Effects of UV-B pre-treatments on physiological properties and accumulation of active ingredients in herbal plant, *Prunella vulgaris*

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Abstract

Aim: The effects of UV-B pretreatment on biosynthesis of active ingredients in *Prunella vulgaris* L. were studied from three aspects: active ingredients, physiological and biochemical parameters and key enzymes of rosmarinic acid metabolism pathway.

Methodology: In greenhouse, the seedlings of *P. vulgaris* were subjected to UV-B treatment for 30 min from 10:30 a.m. to 11:00 a.m. every day for 10 days and then transplanted to field. After ripening, the leaves were harvested for physiological and biochemical estimations and the expression of key enzyme genes and the contents of active ingredients were measured by ear picking.

Results: The results of active ingredient content showed that artificial UV-B radiation increased the contents of phenolic acids and individual flavonoids at mature fruiting stage compared with control plants. Physiological and biochemical results indicated that increase in peroxidase, ascorbate peroxidase, and superoxide dismutase activities seem to be active responses to alleviate the deleterious effects of UV-B in *P. vulgaris*. Under UV-B pre-treatment, genes related to rosmarinic acid/phenolic acid biosynthesis were significantly ($p < 0.05$) over-expressed at seedling stage of *P. vulgaris*.

Interpretation: This study indicated that UV-B pre-treatment in the seedling stage before transplantation to field is effective for inducing phenolic acid and flavonoid accumulation in *P. vulgaris* ears at mature fruiting stage.

Key words: Active ingredients, Gene expression, *Prunella vulgaris* L., Ultraviolet-B

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Introduction

A loss of ozone layer is resulting in depletion of stratosphere and increase in the penetration of ultraviolet-B (UV-B, 280-320 nm) radiation through Earth's surface, with potentially harmful consequences for natural plant ecosystems and agricultural production (Caldwell *et al.*, 2003). Ultraviolet (UV-B) radiation is a key abiotic stress factor with many indirect and direct effects on plants (Singh *et al.*, 2011; Eichholz *et al.*, 2012). Increasing UV-B radiation has shown both favourable and unfavourable effects on the growth and physiology of plants (Liu *et al.*, 2013; Zhang *et al.*, 2017). Generally, increased UV-B radiation is considered harmful to plants (Agrawal and Mishra, 2009; Tossi *et al.*, 2011). Numerous studies have shown that UV-B radiation adversely affects plants, often leading to the production of reactive oxygen species (ROS) (Barta *et al.*, 2004). Some medicinal plants activate various defence mechanisms to resist oxidative stress, including increases in antioxidant enzymes (Yan *et al.*, 2016), production of antioxidant secondary metabolites (mainly phenolic acids and flavonoids) and expression of key enzyme-encoding genes in the phenylpropanoid biosynthetic pathway (Zhang and Bjorn, 2009; Dolzhenko *et al.*, 2010). It is likely that these antioxidant defence mechanisms scavenge ROS to counter the deleterious effects of UV-B radiation.

Prunella vulgaris L. (Family: Labiatae) is a perennial plant that is widely distributed in Europe, North-east Asia and North America (Chen *et al.*, 2018). The aerial parts and dried spicas are used to treat pulmonary tuberculosis, thyroid gland malfunction, infectious hepatitis and mastitis (Bai *et al.*, 2016). In addition to its pharmaceutical industrial use, the fresh leaves of *P. vulgaris* at seedling stage are used as food in China (Chen *et al.*, 2019). *P. vulgaris* dry spicas and fresh leaves contain large amounts of antioxidants and nutritional components, such as flavonoids, phenolics, triterpenes and polysaccharides (Cheung and Zhang, 2008; Wang *et al.*, 2011), which are considered to present potential medicinal and health food functions. Previous studies have reported that abiotic stresses such as drought, different soil nutrient concentrations, and different light spectra significantly enhance the contents of active ingredients (phenolic compounds and flavonoids) in *P. vulgaris* plants (Chen *et al.*, 2013; Fazal *et al.*, 2016). It has been assumed that UV-B pre-treatment of *P. vulgaris* at seedling stage in green house may induce greater ROS production, leading to higher active ingredient content in the plants after transfer to field. On the other hand, it is not completely understood whether synthesis of secondary metabolites is determined by transient metabolic regulation or involves changes in gene expression in the phenylpropanoid synthetic pathway.

