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COMPARISON OF CHLOROPHYLL FLUORESCENCE PARAMETERS OF *CUCUMIS SATIVUS* AND *MENTHA PIPERITA* LEAVES EXPOSED TO SHORT-TERM UV-B IRRADIATION

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Cucumber (*Cucumis sativus* L. cv. Dar) leaves exposed to UV-B irradiation at a biologically effective dose of 9.5 kJ $m^{-2}d^{-1}$ showed decreased chlorophyll fluorescence parameter values versus the control; in peppermint (*Mentha piperita* L. cv. Asia) leaves those values were almost unchanged after treatment. F_v/F_o and Rfd were reduced more than other values, indicating inhibition of the oxygen-evolving complex and cooperation between the light and dark photosynthesis reactions as the primary targets of UV-B. The photosynthetic electron transport rate showed less change directly after irradiation, but after 24 h of recovery it was reduced to 50% of the control. Generally, photosystem II of peppermint leaves appeared more tolerant to the applied UV-B radiation than in cucumber leaves.

Key words: Cucumber, primary photosynthesis reactions, peppermint, ultraviolet-B.

INTRODUCTION

UV-B radiation can affect the structure of chloroplast membranes, hinder photosynthesis, retard plant growth and finally lower crop yields (Bornman, 1989; Jordan, 1996; Jansen et al., 1998). Vulnerability to UV-B radiation varies greatly between plant species (Caldwell et al., 1998; Żuk-Gołaszewska et al., 2003; Kozłowska et al., 2007). Cereals generally are more tolerant as they are monocots with vertical leaves, whereas dicotyledonous plants, including oil rapeseed and cucumber, are more susceptible (Skórska, 2000a,b, 2008; Shinkle et al., 2004; Jansen et al., 2008). There are few published reports on the sensitivity of herbal plants such as peppermint to ultraviolet radiation (Maffei et al., 1999; Maffei and Scannerini, 2000). Those studies have concentrated on photomorphogenesis and the essential oil composition of peppermint plants. The photosynthetic process can be affected by UV-B radiation at different levels: for example, changes in plant and leaf morphology that decrease light interception, changes in stomatal function that limit the availability of CO_2, or enzymes of the carbon fixation pathway. The effects of UV-B radiation on light harvesting and primary photochemical reactions of photosynthetic membranes, particularly on the photosystem II reaction center, have attracted much attention and study (Vass et al. 1996; Jansen et al., 2008). Luminescence tests are a good way to study plant susceptibility to UV-B radiation (Schreiber et al., 1994; Skórska, 2000a). In this work I studied (1) photosynthetic primary reactions of peppermint leaves subjected to briefly applied high-intensity UV-B radiation, as compared with the reactions in cucumber plants as susceptible species, and (2) the recovery capacity of both species after the applied stress.

MATERIAL AND METHODS

Plants of cucumber (*Cucumis sativus* L. cv. Dar) and peppermint (*Mentha piperita* L. cv. Asia) were grown in controlled conditions of light (PPFD 200 μmol $m^{-2}s^{-1}$, 12 h photoperiod)) and temperature (22°C/18°C, day/night). Discs (10 mm diam) were cut out from young, well-developed leaves of both species, placed in Petri dishes with water, and irradiated for 60 min with UV-B (VL-115 M lamp, Vilber Lourmat, France) in the 280–320 nm range with maximum emission at 311 nm (Skórska, 2000a). The emission spectrum of the lamp was recorded with a spectroradiometer (H 2000, Ocean Optics, U.S.A.).

