

Short Communication

Photosynthetic Efficiency, and Photodamage by UV and Visible Radiation, in Red versus Green Leaf Coleus Varieties

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The maximum quantum yield for photosynthetic O₂ evolution in red leaf coleus varieties having anthocyanin in their upper epidermis is much lower in green light and slightly lower in white light than in a green leaf variety lacking anthocyanin. A similar degree of photoinhibition occurred under excess visible light in the red versus green varieties; whereas, the red leaf varieties were less damaged by UV-B and UV-C radiation suggesting protection by anthocyanin in their epidermal tissue.

Key words: Anthocyanin — Coleus — Photosynthesis — Ultraviolet radiation.

There is growing interest in the effects of light stress on plants, including that of excess visible light and ultraviolet radiation. Both photon flux density and wavelength of light alter a plant's response to stress, and are, in themselves, potential sources of damage. With respect to photosynthesis, high levels of visible light cause photoinhibition and primary damage to photosystem II (Baker and Bowyer 1994) while UV light can have multiple detrimental effects on the process (see Cen and Bornman 1990, Strid et al. 1994, Teramura and Sullivan 1994). Thus, both high light and UV radiation can cause considerable damage to the photosynthetic capabilities of plants, though UV radiation has a much greater quantum efficiency for damage than does visible light (Jones and Kok 1966).

The amount of damage from UV radiation is dependent on length and intensity of exposure, as well as the level of supplemental visible lighting, other stress conditions, and the plant's defenses against low wavelength radiation. The 280–320 nm range (UV-B radiation) is of great interest today, as it includes the shortest, most damaging wavelengths to reach the earth's surface. A further decrease in the ozone layer would permit lower wavelengths of UV-B radiation to reach the earth than have in the past (Caldwell et al. 1986). The UV-B part of the spectrum is the only wavelength range that is significantly affected by ozone re-

duction, as higher wavelengths (UV-A: 320–380 nm) are not absorbed differentially in the stratosphere, and lower wavelengths (UV-C: <280 nm) are almost completely absorbed by other gases, such as O₂.

One means of plant protection against UV light is accumulation of screening pigments. There is evidence that colorless flavonoids can accumulate in epidermal tissue of plants and reduce damage from UV radiation because of their absorption of UV light (Robberecht and Caldwell 1978, Shimazaki et al. 1988, Cen and Bornman 1990, Tevini et al. 1991). Anthocyanins, which are a special class of colored flavonoids, absorb in the green and UV region of the spectrum (Harborne 1957, Takahashi et al. 1991). They have multiple functions in different plant tissues (Dakora 1995), but their roles in leaves of plants are largely undetermined. Their production is often stimulated by tissue damage, low temperature, and low-wavelength light (Harborne 1983). About a century ago it was proposed that absorption of solar energy by anthocyanin may help warm cool leaves or serve as a screen to prevent bleaching of leaves (see Onslow 1925). A rise in temperature in tissues containing anthocyanins has been observed in one study (Sturgeon and Mitten 1980); but whether or not this renders a plant more cold hardy is still uncertain. Gould et al. (1995) suggested anthocyanins in leaves may protect against photoinhibition from visible light; however their absorption characteristics would not appear to make them screens for preventing excess absorption of light by chlorophyll. Anthocyanins are diglucosides acylated with a cinnamic acid and they have two absorption peaks in the UV at ca. 290 and 310 nm due to superimposition of the absorption of the cinnamic acid (λ_{\max} 310 nm) upon that of the pigment absorption (Harborne 1957). A possible role for anthocyanin pigments in UV-B screening is supported by a study with suspension cultures of *Centaurea cyanus* in which light induced synthesis of anthocyanin reduces damage to DNA by UV-B and UV-C radiation (Takahashi et al. 1991).

In this study, we have examined the effect of UV radiation on photosynthesis in coleus plants, with and without anthocyanin in the upper epidermis, as compared to the effects of exposure to high levels of visible light in the same varieties. The anthocyanin cyanidin-3,5-diglucoside acylated with *p*-coumaric acid is the major UV absorbing

Abbreviations: PPFD, photosynthetic photon flux density (400–700 nm); ϕ_a , maximum quantum yield on an absorbed light basis.

pigment in red coleus (based on analysis of 17 varieties of *Coleus blumei*) (Lamprecht et al. 1975).