Keeping in view the above, the present study was carried out to investigate the effects of UV-B pre-treatment on the antioxidative defence system, oxidative stress markers, phenylpropanoid biosynthetic pathway gene expression, and active ingredient biosynthesis in *P. vulgaris* plants. This study will contribute to revealing whether UV-B pre-treatment is a potential

stressor for *P. vulgaris* seedlings grown in the greenhouse following transfer to field conditions for production of more active ingredients.

Materials and Methods

Plant material: The experiment was carried out at Chengdu Medical College Botanic Garden, Chengdu, PR. China. Seeds of *P. vulgaris* were collected in June 2017 and disinfected with 9% H₂O₂ for approximately 30 min, then rinsed several times with tap water. On 20 March 2018, twenty seeds were sown in plastic pots with 900 g of nutrient soil, and each pot contained 3 seedlings. All pots were placed in the experimental greenhouse under 14.6 ± 13.08 kilolux of photosynthetically active radiation (PAR), 50-70% relative humidity, and temperatures of 25°C/12°C (day/night). Plant growth was observed daily and the plants were watered as and when required.

UV-B irradiation: On 1 April 2018 seedlings of *P. vulgaris* were divided into four groups: three UV-B-treated groups (lower dose: PAR + 35 $\mu\text{W cm}^{-2} \text{ nm}^{-1}$; intermediate dose: PAR + 55 $\mu\text{W cm}^{-2} \text{ nm}^{-1}$; higher dose: PAR + 75 $\mu\text{W cm}^{-2} \text{ nm}^{-1}$) and a control group (CK). Each group was replicated three times, and each replicate consisted of 24 plants. The PAR and light intensities of UV-B were 25000 ± 100 lux and 700 ± 20 $\mu\text{W cm}^{-2} \text{ nm}^{-1}$ under natural conditions, respectively. UV-B radiation were applied using a portable illuminometer (UV-340A, Lutron, Taiwan) and UV-B fluorescent tubes (Beijing Electric Light Source Research Institute, 40 W, China) for enhanced UV-B radiation. *P. vulgaris* in seedling stage was subjected to UV-B treatment for 30 min every day from 10:30 a.m. to 11:00 a.m. for 10 days. After 10 days of treatment, the control plants and one group of UV-B-treated plants were collected for physiological and biochemical analyses. Another three groups of treated plants and control plants were transferred to field conditions. *P. vulgaris* spicas were harvested at mature fruiting stage to analyse the influence of UV-B pre-treatment on active ingredient biosynthesis.

Anthocyanin and UV-B-absorber contents: Anthocyanin was estimated by the method of Chen *et al.* (2019). Chopped fresh leaves (0.5 g) were soaked in dark with 5 ml of acidified methanol and shaken 4-5 times for 72 hr. UV absorber and anthocyanin contents were estimated by recording the absorbance at 280 and 530 nm, separately. The results were expressed in Units g⁻¹ f.wt. The procedure was repeated three times for each treatment.

Antioxidant enzymes activities: Antioxidant enzymes activities were estimated similar as described by Chen *et al.*, 2019. Briefly, fresh leaves (0.5 g) were placed in 4.5 ml of phosphate buffer (0.1 mol l⁻¹, pH 7.43) solution, ground to a powder in ultra-low temperature liquid nitrogen, and centrifuged at 4°C for complete extraction. Determination of peroxidase (POD) activity by absorbance change at 420 nm. Catalase (CAT) and superoxide dismutase (SOD) activities were measured at 405 nm and 550 nm, respectively. The results of measuring ascorbate peroxidase (APX) activity at 290 nm were expressed in units of mg prot⁻¹, and the glutathione (GSH)

content measured at 420 nm was calculated as milligrams of glutathione per gram of protein.

