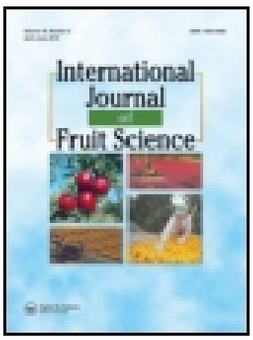

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Impacts of Elevational Changes and Leaf Maturity Stages on Photoprotective Strategies and Biochemical Traits of Wild Fig [*Ficus Carica* Subsp. *Rupestris* (Hausskn)]

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ABSTRACT

Three leaf maturity indices were defined as young, mature and senescence leaves. The effect of leaf phenophase resulted in a significant alteration in photosynthetic pigments, whereas site position had no significant effect on them. FPC values significantly increased along leaf phenophase development. TSP showed a sharp descending trend in the final phase of leaf elasticity. On the contrary, the highest TSS value was observed in senescence stage of leaf development. Such a trend was observed for starch content. The results revealed that FPC values significantly decreased along the elevation rise. RWC was significantly affected by both site position change and leaf phenophase factors, whereas ΨL was just significantly affected by leaf phenophase. The obtained results exhibited that leaf phenophase significantly affected six (F_0, F_m, F_v/F_m, *qP*, NPQ, and ΦPSII) out of seven fluorescence variables. Meanwhile, the drastic effect of site position gradient was observed in F_0, F_v/F_m, and *qP*. Severe decreases in leaf pigment variable values in SL are indicators of damage to chloroplasts due to leaf senescence and reduction of leaf moisture content, depicted through reducing leaf water potential. The FPC and TSS contents exhibited descending trends along site position gradients, which is consistent with the amount of rain and temperature. A remarkable reduction was observed in the values of F_v/F_m and F_m, ΦPSII and qP, suggesting the sufficiency of photochemistry transformation is affected by leaf cycle development, particularly in senescence stage.

KEYWORDS

Fluorescence; phenophase; photosystem; photochemistry; pigment; water potential

Introduction

Leaf phenology can be defined as the arrangement of leaves in time and space that is considered as one of the main elements in any photosynthesis pathway. Based on this definition, patterns of leaf emergence and leaf longevity are controlled by seasonal alterations in environmental variables such as temperature, light, and soil water availability (Kikuzawa, 1995). Among the above-mentioned variables, water deficit stress due to seasonal drought is the main factor restricting the survival and growth of plants in arid and semiarid

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regions (Munné-Bosch et al., 2009). Elevation can climatically affect plant activity by providing a complex of environmental conditions such as low temperatures, potent winds, and low atmospheric pressure (Rangwala and Miller, 2012).

Wild fig or rock-fig tree [*Ficus carica* subsp. *Rupestris* (Hussk)] is a deciduous tree belonging to the Moraceae family that grows in mountainous areas and distributes in arid and semiarid regions. This species is tolerant to drought and cold (Oliveira et al., 2012) such that plants produced from cuttings of this species are used as tolerant rootstocks for production of commercial fig trees in Iran.

Common fig can be found both in the native and domestic habitats (Vemmos et al., 2013). Although this species is generally found at mid-elevations, its growth has been reported between 1000 and 2400 m above sea level (m.a.s.l.). Wild fig trees can tolerate a range of habitats, including infertile rocky land, woodland, scrubland, and even hot dry soil (Keshvari et al., 2013). The shoot growth period varies according to the climatic conditions (Lansky et al., 2010). In Iran, the growth of fig trees initiates with bud-burst in early spring, which coincides with the end of March.

Regional and seasonal water deficit in both soil and air is known to change physiological and biochemical processes such as photosynthesis and solute accumulation (Gholami, et al., 2012). In this relation, chlorophyll fluorescence signals have been extensively used for the assessment of several environmental effects on photosynthetic machinery (Naumann et al., 2008; Paunov et al., 2018; Ranjbarfardoei et al., 2000). For example, the decline in efficiency of photosystem II (ΦPSII) and maximum efficiency of PSII (F_v/F_m) and an increase in non-photochemical quenching (*NPQ*) are promising indicators of the functionality of photosynthetic apparatus of plants (Ranjbar, 2017). In this regard, chlorophyll content is used as a substantial bioindicator of plant vegetation performance under stressed environments. Several reports have shown that drought stress significantly reduces photosynthetic pigments of different native and domestic plants (Gholamin and Khayatnezhad, 2011; Ranjbar, 2015; Ranjbar-Fordoei, 2018). Under drought conditions, plants survival depends on the maintenance of leaf turgor, which may be achieved through the accumulation of solutes in cytoplasms such as proline, soluble carbohydrates, soluble proteins, and ascorbic acid (Queiroz et al., 2016). Among the mentioned solutes, proline acts as a source of energy during recovery from stress (Wang et al., 2007). In addition to the impacts of seasonal drought stress, the responses of plants to alterations in temperature, evaporation, higher levels of radiation, and the partial pressure of carbon dioxide along altitudinal gradients (Bai et al., 2015) have been used to explain the reactions of vegetation, particularly photosynthetic activity, to climatic alterations (Allen and Ort, 2001).

