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THE EFFECT OF UV IRRADIATION ON *IN VITRO* CULTURES DEVELOPMENT OF GOLDEN ROOT – ENDANGERED MEDICINAL PLANT

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Abstract

Increasing production of secondary metabolites from medicinal plants is highly important for food and pharmacology. The plant cell and tissue culture methods offer an integrated approach for valuable substances production. Biosynthesis could be enhanced by different chemical and physical stimuli including UV radiation. Some investigations demonstrated increase of metabolite components in cultivated plant cells and tissues after their irradiation, particularly with UV-B (280-315 nm) and UV-C (200-280 nm) However, in vitro cultures potential to produce secondary metabolites after UV exposure is not extensively studied giving a perspective to enlarge the investigations on the role of UV light as elicitor. In this respect medicinal plants are promising. Golden root is a wildly used medicinal plant, containing a range of antioxidant compounds. The aim of this work was to study the effect of low doses UV radiation on the process of callus formation and in vitro growth.
Plants, calli and explants cultivated in vitro on different nutrient media were irradiated with low doses UV-B (280-315 nm) and UV-C (200-280 nm). Irradiation exposure to UV-B light was for 3, 5 and 7 minutes and to UV-C light for 1 and 2 minutes.
Irradiation caused changes in callus growth, structure and color without influencing calli and plants viability. Lethal effect was observed only for leaf explants exposed to UV-C for 2 min and UV-B for 7 min.

Key words*: medicinal plant, golden root, calli; UV-B; UV-C.*

INTRODUCTION

The use of medicinal plants and their biologically active compounds is increasing worldwide. The plant cell and tissue culture methods offer an integrated approach for valuable substances production. Biosynthesis could be enhanced by different chemical and physical stimuli including UV radiation.
Ultraviolet radiation (UV) is a fraction of sunlight reaching the Earth's surface haring the character of abiotic stress factor. It is arbitrarily divided into UV-A with wave length λ 315-400 nm, UV-B (280-315 nm) and UV-C (200-280 nm). Every type UV radiation affects plant growth and development, such as damage to gradually increase with decreasing wave length. UV-A radiation is poorly absorbed by the ozone because it's the smallest harm, and have not a great interest for ecology, physiology and genetics of plants. Independently of the magnitude and specificity of the impact each type UV radiation changes the growth and metabolic processes.
It is known that the duration of exposure and the dose-response are essential to metabolic reactions. In the natural conditions, UV-A radiation influences sustained at low doses on the plants. The reduction of the stratospheric ozone layer caused by anthropogenic factors leads to a systematic increase in UV-B radiation in the last decades (McKenzie et al., 2007). UV-C rays are absorbed by ozone and oxygen and do not reach the earth's surface, except in the high mountain areas (Häder et al., 2007). Despite the ecological insignificance of UV-C radiation on plants growing in natural conditions their use under controlled conditions is essential for various experimental models. Photons of UV-C radiation are high energy and cause rapid damage to the plant metabolism. However the low doses of UV-C can delay

aging as well as to inhibit the development of phytopathogens which is of practical interest to storage. On the other hand, the effects of prolonged exposure of plants to low doses of UV-B and UV-C have not been well studied, especially those of the UV-C. Some authors presented UV-B and UV-C radiation like a stress agent.

Some investigations demonstrated increase of metabolic components in cultivated plant cells and tissues after irradiation, particularly with small doses UV-B (280-315 nm) and UV-C (200-280 nm) (Schreiner et al., 2014). Mutants of *Arabidopsis* lacking flavonoids are hypersensitive to UV radiation whereas another type of *Arabidopsis* mutant possessing constitutive elevated accumulation of flavonoids and other phenolics is tolerant to lethal UV doses (Bieza and Lois, 2001). Flavonoids strongly absorb light in the range of 220-380 nm and are known to be photo-stable (Stapleton and Walbot, 1994; Ghanati et al., 2013). However, *in vitro* cultures potential to produce secondary metabolites after UV exposure is not extensively studied and giving a perspective to enlarge the investigations on the role of UV light as elicitor. In this respect medicinal plants are promising. Sometimes the antioxidant concentration in a plant production might be dependent on the time of evaluation too, like analyzing immediately after the radiation treatment or after a certain period of time duration (Alothman at al., 2009).

