

Research Article

Performance of chlorophyll a fluorescence parameters in *Dracocephalum kotschy* and *Nepeta crassifolia* plants growing at low and high altitudes

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Abstract

The main focus of the present study was to better understand the effects of altitudinal gradient factors on the photochemical processes as reflected in changes in fast chlorophyll a fluorescence kinetics and related biophysical parameters in low and high-elevation ecotypes of *Dracocephalum kotschy* and *Nepeta crassifolia* (located in Mazandaran, adjacent Central Alborz mountain range, central-northern part of Iran). In both *Dracocephalum kotschy* and *Nepeta crassifolia*, high-altitude plants exhibited higher fluorescence parameters as compared to low-altitude plants, which was coupled with higher levels of carotenoids, suggesting proper functioning of PSII. Increased photochemical activity in high-altitude plants was achieved by increased levels of the activity of the oxygen-evolving complex (F_v/F_o), the performance index (PI_{abs}) and the quantum yield for electron transport (ϕE_o). In addition, a clear increase in the relative amplitude of the IP (F_m) phase from the OJIP curve was observed in high-altitude plants as compared to low-altitude plants. These results indicated a significant variation in the performance of chlorophyll a fluorescence parameter, between low- and high-altitude plants, which is apparently due to eco-physiological differences in relation to their altitudinal distributions. Summarizing, high-altitude plants have developed a series of mechanisms with respect to photosynthetic apparatus efficiency to protect themselves from adverse environmental threats.

Keywords: Altitudinal gradient, Carotenoids, High-altitude plants, Photochemical processes

Introduction

In nature, plants are continually exposed to abiotic and biotic stresses. Under altitudinal gradients, the environmental change in temperature, radiation and moisture negatively affects plant growth, leading to changes in the physiological and ecological adaptability of mountain plants (Cui *et al.*, 2019). Increases in altitude can increase deleterious effects of UVB radiation on the growth, productivity and photosynthesis of higher plants (Singh *et al.*, 2023). Although these stress conditions could retard plant growth, many species have survived and developed different adaptive mechanisms under these circumstances (Elkeilsh *et al.*, 2019). Accumulation of flavonoids and phenols as antioxidants and the increase in proline and soluble protein contents against drought has been reported to play a vital role in tolerance against different environmental biotic and abiotic stresses (Soliman *et al.*, 2020). Indeed, an increased carotenoids compared to chlorophyll reduce high light intensity damage, through upregulation of the xanthophyll-cycle pigment pool

(Saleem *et al.*, 2005). The dissipation of excess light energy as heat within LHC through upregulation of the xanthophyll-cycle protects photosynthetic apparatus against the oxidative damage. Additionally, plants accumulate carotenoids for light harvesting and photoprotection (Cazzonelli and Pogson, 2010; Ashraf and Harris, 2013).

Chlorophyll a fluorescence (ChlF) is a commonly used method to record plant stress conditions in plant research, frequently in association with other morphological, chemical, and physiological variables (Khan *et al.*, 2018). ChlF is a natural phenomenon describing the dissipation and heat radiation or re-emission of the portion of absorbed energy that is not utilized to drive photosynthesis (Kovacs *et al.*, 2014; Kalaji *et al.*, 2016). ChlF measurement prepares information about changes in photosynthetic efficiency and heat dissipation (Kovacic *et al.*, 2020). Apart from evaluating specific parameters, the quantum yield of primary photochemistry F_v/F_m (ϕP_o) and the activity of oxygen-evolving complexes (F_v/F_o) are the most well-

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known and extensively utilized.

Additionally, the interpretation of double normalized curves using the JIP test is a commonly used method to detect photochemical activity in environmental research practices (Szopinski *et al.*, 2019). The O-J-I-P transient is a prime source of observed variations in the efficiency of the chlorophyll antenna in capturing light energy and transferring it to plastoquinone QA (the electron acceptor) (Kovacik *et al.*, 2011). The O-J part of the fluorescence rise is typically attributed to the closure of some of the PSII reaction centers in response to the reduction of QA. The J-I part of the curve corresponds to the reduction of the secondary electron acceptors QB, plastoquinone (PQ), cytochrome (Cyt b6f) and PC. The increase in ChlF in the I-P part of the induction curve is typically related to the reduction of electron transporters (ferredoxin, intermediary acceptors, and NADP) on the PSI acceptor side (Kalaji *et al.*, 2016).