In this study, we aimed to investigate the response of wild fig trees to altitudinal changes and leaf phenotypic plasticity. Field experiments were

carried out with *F. carica* subsp. *Rupestris* to study the effects of physiological and biochemical processes under leaf phenophase and elevation variation throughout the local habitat. We hypothesized that typical shift in physio-biochemical performance during leaves phenological elasticity would depend on-site position such that those with strong elevation gradient would experience drastic changes in photosynthetic activity.

Materials and Methods

Study Area

The study area is situated in the border of Qom and Kashan cities, the central region of Iran (50° 15′ 21″- 50° 51′ 1″ N, 34° 14′ 57″ – 34° 17′ 48″ E). The highest and lowest elevations are 3209 and 792 m.a.s.l., respectively. Due to a wide variation of elevation levels, the climate of the study area varies from hyper-arid in desert-marginal areas to semiarid mountainous regions (Azarakhshi et al., 2011). The average rainfall is 135 mm per year, falling mostly in the period from October to April (110 mm). May to November is the dry period, with as little as 25 mm rainfall. Temperature ranges from a minimum of −5°C in the wet season to a maximum of 45°C in the dry season, with low humidity throughout the year (Darabi et al., 2017).

Plant Material

Ficus carica subsp. *Rupestris* can tolerate a range of habitats, including infertile rocky land, woodland, scrubland, and even dry soils in vast areas of Iran over an elevation range of 1000–2400 m (Keshvari et al., 2013). We hypothesized that typical shift in physio-biochemical performance during leaves phenological elasticity would depend on-site position experiences a strong elevation gradient. In the absence of elevation gradient, we expected a loss of photosynthetic function related to leaf phenological elasticity.

This experiment was carried out during the growing seasons of 2016–2017 for 2 years. The three leaf maturity indices (leaf phenophases) were defined as follows: Young leaves (YL): through this phase, 10–15% of fruits appear. Mature leaves (ML): in this stage, fig seeds develop. Senescent leaves (SL): within this phase, both female and male fruits disperse.

A site was selected approximately every 500 m in the elevation along a transect from 1000 to 2400 m for a total of three sampling sites, at 1268 m (AL1), 1718 m (AL2), and 2325 (AL3) m.a.s.l. At each site, a total of four plots (40 m × 40 m) with five fig-stands in each plot were randomly selected. The precise geographic specifications of the study areas are given in Table 1.

The experimental design was a randomized complete block design (RCBD) with five replicates. In this design, all variables were divided into two groups

Table 1. Geographical coordinates of the selected sits.

A. No	Sampling site	Elevation (m)	Region	Geographical coordinate	Habitat
1.	Yâhyâ-âbâd	AL1	Âb-shirin	51°12′19″N-34°15′17″E	Shrubland northern slopes
2.	Boambâ	AL2	Bire-ghoon	50°57′38′N′-34°22′18″E	Scrubland
3.	Dâre-Gâz	AL3	Fordov	50°51′39″N-34°17′43″E	Rocky walls

namely dependent and independent variables. Three leaf-maturity phases (YL, ML, and SL simultaneous with April, July and end of September, respectively) and sites positions (three altitudinal levels, approximately 1268, 1718 and 2325 m.a.s.l., respectively) were considered as independent variables. Since positions of the sites were completely separated, measurement of fluorescence parameters and leaf sampling were performed in accordance with leaf phenological phases (phenophases) (YL, ML, and SL).

Chlorophyll Fluorescence Analysis

The chlorophyll fluorescent variables were measured using a pulse amplitude modulation fluorometer (PAM-2500, Walz, Effeltrich, Germany). Base fluorescence (F_0) and maximum fluorescence (F_m) were measured in 30 min dark-adapted leaves. Afterward, the same leaves were light-saturated followed by measuring the steady fluorescence (F_s), base fluorescence (F'_0), and maximum fluorescence (F'_m). Based on the measured fluorescent parameters, some basic fluorescence variables like variable fluorescence (F_v), F_v/F_m, ΦPII, qP, and NPQ can be calculated. These variables give insight into the photosynthetic apparatus in chloroplasts (Ranjbar Fordoei et al., 2006).

