Photochemical functioning of *Dracocephalum kotschyi* ecotypes under UVB

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Abstract

The objective of the present study was to determine the efficacy of chlorophyll fluorescence analysis and to understand the specific photosynthetic parameters for detection of UVB radiation and high light (HL)-induced stress in *Dracocephalum kotschyi* plants under low and high altitudes. Seeds of high (3,300 m) and low (2,600 m) altitude ecotypes of *D. kotschyi* were sown in a growth chamber. Following a 3-month acclimation period, independent pots were chosen and exposed to light intensities including 400 and HL (800 µmol m^{-2} s^{-1}) as well as with two levels of UVB (15 and 30 kJ m^{-2} d^{-1}) for further 10 days. High altitude plants displayed more protection to photoinhibition in comparison to low-altitude plants. Under combined stress, only in high altitude plants, the levels of carotenoids correlated well with the maximal quantum yield of photosystem II (F_v/F_m), suggesting that the accumulation of antioxidant metabolites including carotenoids play a key role in enhancing resistance to stresses. Under combined stress condition, low-altitude plants exhibited the occurrence of photoinhibition, which was assessed by the analysis of F_v/F_m. Additionally, in low-altitude plants, under combined stress, IP-phases from the OJIP curve decreased due to a decrease in electron transport towards PSI. To sum up, this study explored the key OJIP parameters that can be used for distinguishing primary mode of action of HL and UVB on photosystem II in different *D. kotschyi* populations.

Keywords: altitudinal gradient, *Dracocephalum kotschyi*, photochemical activity, UVB radiation

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Introduction

To avoid the inhibitory effects of high light stress, plants have developed diverse morpho-physiological and biochemical adaptations which are defined as photoprotection mechanisms (Lingwan et al., 2023; Wu et al., 2023). Photoprotection mechanisms involved in minimizing photoinhibition of photosystem II (PSII) include the movement of chloroplasts, screening of photo radiation through changes in carotenoid and flavonoid biosynthesis, and the dissipation of absorbed light energy (Takahashi and Badger, 2011). Carotenoids, as a structural component of the light harvesting complexes (LHCs), are involved in light harvesting and photoprotection (Demmig-Adams et al., 2020). Under excess light, leaves accumulate carotenoids to alleviate photoinhibition of PSII which are associated with the dissipation of absorbed light energy via xanthophyll cycle (Habibi and Turkan, 2021).

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Moreover, high-light stress is often accompanied by higher doses of UV. In addition, higher dosages of UV radiation on plants lead to accumulation of ROS and DNA damage associated with the changes in metabolites (Yadav et al., 2020). Leaf chlorophyll *a* fluorescence transient (O-J-I-P) provides detailed information associated with status and function of photosystem II (PSII) reaction centers under environmental stresses (Habibi, 2019). The JIP test provides the information about the energy flow in thylakoid membranes, the function of the photosynthetic structures and the trapping of excitation energy and electron transport, to identify plant stress in plant research (Habibi and Turkan, 2021).

Thus, long-term photoprotective mechanisms provide the survival of plants growing at high altitudes under different stresses in their natural habitats. Since previous studies were manipulated in response to individual UVB radiation and high light stress, we recorded, for the first time, the effects of combined stress (HL+UVB) on the regulation of photoprotective mechanisms in medicinal *Dracocephalum kotschyi* plants. There is no information about the possible differences in the role of UVB radiation in *Dracocephalum kotschyi* plants under low and high altitudes. Furthermore, we presumed that the significant difference in photoprotection mechanisms and condition of photosynthetic electron flux exists between low- and high-altitude plants. We, therefore, organized experiments to study the differences in the mechanisms involved in photoprotection of chloroplasts in low- and high-altitude *Dracocephalum kotschyi* plants, in order to recognize the population differentiation of photochemical functioning.

