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The Effects of UV Light and Methyl Salicylate on Phytochemical Constituents and Nutritional Traits in Common Bean (*Phaseolus vulgaris* L.)

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ABSTRACT

Background: Mutagenesis has also been used to improve many desirable traits such as earliness, darkness, resistance or tolerance to biotic and abiotic stress, seed yield and oil quality. There are numerous mutagen agents, both chemical and physical, available to create and obtain valuable mutations in crop plants.

Methods: The experiment was done at the laboratory of the Faculty of Applied Sciences, Ton Duc Thang University in Vietnam from September 2021 to December 2022. This study examined the effects of ultraviolet B-light (UV-B) and methyl salicylate (MeSA) treatment on phytochemical constituents (total phenols and flavonoids) and nutritional characteristics (protein and fat content) in a common bean cultivar, GRIS2.

Result: The results show that UV irradiation for 3 hours increased the phenolic content in M1 seeds when compared to the control and other treatments. Furthermore, compared to the control and other treatments, UV-B irradiation for up to 5 hours increased the phenolic, protein and lipid content in the pod (in the immature pod, mature pod and seed). Alternatively, treatment with MeSA considerably decreased the levels of these same compounds. Treating the seed with 0.05 mM MeSA did increase the flavonoid content. These findings demonstrate the potential of UV-B treatment for improving bean nutritional quality. On the other hand, the negative general implications of the higher MeSA treatment suggest exogenous treatments should be kept at lower concentrations to ensure the minimization of nutritional losses.

Key words: Common bean, Flavonoid, lipid, Mesa, phenolic, Protein, Uv light.

INTRODUCTION

Crops can be treated in a variety of ways to produce desired plant stress responses, defense mechanisms, changes in physiology and enhancements in the production of secondary metabolites (Arbona *et al.,* 2013; Khan *et al.*, 2015; Kusano *et al.*, 2011; Bruno, 2022; Ramegowda and Senthil-Kumar, 2015). Some examples of these treatments include exogenously applied phytohormones, plant extracts and natural compounds, strategically applied nutrients, microbial inoculants, RNAi and physical treatments (Cheng *et al.*, 2015; Ghasemzadeh *et al.*, 2011; Kopriva and Rennenberg, 2004; Glick, 2012; Zhang *et al.*, 2013; Zu *et al.*, 2010).

Ultraviolet light (UV) is a type of physical treatment that affects plant growth, whereby its impact is dependent on the wavelength used (Nasibi and Kalantari, 2005; Hamid and Jawaid, 2011). UV light can be categorized into three different wavelength ranges: UV-A (320-390 nm), UV-B (280-320 nm) and UV-C (254-280 nm). Among the three types of UV radiation, only UV-A and UV-B reach the earth's surface, whereas the ozone layer absorbs UV-C (Bintsis *et al.*, 2000). Plants respond to light through different photoreceptors and previous studies have shown that UV light can either promote or inhibit plant growth and its effects can vary across plant species (Zu *et al.*, 2010; Franklin and Whitelam, 2007; Salama *et al.,* 2011; Escobar-Bravo *et al.*, 2017; Deckmyn and Impens, 1998; Suchar and Robberecht, 2016). UV-B light exposure has been shown to have a positive effect on physiological processes, secondary metabolite abundance and overall growth in several plant species (Ueda and

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Nakamura, 2011; Demkura *et al.*, 2010; Kataria and Guruprasad, 2018; Hao *et al.*, 2022).

The application of methyl salicylate (MeSA) can be considered to be a plant extract treatment. It is a volatile organic compound synthesized from salicylic acid (SA), which plays an important role in pollination, seed germination, plant growth and development and response to abiotic and biotic stressors (Liu *et al.*, 2011; Ha *et al.*, 2019; Ha *et al.,* 2020a; Liu *et al.,* 2018; Brouat *et al.*, 2000). One of the primary functions of MeSA in plants is its role as a signaling molecule in plant defense against pathogens and herbivore attacks (Ren *et al.*, 2020; Shulaev *et al.*, 1997; Zou *et al.,* 2019). In the case of herbivore attack when a plant is impacted, it releases MeSA as a signal to neighboring healthy tissues or other plants, allowing these tissues/plants to produce a systemic acquired resistance (SAR) signal, allowing the activation of defense mechanisms throughout (Park *et al.*, 2007; Thulke and Conrath, 1998). Exogenous MeSA treatments have been utilized as a means to improve a plant's tolerance to salinity, coldness and disease resistance or create a favorable flavor profile (Ha *et al.*, 2019; Ha *et al.,* 2020a; Ding, 2002). MeSA treatments have been found to have a negative influence on nutritional indices in high doses (Kalaivani *et al.,* 2018).

