Agr. Nat. Resour. 54 (2020) 545–552

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Enhancement of health-beneficial compounds of sunflower sprouts using selected elicitors

Intira Koodkaew*, Benjamart Tungkasem, Chalida Urarot

Department of Botany, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

Article Info

Article history:
Received 4 September 2019
Revised 1 May 2020
Accepted 15 May 2020
Available online 30 October 2020

Keywords:
Chitosan,
Phytochemicals,
Salicylic acid,
Sodium chloride,
Sucrose

Abstract

Sunflower sprouts are an excellent source of beneficial health compounds. This study investigated improvement of the health-promoting properties of sunflower sprouts using stressor elicitors—sucrose (25 mM, 50 mM or 100 mM), NaCl (5 mM, 50 mM or 100 mM), chitosan (0.03 mM, 0.05 mM or 0.07 mM) and salicylic acid (0.36 mM, 0.72 mM or 1.44 mM). The experiment was conducted by spraying the sunflower sprouts (1 d after sowing) with 20 ml of each treatment solution per tray in the morning for 5 d while a control group was sprayed with distilled water. The results showed that none of the elicitors significantly changed the fresh weight of sunflower sprouts. Sucrose and NaCl improved the antioxidant property of the sprouts by increasing the phenolic compounds and ascorbic acid. The highest antioxidant activity and amounts of phenolic compounds and ascorbic acid (increases by 75.67%, 43.04% and 353.15%, respectively, compared to the control) were found in the treatment involving 100 mM NaCl. The increase in phenolic compounds might have resulted from an increase in the phenylalanine ammonia-lyase activity. The highest flavonoid and γ-aminobutyric acid contents (increases by 85.63% and 264.66%, respectively, compared to the control) were obtained following the 0.07 mM chitosan treatment. Salicylic acid had the most influence on the protein content in the sprouts (the content was up to 65.39% compared to the control). This research indicated that the pre-harvest application of selected elicitors could increase the nutritional value of sunflower sprouts while limiting yield losses.

Introduction

For many people, their current lifestyle has involved a rapid increase in the consumption of functional foods due to consumer awareness of diet and health. Plant products with a high content of health-promoting compounds are becoming increasingly more commonly planted to satisfy consumer demand (Martin et al., 2011). Recently, great attention has focused on the possibility of using simple and cheap techniques to increase the desirable healthy compounds in plants (Jeong et al., 2018; Koodkaew, 2019). Stress elicitors have been considered because the accumulation of health-promoting bioactive molecules in plants is controlled by environmental growth conditions or is a part of the plant defense response to environmental stress (Ramakrishna and Ravishankar, 2011).

Elicitors are chemical or physical factors that play an important role in the production of phytochemicals and bioactive compounds in plants (Liu et al., 2019). Eliciting of plants has been reported to be a useful technique to enhance compounds beneficial to health and the quality of vegetable foods (Rouphael et al., 2018). Environmental stresses as elicitors (abiotic and biotic), such as sucrose, sodium chloride (NaCl), chitosan and salicylic acid, have been reported to increase the biosynthesis of secondary metabolites in many types

* Corresponding author.
E-mail address: faasirk@ku.ac.th (I. Koodkaew)

https://doi.org/10.34044/j.anres.2020.54.5.12

of sprouts including broccoli (Guo et al., 2011a,b; Guo et al., 2014; Natella et al., 2016), buckwheat (Lim et al., 2012; Jeong et al., 2018), mung bean (Koodkaew, 2019) and Dalia bean (Mendoza-Sánchez et al., 2016).

The sunflower (*Helianthus annuus* L.) belongs to the family Asteraceae and is grown commercially worldwide as sunflower sprouts are an excellent source of beneficial health compounds such as phenolic compounds, flavonoids, alkaloids, carotenoids, tannins, vitamins and trace elements as well as having an antioxidant property which to date represents untapped benefit to human health (Balasaraswathi and Sadasivam, 1997; Jiraungkoorskul, 2016; Guo et al., 2017). The high health-promoting compounds of sunflower sprouts have been shown to contribute to their remedial properties (Guo et al., 2017). Cho et al. (2008) revealed that soaking sunflower seeds in chitosan solution improved the antioxidant activity, amino acid, total phenol, isoflavone and melatonin contents of the sunflower sprouts. However, knowledge about the effects of elicitation in sunflower sprouts is scarce. Therefore, the objective of the current study was to evaluate the effects of elicitors, namely sucrose, NaCl, chitosan and salicylic acid on growth and the health-promoting properties of sunflower sprouts.

Materials and Methods

Plant material and growth conditions

Sunflower seeds were purchased from a commercial agent. The seed germination potential was more than 95%. Healthy seeds were washed and soaked in water for 8 hr and then incubated at room temperature for 12 hr. Afterward, approximately 300 seeds were sown in trays (19.5 cm × 22.0 cm × 6.0 cm) filled with 1:1 ratio of commercial soil to coconut coir. Subsequently, the tray-grown sprouts were transferred to a growth chamber at a constant temperature (25°C) and relative humidity (85%) under dark conditions for 3 d. After 3 d, the sprouts were grown under a photoperiod of 12 hr light (1,500 lux) and 12 hr darkness at the same temperature and relative humidity as earlier. The sprouts were watered twice daily with 50 mL of water.

