



Research article

Improvement of stilbene constituents and nutritional quality of peanut sprouts using abiotic elicitation and germination

Phadtraphorn Chayjarung, Supanich Wongsathan, Chonnikan Tothong, Chanyanut Pankaew, Pakwuan Wongshaya, Apinun Limmongkon*

Department of Biochemistry, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand

Article Info

Article history:

Received 20 April 2023

Revised 15 June 2023

Accepted 9 November 2023

Available online 31 December 2023

Keywords:

Antioxidant activity,
Peanut sprout,
Seed priming,
trans-arachidin-1,
trans-resveratrol

Abstract

Importance of the work: Elicitor treatment has been widely used to improve bioactive compounds in plants. However, very few studies have reported the use of an elicitor treatment in the germination process to improve peanut quality.

Objectives: To evaluate the stilbene compounds and bioactivity of peanut sprouts after seed priming using different elicitors.

Materials & Methods: Peanut seeds were primed using various elicitors: chitosan (CHT), methyl jasmonate (MeJA), methyl- β -cyclodextrin (CD) or a combination of all three (CHT+MeJA+CD). Germination was carried out for 3 d. The antioxidant activity, total phenolic compound, stilbene content and the biochemical profiles in the peanut sprouts were investigated.

Results: After germinating for 1–3 d, the antioxidant activities of all elicitor-treated peanut samples exhibited 1.46–2.12-fold increases compared to the untreated control. The highest contents of *trans*-resveratrol were produced by the peanuts treated with CHT and CHT+MeJA+CD, with 7.72 and 11.48-fold increases, respectively, compared to the untreated control. The highest contents of *trans*-arachidin-1 and *trans*-arachidin-3 were in the peanuts treated with CHT, MeJA and CHT+MeJA+CD on day 3 of germination. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile indicated the protein pattern transformed from a high molecular weight (MW) to a low MW during germination.

Main finding: Combining elicitor priming and germination approaches proved to be an effective strategy that improved the phytochemical profile of peanut sprouts for functional food applications.

* Corresponding author.

E-mail address: apinunl@nu.ac.th (A. Limmongkon)

online 2452-316X print 2468-1458/Copyright © 2023. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), production and hosting by Kasetsart University Research and Development Institute on behalf of Kasetsart University.

<https://doi.org/10.34044/j.anres.2023.57.6.03>

Introduction

The peanut (*Arachis hypogaea*) is an important source of beneficial nutrients such as carbohydrates, proteins and various vitamins (Arya et al., 2016). In addition, the peanut is an efficient nutraceutical agent with a wide variety of bioactive compounds, such as flavonoids, stilbene compounds, phenolic acids and phytosterols (Lopes et al., 2011). One of the most studied stilbene compounds is *trans*-resveratrol, which has been associated with multiple health benefits, such as the prevention of cancer (Aluyen et al., 2012), inflammation, cardiovascular and neurodegenerative diseases (Hung et al., 2000). In addition to *trans*-resveratrol, prenylated forms of stilbenoids, such as *trans*-arachidin-1, and *trans*-arachidin-3, have been investigated in sliced germinated peanuts incubated using artificial aeration. The prenylated stilbenoids can lead to an increase in the lipophilic property of the stilbene molecule, thereby enhancing membrane permeability, resulting in a higher cellular uptake than the non-prenylated form (Valletta et al., 2021). *Trans*-arachidin-1 and *trans*-arachidin-3 have shown favorable bioactivity compared to *trans*-resveratrol, with *trans*-arachidin-1 reported to promote anti-inflammatory activity in endothelial cells by reducing the tumor necrosis factor and increasing the expression of phase II detoxifying enzymes (Chen et al., 2018). *Trans*-arachidin-1, isolated from germinating peanut kernels, exhibits anti-cancer properties by inducing programmed cell death in human leukemia HL-60 cells through both caspase-dependent and caspase-independent pathways (Huang et al., 2010).

The phytoalexin stilbene compound has been reported to provide a defensive response against biotic and abiotic stress (Sák et al., 2021). Peanuts treated with ultraviolet (UV) light and ultrasound had increased resveratrol, piceid and total stilbene compounds compared to the untreated control group (Sales and Resurreccion, 2009). In addition to investigating stilbene compounds directly from peanut plants, their production was achieved through the elicitation of peanut tissue culture, such as callus, cell suspension and peanut hairy root systems, with chitin being used as an elicitor to induce *trans*-resveratrol and *trans*-piceatannol in peanut callus culture, with the results demonstrating that it could induce a higher amount of *trans*-resveratrol compared to sterilized fungi (Yang et al., 2010). The enhancement of *trans*-resveratrol, *trans*-arachidin-1 and *trans*-arachidin-3 was reported in peanut hairy root culture co-treatment with methyl jasmonate (MeJA) and methyl- α -cyclodextrin (CD) (Pilaisangsuee et al., 2018). MeJA is

a phytohormone-like compound and generally considered as a signal transduction for mediating the physiological processes of plants involved in responses against environmental stress (Cheong and Choi, 2003). CD has reported to be an effective elicitor for *trans*-resveratrol production in *Vitis vinifera* cell culture (Bru et al., 2006). This was probably due to its cyclic oligosaccharide structure which resembles a fungal cell wall component and could play a key role in plant elicitation (Bru et al., 2006).

A strategy involving elicitor treatment is considered a powerful tool for increasing the bioactive phytochemical content in germinated seeds. Seed priming by pre-treating seeds with various elicitors, such as MeJA or other stressors, is a promising approach for improving plant adaptation and inducing secondary metabolites in response to stress. For example, rice seed priming using MeJA resulted in a greater increase in total phenolic content, antioxidant activity and rice drought-responsive gene expression compared to unprimed seeds (Samota et al., 2017). Several studies have provided evidence indicating that the germination process facilitates the activation of diverse plant metabolites in kernels (Idowu et al., 2019; Núñez-Gastélum et al., 2023). Sufficient water content is essential for triggering plant phytoalexin production (Paparella et al., 2015). The *trans*-resveratrol content in peanut sprout was reported to significantly increase with an extended germination time (Wang et al., 2005). Limmongkon et al. (2017) investigated the *trans*-resveratrol content in different peanut cultivars, with their results indicating that the antioxidant activity, total phenolic compounds and *trans*-resveratrol content increased with an extended germination period. Liquid chromatography-mass spectrometry/mass spectrometry results indicated the presence of stilbene compounds, such as *trans*-resveratrol, *trans*-arachidin-1 and *trans*-arachidin-3 in peanut kernels germinated during 2 d (Limmongkon et al., 2018).