The common bean (*Phaseolus vulgaris* L.) is a member of the legume family that is highly nutritious, which is why it is consumed worldwide (Ha *et al.,* 2020b). Not only is it a good source of protein, making it a cheap alternative to meat, but the common bean also contains various nutrients such as proteins, starch, unsaturated fatty acids, dietary fibers, vitamins, minerals and phytochemicals, which are beneficial to human health (Ferris and Kaganzi, 2008; Merga, 2020; Chávez-Mendoza *et al.,* 2018). The ever-pressing issues of population growth, climate change, land degradation and resource constraints have led to serious concerns for food security. Concerning the common bean, improving productivity and nutritional quality will help mitigate the effects of these issues. Treatment with exogenous plant hormones and physical treatments has been used to enhance yield, quality and resistance to biotic and abiotic stresses in several plant species (Khan *et al.*, 2015; Escobar-Bravo *et al.*, 2017; Park *et al.*, 2007; Teramura, 1983) and could be used in the common bean. In a recent study, Ha *et al.* (2022a) evaluated the effects of MeSA and UV-B treatments on alpha-amylase activity and phenological and agronomic characteristics of the common bean (GRIS2). The purpose of this study was to investigate the impacts of the same MeSA and UV-B treatments on the phenolic, protein, lipid and flavonoid content in seed pods of the common bean (GRIS2).

MATERIALS AND METHODS

In the current experiment, the common bean GRIS2 cultivar was used as a plant material and the study was conducted at the laboratory of the Faculty of Applied Sciences, Ton Duc Thang University in Vietnam. The seeds of common bean GRIS2 were irradiated with UV light for 3 and 5 hours and two concentrations of MeSA (0.01 and 0.05 mM) as collected by our previous study (Ha *et al.,* 2022a**).** The seeds were sown in the pots to raise the M1 generation and then the number of 30 plants in each treatment was analyzed for phytochemical constituents (total phenols and flavonoids) and nutritional characteristics (protein and fat content).

The phytochemical constituents

The phytochemical constituents such as total phenols and flavonoid content were determined in the pods and seeds of common bean (GRIS2).

Samples collection and plant extraction

500 g of pods and seeds of M1 of common bean GRIS2 were cleaned to remove soil and damaged seeds, dried and ground into a fine powder. Methanol extraction was conducted based on the method described by Obeidat *et al.* (2012). Briefly, extracts of the pods and seeds were each prepared in methanol (plant: solvent ratio [1: 10], w/v) and shaken for 24 hours at room temperature. After that, filter paper was used to separate the extract. The extracts were then dried by removing the solvent by vacuum evaporation. Before being used, the extracts were kept cold at 4 ^{o}C.

Total phenolic content (TPC)

The TPC of each extract of leaves, pods and seeds was determined using the Folin-Ciocalteu reagent, as described by Singleton *et al.* (1999), with minor modifications. First, 0.5 mL of each extract was dissolved in 100 g mL^{-1} methanol and mixed with 2.5 mL of Folin-Ciocalteu reagent (0.2 N). This mixture was then thoroughly shaken and allowed to stand at room temperature for 5 min before adding 2 mL of sodium carbonate solution (75 g L^{-1}). Finally, after 2 hours in the dark, the absorbency at 760 nm was measured against a water blank using a UV-vis spectrophotometer. Gallic acid solutions were used as a standard for the calibration curve and the same procedure was followed in the sample extract. The standard concentrations were set to 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg mL^{-1}. The measurement was repeated three times and the results were expressed in terms of Gallic Acid Equivalent (mg of GAE g^{-1} of extract).

Total flavonoid content (TFC)

The TFC of each pod and seed extract was determined using a slightly modified version of the Dowd method as described by Sawadogo *et al.* (2006). Briefly, 2 mL of 2% $AlCl_3$ in methanol was mixed with 2 mL of each extract (100 g mL^{-1}) and the mixture was held for 10 minutes after vigorous shaking. Using a UV-vis spectrophotometer, the absorbance of a blank sample comprised of 2 mL of methanol and 2 mL of each extract without $AlCl_3$ was measured at 415 nm. The same procedure was repeated with rutin solutions serving as the standards for the calibration curve. The standard concentrations were set to 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg mL^{-1}. The analysis was done in triplicate and the results were expressed as rutin Equivalent (mg of RE g^{-1} of extract).

