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# Variation characteristics of chlorophyll fluorescence of a typical Eremophyte (*Smirnovia Iranica* (Sabeti)) during phenological stages in the sand drift desert (Case study: In Kashan Region)

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## Abstract

*Smirnovia iranica*, a native valuable woody species from *Fabaceae*, is an adaptable plant of central sandy areas of Iran. Changes in chlorophyll fluorescence and photosynthetic pigments characteristics were analyzed in the course of phenological stages including vegetative, flowering, seed ripening, and seed falling stages, respectively (VS, FS, SRS, and SFS). The results obtained from analysis of variance indicated that there was a significant difference among different phonological stages of *S. iranica* in terms of the mentioned characteristics. Extending phenological stages along with increasing water deficiency resulted in significant reduction in $F_v$ /$F_m$ ratio in SRS and SFS. A significant effect of progression in phenological stages on thermal dissipation of light energy (*D*) value was observed in SRS and reached to the highest value in SFS. In all evaluated plants, during phenological development, there were decreases in photochemical efficiency of photosystem II (ΦPSII), and from SRS. A similar influence of the extending phenological stages on ΦPSII was observed for electron transport rate (*ETR*). In SRS to SFS, the decrease of Chl. (a+b) and Car was paralleled with the decrease of $F_v/F_m$, which indicated that pigments breakdown was accompanied by the decreasing of the maximum photochemical efficiency. The results of this study suggest that extending phenological stages along with increasing water deficiency stress resulted in significant alterations in chlorophyll fluorescence parameters and pigment contents in SRS and SFS.

***Keywords***: *E*lectron transport rate; Phenology; Photosynthetic apparatus; Pigment; Quantum yield

## 1. Introduction

One of the most familiar of all natural phenomena is the cycle of events associated with the passage of the seasons. This is especially so in regions where high temperature and low accessibility of water are accompanied by corresponding cycles in the growth and reproduction of the flora. In arid regions, seasons are often marked by differences in rainfall, with life-history events occurring in response to water availability (Janice, 1974; Michael, 1998).

Phenology is the study of the events that lead to the manifestation of phenomena associated with the functioning of some plant organs or of the plant as a whole (Fabio *et al*., 2010).

Phenological studies interpret the reproductive success of a plant population each year, the growth and survival probability of individuals, and their fitness under particular climatic conditions (Cleland *et al*., 2007).

At scales from organs to ecosystems, many processes, particularly those related to the cycling of carbon (productivity and growth), water (evapotranspiration), and nutrients (uptake and mineralization), are directly mediated by phenology (Lianhong *et al*., 2003). The good knowledge of all phenological interactions to the

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environment might lead to better understanding of plant functioning in short-term as well as long-term point of view (Urban *et al*., 2013).

Arid regions are characterized with low annual precipitation, abundant sunshine, and high evaporation rates. In these regions, plants are exposed to a wide variety of abiotic stresses, including excessive light, extreme temperatures, drought stress, and atmospheric pollutants, which can directly or indirectly affect photosynthetic function. Therefore plants associations are composed of such regions are special species (Zhang *et al*., 2011).

Drought stress is one of the most important limitations to photosynthesis and plant productivity. For most plants, water deficit may promote an imbalance between photochemical activity at photosystem II (PSII) and electron requirement for photosynthesis, leading to an over-excitation and damage of PSII reaction centers (Souza *et al*., 2004; Calatayud *et al*., 2006).

The reaction of plants to drought stress depends on the intensity and duration of stress as well as the plant species and its phenological stage (Parameshwarappa and Salimath, 2008). The effect of drought stress on $CO_2$ assimilation rate, transpiration rate, and water use efficiency has been investigated in many plant species (Pireivatlou *et al.*, 2008; Rabiye *et al*., 2010; Ranjbarfordoei *et al*., 2013). Another plant response to drought stress is the change in photosynthetic pigment content. Photosynthetic pigments play important roles in harvesting light. The content of both chlorophyll *a* and *b* changes under drought stress (Farooq *et al*., 2009). The carotenoids play fundamental roles and help plants to resist drought stress (Jaleel *et al*., 2009).