Application of treatments

Solutions were prepared of sucrose (25 mM, 50 mM or 100 mM), NaCl (5 mM, 50 mM or 100 mM), chitosan (0.03 mM, 0.05 mM or 0.06 mM) and salicylic acid (0.36 mM, 0.72 mM or 1.44 mM); distilled water served as the control. These selected concentrations were based on the results of preliminary study that indicated improved antioxidant properties without reducing sprout growth (data not shown). At 1 d after sowing the sunflower seeds, the sprouts were sprayed daily with 20 mL of each treatment solution per tray in the morning (0900–1000 hours) for 5 d. For each treatment, three replicates were prepared (with one tray for each replication). Then, 24 hr after the last spraying, the sunflower sprouts at the two-leaf stage were harvested at soil level, washed and prepared for growth measurement and enzyme analysis. Another part of the sprout samples was immediately put into liquid nitrogen and stored at -20°C until used for phytochemical analysis.

Growth measurement

Samples of sprouts (30 sprouts per replication) were randomly picked from each tray and the stem length was recorded using a ruler. Using 100 sprouts per replication, the fresh and dry weights were measured and the percentage dry matter content (DM) was calculated using the equation DM (%) = (dry weight / fresh weight) × 100.

Total phenolic compounds and flavonoids content

Sunflower sprouts (1 g) were extracted using 10 mL of 80% methanol for 5 min. Then, each sample was centrifuged at 5,000 rpm for 15 min and the supernatant was used for determination of phenolic compounds and flavonoids content.

The total phenolic content of the extracts was determined using the Folin-Ciocalteu colorimetric method (Singleton et al., 1999). Each sample solution (50 µL) was oxidized with the Folin-Ciocalteu reagent (250 µL) and shaken vigorously. After 8 min, 750 µL of 20% Na_2CO_3 and 950 µL of distilled water were added, consecutively. The mixture was allowed to stand at room temperature for 30 min and then the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (UV1800; Shimadzu; Japan). The total phenolic content was expressed as mean milligrams of gallic acid equivalents (GAE) per gram of fresh weight of sample.

The flavonoid content was determined using the method described by Zhishen et al. (1999). Each sample solution (500 µL) was mixed with 2 mL of distilled water and 15 µL of 5% $NaNO_2$ and shaken vigorously. After 6 min, 150 µL of 10% $AlCl_3$, 2 mL of 2 M NaOH and 200 µL of distilled water were added and incubated at 25°C for 30 min. The absorbance was measured at 415 nm using the spectrophotometer. The flavonoids content was expressed as the mean milligrams of quercetin equivalents (QE) per gram of fresh weight of sample.

Phenylalanine ammonia-lyase activity

The phenylalanine ammonia-lyase (PAL) activity was evaluated as described by Wei et al. (2011) with slight modifications. For enzyme extraction, 0.2 g of each fresh sprout sample was ground with 1.7 mL of extraction buffer (1 mM sodium-borate buffer containing 1 mM ethylenediaminetetraacetic acid and 20 mM β-mercaptoethanol at pH 8.8) in an ice bath. The extracts were centrifuged at 10,000 rpm and 4°C for 30 min and the supernatant was collected for enzyme assay. For the PAL assay, 200 µL of the supernatant was incubated with 3 mL of 1 mM sodium-borate buffer (pH 8.8) containing 0.5 mM L-phenylalanine for 1 hr at 37°C. The PAL activity in the extract was determined by the production of cinnamic acid from L-phenylalanine, with detection at 290 nm using the spectrophotometer. One unit of enzyme activity was defined as an increase in absorbance of 0.1 / hr/mL of the enzyme solution (Zhu et al., 2015). PAL activity was expressed as enzyme units per gram of fresh weight (U/g FW).

Ascorbic acid content

Ascorbic acid was analyzed according to the method of Li et al. (2012). Fresh sprouts (1.0 g) were ground with liquid N_2 and extracted with 5 mL of 5% (w/v) trichloroacetic acid for 5 min. After the extract had been centrifuged at 12,000 rpm and 4°C for 10 min, 1.0 mL of the supernatant was collected and mixed with 1.0 mL of ethanol. Then, 0.5 mL of 0.4% (w/v) phosphoric acid, 1.0 mL of 0.5% (w/v) 1, 10-phenanthroline and 0.5 mL of 0.03 mg/mL ferric chloride were added to the mixture and shaken vigorously. The absorbance was measured using the spectrophotometer at 534 nm. Ascorbic acid was used as the standard for the calibration curve.

γ-Aminobutyric acid content

The γ-aminobutyric acid (GABA) content was quantified according to Karladee and Suriyong (2012) with some modifications. Fresh sprouts (0.5 g) were ground with liquid N_2 and extracted with 2.5 mL of 95% ethanol. The extract was filtered through Whatman no. 1 filter paper and the filtrate was boiled at 90°C in a water bath to evaporate the ethanol. Then, the residue was dissolved in 0.5 mL of distilled water and centrifuged at 10,000 rpm for 10 min. The supernatant (0.5 mL) was collected and mixed with 0.2 mL of 0.2 M sodium borate buffer (pH 8.8) and 1.0 mL of 6% (w/v) phenol and shaken vigorously. After the mixture had been cooled in a cooling bath for 5 min, 14.5% NaOCl was added to the solution and shaken for 1 min. Subsequently, the mixture was cooled again in the cooling bath for 5 min and then boiled at 100°C in a water bath for 10 min. After the solution had cooled, the absorbance was measured at 630 nm using the spectrophotometer. GABA was used as the standard for the calibration curve.