Sugar and soluble protein, malondialdehyde (MDA), proline and hydrogen peroxide (H_2O_2) contents: The measurement methods for these compounds were as described by Chen *et al.*, 2019. Briefly, the sample (0.5g) was homogenized with phosphate-buffered saline (PBS) and centrifuged, and the supernatant was determined according to the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Sugar and soluble protein, proline, MDA and H_2O_2 contents were measured at 595 nm, 620 nm, 520 nm, 532 nm and 405 nm, respectively.

Estimation of secondary metabolite: Spicas were collected at mature fruiting stage from two-month-old plants grown under natural conditions. Spicas were dried at $60 \pm 2^\circ\text{C}$ for 4 hr. The extraction and HPLC analysis methods for active ingredient compounds (caffeic acid, salviaflaside, rosmarinic acid, hyperoside, oleanolic acid and ursolic acid) were performed as described by Chen *et al.* (2019)..

RNA extraction and RT-PCR analysis: Mixed leaf samples were stored in at -80°C in liquid nitrogen, and TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total mRNA. Approximately, 100 ng of mRNA was employed for cDNA synthesis using PrimerScript RT kit (TaKaRa Biotechnology (Dalian) Co. Ltd). cDNA was used for RT-PCR, and the primers used are shown in Table 5. RT-PCR was performed in a Piko Real-Time PCR System (PIKORed 96, Thermo Fisher, USA) using the SYBR Premix Ex Taq II kit (TaKaRa Biotechnology (Dalian) Co. Ltd). PCR procedure was as follows: 95°C for 30 sec, followed by 95°C for 5 sec, 55°C for 30 sec, 72°C for 30 sec for 40 cycles. β -actin (Kim *et al.* 2014) was used as an internal standard (not affected by UV-B), and the $2^{-\Delta\Delta\text{CT}}$ method was used to calculate relative expression levels.

Statistical analyses: The data are expressed as the means $\pm$ SDs. SPSS 21.0 software (SPSS, Chicago, IL, USA) was used to determine the one-way analysis of variance (ANOVA).

Results and Discussion

Anthocyanin and UV-B-absorber contents were determined in *P. vulgaris* under UV-B radiation (Table 1).

Anthocyanin content increased due to UV-B radiation, however, the difference was not significant. The levels of UV absorber was also in significantly different between the treated and control groups. The activities of SOD, POD, APX and GSH content showed increasing trends under UV-B radiation as; compared to un-irradiated treatment. APX, POD and GSH showed significant ($p < 0.05$) increase. SOD activities in untreated and treated plants with lower or higher UV-B levels were slightly increased but showed insignificant ($p < 0.05$) difference compared with control plants. CAT activity of plants under UV-B radiation was significantly reduced compared to un-irradiated plants (Table 2).

The soluble protein, H_2O_2 and MDA contents were found to increase under UV-B radiation. Compared with un-irradiated treatment, MDA content was found to increase significantly ($p < 0.05$), however, soluble proteins showed slight insignificant increase. The perusal of data revealed that the levels of H_2O_2 increased significantly ($p < 0.05$) in high UV-B radiation group; however, H_2O_2 levels under lower and intermediate UV-B irradiation showed slight reduction or enhancement with insignificant ($p < 0.05$) difference as compared to un-irradiated plants. The contents of soluble sugars significantly ($p < 0.05$) increased to a peak value under intermediate UV-B radiation but were slightly decreased under lower UV-B treatment and significantly ($p < 0.05$) decreased under higher UV-B treatment. Lower UV-B radiation enhanced the proline content, but intermediate and higher UV-B radiation significantly ($p < 0.05$) reduced the proline content (Table 3).

