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Research Article

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Influence of pectin on phenylpropanoid accumulation in buckwheat (*Fagopyrum esculentum*) sprout

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ABSTRACT

Buckwheat (*Fagopyrum esculentum* Monech) contains several secondary metabolites like phenolic chemicals. Pectin has been demonstrated to be an efficient elicitor from the biotic group for triggering the defensive response, which enhances the production of secondary metabolites. In this study, the effect of pectin on the growth of buckwheat sprouts and the production of phenylpropanoid compounds in common buckwheat sprouts was investigated by using high-performance liquid chromatography (HPLC). Pectin treatments of 0, 2, 4, 6, and 8 mg/L were administered on buckwheat sprouts for ten days to assess the growth characteristics and optimum concentrations. In comparison to the control treatment, 2 mg/L pectin enhances the shoot length by 24%. But when pectin concentration continued to rise, a tendency toward shorter shoots was seen. Pectin treatment decreased the fresh weight of the sprout as compared to the control treatment. The phenylpropanoid accumulation in buckwheat sprouts varied depending on the amount of pectin utilized. Pectin treatment at 6 mg/L resulted in a 15.10% increase in total phenylpropanoid accumulation. The findings of this study indicate that pectin is a possible elicitor, however, more research on how pectin affects the buildup of phenylpropanoids in buckwheat sprouts would be more intriguing to examine the implications of this work.

KEYWORDS: Pectin, Phenylpropanoid, Common buckwheat, *Fagopyrum esculentum* Moench

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INTRODUCTION

An ancient pseudocereal crop belonging to the family and genus Polygonaceae called buckwheat is consumed worldwide and plays a significant role in the human diet (Matsui & Yasui, 2020). *Fagopyrum esculentum* Moench, originating in Southwest China and progressively spreading to every continent, is also known as common buckwheat (Huda *et al.*, 2021). It is regarded as a source of nourishment and medicine for advancing human health (Kreft, 2016).

Phenylpropanoid has pharmacological characteristics that make it valuable. Based on the carbon structure, these chemicals are often divided into six primary subclasses: flavanones, flavones, isoflavones, flavonols, and anthocyanidins (Iwashina, 2000). Different plant components, including fruits, vegetables, leaves, roots, seeds, and nuts, generate phenylpropanoids. It is essential for plant growth and development, structural stability, and responsiveness to stimuli (Cuong *et al.*, 2019; Dong & Lin, 2021; Pratyusha & Sarada, 2022). Additionally, it responds significantly to stressors like high light intensity and mineral deficiency (Clemens & Weber, 2016). Additionally, it served as a mediator for interactions between plants and other creatures (Grover *et al.*, 2022; Ramaroson *et al.*, 2022).

Sprouts are recognized as a special nutritional vegetable and increasing attention as customers demand minimally

processed, additive-free, natural, healthful food (Artés-Hernández *et al.*, 2022; Ebert, 2022). Additionally, buckwheat sprouts have been employed to provide health benefits due to their vital nutritional components. The total phenolic content and antioxidant capacity of buckwheat sprouts are higher than those of seeds (Park *et al.*, 2017; Mansur *et al.*, 2022).

Pectin is a naturally occurring substance (polysaccharide) that is extracted from plant cell walls (Chandel *et al.*, 2022). Pectin is an effective elicitor from the biotic group for producing the defense response that increases the synthesis of secondary metabolites (Zheng *et al.*, 2020).

To our knowledge, there was no previous study that has documented the influence of pectin on the accumulation of phenylpropanoid in common buckwheat sprouts. Thus, this study aimed to optimize the concentration of pectin for the growth and accumulation of phenylpropanoid in common buckwheat sprouts using high-performance liquid chromatography (HPLC) analyses.

MATERIALS AND METHODS

Plant Materials

In this investigation, Yangjeol seeds, one of the popular buckwheat (*Fagopyrum esculentum* Moench) varieties, were obtained from the Rural Development Administration (RDA), Korea. One Hundred seeds were sown on vermiculite in an 11 cm × 11 cm plastic pot and grown in a plant growth chamber at 25 °C under a 16 h light/8 h dark photoperiod. Pectin treatments of 0, 2, 4, 6, and 8 mg/L were administered on Buckwheat sprouts for ten days to assess their growth characteristics and optimum concentrations. The samples were harvested after 10 days of treatment, and all samples were immersed in liquid nitrogen and then freeze-dried for analysis of phenylpropanoid content.