Antioxidant activity

The antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method (Brand-Williams et al., 1995). Shoots of sprouts (1.0 g) were extracted using 10 mL of 80% methanol (equal to a plant sample concentration of 100 mg/mL) and then the mixture was centrifuged at 5,000 rpm for 15 min. The supernatant (200 µL) was mixed with 1.3 mL of 80% methanol and added to 500 µL of 0.5 mM DPPH solution in methanol. The mixture was shaken and allowed to stand for 30 min at room temperature in darkness. Absorbance of the resulting solution was measured at 517 nm using the spectrophotometer. The radical scavenging activity was calculated using the equation: radical scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$ where A_0 is the absorbance of the control (DPPH solution without any sample) and A_1 is the absorbance of the test sample (DPPH solution plus the sample). Trolox (25 µg/mL) was used as the standard.

Protein content

The protein content was measured using the method of Bradford (1976). Fresh sprouts (1 g) were ground on ice and then 10 mL of potassium phosphate buffer (25 mM, pH 7.8) was added. After 5 min homogenization, the sample was centrifuged at 15,000 rpm and 4°C for 20 min. The supernatant was collected and used as crude protein. Bradford reagent (5 mL) was added to the supernatant (1 mL). The mixture was shaken and allowed to react at room temperature for 2 min. Absorbance of the blue-color complex was recorded at 595 nm using the spectrophotometer. Bovine serum albumin was used for the standard curve.

Statistical analysis

Data were expressed as mean ± SD. Data were analyzed using analysis of variance facilitated by the R software program (R Core Team, 2015). Duncan's multiple range test was used to compare means at a significance level of $p < 0.05$. Pearson's correlation coefficient was used for determination of correlation between parameters.

Results

Sunflower sprouts growth

The effects of sucrose, NaCl, chitosan and salicylic acid on the growth of sunflower sprouts are shown in Table 1. Low concentrations of sucrose (25 and 50 mM) and salicylic acid (0.36 mM) significantly increased sprout length by 10.52%, 10.52% and 10.95%, respectively, compared to the control whereas 100 mM NaCl significantly decreased

Table 1 Effects of sucrose, NaCl, chitosan and salicylic acid solutions at different concentrations on stem length, fresh weight, dry weight and dry matter content of sunflower sprouts

Concentration (mM)		Stem length (cm)	Fresh weight (g/100 sprouts)	Dry weight (g/100 sprouts)	Dry matter content (%)
Control	0	13.88 ± 0.31^{cd}	66.94 ± 1.47^{ab}	3.63 ± 0.03^{cde}	5.43 ± 0.15^{ab}
Sucrose	25	15.34 ± 0.80^{ab}	71.94 ± 2.15^{a}	3.71 ± 0.21^{bcd}	5.15 ± 0.15^{ab}
	50	15.23 ± 0.70^{abc}	66.76 ± 4.03^{ab}	3.81 ± 0.12^{bc}	5.72 ± 0.25^{ab}
	100	15.35 ± 0.05^{ab}	67.63 ± 0.42^{ab}	3.76 ± 0.26^{bc}	5.56 ± 0.43^{ab}
NaCl	5	13.01 ± 0.56^{d}	71.81 ± 7.90^{a}	3.81 ± 0.03^{bc}	5.35 ± 0.61^{ab}
	50	13.97 ± 0.57^{bcd}	65.50 ± 3.99^{ab}	3.96 ± 0.28^{ab}	5.73 ± 0.36^{ab}
	100	11.68 ± 0.73^{e}	63.84 ± 0.97^{ab}	4.21 ± 0.25^{a}	6.08 ± 0.54^{a}
Chitosan	0.03	14.54 ± 0.64^{abc}	69.64 ± 2.36^{ab}	3.41 ± 0.12^{e}	4.89 ± 0.20^{b}
	0.05	14.65 ± 0.25^{abc}	70.86 ± 0.21^{a}	3.47 ± 0.08^{de}	5.17 ± 0.57^{ab}
	0.07	14.83 ± 0.92^{abc}	61.23 ± 4.02^{b}	3.58 ± 0.11^{cde}	5.87 ± 0.54^{a}
Salicylic acid	0.36	15.40 ± 1.75^{a}	65.13 ± 6.79^{ab}	3.45 ± 0.06^{de}	5.32 ± 0.48^{ab}
	0.72	15.15 ± 0.40^{abc}	67.97 ± 1.79^{ab}	3.47 ± 0.14^{de}	5.65 ± 0.89^{ab}
	1.44	14.98 ± 0.72^{abc}	65.05 ± 6.30^{ab}	3.37 ± 0.14^{e}	5.60 ± 0.57^{ab}

Values represent mean ± SD of three replications; Different lowercase superscripts in the same column indicate significant difference ($p < 0.05$) between means.

the length by 15.85% compared to the control. However, treatment with sucrose, NaCl, chitosan and salicylic acid had no significant effect on sprout fresh weight. The fresh weight of sunflower sprouts tended to increase after treatment with low concentrations of sucrose, NaCl and chitosan. The highest fresh weight was recorded for the treatment of 25 mM sucrose, with a 7.47% increase compared with the control. Only the treatments of 50 and 100 mM NaCl produced any significant increase in sprouts dry weight by 9.09% and 15.98%, respectively, compared to the control. No significant differences were recorded in the dry matter content based on treatments compared to the control. However, the highest dry matter content was recorded in sprouts treated with 100 mM NaCl.