Biochemical Analyses

Foliar Photosynthetic Pigments

Chlorophylls and carotenoids were extracted from leaf discs in 80% acetone, (Arnon, 1949) . The chlorophyll and carotenoid contents were determined by spectrophotometry according to Gholami, et al. (2012). Following equations were used for the calculation of the concentration of total chlorophyll and carotenoid [mg g^{-1} fresh weight (FW)] in the leaf pigments:

Chl. *a* = $12.25A_{663} - 2.79A_{645}$

Chl. *b* = $21.\ 50A_{645} - 5.10A_{663}$

TCC = $7.15A_{663} - 18.71A_{645}$

Car = (1000A470 – 1.82Ca – 85.02Cb)/198

Where: Chl. *a* = chlorophyll *a*; Chl. *b* = chlorophyll *b*; TCC = total chlorophyll;

Car = carotenoid; A = absorbance spectrum (nm).

Free proline content (FPC) was quantified using the method of Bates et al. (1973) based on the reaction of proline with ninhydrin and glacial acetic acid. The supernatant was read at 520 nm. Results were expressed in M moles of proline per gram of leaf fresh mass.

Total soluble sugar **(TSS)** was determined using the Anthrone method (Salehi et al., 2016). In this procedure, 0.5 g of fresh leaf was mixed with 10 mL distilled water. The sample was heated at 100°C for 1 h and then filtered. Reaction mixture contained 0.5 mL extracts, 0.5 mL mixed reagent (1 g anthrone + 50 mL ethyl acetate), 5 mL H_2SO_4 (98%), and 1.5 mL distilled water. The mixture was heated at 100°C for 1 min and absorbance was read at 630 nm. Sucrose solutions were used as standard samples. Results were expressed in M moles of proline per gram of leaf fresh mass.

Starch Content

The solid residue remaining after removal of all soluble sugars was washed re-extracted and re-centrifuged four times using an 80% (V/V) ethanol. Starch content in the samples was determined calorimetrically using the anthrone method (Mc Cready et al., 1950). The absorbance was read at 630 nm as described by López et al. (2002).

Total Soluble Proteins (TSP)

Leaf tissues were pulverized in liquid nitrogen and mixed with 2 ml of ice-cold 0.02 M Tris-HCl (pH7.5). The homogenate was centrifuged for 10 min and the supernatant was used to determine TSP by the method of Bradford (1976).

Measurement of Leaf Water Status

Predawn leaf water potential (Ψ_L) was measured before the daybreak. Within this time, leaf water potential is in balance with the water potential of the soil. Field measurements were performed using a WP4 dew-point potentiometer (Decagon Devices Inc. USA). Values were determined in leaves close to the same leaves that were applied for the chlorophyll fluorescence monitoring.

Statistical Analysis

Data analysis was carried out using analysis of variance (ANOVA) procedure. The comparisons of means were examined with least significant difference (LSD). The SAS software version 9.4 was used for all the analyses.

Results

Changes in Photosynthetic Pigments Variables along Altitudinal Gradients and Leaf Phenophase Alterations

The ANOVA results related to elevation gradient and leaf phenophase are presented in Table 2. Also, independent and interaction impacts of the mentioned factors are depicted in Table 3, 4, and 5, respectively.

Leaf Chl. *a* Chl. *b*, TCC, and *Car* concentration of *F. carica* subsp. *Rupestris* trees exhibited no significant change in the leaves along the elevation increases.

The effect of leaf phenophase resulted in a significant alteration in photosynthetic pigments ($P < .05$), which were observed in matured and senescence leaves phenophase (Table 3). In contrast to the changes in Chl. *a*, Chl. *b*, and TCC concentrations, the *Car* content significantly increased with an increase in leaf phenologic elasticity, so that the lowest (0.266) and highest (0.348) values were observed in young and senescence phases, respectively. The significant impact of leaf development stage on TCC/*Car* was just observed in the final phase of leaf elasticity (Table 4).

Table 2. Combined analysis of variance results for the impact of site elevation and leaf phenophase on photosynthetic pigment traits in leaves of *F. carica* subsp. *Rupestris*.