Chlorophyll fluorescence measurements have become a widely used method to study the functioning of the photosynthetic apparatus and are a powerful tool to study the plant's response to environmental stress (Stirbet and Govindjee, 2011). One of the most often employed fluorescence parameters in term of detecting stress tolerance is the maximum quantum yield of PSII ($F_v/F_m$) (Maxwell and Johnson 2000). It is well documented that stomatal function and chlorophyll fluorescence parameters depend on the course of leaf development and phenological phases (Romá and Josep, 2004; Nesterenko *et al*., 2006; Čaňová *et al*., 2008).

*Smirnovia iranica* (*Fabaceae*) is one of the valuable shrub species with high resistance to arid conditions and only appears on sand dunes. The plant is important for forage production, soil conservation, and medicinal values (Sabeti, 1994).

Very little information is available on the functioning of the photosynthetic apparatus in *S. iranica* plants during their phenological phases, particularly under arid conditions of growing. Hence, this study was carried out to evaluate the concurrent changes of chlorophyll *a* fluorescence parameters and leaf pigments in the species.

## 2. Materials and Methods

The study was conducted in a typical habitat of *S. iranica* in Maranjab, Isfahan Province, Iran (34°00 –34°10 N, 51°27–51°35 E, 800–950 m a.s.l.). The annual average precipitation, based on 30 years record, is 133 mm, which shows the uneven distribution in the form of storms. The region lies between 2000-2800 is potential evaporation lines and according to ombrothermic diagram, the study area has 9 dry months annually. The region is mostly placed between 15-17.5° isotemperature lines. Also, the climate of this region is extremely warm with dry summers (Azarnivand *et al*., 2006) and experiences drought stress during the most of phenological phases (Fig. 1).

### *2.1. Methodology of research*

The three phenological stages were defined as following. Vegetative: from leaf emergence to first flowering (VS). Flowering: from the commencement of flowering to first sheath at full length (FS). Seed ripening: from full length sheath to physiological maturity of the first sheath (SRP). Seed falling: From fifty percent of sheath mature to initiation of seed dispersal (SFS). Accordingly, the durations of the vegetative, flowering, and seed filling stages were 30, 55, 75, and 100 days, respectively (Azarnivand and Dastmalchii, 2000). In the context of this study, we use phenological phases as a proxy for drought stress treatments (FS, SRS, and SFS, when the plants were gradually exposed to drought stress due to lack of rainfall and low availability of soil water; from mid-April onwards).

Vegetative zones of *S. iranica* species were determined. Then, four areas, within each area five replicates, were selected for sampling purposes. The sampling points were recorded to be tested in the above mentioned phenological stages (Azarnivand and Dastmalchi, 2000; Azarnivand *et al*., 2006; Azarnivand *et al*., 2011).

### *2.2. Chlorophyll fluorescence analysis*

Chlorophyll fluorescence yields were measured using a portable fluorometer *PAM-2500* (*H. Walz*, Effeltrich, Germany). Measurements were made on the uppermost fully expanded leaves. Before measuring chlorophyll fluorescence yields (Chl. FYs), leaves were put in dark-adapted state (DAS) for 30 min (Genty *et al*., 1989) using light exclusion clips. During DAS, all reaction canters and electron carriers of the PSII are re-oxidized; this situation is essential for rapid fluorescence induction kinetics and for recording Chl. fluorescence parameters.