To date, the enhancement has been reported of bioactive compounds in peanut seed using the germination process (Yang et al., 2019). However, very few studies have reported the use of elicitor treatment in the germination process to improve peanut quality. Therefore, the purpose of the current study was to investigate the possible use of various elicitors in the priming treatment to improve stilbene compounds, especially *trans*-arachidin-1 and *trans*-arachidin-3 of peanut sprouts. The exogenous elicitors CHT, MeJA, CD and a combination of all three (CHT+MeJA+CD) improved the stilbene compounds in peanut hairy root culture (Chayjarung et al., 2021), when applied as priming substances in the peanut

germination process. The current study investigated the antioxidant activity, total phenolic compounds, stilbene content and the relevant biochemical substance profiles in peanut sprout crude extract to provide a perspective on the nutritional benefits and improvement of peanut sprouts as potent functional food.

Materials and Methods

Peanut sprout and elicitation

The peanut cultivar Kalasin2 was provided by Mrs Nuntaporn Suksamrahn (Rai Suksamrahn, Kampaeng Phet, Thailand). The surface of the seeds was sterilized using 0.3% Clorox solution for 10 min and subsequently washed with water three times. The seeds were divided into five treatment groups with a total of 600 seeds in each group. All seeds in each treatment group were primed in the following solutions for 10 hr: 1) control group with water; 2) CHT group with 150 mg/L chitosan; 3) MeJA group with 100 μ M methyl jasmonate; 4) CD group with 6.87 mM cyclodextrin; and 5) simultaneous combination of CHT+MeJA+CD group. After priming for 10 hr, a total of 50 seeds were collected on day 0. The germination process was performed in the dark by covering the seeds with a moistened paper towel, followed by irrigation with water for 1 d, 2 d or 3 d of germination. In total, 50 seeds were collected at each time point for every treatment group. All experiments were performed in triplicate. The germinated peanut sprouts were collected, the seed coats peeled off and the radicle length measured. Subsequently, all seeds in each group were dried in an oven at 60°C and pulverized to obtain peanut sprout powder. For each treatment group, 1.5 g of peanut sprout powder was pre-processed by defatting. This was achieved by soaking the powder in 7.5 mL of hexane for 3 hr, followed by filtration to remove the hexane. The defatting process was repeated twice. Then, the filtered peanut cake was extracted using a mixture of ethyl acetate-to-acetone (4:1). The extraction process was repeated three times and the solvent was evaporated using a rotary evaporator at 40°C to obtain the crude peanut extract.

Total soluble sugar assay

The total soluble sugar was determined according to the anthrone method described by Haldar et al. (2017). A 1 mL sample was mixed with 4 mL of anthrone reagent.

The absorbance was measured at 620 nm and the soluble sugar content was calculated against the glucose standard. The soluble sugar content was expressed as milligrams per gram dry weight of sample.

Protein solubility

Soluble proteins in the peanut sprout samples were extracted using the alkaline extraction method by adding 0.1M NaOH to peanut sprout powders (1:20 weight per volume; w/v) and incubating at 60°C with continuous shaking for 2 hr. The soluble protein was determined using the Bio-Rad protein dye reagent (Bio-Rad Laboratories; Hercules, CA, USA). The protein molecular mass was determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie Blue staining used to visualize the abundance of the major soluble protein in all samples.

2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid antioxidant assay

The 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay was performed by mixing crude extract with 7 mM ABTS^{•+} and incubating at room temperature in the dark for 6 min. The absorbance was measured at 734 nm and the antioxidant capacity was expressed as TEAC (micro moles of Trolox per gram dry weight of crude extract).

Total phenolic compound assay

The Folin-Ciocalteu method was used to determine the total phenolic compounds. The reaction mixture, containing the Folin reagent, 20% w/v sodium carbonate and peanut crude extract, was incubated in the dark for 30 min. The absorbance was measured at 765 nm and the total phenolic content was expressed as GAE (micrograms gallic acid per gram dry weight of crude extract).

High-performance liquid chromatography

The stilbene compounds were analyzed using high-performance liquid chromatography (HPLC), as described by Somboon et al. (2019). A reverse phase C18 (2) 100 Å column, 4.6×250 mm, 5 μ m (Phenomenex; Torrance, CA, USA) was used for peanut crude extract separation. The *trans*-resveratrol was detected using a UV detector at a wavelength of 306 nm, while the prenylated forms of *trans*-arachidin-1 and *trans*-arachidin-3 were detected at the longer wavelength of 340 nm.

Statistical analysis

All experiments were performed in triplicate and data were presented as mean \pm SD. Statistical analysis was performed employing one-way analysis of variance. Significant differences were tested at $p < 0.05$ based on Fisher's least significant difference (LSD) test. The analyses were conducted employing the SPSS software (version 23.0; SPSS Inc.; Chicago, IL, USA).

Results

Seed germination rate and root length

After priming the peanut seeds with water, CHT, MeJA, CD or the combination of all three (CHT+MeJA+CD) for 10 hr, they were then germinated and samples were collected at 1 d, 2 d and 3 d, as shown in Fig. 1. The percentage of seed germination in all treatment groups was not significantly different from the control group during 1–2 d. However, the treatment involving a combination of CHT+MeJA+CD significantly reduced the germination percentage to 57.33 ± 7.57 after 3 d compared to the control group (Fig. S1). Growth was evaluation based on root length measurement. After 3 d of germination, the root lengths decreased by 13.8% and 12% for the MeJA and CHT treatments, respectively, with a marked decrease of 42.4% after treatment with the combination of CHT+MeJA+CD compared to the control group (Fig. S1).

Total soluble sugar

The sprouting process tended to decrease the total soluble sugar in the control and the treatment groups, except for the MeJA group, where the soluble sugar content remained constant throughout the entire germination period. The total soluble sugar decreased significantly from 0.91 ± 0.20 mg/g dry weight to 0.54 ± 0.17 mg/g dry weight in the CHT+MeJA+CD group after germination for 3 d. There were no significant differences between the control and treatment groups in each germination period (Fig. 2A).

Soluble protein

Soluble proteins tended to increase during the germination process. As shown in Fig. 2B, there was a significant increase in soluble protein in the control group on the second germination day, rising from 120.52 ± 36.00 mg/g dry weight to 210.85 ± 75.49 mg/g dry weight. For all treatment groups, the soluble protein significantly increased after germination for 3 d in the range 152.24–200.68 mg/g dry weight compared to day 0. After the seeds had been pre-soaked with elicitors for 10 hr (day 0), the soluble protein detected in the treatment group was lower than for the untreated control group. However, there were no significant differences between the control and treatment groups during each germination period.

