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Effects of UV irradiation on cell viability, anthocyanin, and flavonoid contents of callus-cultured *Malva neglecta* cells

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***Abstract*— Callus cultures from leaf explants of *Malva neglecta* were initiated in vitro, and their capacity to produce UV absorbing compounds was analysed. The objective of this study was to evaluate the effects of UV-B and UV-C on UV absorbing compounds (e.g., flavonoid, anthocyanin, tannin) of callus-cultured *Malva neglecta* cells. The calli were exposed to different doses of UV irradiation as follows: 144, 288, 432, 576, 720, 864, 1296, and 1728 j/m^2 for UV-B and 204, 408, 612, 816, 1020, 1284, 1836, and 2448 j/m^2 for UV-C. The results showed that the flavonoids and anthocyanins were increased significantly, compared with the control cells. In addition, tannin such as catechin was increased by longer exposure to UV-C and UV-B, compared to control. The viability percent of cells under UV treatment decreased, compared with those of the control cells.**
Keywords- Anthocyanin; Flavonoide; Malva neglecta; Tannin; Ultraviolet irradiation

I. INTRODUCTION

Malva (from Malvaceae) is composed of 25–30 species of herbaceous annual, biennial, and perennial plants. The genus is widespread throughout the temperate, subtropical and tropical regions of Africa, Asia and Europe. *Malva neglecta,* also known as common mallow, button weed, cheese plant, cheese weed, dwarf mallow and round leaf mallow is usually consumed as a food. This is especially true of the seeds, which contain 21% protein and 15.2% fat. Because of its high mucilage content, *Malva* tea helps to alleviate inflammation and mouth irritation. The plant is also useful because of its expectorant and cough-suppressing action. In addition, the plant contains tannins in its leaves and blossoms, which can relive the pain of stomach upset, relax intestinal spasms and soothe irritated skin [1- 6].

Plants use sunlight for photosynthesis and, as a consequence, are exposed to the ultraviolet (UV) radiation that is present in sunlight [7]. Ultraviolet radiation (UV) is a part of the non-ionizing region of the electromagnetic spectrum which comprises approximately 8-9% of the total solar radiation [8-9]. UV is traditionally divided in to three wavelengths: UV-C (200-280 nm) is extremely harmful to living organisms, but not relevant under natural conditions of solar irradiation; UV-B (280-320 nm) is of particular interest because although this wavelength represent only approximately 1.5% of the total spectrum, but can induce a variety of damaging effects in plants; UV-A (320-400 nm) represents approximately 6.3% of the incoming solar radiation and is the least hazardous part of UV radiation [10]. The stratospheric ozone layer efficiently filters out most of the detrimental, shortwave UV radiation, shorter than 280 nm. The absorption coefficient of ozone decreases rapidly at wavelength longer than 280 nm and approaches zero at about 330 nm [11]. Therefore UV-A radiation is virtually unaffected by changes of ozone concentration [12, 13]. A small decrease in ozone levels may cause a large relative increase in biologically effective UV radiation [14, 15]. In general, each 1% reduction in ozone causes of 1.3-1.8% in UV-B radiation reaching the biosphere. The decrease in ozone level originates from human activities such as the release of chlorofluorocarbons and nitrogen oxides, which act as ozone antagonists [16-18]. Plants, which use sunlight for photosynthesis, are unable to avoid exposure to enhanced levels of UV-B radiation [10].

Ultraviolet sensitive Plants may also respond by accumulating UV-absorbing compounds in their outer tissue layers, which presumably protect sensitive targets from UV damage [18]. UV radiation is readily absorbed by biomolecules such as amino acids, polypeptides and nucleic acids [10, 19]. The role of UV absorbing compounds (flavonoid, anthocyanin) in providing stable blue flower colours in the angiosperms is outlined. By contrast with the very visible flavonoids in flower petals, the flavonoids present in leaves are completely hidden by the ubiquitous green of the chlorophylls. Nevertheless, there is increasing evidence that these flavonoids, particularly when they are located at the upper surface of the leaf or in the epidermal cells, have a role to play in the physiological survival of plants. Perhaps the most active area of flavonoid research at the present time is in the possible medicinal contribution that flavonoids make to human health. Medicinal properties of flavonoids are including to antioxidant activity, inhibition of enzymes, dietary, healing of coronary heart disease, anti-inflammatory, and oestrogenic activity [20- 22].

