, total organic acid content increased by 9.19% in treatment with a concentration of 6 mg L^{-1} and by 24.55% in treatment with a concentration of 12 mg L^{-1} compared to the control. When it comes to P_{375} variety, at the stage of 5-leaf, total organic acid content increased by 8.14% in treatment with a concentration of 6 mg L^{-1} and by 13.62% in treatment with a concentration of 12 mg L^{-1} compared to the control. At the flowering stage, total organic acid content increased by 8.19% in treatment with a concentration of 6 mg L^{-1} and by 20.83% in treatment with a concentration of 12 mg L^{-1} compared to the control. Data collected in P_n variety showed that at the stage of 5-leaf, total organic acid content increased by 0.12% in treatment with a concentration of 6 mg L^{-1} and by 2.33% in treatment with a concentration of 12 mg L^{-1}. While at the flowering stage, it increased by 30.39% in treatment with a concentration of 6 mg L^{-1} and by 58.91% in treatment with a concentration of 12 mg L^{-1}. It can be seen that 6-BA at two concentrations of 6 and 12 mg L^{-1} increased the total organic acid content in tomato leaves; the impacts of 6-BA on total organic acid content of different varieties also considerably varied in descending order of $P_n>F>P_{375}>CS_1$. Similar results are found in the research by Merewitz *et al.*[18], stating that CK treatment causes an increase in the total organic acid content of *Agrostis stolonifera*.

Impacts of 6-BA on catalase enzyme activity and peroxidase enzyme activity: An amount of hydrogen peroxide (H_2O_2) is produced during the metabolic processes in tomato plants, which renders toxic stress to the plants. To counteract the stress, plants use heme enzymes such as peroxidase and catalase to metabolize H_2O_2. As a result, these two enzymes' activities affect the physiological activities of plants[28]. The application of 6-BA had some effects on catalase enzyme activity and peroxidase enzyme activity in tomato plants (Table 4). Increased catalase and peroxidase enzyme activity are essential in maintaining a higher photosynthesis rate as well as detoxifying the toxic byproducts of photosynthesis. For catalase enzyme activity, 6-BA exerted a significant impact, especially at the concentration of 12 mg L^{-1}. At the 5-leaf stage, under the treatment of 6-BA at a concentration of 6 mg L^{-1}, the highest catalase enzyme activity was recorded in P_{375} with an increase by 51.21%, when compared to the control, followed by F variety with an increase by 20.79%, then CS_1 with 20.16% and the lowest was collected in P_n with an increase by 8.17%, when compared to the control. Meanwhile, under the application of 6-BA at 12 mg L^{-1}, the highest data was recorded in P_n variety with an increase by 161.13%, when compared to the control, followed by P_{375} with 102.42%, then F variety with 49.71% and finally CS_1 with 34.04%, when compared to the control. At the flowering stage, under the treatment of 6-BA at a concentration of 6 mg L^{-1}, the highest catalase enzyme activity was recorded in P_n with an increase

Table 4: Impacts of 6-BA on catalase enzyme activity and peroxidase enzyme activity

Variety	Stage	Concentration (mg L^{-1})	Catalase (mM H_2O_2 g^{-1} min^{-1})	Compared to the control (%)	Peroxidase (UI g^{-1} sec^{-1})	Compared to the control (%)
P_{375}	5-leaf	Control	10.35±0.333	100.00	0.97±0.023^{b}	100.00
		6	15.65±0.337^{b}	151.21	1.05±0.043^{b}	108.25
		12	20.95±0.333^{a}	202.42	1.82±0.042^{a}	187.63
	Flowering	Control	16.67±0.412^{c}	100.00	1.27±0.071^{b}	100.00
		6	20.33±0.452^{b}	121.20	1.55±0.024^{b}	112.05
		12	25.00±0.157^{a}	150.00	2.25±0.042^{a}	177.17
CS_1	5-leaf	Control	25.00±0.253^{c}	100.00	1.33±0.113^{c}	100.00
		6	30.04±0.257^{b}	120.16	2.03±0.033^{b}	152.63
		12	33.51±0.253^{a}	134.04	2.73±0.058^{a}	205.26
	Flowering	Control	27.08±0.384^{c}	100.00	2.22±0.025^{c}	100.00
		6	30.58±0.333^{b}	112.92	2.33±0.032^{b}	104.96
		12	37.50±0.105^{a}	138.48	2.53±0.032^{a}	113.96
P_n	5-leaf	Control	7.59±0.225^{b}	100.00	0.98±0.014^{b}	100.00
		6	8.21±0.059^{b}	108.17	1.09±0.024^{b}	111.22
		12	19.82±0.415^{a}	261.13	1.90±0.027^{a}	193.88
	Flowering	Control	8.33±0.035^{c}	100.00	1.33±0.005^{c}	100.00
		6	17.20±0.257^{b}	206.48	1.86±0.010^{b}	139.85
		12	23.33±0.037^{a}	280.07	2.26±0.010^{a}	169.92
F	5-leaf	Control	41.66±0.039^{c}	100.00	1.05±0.033^{c}	100.00
		6	50.32±0.055^{b}	120.79	1.99±0.023^{b}	189.52
		12	62.37±0.057^{a}	149.71	2.32±0.033^{a}	220.95
	Flowering	Control	58.33±0.050^{b}	100.00	2.15±0.021^{c}	100.00
		6	60.12±0.049^{b}	103.07	2.26±0.004^{b}	105.12
		12	66.67±0.075^{a}	114.30	2.36±0.040^{a}	109.77

