



ISSN: 2455-9377

Effect of cytokinins on growth and phenylpropanoid accumulation in Tartary buckwheat sprouts (*Fagopyrum esculentum* Moench)

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ABSTRACT

This study analyzed the effect of plant hormones, zeatin, 6-benzyl amino purine (BAP), kinetin, and thidiazuron (TDZ) on the growth of Tartary buckwheat sprouts and analyzed the fresh weight, shoot and root length, and production of phenolic compounds. All the hormone-treated plants at the lowest concentration (0.1 mg/L) showed the highest levels of growth parameters (fresh weight, shoot, and root length) when compared to the control. Among the various hormones treatment, the plant treated with 1 mg/L of BAP, kinetin, and zeatin showed the highest total phenolic level, whereas the TDZ showed the highest accumulation of total phenolic at the lowest concentration (0.1 mg/L). A total of 6 compounds were identified (4-hydroxybenzoic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, rutin, and trans-cinnamic acid) were quantified by high liquid performance chromatography (HPLC) after treatment of plant with different concentrations of hormones. Among these individual phenolic compounds, at the higher hormonal concentration (1 mg/L) the rutin showed the highest accumulation in BAP, zeatin, and kinetin treated sprout, whereas in the TDZ treated sprout the rutin content was highest at the lowest concentration (0.1 mg/L). From these results, it is suggested that BAP, zeatin, and kinetin at the lowest concentrations might positively enhance the growth of buckwheat sprouts, whereas at the highest hormonal treatment the accumulation of the phenolic compounds was higher. However, in TDZ treatment the growth and phenolic compound accumulation were highest at the lowest concentration. From these results, it is showed that suitable concentrations might enhance the growth and phenolic compound accumulation in Tartary buckwheat sprout.

KEY WORDS: Tartary buckwheat, *Fagopyrum esculentum* Moench, phenylpropanoid, zeatin, 6-benzyl amino purine, kinetin, thidiazuron

Received: March 07, 2021
Revised: July 26, 2021
Accepted: July 27, 2021
Published: August 05, 2021

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INTRODUCTION

Fagopyrum esculentum Moench is called common buckwheat, and it is mainly grown and consumed in the countries such as China, South Korea, and Japan (Jeon *et al.*, 2007). The buckwheat is considered as one of the most important pseudocereals due to its agricultural and various medicinal properties such as anti-allergic (Kim *et al.*, 2003), antibacterial (Amarowicz *et al.*, 2008), biological activities, anti-hypertensive (Kim *et al.*, 2009), antioxidant (Ren & Sun, 2014), and cytotoxic (Daníhelová *et al.*, 2013), and allelopathic effect (Golisz *et al.*, 2007). These properties prove that the common buckwheat consists of various nutrients such as

amino acids, dietary fibers, dietary flavonoids, minerals, fatty acids, phenolic compounds, and vitamins (Golisz *et al.*, 2007; Kim *et al.*, 2009).

The phenylpropanoid pathway is one of the largest groups of benzo- γ -pyrone derivatives and ubiquitously distributed in plant species (Kumar & Pandey, 2013). This pathway byproduct is mainly involved in the growth and development of the plant, disease protection, pigment generation, and photoprotection (Dixon & Paiva, 1995; Winkel, 2001). Previously several studies reported that the common buckwheat contains phenolic compounds such as (-)-epicatechin, (+)-catechin, caffeic acid, chlorogenic acid,

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ferulic acid, quercetin, gallic acid, and rutin (Golisz *et al.*, 2007). Among these individual carotenoids, rutin is the main component present in common buckwheat (Kalinova *et al.*, 2006; Kreft *et al.*, 2006; Golisz *et al.*, 2007; Jiang *et al.*, 2007; Kim *et al.*, 2008). Rutin obtained from buckwheat species such as *F. homotropicum*, *F. tataricum*, and *F. esculentum* showed several health benefits such as antidiabetic (Watanabe & Ayugase, 2010), anti-neuroprotective (Gulpinar *et al.*, 2012), anti-inflammatory (Lee *et al.*, 2013), antioxidant (Jiang *et al.*, 2007), cytotoxic effects (Kim *et al.*, 2007), and cardiac hypertrophy inhibition (Han *et al.*, 2010).

Plant hormones are otherwise called phytohormones, are well-defined as organic molecules and even at a low concentration, it has a positive effect on plant physiological processes such as growth and development, and differentiation (Davies, 2010). In-plant tissue culture and cell culture, the phytohormones are divided into 5 main classes namely, abscisic acid (ABA), auxins, ethylene, cytokinins, and gibberellins (GAs). Mostly, the interaction of auxin and cytokinin is essential for the regulation of physiological developments in plant tissue and organ cultures. However, the ABA, GAs, and ethylene play regulatory roles in the cultures (Gaspar *et al.*, 1996).

