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Research Article
Effects of *p*-Coumaric Acid on the Morphological Characteristics of Soybean Sprouts

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Abstract
Background and Objective: Soybean is an important legume commodity that contains bioactive isoflavones. The increase in bioactive compounds during germination can be manipulated by exogenous precursor treatment to stimulate the synthesis of certain bioactive compounds, one of which is coumaric acid. This study aimed to identify the role of coumaric acid in the growth of soybean sprouts. **Materials and Methods:** The study used a Factorial Completely Randomized Design with 2 factors. The first factor was precursor treatment (no precursor; 250 ppm of *p*-coumaric acid; 500 ppm of *p*-coumaric acid). The second factor was soybean variety (Dering 1, Devon 1, and Devon 2). The data obtained were analyzed using analysis of variance. If significant differences were found, Duncan's Multiple Range Test was performed at α = 5%. **Results:** The difference in varieties significantly affected the percentage of germination, radicle length, hypocotyl length and fresh weight of seedlings. Dering 1 variety has the highest germination percentage, but for the fresh weight of the sprouts, the Devon 1 variety is the highest compared to Dering 1 and Devon 2. *The p*-coumaric acid treatment (0-500 ppm) significantly reduced the percentage of germination, radicle length, hypocotyl length and fresh weight of seedlings. **Conclusion:** The *p*-coumaric acid treatment (0-500 ppm) reduced the percentage of germination, radicle length, hypocotyl length and fresh weight of soybean sprout.

Key words: *p*-coumaric acid, soybean, sprout, morphological

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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

The positive effects of soybeans on human health have led to increased interest in soybeans in recent years. The low incidence of cancer and cardiovascular disease in Asia is thought to be related to the habit of Asian populations consuming soybeans, an important legume source of isoflavones[1].

In soybeans, isoflavones are classified as secondary metabolites that produce antioxidants and act as chemopreventive agents, reducing the risk of cardiovascular disease and menopausal syndrome in women, as well as preventing osteoporosis and premature aging[2-5]. Daidzein, genistein and glycitein are major isoflavones in soybean seeds and all soybean seedling organs, synthesized through the phenylpropanoid biosynthesis pathway[6,7]. Higher levels of isoflavones are found in soybean seeds, especially in the hypocotyls, which will grow into plants. In addition, isoflavones are also found in the cotyledons, which will grow into the first leaves of soybean plants[8].

During germination, bioactive compounds can be enhanced through exogenous precursor treatment so that the synthesis of certain bioactive compounds can be stimulated. Target bioactive compounds can be metabolized and produced in plant systems through the role of precursors[8]. In the phenylpropanoid pathway, *p*-coumaric acid ($C_9H_8O_3$) is an upstream metabolite that plays a role in the biosynthesis of various derivatives, including anthocyanins, flavonoids and stilbenes[9,10]. Phenylalanine, tyrosine and tryptophan, as amino acids produced also function as functional precursors for various secondary metabolites needed by plants and humans[11]. The *p*-coumaric acid acts as a precursor in the biosynthesis of secondary metabolites, such as polyphenols, flavonoids and some polyketides[12].

Research on the role of *p*-coumaric acid in the morphological characteristics of soybean sprouts is still limited. Based on this background, this research aims to identify the role of *p*-coumaric acid on the morphological characteristics of sprouts of several soybean varieties.

MATERIALS AND METHODS

Planting of soybean seeds and morphological analysis of soybean sprouts were carried out at the Biotechnology Laboratory, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.

Materials and tools: The materials used were soybean varieties, *p*-coumaric acid, NaOH and distilled water. The tools used were seedling trays, cotton, a hand sprayer, treatment markers, an analytical balance, a magnetic stirrer, a ruler and office stationery.

Research methods: The study used a Factorial Completely Randomized Design with 2 factors. The first factor was precursor treatment (no precursor; 250 ppm *p*-coumaric acid; 500 ppm *p*-coumaric acid). The second factor was soybean variety (Dering 1, Devon 1 and Devon 2). The *p*-coumaric acid, according to the treatment concentration, was weighed and placed into a beaker. Then, 1 drop of NaOH was added and stirred until the coumaric acid dissolved, then water was added to reach 100 mL. To ensure a homogeneous solution, stirring was carried out with a magnetic stirrer for 2 min. The coumaric acid precursor solution treatment was carried out by soaking the seeds for 24 hrs at the appropriate concentration for the treatment. The seeds were then planted in seedling trays for 7 days.

During germination, humidity was maintained by spraying the seeds with a hand sprayer. The morphological characteristics observed included the percentage of germination, hypocotyl length, radicle length, fresh weight and dry weight of soybean seedlings 7 days after planting.

