International Journal of Horticultural Science and Technology
Journal homepage: http://ijhst.ut.ac.ir

Production of Phenolic Acids Improved in Callus Cultures of *Lactuca undulata* by Ultraviolet-B Irradiation

Morteza Mofid Bojnoordi, Rezvan Ramezannejad, Mahnaz Aghdasi*, Mohammad Fatemi

Department of Biology, Faculty of Sciences, Golestan University, Gorgan, Iran

ARTICLE INFO

Article history:

Received: 23 October 2021,
Received in revised form: 28 February 2022,
Accepted: 15 March 2022

Article type:

Research paper

Keywords:

Cichoric acid,
Chlorogenic acid,
Exposure time,
Lactuca undulata,
PAL

ABSTRACT

As a valuable industrial compound, cichoric acid has antiviral, antioxidant, and anti-inflammatory properties. It is effectively used in obesity prevention and for neuroprotective purposes. Here, we aimed to compare the effects of plant growth regulators and UV-B radiation on the induction of cichoric acid production under tissue culture conditions. For this purpose, *Lactuca undulata*, an herbaceous medicinal plant, was used as a new source of cichoric acid. Leaf explants were initially cultured on ½ MS (Murashige and Skoog) medium containing 0.1, 1 mg/L Kinetin (Kin) and 2,4-Dichlorophenoxyacetic acid (2,4-D). The callus was then exposed to different UV-B exposure times (0, 20, 40, and 60 min) and was harvested after 5, 10, and 15 days. The highest amounts of total phenol, cichoric acid, chlorogenic acid, and caffeic acid were achieved in callus samples that were treated with 20 and 40 minutes of UV-B radiation for 10 days. Cichoric acid content reached an amount of 6.15±0.292 mg/g DW after 20 min of radiation. Pearson's correlation coefficient analysis (PCC) showed that Phenylalanine Ammonia Lyase (PAL) activity did not correlate with the production of caffeic acid derivatives. These results suggested that UV-B irradiation can be used as an elicitor to induce the production of cichoric acid in *Lactuca undulata* callus cultures.

Introduction

The production of secondary metabolites in large scales is usually difficult and expensive. These valuable compounds cannot be produced using plants grown in the field due to low concentrations of secondary metabolites, as well as the difficulties that are associated with domesticating and cultivating these plants. So far, different strategies have been developed to increase secondary metabolite production in tissue culture conditions. Ultraviolet (UV) radiation is one of the most important elicitors which can be used for stimulating secondary metabolites biosynthesis in tissue culture conditions (Kumar et al., 2008; Robson et al., 2015). Sunlight naturally has a UV-B range (290-320 nm) that accounts for less than 0.5% of its spectrum. Depending on the angle of sunlight and the thickness of the ozone layer, all living organisms are subjected to UV-B radiation (Zlatev and Lidon, 2012). Plants usually receive UV-B radiation through their specific receptors and then they respond accordingly. UV-B radiation can alter gene expression, plant physiology, morphology, and accumulation of secondary metabolites (Heijde and Ulm, 2012; Schreiner et al., 2012). Due to its sufficient energy to break chemical bonds, UV-B radiation has many destructive effects on cellular structures and metabolic processes. Reactive oxygen species (ROS) production, along with damaged forms of DNA, proteins, lipids, chloroplasts, and

* Corresponding author's email:
Aghdasi1346@gmail.com

photosynthetic pigments are the most destructive effects of UV-B radiation. To reduce these destructive effects, plants produce and accumulate UV-absorbing compounds in their epidermal cells (Treutter, 2005). There are several sources and documents that demonstrate UV-B radiation induces the production of phenolic compounds in plants (Sun et al., 2010, Harbaum-Piayda et al., 2010). Ghasemzadeh et al. (2016) showed that the production of phenolic compounds (catchiness, cinnamic acid, quercetin, rutin, gallic acid) increased in response to UV-B treatment in basil plants. Furthermore, they showed that Chalon synthase and antioxidant enzyme activity increased as a result of the UV-B treatment (Ghasemzadeh et al., 2016).

Lactuca undulata is an annual herb from the Asteraceae family which is distributed throughout central and west Asia (Safavi et al., 2013). It contains industrially-valuable medicinal compounds such as caffeic acid and relevant derivatives. One of the most important phytochemical compounds of this plant is chicoric acid (Ramezannezhad et al., 2019). Cichoric acid is a caffeic acid derivative that has various pharmacological and biological properties (Lee and Scagel, 2013). The amount of cichoric acid in *Lactuca undulata* reportedly ranged from 1.38 to 3.5 mg/g DW, depending on populations (Mofid Bojnoordi et al., 2020). The presence of caffeic acids and their derivatives in *Lactuca undulata* showed a great potential of this species for the production of valuable metabolites (Ramezannezhad et al., 2019). This herbaceous species can be easily grown in the field and in vitro. Therefore, *Lactuca undulata* was selected as a suitable model for the production of cichoric acid under tissue culture conditions.