In further, the production of medicinally valuable secondary metabolites has been made through various strategies such as culture systems, elicitation, nutrient conditions, plant hormones, precursor feeding, and removal of toxic products (Cho *et al.*, 1988; Yeoman & Yeoman, 1996; Rao & Ravishankar, 2002; Vanisree *et al.*, 2004; Karuppusamy, 2009). Specifically, treatment of plants with hormones will enhance the secondary metabolites production, for example, treatment of buckwheat plantlets with 6-benzyl adenine increases the concentrations of rutin (Hou *et al.*, 2015); in *Brassica rapa* spp. *pekinensis* the ABA increases the phenolic and glucosinolates content (Thiruvengadam *et al.*, 2015) and in *Vitis rotundifolia* the ABA treatment enhances the production of anthocyanin and flavonol compounds (Sandhu *et al.*, 2011). In cell cultures of *Catharanthus roseus*, ethylene hormone improved the accumulation of alkaloids (Yahia *et al.*, 1998). In addition, the adventitious roots of *Periploca sepium* Bunge treated with indolebutyric acid, increase the production of Periplocin (Zhang *et al.*, 2012); Moreover, treatment of Tartary buckwheat “Hokkai T10” cultivar with appropriate concentration of auxins (indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid, indolebutyric acid, naphthaleneacetic acid) will enhance the production of the anthocyanin in hairy root cultures (Park *et al.*, 2016).

To the best of our knowledge, there were no previous reports on the effect of zeatin, benzyl amino purine (BAP), kinetin, and thidiazuron (TDZ) on the accumulation of phenolic compounds in common buckwheat sprouts. Thus, in this study, we aim to optimize the suitable concentration of BAP, kinetin, zeatin, and TDZ to enhance the growth and production of phenolic compounds especially the rutin content in common buckwheat sprouts.

MATERIALS AND METHODS

Plant Materials

Tartary buckwheat seeds were purchased from Jeju, and it was soaked into the water for 16 h at room temperature. The next day, the soaked seeds were sowed into the pot containing vermiculites and germinated at 25 °C in a plant growth chamber consist of white cool fluorescent lights (35 $\mu\text{mol s}^{-1}\text{m}^{-2}$) with 16/8-h light/dark period. For hormonal treatment, the plants were treated with different concentrations (0.1, 0.5, and 1.0 mg/L) of BAP, kinetin, zeatin, and TDZ. The grown sprout was harvested after 10 days and measured the fresh weight, shoot, and root length. Then the samples were immediately frozen by using -196 °C liquid nitrogen and stored at deep freezer (-80 °C). After freeze-dried the samples were ground into powdered and 100 mg of each powdered sample were taken for HPLC analysis.

Chemical and Standards

All the plant hormones (Zeatin, BAP, kinetin, and TDZ) and the HPLC external standards such as caffeic acid, chlorogenic acid, *p*-coumaric acid, *trans*-cinnamic acid, and rutin were purchased from Sigma-Aldrich Co., Ltd., St. Louis, MO, USA, whereas hydroxybenzoic acid purchased from Extrasynthese, Genay, France.

Extraction and Analysis of Phenylpropanoid Compounds

The extraction and analysis of phenylpropanoid compounds in the common buckwheat sprouts were carried out with little modification as previously described by Yeo *et al.* (2021). A total of 0.1g of finely powdered Tartary buckwheat sprout were mixed with 3 ml of methanol solution (80%). Then vortexed well for 1 min and sonicated it for 1 h at 37 °C. The mixture was centrifuged at 4 °C for 15 min at 10,000 rpm. The supernatant was passed through the PTFE syringe filter into a glass vial. The HPLC machine, mobile phase, HPLC condition, gradient program, identification, and quantification of phenolic compounds were similar to that of the protocol of Yeo *et al.* (2021).

Statistical Analysis

Data were analysis was done by using the SPSS statistics, version 26 (IBM Corp, Armonk, NY, USA), and Duncan’s multiple range test was performed to determine significant differences. All experimental analyses were analyzed in triplicates.