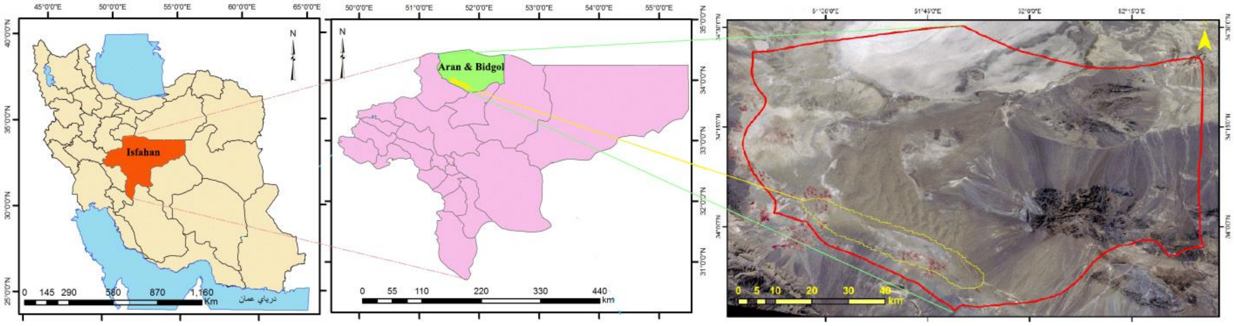

Fig. 1. Geographical location of *Smirnovia iranica* habitat in Maranjab area

The following Chl. FYs were measured: minimum Chl. FY in the dark and light-adapted states ($F_0$ and $F'_0$), maximum Chl. FY in the dark and light-adapted states ($F_m$ and $F'_m$), and steady-state Chl. FY in the light-adapted state ($F_s$) (Zhang *et al*., 2011). Some basic, mutually independent, chlorophyll fluorescence parameters (FPs), such as maximum quantum yield of PSII photochemistry ($F_v/F_m$), photochemical efficiency of photosystem II, (ΦPSII), effective quantum yield ($F'_V/F'_m$), dissipation (*quenching*) of absorbed light energy to heat ($D = 1 - F_V'/F_m'$), and electron transport rate (*ETR*), can be calculated from Chl. FYs, that give insight into the photosynthetic processes in chloroplasts and can be used effectively in photosynthesis research (Ranjbarfordoei *et al*., 2006).

### *2.3. Pigment contents*

The measurements of pigment contents in leaves of *S. iranica* plants were performed during the experimental period, from VS until SFS (30, 55, 75 and 100 days). Analyses were accomplished in samples collected from the same leaves upon which the chlorophyll fluorescence parameters were determined. Chlorophyll concentrations were determined according to the methodology of Arnon (1949). Half a gram of fresh leaf material was ground with 10 ml of 80 percent acetone at 4 °C and centrifuged at 2500xg for 10 minutes at 4 °C. This procedure was repeated until the residue became colorless. The extract was transferred to a graduated tube and made up to 10 ml with 80 percent acetone and assayed immediately.

Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663, and 480 nm with a spectrophotometer (U-2001-Hitachi) against 80% acetone as blank. Carotenoid content was estimated using the formula of Kirk and Allen (1965). Pigment contents were calculated and expressed in milligram per gram fresh weight (mg $g^{-1}$ FW).

## 3. Results and Discussion

In terms of physiology, the stages VS and FS can be classified as stages with sufficient water supply and desired temperatures during the vegetation period at the location of study. Precipitation deficit concomitant with high temperatures in stages SRS and SFS was mainly reflected in the parameters of chlorophyll fluorescence. Table 1 shows a statistically highly significant negative effect of the water deficiency stress on the values of chlorophyll fluorescence parameters in the assimilatory organs of *S. iranica* bushes in SRS and SFS stages.