So far, few cases of research have reported on the effects of UV-B radiation on the production of caffeic acid derivatives. Luis et al. (2007) showed that UV-B radiation enhanced the accumulation of caffeic acid, rosmarinic acid, and carnosic acid in *Rosmarinus officinalis* (Luis et al., 2007). The UV-B treatment reportedly increased the accumulation of phenolic compounds and caffeic acid derivatives in *Echinacea purpurea* cell suspension culture (Manaf et al., 2016).

The objective of the current research was to explore the effects of various UV-B exposure durations on *Lactuca undulata* under tissue culture conditions, such that the production of cichoric acid and its related compounds were affected in the plant. This would be the first study, to appear in the available literature, that aims to induce and improve cichoric acid production in Lactuca undulata callus culture.

Materials and Methods

Chemicals and reagents

Acetonitrile was purchased as HPLC grade. Cichoric acid, chlorogenic acid, caffeic acid, and Folin-Ciocalteu were purchased from Sigma-Aldrich (Steinheim, Germany). UV-B lamps were purchased from Philips (Germany).

Plant materials

Lactuca undulata samples were collected from a mountain region in Firoozkooh, Iran (S: 52°49'17.2", E: 35°47'12.1"). An herbarium voucher (GU6962) was lodged with Golestan University Herbarium, Golestan University, Gorgan, Iran.

Sterile seedlings

Seeds of *Lactuca undulata* were collected from plants growing in their natural habitats. Following surface sterilization with tap water (30 min), the seeds were pretreated with 70% ethanol for 1 min, and 5% commercial Clorox for 10 min. Then, they were planted on ½ MS medium (Murashige and Skoog, 1962) containing 15 g/L sucrose and 8 g/L agar (pH 5.7). The cultures were incubated in a growth chamber at 25°C under 16/8 h photoperiod conditions.

Callus induction

Explants were obtained from 2-month-old sterile seedlings. Leaf explants were cultured on ½ MS medium, supplemented with 0.1, 1 mg/L Kin, and 2,4-D. Then, the cultures remained in the growth chamber at 25 ± 2°C and 16/8 h photoperiod. They were sub-cultured every 14 days.

UV-B treatment

Forty-day-old callus were used in this experiment. After removing the petri dish cover under a sterilized laminar airflow, the cultures were exposed to UV-B radiation. A UV-B lamp (230-330 nm) with TL 40 W/12 RS (Philips-Germany) was placed at 30 cm above the cultures. The exposure time was 0, 20, 40, and 60 min. After the treatments, the cultures were transferred to the growth chamber with the same conditions as mentioned above. The callus was harvested after 5, 10, and 15 days to evaluate their morphology, to measure the fresh and dry weights and to analyze phytochemical compounds.

Fresh and dry weight measurements

The fresh weight of the harvested callus was measured immediately. Then, the callus was dried at 37°C for 3 days. The dried callus was used for measuring the amounts of total phenol and caffeic acid derivatives.

Total phenol content (TPC) extraction and measurement

One gram of dried and powdered callus was dissolved in 20 ml of 80% (v/v) aqueous methanol for 48 hours. It was filtered through filter paper and the extraction was repeated two times. The filtrate was then centrifuged at 13,000×g for 10 min at 0°C. Then, the supernatants were evaporated at 40°C (Pourmorad et al., 2006). TPC was measured by a spectrophotometer using the Folin-Ciocalteu method (Meda et al., 2005) and was calculated using a standard quercetin curve.

Dry powdered callus (1 g) was dissolved in 7 mL aqueous acetonitrile (20%). The extracts were placed in a shaker under full darkness for 24 h. They were filtered and centrifuged at 13,000×g for 10 min. The supernatant solution was assayed for caffeic acid derivatives (Luo et al., 2003).