Dracocephalum kotschyi and *Nepeta crassifolia* plants are traditional Iranian medicinal plants belonging to the Labiatae family (Naghibi *et al.*, 2005), growing at low and high altitudes. In Mediterranean ecosystems, increases in altitude can increase the deleterious effects of drought (Gimenez-Benavides *et al.*, 2008) and UV radiation on the growth, productivity and photosynthesis of higher plants. *Dracocephalum kotschyi* and *Nepeta crassifolia* plants grown at high altitudes have shorter heights and thicker leaves than those grown at low altitudes. The reduced height of plants shows an adaptive strategy to avoid the damaging effect of winds at high elevations (Hashim *et al.*, 2020). Since biochemical adaptations to avoid the inhibitory effects of the higher intensities of light radiation are accompanied by photosynthetic efficiency and photoprotection mechanisms, we analyzed, for the first time, the effects of altitudinal distributions in the regulation of photochemical activity in medicinal *Dracocephalum kotschyi* and *Nepeta crassifolia* plants. In addition, whether altitudinal distributions can up-regulate leaf concentrations of chlorophyll and carotenoids in *Dracocephalum kotschyi* and *Nepeta crassifolia* plants has not previously been examined. In the present study, we evaluate mechanisms determining the photochemical processes, as reflected in changes in fast ChlF kinetics and related biophysical parameters, in low and high-elevation ecotypes of *Dracocephalum kotschyi* and *Nepeta crassifolia*, in order to better understand the eco-physiological differences in relation to their altitudinal distributions.

Materials and methods

Plant material: The research was conducted in 2021. High and low-elevation ecotypes of *Dracocephalum kotschyi* were collected from high (36°13'N, 51°27'W; 3,300 m) and low (36°13'N, 52°32'W; 2,600 m) elevation sites, located in Mazandaran (in the adjacent Central Alborz mountain range, in central-northern part of Iran). High and low-elevation ecotypes of *Nepeta*

crassifolia were collected from high (36°13'N, 51°27'W; 3,200 m) and low (36°19'N, 51°23'W; 2,300 m) elevation sites, located in Mazandaran (in the adjacent Central Alborz mountain range, in central-northern part of Iran). Three to five seeds of *Dracocephalum kotschyi* and *Nepeta crassifolia* were transferred and sown on the surface of the cylindrical plastic pots (30 cm in diameter and 40 cm in depth) containing sandy soil mixed with peat moss and perlite for 12 weeks, and they were irrigated with distilled water every 7 days. Plants were grown at 25 °C day/14 °C night temperatures with 16 h photoperiods and a daytime photon flux density of 350-400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (measured by a quantum sensor attached to the leaf chamber of the gas exchange unit) for a period of three months prior to the start of experiments. Following the 3 months acclimation period, when the plants had developed 4-5 pairs of leaves, independent pots were selected randomly and assigned to measurements.

Determination of total carotenoids and chlorophylls a and b: Leaf concentration of chlorophyll and carotenoids was determined after extraction of pigments in the cold acetone and allowing the samples to stand for 24 h in the dark at 4 °C (Lichtenthaler and Wellburn, 1985).

Chlorophyll a fluorescence: The chlorophyll a fluorescence was measured on leaves from in vitro cultured shoots using daily with a 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. The JIP-test-method has been developed, by which several selected phenomenological and biophysical parameters quantifying the PSII and PSI behaviors are calculated. Several parameters can be derived from the polyphasic ChlF rise (OJIP curve), which provides information about photosynthetic fluxes (Strasser *et al.*, 2004; Kumar *et al.*, 2020). To visualize functional and structural changes of photosystem II (PSII) selected parameters were calculated according to Jiang *et al.* (2009) and Kalaji *et al.* (2011), which are described in the following section.

F_o : Minimum fluorescence, when all PSII reaction centers (RC) are open.

F_m : Maximum fluorescence, when all PSII reaction centers are closed.

F_v : Variable fluorescence.

F_v/F_m : Maximum quantum yield of PSII.

F_v/F_o : Efficiency of the oxygen-evolving complex on the donor side of the PSII.

V_j : Relative variable fluorescence at 2 ms (J-step), that refers to the number of closed RCs relative to the total number of RCs.

