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Glutamic acid and L-phenylalanine as potential elicitors for quality soybean sprouts

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ABSRACT
Elicitation seems to be a promising alternative to other conventional biotechnological techniques used for improving bioactive compounds and biological activities of sprouts. The purpose of this study was to evaluate the effect of amino acids as elicitors such as glutamic acid (1 mM, 5 mM, 10 mM) and L-phenylalanine (0.1 mM, 0.5 mM, 1.0 mM) on oil content, nutritional parameters such as ascorbic acid and beta-carotene; and anti-nutritional parameters such as saponin and phytic acid for soybean sprouts. The results revealed that maximum recovery of oil content in soybean sprouts were obtained at 1 mM glutamic acid (24.40%). The effect of elicitation on ascorbic acid and beta-carotene during the sprouting of soybean was found highest at 1.0 mM L-phenylalanine elicitor as 54.42 mg/100g and 4.88 µg/g respectively. The anti-nutritional parameters such as saponin and phytic acid were reduced after the treatment of elicitor during the sprouting process in soybean sprouts. The lowest values of saponin content and phytic acid were examined as 3.27 mg/g and 1.34 mg/g at the highest concentration of L-phenylalanine and glutamic acid respectively. The use of these amino acids as chemical elicitors significantly improves the quality of soybean sprouts.
Key words: Elicitation, glutamic acid, L-phenylalanine, soybean, sprouts

INTRODUCTION

Plants are a crucial component of our diet because they provide vitamins, minerals, and other nutrients or bioactive that are needed for healthy physiological function. To promote the production of secondary metabolites in plants, many biotechnological tools have been employed, such as the enhancement of culture conditions, cell cultures, micropropagation, hairy root culture, elicitation, precursor feeding, or biotransformation. Among all of them, the elicitation strategy stands out as a potent method for influencing metabolic pathways to boost the production of bioactive compounds (Halder*et al.*, 2019). Elicitation appears to be a promising alternative to other traditional biotechnological techniques used to improve sprout bioactive compounds and biological activities.

Elicitors are substances that cause physiological and morphological changes in the target plant. As a result, elicitors are commonly used to increase the yield of secondary metabolites with intriguing bioactivities in plant cells, tissues, or the entire plant. According to research by Gan*et al.*, (2017) during seed germination, the concentration of various bioactive compounds increases, giving sprouts access to a variety of bioactivities including antioxidant, antidiabetic, anti-inflammatory, hypolipidemic, and anticarcinogenic activities. Germination can cause catabolism, degradation of major macronutrients, and a decrease in anti-nutritional and non-digestible factors.The year round growth in demand for sprouts forces providers to look for new ways to provide supply for fresh sprouts around the year(Bishnoi and Ray.,2023).

MATERIALS AND METHODS

The study was conducted in the Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India. The seeds of the soybean (*Glycine max*) variety SL-688 were procured from the Department of Plant Breeding and Genetics, Punjab Agriculture University, Punjab. For further work, the seeds were manually cleaned to remove spoiled, immature, or broken seeds, stone, dust, dirt, sand, stubbles, and other extraneous material and stored in a deep freezer (-18℃). The seeds were initially sterilized in 1% (v/v) sodium hypochlorite for 30 minutes, then drained and washed three times with distilled water so they reached a neutral pH. The seeds were then placed in distilled water (1:5) and soaked for 2 h at 26℃. Seeds were germinated in a Seed Germinator (Dewsil Company) on Petri dishes (Diameter 125 mm) lined with absorbent paper at 24℃ temperatures, 83% relative humidity (RH), for 3.5 days. Seedlings were watered daily two times with distilled water for control samples and glutamic acid (1 mM, 5 mM, 10 mM) and L-phenylalanine (0.1 mM, 0.5 mM, 1.0 mM) for elicitor samples. The content of oil, ascorbic acid, beta-carotene, saponin and phytic acid for sprouts was estimated using standard methods.