HPLC analysis

The amounts of caffeic acid derivatives were measured by HPLC-UV. The analysis was done at room temperature by using a C18 (250 × 4 mm) column and a V/VIS detector (Merck, Hitachi). The chromatographic conditions comprised isocratic mode, mobile phase: acetonitrile (solvent a) and acetic acid 0.1% with deionized water (solvent b), a flow rate of 1 mL/min, and a detection wavelength of 330 nm for cichoric acid and 278 nm for both chlorogenic and caffeic acid. Equal amounts (20 µl) of each sample were injected into the HPLC system. Reference standards of cichoric, chlorogenic, and caffeic acid were purchased from Merck (Germany). To quantify the concentrations of caffeic acid derivatives, a calibration curve was obtained by injecting known concentrations of reference standard compounds. The amounts of caffeic acid derivatives were expressed as mg/g DW of the sample.

PAL assay

Phenylalanine Ammonia Lyase (PAL) (EC 4.3.1.5) activity was quantified according to a method described by Syklowska-Baranek et al. (2012). Fresh callus (0.05 gr) was frozen and ground in 1 mL of Tris- HCl buffer (0.05 M adjusted to pH: 8) and consisted of mercaptoethanol (0.8 mM) and Polyvinyl polypyrrolidone (PVPP) (1% W/V). After centrifugation at 14000 g for 15 min at 4°C, the clear supernatant was used for measurements of enzyme activity. The reaction mixture remained in a final volume of 3 mL and consisted of Tris-HCL buffer (pH=8), 0.1 ml supernatant, and 10 mM phenylalanine (0.1 ml). Then, the reaction mixture was incubated at 37°C for 1 hour. The activity of PAL was recorded by a spectrophotometer at 290 nm and was expressed as free cinnamic acid (µmol/g FW.min).

MDA measurement

Malondialdehyde (MDA) content was measured according to Prochazkova et al. (2001). In brief, fresh callus (0.5 gr) was extracted with 0.1% (v/v) trichloroacetic acid. The homogenate was then centrifuged at 15000 g for 15 min at 4°C. After removal of the upper lipid layer, MDA content was measured in the clear supernatant. The reaction mixture consisted of 1 mL supernatant extract and 4 mL TBA (0.5%). Then, the mixture was incubated in a boiling water bath for 30 min and was immediately relocated onto ice to remain there for 15 min. After cooling, the mixture was centrifuged at 10000 g for 10 min at 0°C. The absorbance was measured at 440, 532, and 600 nm. The MDA content was then calculated according to the following formula (Du and Bramlage, 1992).

[LP](nm.ml-1) = [[(A532 - A600) - [(A440 - A600) (MA of sucrose at 532nm/MA of sucrose at 440nm)]]/157000]106

Statistical analysis

The amount of exposure time to UV-B and the amount of time to harvest were considered as two independent factors. Data were analyzed using a two-factor analysis of variance, with three replicates. The Duncan's test was also employed for calculating significant differences in the mean values.

Results

Callus morphology and biomass under UV treatment

The effects of UV-B exposure time on callus morphology and biomass production were evaluated. As shown in Table 1 and Fig. 1, there was a notable difference among various UV-exposure times on callus morphology. The data showed that the color of callus varied from light green to yellow. In response to a short incubation period (5 days) and different UV-B exposure times, callus tissue became friable in texture and light green in color. But by increasing the incubation period and exposure time (40 and 60 min), the callus changed to yellow and became dry. The results also indicated that different UV-B exposure times increased the dry and fresh weights after a 5-day incubation period. Meanwhile, the increase in fresh weight was obtained in the callus and in response to 20 and 40 min UV-B radiation after 10 and 15 days of incubation.

Table 1. The effects of different UV-B exposure time and incubation period on quality, fresh and dry weights of leaf-derived callus with optimized concentrations of plant growth regulators. Values are means ± SE of three independent experiments. Data are mean values ± SE. Different letters in each column indicate significance differences ($P<0.01$), according to Duncan's multiple range test

Days after treatment	Exposure time (min)	Callus morphology	Fresh weight (gr)	Dry weight (gr)
	0	Light green/ Friable	1.55±0.14cd	0.10±0.01e
	20	Light green/ Friable	2.46±0.27a	0.27±0.01ab
5	40	Light green/ Friable	2.46±0.17a	0.30±0.01a
	60	Light green/ Friable	2.44±0.20a	0.30±0.01a
	0	Light green/ Friable	1.72±0.19cd	0.14±0.02e
	20	Light green/ Friable	1.88±0.18cd	0.19±0.02d
10	40	Yellow/ Dried	2.04±0.04bc	0.21±0.00cd
	60	Yellow/ Dried	1.66±0.09cd	0.23±0.01bcd
	0	Light green/ Friable	1.80±0.08cd	0.10±0.02e
	20	Light green/ Friable	2.34±0.29b	0.24±0.02bc
15	40	Yellow/Dried	2.07±0.14bc	0.25±0.02bc
	60	Yellow, Dried/ Necrosis	1.68±0.10cd	0.26±0.02bc