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The intensity of UV-B irradiation (7.0 $W \cdot m^{-2}$) was measured with an IL 1403 radiometer with a SEL 240-UVB1 calibrated detector (International Light Inc., U.S.A.). The daily biologically effective dose of ultraviolet-B radiation (9.5 $kJm^{-2}d^{-1}$) was calculated according to Caldwell (1977). After irradiation, the photochemical efficiency of photosystem II was measured. Chlorophyll fluorescence was measured with a fluorometer (PAM-210, Heinz Walz GmbH, Germany). Before measurements the plants were dark-adapted for ~15 min. The leaf disc was placed on the head (adaxial surface down on the head) and covered with a magnetic clip. A weak measuring beam (0.04 µmol m^{-2} s^{-1}, 650 nm), pulse saturating light (3200 µmol m^{-2} s^{-1}, 665 nm) and actinic light (120 µmol m^{-2} s^{-1}, 665 nm) were used for measurements. The fluorescence signal was recorded for 3 min. The following parameters were estimated: the intensity of initial (F_o), maximum (F_m), stationary (F_s) and variable ($F_v = F_m - F_o$) fluorescence, F_v/F_m, F_v/F_o, ETR (electron transport rate in photosystem II), the vitality index [$Rfd = (F_m - F_s)/F_s$], and the coefficients of photochemical (qP) and nonphotochemical (qN) quenching, according to van Kooten and Snel (1990) and Lichtenthaler et al. (1986). Measurements were made from 6–8 plants. The results are presented as means of 6–8 replicates (different leaves). All data were analyzed with Statistica 8.0 (Statsoft, U.S.A.-PL) by two-way ANOVA. The multiple range Newman-Keul test at $p < 0.05$ was used to separate homogenous groups of means. For relative values (quotients, Fig. 1) the standard uncertainties were calculated for combined values (ISO, 1993).

RESULTS AND DISCUSSION

After 60 min of UV-B irradiation the values of chlorophyll fluorescence parameters for cucumber leaves decreased by 4% to 44% versus the control (Tab. 1). There were large decreases in F_v/F_o (20%) and Rfd (33%). The decrease of F_v/F_o indicates effects on the oxygen-evolving complex in photosystem II. Reduction of Rfd shows disruption of cooperation between light phase and dark phase reactions of photosynthesis (Lichtenthaler et al., 1986). A decreased F_v/F_m ratio indicates down-regulation of photosynthesis or photoinhibition (Krause and Weis, 1984). However, the slight change of the F_v/F_m ratio in this experiment was accompanied by a much stronger decrease of F_m values. In peppermint most of the measured parameters remained almost the same or even increased as in the F_v/F_m and F_v/F_o values. The photosynthetic apparatus of peppermint leaves showed more tolerance to short-term high-intensity UV-B radiation than that of the cucumber leaves. Only one measured

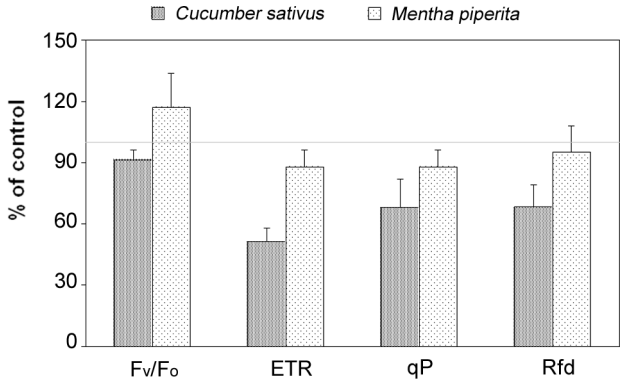

Fig. 1. Relative values of chlorophyll fluorescence parameters after 24 h of recovery after UV-B irradiation of *Cucumis sativus* and *Mentha piperita* leaves, expressed as % of control (without UV-B). All means of both species differ significantly at $p < 0.05$. Vertical segments present standard uncertainties of means.

parameter, the photochemical quenching coefficient (qP), did not change in cucumber leaves but in peppermint it decreased 13%. The nonphotochemical quenching coefficient (qN) was reduced by 44% in cucumber leaves while in peppermint it remained unchanged.

At 24 h after the end of UV-B stress there was no observed recovery of disturbed photosynthetic function in peppermint leaves, but in cucumber leaves this negative effect intensified (Fig. 1). Interestingly, the biggest decrease was in ETR, by almost 50%, but directly after UV-B stress it was only 6% lower. This indicates persisting damage to electron transport in photosystem II in cucumber leaves. In peppermint leaves this change was very small. The photochemical quenching coefficient decreased 32% in cucumber leaves versus the control. The nonphotochemical quenching coefficient increased by 62% (0.55) in cucumber, while in peppermint it decreased by 22% (0.31) versus the control.

Quenching analysis can distinguish two fundamentally different pathways of absorbed light energy conversion: qP quenching reflects the action of open photosystem II reaction centers and denotes the proportion of excitation energy trapped by them, while qN determines nonphotochemical fluorescence quenching (van Kooten and Snel, 1990; Krause and Weis, 1991). A decrease of photochemical quenching indicates that the fluorescence yield is lowered because the excitation energy is being used for photochemical reactions (Schreiber et al., 1994).