RESULTS AND DISCUSSION

Effect of BAP, Kinetin, Zeatin, and TDZ on Growth Characteristics

The growth pattern of buckwheat sprouts at different growth regulators (BAP, kinetin, zeatin, and TDZ) was observed by

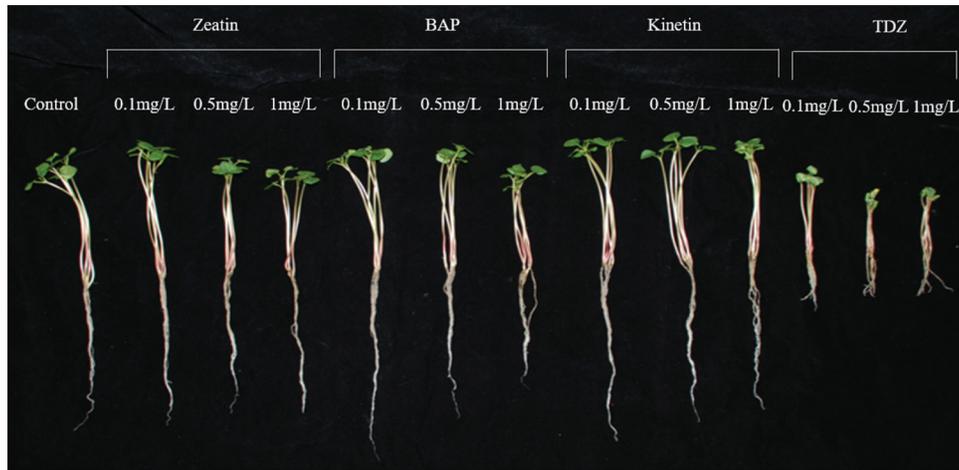


Figure 1: The effect of zeatin, BAP, kinetin, and TDZ on Tartary buckwheat sprout growth

measuring its fresh weight (g), shoot, and root length (cm). The result showed that different growth regulators exhibit similar growth patterns (Figure 1). At the lowest concentration (0.1 mg/L), in all the exogenous supplied plant hormones (BAP, kinetin, zeatin, and TDZ) the fresh weight, shoot, and root length were highest when compared to the control and other concentrations (0.5 and 1.0 mg/L). The control represents that without the addition of exogenous hormones for 10 days. Among the different growth regulators, the highest fresh weight was achieved in 0.1 mg/L BAP, and it was 1.24-, 1.06-, 1.05-, and 1.69- times higher than that of the control, 0.1 mg/L of zeatin, kinetin, and TDZ, respectively (Figure 2). Similar results were obtained in the shoot and root length; both lengths were highest in the 0.1 mg/L BAP treatment, whereas at the highest concentrations (0.5 and 1.0 mg/L) the shoot and root length were lowered in all the hormonal treatments. The shoot and root length of 0.1 mg/L BAP treated sprout was 11.24 ± 0.25 and 16.20 ± 0.57 , which was 1.06- & 1.31-, 1.07- & 1.09-, and 1.56- & 3.29- higher than that of the other 0.1 g/L hormonal treatments (zeatin, kinetin, and TDZ), respectively. Among all the growth regulators, the TDZ treatment at different concentrations showed the lowest fresh weight, shoot and root length. From these results, it is showed that BAP at the lowest concentration (0.1 mg/L) significantly enhances the fresh weight, shoot and root length in the Tartary buckwheat sprout.

From the growth characteristic results, it is showed that the BAP enhances the growth of buckwheat sprouts. Several studies have been reported that BAP enhances the growth of plants. El Kbiach *et al.* (2002) reported that BAP enhanced the axillary bud sprouting in *Quercus suber* L. and they found that BAP is one of the best growth regulators. In addition, they have reported that the absence of stem on different control culture medium proves that cytokinins are important for the development of aerial organs in plants when compared to auxins (El Kbiach *et al.*, 2002). In strawberries, the BAP treatment enhances the fresh matter weight (Hunter *et al.*, 1984). This result was an agreement with the previous study, in nature the differences of growth on the various genotype of *Dioscorea* sp. is determined by the amount of cytokinin (Saleil *et al.*, 1990). In addition, it