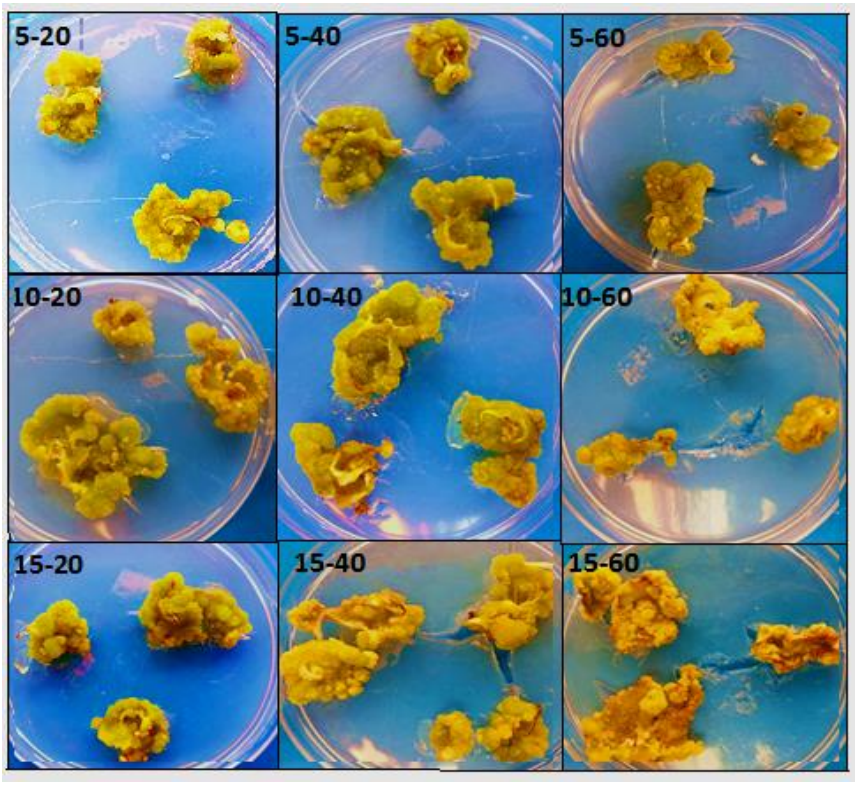

Fig. 1. The effects of different UV-B exposure times and incubation periods on leaf-derived callus of *Lactuca undulata* grown on ½ MS medium supplemented with 1 and 0.1 mg/l Kin and 2,4-D, respectively. Callus was exposed to three periods of UV-B (20, 40, and 60 min) for 5, 10, and 15 days.

Secondary metabolites production

As shown in Table 2, UV-B irradiation induced total phenol production in the callus. The results indicated higher production of TPC in the treated callus, compared to the control. The highest TPC was achieved in response to 20 and 40 min UV-B radiation after 10 and 15 days of incubation, respectively. However, 60 min of UV-B exposure during 15 days of incubation reduced the amount of TPC, compared to the control level.

While the amount of caffeic acid was 0.152±0.0 mg/g DW in untreated callus, it decreased after different exposure times during the 5-day incubation period. The highest amount of caffeic acid was obtained in response to 20 and 40 min UV-B radiation after a 10-day incubation period. But a significant reduction in caffeic acid production was observed in response to the 15-day incubation period. There was no significant difference in chlorogenic acid levels among different exposure times after the 5- and 15-day incubation periods. Nonetheless, the amount of this metabolite increased by up to 3 times when

the callus was treated with 20 and 40 min UV-B radiation during the 10-day incubation period. UV-B radiation reduced the amount of cichoric acid in the corresponding callus after 20 and 40 min during the 5-day incubation period, compared to the control, whereas the level of cichoric acid in callus exposed to UV-B for 60 min did not differ significantly from the control. Meanwhile, 20 min of UV-B exposure after 10 days of incubation caused cichoric acid accumulation to become twice as much as the amount in the control. Longer incubation periods of up to 15 days significantly reduced cichoric acid accumulation (Table 2).