V_i : Relative variable fluorescence at 30 ms (I-step); that reflects the ability of PSI and its acceptors to oxidize reduced plastoquinone.

S_m : Normalized total complementary area above the OJIP transient (reflecting multiple-turnover QA

reduction events) or total electron carriers per RC.

ABS/RC: Absorption flux per RC; That reflects the proportion between chlorophyll *a* molecule amounts in fluorescence-emitting antenna complexes and the active reaction centers.

TR_o/RC: Trapped energy flux per RC.

ET_o/RC: Electron transport flux per RC.

DI_o/RC: Dissipated energy flux per RC.

ABS/CS: Absorption flux per CS; Represents the amount of photon energy absorbed by the antenna associated with active and inactive reaction centers of PSII.

TR_o/CS_m: Trapped energy flux per CS.

ET_o/CS_m: Electron transport flux per CS.

DI_o/CS_m: Dissipated energy flux per CS.

RC/C_m: Amount of active PSII RCs per CS.

ABS/CSM = $\approx F_0$: Absorbed photon flux per excited PSII cross-section at time zero.

ϕP_0 : Maximum quantum yield of primary photochemistry; That indicates the probability of trapping the energy of absorbed photons by PSII reaction centers.

ϕE_0 : Quantum yield for the reduction of end acceptors of PSI per photon absorbed.

ψE_0 : Probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond QA.

PI_{abs}: The performance index.

Statistical analysis: The experiments were carried out in a completely randomized design with four independent replications. All data were subjected to ANOVA to compare the data means at the same time point, and significant differences between means were concluded by the Tukey test ($P < 0.05$). The achieved data on Chl fluorescence were assessed using the PEA Plus ver. 1.10 software.

Results

Impacts of altitudinal gradient on photosynthetic pigment:

Results revealed that the calculated value of chlorophyll *a+b* to carotenoids depended on ecotype (Table 1). It is noteworthy that the trend of changes in the photosynthetic pigment concentration in both *Dracocephalum kotschyi* and *Nepeta crassifolia* plants growing at low and high altitudes was the same. Beside a significant difference in carotenoid accumulation between low and high-altitude plants, an increase in the carotenoid content in high-altitude plants as compared to low-altitude plants was obtained (Table 1).

Chlorophyll a fluorescence transient rise: ChlF rise of plants was measured, and a typical OJIP induction curve was obtained when plotted on the logarithm timescale (Figures 1 and 2). According to the results, a clear increase in the relative amplitude of the IP (F_m) phase from the OJIP curve was observed in high-altitude plants as compared to low-altitude plants (Figures 1 and 2). In addition, a slight decrease in the OJ phase of the fluorescence rise only in the high-altitude *Dracocephalum kotschyi* leaves was detected as compared to the low-altitude ones (Figure 1).

Biophysical parameters derived by the 'JIP-test'

equations: The maximal variable fluorescence (F_v) and the maximum fluorescence intensity (F_m) both increase with increasing the altitudinal gradient (Figures 3 and 4). Further, a significant increase in the activity of the oxygen-evolving complex (F_v/F_0) was recorded at a high level in high-altitude leaves of both species as a result of a significant decrease in F_v as shown in Figures 3 and 4.

As compared to low-altitude plants, high-altitude *Dracocephalum kotschyi* leaves exhibited a significant decline in minimal fluorescence intensity (F_0). However, this parameter in both low and high-altitude *Nepeta crassifolia* leaves was the same. As compared to low-altitude plants, in high-altitude leaves of both species, a slight decrease in the value of V_j was observed, but with increasing altitude, the V_i (relative variable fluorescence at I step) value was decreased in high-altitude leaves of *Nepeta crassifolia*. As compared to low-altitude plants, in high-altitude leaves of both species, a slight decrease in the value of turnover number (N), de-excitation rate constants for nonphotochemical reaction (K_N) and de-excitation rate constants for photochemical reaction (K_P) was observed. As compared to low-altitude plants, in high-altitude *Dracocephalum kotschyi* leaves, the values of S_m decreased; However, this parameter in high-altitude *Nepeta crassifolia* leaves showed an increase as compared to low-altitude plants.