		MSS				
source	df	Chl.*a*	Chl.*b*	TCC	Car	TCC/*Car*
Site position (S)	2	0.0002	0.00004	0.0006	0.000017	0.0003
Phenophase (P)	2	0.647*	0.502*	1.037*	0.059*	0.17*
S × P	4	0.00008	0.00005	0.00002	0.00003	0.00008
CV		4.73	11.46	5.02	7.18	7.480

*Significantly different at $P < 0.001$.

Table 3. Comparison of different photosynthetic pigments through various elevation levels indices in leaves of *F. carica* subsp. *Rupestris*.

	Mean				
site position	Chl. *a*	Chl. *b*	TCC	*Car*	TCC/*Car*
AL1	0.963a	0.273a	1.224a	0.281a	0.244a
AL2	0.956a	0.270a	1.226a	0.279a	0.242a
AL3	0.957a	0.272a	1.236a	0.275a	0.236a

Different letters in the table characterize significant difference among the site position indices.

Table 4. Comparison of different photosynthetic pigments through leaf phenophases in leaves of *F. carica* subsp. *Rupestris*.

	Mean				
Leaf phenophase	Chl. *a*	Chl. *b*	TCC	*Car*	TCC/*Car*
YL	0.973b	0.281b	1.254b	0.226a	0.181b
ML	1.139a	0.325a	1.478a	0.261b	0.177b
SL	0.744c	0.210c	0.954c	0.348c	0.365a

Different letters in the table characterize significant difference among the phenophase indices.

No significant difference was found for the effect of site position and its interaction with leaf phenophase on pigments concentration of leaves (Table 5).

Changes in Biochemical Variables along Altitudinal Gradients and Leaf Phenophase Alterations

The ANOVA results revealed that leaf phenophase, elevation gradient, and their interaction (S × P) significantly ($P < .05$) affected biochemical variables (i.e., FPC, TSS, TSP, and starch). The differences in the response of the variables to the selected factors indicate that each factor and their interaction have a different impact on the biochemical parameters.

For instance, all of the biochemical parameters significantly changed along leaf phenophase development whereas the effects of the elevation gradient and its interaction with leaf phenophase were significant for FPC and TSS (Table 6).

Based on Table 7, FPC values significantly increased along leaf phenophase development. This variable achieved the highest value (8.741) at senescence phase. A steadily significant ascending trend was observed in this variable from the first stage of leaf phenophase to the second stage, and then the highest value was observed in senescence leaf phenophase. TSP showed a non-significant ascending trend from young phase to a mature phase, and then a sharp descending trend appeared in the final phase of leaf elasticity with the lowest TSP value (0.233). On the contrary, the highest TSS value (21.68) was observed in senescence stage of leaf development.

The starch content significantly decreased with an increase in the development of leaf elasticity. The lowest value (69) for this variable was observed in senescence leaves (Table 7).

Data presented in Table 8 revealed that FPC values significantly decreased along the elevation rise. This variable achieved the lowest value (7.043) at AL3. An effective reduction of TSS was observed at AL2 and reached the lowest value (16.17) at AL3.

Table 5. Comparison of different photosynthetic pigments through interaction effects of site position and leaf phenophase indices (S × P) in leaves of *F. carica* subsp. *Rupestris*.

		Mean				
Site position (S)	Phenophase (P)	Chl. *a*	Chl. *b*	TCC	*Car*	TCC/*Car*
AL1	YL	0.97b	0.28c	1.25b	0.23c	0.184b
	ML	1.16a	0.33a	1.47a	0.26b	0.178b
	SL	0.74c	0.21d	0.95c	0.35a	0.371a
AL2	YL	0.97b	0.28c	1.25b	0.23c	0.181b
	ML	1.16a	0.32a	1.48a	0.26b	0.176b
	SL	0.74c	0.21d	0.95c	0.35a	0.369a
AL3	YL	0.98b	0.28c	1.26b	0.22c	0.177b
	ML	1.16a	0.32a	1.48a	0.26b	0.176b
	SL	0.75c	0.21d	0.96c	0.34a	0.355a

Different letters in the table characterize significant difference among the S × P indices.

Table 6. Combined analysis of variance results for impact of site position and leaf phenophase on biochemical traits in leaves of *F. carica* subsp. *Rupestris.*

		MSS			
source	df	FPC	TSP	TSS	Starch
Site position (S)	2	0.53*	0.0004	2.352*	134.82
Phenophase (P)	2	26.53*	0.055*	303.719*	9997.36*
S × P	4	0.96*	0.00003	0.505*	23.06
CV		1.26	13.35	1.75	6.39

*Significantly different at $P < 0.01$.