Table 2. The effects of different treatments of UV-B on the accumulation of secondary metabolites in leaf-derived callus of *Lactuca undulata*. Callus was exposed to three periods of UV-B irradiation (20, 40, and 60 min) during 5, 10, and 15 days. Values are means ±SE of three independent experiments. Different letters within each column indicate significant differences ($P<0.01$), according to Duncan's multiple range test

Days after Treatment	Exposure time (min)	Total phenol (mg/g DW)	Caffeic acid (mg/g DW)	Chlorogenic acid (mg/g DW)	Cichoric acid (mg/g DW)
5	0	34.35±1.13 c	0.152±0.000bc	0.134±0.034 bcde	3.16±0.166 d
	20	41.41±4.13 bc	0.144±0.004d	0.159±0.028 bcd	1.90±0.347 ef
	40	46.07±3.73ab	0.102±0.019de	0.118±0.008 cde	2.13±0.381 ef
	60	42.20±1.61 bc	0.082±0.009de	0.118±0.008 cde	3.59±0.315 d
10	0	36.07±0.79 c	0.141±0.017c	0.088±0.012 e	3.53±0.053 d
	20	51.99±2.15a	0.254±0.020a	0.302±0.009 a	6.15±0.292 a
	40	49.19±4.97 ab	0.265±0.005 a	0.309±0.006 a	5.35±0.233 b
	60	45.71±1.80 ab	0.189±0.001 b	0.180±0.012 bc	4.60±0.089 c
15	0	34.89±0.93c	0.154±0.018 bc	0.140±0.023 bcde	2.16±0.299 efg
	20	48.08±1.21 ab	0.046±0.008 e	0.185±0.030 b	1.33±0.194 fg
	40	51.02±1.18a	0.093±0.007 d	0.135±0.013 bcde	1.71±0.033 efg
	60	34.60±1.55c	0.081±0.005 de	0.095±0.004 e	1.06±0.006 g

MDA production and PAL activity assay

The UV-B radiation changed MDA production in the corresponding callus during different incubation periods. The highest amount of MDA was obtained in 60 min UV-B radiation after 10 and 15 days of incubation (Fig. 2A).

The results indicated that different UV-B exposure times caused a gradual increase in PAL activity after 5 and 10 days of incubation. Maximum PAL activity was detected against 60 min exposure to UV-B after 10 days of incubation, compared to the other treatments (Fig. 2B).

Pearson's correlation coefficient (PCC) analysis showed a significant correlation between TPC and PAL activity, although PAL activity did not correlate with cichoric acid, chlorogenic acid, and caffeic acid production (Table 3). The current results indicated that PAL activity and phenol concentrations correlated with each other significantly. It was also shown that the production and accumulation of caffeic and cichoric acids in the different treatment groups had significant correlations among each other.

Discussion

In the current study, leaf derivate callus of *Lactuca undulata* was exposed to different (20, 40, and 60 min) periods of UV-B radiation for 5, 10, and 15 days. This enabled evaluations of the positive effects of UV-B radiation on the production of phytochemical components in the plant. The results revealed that UV-B radiation caused changes in the callus in terms of morphology, growth, and TPC. By increasing the exposure time and incubation period, the color of callus changed from green to yellow which can be due to the destructive effects of UV radiation on chlorophyll, as well as the accumulation of phenolic compounds (Karvansara and Razavi, 2019). The results also indicated that UV-B radiation increased callus dry weight in all treatments. These findings are in agreement with previous results by Manaf et al. (2016) that UV-B radiation increased the amount of dry weight in the callus culture of *Echinacea purpurea*. In contrast, Zagoskina et al. (2003) reported that UV-B radiation reduced the dry weight of *Camellia sinensis* callus. There is a high genetic diversity among different plant species in response to UV-B radiation, so much so that some species are affected negatively by the treatment, whereas others receive a stimulating effect on their growth and secondary metabolite accumulation (Zlatev and Lidon, 2012). According to our results, in the control samples, TPC did not show any significant change throughout the 15 days of incubation, whereas UV-B radiation-induced TPC accumulation in the corresponding callus. Meanwhile, TPC decreased when the exposure time was extended to 60 min in the 15-day incubation period. These findings are in

agreement with previous results by Namli et al. (2014) in an experiment where *Hypericum perforatum* was treated with 15, 30, and 45 min UV-B radiation for 5 days, thereby inducing higher productions of TPC and total flavonoid content, whereas 60 min of UV-B radiation reduced the degree of secondary metabolite accumulation.