Under pre-treatment with UV-B radiation, the contents of caffeic acid, rosmarinic acid, hyperoside and salviaflaside showed an increasing trend, which was significantly ($p < 0.05$)

Table 1: Effect of UV-B pre-treatment for 10 days on anthocyanin and UV absorber contents in *P. vulgaris* leaves at seedling stage

Treatments	Anthocyanin	UV absorber
Control	3.01 ± 0.07^a	2.77 ± 0.00^a
$35 \mu\text{W cm}^{-2} \text{ nm}^{-1}$	3.07 ± 0.06^a	2.77 ± 0.00^a
$55 \mu\text{W cm}^{-2} \text{ nm}^{-1}$	3.12 ± 0.07^a	2.77 ± 0.00^a
$75 \mu\text{W cm}^{-2} \text{ nm}^{-1}$	3.12 ± 0.02^a	2.76 ± 0.00^a

Values are mean of three replicates $\pm$ S.D. The same letter a in each column indicate insignificant difference at $p < 0.05$

Table 2: Effect of UV-B pre-treatment for 10 days on SOD, POD, CAT and APX activities and GSH content at seedling stage in *P. vulgaris* leaves

Treatments	POD activity ($\text{U mg}^{-1} \text{ protein}$)	CAT activity ($\text{U mg}^{-1} \text{ protein}$)	SOD activity ($\text{U mg}^{-1} \text{ protein}$)	APX activity ($\text{U mg}^{-1} \text{ protein}$)	GSH content ($\text{mg GSH g}^{-1} \text{ protein}$)
CK	3.62 ± 7.51^d	29.97 ± 2.26^a	27.31 ± 3.54^b	0.14 ± 0.11^c	25.11 ± 3.72^b
$35 \mu\text{W cm}^{-2} \text{ nm}^{-1}$	7.18 ± 4.02^a	17.80 ± 1.87^b	27.47 ± 4.50^b	0.23 ± 0.08^a	27.20 ± 7.63^a
$55 \mu\text{W cm}^{-2} \text{ nm}^{-1}$	6.58 ± 8.15^b	14.85 ± 1.30^b	41.96 ± 2.67^a	0.21 ± 0.10^b	27.37 ± 6.41^a
$75 \mu\text{W cm}^{-2} \text{ nm}^{-1}$	5.23 ± 9.63^c	19.08 ± 2.98^b	29.16 ± 6.86^b	0.15 ± 0.00^c	26.99 ± 3.25^a

Values are mean of three replicates $\pm$ S.D. The letters a, b, c, and d indicate that different letters in each column are significantly different at $p < 0.05$ and vice versa

Table 3: Effect of UV-B pre-treatment for 10 days on soluble protein, soluble sugar, proline, H₂O₂ and MDA contents in *P. vulgaris* leaves at seedling stage

Treatments	Soluble protein (g l ⁻¹ f.wt.)	Soluble sugar (mg mg ⁻¹ protein)	Proline (μg g ⁻¹ f.wt.)	H ₂ O ₂ (mmol g ⁻¹ protein)	MDA (nmol mg ⁻¹ protein)
Control	4.3 ± 0.02 ^a	6.33 ± 0.10 ^{bc}	35.24 ± 2.65 ^a	31.38 ± 1.06 ^b	9.82 ± 2.11 ^b
35 μW cm ⁻² nm ⁻¹	4.5 ± 0.02 ^a	6.10 ± 0.25 ^c	38.27 ± 1.05 ^a	29.29 ± 0.58 ^c	11.76 ± 1.98 ^b
55 μW cm ⁻² nm ⁻¹	4.4 ± 0.03 ^a	7.07 ± 0.12 ^a	23.12 ± 2.28 ^b	31.62 ± 0.30 ^b	17.84 ± 1.80 ^a
75 μW cm ⁻² nm ⁻¹	4.6 ± 0.03 ^a	4.72 ± 0.14 ^d	26.82 ± 2.08 ^b	45.37 ± 1.59 ^a	20.27 ± 4.11 ^a