The germination percentage was calculated using the formula as described by Onofri *et al*.[13].
as follows:

$$\text{Germination (\%)} = \frac{\text{Final number of seeding emerged}}{\text{Total number of seeds sown}} \times 100$$

Hypocotyl length was measured by placing the seedling on a flat surface, then measuring from the base of the hypocotyl (the point where the hypocotyl meets the root or the lowest point of the stem below the cotyledons) to the base of the cotyledons (the point where the hypocotyl meets the seed leaves) using a ruler[14]. To measure radicle length, the seedling was first cleaned of the growing medium by spraying it with water. The radicle was measured from the base of the stem (the root boundary) to the tip of the longest radicle using a ruler or precision measuring instrument[15]. The data obtained were analyzed using analysis of variance. If significant differences were found, Duncan's Multiple Range Test was performed at $\alpha = 5\%$.

RESULTS AND DISCUSSION

Germination percentage: Variety and *p*-coumaric acid significantly affected the germination percentage of several soybean varieties, while the interaction between variety and

Table 1: Germination percentage of several soybean varieties with the application of *p*-coumaric acid

	p-Coumaric acid (C)			
Variety (V)	0 ppm (C_0) (%)	250 ppm (C_1) (%)	500 ppm (C_2) (%)	Mean
Dering-1 (V_1)	92.1	90.2	72.6	85.0[b]
Devon-1 (V_2)	95.1	92.1	80.3	89.1[a]
Devon-2 (V_3)	84.3	85.3	65.7	78.3[c]
Mean	90.5[a]	89.2[a]	72.8[b]	

Numbers followed by the same letter at the same column or row indicate no significant difference based on Duncan's Multiple Range Test at α=5%

p-coumaric acid had no significant effect on germination percentage (Table 1). The Devon 1 variety had a higher germination percentage than Dering 1 and Devon 2. This indicates that genetic differences (genetic variability) influence the physiological and morphological characteristics of seeds, which are caused by differences in seed vigor and viability. Seed vigor is the ability of seeds to germinate quickly and uniformly and produce healthy plants under various environmental conditions. Different varieties have different genes that control seed quality. In this study, the Devon 1 variety, which had the highest germination percentage (89%), may have genetically higher viability and vigor. This means its seeds are healthier, more resistant to stress and have more readily available food reserves for mobilization during germination. The other varieties (Dering 1 and Devon 2) may have genetically lower vigor.

In addition, the genetically inherited physical and chemical characteristics of seeds significantly influence germination, related to the permeability of the seed coat, as soybeans must absorb water (imbibition) to initiate germination. Varieties with more water-permeable seed coats will germinate more quickly and uniformly. Meanwhile, varieties with harder or more water-resistant seed coats (called hard seeds) will have a lower or slower germination percentage. In addition, each variety has a slightly different profile of hormones and chemical compounds. Varieties that contain more natural inhibitory compounds or require a longer inactivation time (e.g., internal phenolic acids, such as coumaric acid in this context) will have a lower germination percentage[15,16].

The Dering 1, Devon 1 and Devon 2 varieties are genetically distinct. The superiority of the Devon 1 variety in germination percentage is a manifestation of its superior genetic traits, which include high seed vigor, optimal seed coat and good metabolic efficiency compared to Dering 1 and Devon 2. The efficient utilization of food reserves also influences germination percentage because the germination process requires the mobilization of food reserves (protein, fat and carbohydrates) stored in the cotyledons to support embryo growth. Different varieties have different levels and rates of enzyme synthesis and activity (such as amylase, protease and lipase) during imbibition. The Dering variety may have a more efficient and faster enzyme system in breaking down food reserves into forms usable by the embryo, ultimately supporting a higher germination percentage. Table 1 also shows that increasing *p*-coumaric acid application (0-500 ppm) significantly reduced germination percentage (90.5-72.8%).

This phenomenon demonstrates that *p*-coumaric acid, at higher concentrations, acts as a germination inhibitor in soybeans. The decrease in soybean germination percentage due to increasing *p*-coumaric acid concentrations is due to the phytotoxic nature of this phenolic compound, which acts as an allelopathic agent. The mechanism that can explain the inhibition of germination due to increasing coumaric acid concentrations is the disruption of enzymatic metabolism; it inhibits water absorption and damages cell structure and root growth.

The *p*-coumaric acid causes enzymatic metabolic disruption because coumaric acid, as a phenolic compound, can disrupt important metabolic processes that occur during germination, such as inhibiting or deactivating the activity of key enzymes needed to break down food reserves (such as starch, protein and fat) in seeds. The breakdown of these food reserves is crucial for supplying energy and raw materials for the growth of the embryo and the emerging young root (radicle).