RESULTS AND DISCUSSION

In this work, the effect of various elicitors on the nutritional and anti-nutritional properties of soybean sprouts was examined. The elicitation chemicals used were glutamic acid at concentration of 1 mM, 5 mM, 10 mM and L-phenylalanine at concentration of 0.1 mM, 0.5 mM, 1.0mM. This study aimed to systematically evaluate the effect of different concentrations of amino acid elicitors, on the oil content, nutritional parameters(ascorbic acid and beta-carotene); and anti-nutritional parameters (saponin and phytic acid) for soybean sprouts.

As illustrated in Table 1 different concentrations of elicitors have been shown a significant effect on the oil content of sprouted soybeans. Glutamic acid concentration at 1 mM showed 24.40 % of oil content. With increase in concentration of glutamic acid, there was a non-significant effect found on the oil content. In the case of L-phenylalanine, a slight reduction has been examined, at 0.1 mM, 0.5 mM, and 1.0 mM concentration the value of oil content was reported as 23.14 %, 20.52 %, and 19.93 %, respectively.

Table 1: Effect of using chemical elicitation on quality parameters during sprouting of soybean

Sample	Concentration of chemical elicitations	Oil (%)	Ascorbic acid (mg/100g)	Beta carotene (µg/g)	Saponin (mg/g)	Phytic acid (mg/g)
Control	-	20.31 ± 0.06^{b}	27.80 ± 0.02^{a}	4.14 ± 0.03^{a}	4.70 ± 0.02^{f}	2.89 ± 0.02^{g}
Glutamic acid	1 mM	24.40 ± 0.08^{f}	30.34 ± 0.02^{b}	4.19 ± 0.01^{b}	4.23 ± 0.03^{e}	2.43 ± 0.02^{e}
	5 mM	24.29 ± 0.06^{f}	39.71 ± 0.35^{d}	4.34 ± 0.03^{c}	3.96 ± 0.04^{d}	1.87 ± 0.02^{c}
	10 mM	23.55 ± 0.13^{e}	51.94 ± 0.11^{f}	4.53 ± 0.02^{d}	3.44 ± 0.02^{b}	1.34 ± 0.02^{a}
L-phenylalanine	0.1 mM	23.14 ± 0.04^{d}	35.34 ± 0.01^{c}	4.34 ± 0.02^{c}	4.18 ± 0.02^{e}	2.53 ± 0.02^{f}
	0.5 mM	20.52 ± 0.03^{c}	43.23 ± 0.01^{e}	4.63 ± 0.03^{e}	3.86 ± 0.02^{c}	2.13 ± 0.03^{d}
	1.0 mM	19.93 ± 0.02^{a}	54.42 ± 0.02^{g}	4.88 ± 0.02^{f}	3.27 ± 0.06^{a}	1.63 ± 0.03^{b}

The values are represented in mean ± standard deviation (SD) of triplicate data, superscripts represent significant difference at $p<0.05$

The ascorbic acid was significantly influenced by amino acid elicitors used during the sprouting process as represented in Table 1. The results demonstrated that the glutamic acid at 1 mM, 5 mM, and 10 mM showed 30.34 %, 39.71 %, and 51.94 % of ascorbic acid content respectively. A similar trend was found in the case of L-phenylalanine, at 0.1 mM, 0.5 mM, and 1.0 mM concentrations in which 35.34 %, 43.23 %, and 54.42 % of ascorbic acid were examined that were significantly ($p<0.05$) higher as compared to the control sample. The results indicated that the value of ascorbic acid after using amino acids as elicitors was significantly two-fold of the control of soybean sprouts at their higher concentrations (10 and 1.0 mM).The results were in agreement with Koodkaew*et al.* (2020) who explained that ascorbic acid content was increased in the case of sunflower sprouts in various elicitors. Lobiuc*et al.* (2017) report that ascorbic acid is a primary metabolite and is considered a water-soluble antioxidant compound, and Liu *et al.* (2019) stated that an increase in ascorbic acid content might be due to the induction of some genes that are related to ascorbic acid biosynthesis when sprouts were treated with elicitors. During the sprouting process, L-galactono-lactone dehydrogenase (GLDH) is considered one of the key enzymes which support the synthesis of ascorbic acid, so this enzyme was induced by various elicitation treatments.