Specific energy flux parameters: The specific energy fluxes such as absorption flux per reaction center (ABS/RC), trapped energy flux per reaction center (TR_o/RC), electron transport flux per reaction center (ET_o/RC), and dissipated energy flux per reaction center (DI_o/RC) were analyzed to determine the photosynthetic performance of active PSII reaction centers (Figures 3 and 4). In high-altitude leaves of both species, no significant variations in ET_o/RC were recorded while a remarkable decrease in ABS/RC, TR_o/RC and DI_o/RC was recorded as compared to low altitude plants.

Phenomenological energy flux parameters:

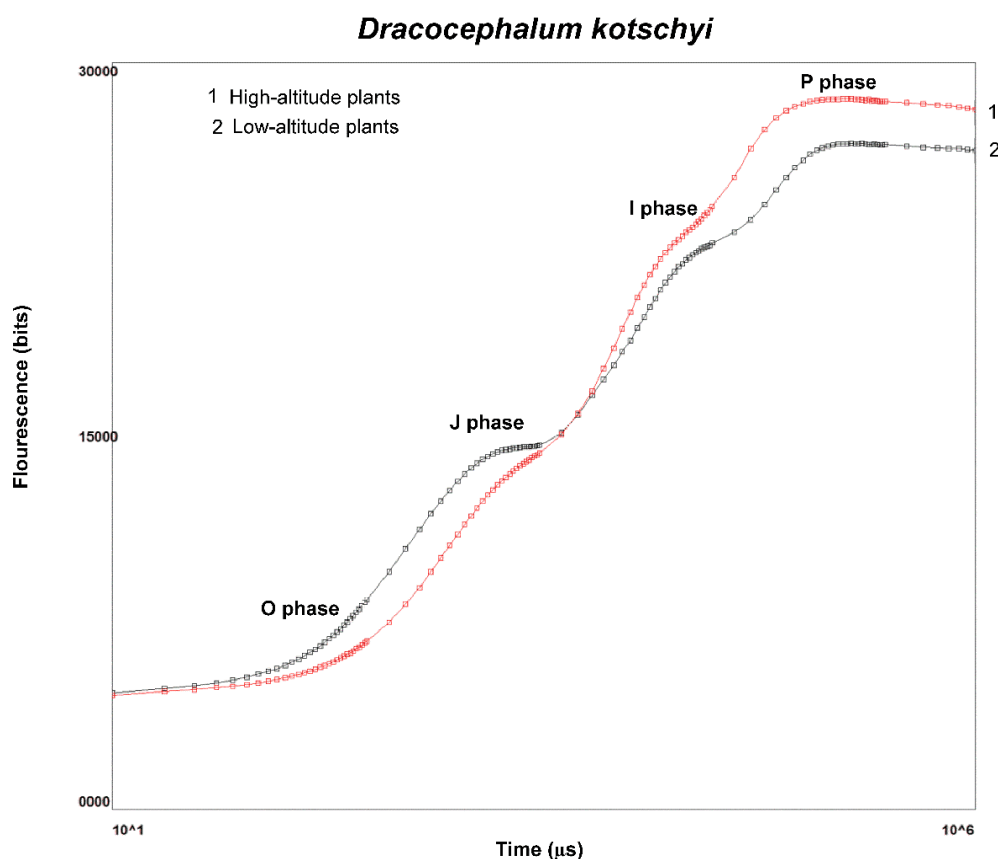
Phenomenological energy fluxes mean absorption flux per cross-section (ABS/CS_m), trapped energy flux per cross-section (TR_o/CS_m), electron transport flux per cross-section (ET_o/CS_m), and dissipated energy flux per cross-section (DI_o/CS_m) significantly affected by altitudinal gradient (Figures 3 and 4). The highest values of phenomenological energy flux parameters were recorded in high-altitude leaves of both species, as compared to low-altitude plants.

Quantum yield and performance index: The quantum yield of primary photochemistry F_v/F_m (ϕP_0), which reflects the overall photosynthetic potential of active PSII reaction centers, was not affected by altitudinal gradient in leaves of both species (Figure 3 and 4). In contrast, ϕE_0 (quantum yield for electron transport) and ψE_0 (efficiency/probability that an electron moves further than QA) exhibited an increase in high-altitude leaves of both species in comparison to low-altitude plants. To analyze the effects of altitudinal gradient on overall photosynthesis performance, PI_{abs}

Table 1. Effect of altitudinal gradient on the chlorophyll and carotenoids contents in *Dracocephalum kotschyi* and *Nepeta crassifolia* plants growing at low and high altitudes.