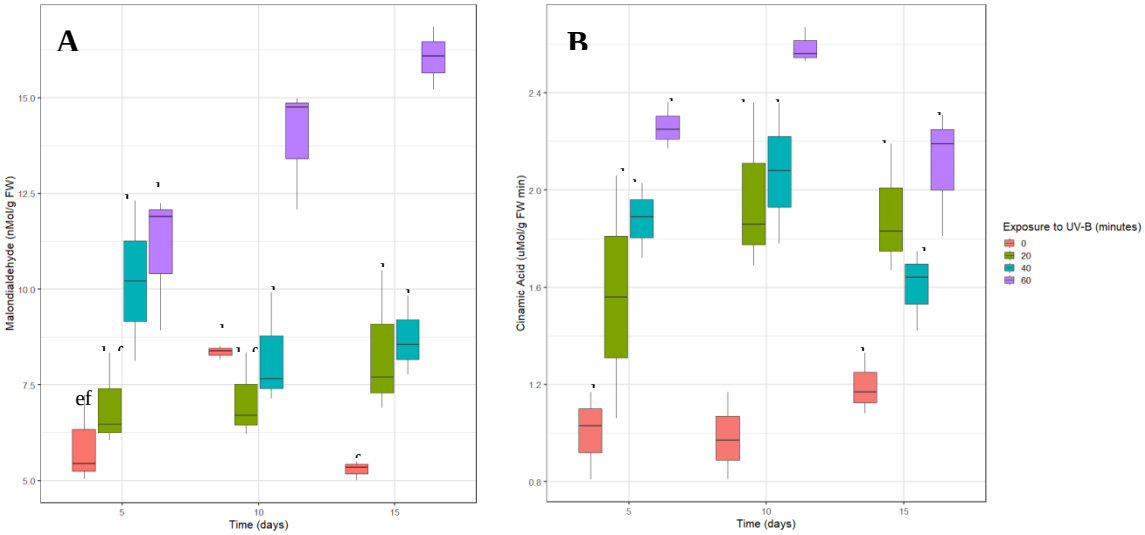

Fig. 2. A) Malondialdehyde (MDA) contents and B) PAL activity in leaf-derived callus of *Lactuca undulata* in response to different treatments of UV-B. Callus was exposed to three periods of UV-B irradiation (20, 40, and 60 min) for 5, 10, and 15 days. Box plots show median (horizontal line), and an interquartile range (hinges). Different letters on each box indicate significant differences ($P<0.01$), according to Duncan's multiple range test.

Table 3. Correlation analysis using Pearson's correlation coefficient (PCC) between PAL activity and secondary metabolites in leaf-derived callus of *Lactuca undulata* in response to the UV-B treatment

		Total Phenol	Cichoric acid	Caffeic acid	Chlorogenic acid	PAL
Total Phenol	Pearson's Correlation	1	0.342*	0.243	0.552**	0.443**
Cichoric acid	Pearson's Correlation	0.342*	1	0.808**	0.660**	0.218
Caffeic acid	Pearson's Correlation	0.243	0.808**	1	0.707**	0.070
Chlorogenic acid	Pearson's Correlation	0.552**	0.660**	0.707**	1	0.278
PAL	Pearson's Correlation	0.443**	0.218	0.070	0.278	1

**. Correlation is significant at the 0.01 level (2-tailed)

The data of the current research showed that UV-B radiation induced an accumulation of caffeic acid in the callus culture of *Lactuca undulata*. This can be confirmed by similar results in the available literature. For instance, according to Manaf et al. 2016, 2 hours of UV-B radiation after 2 weeks of incubation induced caffeic acid-derived contents in *Echinacea purpurea*. They observed that secondary metabolites decreased in response to an increase in radiation time and incubation period. Yildirim et al. (2020) reported a reduction in chlorogenic acid and an induction in rosmarinic acid content in *Echium orientale* when exposed to UV-B radiation. Thus, it can be concluded that various parameters, such as exposure time, incubation period, and age of callus tend to play an important role in the induction of secondary metabolite production via the effects of UV radiation.