Plant material—*Coleus* varieties were grown under greenhouse conditions at a daily maximum photosynthetic photon flux density (PPFD) of 140–480 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ depending upon cloud cover, and day/night temperatures of approximately 25°C/18°C. The PPFD was measured between 400–700 nm with a quantum sensor (Li-Cor LI-185 from Li-Cor Instrument Corp., Lincoln, Nebraska, U.S.A.). Varieties used were of the green Rainbow, red Wizard, and red Dragon series. Red coleus types accumulated anthocyanin in the upper epidermal tissue. In most experiments with UV radiation, green Rainbow and red Wizard plants were used since their leaves are flatter and less undulated than the red Dragon variety, which allows for more even exposure of the leaf tissue to light treatments. However, similar trials with the red Dragon variety gave results which were consistent with those of the red Wizard plants.

Spectral characteristics and pigment analysis—Chlorophyll content was measured spectrophotometrically from extracts in absolute ethanol and calculated according to Wintermans and De Mots (1965). Carotenoid content was measured from absorbance at 480 nm in absolute ethanol extracts with correction for chlorophyll absorption according to Kirk and Allen (1965), using an extinction coefficient, $a_{480 \text{ nm}} = 200 \text{ g}^{-1} \text{ cm}^{-1}$. Anthocyanin was quantified based on absorbance at 530 nm in methanol : H₂O : HCl (70 : 29 : 1; Robberecht and Caldwell 1978).

Reflectance, transmittance, and absorptance of leaves between 400–700 nm were determined for leaf discs using an integrating sphere with a silicon detector (LabSphere IS-040-SF and SDA-050-U, North Sutton, New Hampshire) connected to a monochromator (Leitz IKESY containing a Xenon arc light source, Leitz, West Germany).

For calculating quantum yields for photosynthesis on an absorbed light basis (ϕ_a), leaf absorptance of light from the white, green or red light sources was determined with an integrating sphere. A lamp (designed by Ö. Björkman, Carnegie Institute) containing a 100 W tungsten filament lamp with a 750 nm heat filter (Optics 6174 series), was used as the source of white light. The same lamp served as a green light source when 450 nm and 550 nm quartz cutoff filters (Optics 6174 series) were inserted together in place of the heat filter. Red light was provided by LED with peak emittance at 660 nm (LH36 from Hansatech Instruments, King's Lynn, Suffolk, England).

Photosynthetic measurements—A leaf disc O₂ electrode cuvette system (Model LD2, Hansatech Instruments), was used to measure photosynthesis in $\mu\text{mol O}_2$ evolved $\text{m}^{-2} \text{s}^{-1}$, plot light response curves, and calculate quantum yield under 1% CO₂ (Lal and Edwards 1995). A Björkman tungsten light source with heat filter was used to generate response curves under white and green light (intensity con-

trolled by Esco and Ealing neutral density filters). The leaf disc was kept under 200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for 4 to 6 min for induction of photosynthesis, prior to running the light response curve from high to low light.

Plant treatments—Leaves treated with UV light or high intensity white light were positioned horizontally to the light source. One leaf at the third node from the apex was treated while the adjacent leaf (and the rest of the plant) was protected and served as a control. To test for photoinhibition, high white light treatments (1,800 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) were made at 15°C in growth chambers under 1,000 W metal halide lamps (leaves shaded with cheese cloth receiving 130 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ served as controls). After treatment, plants were incubated in a growth chamber for 15 min at room temperature under a PPFD of ca. 500 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Then, the light dependence of photosynthesis was measured with the leaf disc system as described above.

For UV-C treatments, an unfiltered lamp with max. emission at 254 nm was used (Philips Sterilamp, Seattle, Washington, U.S.A.) at room temperature. The UV-C irradiance was ca. 740 $\mu\text{W cm}^{-2}$, equivalent to ca. 16 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (measured with a Blak-Ray Model J225 ultraviolet meter San Gabriel, California, U.S.A., not biologically weighted); during UV-C treatment plants were exposed to low levels of room light from fluorescent lamps (PPFD of 8 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$). One leaf at the third node was exposed to UV-C radiation (27 cm from UV-C source); a mylar sheet, which absorbs under 320 nm, and an additional layer of thin plastic were used to protect the adjacent control leaf and the rest of the plant from UV radiation.