Values are mean of three replicates ± S.D. The letters a, b, c, and d indicate that the different letters in each column are significantly different at $p < 0.05$ and vice versa

Table 4: Effect of UV-B pre-treatment on caffeic acid, salviaflaside, rosmarinic acid, hyperoside, oleanolic acid and ursolic acid contents at mature-fruited stage in *P. vulgaris* ears

Treatments	Rosmarinic acid (%)	Caffeic acid (%)	Salviaflaside (%)	Hyperoside (%)	Ursolic acid (%)	Oleanolic acid (%)
Control	0.20 ± 0.02 ^c	0.017 ± 0.002 ^b	0.14 ± 0.02 ^c	0.01 ± 0.00 ^b	0.27 ± 0.00 ^a	0.05 ± 0.00 ^a
35 μW cm ⁻² nm ⁻¹	0.27 ± 0.07 ^a	0.023 ± 0.007 ^a	0.24 ± 0.06 ^a	0.02 ± 0.00 ^a	0.17 ± 0.00 ^b	0.04 ± 0.00 ^b
55 μW cm ⁻² nm ⁻¹	0.28 ± 0.06 ^a	0.024 ± 0.004 ^a	0.23 ± 0.04 ^a	0.02 ± 0.00 ^a	0.15 ± 0.00 ^c	0.03 ± 0.00 ^c
75 μW cm ⁻² nm ⁻¹	0.23 ± 0.03 ^b	0.016 ± 0.001 ^b	0.20 ± 0.03 ^b	0.02 ± 0.00 ^a	0.15 ± 0.00 ^c	0.04 ± 0.00 ^b

Values are mean of three replicates ± S.D. The letters a, b, c and d indicate that the different letters in each column are significantly different at $p < 0.05$ and vice versa

Table 5: Primers used in RT-PCR analysis

Gene	Primer sequence (5'→3')
Phenylalanine ammonia lyase (PAL)	F: TCCGTGCTTGTGTGTT TGTGCCTGTC R: GGCTTCCTGAACCTCT CCACATCCT
Cinnamate acid 4-hydroxylase (C4H)	F: GCGAACACCCCTGCCAATGGAAGA R: TGCCCTCCTTTCTCCGACGTACCAAT
4-coumarate-CoA ligase (4CL)	F: CCACAAGAGCCTCATCACCAGCATCG R: TGAGCGAGCAGAGCAGGACGGAAT
Chalcone synthase (CHS)	F: AACAGCGAGCATAAGACCGACCTCAA R: CGCCGTGATGTTTGGGTTCTCCTTC
Chalcone isomerase (CHI)	F: CTTCACTCAGTCACCTGTGGCTCAC R: AACTCTGCCTTGCCGTGGGAGACA
Rosmarinic acid synthetase (RAS)	F: CGTC TCCG CTCT CCAC TTCA TCAA CA R: GGAGT AGGC GATC TCGG TGTC TTGG
β-actin	F: TGGTCGGAATGGGACAGAAG R: CTCAGTCAGGAGAACAGGGT

Remark: accession numbers of genes: PAL (KJ010815.1), C4H (KJ010816.1), 4CL (KJ010814.1), CHS (KF255834.1), CHI (MH447677.1), RAS (KM575933.1)

different from the results un-irradiated treatment, except for slight but insignificant ($p < 0.05$) decrease in caffeic acid under higher UV-B. The contents of oleanolic acid and ursolic acid were significantly ($p < 0.05$) decreased when plants were subjected to UV-B radiation as compared to un-irradiated treatment (Table 4). The results of analysis of *C4H*, *PAL*, *4CL*, *CHS*, *RAS* and *CHI* gene expression are shown in Fig. 1. Compared with un-irradiated plants, the expression of six genes in UV-B-treated *spicas* sharply increased and reached peak under lower UV-B