Total phenolic compounds and flavonoids contents and phenylalanine ammonia-lyase activity

The total phenolic compounds increased significantly after treatment with 50 mM and 100 mM sucrose and 5–100 mM NaCl. The percentage increase ranged from 27.77% to 43.04% compared to the control. No significant differences were found in the treatments using chitosan and salicylic acid (Fig. 1A). On the contrary, the flavonoid content was significantly enhanced in the treatments using chitosan (increases by 59.87%, 76.58% and 85.63% in sprouts treated with 0.03 mM, 0.05 mM and 0.07 mM chitosan, respectively, compared to the control) and using 1.44 mM salicylic acid (43.85% compared to the control). No significant differences were recorded for the control and treatments using sucrose and NaCl (Fig. 1B). Consistent with the changes in the total phenolic compounds content, the PAL activity was significantly induced with 50 mM and 100 mM sucrose and 5–100 mM NaCl (the percentage increase ranged from 65.19% to 159.46% compared to the control). A significant increase in the PAL activity also was found in the treatment with 0.07 mM chitosan (Fig. 1C).

Ascorbic acid and γ-aminobutyric acid contents

As shown in Fig. 2, there was a significant difference in the ascorbic acid content between the sprouts treated with sucrose and NaCl and the control sprouts. The increment in the ascorbic acid content was up to 353.15% compared to the control in the treatment with 100 mM NaCl.

The results demonstrated that treatment with 50–100 mM NaCl, chitosan and salicylic acid significantly enhanced the GABA content. Sprouts treated with chitosan had a high accumulation of GABA (206.61–264.66% compared to the control) whereas there was no significant difference in the GABA content in sprouts treated with sucrose (Fig. 3).

Antioxidant activity

The antioxidant activity was measured using the DPPH method and the results are shown in Fig. 4. The DPPH radical scavenging ability of sunflower sprouts (100 mg/mL) after treatment with various elicitors ranged from 28.41 ± 1.43% to 66.04 ± 0.51% while the radical scavenging ability of the standard trolox (25 µg/mL) was 64.50 ± 2.79%. The treatments with 100 mM sucrose, 5–100 mM NaCl and 0.05–0.07 mM chitosan resulted in significant increases in the antioxidant activity in the sunflower sprouts. The radical scavenging was highest for the treatment with 100 mM NaCl (an increase of 75.67% compared to the control).

Protein content

The accumulation of protein in the elicitor-treated sunflower sprouts is shown in Fig. 5. There was a significant increase in the protein content for the treatments with 50–100 mM sucrose, 0.05–0.07 mM chitosan and 0.36–1.44 mM salicylic acid. The percentage increase ranged from 35.33% to 65.39% compared to the control.

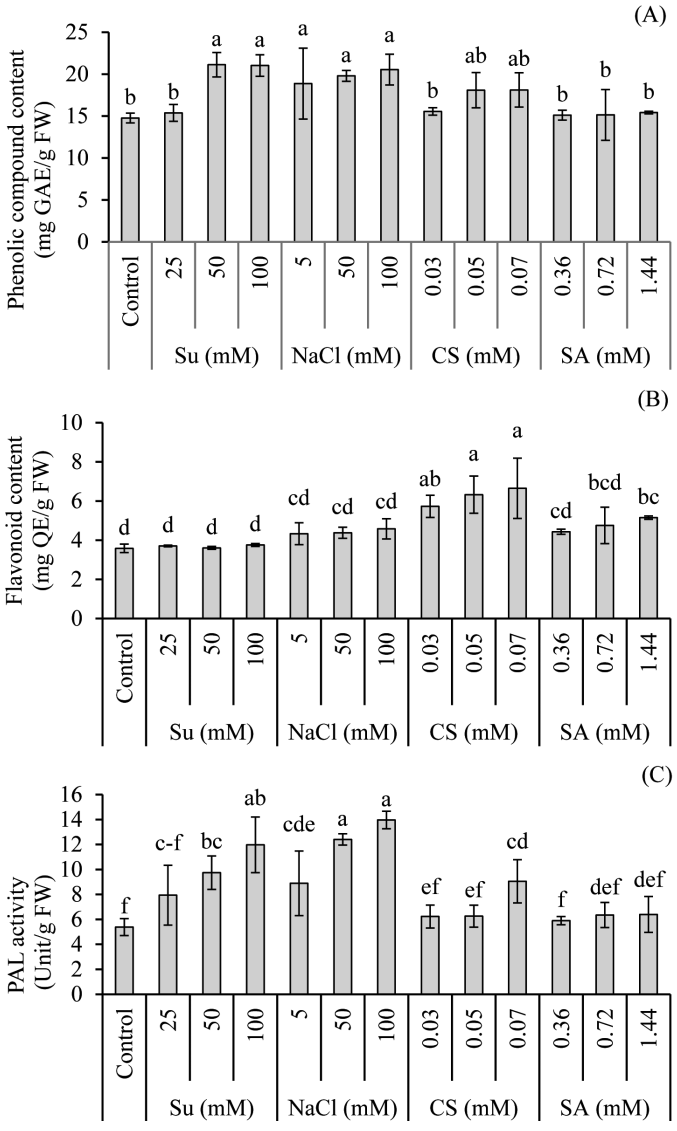

Fig. 1 Effects of different sucrose (Su), NaCl, chitosan (CS) and salicylic acid (SA) concentrations on: (A) phenolic compound content; (B) flavonoid content; (C) PAL activity of sunflower sprouts; Data are expressed as the mean ± SD; Bars with different lowercase letters are significantly different ($p < 0.05$); GAE = gallic acid equivalents; QE = quercin equivalents; PAL = phenylalanine ammonia-lyase; FW = fresh weight