Materials and Methods

Plant material and treatments

High- and low-altitude ecotypes of *Dracocephalum kotschyi* were prepared from high (36°13′N, 51°27′W; 3,300 m) and low (36°13′N, 52°32′W; 2,600 m) altitude sites, located in Mazandaran (in central-northern part of Iran). Three to five seeds of *Dracocephalum kotschyi* were sown in plastic pots, which was prepared by mixing of sandy soil with peat moss and perlite, and were irrigated with distilled water every 7 days. Plants were grown under day/night temperature of 25 °C/14 °C, 16/8 h day/night cycle, and a daytime photon flux density of 400 $\mu mol\ m^{-2}\ s^{-1}$ for a period of three months prior to the start of experiments. Following the 3 months acclimation period, independent pots were chosen randomly and allocated to measurements. The mature plants were then grown under irradiation with UVB and high-intensity light. Thereafter, plants were exposed to light intensities including 400 and high light (HL, 800 $\mu mol\ m^{-2}\ s^{-1}$) as well as with two levels of ultraviolet-B irradiation (UVB 15 and 30 $kJ\ m^{-2}\ d^{-1}$) for further 10 days. The photosynthetic active radiation (PAR, 400-700 nm) was supplied by cool white fluorescent lamps, and for UV radiation treatments, UVB fluorescent lamps (40 W, Philips, Germany) were used. The spectral outputs of the three lighting conditions were recorded using calibrated spectrophotometer and biologically effective UV doses employed were 15 and 30 $kJ\ m^{-2}\ d^{-1}$ calculated according to Caldwell (1971). Fully expanded leaves were assigned to measurements of chlorophyll fluorescence and other analysis.

Measurements of total carotenoids, chlorophyll *a* and *b*

To estimate chlorophylls and carotenoids concentrations, after extraction of pigments in the cold acetone and permitting the samples to stand for 24 h in the dark at 4 °C, the homogenate was filtered, and then measurements were made spectrophotometrically at 400–700 nm (Lichtenthaler and Wellburn, 1983).

Determination of chlorophyll *a* fluorescence parameters

Chlorophyll *a* fluorescence (OJIP transient) was estimated daily by Pocket-PEA chlorophyll fluorimeter (Plant Efficiency Analyzer, Hansatech Instruments Ltd., King’s Lynn, Norfolk, PE 32 1JL, England) between 09 00 hours and 11 00 hours in dark-adapted leaves for at least 30 min. JIP-test method has been developed for the determination of phenomenological and biophysical parameters, quantifying the PSII and PSI behaviors. To visualize functional and structural changes of photosystem II (PSII) selected parameters were recorded according to Kalaji et al.(2011), purported in the following section.

F_o: A parameter that describes minimum fluorescence, when all PSII reaction centers (RC) are open

F_m: A parameter that describes maximum fluorescence, when all PSII reaction centers are closed

F_v: Variable fluorescence

F_v/F_m: A parameter that characterizes the maximum quantum yield of PSII

F_v/F_o: The oxygen-evolving complex efficiency on the donor side of the PSII

ϕE_o: A parameter that describes the quantum yield related to the reduction of end acceptors of PSI per photon absorbed

PI_{abs}: A parameter that represents the performance index

Statistical Analysis

Experiments were performed using the completely randomized design with four independent replications. Analysis of variance (ANOVA) was employed to compare the data means at the same time point, and Tukey test (P<0.05) was used to record significant differences between means. The achieved data on Chl fluorescence were analyzed and conducted using the PEA Plus ver. 1.10 software. Correlation analysis using Spearman Rank Order Correlation in Sigma Stat (3.5) software was employed to assess the relationship between parameters.