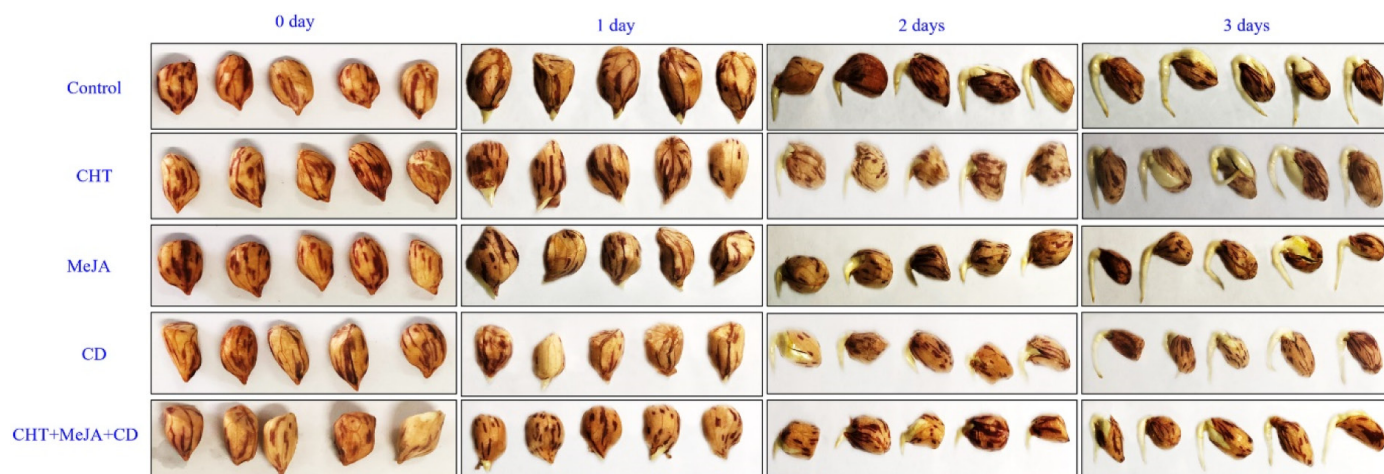


Fig. 1 Seeds and sprouts of peanut cultivar Kalasin2 primed using different elicitors, where seeds in each treatment group were primed for 10 hr using solutions of control group (with water) or with chitosan (CHT), methyl jasmonate (MeJA), methyl- β -cyclodextrin (CD) or a combination of all three (CHT+MeJA+CD), followed by sampling on day 0 and subsequently germinated for 1 d, 2 d or 3 d

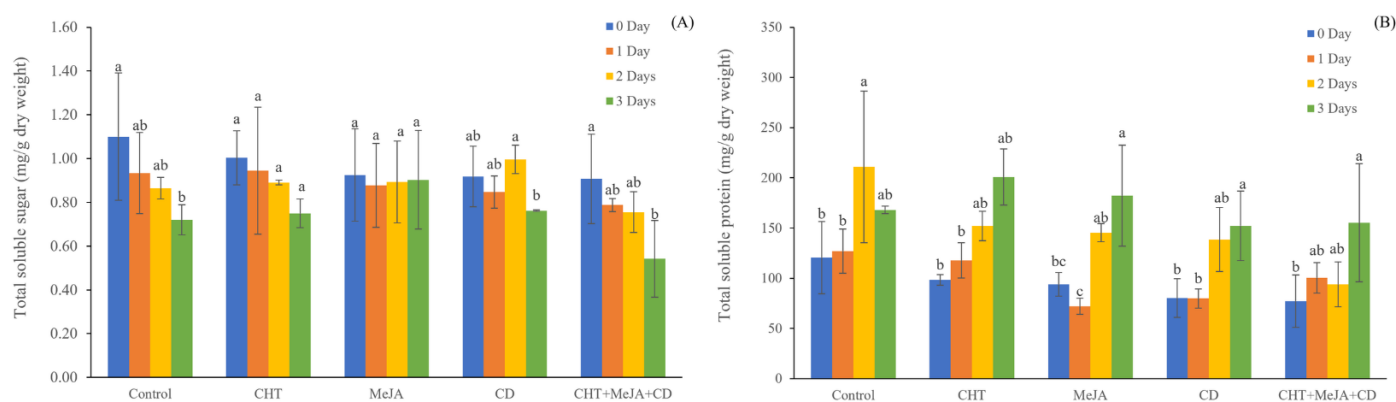


Fig. 2 Peanuts treated with chitosan (CHT), methyl jasmonate (MeJA), methyl- β -cyclodextrin (CD) or a combination of CHT+MeJA+CD compared to the control group for 0–3 d germination: (A) total soluble sugar; (B) total soluble protein, where data represented as mean \pm SD ($n = 3$) and error bars with different lowercase letters are significantly ($p < 0.05$) different between germination days within same treatment group.

Protein profile based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The profiles were investigated of the crude protein extracts from the various treatments during the different germination periods using SDS-PAGE, as illustrated in Fig 3. Protein bands with high molecular weights, approximately 45 kDa, 55 kDa, and 82 kDa, were detected in both the control and treatment groups on day 0 and day 1. These bands gradually lightened over 2–3 d germination. The 82 kDa protein band had almost disappeared after 3 d germination in both the control and treatment groups. The high-intensity protein molecular weight of approximately 12 kDa and 26 kDa substantially increased with germination time. On day 2 of germination, an approximate 47 kDa band was clearly visible in all treatment

samples but not in the control group. Additionally, a band with a molecular weight close to 55 kDa was clearly present in the CHT, CD and CHT+MeJA+CD groups on day 2 of germination. On day 3 of germination, the band with a molecular weight close to 62 kDa had almost disappeared in the CHT+MeJA+CD group compared to the other samples.