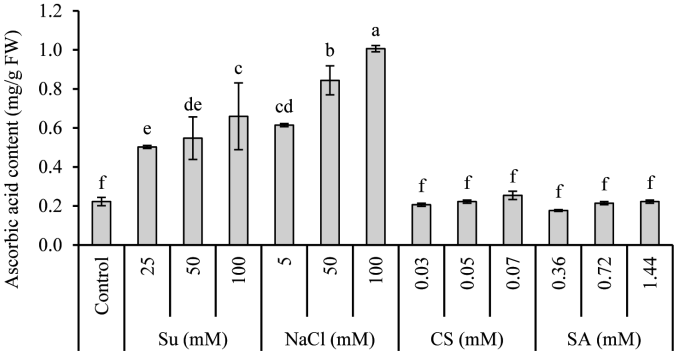

Fig. 2 Effect of different sucrose (Su), NaCl, chitosan (CS) and salicylic acid (SA) concentrations on ascorbic acid content of sunflower sprouts; Data are expressed as the mean ± SD; Bars with different lowercase letters are significantly different ($p < 0.05$); FW = fresh weight

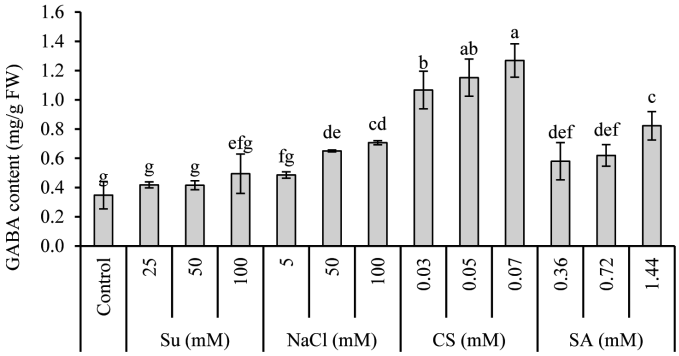

Fig. 3 Effect of different sucrose (Su), NaCl, chitosan (CS) and salicylic acid (SA) concentrations on GABA content of sunflower sprouts; Data are expressed as the mean ± SD; Bars with different lowercase letters are significantly different ($p < 0.05$); FW = fresh weight

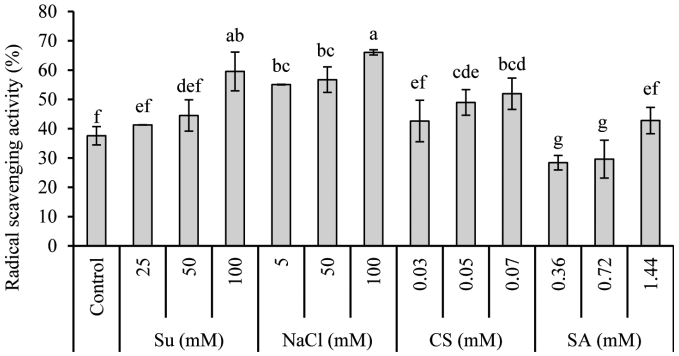

Fig. 4 Effects of different sucrose (Su), NaCl, chitosan (CS) and salicylic acid (SA) concentrations on radical scavenging activity of sunflower sprouts; Data are expressed as the mean ± SD; Bars with different lowercase letters are significantly different ($p < 0.05$); Radical scavenging activity of trolox (25 µg/mL) was 64.50 ± 2.79%

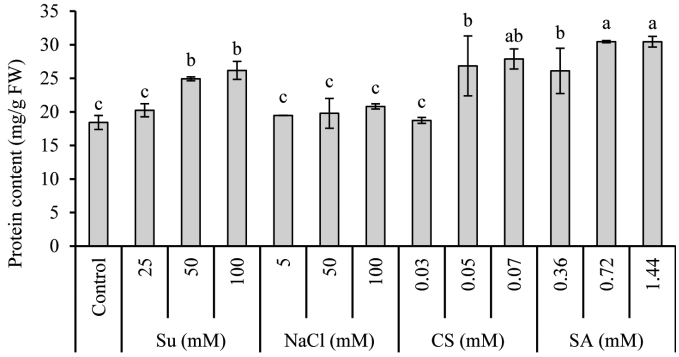

Fig. 5 Effect of different sucrose (Su), NaCl, chitosan (CS) and salicylic acid (SA) concentrations on protein content of sunflower sprouts; Data are expressed as the mean ± SD; Bars with different lowercase letters are significantly different ($p < 0.05$); FW = fresh weight

Discussion

From the human nutrition viewpoint, added value to plant-based foods due to their health-promoting quality is desirable. Over recent decades, an increasing emphasis has been placed on producing plants enriched with health-promoting compounds (Lim et al., 2012; Guo et al., 2014; Natella et al., 2016; Rouphael et al., 2018; Koodkaew, 2019). Consequently, this study evaluated the effects from the exogenous spraying of different elicitors (sucrose, NaCl, chitosan and salicylic acid, at different concentrations) on the growth and health-promoting properties of sunflower sprouts. The current study found no significant change in the fresh weight of sunflower sprouts after spraying with any of the elicitor treatments. This is desirable as growers would not want any negative influence on sprout yield after any stress elicitor treatment. However, a reduction of sunflower sprout length and an increment in the dry weight and the dry matter content of the sprouts were observed in the treatment with 100 mM NaCl. A high concentration of NaCl can cause salinity stress to the sprouts resulting in growth reduction and this condition may activate the accumulation of stress defensive compounds resulting in an increase in the dry weight (Koodkaew, 2019).