Extraction and HPLC Analysis of Phenylpropanoid Compounds

A hundred milligrams of dried powder samples were mixed with 2 mL 80% methanol and sonicated at 37°C for 1 h. After that, centrifuge the sonicated samples at 12,000 rpm for 15 min, and the collected supernatant was filtered-sterilized through a 0.45 µm hydrophilic PTFE syringe filter (Ø, 13 mm, Advantec, Tokyo, Japan). Phenylpropanoids were detected using an HPLC device (Futecs model NS-4000, Daejeon, Korea) coupled with a C_{18} column (250 × 4.6 mm, 5 µm; RStech, Daejeon, Korea) at a wavelength of 280 nm. The mobile phase, column temperature, flow rate, and injection volume were similar to the protocol described by Sathasivam *et al.* (2021). All the compounds were identified and quantified based on peak areas, and the concentrations were calculated as equivalents of representative standard compounds.

Statistical Analysis

Statistical analysis was done with SPSS 22 (IBM Corp., Armonk, NY, USA) using an analysis of variance (ANOVA) with Tukey's honestly significant difference test. All values were calculated as the mean values ± standard deviation of triplicate experiments.

RESULTS

Effect of Pectin on Morphological Differentiation of Buckwheat Sprout

Both the shoot length and fresh weight of buckwheat sprouts significantly changed at different pectin concentrations. A distinguished morphological variation was observed from the growth pattern as influenced by different pectin concentrations (Figure 1). The buckwheat sprout's shoot length ranged from 4.76 cm to 13.45 cm. Pectin application at 2 mg/L resulted in a 24% increase in shoot length as compared to the control treatment (10.84 mg/L). But when pectin concentration

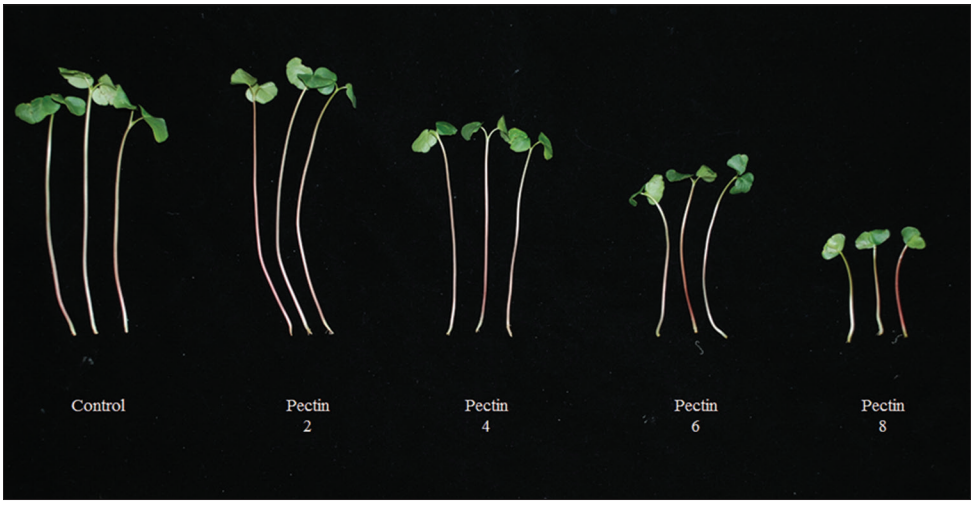

Figure 1: Effect of different concentrations of pectin on sprout growth

continued to rise, a tendency toward shorter shoots was seen. In fact, the lowest shoot length was observed at the maximum pectin concentration of 8 mg/L. In the control treatment, buckwheat sprouts had the greatest fresh weight (0.26 g). When compared to the control treatment, pectin treatment dramatically decreased the fresh weight of the sprout. Pectin application at 8 mg/L resulted in the lowest fresh weight (0.08 g), followed by pectin 6 (0.13 g), pectin 4 (0.16 g), and pectin 2 (0.24 g) (Table 1).