The nutritional traits

The nutritional traits such as protein content and crude lipid content were determined in the immature pods, filled pods and seeds derived from the M1 generation of common bean (GRIS2).

Quantification of protein content (PC)

PC was quantified by the dye-binding assay method as described by Bradford (1976) with slight modification. The principle of this test is the existence of a bond between Coomassie Brilliant Blue G250 (CBBG) with protein in acidic conditions. Briefly, the test begins with standard curve-making using Bovine Serum Albumin, each at concentrations of 0, 10, 20, 30, 40 and 50 µg mL^{-1}. Furthermore, each 0.1 mL of standard protein and protein extract were dissolved in 5 mL of Bradford reagent (0.01 g CBBG in 5 mL of 95% ethanol (v/v), adding 10 mL of 85% phosphoric acid (v/v) and homogenized. 0.1 mL of distilled water and 5 mL of Bradford reagent were used as a reference. After incubating at room temperature for 15 min, absorbance was read at a wavelength of 595 nm, the analysis was done in triplicate. The standard curve equation obtained was used to calculate the protein content of the sample extract. Protein concentration was expressed as g protein $100g^{-1}$ of fresh weight.

Quantification of crude lipid content (LC)

The crude LC was determined using the Soxhlet extraction method described by Felix and Francis (2019) with slight modifications. Briefly, weighing 1 g of each sample was placed in a porous thimble of a Soxhlet extractor with a cotton plug at its mouthed and the thimble was placed in an extraction chamber which was suspended to a previously weighed flask containing petroleum ether. The whole assembly was adjusted and extraction was carried out for 5 h at 40 °C. After the extraction, the thimble was removed from the Soxhlet apparatus and the solvent was removed under reduced pressure to afford crude lipid. The flask containing lipids was placed in the oven till drying was complete then cooled in a desiccator and weighed. The fat content in each sample was calculated using the following equation:

$$FC\ (\%) = [W_2 - W_1 \times 100] \div W_5$$

Where:

FC (%) = Fat content.

W_1 = Weight of extraction flask.

W_2 = Weight of extract ion flask with oil.

W_5 = Weight of sample.

Statistical analysis

The results of at least three duplicate measurements were expressed as mean and standard deviation. Analysis of variance (ANOVA) and Duncan's multiple range test were used to identify differences that were significant at P< 0.01. The statistical software SAS version 8.0 and Microsoft Excel 2019 were used for all statistical analysis.

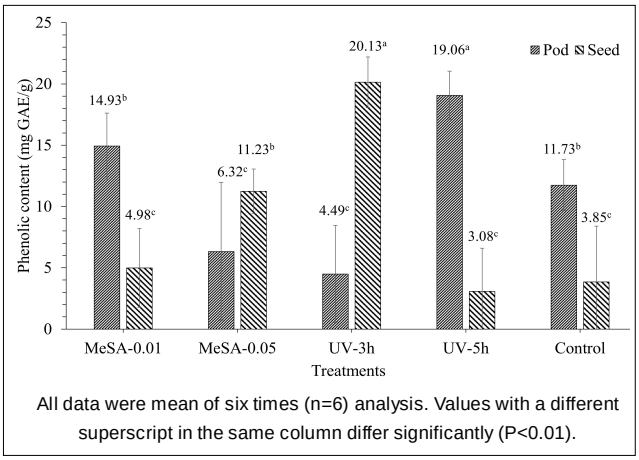
RESULTS AND DISCUSSION

The effect of MeSA and UV light on TPC

The content of total phenols in different extracts (Fig 1) calculated from the Gallic acids calibration curve (y = 10.289x + 0.0046, R^2 = 0.9995) ranged between 4.49 and 19.06 mg GAE g^{-1} and 3.08 to 20.13 mg GAE g^{-1} in bean pod and seed, respectively. In the bean pod, total phenolic levels decreased when treated with MeSA-0.05 and UV-3h (6.32 and 4.49 mg GAE g^{-1}, respectively) as compared to control (11.73 mg GAE g^{-1}). However, treatment treated with UV-5h (19.06 mg GAE g^{-1}) was found to have the highest total phenolic content in pods. While with UV-3h treatments, in seeds, the highest TPC (20.13 mg GAE g^{-1}) as compared to control (3.85 mg GAE g^{-1}) was observed. The effects when seeds were treated with MeSA and UV light were likely to be positive and negative depending on the doses used and plant species studied. Mutations or changes in the genetic material are the ultimate source of all genetic variation between individuals (Begna, 2021). Many mutants have been released directly as new varieties and many others used as parents to create varieties with improved traits like yield, quality of seed propagated crops, modified oil, protein and starch quality, enhanced uptake of specific metals, deeper rooting system and resistance to biotic and abiotic stresses. The results were consistent with those of many previous studies that showed the effects of UV light (Younis *et al.,* 2010; Papoutsis *et al.,* 2016) and MeSA (Ha *et al.,* 2020a; 2022b) on flavonoid and phenolic content in plants. Previous studies reported that UV-C light increased phenolic compound levels and antioxidant capacity in tomatoes (Barka, 2001; Bravo *et al.,* 2012).