Damage to cell structure and root growth, such as at higher concentrations (concentrations up to 500 ppm), *p*-coumaric acid acts as a toxin to rapidly dividing cells. The direct phytotoxicity of *p*-coumaric acid is that this compound can damage cell membranes, interfere with cell division and specifically inhibit root elongation and seedling growth. If root (radicle) growth is significantly impaired, seeds are considered to have failed to germinate or produce abnormal seedlings[17,18].

Hypocotyl length: Based on Table 2, it can be seen that variety and *p*-coumaric acid significantly affect the hypocotyl length of several soybean varieties, while the interaction between variety and coumaric acid does not significantly affect hypocotyl length. Dering variety has the highest hypocotyl length, which is significantly different from the Devon 1 and Devon 2 varieties. Increasing coumaric acid from 0 to 500 ppm decreases the length of the soybean hypocotyl.

Table 2: Hypocotyl length of several soybean varieties sprouts with the application of *p*-coumaric acid

	p-Coumaric acid (C)			
Variety (V)	0 ppm (C_0) (cm)	250 ppm (C_1) (cm)	500 ppm (C_2) (cm)	Mean
Dering- 1(V_1)	8.75	8.60	4.81	7.39[a]
Devon-1 (V_2)	7.45	6.56	2.96	5.66[b]
Devon-2 (V_3)	7.48	4.75	3.52	5.25[b]
Mean	7.89[a]	6.64[b]	3.76[c]	

Numbers followed by the same letter at the same column or row indicate no significant difference based on Duncan's Multiple Range Test at α=5%

Table 3: Radicle length of several soybean varieties sprouts with the application of *p*-coumaric acid

	p-Coumaric acid (C)			
Variety (V)	0 ppm (C_0) (cm)	250 ppm (C_1) (cm)	500 ppm (C_2) (cm)	Mean
Dering-1 (V_1)	4.65	4.64	2.43	3.91[a]
Devon-1 (V_2)	4.70	3.93	3.16	3.93[a]
Devon-2 (V_3)	3.50	2.75	2.07	2.78[b]
Mean	4.29[a]	3.77[a]	2.55[b]	

Numbers followed by the same letter at the same column or row indicate no significant difference based on Duncan's Multiple Range Test at α=5%

The difference in hypocotyl length between Dering (the tallest), Devon 1 and Devon 2 is due to genetic variability that affects seedling characteristics in the form of genetic growth potential.

Dering 1 variety genetically has a stronger growth capacity (including cell elongation potential) than the two Devon varieties. In addition, stress tolerance in the Dering 1 variety may have better biochemical defense mechanisms, such as antioxident systems or detoxification capabilities, which make it more tolerant to the toxic effects of *p*-coumaric acid (although the effect remains significant) and able to maintain hypocotyl length better. Regarding seed quality (vigor), it is suspected that the Dering 1 variety has seeds with superior vigor, which allows for faster and more efficient mobilization of food reserves, thereby supporting longer hypocotyl growth from the outset. Increasing coumaric acid concentrations (from 0 to 500 ppm) reduced soybean hypocotyl length due to its properties as an allelochemical or phytotoxin that disrupts cellular and hormonal growth in seedlings. The mechanism of hypocotyl growth inhibition due to coumaric acid, a phenolic compound, occurs through several mechanisms: Interference with growth hormone (auxin); disruption of cell walls and water uptake; and direct toxicity and oxidative stress[19,20].

Hypocotyl elongation growth is highly dependent on the hormone auxin. The *p*-coumaric acid is known to disrupt auxin metabolism because it can act as an inhibitor of auxin cofactors or affect auxin transport, thereby reducing the effectiveness of this hormone in promoting cell elongation. Furthermore, coumaric acid can induce oxidase enzymes by increasing the activity of auxin oxidase, which breaks down or inactivates auxin. Reducing active auxin levels directly inhibits hypocotyl elongation.

Radicle length: Based on Table 3, it can be seen that variety and *p*-coumaric acid significantly affected the radicle length of several soybean varieties, while the interaction between variety and coumaric acid did not significantly affect radicle length. The Dering 1 variety had the highest radicle length, which was significantly different from the Devon 1 and Devon 2 varieties. Increasing coumaric acid from 0 to 500 ppm decreased the length of soybean radicles.

The Dering 1 and Devon 1 varieties genetically have an inherently higher radicle growth potential (vigor) in normal or mildly stressed environments, compared to the Devon 2 variety. This difference is a genetic trait. The radicle (potential root) is the most sensitive part of the seedling to allelochemical compounds in the environment. The decrease in radicle length due to increased coumaric acid occurs due to this compound's phytotoxin properties, which directly interfere with root cell division and elongation. The *p*-coumaric acid, a phenolic compound, is known to inhibit mitosis (cell division) in meristem tissue, which is the active growth zone at the root tip.