When amino acids (glutamic acid and L-phenylalanine) were used as elicitors for soybean sprouts, as observed in Table 1, at lower concentrations (1 and 0.1 mM), 4.19 µg/g and 4.34 µg/g beta-carotene was observed which showed 1.20% and 4.83% enhancement as compared to control. As the concentration of elicitors was increased, the content of beta-carotene was significantly increased. The present results were in agreement with the research conducted by Jeong*et al.* (2018), who conducted a study on the effect of elicitors on buckwheat sprouts that demonstrated treatment of sucrose as elicitor had a significant increase the content of beta-carotene with respect to control. This might be due to the impact of elicitors which induced the biosynthesis process of abscisic acid from carotenoids to regulate the plant growth and raise amount of carotene. According to Natella*et al.* (2016), their results were contrary to the present findings, mannitol (88 mM) and sucrose (176 mM) treatment indicated a significant reduction of beta-carotene content at 30% and 61%, respectively.

The processes of germination with elicitor show that anti-nutritional parameters such as saponin and phytic acid were reduced significantly ($p<0.05$) as can observed in Table 1. The saponins are natural complex compounds present in legumes and pulses. Glutamic acid and L-phenylalanine have been indicating a significant impact on the saponin content in soybean sprouts. The saponin content in soybean sprouts was found 4.23 mg/g, 3.96 mg/g, and 3.44 mg/g at 1 mM, 5 mM, and 10 mM concentrations of glutamic acid, with gradual increases in the concentration of glutamic acid there were constant decreases in the value of saponin content. The impact of the L-phenylalanine elicitor was also found as identical, a lower concentration (0.1 mM) of this elicitor showed low impact and with an increase in its concentration from 0.5 to 1.0 mM, the value of saponin content was gradually decreased (3.86 mg/g and 3.27 mg/g).

Phytic acid is considered the primary storage compound of phosphorus in legume seeds, which binds many elements like calcium, iron, zinc, potassium, etc., and makes them insoluble and unavailable as nutrition. However, the germination process hydrolyses phytic acid by endogenous phytase to release phosphate and other micronutrients (Sharma*et al.*, 2022).

During the germination process, inositol hexa-phosphate is hydrolyzed into the lower molecular weight compound, which reduces the level of phytic acid.

Treatment with glutamic acid and L-phenylalanine, decreased the value of phytic acid with an increase in the concentration of these elicitors. The seed treatment with the lowest concentration (1 and 0.1 mM) has shown a slight impact on phytic acid for glutamic acid and L-phenylalanine at 2.43 mg/g and 2.53 mg/g compared to control. When the concentration of glutamic acid and L-phenylalanine increased, the phytic acid values were significantly ($p<0.05$) reduced.

CONCLUSION

The findings of this research concluded that the various amino acids as elicitors (glutamic acid, and L-phenylalanine) have shown a significant($p<0.05$) impact on nutritional and antinutritional parameters (oil content, ascorbic acid, beta-carotene, saponin andphytic acid) during the elicitation process of the soybean sprouts. It was concluded that maximum recovery of oil content in soybean sprouts was obtained at 1 mM glutamic acid (24.40%). The effect of elicitation on ascorbic acid and beta-carotene during the sprouting of soybean was found highest at 1.0 mM L-phenylalanine elicitor as 54.42 mg/100g and 4.88 µg/g respectively. The anti-nutritional parameters such as saponin and phytic acid were reduced after the treatment of elicitor during the sprouting process in soybean sprouts. The lowest values of saponin content and phytic acid were examined as 3.27 mg/g and 1.34 mg/g at the highest concentration of L-phenylalanine and glutamic acid respectively. Therefore, the application of chemical elicitors significantly improves the quality of sprouts and it can be concluded from the present finding that amino acids such as glutamic acid and L-phenylalanine as an elicitor enhanced the nutritional factor and reduced the anti-nutritional factors in soybean sprouts.

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