For UV-B treatments, plants were exposed at 26°C to a combination of UV-B radiation and visible light by an alternate arrangement of Westinghouse FS40 fluorescent lamps (Candela Corp. Santa Ana, California, U.S.A.) having a radiant flux of ca. 450 $\mu\text{W cm}^{-2}$ (not biologically weighted, estimated with an Eppley radiometric sensor, Newport, Rhode Island, U.S.A., equivalent to ca. 12 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$; peak emission was at 310 nm measured with an Optronics Model OL754 spectroradiometer) and Agrolux fluorescent bulbs providing a PPFD of 120 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Cellulose acetate film was used to eliminate the small amounts of UV-C radiation generated by the FS40 lamps. The untreated part of the plant was protected from UV-B radiation with mylar.

Leaf spectral qualities and pigment content—Red Dragon and green Rainbow coleus had similar visible spectral characteristics in all but the green anthocyanin-absorbing region (ca. 500–600 nm, Fig. 1), and both varieties had similar chlorophyll and carotenoid contents per unit area (Table 1). The red Dragon variety had a lower reflectance (also see study on coleus by Grant et al. 1987) and transmittance, and a higher absorptance in the green part of the

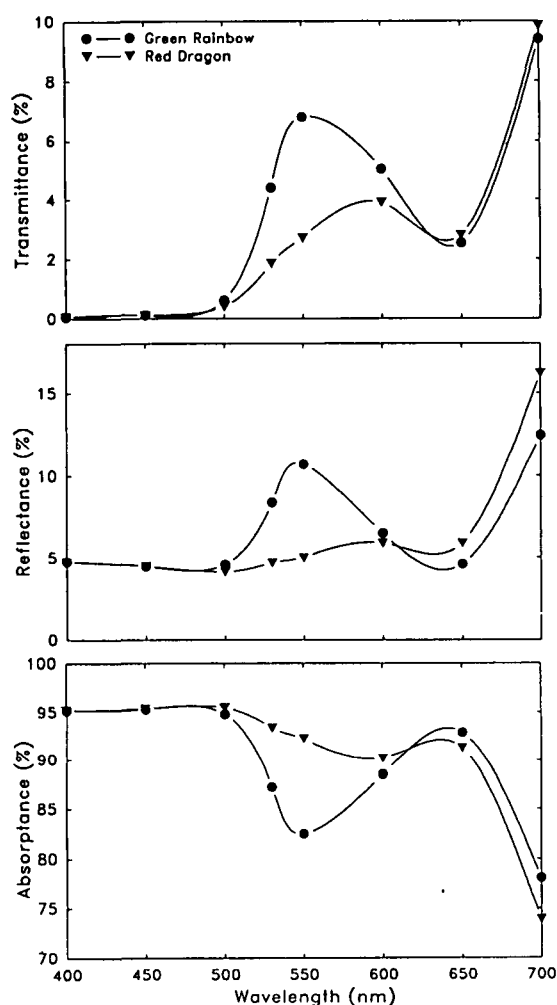


Fig. 1 Mean leaf reflectance, transmittance, and absorbance (%) of untreated red Dragon and green Rainbow coleus.

spectrum than did the green Rainbow variety. Red Wizard leaves also absorbed more in this region than the green Rainbow leaves, but had lower absorbance in the red part of the spectrum (results not shown), due to a lower chlo-

Table 2 Quantum yields (ϕ_a) of coleus varieties under different qualities of light

| Variety | Quantum yield (ϕ_a) | | |
|---------------|----------------------------|-------------|-------------|
| | Red light | White light | Green light |
| Green Rainbow | 0.087 | 0.083 | 0.063 |
| Red Dragon | 0.089 | 0.071 | 0.017 |
| Red Wizard | 0.082 | 0.061 | 0.015 |

ϕ_a values represent mol O₂ mol quanta⁻¹ on an absorbed basis and are an average of two or more replications.

rophyll content (Table 1). Absorbance measurements by leaves were not made in the UV range as the degree of absorption by anthocyanin in the upper epidermis of red Wizard can not be evaluated against the high absorption of UV radiation by various macromolecules throughout the leaf tissue.