At high concentrations (500 ppm), *p*-coumaric acid can damage cellular structures and disrupt the cell cycle, drastically reducing the number of new cells produced for root elongation. The primary function of *p*-coumaric acid in plant metabolism is as a precursor (raw material) for lignin biosynthesis. Excessive coumaric acid concentrations will stimulate increased lignin production and deposition in root cell walls. Lignin makes cell walls rigid and strong, but effectively prevents root cells from elongating. Radicles that cannot elongate will become shorter.

The *p*-coumaric acid can induce root stress by increasing the production of Reactive Oxygen Species (ROS), such as free radicals, which damage cell membranes,

Fig. 1: The appearance of sprouts from several soybean varieties treated with *p*-coumaric acid
V1: Dering 1 variety, V2: Devon 1 variety, V3: Devon 2 variety, C0: Without *p*-coumaric acid, C1: 250 ppm of *p*-coumaric acid and C2: 500 ppm of *p*-coumaric acid

Table 4: Fresh weight of several soybean varieties sprouts with the application of *p*-coumaric acid

	p-Coumaric acid (C)			
Variety (V)	0 ppm (C_0) (g)	250 ppm (C_1) (g)	500 ppm (C_2) (g)	Mean
Dering-1 (V_1)	0.41	0.39	0.27	0.36[c]
Devon-1 (V_2)	0.59	0.55	0.44	0.53[a]
Devon-2 (V_3)	0.51	0.44	0.39	0.45[b]
Mean	0.50[a]	0.46[a]	0.37[b]	

Numbers followed by the same letter at the same column or row indicate no significant difference based on Duncan's Multiple Range Test at α=5%

DNA and proteins. This damage disrupts water and nutrient uptake and inhibits normal metabolic functions necessary for growth. Furthermore, root growth is tightly regulated by the balance of hormones, particularly auxins and cytokinins. Phenolic compounds, including coumaric acid, can interfere with root transport[21].

Fresh weight of soybean sprout: Table 4 showed that the differences in varieties and *p*-coumaric acid treatment significantly affected the fresh weight of soybean sprouts. The Devon-1 variety had the highest fresh weight of sprouts, which was significantly different from the fresh weight of sprouts of Devon-2 and Dering 1. This was caused by differences in seed size, which is related to seed nutrient reserves. Devon-1 soybeans, which are classified as large seeds, have higher seed nutrient reserves than Dering 1 and Devon-2, resulting in a higher seed weight for Devon-2. It can be said that seed weight is greatly influenced by seed size, composition

Increased concentrations of *p*-coumaric acid cause a decrease in the fresh weight of soybean seedlings because this compound is allelopathic and phenolically toxic, which can inhibit root growth, water absorption and enzyme and growth hormone activity. The *p*-coumaric acid is one of the phenolic acids released by roots or plant residues. In high concentrations, it can act as an inhibitor to other plants, including soybean seedlings. The mechanism is similar to abiotic stress that causing disruption of primary and secondary metabolism. In addition, coumaric acid can alter the permeability of root plasma membranes, thereby disrupting water and mineral absorption and also inhibiting aquaporin function (water transport proteins), causing cell turgor to decrease, thereby inhibiting tissue growth. Phenolic acids, including *p*-coumaric acid, can inhibit respiratory enzymes such as dehydrogenase, thereby reducing the energy (ATP) available for growth and inhibiting the activity of amylase and protease during germination, which serve to break down food reserves in seeds. As a result, the supply of energy and raw materials for the formation of new tissue is disrupted[22,23].

The appearance of sprouts from several soybean varieties treated with coumaric acid can be seen in Fig. 1, which shows variations in the morphology of soybean sprouts due to *p*-coumaric acid treatment.

CONCLUSION

The results showed that the difference in varieties significantly affected the percentage of germination, radicle length, hypocotyl length and fresh weight of sprouts. Devon 1 variety has a higher germination percentage, radicle length and fresh weight of sprouts compared to Devon 2 and Dering 1. The increasing dose of coumaric acid treatment (0-500 ppm) significantly reduced the percentage of germination, radicle length, hypocotyl length and fresh weight of seedlings.

SIGNIFICANCE STATEMENT

This study highlights the influence of *p*-coumaric acid on the early growth performance of multiple soybean varieties. By determining the concentration ranges that suppress or support germination-related parameters, the findings provide valuable insights for future research aiming to optimize precursor use in soybean sprout enhancement. These results are particularly relevant for studies investigating p-coumaric acid as a biochemical precursor in isoflavone synthesis and its potential role in improving the nutritional value of soybean-based products.

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