Plants	Chlorophyll <i>a</i> + <i>b</i> /carotenoids
<i>Dracocephalum kotschyi</i>	
Low-altitude plants	7.14±0.08 ^a
High-altitude plants	5.50±0.07 ^b
<i>Nepeta crassifolia</i>	
Low-altitude plants	7.21±0.04 ^a
High-altitude plants	5.33±0.02 ^b

Data of each row within each parameter indicated by the same letter are not significantly different ($P < 0.05$, Tukey test). Values are the mean \pm SD ($n=4$).

**Fig. 1.** Effect of the altitudinal gradient on the chlorophyll *a* fluorescence induction curve of *Dracocephalum kotschyi* plants growing at low and high altitudes.

was determined. Altitudinal gradient led to a significant effect on the performance index in high-altitude leaves of both species. The lowest performance index was recorded in low-altitude leaves of both species.

Discussion

Combinations of high-altitude environmental stress increase the risk of oxidative damage. Under these conditions, plants have developed a series of mechanisms, including high antioxidant and carotenoid levels, to protect themselves from these adverse environmental threats (Oncel *et al.*, 2004; Soliman *et al.*, 2020). As demonstrated by previous studies,

carotenoids play a major role in the maximal photochemical efficiency of PSII (Habibi, 2020; Habibi, 2021; Muszynska *et al.*, 2021). Since carotenoids play a major role in the maximal photochemical efficiency of PSII, we looked more closely at the accumulation of these pigments at both studied altitudes. In both *Dracocephalum kotschyi* and *Nepeta crassifolia*, high-altitude plants showed a higher accumulation of carotenoids as compared to low-altitude plants (Table 1). This higher carotenoids accumulation can represent a mechanism that increases the light harvesting and photoprotection processes (Singh *et al.*, 2022). Moreover, the increased carotenoid content in high-

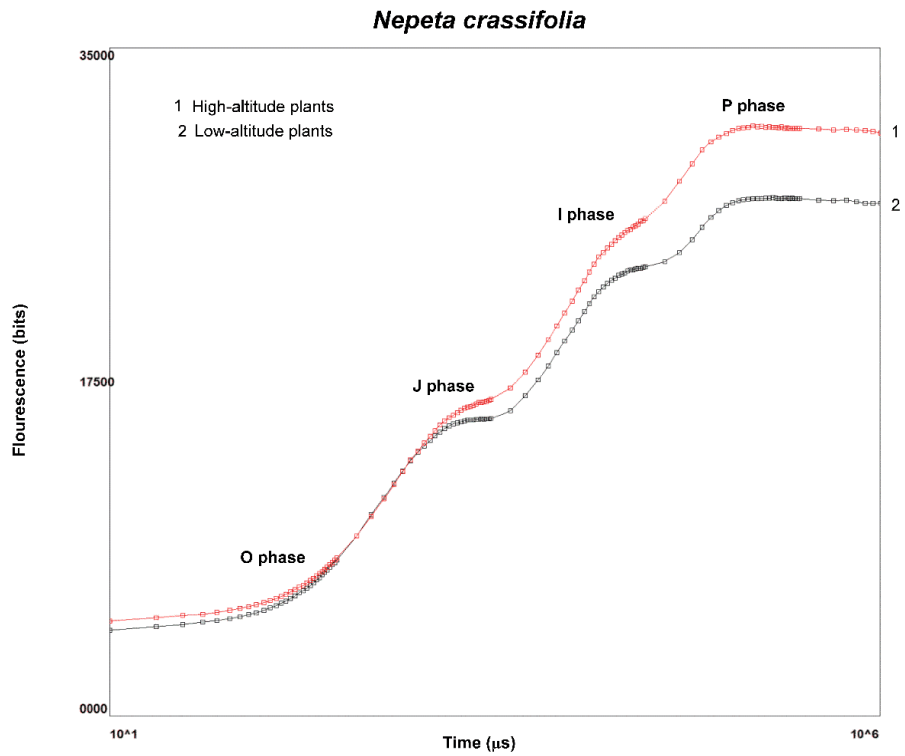


Fig. 2. Effect of the altitudinal gradient on the chlorophyll a fluorescence induction curve of *Nepeta crassifolia* plants growing at low and high altitudes.