The effect of MeSA and UV light on TFC

Results for TFC (Fig 2), calculated from the Rutin calibration curve (y = 6.4957x + 0.0087, R^2 = 0.9989), ranged between 6.75 and 9.24 mg RE g^{-1} and 6.26 and 11.74 mg RE g^{-1} in bean pod and seed, respectively. In the seeds, all the treatments had significant effects on TFC at P<0.01 (Fig 2).



All data were mean of six times (n=6) analysis. Values with a different superscript in the same column differ significantly (P<0.01).

Fig 1: The effects of MeSA and UV light on the total phenolic content of common bean (GRIS2).

Table 1: The effects of MeSA and UV light on the protein content in the M1 generation of common bean (GRIS2).

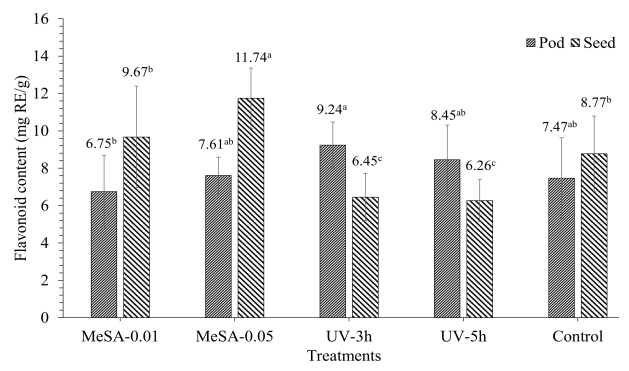
Treatments	Protein content (g 100g^{-1})		
	Immature pod	Pod fill	Seed
MeSA-0.01	16.20±0.73^{d}	16.02±0.87^{e}	16.72±0.76^{d}
MeSA-0.05	22.03±0.87^{b}	24.34±0.56^{b}	23.92±0.69^{b}
UV-3h	21.90±0.96^{b}	16.93±0.63^{d}	23.90±0.83^{b}
UV-5h	30.19±0.49^{a}	25.98±0.65^{a}	29.59±0.80^{a}
Control	20.20±0.83^{c}	19.02±0.81^{c}	21.02±0.76^{c}

All data are mean ± SD of six times (n=6) analyses. Values with a different superscript in the same column differ significantly (P<0.01).

Table 2: The effects of MeSA and UV light on the lipid content in the M1 generation common bean (GRIS2).

Treatments	Lipid content (%)		
	Immature pod	Pod fill	Seed
MeSA-0.01	2.22±0.27^{c}	1.37±0.87^{c}	1.39±0.85^{c}
MeSA-0.05	2.86±0.66^{b}	3.17±0.94^{b}	2.43±0.56^{b}
UV-3h	3.37±0.48^{b}	4.44±0.55^{a}	1.55±0.35^{c}
UV-5h	4.08±0.36^{a}	4.52±0.86^{a}	3.37±0.67^{a}
Control	3.35±0.22^{b}	2.32±0.41^{b}	2.32±0.33^{b}

All data are mean ± SD of six times (n=6) analyses. Values with a different superscript in the same column differ significantly (P<0.01).



All data were mean of six times (n=6) analyses. Values with a different superscript in the same column differ significantly (P<0.01).

Fig 2: The effects of MeSA and UV light on the total flavonoid content of common bean (GRIS2).

Lesser TFC was observed in the seeds treated with UV-3h (6.45 mg RE g^{-1}) and UV-5h (6.26 mg RE g^{-1}). While the highest TFC was observed when the seeds were treated with MeSA-0.05 (11.74 mg RE g^{-1}) as compared to the control (8.77 mg RE g^{-1}). Gurdon *et al.* (2019) examined UV-induced changes in flavonoid and total phenol concentration in lettuce. Rivera-Pastrana *et al.* (2013) observed indeed that flavonoids accumulated more in the peel than in any other plant parts of papaya fruits submitted to UV-C light. Li *et al.* (2019) showed that exogenous methyl salicylate increased flavonoid concentration in tea leaves in a dose-dependent manner. While 1 mM MeSA resulted in the highest increase in flavonoid concentration and a high concentration of 5 mM MeSA decreased flavonoid concentration in tea leaves (Li *et al.,* 2019). These results show the role of MeSA and UV light in regulating flavonoid and phenolic biosynthesis in the common bean, which may have potential significance for improving plant varieties.