treatment. UV-B irradiation resulted in significant ($p < 0.05$) accumulation of *PAL*, *CHS* and *CHI* transcripts at seedling stage. The expression levels of *C4H* and *RAS* were significantly ($p < 0.05$) increased under lower UV-B treatment, but under intermediate and higher UV-B treatment, there was little difference compared with the control plants. *4CL* transcript abundance significantly ($p < 0.05$) increased at lower and intermediate UV-B treatments and decreased under higher UV-B treatment compared with the control treatment.

Fig.1: Expression levels of PAL, 4CL, C4H, CHI, CHS and RAS genes at seedling stage of *P. vulgaris* under UV-B and control treatments. Values are mean of three replicates $\pm$ S.D. The letters a, b, c, and d indicate that different letters above same pattern indicate a significant difference at $p < 0.05$.

The present study showed that anthocyanin contents increased under UV-B radiation (Table 2). This study revealed that UV-B radiation stimulates *P. vulgaris* leaves to produce anthocyanins, which is similar to the findings of previous studies (Kataria et al., 2014). Anthocyanin mainly occurs in the epidermal cells of leaves or other plant parts (Feild et al., 2001) and modulates damage by decreasing the permeation UVB (Winkel-Sherley, 2002). Thus, anthocyanin content in *P. vulgaris* increased under UV-B irradiation. In contrast, UV absorber compounds did not show significant changes under different intensities of UV-B radiation, consistent with previous studies (Shi et al., 2010). A reasonable explanation for this finding is that the mature leaves of *P. vulgaris* already have high contents of UV-absorbing substances in the epidermis that prevents possible damage by UV-B radiation and maintain a stable level of UV-absorbing substances. This relative stability of UV-B-absorbing substances may also be related to the longer metabolic period in mature leaves.

Plants activate non-enzymatic and antioxidant enzyme system to quench ROS induced by UV-B radiation (Barta et al., 2004). In this work, the activities of POD, APX and SOD and GSH content all increased under UV-B radiation, but CAT activities were significantly decreased under different UV-B radiation intensities; these results are in confirmation with the previous reports of (Rai et al., 2011). Enhanced SOD, POD and APX level increases the oxygen radical scavenging capacity of plants and maintains cellular membrane integrity and sub-cellular structures (Inostroza-Blancheteau et al., 2016). When GSH content increases, UV-B

radiation injury is alleviated (Yan et al., 2016). In *P. vulgaris* leaves, CAT activities significantly reduced under varied UV-B radiation intensities of UV-B causes ROS and lipid peroxidation damage to plant cells, reducing their ability to respond to oxidative stress (Gao et al., 2008). On the other hand, *P. vulgaris* produces antioxidants such as glutathione, ascorbate and α -tocopherol to offset the negative effects of UV-B exposure (Jain et al., 2004). Under UV-B stress, the contents of soluble sugar and soluble protein in *P. vulgaris* leaves increased, similar to previous results. Soluble sugars and proteins are osmotic regulatory substances involved in adaptation to UV-B stress through osmotic regulation (Wu et al., 2016). Interestingly, the soluble sugar contents of *P. vulgaris* leaves were significantly reduced due to higher UV-B stress, similar to previous results. Hydrolase activity was inhibited under UV-B stress, thereby reducing the hydrolysis rate in the conversion of macromolecular components into small molecules like soluble sugars. Additionally due to oxidation stress inducing more ROS under higher UV-B levels, reducing sugars might be oxidized, thus decreasing their levels (Jiao et al., 2015). Proline accumulation under UV-B stress presents great protective potential against ROS induced by oxidative stress in plant cells (Kumar and Bhardwaj, 2019). In contrast to the increased proline content of *P. vulgaris* leaves under lower UV-B irradiation, a decrease was observed under both intermediate and higher UV-B treatments (Katerova et al., 2009). reported a similar trend in proline contents in pea under UV-B treatments. MDA is used as an indicator of oxidative stress (Chen et al., 2019), and UV-B stress has been found to increase MDA content in previous studies, indicating oxidative damage (Yan et al., 2016).