Table 7. Comparison of different biochemical variables through leaf phenophases in leaves of *F. carica* subsp. *Rupestris.*

	Mean			
Leaf phenophase	FPC	TSP	TSS	Starch
YL	6.353c	0.324a	13.919b	105.73b
ML	6.533b	0.347a	13.859b	118.73a
SL	8.710a	0.233b	21.683a	96.00c

Different letters in the table characterize significant difference among the phenophase indices.

Table 8. Comparison of different biochemical variables through site positions in leaves of *F. carica* subsp. *Rupestris.*

	Mean			
Site position	FPC	TSP	TSS	Starch
AL1	7.413a	0.306a	16.933a	101.27a
AL2	7.171b	0.302a	16.352b	95.80a
AL3	7.043c	0.295a	16.176b	96.40a

Different letters in the table characterize significant difference among the site position indices.

TSP and starch revealed no significant difference through variation in site elevation (Table 8).

The interaction between leaf phenophase and site position was significant for FPC and TSS (Table 6). The highest TSS and FPC values were recorded in AL1 and senescence leaf phase. In addition, the lowest values of these two variables were observed in AL3, matured, and young phases of leaves development, respectively.

No significant difference was observed in TPS and Starch values through interaction effects of various site positions and leaf phenophase indices (S × P) (Table 9).

Changes in Leaf Water Status along Altitudinal Gradients and Leaf Phenophase Alterations

The ANOVA results showed that RWC significantly was affected by both site position change and leaf phenological elasticity factors, whereas ΨL was just significantly affected through leaf phenophase (Table 10).

Table 9. Comparison of different biochemical variables through interaction effects of site position and leaf phenophase indices (S × P) in leaves of *F. carica* subsp. *Rupestris*.

		Mean			
Site position	Leaf phenophase	FPC	TSP	TSS	Starch
AL1	YL	6.446^{e}	0.330^{a}	13.970^{d}	111.b^{c}
	ML	6.670^{d}	0.350^{a}	14.406^{c}	120.2^{a}
	SL	9.124^{a}	0.238^{b}	22.422^{a}	72.6^{e}
AL2	YL	6.380^{e}	0.322^{a}	13.906^{d}	104.0^{c}
	ML	6.496^{e}	0.350^{a}	13.664d^{e}	116.2^{a}
	SL	8.638^{b}	0.234^{b}	21.486^{b}	67.2^{e}
AL3	YL	6.234^{f}	0.320^{a}	13.880^{de}	102.2^{d}
	ML	6.434^{e}	0. 340^{a}	13.508^{e}	119.8^{a}
	SL	8.462^{c}	0.226^{b}	21.140^{b}	67.2^{e}

Different letters in the table characterize significant difference among the S × P indices.

Table 10. Combined analysis of variance results for impact of site position and leaf phenophase on ΨL and RWC in leaves of *F. carica* subsp. *Rupestris*.

		MSS	
Source	df	ΨL	RWC
Site position (S)	2	0.00009	7.2*
Leaf phenophase (P)	2	3.54*	736.86*
S × P	4	0.0001	0.64
CV		−4.02	1.32

*Significantly different at $P < 0.01$.

As Table 11 shows, ΨL changed along site position gradient, but RWC increased gradually across position changing, with a significant increase in the AL3. A marked difference in RWC was not observed between AL1 and AL2. This variable was highest at AL3 and vice versa.

As shown in Table 12, both variables are significantly affected through leaf phenophase and thus exhibited a descending trend along leaf phenophase development.

ΨL decreased significantly from young phase of leaves to the senescence phase and reached its lowest value. A similar trend was observed in RWC with developing leaf phenophase. This variable was highest (78.35%) in young phase and lowest (64.57%) in the senescence phase.

Table 11. Comparison of ΨL and RWC through various site positions in leaves of *F. carica* subsp. *Rupestris*.

	Mean	
Site position	ΨL (MPa)	RWC (%)
AL1	−1.907^{a}	71.28^{b}
AL2	−1.902^{a}	71.78^{b}
AL3	−1.905^{a}	72.65^{a}

Different letters in the table characterize significant difference among the site position indices.

Table 12. Comparison of ΨL and RWC through various leaf phenophase stages in leaves of *F. carica* subsp. *Rupestris*.

	Mean	
Leaf phenophase	ΨL (MPa)	RWC (%)
YL	−1.474[a]	78.533[a]
ML	−1.809[b]	72.613[b]
SL	−2.431[c]	64.569[c]

Different letters in the table characterize significant difference among the leaf phenophase indices.