Antioxidant activity and total phenolic compounds

The ABTS antioxidant activity of the untreated control showed a decreasing trend after 2 d of germination, while most of the elicitor-treated groups showed an increasing tendency toward ABTS antioxidant activity with increasing germination time (Fig. 4A). The highest ABTS antioxidant activity was on day 3 of germination in the peanut sprouts that had been treated with CHT,

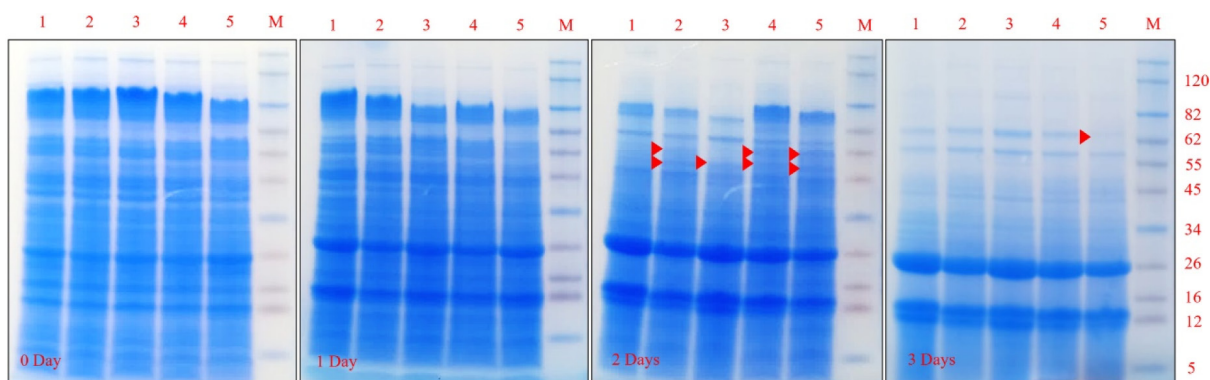


Fig. 3 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles of crude protein extracts from peanuts treated with chitosan (CHT), methyl jasmonate (MeJA), methyl- β -cyclodextrin (CD) or a combination of CHT+MeJA+CD compared to control group for 0–3 d germination, where lane 1 = untreated control group, lane 2 = CHT group, lane 3 = MeJA group, lane 4 = CD group, lane 5 = combination of CHT+MeJA+CD group, M = molecular weight of protein marker and red arrows indicate different protein band patterns among the treatment group in SDS-PAGE.

MeJA, CD and CHT+MeJA+CD with values of 0.52 ± 0.03 $\mu\text{mol Trolox/g dry weight}$, 0.36 ± 0.14 $\mu\text{mol Trolox/g dry weight}$, 0.44 ± 0.07 $\mu\text{mol Trolox/g dry weight}$ and 0.49 ± 0.05 $\mu\text{mol Trolox/g dry weight}$, respectively, representing 1.46–2.12-fold increases compared to the untreated control during the same germination period.

The total phenolic content of the untreated peanut sprouts increased on day 2 of germination to 738.22 ± 27.88 $\mu\text{g gallic acid/g dry weight}$, decreasing slightly thereafter (Fig. 4B). The phenolic content of all treated peanuts increased with within the range 635.89 ± 171.35 $\mu\text{g gallic acid/g dry weight}$ to 662.48 ± 95.02 $\mu\text{g gallic acid/g dry weight}$; however, the increased values were not significantly different from the ungerminated control group on day 3 of germination.

Stilbene compound determination

The major stilbene compounds (*trans*-resveratrol, *trans*-arachidin-1 and *trans*-arachidin-3) were in the control and treated peanut groups during days 0–3 of germination, as demonstrated by HPLC chromatograms of the control and CHT+MeJA+CD treatments in Fig. 5. The HPLC chromatogram of the individual treatments using CHT, MeJA and CD are provided in Figs. 2S–5S. As can be observed from Fig. 6, the detected stilbene content was lower for all stilbene compounds on day 0 (pre-soaked), with all samples increasing after germination. The *trans*-resveratrol detected in the untreated control group on the first day of germination increased from 0.19 ± 0.05 $\mu\text{g/g dry weight}$ to the highest level of 3.09 ± 0.32 $\mu\text{g/g dry weight}$ and significantly decreased after 2–3 d germination. Notably, the *trans*-resveratrol content

gradually increased with germination time in the peanuts treated with CHT and CHT+MeJA+CD. The highest *trans*-resveratrol contents of 10.74 ± 1.71 $\mu\text{g/g dry weight}$ and 15.97 ± 1.26 $\mu\text{g/g dry weight}$ were recorded for the peanuts treated with CHT and CHT+MeJA+CD, respectively, on day 3 of germination, representing a significant increase in the range 7.72–11.48-folds compared to the untreated control group (Fig. 6A).

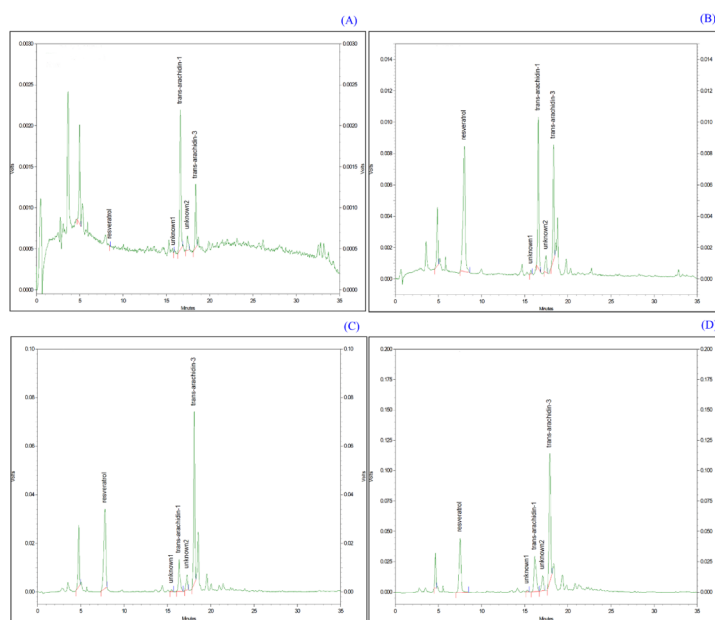


Fig. 5 High-performance liquid chromatograms of K2-K599 peanut crude extracts for 0–3 d germination following treatments with: (A) chitosan (CHT)+methyl jasmonate (MeJA)+methyl- β -cyclodextrin (CD) at 0 d; (B) CHT+MeJA+CD at 1 d; (C) CHT+MeJA+CD at 2 d; (D) CHT+MeJA+CD at 3 d