Rhodiola rosea is an indigenous plant grown in the cold regions and high mountains area about 1800 m altitude. The species is protected by Low in Bulgaria and other countries due to is it poor germination and over exploitation. Golden root is a wildly used medicinal plant, containing a range of antioxidant compounds. The stimulating and adaptogenic properties of *Rhodiola rosea* are attributed to p-tyrosol, salidroside, rhodioniside, rhodiolin, rosin, rosavin, rosarin, and rosiridin. The plant extracts have a positive influence on memory, nervous system and brain. They have anti-stress and anti-depressant properties, and increase antitumor activity of the body etc.

The aim of this work was to study the effect of low doses UV radiation on some *in vitro* process like callus formation, plant *in vitro* growth and development.

MATERIALS AND METHODS

In vitro culture. In vitro propagated plants *Rhodiola rosea* were used in the experiments (Tasheva and Kosturkova, 2010, 2012a, 2013), leaf explants obtained from these plants and calli. Calli was obtained from leaf explants of *in vitro* propagated plants and cultivated on solidified Murashige and Skoog medium (1962) with added different combination and concentration of growth regulators N^6-benzylaminopurine and 2,4-dichlorophenoxyacetic acid (BAP and 2.4-D) and casein hydrolysate, pH 5.8 (Tasheva and Kosturkova, 2012a, b). The contain of nutrient media for callus induction was as follows:

Variant 1 - BAP (1.0 mg/l) and 2.4-D (1.0 mg/l); Variant 2 – BAP (1.0 mg/l) and 2.4-D (0.5 mg/l); Variant 3 – BAP (0.5 mg/l) and 2.4 –D (1.0 mg/l); Variant 4 – BAP (1.0 mg/l), 2.4-D (1.0 mg/l) and casein hydrolisate 1000 mg/l

However, content of sucrose was 20 g/l, content of agar-agar was constant 6.0 g/l. Culture media for plant propagation was MS medium containing zeatin (2.0 mg/l) and IAA (0.2 mg/l). Culture media were autoclaved at 1.1 $kg.cm^{-2}$, 121^o C for 20 min.

Irradiation of the explants, calli and *in vitro* propagated plants were held 7 days after their transfer and cultivation on the fresh nutrient medium.

Irradiation by UV light/rays. Effect of UV on the growth of *Rhodiola* cells and tissues was monitored by comparing percent of survival of treated with the control ones. As a source of UV rays **(**280-315nm) were used UV-B and UV-C lamps.

Parameters of lamps. As a source of UV-B (200-280nm) radiation was used mercury lamp (Philips TL 20W/12 UV-B c λmax=312±25nm, 2*20W=40W) and UV-C lamps (STYLO STY 115, GE Lighting, c λmax 254 nm, 15 W). The distance between UV lamps and irradiated tissues were 0.31±0.01 m. The irradiation by UV-B light were applied for 1, 5 and 7 minute, and with UV-C for 1 and 2 minutes. The UV-C is a most power and is expected to have stronger effect on plant material.

Characteristics of in vitro cultures. During the study have been monitored morphological characteristics, such as development, shape, texture and pigmentation of callus tissue.

Control observations were conducted on the 3rd, 7th, 15th, 20th day. For the main signs were evaluated qualitative and quantitative characteristics.The physiological characteristics such as period of time needed to induce dedifferentiation in tissues in the formation of callus and time to form non differentiated and differentiated structures such as buds and roots in regenerants, etc. were monitored.

In vitro cultivation condition. Callus cultures were induced and maintained in cultivation room at temperature of 23-24°C and dim light (20 $\mu Mm^{-2}s^{-1}$). The *in vitro* plants were cultivated in a cultivation room at temperature of 23-24°C and 40 $\mu Mm^{-2}s^{-1}$ light intensity. Response of 30 to 50 explants was examined for each variant of media and UV – irradiation. Sigma Plot 11.1 was applied for statistics.

RESULTS AND DISCUSSIONS

In vitro cultures. In vitro propagated plants were used like a source for the leaf explants, reported in our previously study (Tasheva and Kosturkova, 2010).