Phenolic compounds are important phytochemicals in plants and their activity, including as antioxidants, can be beneficial to human health (Babbar et al., 2011). Sucrose and NaCl induced the production of phenolic compounds in sunflower sprouts, similar to the results from Guo et al. (2011b) who found that 176 mM sucrose increased the total phenolic content in broccoli sprouts and from Lim et al. (2012) who reported that 10–200 mM NaCl enhanced phenolic compounds in buckwheat sprouts. Similar results were obtained by increasing the PAL activity through sucrose and NaCl applications to the sunflower sprouts. PAL is a key enzyme in the phenylpropanoid pathway, functioning as a catalyst for the conversion of L-phenylalanine to trans-cinnamic acid for phenolic compounds synthesis (Vogt, 2010). The activity of PAL could be induced by different stress conditions (Zhang and Liu, 2015). In the current study, there was a highly significant positive correlation between the phenolic compounds

content and PAL activity (r = 0.63, $p < 0.001$). Therefore, it can be claimed that treating sunflower sprouts with sucrose and NaCl stimulated the enzymatic activity of PAL for phenolic compounds biosynthesis through the phenylpropanoid pathway. These results were in agreement with the increase in the PAL activity observed in broccoli sprouts after sucrose treatment (Guo et al., 2011b) and in mung bean sprouts after NaCl treatment (Koodkaew, 2019).

Flavonoids are a class of phenolic compounds. The flavonoid content in the sunflower sprouts after treatment with different elicitors did not show the same trend as for phenolic compounds. A significant increase in the flavonoid content was recorded with the treatment of chitosan and 1.44 mM salicylic acid. This result was in agreement with the increase in total flavonoids of bean sprouts with 7 µM chitosan and 2 mM salicylic acid (Mendoza-Sánchez et al., 2016). Furthermore, soaking the seeds in chitosan solution increased the total isoflavone content of sunflower sprouts (Cho et al., 2008) and the total flavonoid content in yellow soybean sprouts (Yang et al., 2019). In the current study, all tested concentrations of chitosan induced the accumulation of total flavonoids in the sunflower sprouts but only the highest concentration significantly increased the PAL activity, whereas salicylic acid did not produce a significant change in the PAL activity. This could suggest that the increment in the flavonoids in the sprouts was not only related to the activity of PAL but also may result from the release of cell wall-bound phenolic compounds and this might result in an increase in the flavonoids in sprouts (Randhir et al., 2009).

Ascorbic acid, also known as vitamin C, is a water soluble antioxidant compound and elicitation can distinctly change its content in sprouts (Liu et al., 2019). The current study produced an improvement in the ascorbic acid contents in the sunflower sprouts with the sucrose and NaCl treatments. Enhancement of ascorbic acid by sucrose and NaCl in different sprouts has been reported elsewhere. For example, pre-treatments with 88 mM and 176 mM sucrose resulted in an increase in the ascorbic acid content of broccoli sprouts (Natella et al., 2016). NaCl caused a significant increase in the ascorbic acid content in mung bean sprouts (Koodkaew, 2019). The possible mechanism of elicitation in changing the amount of ascorbic acid in sprouts might be related to the activity of L-galactono-γ-lactone dehydrogenase, the key enzyme involved in ascorbic acid biosynthesis (Xu et al., 2005).

Antioxidant activity in the sunflower sprouts was evaluated using DPPH radical scavenging assay and the results showed that NaCl and a high concentration of sucrose and chitosan increased the radical scavenging activity in the sprouts. Similar results have been reported in other target plants such as broccoli sprouts with the treatment of NaCl (Guo et al., 2014) and sucrose (Natella et al., 2016), buckwheat sprouts after treatment with NaCl (Lim et al., 2012) and sucrose (Jeong et al., 2018) and bean sprouts after treatment with chitosan (Mendoza-Sánchez et al., 2016). The highest radical scavenging activity was observed in the treatment with 100 mM NaCl (66.04 ± 0.51%) followed by 100 mM sucrose (59.53 ± 3.82%) which nearly to the standard trolox (64.50 ± 2.79%). This suggested that NaCl and sucrose could improve the antioxidant property of sunflower sprouts. Basically, the antioxidant activity is related to the presence of bioactive compounds that quench free radicals and protect against oxidative damage (Forni et al., 2019). The current study showed a highly significant positive correlation between the antioxidant activity and phenolic compounds (r = 0.63, $p < 0.001$) and ascorbic acid (r = 0.75, $p < 0.001$) contents. Therefore, the increment in the antioxidant activity of the sunflower sprouts after the sucrose, NaCl and chitosan treatments could be attributed to the increase in phenolic compounds, flavonoids and ascorbic acid.

Previous research reported that pre-harvest treatment with salicylic acid increased the total phenolic compounds and antioxidant activity in broccoli sprouts (Natella et al., 2016) and bean sprouts (Mendoza-Sánchez et al., 2016), with similar results reported using chitosan in sunflower sprouts (Cho et al., 2008), while No et al. (2003) reported the enhancement of ascorbic acid in soybean sprouts after treatment with chitosan. In contrast to these reports, the current study found that chitosan and salicylic acid had no significant effect on phenolic compounds and ascorbic acid; furthermore, the antioxidant activity was reduced after salicylic acid treatment. The different results could have been due to the genetic characteristics of the sprouts, the growth phase, the growing conditions of sprouts, the elicitor dose or the method of elicitor application (Liu et al., 2019).