Table 1. Parameters of chlorophyll *a* fluorescence measured in four different phenological stages

| Chl. FPs<br>Phenological stage | $F_0$ | $F_m$ | $F_v/F_m$ | $F_V'/F_m'$ | *D* | ΦPSII | *ETR* |
|---|---|---|---|---|---|---|---|
| VS | $166 \pm 21^a$ | $1211 \pm 36^a$ | $0.86^a$ | $0.62^a$ | $0.35^a$ | $0.100^a$ | $49.5^a$ |
| FS | $173 \pm 17^a$ | $1106 \pm 45^a$ | $0.84^a$ | $0.58^a$ | $0.42^a$ | $0.096^a$ | $48.6^a$ |
| SRS | $256 \pm 22^b$ | $927 \pm 28^b$ | $0.72^b$ | $0.51^b$ | $0.49^b$ | $0.080^b$ | $42.4^b$ |
| SFS | $283 \pm 27^c$ | $772 \pm 30^c$ | $0.63^c$ | $0.44^c$ | $0.56^c$ | $0.053^c$ | $24.6^c$ |

Different letters in each column represent statistically significant at $P \leq 0.05$ (DMRT); Means±S.D., $n=5$; chlorophyll fluorescence parameters (Chl. FPs)

The plants showed increases in $F_0$ with progressing phenological stages ($P \leq 0.05$). However, in VS and FS, the photosynthetic apparatus was significantly not altered. Significant increase in $F_0$ was initiated in SRS and reached to the highest value (283) in SFS. The $F_0$ value may increase if the transfer of excitation energy from the antenna to the reaction centers is impaired (Schreiber *et al*., 1998). Thus, the increase in $F_0$ observed in *S. iranica* plants studied may be associated with damage to the photosynthetic apparatus, such as inactivation reaction centers of *PSII* (Ranjbarfordoe *et al*., 2013).

In contrast, $F_m$ value decreased with progressing phenological stages ($P < 0.05$). However, in VS and FS the efficiency of energy capture and conversion into chemical energy in photosystem II was significantly not changed. Significant decrease in $F_m$ was initiated in SRS and reached to the lowest value (772) in SFS. The reduction of $F_m$ can be associated with increased non-photochemical dissipation as heat or may be related to the decrease in the activity of the water-splitting enzyme complex (Cicero *et al*., 2012). Our results on $F_0$ and $F_m$ agree with the findings of Li *et al*. (2015) on *Medicago sativa* when exposed to drought.

The increase of water deficit concomitant with progressing of phenological stages provided the decline in $F_v/F_m$. Plants in VS and FS showed $F_v/F_m$ ratio within the range of healthy plants (values between 0.750 and 0.850) (Bolhar-Nordenkampf and Oquist, 1993). However, extending phenological stages along with increasing water deficiency stress resulted in significant reduction in $F_v/F_m$ ratio in SRS and SFS. Our results on $F_v/F_m$ can be interpreted in the view of the arguments of Rong-Hua *et al*. (2006) who suggested that any decrease in $F_v/F_m$ indicates that PSII suffers from damage and that the key reactions of photosynthesis are inhibited. A similar result was also reported by Zhao *et al*. (2014) in *Lotus corniculatus*.

A similar influence of the extending phenological stages on $F_v/F_m$ was observed for $F_v'/F_m'$. The increase in water deficit brought the decline of $F_v'/F_m'$ ($P < 0.05$). Significant decrease in $F_v'/F_m'$ was initiated in SRS (0.51) and reached to the lowest value (0.44) in SFS. The reduction of $F_v'/F_m'$ can be associated with decrease in conversion efficiency of electron energy in PII into chemical energy (Schreiber *et al*., 1994). A similar result was also reported by Cicero *et al*. (2012) in mango.

Significant effect of water deficit stress on thermal dissipation of light energy (*D*) value initiated in SRS and reached to the highest value (0.56) in SFS. So the lowest measured value (0.35) was recorded in FS (Table 1). The increase in *D* reflects the activation of several processes of non-photochemical nature during the light period and mostly leading to non-radiative dissipation of the excitation energy as heat (Krause and Johns, 2004). Our findings on *D* are consistent with those previously reported by Ping *et al*. (2014) in apple trees.