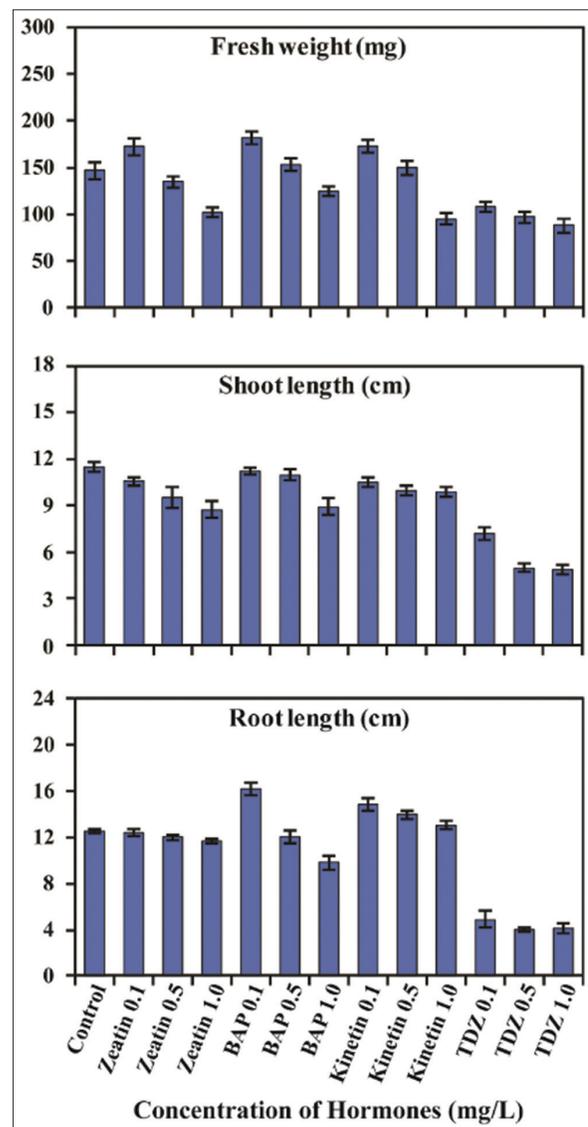


Figure 2: Effect of different hormones in Tartary buckwheat sprout growth characteristics at different concentrations. Data were analysed after 10 days of growth.

Table 1: Effect of zeatin, BAP, Kinetin, and TDZ on accumulation of phenolic compounds in common buckwheat sprouts.

Plant Hormones	Concentrations (mg/L)	Phenolic compounds (mg/g DW)						
		4-hydroxybenzoic acid	Chlorogenic acid	Caffeic acid	<i>p</i> -coumaric acid	Rutin	<i>trans</i> -cinnamic acid	Total
Control	0	0.19±0.01a	0.01±0.00b	0.54±0.02a	0.03±0.00c	23.15±0.74b	0.06±0.01a	23.99±0.75b
Zeatin	0.1	0.14±0.01d	0.01±0.00b	0.39±0.06d	0.04±0.00b	20.49±0.46c	0.05±0.01b	21.12±0.38c
	0.5	0.16±0.00c	0.02±0.00a	0.46±0.00b	0.05±0.00a	23.95±1.36b	0.06±0.00a	24.68±1.35a
	1.0	0.18±0.01b	0.02±0.00a	0.45±0.02c	0.04±0.00b	24.22±2.41a	0.05±0.00b	24.95±2.39a
Control	0	0.19±0.01b	0.01±0.00a	0.54±0.02b	0.03±0.00b	23.15±0.74c	0.06±0.01a	23.99±0.75c
BAP	0.1	0.25±0.00a	0.01±0.00a	0.53±0.01c	0.03±0.00b	22.60±1.72d	0.06±0.00a	23.48±1.72c
	0.5	0.18±0.01c	0.01±0.00a	0.50±0.01d	0.03±0.00b	24.79±0.55b	0.04±0.00b	25.55±0.56b
	1.0	0.17±0.01d	0.01±0.00a	0.61±0.02a	0.04±0.01a	26.49±0.61a	0.04±0.00b	27.36±0.56a
Control	0	0.19±0.01a	0.01±0.00a	0.54±0.02b	0.03±0.00a	23.15±0.74c	0.06±0.01b	23.99±0.75c
Kinetin	0.1	0.16±0.01b	0.01±0.00a	0.46±0.03c	0.03±0.00a	24.46±1.28b	0.04±0.00c	25.17±1.26b
	0.5	0.16±0.01b	0.01±0.00a	0.42±0.02d	0.03±0.00a	22.99±2.46d	0.06±0.00b	23.67±2.49c
	1.0	0.16±0.01b	0.01±0.00a	0.58±0.02a	0.03±0.00a	28.73±1.07a	0.08±0.00a	29.59±1.04a
Control	0	0.19±0.01a	0.01±0.00a	0.54±0.02a	0.03±0.00b	23.15±0.74d	0.06±0.01b	23.99±0.75d
TDZ	0.1	0.13±0.00b	0.01±0.00a	0.46±0.00b	0.04±0.00a	29.16±0.27a	0.06±0.00b	29.86±0.26a
	0.5	0.10±0.00c	0.01±0.00a	0.36±0.00d	0.03±0.00b	26.89±0.29b	0.10±0.00a	27.49±0.29b
	1.0	0.09±0.00d	0.01±0.00a	0.39±0.02c	0.03±0.00b	25.58±0.29c	0.06±0.00b	26.16±0.27c