Growth and characteristics of the callus. Calli were obtained from leaf on *in vitro* propagated plants and cultivated on different nutrient medium. Callus growth rate and tissue characteristics varied depending on the culture media composition (Tasheva and Kosturkova, 2012a, 2013). Characteristics of callus tissues used in the experiments was as follows:

Calli variant 1: soft calli pale in colour, 20% loosy calli with liquid like texture and brownish color;

Calli variant 2: grain, compact calli pale in color;

Calli variant 3: 62 % more compact calli; 38 % loose, liquid like calli

Calli variant 4: compact, grain calli, pale, yellowish-beige in color.

The type of calli tissue affects their survival after irradiation.

Irradiation by UV light/rays. Callus tissue was irradiated to stimulate the growth and biomass accumulation, followed by biologically active substances production. The calli and explants cultivated for callus induction were obtained on various nutrient media. Plants regenerants grown in *in vitro* conditions were irradiated, too. The irradiation was with different UV light (B and C) and time of duration.

On the 3rd day after irradiation leaf explants, calli and *in vitro* propagated plants survived 100 % with exception of calli variant 1 with survival rate 60 %. The necrosis in plant material began to occur later.

Observation on the 15th day after irradiation showed the following changes: high survival rate was observed in the calli tissues and in *in vitro* propagated plants in all case of UV irradiations.

On the 15th day after irradiation with low doses UV-B for 3 minute and UV-C for 1 minute the percentage of survived calli is almost from 70.0% to 95.4 % for UV-B and 64.4 % to 100% for UV-C, respectively (Table 1).

Table 1. Survival rate (%) on explants, calli and plants on 15th and 20 days after UV irradiation.

UV irradiation	Survival [%]											
	15 day						20 day					
	Leaf expl	*In vitro* plants	Calli				Leaf expl	*In vitro* plants	Calli			
			var. 1	var. 2	var. 3	var. 4			var. 1	var. 2	var. 3	var 4
UV – B												
3 minute	42.2	75.5	70.0	82.2	95.4	84.4	0	31.1	62.5	46.6	59.1	72.7
5 minute	26.6	71.1	42.5	75.5	81.8	6.6	0	0	0	48.8	40.9	0
7 minute	0	0	23.0	65.9	48.8	4.0	0	0	0	11.1	0	0
UV – C												
1 minute	17.8	48.8	64.4	100 green 20 %	93.3	96.0	0	12.0	40.0	73.3	42.2	66.0
2 minute	2.0	0	22.2	0	0	0	0	0	0	0	0	0
Control	100	100	100	100	100	100	100	100	100	100	100	100

The control variant were calli, explant and plants cultivated on the same medium without irradiation.

On the 20th day after UV-B irradiation the survival rate of the calli was almost equally. Three minute UV-B irradiation caused survival rate from 46.6 % (variant 2) to 72.7 % (variant 4), while 5 min irradiation resulted 0 % for variant 1 and 4 and 48.8 % survival rate for variant 3. UV-B irradiation for 7 min caused lethal effect on calli variant 1, 2 and 4, while variant 3 hat survival rate 11.1 %. Irradiation with UV-C for 1 min resulted survival rate from 40.0 % (variant 1) to 66 % for variant 4, while 2 min UV-C irradiation caused 100 % necrosis in all type of calli tissue.

Leaf explants were more sensitive to UV irradiation in comparison to *in vitro* propagated plants and calli. On the 15th day their survival rate after 3 and 5 min of UV-B irradiation was 42.2 % and 26.6 %, respectively, while 7 min irradiation caused 100 % lethal effect. One minute irradiation with UV-C resulted in 17 % survival of explants, while irradiation for 2 min caused extremely low survival – 2 %. Lethal effect on the explants was observed on the 20th day after irradiation with any of the different doses UV-B and UV-C light (Table 1).

The survival rate of *in vitro* propagated plants varied depending on the type of UV rays. UV-C irradiation of plants with the minimal time of 1 minute reduced twice survival rate (48 %), while in the case of UV-B longer irradiation for 3 min and 5 min caused less damages (survival of 75.5 % and 71.1 %, respectively). On the 20th day necrosis was observed in the case with UV-B irradiation for 5 and 7 min and UV-C for 2 min, while 3 min UV-B irradiation and 1 min UV-C caused survival plants 31.1 % and 12.0%, respectively. There was not obtained the changes in the structure and texture at the all of the irradiated plant material (Figure 1 – 5).