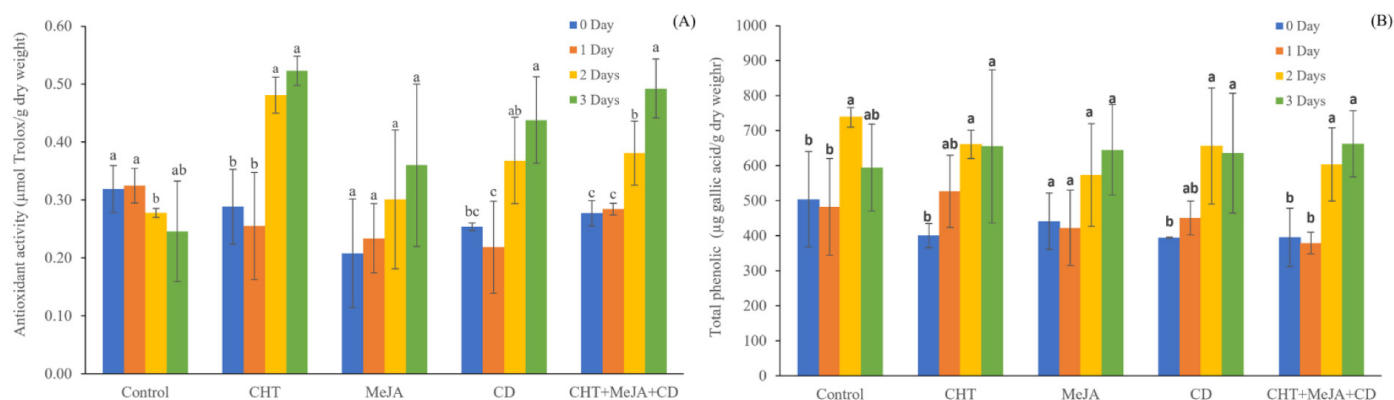


Fig. 4 Peanuts treated with chitosan (CHT), methyl jasmonate (MeJA), methyl- β -cyclodextrin (CD) or a combination of CHT+MeJA+CD compared to control group for 0–3 d germination: (A) 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid antioxidant activity; (B) total phenolic content, where data represented as mean \pm SD ($n = 3$) and error bars with different lowercase letters are significantly ($p < 0.05$) different between germination days within same treatment group.

As indicated by the results in Fig. 6B, *trans*-arachidin-1 rapidly increased on day 1 of germination and slightly decreased on the second germination day in both the control and treatment groups. The highest *trans*-arachidin-1 content in the CD-treated peanuts was detected on the first day of germination with a values of 16.46 ± 1.05 $\mu\text{g/g}$ dry weight, while for CHT, MeJA and CD the maximum was on day 3 of germination with values of 13.07 ± 2.34 $\mu\text{g/g}$ dry weight, 17.77 ± 12.59 $\mu\text{g/g}$ dry weight and 13.41 ± 5.96 $\mu\text{g/g}$ dry weight, respectively.

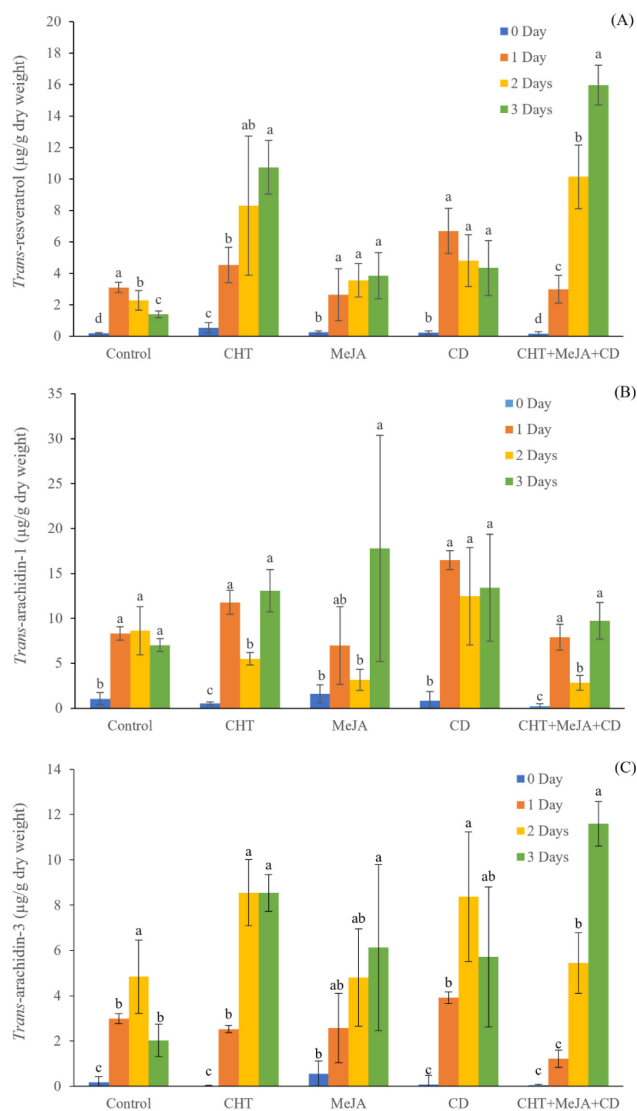


Fig. 6 Peanuts treated with chitosan (CHT), methyl jasmonate (MeJA), methyl- β -cyclodextrin (CD) or a combination of CHT+MeJA+CD compared to control group for 0–3 d germination: (A) *trans*-resveratrol content; (B) *trans*-arachidin-1 content; and (C) *trans*-arachidin-3 content, where data represented as mean \pm SD ($n = 3$) and error bars with different lowercase letters are significantly ($p < 0.05$) different between germination days within same treatment group.

Most of the *trans*-arachidin-3 was expressed at high levels on day 3 of germination after elicitor treatment (Fig. 6C). There were significant increases in the CHT, MeJA and CHT+MeJA+CD groups with values of 8.54 ± 0.81 $\mu\text{g/g}$ dry weight, 6.13 ± 3.67 $\mu\text{g/g}$ dry weight and 11.59 ± 0.98 $\mu\text{g/g}$ dry weight, respectively. These values were 4.20-fold, 3.01-fold and 5.70-fold higher than for the control group during the same germination period.

Discussion

This study investigated the use of different priming substances and germination periods to enhance the stilbene compounds and bioactivity of peanut sprouts. The results showed that there was slight decreases in the seed germination percentage and root length following treatment with CHT and MeJA, and a significant reduction when treated with CHT+MeJA+CD on day 3 of germination compared to the untreated control group. Plant growth and development were correlated with various types and doses of regulators (Kanto et al., 2014). The application of a high concentration of polymeric chitosan/tripolyphosphate nanoparticles had a negative effect on maize and inhibited the germination process (Nakasato et al., 2017). A decrease in *Brassica oleracea* L. var. *italica* shoot length was observed following treatment with $1 \mu\text{M}$ exogenous MeJA, while an increase in shoot length was detected using 1 pM diluted MeJA compared to the control group (Sirhindi et al., 2020). The results of the current study were in agreement with Kępczyńska and Król (2012) who demonstrated the negative effect of a high concentration of MeJA on seed germination and seedling emergence.