II. MATERIAL AND METHODS

A. *plant cell material and UV treatment*

The calli were established from leaf surface-sterilized explants of *Malva neglecta* on modified B5 media. After 7 days, the calli were emerged and were sub cultured every 10 days. After 11 subcultures by reaching to a cell line with a fast and stabilized growth rate, the cells were exposed to

different doses of UV irradiation, using UV-B and UV-C lamps providing 144, 288, 432, 576, 720, 864, 1296, and 1728 j/m2 for UV-B and 204, 408, 612, 816, 1020, 1284, 1836, and 2448 j/m2 for UV-C . The applied doses UV for 10, 20, 30, 40, 50, 60, 90, and 120 min, in tandem.

B. *Growth curve*

In order to determine the growth rate of the cells along with the time, 2 g of the cells were transferred to new media and were weighted every 4 days, in triplicate.

C. *Viability assay*

For analysis of viability percent, 0.2 g calli were put in 1 mL of aquatic Evans blue solution (0.25%) for 5 min. The samples were then washed with distilled water. The cells were then put in SDS (sodium dodecyl sulfate) 0.5% and incubated in a water bath at 70 ºC for 10 min. The absorbance of the supernatant of each sample was measured at 600 nm by spectrophotometer (Cintra 6, GBC, Australia).

D. *Flavonoid assay*

For measurement of *flavonoid* contents, 0.1 g calli were ground in 3 mL of acidified ethanol (99: 1, ethanol: HCl). Samples were then centrifuged at 12000 rpm for 20 min and the supernatant of each sample was gently boiled for 10 minutes in a water bath at 80 ºC. The absorbance was measured at 270, 300 and 330 nm, and the flavonoid content was calculated using an extinction coefficient of 33000 $M^{-1}cm^{-1}$ [23].

E. *Anthocyanin assay*

For measurement of anthocyanin contents, 0.1 g were ground in 3 mL of acidified methanol (99: 1, methanol: HCl). Samples were then centrifuged at 12000 rpm for 20 min and the supernatant of each sample incubated in the dark at room temperature for 24 h. The absorbance was recorded at 550 nm, and the anthocyanin content was calculated using an extinction coefficient of 33000 $M^{-1}cm^{-1}$ [24-25].

F. *HPLC analysis of tannin*

The cells (1.5 g) were homogenized in 8 mL of pure methanol, and were sunicated for 90 min. After centrifugation at 15000 rpm for 20 min, the supernatant of each sample was air-dried, re-dissolved in methanol before determination by HPLC system (Knauer, Germany), which was equipped with a C-18 column (Perfectsil Target ODS-3 (5µm), 250 × 4.6 mm) MZ-Analysentechnik, Mainz, Germany). Tannin was eluted at a flow rate of 1 $mL.min^{-1}$ with a linear gradient of 30–100% acetonitril and were detected at 340 nm, using commercially available authentic catechin as standard.

III. RESULTS AND DISCUSSIONS

A. Growth curve and viability percent

The growth curve of *Malva neglecta* cells is shown in Fig.1. As seen in this figure the cells were in logarithmic growth phase, when they were exposed to UV.

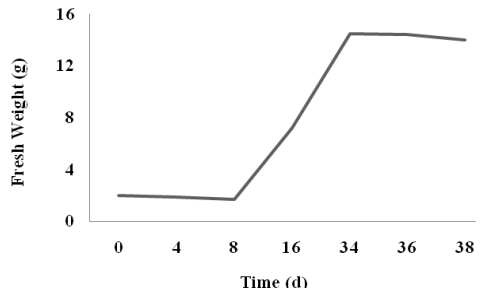

Figure 1.Growth curve.

The viability percent of calli after 120 min UV-B and C exposure reduced to 28.68% , 15.63% of the control, respectively (Figs. 2-3). This reveals that UV-C was more detrimental than UV-B for *Malv*a cells.

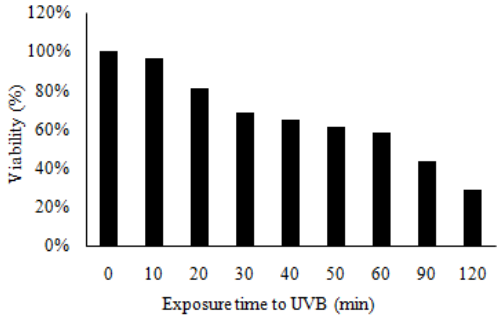

Figure 2. Viability percent of calli in response to UV-B.

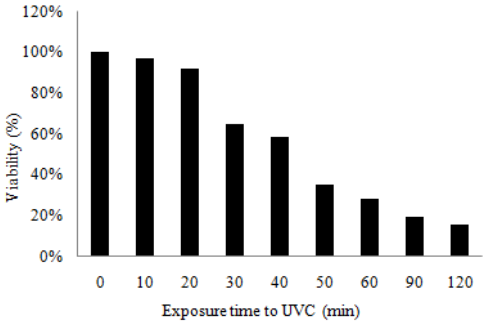

Figure 3. Viability percent of calli in response to UV-C.