The overall quantum yield (Y) of photochemical energy conversion is a measure of the actual photochemical efficiency of photosystem II in illuminated leaves. In the UV-B-treated cucumber leaves the

TABLE 1. Chlorophyll fluorescence parameter values from cucumber and peppermint leaf discs subjected to UV-B irradiation

Parameter	*Cusumis sativus*		*Mentha piperita*	
	0	UV-B	0	UV-B
F_o	276 ± 31a	247 ± 13b	276 ± 9a	233 ± 16b
F_m	1087 ± 100a	891 ± 22b	1058 ± 105a	1122 ± 102a
F_v/F_m	0.757 ± 0.010b	0.727 ± 0.011c	0.737 ± 0.017c	0.790 ± 0.026a
F_v/F_o	3.15 ± 0.15b	2.54 ± 0.16c	2.90 ± 0.25b	3.96 ± 0.47a
Y	0.63 ± 0.01a	0.59 ± 0.03b	0.58 ± 0.01b	0.56 ± 0.05b
ETR	28.8 ± 0.5a	27.2 ± 1.4b	26.9 ± 0.7b	25.9 ± 1.5b
qP	0.93 ± 0.06a	0.87 ± 0.05a	0.92 ± 0.06a	0.80 ± 0.05b
qN	0.34 ± 0.05a	0.19 ± 0.03b	0.40 ± 0.06a	0.39 ± 0.10a
Rfd	2.66 ± 0.13a	1.78 ± 0.16b	2.38 ± 0.14a	2.32± 0.31a

Means with the same letter belong to the same homogenous group at $p < 0.05$ (Newman-Keul test).

Y value slightly decreased immediately after and especially 24 h after the end of the stress treatment. Similar changes were observed for electron transport rate ETR. That is due mainly to nonphotochemical conversion of absorbed light energy for thylakoid membrane energization. In this light condition nearly all reaction centers of photosystem II in control and UV-B-treated leaves remain oxidized (opened), as indicated by the qP values. Decreases in Y are associated with increases in excitation energy quenching in photosystem II antennae, and are generally considered to indicate down-regulation of electron transport. Consequently, the decreases in Y and ETR 24 h after the end of the UV-B treatment in both species can be understood as manifesting physiological regulation of electron transport by increasing excitation energy quenching in photosystem II antennae.

Shinkle et al. (2004) examined the influence of short-term exposure to different UV wavebands on the fine-scale kinetics of hypocotyl growth of cucumbers (*Cucumis sativus* L.) grown in dim red light. The response to short-wavelength UV-B persisted for at least 24 h, while the response to long-wavelength UV-B lasted only 3 h. They concluded that different photosensory processes are involved in mediating the growth and morphological responses to short-wavelength UV-B (280–300 nm) and long wavelength UV-B (essentially 300–320 nm) (Shinkle et al., 2004). My results from this experiment using a source of broadband UV-B did not confirm this conclusion. Another way of interpreting it is in terms of van Rensen et al.'s (2007) assertion that damage caused by UV-B radiation occurs first on the acceptor side of photosystem II and only later on the donor side. The decrease of F_v/F_o, attributed to inhibition of photosynthetic electron transport at the acceptor side, was observed only in the cucumber leaves subjected to UV-B. In peppermint leaves it increased, probably due to the higher tolerance of this species to UV-B. It is worth pointing out that changes indicating recovery were observed 24 h after the end of the UV-B stress treatment, suggesting that the damage to the acceptor side of photosystem II was reversible. On the other hand, damage to the donor side, reflected by the Y, ETR and Rfd parameters, seemed irreversible.

CONCLUSIONS

1. Brief UV-B irradiation caused a decrease in fluorescence parameter values in cucumber leaves versus control leaves not exposed to UV-B, particularly F_v/F_o and Rfd; peppermint leaves showed less change in the measured parameters.
2. At 24 h after the end of the UV-B stress treatment, adverse changes of ETR, qP and Rfd intensified, especially in the cucumber leaves, indicating irreversible damage on the donor side of photosystem II.
3. Generally, photosystem II of the peppermint leaves seemed more tolerant of the applied UV-B radiation than that of the cucumber leaves.

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