The interaction between leaf phenophase and elevation gradient was significant for ΨL and RWC (Table 13). The highest ΨL and RWC values were recorded in the young phase of leaf development. A gradual increase in RWC was observed with increasing elevation. The highest value (79.55) of this variable was recorded at AL3 and in the young phase of leaf development. This trend was accompanied for the decrease in ΨL value by the development of leaf phenophase.

The highest (−1.47 MPa) and lowest (−2.43 MPa) values were related to the young and senescence stages of leaf phenophase, respectively. There was no significant difference in ΨL values among various site positions (Table 13).

Changes in Fluorescence Variables along Elevational Gradients and Leaf Phenophase Alterations

The ANOVA results exhibited that leaf phenophase significantly affected six (F_0, F_m, F_v/F_m, qP, NPQ, and ΦPSII) out of seven fluorescence variables. In addition, the drastic effect of site position gradient was observed in F_0, F_v/F_m, and qP variables (Table 14).

Table 13. Comparison of leaf water potential (ΨL) and (RWC %) through interaction effects of site position and leaf phenophase indices (S × P) in leaves of *F. carica* subsp. *Rupestris*.

		Mean	
Site position	Leaf phenophase	ΨL (MPa)	RWC (%)
AL1	YL	−1.48[a]	77.44[b]
	ML	−1.81[b]	72.17[c]
	SL	−2.43[c]	64.23[d]
AL2	YL	−1.47[a]	78.61a[b]
	ML	−1.80[b]	72.39[c]
	SL	−2.43[c]	64.36[d]
AL3	YL	−1.47[a]	79.55[a]
	ML	−1.81[b]	73.28[c]
	SL	−2.43[c]	65.12[d]

Different letters in the table characterize significant difference among the S × P indices.

Table 14. Combined analysis of variance results for impact of site position and leaf phenophase on chlorophyll *a* fluorescence traits in leaves of *F. carica* subsp. *Rupestris*.

		MSS						
source	df	F_0	F_m	F_v/F_m	F_m/F_0	qP	NPQ	ΦPSII
Site position (S)	2	1943.89**	99.09	0.001**	0.00002	0.004**	0.005	0.0005
Phenophase (P)	2	59475.56**	728637.1**	0.081**	0.0029	0.129**	1.7488**	1.07*
S × P	4	1310.56**	235.16	0.0006**	0.008	0.0007	0.002	0.011
CV		2.42	0.58	0.9	30.29	3.33	9.81	18.91

Significantly different at $^{**}P < 0.01$, $^{*}P < 0.05$

For F_0 and F_v/F_m, a significant interaction between site position and leaf phenophase was observed.

Regarding the data depicted in Table 15, F_0 and NPQ variables showed an ascending trend in all leaf phenological stages. For, both variables, the lowest value was initiated in YL and reached the highest value in SL (446.67 and 1.547, respectively).

On the contrary, F_m, F_v/F_m, qP, and ΦPSII variables showed a descending trend with a development in leaf phenophase with the highest and the lowest values in YL and SL phases, respectively. There was no significant difference in F_m/F_0 values among the various phenological phases (Table 15).

In the current study, a decrease in the *qP* was observed in response to the summer drought stress and development of phenological phases, indicating that a large section of the PSII reaction centers was damaged. It also indicated that the balance between excitation rate and electron transfer rate had collapsed.

A drastic decrease in F_v/F_m and increase in F_0 indicate the occurrence of damage to photosystem II, which reduces the efficiency of absorbed light energy transfer from the light-harvesting complex.

Table 15. Comparison of different fluorescence variables through leaf phenophases in leaves of *F. carica* subsp. *Rupestris*.

	Mean						
Leaf phenophase	F_0	F_m	F_v/F_m	F_m/F_0	qP	NPQ	ΦPSII
YL	321.33^c	1820.72^a	0.822^a	0.218^a	0.544^a	0.915^c	0.502^a
ML	373.33^b	1779.07^b	0.786^b	0.238^a	0.513^a	1.013^b	0.467^b
SL	446.67^a	1419.87^c	0.681^c	0.244^a	0.370^c	1.547^a	0.454^b

Different letters in the table characterize significant difference among the phenophase indices.

Table 16. Comparison of different fluorescence variables through sit positions in leaves of *F. carica* subsp. *Rupestris*.