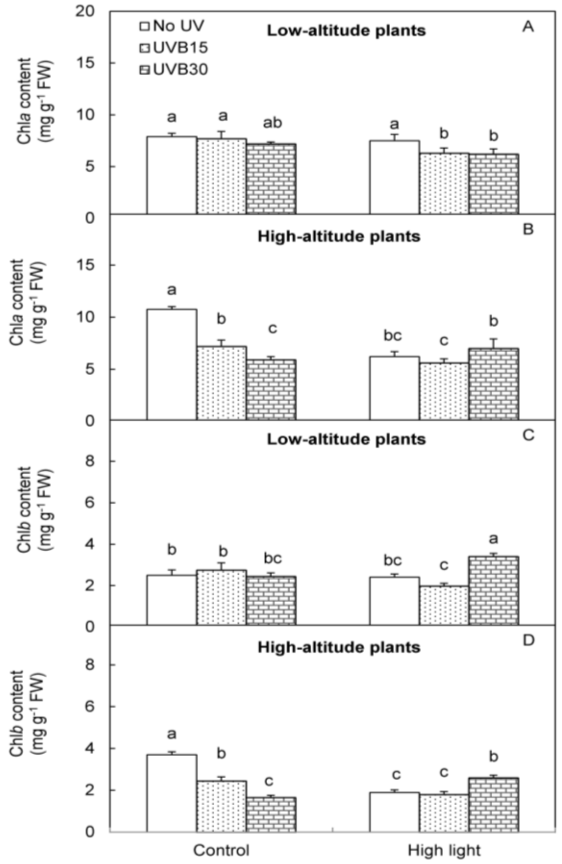

Fig. I. The effects of UVB radiation or high light alone and their combination on the chlorophyll contents in leaves of *Dracocephalum kotschyi* plants growing at low and high altitudes; bars indicated with the *same letter* within each altitude site are not significantly different ($p<0.05$, Tukey test). Values are the mean ± SD (n=4).

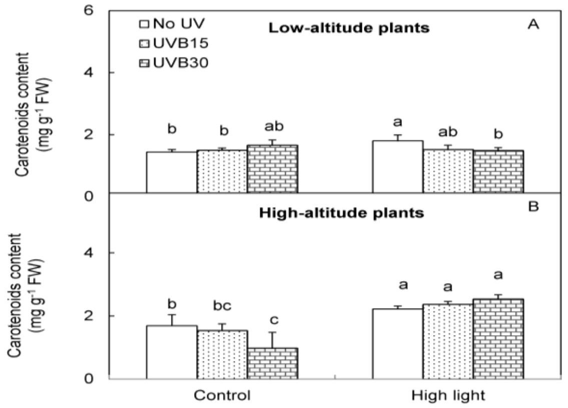

Fig. II. The effects of UVB radiation or high light alone and their combination on the carotenoid contents in leaves of *Dracocephalum kotschyi* plants growing at low and high altitudes; *bars* with the *same letter* within each altitude site are not significantly different ($p<0.05$, Tukey test). Values are the mean ± SD (n=4).

Results

High altitude plants treated with UVB-alone, HL-alone or combined stress (UVB+HL) exhibited the highest level of stress leading to lower Chl *a* and Chl *b* content (Fig. I). In contrast, in low-altitude plants, despite a reduction in Chl values with UVB15+HL, no major differences were detected in Chl content in response to other treatments. Beside a small difference in carotenoid accumulation between low- and high-altitude plants, we showed an increase in the carotenoid content in HL-treated samples of high-altitude plants (Fig. II). In low altitude plants, F_v/F_m