An *in vivo* study conducted on Sprague-Dawley rats revealed no apparent growth hazards or health toxicities associated with the consumption of peanut sprouts. Furthermore, the dietary supplementation of peanut sprouts was observed to lead to a reduction in serum triglyceride levels, indicating the potential advantages of utilizing peanut sprouts for the development of functional food (Lin et al., 2008). Chiou et al. (1997) demonstrated the changes in the composition of peanut kernels after germination for various durations. The sucrose content and lipoxygenase activity decreased during the initial stages of germination, whereas the total free amino acid and free fatty acid contents increased over time. Germination is a complex process, involving physiological and biochemical changes in seeds and contributing to the development of young seedlings

(Wolny et al., 2018). A decrease in the total soluble sugar was observed in the control and treatment groups in the current study, particularly for the CHT+MeJA+CD treatment. The breakdown of storage protein and carbohydrate during seed germination can supply energy for seedling growth and development. It has been suggested that the germination process leads to an increase in protein and carbohydrate digestibility that improves the nutritional quality of seeds (Mikola et al., 2001; You et al., 2016). By increasing the germination time, the current results demonstrated an increase in the soluble protein in both the control and elicitor-treated groups. These results were in accordance with Elkhailifa and Bernhardt (2010), who reported higher protein solubility in germinated sorghum compared to the control group. This increase in protein solubility could have been due to an increase in the proteolytic enzyme activity that broke down storage proteins into more soluble proteins during the germination process. Notably, the soluble protein levels in the treated peanuts were lower than for the untreated control group, although there were no significant differences at each time point. The lower soluble protein content could have been due to the inhibition of proteolytic enzymes after elicitor treatment, thus slowing the process of proteolytic cleavage from the storage protein. In the plant defense mechanism, MeJA has been reported to induce pepper proteinase inhibitor proteins which function as insect or microbial proteolytic enzyme inhibitors (Moura and Ryan, 2001). In the current study, the protein profile was investigated using SDS-PAGE electrophoresis. During germination, the high molecular weight protein pattern changed considerably, resulting in a low molecular weight in the range of approximately 12–26 kDa, thereby indicating that the proteins had been broken down into short chain, more soluble components. The diversity of the seed storage protein profile, including the high molecular weight of albumins and globulins and the changing patterns of SDS-PAGE protein were in agreement with the findings reported by Wang et al. (2005).

The treatment of peanuts with CHT, MeJA, CD or CHT+MeJA+CD improved the antioxidant capacity, based on the increase in the germination time compared to the control group. The exogenous application of 100 μ M MeJA on *Stevia* plants increased their antioxidant capacity and phenolic compounds (Lucho et al., 2019). Pre-soaking of chickpea seeds with 30 mM H_2O_2 for 24 hr prior to germination revealed that after 72 hr, there were higher levels of total phenolic compounds and antioxidant activity compared to the control group (León-López et al., 2020). The current results were

consistent another report on elicitor treatment in peanut hairy root culture that demonstrated an increase in ABTS antioxidant activity following CHT, MeJA, CD or CHT+MeJA+CD treatments (Chayjarung et al., 2021). However, the antioxidant activity detected in the treated peanut sprouts was much lower than in the hairy root culture system.

The effect of elicitation on stilbene compounds was evaluated. After pre-soaking on day 0, a small number of stilbene compounds, such as *trans*-resveratrol, *trans*-arachidin-1 and *trans*-arachidin-3, were detected in all the treated and untreated samples. These results for the untreated peanut sprouts were in accordance with another report showing an increase in *trans*-resveratrol during the early stage of germination that decreased with an increase in the germination period (Limmongkon et al., 2017). Notably, the elicitation of peanuts resulted in increases in *trans*-resveratrol, *trans*-arachidin-1 and *trans*-arachidin-3 throughout the 3 d after germination. The highest content of *trans*-resveratrol and *trans*-arachidin-3 was in the CHT+MeJA+CD treatment on day 3 of germination, with the highest amount of *trans*-arachidin-3 detected on day 3 of germination in the MeJA treatment. Several strategies have been applied to increase the nutritional ingredients for functional food improvement (Nakorn and Kaewsorn, 2021). A combination of elicitor treatment and germination could provide an effective approach for stimulating higher bioactive compounds in plants. Soybean seeds soaked in 0.01% of chito-oligosaccharide and germinated for 3 d showed increases in the vitamin C content, total phenolic compounds, flavonoid contents and antioxidant activity, effectively improving the nutritional value of the soybean sprouts (Tang et al., 2021). The soaking of peanut seeds with a $ZnCl_2$ solution throughout the germination process improved the antioxidant and vitamin contents (Zhao et al., 2020). Treatment with a combination of 2 mM mannitol and 40 μ M jasmonic acid significantly increased *trans*-resveratrol biosynthesis in *Vitis vinifera* callus culture (Mihai et al., 2011). Batch-fed *V. vinifera* cell suspension culture treated with 50 mg/L of chitosan increased mono-glucosylated stilbene production (Ferri et al., 2011). Another study on hairy root peanut culture demonstrated the promising outcome of enhancing *trans*-resveratrol, *trans*-arachidin-1 and *trans*-arachidin-3 with high antioxidant activity using elicitor treatments of CHT, MeJA, CD and simultaneously CHT+MeJA+CD (Chayjarung et al., 2021). The use of an external elicitor treatment could be a promising strategy for increasing the production of phytochemicals and bioactive compounds in plants.

Conclusions

Peanut sprouts were a better source of stilbene compounds compared to ungerminated peanut seeds. This was the first known report to apply a combination of elicitors during seed priming to enhance the quality of peanut sprouts. Pre-soaking peanut seeds with different elicitors for subsequent germination increased the antioxidant activity and the stilbene compounds (*trans*-resveratrol, *trans*-arachidin-1 and *trans*-arachidin-3) with increased germination time compared to the untreated control group. Adversely, the germination profile (root length, germination rate, soluble protein and total soluble sugar) of the group receiving the elicitor treatment tended to be lower than for the untreated control group during each germination period. The profiles of these biochemical substances could be related to the germination process and the response against elicitor treatment. Accordingly, a combination of elicitor treatment and germination strategies could provide an effective approach for improving the nutritional value and phytochemical profiles of peanuts. However, before considering the application of elicited peanut sprouts as a functional vegetable, further investigations are needed involving *in vitro* and *in vivo* toxicological assessments to address food safety concerns.