	Mean						
Site position	F_0	F_m	F_v/F_m	F_m/F_0	*qP*	NPQ	ΦPSII
AL1	390.3^a	1672.5^a	0.75^b	0.234^a	0.464^b	1.164^a	0.472^a
AL2	383.0^b	1676.0^a	0.76^b	0.234^a	0.469^b	1.172^a	0.483^a
AL3	368.0^c	1671.0^a	0.77^a	0.232^a	0.493^a	1.139^a	0.476^a

Different letters in the table characterize significant difference among the site position indices.

The results demonstrated F_0, F_v/F_m, and *qP* variations were significantly associated with elevation gradient with the highest and lowest values in AL1 and AL3 (respectively), but F_0 exhibited a descending trend against elevation variation. On the contrary, *qP* and F_v/F_m values increased with an increase in the elevation of site position. A drastic effect of site position was not observed in F_m, F_m/F_0, NPQ, and ΦPSII variables.

Based on Tables 16 and 17, the significant S × P interaction indicates that the response of both F_0 and F_v/F_m variables to site position gradient differed among the leaf phenological phases. As shown in Table 17, the F_0 drastically decreased in response to developing phenological phase and increasing the elevation level so that the highest (477) and lowest (371) values were found in SL and AL3, respectively. On the contrary, F_v/F_m value decreased with developing the leaf phenophase with an elevation gradient.

The highest (0.82) and lowest (0.66) values for F_v/F_m were obtained in the first (YL) and third (SL) phases of leaf development and in AL3, respectively. Mean comparing values of interaction between phenological amplitudes and site position range revealed no significant differences in F_m, F_m/F_0, qP, NPQ, and ΦPSII (Table 17).

Discussion

The lack of a significant response of the photosynthetic pigments to elevation gradient (Table 3) might affect maintaining a high photosynthetic capability at high elevations (Shi et al., 2006). In this case, our results are in agreement with findings of Li et al. (2013) on some woody species.

However, the drastic effect of leaf phenological amplitude on photosynthetic pigments was noticed. The increase in Chl. *a*, Chl. *b*, and TCC values in ML suggests that young leaves were not matured while the structure of mesophyll has fully progressed with high pigment content in mature leaves (Pramod et al., 2015). Severe decreases in the mentioned variables in SL are

Table 17. Comparison of fluorescence variables through interaction effects of site position and leaf phenophase indices (S × P) in *F. carica* subsp. *Rupestris* trees.

		Mean						
Site position	Leaf phenophase	F_0	F_m	F_v/F_m	F_m/F_0	qP	NPQ	ΦPSII
AL1	YL	321^{e}	1816^{a}	0.82^{a}	0.189^{a}	0.534^{bc}	0.916^{b}	0.527^{a}
	ML	372^{d}	1781^{b}	0.79^{bc}	0.254^{a}	0.510^{d}	1.04^{b}	0.434^{a}
	SL	477^{a}	1420^{cd}	0.70^{d}	0.258^{a}	0.348^{f}	1.536^{a}	0.456^{a}
AL2	YL	326^{e}	1823^{a}	0.82^{a}	0.267^{a}	0.542^{ab}	0.918^{b}	0.451^{a}
	ML	378^{d}	1776^{b}	0.78^{c}	0.231^{a}	0.506^{d}	1.02^{b}	0.494^{a}
	SL	445^{b}	1425^{d}	0.68^{e}	0.204^{a}	0.360^{f}	1.58^{a}	0.505^{a}
AL3	YL	317^{e}	1823^{a}	0.82^{a}	0.198^{a}	0.556^{a}	0.91^{b}	0.527^{a}
	ML	369^{d}	1780^{b}	0.79^{b}	0.229^{a}	0.522^{cd}	0.98^{b}	0.473^{a}
	SL	418^{c}	1410^{d}	0.66^{f}	0.271^{a}	0.402^{e}	1.53^{a}	0.429^{a}

Different letters in the table characterize significant difference among the S × P indices.

indicators of damage to chloroplasts due to leaf senescence and reduction of leaf moisture content, depicted through reducing leaf water potential (Wang et al., 2014). Our results on *Car* content are in agreement with findings of Field and Mooney, (1983), who reported, with the initiation of leaf senescence, chlorophylls (*a* and *b*) are degraded, giving leaves dark-green color and carotenoids are increased and appeared in the senescent leaves. In SL phase, a drastic increase in the TCC/*Car* ratio was observed that might be attributed to the fast chlorophyll degradation in response to abiotic stresses as water stress deficiency (Lee et al., 2003).