Effect of Pectin on the Accumulation of Phenylpropanoid

The administration of pectin at various concentrations caused a wide range of variations in phenylpropanoid accumulation in buckwheat sprouts. The buckwheat sprout accumulated the highest amount of chlorogenic acid (74.06 µg/g DW) when pectin was administered at a concentration of 6 mg/L, which was statistically equivalent to the amounts accumulated at pectin 2 (68.40 µg/g DW), 4 (67.88 µg/g DW), and 6 (74.06 µg/g DW). The lowest accumulation of chlorogenic acid was documented in pectin 8 (50.12 µg/g DW). The amount of caffeic acid was maximum (51.84 µg/g DW) in buckwheat sprouts treated with pectin 4 (4 mg/L), while it was lowest (36.38 µg/g DW) in pectin 8 (8 mg/L). Accumulation of *p*-coumaric acid by buckwheat sprout spanned from 0.93 µg/g DW to 5.04 µg/g DW where the highest was accumulated at pectin 8 and lowest was at control treatment. Ferulic acid was shown to accumulate to its highest level (15.09 µg/g DW) in the control treatment, whereas this level was seen to drop to its lowest (12.11 µg/g DW) in the pectin 2 treatment. The greatest rutin accumulation (2309 µg/g DW) was achieved with the administration of pectin at 6 mg/L, however, pectin 8 treatment was shown to have the lowest accumulation (1821.05 µg/g DW). About 1926.46 µg/g DW- 2451.89 µg/g DW total phenylpropanoid was accumulated by the Buckwheat sprout due to the application of pectin. Comparing the application of pectin at 6 mg/L to the control (2130.07 µg/g DW), there was a 15.10% increase in the accumulation of total phenylpropanoid. However, 9.55% less phenylpropanoid accumulation was seen with the administration of pectin at 8 mg/L compared to the control treatment (Figure 2).

DISCUSSION

Pectin governs the dynamics of cell development and separation in plants, as well as cell adhesion and growth. Pectin plays crucial functions in plant growth and stress responses that are supported by its dynamics, changes, and interactions at the molecular and cellular levels.

Table 1: Morphological differentiation of common buckwheat sprouts after exposed pectin at different concentrations. Data was recorded after 10 days of growth

	Shoot (cm)	Fresh weight (g)
Control	10.84 ± 0.59b	0.26 ± 0.03a
Pectin 2 (mg/L)	13.45 ± 1.07a	0.24 ± 0.01a
Pectin 4 (mg/L)	9.54 ± 0.54c	0.16 ± 0.03b
Pectin 6 (mg/L)	8.02 ± 0.38d	0.13 ± 0.01b
Pectin 8 (mg/L)	4.76 ± 0.23e	0.08 ± 0.01c

In this study, pectin application at 2 mg/L resulted in a 24% increase in shoot length as compared to the control treatment. However, a trend toward shorter shoot was seen as pectin concentration increased. On the other hand, pectin treatment substantially lowered the sprout's fresh weight as compared to the control treatment. Pectin is an abundant polysaccharide found in cell walls that plays crucial parts in several biological processes. Plant cell differentiation frequently results in differential development to create a particular cell shape, which necessitates significant modifications in the cell wall. The size of the central vacuole also grows as a result of this process. The consensus is that the cell wall, turgor pressure, and vacuole all play crucial roles in regulating how long cells are and how they are shaped (Chen *et al.*, 2021). The cell wall is an active participant in this process, as opposed to serving as a static structure, according to evidence, and the interactions between these elements are crucial for controlling cell shape. Pectin, which exists as a calcium (Ca) pectate gel between the load-bearing cellulose microfibrils and xyloglucan (XG) chains, is presented as a model that regulates the pace of cell expansion (Blamey, 2003). Using immunohistochemistry, it has recently been discovered that primordia development is also preceded by a local de-esterification of pectins (Peaucelle *et al.*, 2008). This shows that pectin is crucial for the development of organs. Pectin serves as a binder in plants and holds plant parts together, guaranteeing the cohesiveness of the fibers and giving the plant tissue stability. The chain orientation, mechanical characteristics, and cross-links between pectin and cellulose fibers all contribute to the plant cell wall's strength which regulates the shoot length.

Pectin is an effective elicitor from the biotic group for producing the defensive response that increases the synthesis of secondary metabolites (Zheng *et al.*, 2020). The crucial function that phenylpropanoid plays in plant growth and development, structural support, and responsiveness to stimuli makes it an essential secondary metabolite.

In the current study, the administration of pectin at various concentrations caused the accumulation of phenylpropanoid in buckwheat sprouts to vary. Pectin stimulated the accumulation. This might be due to the tensile strength to maintain the turgor pressure within the cell and increase the cell growth (Wolf & Greiner, 2012). Pectin's stimulatory properties were in line with the findings of Wiktorowska *et al.* (2010), which showed that *Calendula officinalis* cell cultures boosted cell proliferation and oleanolic acid buildup at low pectin concentrations. Pectin was discovered to be a viable option for enhancing the production of phenylpropanoids in *Hypericum perforatum* cultures (Shakya *et al.*, 2019). According to Cai *et al.* (2012), pectin treatment increased the synthesis of phenolic acids and anthocyanin in *Vitis vinifera* cell cultures. Pectin has been shown to have favorable effects on cell proliferation and amaranthin accumulation in *Chenopodium rubrum* cells when added to chitosan-treated cell cultures (Dornenburg & Knorr, 1995). Ullah *et al.* (2021) reported that the micro-shoot cultures of *Ajuga integrifolia* treated with pectin and yeast extract might be an efficient bio-factory to produce specific commercial secondary metabolites.