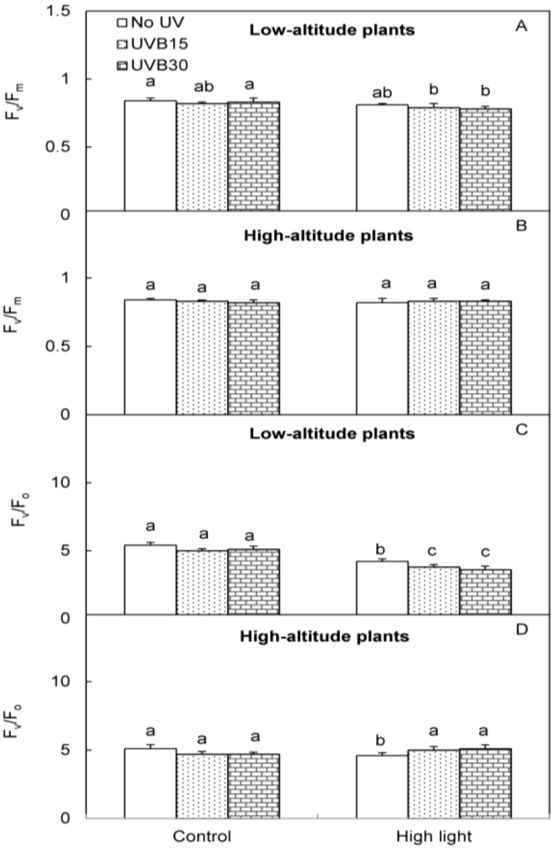

Fig. III. The effects of UVB radiation or high light alone and their combination on the maximum quantum yield (F_v/F_m) and oxygen-evolving complex efficiency of PSII (F_v/F_o) in leaves of *Dracocephalum kotschyi* plants growing at low and high altitudes; bars with the *same letter* within each altitude site are not significantly different (p<0.05, Tukey test). Values are the mean ± SD (n=4).

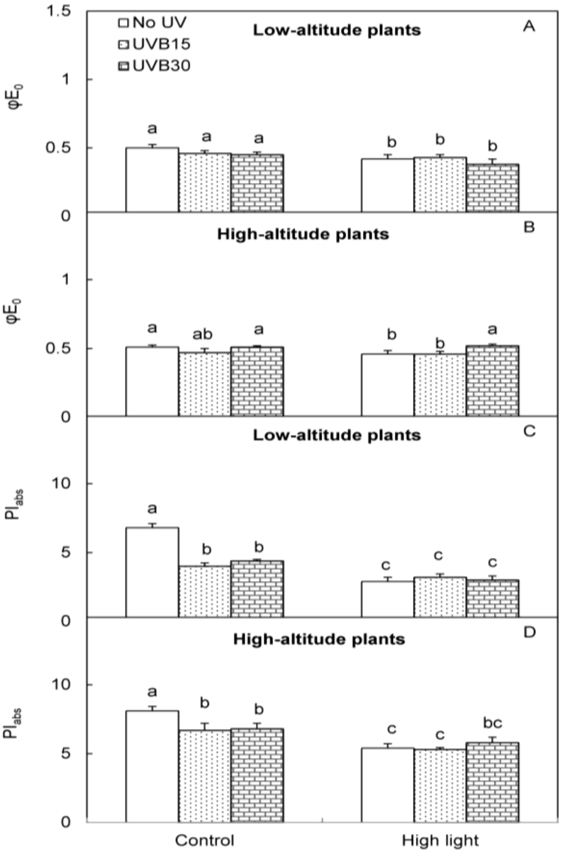

Fig. IV. The effects of UVB radiation or high light alone and their combination on the quantum yield of electron transport (ϕE_o) and performance index (PI_{abs}) in leaves of *Dracocephalum kotschyi* plants growing at low and high altitudes; bars with the *same letter* within each altitude site are not significantly different (p<0.05, Tukey test). Values are the mean ± SD (n=4).

decreased when UVB stress were combined with high light intensity in this study (Fig. III). In contrast, in high altitude plants, the decline in F_v/F_m was not significant under HL and UVB stress. Although, the oxygen-evolving complex (OEC) efficiency of PSII (F_v/F_o) and quantum yield of electron transport (φE_o) of low altitude leaves was not influenced by UVB-alone treatments (Fig. IV), we observed a significant decline in the F_v/F_o and φE_o under combined treatment, as compared to individual stresses (Fig. IV). High altitude plants maintained the F_v/F_m and F_v/F_o at equivalent levels to those determined in the control leaves, as compared to low-altitude plants (Fig. III). The UVB irradiance reduced the performance index of photosystems (PI_{abs}) in both altitudes (Fig. IV). However, the greatest decrease in PI_{abs} was recorded under HL+UVB radiation in both low- and high-altitude plants.

In low-altitude plants, a clear reduction in the relative amplitude of the IP (F_m) phase was recorded in leaves treated with UVB or HL alone and their combination (Fig. V). However, the OJ part of the fluorescence rise was not affected by UVB and HL compared to IP part of the fluorescence rise. Similar results were found only after UVB15 or HL alone and their combination in high-altitude plants with respect to control plants. Interestingly, we observed that the UV-B irradiance at a dose of 30 caused an increase in the OJ phase of the fluorescence rise under both control and HL conditions (Fig. V).