Rosmarinic acid is the main active ingredient in *P. vulgaris* spicas and is the component employed for quality control in the spicas of *P. vulgaris* in the Chinese Pharmacopoeia (2015). In this study, the rosmarinic acid content under different UV-B intensity radiation treatments was significantly increased compared to the control group, and similar results were reported Zhang *et al.* (2017). Some studies have shown that rosmarinic acid presents a superior ROS-scavenging ability and is a photoprotective agent, and suppress UV radiation-induced ROS production in the cells of human skin in pharmacological studies (Sánchez-Campillo *et al.*, 2009). These findings provide enough evidence to prove that the increase rosmarinic acid in *P. vulgaris* spicas can eliminate ROS induced by UV-B stress. Hyperoside and caffeic acid are the main active ingredients in the spicas of *P. vulgaris* (Chen *et al.*, 2013).

It has been observed that hyperoside and caffeic acid are more abundant in plants coping with abiotic stress of high UV-B radiation (Cirak *et al.*, 2017). Previous studies have indicated that hyperoside and caffeic acid are effective ROS scavengers that can protect the phospholipid bilayer of cell membrane from UV-B irradiation (Camas *et al.*, 2014). Thus, the increase in hyperoside and caffeic acid contents found in *P. vulgaris* under pre-treatment with UV-B may help to remove ROS in the cells of *P. vulgaris*. Salviaflaside is the main phenolic glycoside found in *P. vulgaris*. In our study, pre-treatment with UV-B radiation significantly increased salviaflaside contents. It was found that the expression of key genes related to phenolic acid biosynthesis (e.g., *PAL*, *4CL*, *C4H*, *RAS*, *CHS* and *CHI*) increased in *P. vulgaris* leaves under UV-B stress, which may be the reason for increased salviaflaside content found in *P. vulgaris* under pre-treatment with UV-B radiation. Oleanolic acid and ursolic acid are commonly found in the spicas of *P. vulgaris* (Bai *et al.*, 2016). This study reveals that the contents of oleanolic acid and ursolic acid significantly decreased under pre-treatment with UV-B radiation, similar to the results previously obtained by others (Ambasht and Agrawal, 1998). Another study indicated that the expression of triterpenoid synthesis genes was not affected by UV-B irradiation (Zhao *et al.*, 2013). This may be the reason for decreased oleanolic acid and ursolic acid contents observed in *P. vulgaris* under pre-treatment with UV-B radiation.

In this study, when *P. vulgaris* was pre-treated with UV-B radiation, six kinds of critical phenylpropanoid pathway enzymes (e.g., *PAL*, *4CL*, *C4H*, *CHI*, *CHS* and *RAS*) showed higher transcript accumulation. Increased expression of genes encoding enzymes involved in the phenylpropanoid pathway, especially *PAL*, is induced by UV-B radiation and plays a key role in the first and most important step of the phenylpropanoid pathway, which results in increases in individual phenolic acids (Yu *et al.*, 2015). *CHS* is the first flavonoid enzyme in the flavonoid pathway (Samuelian *et al.*, 2009). This study indicated that UV-B induced significant accumulation of *CHS* transcripts, which explains the observed increase in the contents of individual flavonoids. In conclusion, this study demonstrated that UV-B pre-treatment in the seedling stage before transplantation to the field is effective for inducing phenolic acid and flavonoid accumulation in the

mature fruiting stage of *P. vulgaris* ears. However, further studies should be carried out to clarify the multiple metabolic pathways of UV-B radiation regulating *P. vulgaris*.

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