UV-B exposure usually activates defense

mechanisms and productions of essential phytochemical components in plants (Yin et al., 2016). Our data demonstrated that the general pattern of TPC accumulation and phenolic acid enhancement correlated with PAL activity in the corresponding callus, which indicates the stimulatory effects of UV-B on the phenylpropanoids pathway. PAL is a key enzyme in the metabolism and synthesis of phenylpropanoids, which converts L-phenylalanine to trans-cinnamic acid. It facilitates a key biochemical reaction in plant growth and development (Chang et al., 2008). In agreement with the current findings, several reports exist on the effects of UV-B on PAL activity and phenolic components (Lee et al., 2014; Manaf et al., 2016; Papoutsis et al., 2016). PAL activity is reportedly regulated at both transcriptional and post-transcriptional levels in response to UV-B radiation. Also, UV-B radiation usually suppresses the expression of KFBS, belonging to F-box proteins, which is a negative regulator of PAL expression (Zhang et al., 2013; Zhang et al., 2015). When plants are exposed to stress, the MDA content increases as an indication of lipid peroxidation (Tsikas, 2017). In the current study, the amount of MDA increased in response to extending UV-B exposure time and the duration of the incubation period, specifically when exceeding 60 min of UV-B radiation. Under oxidative stress, MDA is produced as a result of a reaction between cell membrane fatty acids, specifically arachidonic acid, and ROS (Tsikas, 2017). The current results showed that the highest amount of MDA and the lowest amount of TPC were observed in callus that were exposed to 60 min UV-B radiation. In contrast, the exposure of callus to 20 and 40 min UV-B radiation caused the lowest amount of MDA and the highest amount of TPC, suggesting that phenols can serve as an antioxidant compound in reducing the destructive effects of oxidative stress caused by the UV-B radiation. As Ibrahim and Jaafar (2012) noted, there was a negative correlation between MDA production and TPC accumulation in *Labisia pumila* plants when exposed to UV-radiation. UVR8 is known as a specific UV-B receptor in *Arabidopsis thaliana*. However, it is not as yet clear which plant species in particular respond to UV-B radiation through this special receptor, and which species initiate a response from the general pathway when encountering a variety of stress factors (Rácz et al., 2020).

Conclusion

The UV-B elicitor-effect essentially targets phenolic compound production. Treating the callus with UV irradiation increased the production of secondary metabolites. In conclusion, exposure to a short-time radiation (i.e. 20 min for a total 10-day incubation period) resulted in a higher concentration of secondary metabolites in the callus. Therefore, the process of callus culture in association with UV-B irradiation can be a viable option for researchers in their aims to produce cichoric acid.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Acknowledgment

We thank the Golestan University Deputy of Research and Office of High Education and Iran National Science Foundation (INSF) for financial support (Project No: 96014549).

References

Chang A, Lim MH, Lee SW, Robb EJ, Nazar RN. 2008. Tomato PAL gene family: highly redundant but strongly underutilized. Journal of Biological Chemistry 283, 33591-33601.

Ghasemzadeh A, Ashkani S, Baghdadi A, Pazoki A, Jaafar HZE, Rahmat A. 2016. Improvement in Flavonoids and Phenolic Acids Production and Pharmaceutical Quality of Sweet Basil (*Ocimum basilicum* L.) by Ultraviolet-B Irradiation. Molecules 21, 1203-1210.

Harbaum-Piayda B, Walter B, Bengtsson GB, Hubbermann EM, Bilger W, Schwarz K. 2010. Influence of pre-harvest UV-B irradiation and normal or controlled atmosphere storage on flavonoid and hydroxycinnamic acid contents of pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*). Postharvest Biology and Technology 56, 202–208.

Heijde M, Ulm R. 2012. UV-B photoreceptor-mediated signaling in plants. Trends in Plant Science 17, 230–237.

Ibrahim MH, Jaafar HZE. 2012. Primary, secondary metabolites, H2O2, malondialdehyde and photosynthetic responses of *Orthosiphon stimaneus* Benth. To different irradiance levels. Molecules 17, 1159–1176.

Karvansara PR, Razavi SM. 2019. Physiological and biochemical responses of sugar beet (Beta vulgaris L) to UV-B radiation. Peer Journal 7, 6790-67106.

Kumar J, Gupta PK. 2008. Molecular approaches for improvement of medicinal and aromatic plants. Plant Biotechnology Report 2, 93-112.

Lee J, Scagel CF. 2013. Cichoric acid: chemistry, distribution, and production. Frontiers in Chemistry 1, 117-123.

Lee MJ, Son JE, Oh MM. 2014. Growth and phenolic compounds of *Lactuca sativa* L. grown in a closed-type plant production system with UV-A, -B, or -C lamp.

Science Food Agriculture 94, 197-204.

Luo XB, Chen B, Yao SZ, Zeng JG. 2003. Simultaneous analysis of caffeic acid derivatives & alkamides in roots and extracts of *Echinacea purpurea* by HPLC-photodiode array detection-electrospray mass spectrometry. Journal of Chromatography 986, 73-81.

Luis JC, Perez RM, Gonzalez FV. 2007. UV-B radiation effects on foliar concentrations of rosmarinic and carnosic acids in rosemary plants. Food Chemistry 101, 1211–1215.