We showed a linear correlation between the levels of carotenoids and the maximal quantum yield of photosystem II (F_v/F_m) under HL (r = 0.72, p<0.01), HL+UVB15 (r = 0.60, p<0.05), and HL+UVB30 (r = 0.62, p<0.05) conditions (Fig. V) in plants growing at high altitudes. In addition, no correlation was

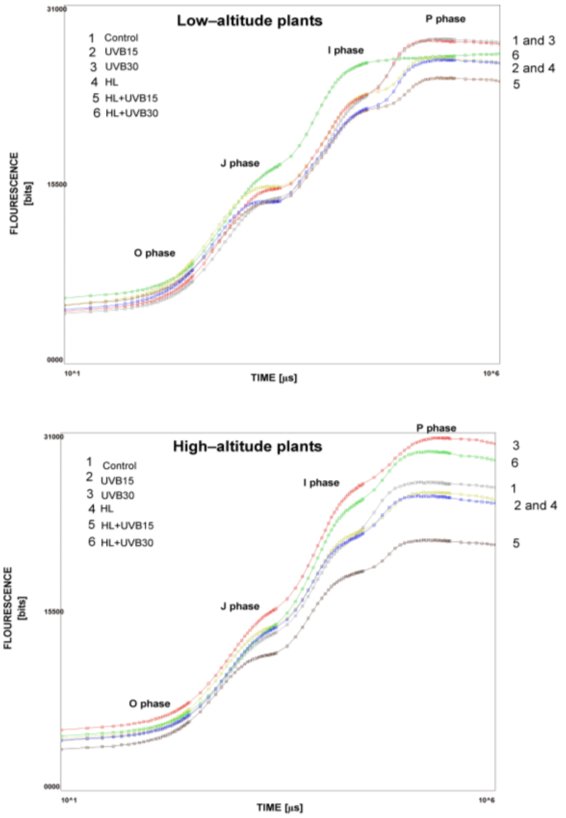

Fig. V. The effects of UVB radiation or high light alone and their combination on the chlorophyll *a* fluorescence induction curve of *Dracocephalum kotschyi* leaves growing at low and high altitudes; *bars* with the *same letter* within each altitude site are not significantly different (*p*<0.05, Tukey test). Values are the mean ± SD (n=8).

detected between these parameters in plants under low altitudes.

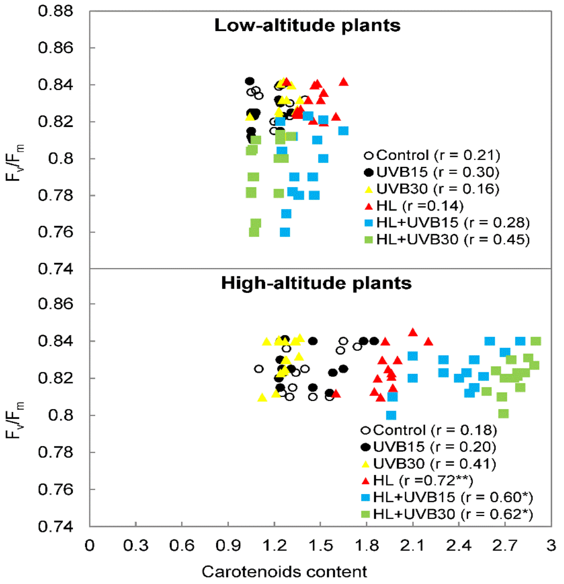

Fig. VI. Correlation between values of total phenolic and malondialdehyde (MDA) recorded in Dracocephalum kotschyi plants growing at low and high altitudes under UVB radiation or high light stress; ns: non-significant, * and **: significant at the 5% and 1% levels of probability, respectively.