The survived cultures were transferred on the fresh nutrient medium for mass propagation, followed by the analysis of the biologically active complex.

Exposure to UV adversely affected the growth of *Rhodiola* cells and tissue and this was more pronounced in UV-C treatments. Similarly viability of the cells significantly decreased after exposure to UV-B and UV-C, compared to the control cells. The most adverse effect of UV on cell viability was observed in prolonged exposure periods. Decrease of the viability of the cells was more pronounced under UV C treatments.

The high doses of UV-C and UV-B radiation leads to destructive changes in the organism, as a part of them are similar, like inhibition of growth, decrease the content of nonspecific (protective) substances, etc. From the other side Schreiner et al. (2014) reported that often the treatment with UV light, particularly from the UV-B range (280–320 nm), is an example for effective elicitor application.

Some authors showed that the UV-B radiation had negatively affected the growth of the bean plants, and reduced total biomass with 61.6% (Singh et al., 2011). The short –term irradiation of *Pisum sativum, Triticum aestivum* and *Hordeum vulgare* with 49 kJ m-2d-1 UV-B increased the prolin quality and UV absorbed substances (Alexieva et al., 2001; Fedina et al., 2007).

Figure 1. *In vitro* propagated plants on the 3-th day after UV-B irradiation for 3 minutes.

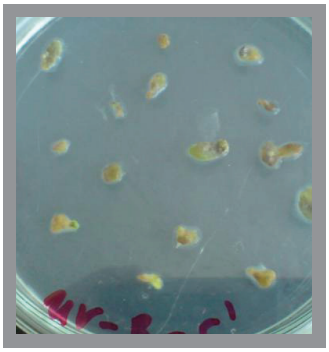

Figure 2. Leaf explants irradiated on the 3th day with UV-B for 5 minutes

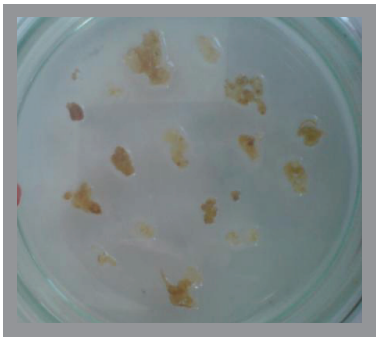

Figure 3. Calli Variant 1 on the 3th day after UV-B irradiation for 3 minutes

Change in biomass accumulation is an important measure to assess s UV-B sensitivity, since this parameter reflects the cumulative effect of many small disruptions in plant function. UV-B radiation exclusion studies have also indicated that UV-B radiation reduced biomass accumulation in cucumber (Krizek et al., 1997), spinach (Mishra and Agrawal, 2006) and mung bean (Agrawal et al., 2006).

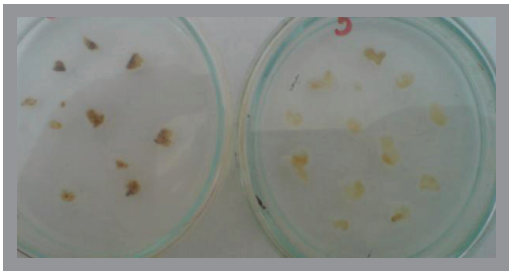

Figure 4. Calli (Variant 1 (left) and Varint 3 (right)) on the 3th day after UV-B irradiation for 5 minute

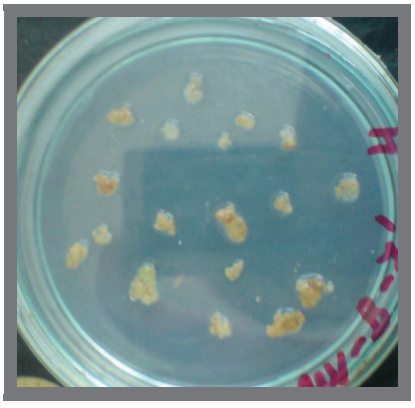

Figure 5. Calli (Variant 2) on the 3th day after UV-B irradiation for 7 minute.

CONCLUSIONS

Obtained results contributed to deeper understanding of influence of UV - rays on plant growth, development and metabolism.The experiments can serve as a basis for future research in the field of increase biologically active substances production using UV irradiation. This would also allow synthesis of valuable substances in laboratory conditions for protection of species from overharvest from their natural habitats.

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