Photosynthesis measurements without photoinhibitory treatments—Anthocyanin pigments, in addition to absorbing light in the UV region, absorb in the green (ca. 520–550 nm) region (Harborne 1957), whereas chlorophyll is most efficient in absorbing in the blue (ca. 435–450 nm) and red (ca. 650–680 nm) parts of the spectrum. Quantum yields were very similar for the three varieties under red light when compared on an absorbed basis, which is typical for terrestrial C₃ plants under unstressed conditions (Björkman and Demmig 1987). However, quantum yields (ϕ_a) were reduced much more under green light for the red varieties (ca. 76% reduction) than for the green specimen (ca. 24% reduction) (Table 2), which can be explained by the anthocyanin-containing upper epidermal cells of red varieties absorbing green light and reducing its penetration to photosynthetic tissue. Thus, screening of green light by anthocyanin in the upper epidermis reduces the efficiency of its use for photosynthesis. However, the green and red varieties of coleus both had high quantum yields under white light, although photosynthetic efficiencies for both red varieties were slightly lower than for the green coleus.

Table 1 Pigment content of coleus varieties

| Variety | Chlorophyll | | | Carotenoids (mg cm ⁻²) | Anthocyanin ^a (A ₅₃₀ cm ⁻²) |
|---------------|-------------|---------------------------------|-------|------------------------------------|---|
| | <i>a</i> | <i>b</i> (mg cm ⁻²) | Total | | |
| Green Rainbow | 29 | 10 | 39 | 7.5 | 0.09 |
| Red Dragon | 28 | 11 | 39 | 6.5 | 0.59 |
| Red Wizard | 16 | 4 | 20 | 4.0 | 0.46 |

The results are averages of four or more replications.

^a The relative content of anthocyanin was determined based on absorbance at 530 nm in a methanol : H₂O : HCl leaf extract (see Methods) and results were expressed per unit leaf area.

The larger quantum yields in the red varieties under red light compared to white light are considered due to the lower efficiency in utilizing light for photosynthesis in the anthocyanin-absorbing region of the spectrum.

Although there is some reduction in ϕ_a under white light in the anthocyanin-containing varieties compared to the green variety (Table 2), rates of photosynthesis under saturating white light were similar for all three varieties (results not shown). Thus, the absorbance of light by anthocyanin in the visible region of the spectrum causes some reduction in net carbon gain under limiting light. An even larger reduction could occur in shady environments which receive a higher proportion of green, than red or blue light. This potential loss could be a negative factor relative to the possible benefits of accumulating anthocyanins. Indeed, in shady environments, few plants accumulate anthocyanins on the upper surfaces of their leaves, where they could interfere with light harvesting (see Onslow 1925).

Treatment with high levels of white light or UV light—

When red Dragon and green Rainbow coleus were treated with high levels of visible light for 2 h at low temperature (15°C), both varieties were photoinhibited to a similar degree based on reduction of ϕ_a (Fig. 2A). Red Wizard responded similarly in an experiment with a slightly longer exposure time (data not shown). From these treatments, there was no evidence that anthocyanin reduces photoinhibition by white light.

Following exposure to UV-C radiation, the green Rainbow variety had a lower quantum yield, a higher rate of dark respiration, a higher light compensation point, and lower photosynthesis rates at near-saturating light intensities than did the red Wizard variety (Fig. 2B). Exposure to UV-B light (Fig. 2C) produced effects which were very similar to those obtained from UV-C exposure. The green-leaved variety was more susceptible to damage than the red-leaved variety under several different times of exposure to UV-B and UV-C radiation (data not shown). It is evident from the photosynthetic light response curves that red coleus varieties having anthocyanin stored in the upper epidermal tissue of the leaf are less damaged by UV radiation. The results suggest that anthocyanin provides some protection from lower wavelength solar radiation.