The values represent the Mean±SD of three independent measurements, For data of the same variety, at the same growth stage and data column, values with the same letter represent non-significant differences, values with different letters represent a significant difference at the significance level $p \leq 0.05$

by 106.48% when compared to the control, followed by P_{375} variety with an increase by 21.20%, then CS_1 with 12.92% and the lowest was collected in F variety with an increase by 3.07% when compared to the control. Meanwhile, under the application of 12 mg L^{-1} 6-BA, the highest data was recorded in P_n variety with an increase by 180.07% when compared to the control, followed by P_{375} with 50.00%, then CS_1 variety with 38.48% and finally F with 14.30% when compared to the control. It can be seen that 6-BA does have impacts on catalase enzyme activity of all four tomato varieties, especially in P_n and P_{375}. The impacts of 6-BA on catalase enzyme activity of tomato varieties can be arranged in descending order of P_n>P_{375}>CS_1>F.

6-BA of different concentrations also had impacts on peroxidase enzyme activity of different tomato varieties at different rates (Table 4). At the 5-leaf stage, 6-BA at a concentration of 6 mg L^{-1} increased the peroxidase enzyme activity remarkably. Peroxidase enzyme activity was recorded to increase by 89.52% in F variety when compared to that of the control, 52.63% in CS_1 variety, 11.22% in P_n variety and finally 8.25% in P_{375} variety. Meanwhile, under the application of 6-BA at 12 mg L^{-1}, the highest data was recorded in F variety with an increase by 120.95% when compared to the control, 105.26% in CS_1 variety, 93.88% in P_n variety and finally with 87.63% in P_{375} when compared to the control. At the flowering stage, under the treatment of 6-BA at a concentration of 6 mg L^{-1}, the highest peroxidase enzyme activity was recorded in P_n with an increase by 39.85% when compared to the control, followed by P_{375} variety with an increase by 12.05%, then F with 5.12% and CS_1 with 4.96% when compared to the control. Meanwhile, under the application of 12 mg L^{-1} 6-BA, the highest data was recorded in P_{375} variety with an increase by 77.17% when compared to the control, followed by P_n with 69.92%, then CS_1 variety with 13.96% and finally F with 9.77% when compared to the control. Results show that the impacts of 6-BA on peroxidase enzyme activity at the 5-leaf stage are more remarkable than those at the flowering stage and those impacts in different varieties might be arranged in descending order of F> P_n>P_{375}>CS_1. These results agree with those of previous research, stating that CK contributes to increases in catalase enzyme activity and peroxidase enzyme activity[29,30].

CONCLUSION

6-BA whether at a concentration of 6 or 12 mg L^{-1} still increased plant height and promoted the dry matter accumulation capacity of tomato varieties, in which the

impacts of 6-BA at the 5-leaf stage were profound than at the flowering stage. The application of 6-BA at 6 and 12 mg L^{-1} increased chlorophyll and carotenoid contents stimulated metabolic activities in plants and retarded tomato plant senescence. 6-BA at both concentrations of 6 and 12 mg L^{-1} affected vitamin C and total organic acid contents in leaves of different tomato varieties, of which the highest data were recorded at the flowering stage. Besides, 6-BA also promoted catalase enzyme activity and peroxidase enzyme activity, thus improving tomato plant resistance against environmental conditions, promoting plant growth and productivity.

SIGNIFICANCE STATEMENT

This study discovers the impacts of cytokinin on the physiological and biochemical parameters of tomato varieties at the 5-leaf stage and the flowering stage cultivated in Vietnam. The study also confirmed that the impacts of cytokinin on tomato physiological and biochemical parameters depend not only on the concentration of cytokinin but also on the developmental stages and plant varieties. This study will help the researcher to uncover the critical areas of cytokinin to physiological and biochemical parameters of tomato varieties that many researchers were not able to explore.

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