Mean with different letters (a, b, c, and d) in the same column differ significantly ($p < 0.05$, ANOVA, DMRT)

has been reported that cytokinins trigger and enhance the dry matter mass of the tubers of *Dioscorea bulbifera* (Mantell & Hugo, 1989). Moreover, in the micropropagation of banana plants, the BAP showed high efficiency than the zeatin (De Langhe & Vuylstek, 1985). A similar result was obtained in the Afassie Blanche, a Togolese variety (Toklo, 2000). In another study, when compared to kinetin, the highest shoot growth for other yam varieties of Benin was obtained in BAP (Montcho, 2004). Ahanhanzo *et al.* (2010), studied the effect of two growth regulators (BAP and Zeatin) on yams varieties and they found that BAP showed the highest bud sprouting and development of shoot. In addition, they concluded that when compared to zeatin the BAP is considered to be one of the important cytokinins which have the best morphogenic aptitude in yam micropropagation (Ahanhanzo *et al.*, 2010). Based on these, the response of explants and mode of action of cytokinins is mainly dependent on the genotype of the plants. From these overall results it is showed that among the different growth regulators, the BAP is useful for enhancing the growth of plants.

Effect of BAP, Kinetin, Zeatin, and TDZ on Phenolic Compounds

In the HPLC analysis, a total of 6 phenolic compounds were identified and quantified in the Tartary buckwheat sprouts supplement with exogenous growth regulators (BAP, kinetin, zeatin, and TDZ) which was shown in Table 1. The treatment of TDZ resulted in the highest accumulations of the total phenolic compounds at the lowest concentrations (0.1 mg/L of TDZ), whereas 0.5 and 1.0 mg/L of TDZ showed a slight decrease in the accumulations (Table 1). The total phenolic compounds were observed after supplement of 0.1 mg/L TDZ (29.86 ± 0.26 mg/g DW), which is 1.24-, 1.27-, 1.19-, and 1.41- times higher than the control, 0.1 mg/L of BAP, kinetin, zeatin treatment. Particularly, the levels of 4-hydroxybenzoic acid, caffeic acid, and rutin content were significantly increased in all the growth regulator treatments. The highest levels of rutin obtained after treatment of 0.1 mg/L TDZ, which is 1.26- times higher than the control. However, the TDZ treatment led

to a gradually decreased accumulation of chlorogenic acid, *trans*-cinnamic acid, and *p*-coumaric acid depending on all the concentrations. The second-highest total phenolic content after growth regulator treatment was obtained 1.0 mg/L of kinetin (29.59 ± 1.04 mg/g DW). In the kinetin treatment, the individual phenolic compound (mg/g DW) is highest in the following orders rutin (28.73 ± 1.07), caffeic acid (0.58 ± 0.02), 4-hydroxybenzoic acid (0.16 ± 0.01). The third-highest total phenolic content was achieved in BAP hormonal treatment, among the individual phenolic compound levels the 4-hydroxybenzoic acid, caffeic acid, and rutin content. From these result, it is showed that different growth regulator has a significant effect on the different phenolic compound accumulation in the hormonal treatment, especially in higher TDZ treatment.

The result of different growth regulators on the accumulation of phenolic compounds showed that the highest total phenolic content was obtained in the 0.1 mg/L TDZ (29.86 ± 0.26), followed by 1 mg/L kinetin (29.59 ± 1.04), 1 mg/L BAP (27.36 ± 0.56), and 1 mg/L zeatin (24.95 ± 2.39). A similar result was obtained in the callus culture of *Artemisia absinthium* which showed that TDZ positively enhances the production of phenolic content. In addition, they found that at lowest TDZ concentration (0.5-3.0 mg/L) exhibited the highest total phenolic compounds and antioxidant activity (Ali & Abbasi, 2014).

CONCLUSIONS

This study shows the effect of various growth regulators (BAP, kinetin, zeatin, and TDZ) on the growth development of Tartary buckwheat sprouts. Among these, BAP showed strong improvement in the growth parameters when compared to the control and other growth regulators. Hence, BAP is one of the good cytokinins which has the best morphogenic ability when compared to other plant growth regulators in the Tartary buckwheat sprout. HPLC analysis shows that when compared to BAP, kinetin, and zeatin, the TDZ is highly effective in

the accumulation of the phenolic compounds during Tartary buckwheat sprout development.

ACKNOWLEDGEMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2019R1F1A1061916) and National Research Foundation of Korea (NRF-2019K2A9A2A06024347).

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