Production of screening pigments may enable a plant to acclimate quickly to exposure to excess light or to increased levels of low wavelength light. Massive amounts of accumulated β -carotene have been shown to be effective in protecting the photosynthetic apparatus of *Dunaliella bardawil* from damage by high intensity blue light, its region of peak absorption (Ben-Amotz et al. 1989). In the present study, the presence of anthocyanin in the upper epidermal tissue of coleus caused no apparent loss in photosynthetic capacity under high light, and only a moderate loss in quantum yield under white light. We saw no evidence that anthocyanin reduces photoinhibition by screening excess white

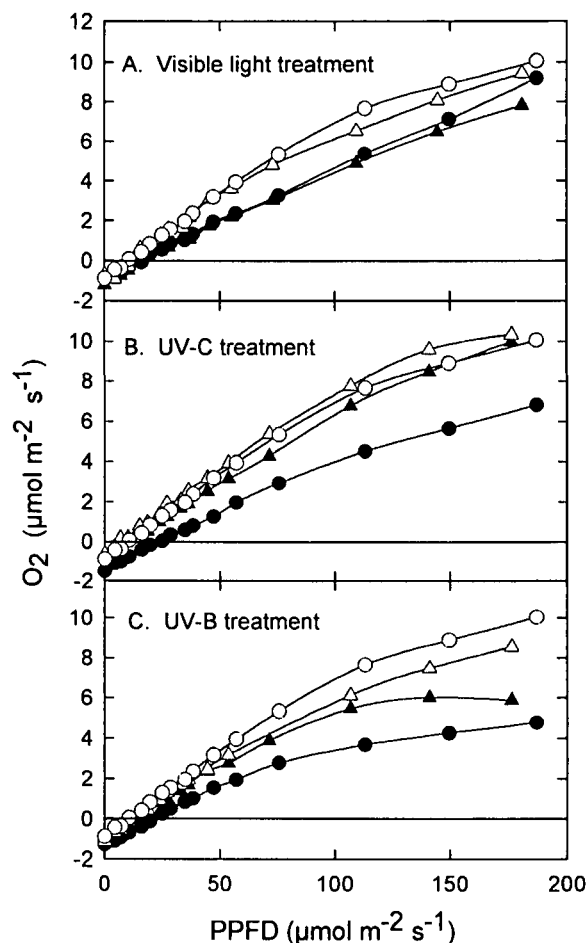


Fig. 2 Effect of treatment with high levels of white light (Panel A), UV-C (Panel B) and UV-B (Panel C) on photosynthesis of red versus green leaf coleus. Open symbols are control plants, closed symbols are treated plants. Panel A. Plants treated with high levels of white light ($1,800 \mu\text{mol m}^{-2} \text{s}^{-1}$ white light at 15°C for 2 h). (Δ , control; \blacktriangle , treated) Red Dragon; (\circ , control; \bullet , treated) Green Rainbow. Calculated ϕ_a values for red Dragon are 0.084 for control and 0.061 for treated. Calculated ϕ_a values for green Rainbow are 0.083 for control and 0.059 for treated. Panel B. Plants treated with UV-C (45 min at room temperature). (Δ , control; \blacktriangle , treated) Red Wizard; (\circ , control; \bullet , treated) Green Rainbow. Calculated ϕ_a values for red Wizard are 0.084 for control and 0.073 for treated. Calculated ϕ_a values for green Rainbow are 0.083 for control and 0.057 for treated. Panel C. Plants treated with UV-B (3 h at 26°C). (Δ , control; \blacktriangle , treated) Red Wizard; (\circ , control; \bullet , treated) Green Rainbow. Calculated ϕ_a values for red Wizard are 0.083 for control and 0.073 for treated. Calculated ϕ_a values for green Rainbow are 0.083 for control and 0.062 for treated.

light in contrast to the suggestion by Gould et al. (1995) that anthocyanin on the lower side of leaves of tropical species protect against excess visible light. The lack of protection against photoinhibition in the present study is consistent with the lack of screening by anthocyanin in the blue and red part of the spectra where chlorophyll absorption is

maximum. Its presence in many plants at low latitudes and high altitudes, in fact, seems to have little relation to light in the visible spectrum. However, the results suggest it may provide a means of adaptation against the damaging effects of UV radiation.