In the present study, ΦPSII decreased significantly due to progressing water deficit stress. SF was the highest in ΦPSII (0.100) than the other phenological stages, while SFS was the lowest (0.053) in this attribute at the highest water deficit conditions. The decrease in ΦPSII can be ascribed to the increased rate of dissipation of light energy to heat in the PII antenna complexes as well as a decrease in proportion of photons and excitation of electrons of the chlorophyll (Schreiber *et al*., 1998). Our findings are in agreement with those of Cousins *et al*. (2002), who reported declines in ΦPSII could be due to progressing low water availability.

The *S. iranica* plants showed a reduction in *ETR* with progressing of phenological stages concomitant with increasing low water availability ($P < 0.05$). However, significant reduction in *ETR* value occurred in SRS (42.4) and reached to the lowest value in SFS (24.6).

ETR value shows the sum total of all electrons sinks in chloroplast such as carbon fixation, photorespiration, nitrate assimilation, and Mehler reaction. A perturbation or change in any of these parameters affects ETR (Vladkova *et al*., 2011). Similar to the findings of the present study, previous authors have reported either stable or decreased ETR for *Mangifera indica* (Cicero *et al*., 2012) *Vitis vinifera* (Qing-Ming *et al*., 2008; Iacono and Sommer, 2000).

The results on the effect of gradual water deficit on the pigment parameters in leaves of *S. iranica* are presented in (Table 2). A significant alteration in chlorophyll content of the leaf was not noticed with increasing low available water up to VS and thereafter, it significantly declined in SRS. Reduction of total pigments content, as a result of either slow synthesis or fast breakdown, has been considered as a typical symptom of oxidative stress (Mafakheri *et al*., 2010). In SRS to SFS, the decreases of Chl. (*a*+*b*) and *Car* were paralleled with decrease of $F_v/F_m$, which indicated that pigments breakdown was accompanied by the decreasing of the maximum photochemical efficiency.

Table 2. Concentration of photosynthetic pigments in the leaves of *S. iranica* measured in four different phenological stages

| photosynthetic pigments | Chl. *a* (mg $g^{-1}$) | Chl. *b* (mg $g^{-1}$) | *Car* (mg $g^{-1}$) | Chl. (*a*+*b*) (mg $g^{-1}$) | Chl. (*a*/*b*) |
|---|---|---|---|---|---|
| Phenological stage | | | | | |
| VS | $3.97^{a}$ | $1.94^{a}$ | $0.36^{a}$ | $5.91^{a}$ | $2.04^{a}$ |
| FS | $3.88^{a}$ | $1.89^{a}$ | $0.36^{a}$ | $5.77^{a}$ | $2.05^{a}$ |
| SRS | $2.84^{b}$ | $1.38^{b}$ | $0.28^{b}$ | $4.22^{b}$ | $2.06^{a}$ |
| SFS | $2.41^{c}$ | $1.15^{c}$ | $0.23^{b}$ | $3.56^{c}$ | $2.09^{a}$ |

Different letters in each column represent statistically significant at $P \leq 0.05$ (DMRT), Means ± S.D., $n = 5$

Other authors explained this phenomenon as a photoprotection mechanism through reducing light absorbance by decreasing pigments content (Galmes *et al*., 2007; Elsheery and Cao, 2008). The ratio Chl. (*a*/*b*) tended to increase with progressing of phenological stages, although significant differences did not occur. Lack of significant effects of water deficiency on the Chl. (*a*/*b*) indicates that the size of photosystems has not changed (Fahl *et al*., 1994).

The results of this study suggest that extending phenological stages along with increasing water deficiency stress resulted in significant alterations in chlorophyll fluorescence parameters and pigments content in SRS and SFS. In addition to these results, chlorophyll fluorescence, as a measure of photosynthetic performance, is a potential tool to assess stress in *S. iranica* due to soil water deficit.

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