GABA is a non-protein amino acid common in most plants and animals whose main function is as an inhibitory neurotransmitter in the nervous system (Bowery and Smart, 2006). GABA is accumulated when a plant faces environmental stresses (Kinnersley and Turano, 2000; Bouché et al., 2003). The current study showed chitosan, salicylic acid and a high concentration of NaCl enhanced the amount of GABA in the sunflower sprouts. Similar results were found in lentil sprouts after treatment with chitosan in glutamic acid (Peñas et al., 2015). However, the current study is the first to report the effect of salicylic acid and NaCl on the GABA content in sprouts. These chemical stress elicitors could induce the physiological mechanism of GABA synthesis. Two possible mechanisms for accumulation of GABA in plants under environmental stresses have been reported: 1) stress causing metabolic disruptions followed by cytosolic acidification resulting in an acidic pH-dependent activation of glutamate decarboxylase and GABA synthesis; and 2) stresses elevating cellular levels of Ca^{2+} which induces calmodulin-dependent glutamate decarboxylase activity and GABA synthesis (Kinnersley and Turano, 2000).

Sunflower sprouts are a source of proteins with good nutritional quality (Balasaraswathi and Sadasivam, 1997). The current study found that salicylic acid and a high concentration of sucrose and chitosan increased the protein content in sunflower sprouts. This was in agreement with Koodkaew (2019) who reported that glucose treatment enhanced the protein content in mung bean sprouts and with Cho et al. (2008) who reported that soaking sunflower seeds in chitosan solution improved the amino acid content (especially the glutamic acid content) in sunflower sprouts compared to raw seeds.

The amounts of phytochemicals in sunflower sprouts exposed to different elicitors were concentration- and elicitor-dependent specific. Based on the current study, it was possible to enhance the

health-promoting properties of sunflower sprouts using sucrose, NaCl, chitosan and salicylic acid without reducing the sprout yield, which added little cost and increased the benefits to the consumer.

Conflict of Interest

The authors confirm that this article content involves no conflict of interest.

Acknowledgement

This work was supported by the Kasetsart University Research and Development Institute (KURDI), Kasetsart University, Bangkok, Thailand.

References

Babbar, N., Oberoi, H.S., Uppal, D.S., Patil, R.T. 2011. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. Food Res. Int. 44: 391–396. doi: 10.1016/j.foodres.2010.10.001

Balasaraswathi, R., Sadasivam, S. 1997. Changes in oil, sugars and nitrogenous components during germination of sunflower seeds, *Helianthus annuus*. Plant Food Hum. Nutr. 51: 71–77. doi: 10.1023/a:1007924026633

Bouché, N., Lacombe, B., Fromm, H. 2003. GABA signaling: A conserved and ubiquitous mechanism. Trends Cell Biol. 13: 607–610. doi.org/10.1016/j.tcb.2003.10.001

Bowery, N.G., Smart, T.G. 2006. GABA and glycine as neurotransmitters: A brief history. Br. J. Pharmacol. 147: S109–S119. doi: 10.1038/sj.bjp.0706443

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254. doi.org/10.1016/0003-2697(76)90527-3

Brand-Williams, W., Cuvelier, M.E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28: 25–30. doi.org/10.1016/ S0023-6438(95)80008-5

Cho, M.H., No, H.K., Prinyawiwatkul, W. 2008. Chitosan treatments affect growth and selected quality of sunflower sprouts. J. Food Sci. 73: S70–S77. doi.org/10.1111/j. 1750-3841.2007.00607.x

Forni, C., Facchiano, F., Bartoli, M., et al. 2019. Beneficial role of phytochemicals on oxidative stress and age-related diseases. Biomed Res. Int. 2019: 1–16. https://doi.org/10.1155/2019/8748253

Guo, S., Ge, Y., Jom, K.N. 2017. A review of phytochemistry, metabolite changes, and medicinal uses of the common sunflower seed and sprouts (*Helianthus annuus* L.). Chem. Cent. J. 11: 95. doi: 10.1186/s13065-017-0328-7

Guo, L., Yang, R., Wang, Z., Guo, Q., Gu, Z. 2014. Effect of NaCl stress on health-promoting compounds and antioxidant activity in the sprouts of three broccoli cultivars. Int. J. Food Sci. Nutr. 65: 476–481. doi: 10.3109/09637486.2013.860583

Guo, R., Yuan, G., Wang, Q. 2011a. Sucrose enhances the accumulation of anthocyanins and glucosinolates in broccoli sprouts. Food Chem. 129: 1080–1087. doi.org/10.1016/j. foodchem.2011.05.078

Guo, R., Yuan, G., Wang, Q. 2011b. Effect of sucrose and mannitol on the accumulation of health-promoting compounds and the activity of metabolic enzymes in broccoli sprouts. Sci. Hortic. 128: 159–165. doi.org/10.1016/j.scienta.2011.01.014

Jeong, H., Sung, J., Yang, J., Kim, Y., Jeong, H.S., Lee, J. 2018. Effect of sucrose on the functional composition and antioxidant capacity of buckwheat (*Fagopyrum esculentum* M.) sprouts. J. Funct. Foods. 43: 70–76. doi.org/10.1016/j.jff.2018.01.019

Jiraungkoorskul, W. 2016. Review of nutraceutical uses of an antioxidant sunflower sprout, *Helianthus annuus*. Asian J. Pharm. Clin. Res. 9: 21–23. doi: 10.22159/ajpcr.2016. v9i6.12874