FPC and TSS are reducing agents that play effective roles in controlling osmotic adjustments in plants suffering from abiotic stress, which can be induced directly or indirectly by various environmental factors (Manivannan et al., 2007). The FPC and TSS contents of *F. carica* subsp. *Rupestris* were affected by elevation and exhibited descending trends along elevation gradient from low to high elevation. This trend is consistent with the amount of rain and temperature. Thus, with decreasing elevation level, ambient temperature and the evapotranspiration rates increase. That is why the highest values of both variables appeared at AL1. An increase in soluble sugar contents in plants can be attributed to the role of this osmolyte as a defense mechanism and maintenance osmotic adjustment (Bartels and Sunkar, 2005; Manivannan et al., 2007). Our results were consistent with the findings of Cui et al. (2018) on *Leymus secalinus*.

Regarding the data presented in Table 7, the highest values of FPC and TSS were observed in the last step of leaf development while, on the contrary, the lowest values of TSP and starch appeared in the same leaf phenophase-step. Several reports have shown an increase in the FPC and TSS values along the phenological phase changing, particularly the onset of senescence (Bandurska, 2000; Paul and Pellny, 2003; Yuanyuan et al., 2009). The accumulation of TSS and reduction of starch is associated with the hydrolysis of starch and its reduction during aging (Lim et al., 2007). Our results were consistent with the findings of Wehner et al. (2015) on barley.

The highest value of RWC% was observed in AL3 that may due to lower temperature and consequently lower evapotranspiration rate than in AL1 and AL2. As Table 12 exhibited, both leaf water status variables were drastically affected by leaf aging. The association of leaf phenological stages with environmental conditions such as water deficit may suggest that leaf aging through changes in internal leaf structure decrease values of the leaf water status variables (ΨL and RWC) and elasticity of cell walls. Another possibility for the lower ΨL observed in senescence leaves can be ascribed to down-regulation or inactivation of aquaporin channels in living cells (Locke and Ort, 2014).

The data presented in Table 15 exhibit a substantial change in PSII activity, reflected by fluorescence variables. F_v/F_m was affected drastically through leaf phenophase, particularly in the last stage of leaf cycle

(senescence), which was previously reported by Zhao et al. (2014) in *Lotus corniculatus* and Ranjbar (2017) in *Pistacia vera*. Any reduction in F_v/F_m values can be ascribed to damage in photosynthetic apparatus, indicating more energy dissipated as heat (Hazrati et al., 2016). Our results also showed significant changes in F_0 and in F_m during leaf phenophases, particularly in senescence phase. The greatest and lowest values of F_0 and F_m were observed in senescent leaves, which represent the dysfunction of PSII reaction centers. A drastic decrease in F_v/F_m, F_m, and increase in F_0 can be attributed to a reduction in the efficiency of absorbed light energy transfer from the light-harvesting complex (Maxwell and Johnson, 2000). The decrease in F_m values through leaf cycle development confirm the results of Müller et al. (2001), who reported that low levels of Fm can be associated with increased non-photochemical dissipation and with the xanthophyll cycle. A significant increase in NPQ was observed in response to leaf phenophase development. This fluorescence parameter reflects the thermal dissipation of excess excitation energy in the chloroplasts and has a link with F_v/F_m (Girma, 2016). Our results agree with those of Alves et al. (2013), who reported a significant increase in NPQ in response to drought stress. Remarkable reduction values of ΦPSII and qP (Table 15) suggest that the sufficiency of photochemistry transformation and electron flow are affected by leaf cycle development, particularly in senescence stage (Ranjbar-Fordoei, 2018). A reduction in ΦPSII value can be attributed directly or indirectly to leaf senescence, which plays an important role in photosynthesis response (Atlassi et al., 2009). A decrease in qP, due to the extension of leaf phenophase, indicates that a large section of PSII reaction system was damaged (Ranjbar-Fordoei, 2018).

Conclusion

The obtained results showed that leaf phenophase progress resulted in a significant alteration in chlorophyll fluorescence parameters, RWC, ΨL, TSP, FPC, and TSS wild fig plants. These resulted indicated that the examined plants responded to leaf phenological elasticity through changing physiological and biochemical parameters. Also, the FPC and TSS contents were affected by site elevation, exhibiting descending trends along elevation gradient from low to high elevation. This trend is consistent with the amount of rain and temperature. Thus, with decreasing elevation level, ambient temperature and the evapotranspiration rates increase. Generally, the manifested alterations were mainly associated with leaf senescence and reduction of leaf water potential.

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