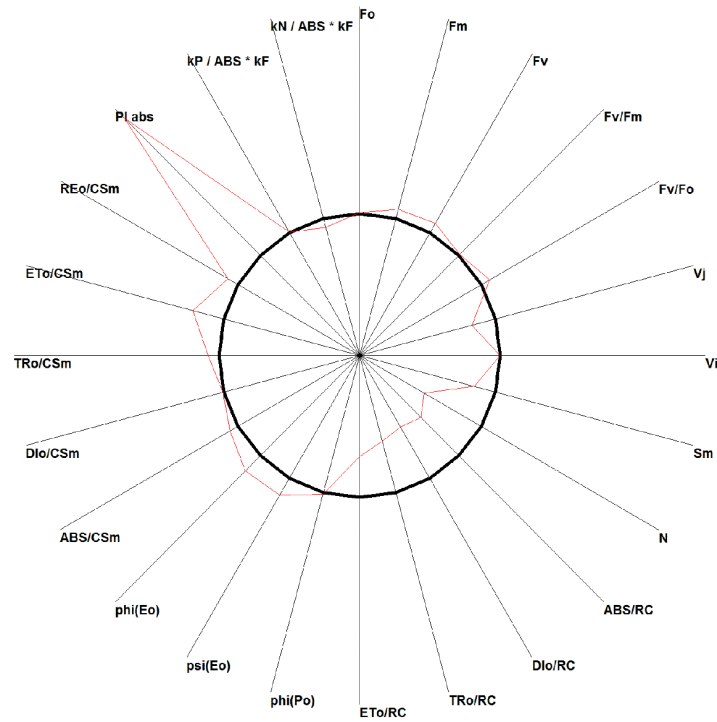


Fig. 3. Radar plots showing various technical fluorescence parameters in *Dracocephalum kotschy* plants growing at low and high altitudes. Each line represents the average of 6 measurements per treatment.

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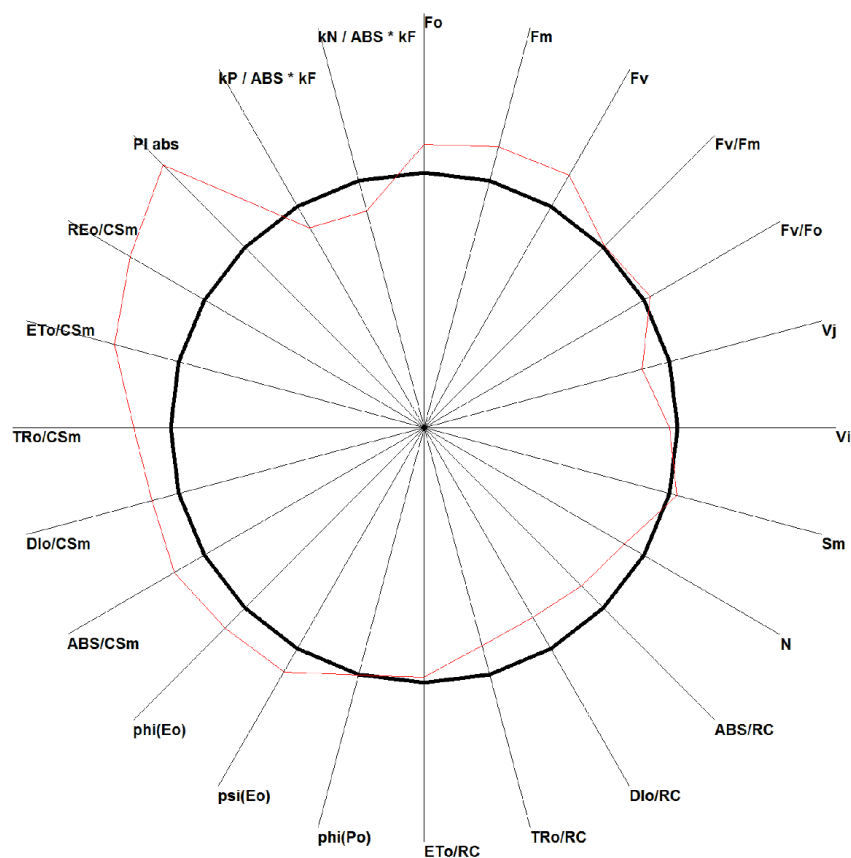


Fig. 4. Radar plots showing various technical fluorescence parameters in *Nepeta crassifolia* plants growing at low and high altitudes. Each line represents the average of 6 measurements per treatment.

altitude plants could also explain the increased photochemical activity. Indeed, carotenoid accumulation is highly regulated by several abiotic stresses, seemingly due to the protective effect of carotenoids against environmental stresses (Cazzonelli and Pogson, 2010; Habibi and Ajory, 2015).