Manaf HH, Rabie KAE, Abd El-Aal MS. 2016. Impact of UV-B radiation on some biochemical changes and growth parameters in *Echinacea purpurea* callus and suspension culture. Annals of Agricultural Science in press. Annual Agricultural Science 61, 207–216.

Meda A, Euloge C, Romito M, Millogo J, Germaine O. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry 91, 571–577.

Mofid Bojnoordi M, Aghdasi M, Fatemi M. 2020. An investigation on phytochemical components and antioxidant activity of *Luctuca undulata in 5 natural habitats of Iran. Journal of Medical Plants* 75, 65-75.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Plant Physiology 15, 473–497.

Namli S, Işıkalan C, Akbaş F, Toker Z, Tilkat EA. 2014. Effects of UV-B radiation on total phenolic, flavonoid and hypericin contents in *Hypericum retusum* Aucher grown under in vitro conditions. National Product Research 28, 2286-2292.

Papoutsis K, Vuong QV, Pristijono P, Golding JB, Bowyer MC, Scarlett CJ, Stathopoulos CE. 2016. Enhancing the total phenolic content and antioxidants of lemon pomace aqueous extracts by applying UV-C irradiation to the dried powder. Foods 10, 512–516.

Pourmorad F, Hosseinimehr SJ, Shahabimajd N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology, 11,1142–1145.

Prochazkova D, Sairam RK, Srivastava GC, Singh DV. 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Science 161, 765–771.

Rácz A, Czégény G, Csepreg K, Hideg E. 2020. Ultraviolet-B acclimation is supported by functionally heterogeneous phenolic peroxidases. Scientific Reports 10, 16303-16315.

Ramezannezhad R, Aghdasi M, Fatemi M. 2019. An investigation on cichoric acid content and antioxidant activity in some Iranian native species compared to *Echinacea purpurea* L. in different developmental stages. Iranian Journal of Medical Aromatic Plants 34, 909-923.

Robson TM, Klem K, Urban O, Janso MAK. 2015. Re-interpreting plant morphological responses to UV-B radiation. Plant, Cell and Environment 38, 856–866.

Safavi SR, Asadi R, Naseh Y, Jafari E, Heydarnia N. 2013. Flora of Iran, Asteraceae Tribe Cichorieae. Research Institute of Forests and Rangelands Press.

Schreiner M, Mewis I, Huyskens-Keil S, Jansen MAK, Zrenner R, Winkler JB, Krumbein, A. 2012. UV-B-induced secondary plant metabolites–potential benefits for plant and human health. Critical Reviews in Plant Sciences 31, 229–240.

Sun M, Gu X, Fu H, Zhang L, Chen R, Cui L, Zheng L, Zhang D, Tian J. 2010. Change of secondary metabolites in leaves of Ginkgo biloba L. in response to UV-B induction. Innovative Food Science and Emerging Technologies 11, 672–676.

Treutter D. 2005. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. Plant Biology 7, 581-591.

Tsikas D. 2017. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges. Anal of Biochemistry 524, 13–30.

Yildirim AB. 2020. Ultraviolet-B-induced changes on phenolic compounds, antioxidant capacity and HPLC profile of in vitro grown plant materials in *Echium orientale* L. Industrial Crops and Products 153, 112584- 112590.

Yin X, Sunger SD Qiao H, Liu Y, Jia C, Wang H, Fei Z, Wang Y, Fan C. 2016. Insights into the mechanism underlying Ultraviolet-C induced resveratrol metabolism in grapevine (*Vitis anurensis* Rupr.) Cv. "Tonghua-3". Frontiers in Plant Science 7, 503-510.

Zhang X, Gou M, Liu CJ. 2013. Arabidopsis Kelch repeats F-box proteins regulate phenylpropanoid biosynthesis via controlling the turnover of phenylalanine ammonia-lyase. Plant Cell 25, 4994–5010.

Zhang X, Gou M, Gou C, Yang H, Liu CJ. 2015. Down-Regulation of Kelch Domain-Containing F-Box Protein in *Arabidopsis* Enhances the Production of (Poly) phenols and Tolerance to Ultraviolet Radiation. Plant Physiology 167, 337–350.

Zagoskina NV, Dubravina GA, Alyavina AK, Goncharuk EA. 2003. Effect of ultraviolet (UV-B) radiation on the formation and localization of phenolic compounds in tea plant callus cultures. Russian Journal of Plant Physiology 50, 270–275.

Zlatev Z, Lidon FC. 2012. Plant physiological responses to UV-B radiation. Emirate Journal of Food and Agriculture 24, 481-501.