The effect of MeSA and UV light on protein content (PC)

PC in the immature pod, pod fill and seed was determined by the Bradford method using bovine serum albumin as the standard curve. This method was simple, fast, easy to perform, less susceptible to interference by contaminants and inexpensive for multiple applications in experimental sciences. The PC (Table 1), calculated from the Bovine Serum Albumin calibration curve (y = 0.0049x + 0.0102, R^2 = 0.9944), ranged between 16.20±0.73 and 30.19 0.49 g 100 g^{-1} for the immature pod, 16.02±0.87 and 25.98±0.65 g 100 g^{-1} for pod fill and 16.72±0.76 and 29.59±0.80 g 100 g^{-1} for seed. In the immature pod, the highest PC was found in the seeds treated with UV-5h (30.19±0.49 g 100 g^{-1}) compared to the control, while the lowest protein content was observed in the seeds treated with MeSA-0.01 (16.20±0.73 g 100 g^{-1}) as compared to control (20.20±0.83 g 100 g^{-1}). In the filled pods, all the UV light and MeSA treatments had significant effects on PC at P<0.01. Low PC was observed in the seeds treated with MeSA-0.01 (16.02±0.87 g 100 g^{-1}) as compared to the control (19.02±0.81 g 100 g^{-1}). Meanwhile, the highest PC was found in the seeds treated with UV-5h (25.98±0.65 g 100 g^{-1}). Like in filled pods, in the seeds, low PC was observed in the seeds treated with MeSA-0.01 (16.72±0.76 g 100 g^{-1}) as compared to the control (21.02±0.76 g 100 g^{-1}). The highest PC was found in the seeds treated with UV-5h (29.59±0.80 g 100 g^{-1}).

The effect of MeSA and UV light on lipid content (LC)

The results of the crude LC using the Soxhlet extraction method are represented in Table 2. In the immature pod, the highest LC was found in the seed treated with UV-5h (4.08%) compared to the control, while the lowest LC was observed in the seed treated with MeSA-0.01 (2.22%) as compared to the control (3.35%). In the filled pods, low LC was observed in the seed-treated MeSA-0.01 (1.37%) as

compared to the control (2.32 ± 0.41%) whereas the highest LC was found in the seed treated with UV-5h and UV-3h (4.52% and 4.44%, respectively). On the other hand, in the seed, low LC was observed in the seed-treated MeSA-0.01 (1.39%) and UV-3h (1.55%) as compared to the control (2.32%). Meanwhile, the highest LC was found in the seed treated with UV-5h (3.37%).

Kalaivani *et al.* (2018) reported that MeSA influenced nutrition indices negatively in a dose-dependent manner. In this study, compared to the control treatment, UV-5h treatment of M1 generation of common bean (GRIS2) resulted in significant increases in protein and lipid contents. Also, seeds treated with MeSA-0.05 had high protein and lipid contents, although it was lower than for the treatment with UV-5h. In contrast, seeds treated with MeSA-0.01 had an impact negative on protein and lipid contents in the M1 common bean population (GRIS2). This showed that both UV light and MeSA had affected the bean positively or negatively. Hence, the effect depends on the type of mutagen and doses used to improve nutrients for the crop.

CONCLUSION

This study examined the effect of UV light radiation and MeSA on phytochemical constituents (total phenolic and flavonoid contents) and nutritional traits (protein and lipid contents) in the M1 generation of common bean GRIS2. Results show that the UV-5h and MeSA 0.05 mM treatments had a significant positive effect on protein and lipid contents in the M1 generation of common bean GRIS2 when compared with the control treatment ($P<0.05$). Furthermore, UV-3h treatment increased the TPC of seeds and TFC of pods, whereas UV-5h treatment increased the TPC of pods more effectively in M1 generation common bean GRIS2. Also, the MeSA-0.05 mM treatment increased the TFC of seeds in M1 common bean GRIS2. This experiment provides useful information for improving the nutritional quality of the common bean and these results need to further be encouraged and explored in the mutation breeding programs of the common bean.

Conflict of interest: None.

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