Karladee, D., Suriyong, S. 2012. γ-Aminobutyric acid (GABA) content in different varieties of brown rice during germination. Sci. Asia. 38: 13–17. doi: 10.2306/scienceasia 1513-1874.2012.38.013

Kinnersley, A.M., Turano, F.J. 2000. Gamma aminobutyric acid (GABA) and plant responses to stress. Crit. Rev. Plant Sci. 19: 479–509. doi.org/10.1080/07352680091139277

Koodkaew, I. 2019. NaCl and glucose improve health-promoting properties in mung bean sprouts. Sci. Hortic. 247: 235–241. doi.org/10.1016/j.scienta.2018.12.022

Li, H., Tang, C., Xu, Z., Lin, X., Han, X. 2012. Effects of different light sources on the growth of non-heading Chinese cabbage (*Brassica campestris* L.). J. Agri. Sci. 4: 262–273. doi: 10.5539/jas.v4n4p262

Lim, J.H., Park, K.J., Kim, B.K., Jeong, J.W., Kim, H.J. 2012. Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout. Food Chem. 135: 1065–1070. doi: 10.1016/j.foodchem.2012.05.068

Liu, H., Kang, Y., Zhao, X., Liu, Y., Zhang, X., Zhang, S. 2019. Effects of elicitation on bioactive compounds and biological activities of sprouts. J. Funct. Foods. 53: 136–145. doi: 10.1016/J.JFF.2018.12.019

Martin, C., Butelli, E., Petroni, K., Tonelli, C. 2011. How can research on plants contribute to promoting human health? Plant Cell. 23: 1685–1699. doi: 10.1105/tpc.111.083279

Mendoza-Sánchez, M., Guevara-González, R.G., Castaño-Tostado, E., Mercado-Silva, E.M., Acosta-Gallegos, J.A., Rocha-Guzmán, N.E., Reynoso-Camacho, R. 2016. Effect of chemical stress on germination of cv Dalia bean (*Phaseolus vulgaris* L.) as an alternative to increase antioxidant and nutraceutical compounds in sprouts. Food Chem. 212: 128–137. doi: 10.1016/j.foodchem.2016.05.110

Natella, F., Maldini, M., Nardini, M., et al. 2016. Improvement of the nutraceutical quality of broccoli sprouts by elicitation. Food Chem. 201: 101–109. doi: 10.1016/j.foodchem. 2016.01.063

No, H.K., Lee, K.S., Kim, I.D., Park, M.J., Kim, S.D., Meyers, S.P. 2003. Chitosan treatment affects yield, ascorbic acid content, and hardness of soybean sprouts. J. Food Sci. 68: 680–685. doi.org/10.1111/j.1365-2621.2003.tb05731.x

Peñas, E., Limon, R.I., Martinez-Villaluenga, C., Restani, P., Pihlanto, A., Frias, J. 2015. Impact of elicitation on antioxidant and potential antihypertensive properties of lentil sprouts. Plant Foods Hum. Nutr. 70: 401–407. doi: 10.1007/s11130-015-0508-3

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/, 15 March 2015.

Ramakrishna, A., Ravishankar, G.A. 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6: 1720–1731. doi: 10.4161/psb.6.11.17613

Randhir, R., Kwon, Y.I., Shetty, K. 2009. Improved health-relevant functionality in dark germinated *Mucuna pruriens* sprouts by elicitation with peptide and phytochemical elicitors. Bioresour. Technol. 100: 4507–4514. doi: 10.1016/j.biortech.2009.01.078

Rouphael, Y., Petropoulos, S.A., Cardarelli, M., Colla, G. 2018. Salinity as eustressor for enhancing quality of vegetables. Sci. Hortic. 234: 361–369. doi.org/10.1016/j. scienta.2018.02.048

Singleton, V.L., Orthofer, R., Lamuela-Ravenros, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method. Enzymol. 299: 152–178. doi.org/10.1016/ S0076-6879(99)99017-1

Vogt, T. 2010. Phenylpropanoid biosynthesis. Mol. Plant 3: 2–20. doi. org/10.1093/mp/ssp106

Wei, J., Miao, H., Wang, Q. 2011. Effect of glucose on glucosinolates, antioxidants and metabolic enzymes in *Brassica* sprouts. Sci. Hortic. 129: 535–540. doi.org/10.1016/ j.scienta.2011.04.026

Xu, M.J., Dong, J.F., Zhu, M.Y. 2005. Effects of germination conditions on ascorbic acid level and yield of soybean sprouts. J. Sci. Food Agric. 85: 943–947. doi.org/10.1002/ jsfa.2050

Yang, R., Jiang, Y., Xiu, L., Huang, J. 2019. Effects of chitosan pre-soaking on the growth and quality of yellow soybean sprouts. J. Sci. Food Agric. 99: 1596–1603. doi.org/ 10.1002/jsfa.9338

Zhang, X., Liu, C.J. 2015. Multifaceted regulations of gateway enzyme phenylalanine ammonia-lyase in the biosynthesis of phenylpropanoids. Mol. Plant. 8: 17–27. doi: 10.1093/mp/ssu134.

Zhishen, J., Mengcheng, T., Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555–559. doi.org/10.1016/S0308-8146(98)00102-2

Zhu, Q., Xie, X., Lin, H., et al. 2015. Isolation and functional characterization of a phenylalanine ammonia-lyase gene (*SsPAL1*) from Coleus (*Solenostemon scutellarioides* (L.) Codd). Molecules 20: 16833–16851. doi: 10.3390/ molecules 200916833