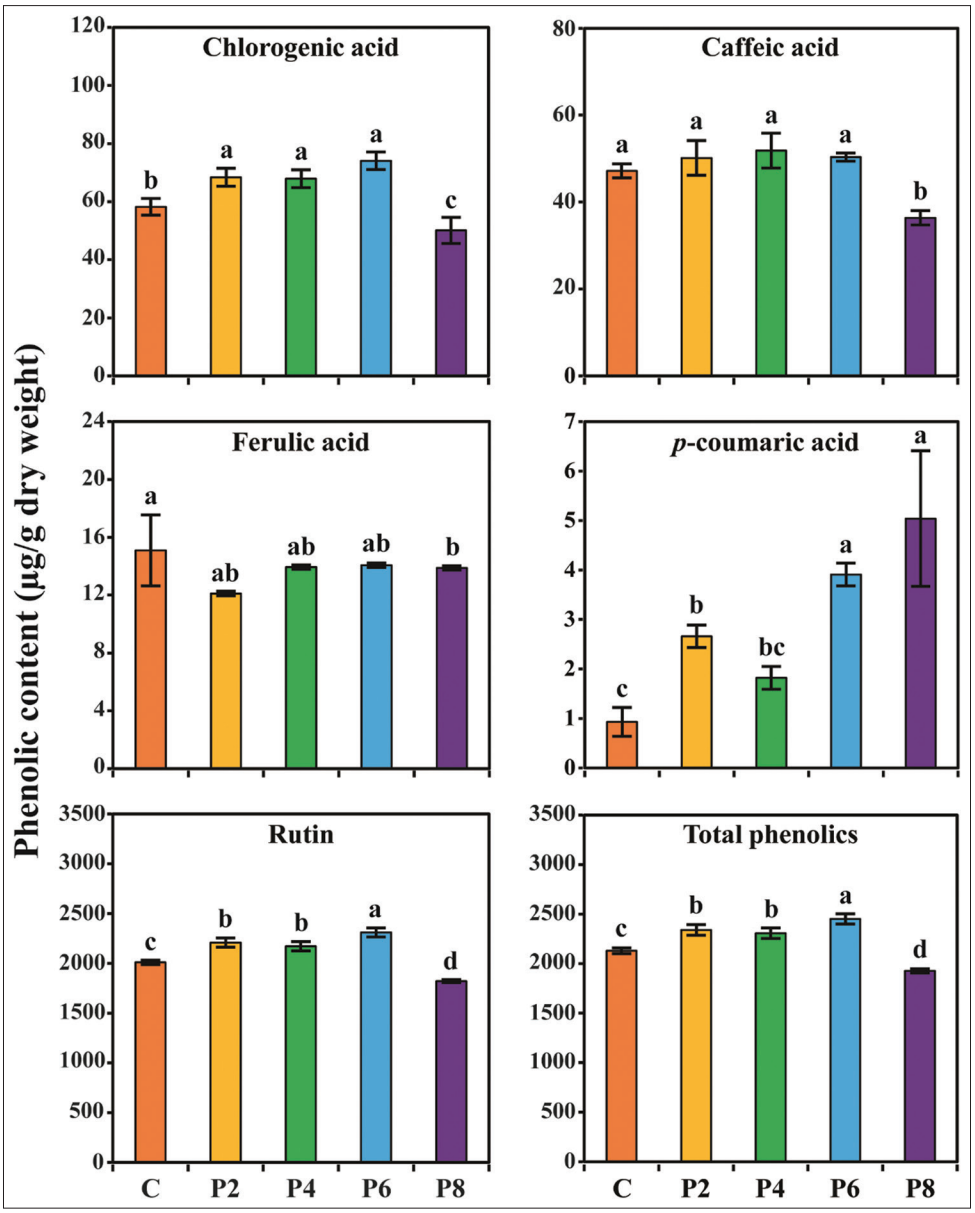

Figure 2: Effect of pectin on phenylpropanoid accumulation in common buckwheat sprouts. C-Control; P2-Pectin 2 mg/L, P4-Pectin 4 mg/L; P6-Pectin 6 mg/L; P8-Pectin 8 mg/L

CONCLUSION

The results obtained from this study reveal that pectin is a potential elicitor and application of pectin at various concentrations caused the accumulation of phenylpropanoid in buckwheat sprouts to vary. However, further studies regarding the effect of pectin on the accumulation of phenylpropanoids in buckwheat sprouts would be more interesting to explore the insight of this present work.

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