Discussion

Our results revealed that UVB stress caused further decreases in chlorophyll content of HL-exposed plants, which may lead to a decline in the photosynthetic capacity of low- and high-altitude plants. Dou et al., (2019)found similar results in basil (*Ocimum basilicum*) plants and observed a significant decrease in the chlorophyll content after UVB radiation, probably via degradation or retardation of enzymes involved in the chlorophyll biosynthesis (Yadav et al., 2020). In addition, priming of plants with UVB15 significantly increased the leaf content of carotenoids under the HL stress. However, in high altitude plants, the degree of increase in carotenoid contents was higher than the low altitude plants. Owing to the protective effects of carotenoids in the dissipation of absorbed light energy as thermal energy (Habibi, 2019; Habibi and Ajory, 2015), this higher accumulation of carotenoids plays a crucial role in plant photoprotection under HL and UVB stress (Demmig-Adams et al., 2020). In low altitude plants, the decrease in F_v/F_m showed the occurrence of photoinhibition of PSII (Chen et al., 2023; Guidi et al., 2019) under UVB+HL treatments, which was coincident with the largest decrease in F_v/F_o, quantum yield of φE_o and PI_{abs}. This decrease in F_v/F_o may reflect the decline in the water-splitting complex activity at the donor site of PSII (Habibi and Ajory, 2015; Kalaji et al., 2016). As compared to low altitude plants, the F_v/F_m and F_v/F_o were not affected by UVB+HL treatments in high altitude plants, characterizing proper functioning of PSII as well as more tolerance to photoinhibition. This intensification of photoprotection activity corresponded with a significant accumulation of carotenoids. High carotenoids content has a role in the light harvesting and photoprotection processes via xanthophyll cycle (Chouhan et al., 2023; Demmig-Adams et al., 2020; Faria-Silva et al., 2019). In fact, higher level of carotenoids can provide protection of chloroplasts under high light stress conditions (Sandmann, 2019).

To further study the effects of HL and UVB on photosystem II (PSII) electron transport chain components of leaves, we assayed the typical OJIP chlorophyll *a* fluorescence transient. We also recorded changes in the OJIP curve in response to environmental stress, to record the status of the electron transport activity (Liang et al., 2019; Maxwell and Johnson, 2000). The changes in OJIP transient reflect differences in the efficiency of the chlorophyll antenna involved in capturing light energy and conduct to the electron acceptor, plastoquinone QA (Küpper et al., 2019). In both low- and high-altitude plants, after exposure of the leaves to HL alone, UVB15 alone, or their combination, a clear reduction in the IP (F_m) phase was recorded, which may be due to the suppression of the content of reaction centers in PSI (Gupta et al., 2017). Interestingly, UVB30 either with or without HL increased the IP phase only in high altitude plants. Indeed, the IP phase may be related to the electron transport carriers to the (electron) acceptor side of PSI (Hamdani et al., 2015; Muszyńska et al., 2021) as well as to the amount of PSI (Muszyńska et al., 2021). Here, we showed that there is a higher IP phase in high-altitude plants in comparison to low-altitude plants , which may correlate to the higher PSI/PSII ratio in high-altitude plants in comparison with low-altitude plants (Zhang et al., 2017); however, a higher IP phase could have multiple causes, and further analyses are required to understand the effects of altitude on the chlorophyll *a* fluorescence transient. In the high-altitude leaves, we assume that the prevention of stress-induced reduction of photosynthetic activity under HL and UVB conditions was due to the increased content of carotenoids and UV-absorbing pigments (Kreslavski et al., 2021)as compared with the low-altitude plants. Indeed, high-altitude plants exhibited more tolerance to combined stress through maintenance non-enzymatic antioxidant pools, and this may lead to the conservation of F_v/F_m and F_v/F_o, as indices of photochemical activity (Kamran et al., 2020).

Conclusion

In conclusion, significant variation in stress tolerance was detected between low- and high-altitude plants, which was probably due to their altitudinal distributions. The exposure of low altitude plants to combined stress resulted in the occurrence of photoinhibition, which was correlated with the largest decrease in F_v/F_o, φE_o and PI_{abs}. In comparison, when high-altitude plants were exposed to stress conditions (UVB+HL), photoprotective mechanisms were activated, leading to acclimation to excess light through the accumulation of antioxidant metabolites including carotenoids and consequently, higher photochemical functioning. Since in low-altitude plants, under HL and UVB stress, the OJIP curve has been flattened because of reduction of electron transport towards PSI, we explored the key OJIP parameters that can be used for distinguishing primary mode of action of HL and UVB on photosystem II in different *D. kotschyi* populations.

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