We estimated the changes in the OJIP curve to determine the impacts of altitudinal gradient on photosynthesis. The O-J is a photochemical phase (light-dependent) while J-I-P is a thermal phase (Stirbet and Govindjee, 2012). In high-altitude plants, there was a clear increase in the relative amplitude of the IP (F_m) phase of the fluorescence rise. The increase in ChlF in the IP part of the induction curve is typically attributed to the reduction of electron transporters (ferredoxin, intermediary acceptors, and NADP) of the PSI acceptor side (Kalaji *et al.*, 2016). In high-altitude *Dracocephalum kotschyi* leaves, there was a clear increase in the relative amplitude of the IP (F_m) phase in parallel with the decline in the OJ (F_o) phase of the fluorescence rise, suggesting that the altitudinal gradient affected all the components of electron transport chain from PSII towards PSI. This lower OJ phase revealed the all primary quinone acceptor (QA) is in the open (oxidized) state (Singh *et al.*, 2022). However, further analysis will be needed to understand the effect of

altitudinal gradient on the photochemical processes as well as fast chlorophyll *a* fluorescence kinetics.

The activity of the oxygen-evolving complex (F_v/F_o) is related to the photosynthetic efficiency of plants, and an increased value of F_v/F_o in high-altitude plants indicated proper functioning of PSII (Singh *et al.*, 2022). The relative variable fluorescence at the J step is denoted as V_j which is defined as the fraction of the primary quinone electron acceptor of PSII in its reduced state (QA^- / QA (total)) (Strasser *et al.*, 1995). Complimentary area (S_m) is defined as an important parameter that is directly proportional to the number of reductions and oxidations of one QA^- molecule during the fast OJIP transient, or the number of electrons passing through the electron transport chain. The turnover number (N) is characterized as the number of times QA becomes reduced and re-oxidized another time until the F_m (Maximum fluorescence intensity) is reached (Bhatt *et al.*, 2020). The increased values of S_m in high-altitude leaves of *Nepeta crassifolia* displayed the reduced electron transport between these photosystems. Based on the present results, TR_o/CSm and ET_o/CSm increased with increasing the altitudinal gradient in high-altitude leaves of both species because RCs are active, consequently increasing the trapping efficiency and electron transport from PSII (Tsimilli-

Michael, 2020). In high-altitude leaves of both species, a decrease in ET_o/RC revealed that the re-oxidation of reduced QA through electron transport in an active RC is decreased because a greater number of active RCs are available. The quantum yield of primary photochemistry F_v/F_m (ϕP_o), as a stress indicator in numerous photosynthetic studies (Kalaji *et al.*, 2012) was not affected by the altitudinal gradient in the leaves of both species. Since this quantitative parameter is not usually sensitive enough to detect alteration across samples, the performance index (PI_{abs}) is employed as a novel, more responsive, and significant parameter to measure photosynthetic efficiency under stress (Tsimilli-Michael, 2020). Based on the results of this study, the performance index in high-altitude leaves of both species increased due to proper primary photochemistry and increased electron transport (Kumar *et al.*, 2020).

Conclusion

In both *Dracocephalum kotschyi* and *Nepeta crassifolia*, high-altitude plants exhibited higher fluorescence parameters, including the activity of the oxygen-evolving complex (F_v/F_o), the performance index (PI_{abs}), the quantum yield for electron transport (ϕE_o), phenomenological energy fluxes mean absorption flux per cross-section (ABS/CS_m), trapped energy flux per cross-section (TR_o/CS_m), electron transport flux per cross-section (ET_o/CS_m) and dissipated energy flux per cross-section (DI_o/CS_m) as compared to low-altitude plants, which coupled with higher levels of carotenoids, suggesting proper functioning of PSII. However, an increase in the F_o phase (O) of the fluorescence rise was observed only in the high-altitude leaves of *Nepeta crassifolia* compared to low-altitude plants. In summary, significant variation in the performance of chlorophyll a fluorescence parameters exists between low and high-altitude plants, which is related to their altitudinal distributions.

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