Acknowledgements

Asst. Prof. Suwaree Saijeen provided expertise regarding peanut germination process. Mrs. Nuntaporn Suksamrahn (Rai Suksamrahn, Kampaeng Phet, Thailand) kindly provided the peanut cultivar Kalasin2 seeds for the experiment.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

- Aluyen, J.K., Ton, Q.N., Tran, T., Yang, A.E., Gottlieb, H.B., Bellanger, R.A. 2012. Resveratrol: Potential as anticancer agent. *J. Diet. Suppl.* 9: 45–56. doi.org/10.3109/19390211.2011.650842
- Arya, S.S., Salve, A.R., Chauhan, S. 2016. Peanuts as functional food: A review. *J Food Sci Technol.* 53: 31–41. doi.org/10.1007/s13197-015-2007-9
- Bru, R., Sellés, S., Casado-Vela, J., Belchí-Navarro, S., Pedreño, M.A. 2006. Modified cyclodextrins are chemically defined glucan inducers of defense responses in grapevine cell cultures. *J. Agric. Food Chem.* 54: 65–71. doi.org/10.1021/jf051485j
- Chayjarung, P., Poonsap, W., Pankaew, C., Inmano, O., Kongbangkerd, A., Limmongkon, A. 2021. Using a combination of chitosan, methyl jasmonate, and cyclodextrin as an effective elicitation strategy for prenylated stilbene compound production in *Arachis hypogaea* L. hairy root culture and their impact on genomic DNA. *Plant Cell Tiss. Organ Cult.* 147: 117–129. doi.org/10.1007/s11240-021-02112-4
- Chen, L.G., Zhang, Y.Q., Wu, Z.Z., Hsieh, C.W., Chu, C.S., Wung, B.S. 2018. Peanut arachidin-1 enhances Nrf2-mediated protective mechanisms against TNF- α -induced ICAM-1 expression and NF- κ B activation in endothelial cells. *Int. J. Mol. Med.* 41: 541–547. doi.org/10.3892/ijmm.2017.3238
- Cheong, J.-J., Choi, Y.D. 2003. Methyl jasmonate as a vital substance in plants. *Trends Genet.* 19: 409–413. doi.org/10.1016/S0168-9525(03)00138-0
- Chiou, R.Y.Y., Ku, K.L., Chen, W.L. 1997. Compositional characterization of peanut kernels after subjection to various germination times. *J. Agric. Food Chem.* 45: 3060–3064. doi.org/10.1021/jf970082j
- Elkhalifa, A.E.O., Bernhardt, R. 2010. Influence of grain germination on functional properties of sorghum flour. *Food Chem.* 121: 387–392. doi.org/10.1016/j.foodchem.2009.12.041
- Ferri, M., Dipalo, S.C.F., Bagni, N., Tassoni, A. 2011. Chitosan elicits mono-glucosylated stilbene production and release in fed-batch bioreactor cultures of grape cells. *Food Chem.* 124: 1473–1479. doi.org/10.1016/j.foodchem.2010.07.114
- Haldar, D., Sen, D., Gayen, K. 2017. Development of spectrophotometric method for the analysis of multi-component carbohydrate mixture of different moieties. *Appl. Biochem. Biotechnol.* 181: 1416–1434. doi.org/10.1007/s12010-016-2293-3
- Huang, C.P., Au, L.C., Chiou, R.Y.Y., et al. 2010. Arachidin-1, a peanut stilbenoid, induces programmed cell death in human Leukemia HL-60 cells. *J. Agric. Food Chem.* 58: 12123–12129. doi.org/10.1021/jf102993j
- Hung, L.M., Chen, J.K., Huang, S.S., Lee, R.S., Su, M.J. 2000. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovasc. Res.* 47: 549–555. doi.org/10.1016/S0008-6363(00)00102-4
- Idowu, A.T., Olatunde, O.O., Adekoya, A.E., Idowu, S. 2019. Germination: An alternative source to promote phytonutrients in edible seeds. *Food Qual. Saf.* 4: 129–133. doi.org/10.1093/fqsaf/fyz043
- Kanto, U., Jutamane, K., Osotsapar, Y., Chai-arree, W., Jattapornpong, S. 2014. Rice seed priming with swine manure extract to ameliorate vigor and nutrient status of seedlings. *Agr. Nat. Resour.* 48: 333–347.
- Kępczyńska, E., Król, P. 2012. The phytohormone methyl jasmonate as an activator of induced resistance against the necrotroph *Alternaria porri* f. sp. *solani* in tomato plants. *J. Plant Interact.* 7: 307–315. doi.org/10.1080/17429145.2011.645169
- León-López, L., Escobar-Zúñiga, Y., Salazar-Salas, N.Y., Mora Rochin, S., Cuevas-Rodríguez, E.O., Reyes-Moreno, C., Milán-Carrillo, J. 2020. Improving polyphenolic compounds: Antioxidant activity in chickpea sprouts through elicitation with hydrogen peroxide. *Foods* 9: 1791. doi.org/10.3390/foods9121791