B. Flavonoid, anthocyanin and catechin contents

A remarkable increase was observed in flavonoid contents of UV-irradiated cells, compared to those of the control ones (Figs. 4-5).

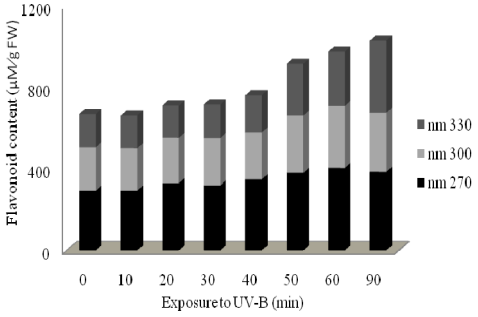

Figure 4. Flavonoid content of calli in response to UV-B.

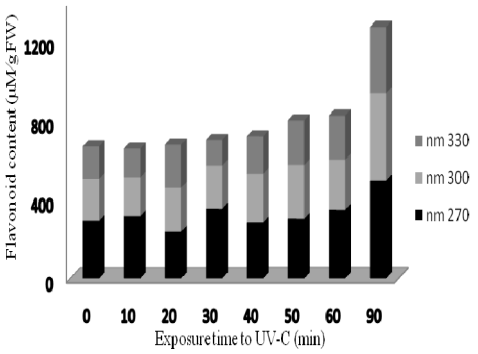

Figure 5. Flavonoid content of calli in response to UV-C.

The anthocyanin contents of UV-exposed *Malva* cells were higher than those of the control cells. In control samples anthocyanin content was 20.13636 µM/g FW but increased up to 20.13636 µM/g FW and 22.78788 µM/g FW, after exposure to after to UV-B and UV-C for 90 min, respectively (Figs. 5-6). The results suggest that increase of anthocyanin content was more pronounced by UV-B than UV-C irradiation (Figs. 6-7).

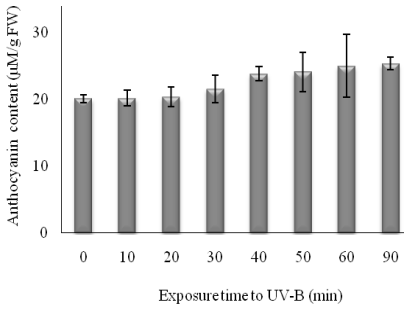

Figure 6. Anthocyanin content of calli in response to UV-B.

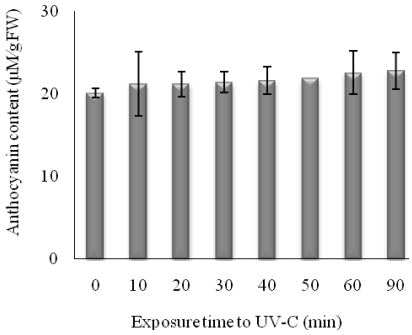

Figure 7. Anthocyanin content of calli in response to UV-C.

Catechin contents also increases in calli after 90 min exposure to UV irradiation, so that 10 and 17 folds increase were observed in those cells which were treated 90 min with UV-B and C, respectively (Fig. 8-9).

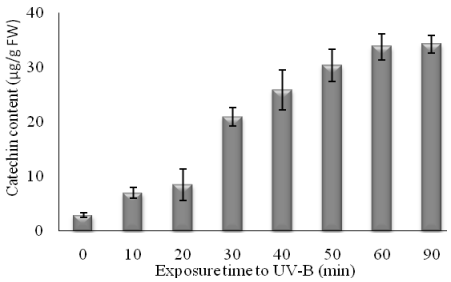

Figure 8. Catechin content of calli in response to UV-B.

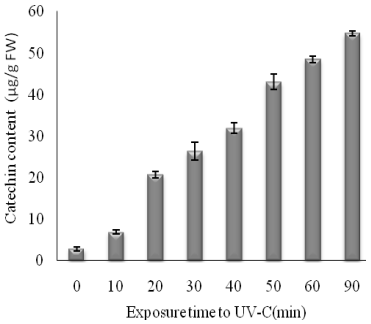

Figure 9. Catechin content of calli in response to UV-C.

IV. CONCLUSIONS

In conclusion the result presented here, suggests that UV-C exerted more detrimental effects on *Malva* cells and reduced their viability. Although both kinds of UV increased UV absorbing compounds of the cells, but UV-B irradiation more remarkably resulted in increase of anthocyanins, while UV-C more increased the contents of tannins and flavonoids

of *Malva* cells, suggesting a differential response of these cells to different kinds of UV.

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