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References

- Baker, N.R. and Bowyer, J.R. (1994) *Photoinhibition of Photosynthesis*. Bios Scientific Publisher, Oxford.
- Ben-Amotz, A., Shaish, A. and Avron, M. (1989) Mode of action of the massively accumulated β -carotene of *Dunaliella bardawil* in protecting the alga against damage by excess irradiation. *Plant Physiol.* 91: 1040-1043.
- Björkman, Ö. and Demmig, B. (1987) Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. *Planta* 170: 489-504.
- Caldwell, M.M., Camp, L.B., Warner, C.W. and Flint, S.D. (1986) Action spectra and their key role in assessing biological consequences of UV-B radiation change. In *Stratospheric Ozone Reduction, Solar Ultraviolet Radiation and Plant Life*. Edited by Worrest, R.C. and Caldwell, M.M. pp. 87-111. Springer-Verlag, Berlin.
- Cen, Y. and Bornman, J.F. (1990) The response of bean plants to UV-B radiation under different irradiances of background visible light. *J. Exp. Bot.* 41: 1489-1495.
- Dakora, F.D. (1995) Plant flavonoids: biological molecules for useful exploitation. *Aust. J. Plant Physiol.* 22: 87-99.
- Gould, K.S., Kuhn, D.N., Lee, D.W. and Oberbauer, S.F. (1995) Why leaves are sometimes red. *Nature* 378: 241-242.
- Grant, L., Daughtry, C.S.T. and Vanderbilt, V.C. (1987) Polarized and non-polarized leaf reflectances of *Coleus blumei*. *J. Exp. Bot.* 27: 139-145.
- Harborne, J.B. (1957) Spectral methods of characterizing anthocyanins. *Biochem. J.* 70: 22-28.
- Harborne, J.B. (1983) *Current Advances in the Biochemistry of Flavonoids*. pp. 44-47. Academic Press, London.
- Jones, L.W. and Kok, B. (1966) Photoinhibition of chloroplast reactions. I. Kinetics and action spectra. *Plant Physiol.* 41: 1037-1043.
- Kirk, J.T.O. and Allen, R.L. (1965) Dependence of chloroplast pigment synthesis on protein synthesis: effect of actidione. *Biochem. Biophys. Res. Commun.* 21: 523-530.
- Lal, A. and Edwards, G.E. (1995) Maximum quantum yields of O_2 evolution in C_4 plants under high CO_2 . *Plant Cell Physiol.* 36: 1311-1317.
- Lamprecht, W.O., Applegate, H. and Powell, R.D. (1975) Pigments of *Coleus blumei*. *Phyton* 33: 157-163.
- Onslow, M.W. (1925) *The Anthocyanin Pigments of Plants*. pp. 314. Cambridge University Press, London.
- Robberecht, R. and Caldwell, M.M. (1978) Leaf epidermal transmittance of ultraviolet radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. *Oecologia* 32: 227-287.
- Shimazaki, K., Igarashi, T. and Kondo, N. (1988) Protection by the epidermis of photosynthesis against UV-C radiation estimated by chlorophyll *a* fluorescence. *Physiol. Plant.* 74: 34-38.
- Strid, A., Chow, W.S. and Anderson, J.M. (1994) UV-B damage and protection at the molecular level in plants. *Photosynth. Res.* 39: 475-489.
- Sturgeon, K.B. and Mitten, J.B. (1980) Cone color polymorphism associated with elevation in white fir, *Abies concolor*, in southern Colorado. *Amer. J. Bot.* 67: 1040-1045.
- Takahashi, A., Takeda, K. and Ohnishi, T. (1991) Light-induced anthocyanin reduces the extent of damage of DNA in UV-irradiated *Centaurea cyanus* cells in culture. *Plant Cell Physiol.* 32: 541-547.
- Teramura, A.H. and Sullivan, J.H. (1994) Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. *Photosynth. Res.* 39: 463-473.
- Tevini, M., Braun, J. and Fieser, G. (1991) The protective function of the epidermal layer of rye seedlings against ultraviolet-B radiation. *Photochem. Photobiol.* 53: 329-333.
- Wintermans, J.F.G.M. and De Mots, A. (1965) Spectrophotometric characteristics of chlorophylls *a* and *b* and their pheophytins in ethanol. *Biochim. Biophys. Acta* 109: 448-453.

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