- Limmongkon, A., Janhom, P., Amthong, A., et al. 2017. Antioxidant activity, total phenolic, and resveratrol content in five cultivars of peanut sprouts. *Asian Pac. J. Trop. Biomed.* 7: 332–338. doi.org/10.1016/j.apjtb.2017.01.002
- Limmongkon, A., Nopprang, P., Chaikeandee, P., Somboon, T., Wongshaya, P., Pilaisangsuree, V. 2018. LC-MS/MS profiles and interrelationships between the anti-inflammatory activity, total phenolic content and antioxidant potential of Kalasin 2 cultivar peanut sprout crude extract. *Food Chem.* 239: 569–578. doi.org/10.1016/j.foodchem.2017.06.162
- Lin, B.S., Lien, T.F., Chao, M.R., Lai, T.-Y., Chang, J.-C., Chou, S.-J., Liao, H.-F., Chiou, R.-Y.Y. 2008. Toxicological and nutraceutical assessments of peanut sprouts as daily supplements to feed Sprague-Dawley rats for 18 weeks. *J. Sci. Food Agric.* 88: 2201–2207. doi.org/10.1002/jsfa.3335
- Lopes, R.M., Agostini-Costa, T.d.S., Gimenes, M.A., Silveira, D. 2011. Chemical composition and biological activities of *Arachis* species. *J. Agric. Food Chem.* 59: 4321–4330. doi.org/10.1021/jf104663z
- Lucho, S.R., do Amaral, M.N., López-Orenes, A., Kleinowski, A.M., Amarante, L.d., Ferrer, M.Á., Calderón, A.A., Braga, E.G.B., 2019. Plant growth regulators as potential elicitors to increase the contents of phenolic compounds and antioxidant capacity in *Stevia* plants. *Sugar Tech* 21: 696–702. doi.org/10.1007/s12355-018-0673-4
- Mihai, R., Cristina, S., Helepciuc, F., Brezeanu, A., Stoian, G. 2011. Biotic and abiotic elicitors induce biosynthesis and accumulation of resveratrol with antitumoral activity in the long-term *Vitis vinifera* L. callus cultures. *Rom. Biotechnol. Lett.* 16: 6883–6889.
- Mikola, M., Brinck, O., Jones, B.L. 2001. Characterization of oat endoproteases that hydrolyze oat avenins. *Cereal Chem.* 78: 55–58. doi.org/10.1094/CCHEM.2001.78.1.55
- Moura, D.S., Ryan, C.A. 2001. Wound-inducible proteinase inhibitors in pepper. Differential regulation upon wounding, systemin, and methyl jasmonate. *Plant Physiol.* 126: 289–298. doi.org/10.1104/pp.126.1.289
- Nakasato, D.Y., Pereira, A.E.S., Oliveira, J.L., Oliveira, H.C., Fraceto, L.F. 2017. Evaluation of the effects of polymeric chitosan/tripolyphosphate and solid lipid nanoparticles on germination of *Zea mays*, *Brassica rapa* and *Pisum sativum*. *Ecotoxicol. Environ. Saf.* 142: 369–374. doi.org/10.1016/j.ecoenv.2017.04.033
- Na nakorn, P., Kaewsorn, P. 2021. Effects of KNO₃ concentration and aeration during seed priming on seed quality of wax gourd (*Benincasa hispida* [Thunb.] Cogn.). *Agr. Nat. Resour.* 55: 873–881.
- Núñez-Gastélum, J.A., Arguijo-Sustaita, A.A., López-Díaz, J.A., Díaz-Sánchez, Á.G., Hernández-Peña, C.C., Cota-Ruiz, K. 2023. Seed germination and sprouts production of *Moringa oleifera*: A potential functional food? *J. Saudi Soc. Agric. Sci.* 22: 223–230. doi.org/10.1016/j.jssas.2022.12.002
- Paparella, S., Araújo, S.S., Rossi, G., Wijayasinghe, M., Carbonera, D., Balestrazzi, A. 2015. Seed priming: State of the art and new perspectives. *Plant Cell Rep.* 34: 1281–1293. doi.org/10.1007/s00299-015-1784-y
- Pilaisangsuree, V., Somboon, T., Tonglairoum, P., Keawracha, P., Wongs, T., Kongbangkerd, A., Limmongkon, A. 2018. Enhancement of stilbene compounds and anti-inflammatory activity of methyl jasmonate and cyclodextrin elicited peanut hairy root culture. *Plant Cell Tiss. Organ Cult.* 132: 165–179. doi.org/10.1007/s11240-017-1321-5
- Sales, J.M., Resurreccion, A.V.A. 2009. Maximising resveratrol and piceid contents in UV and ultrasound treated peanuts. *Food Chem.* 117: 674–680. doi.org/10.1016/j.foodchem.2009.04.075
- Sák, M., Dokupilová, I., Kaňuková, Š., Mrkvová, M., Mihálik, D., Hauptvogel, P., Kraic, J. 2021. Biotic and abiotic elicitors of stilbenes production in *Vitis vinifera* L. cell culture. *Plants* 10: 490. doi.org/10.3390/plants10030490
- Samota, M.K., Sasi, M., Awana, M., et al. 2017. Elicitor-induced biochemical and molecular manifestations to improve drought tolerance in rice (*Oryza sativa* L.) through seed-priming. *Front. Plant Sci.* 8: 934. doi.org/10.3389/fpls.2017.00934
- Sirhindi, G., Mushtaq, R., Gill, S.S., Sharma, P., Abd Allah, E.F., Ahmad, P. 2020. Jasmonic acid and methyl jasmonate modulate growth, photosynthetic activity and expression of photosystem II subunit genes in *Brassica oleracea* L. *Sci. Rep.* 10: 9322. doi.org/10.1038/s41598-020-65309-1
- Somboon, T., Chayjarung, P., Pilaisangsuree, V., Keawracha, P., Tonglairoum, P., Kongbangkerd, A., Wongkrajang, K., Limmongkon, A. 2019. Methyl jasmonate and cyclodextrin-mediated defense mechanism and protective effect in response to paraquat-induced stress in peanut hairy root. *Phytochemistry* 163: 11–22. doi.org/10.1016/j.phytochem.2019.03.017
- Tang, W., Lei, X., Liu, X., Yang, F. 2021. Nutritional improvement of bean sprouts by using chitooligosaccharide as an elicitor in germination of soybean (*Glycine max* L.). *Appl. Sci.* 11: 7695. doi.org/10.3390/app11167695
- Valletta, A., Iozia, L.M., Leonelli, F. 2021. Impact of environmental factors on stilbene biosynthesis. *Plants* 10: 90. doi.org/10.3390/plants10010090
- Wang, K.H., Lai, Y.H., Chang, J.C., Ko, T.F., Shyu, S.L., Chiou, R.Y. 2005. Germination of peanut kernels to enhance resveratrol biosynthesis and prepare sprouts as a functional vegetable. *J. Agric. Food Chem.* 53: 242–246. doi.org/10.1021/jf048804b
- Wolny, E., Betekhtin, A., Rojek, M., Braszewska-Zalewska, A., Lusinska, J., Hasterok, R. 2018. Germination and the early stages of seedling development in *Brachypodium distachyon*. *Int. J. Mol. Sci.* 19. doi.org/10.3390/ijms19102916
- Yang, M.H., Kuo, C.H., Hsieh, W.C., Ku, K.L. 2010. Investigation of microbial elicitation of trans-resveratrol and trans-piceatannol in peanut callus led to the application of chitin as a potential elicitor. *J. Agric. Food Chem.* 58: 9537–9541. doi.org/10.1021/jf1022725
- Yang, Q.Q., Cheng, L., Long, Z.-Y., Li, H.B., Gunaratne, A., Gan, R.Y., Corke, H. 2019. Comparison of the phenolic profiles of soaked and germinated peanut cultivars via UPLC-QTOF-MS. *Antioxidants* 8: 47. doi.org/10.3390/antiox8020047
- You, S.Y., Oh, S.G., Han, H.M., Jun, W., Hong, Y.S., Chung, H.J. 2016. Impact of germination on the structures and in vitro digestibility of starch from waxy brown rice. *Int. J. Biol. Macromol.* 82: 863–870. doi.org/10.1016/j.ijbiomac.2015.11.023
- Zhao, K., Zhao, C., Yang, M., Yin, D. 2020. ZnCl₂ treatment improves nutrient quality and Zn accumulation in peanut seeds and sprouts. *Sci. Rep.* 10: 2364. doi.org/10.1038/s41598-020-59434-0