



The Study of Different Water Regimes on Photosynthetic Performance and Leaf Water Status of Pistachio Trees (*Pistacia vera* L.)

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ARTICLE INFO

Keywords:

Chlorophyll;
Drought;
Photoinhibition;
Photosynthesis;
Photosystem

ABSTRACT

Water deficiency is one of the most important environmental stresses that limit plant growth and crop production. Measurement of chlorophyll fluorescence parameters is considered as an important indicator to evaluate the photosynthetic apparatus. In the present study, the effects of regulated water deficit, investigated in four water-regimes in pistachio orchard with 12-year-old trees of Akbari cultivar (*Pistacia vera* cv. Akbari). The water regime treatments included 20, 15.6, 10.4, and 6% of field capacity (FC) equivalent and irrigation intervals of 4, 12, 18 and 24 days. Chlorophyll fluorescence indices of photosystem 2 (PSII), photosynthetic pigments, and leaf water status parameters were studied. Drought stress inhibited PSII activity and induced alterations in thylakoid proteins. The results showed significant effects on effective quantum yield of PSII (Φ_{PSII}), maximum quantum efficiency of PSII (F_v/F_m), electron transport rate (ETR), quantum yield baseline (F_0/F_m), non-photochemical quenching (NPQ), and photoinhibition (PIHN) rate. Such changes may be due to either degradation of photosynthesis apparatus function or photoinhibition process. From the results of the present study, it can be concluded that exposure of pistachio trees (*P. vera* cv. Akbari) to an interval of 18-days (T18) may not significantly affected the parameters measured. This means that with an increase in irrigation intervals to 16-days, compared to the traditional interval (varies from 8 to 10-days), soil may be able to provide enough moisture for the pistachio trees to complete metabolic activities.

Introduction

Water deficiency is one of the most important environmental stresses that limit plant growth and crop production. There are several methods for investigating of plant reactions in to soil water deficiency. Measurement of pulse-amplitude-modulated (PAM) chlorophyll fluorescence parameters is considered as an important approach to evaluate safety and efficiency of photosynthetic apparatus (Lichtenthaler *et al.*, 2005). Chlorophyll fluorescence technique presents an appropriate

method for obtaining non-invasive estimates of photosynthetic performance. Several studies have confirmed that chlorophyll fluorescent parameters are appropriate to characterize the performance of plant photosynthetic apparatus under water deficit conditions (Lichtenthaler *et al.*, 2005; Catalina *et al.*, 2011; Hailemichael *et al.*, 2016).

For instance, the intensity of water deficit in most studied plants has been significantly connected with the maximum quantum efficiency of PSII (F_v/F_m) and

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Received: 17 May 2018; Received in revised form: 14 January 2019; Accepted: 21 May 2019

DOI: 10.22034/jon.2019.665034

effective quantum yield of PSII (Φ_{PII}) (Šajbidorova *et al.*, 2015). F_v/F_m is the most important property used for evaluation of plant's reaction to drought stress. However, the dark-adapted fluorescence variables measured in leaves such as base fluorescence (F_0), maximum fluorescence (F_m) and the difference between them ($F_m - F_0$), variable fluorescence (F_v), steady-state fluorescence (F_s), and the F_s to F_0 ratio (F_s/F_0) have been reported as beneficial factors in the early detection of water deficit stress (Flexas *et al.*, 2002b; Afrousheh *et al.*, 2010; Ranjbar 2015). Under stress conditions, the light-harvesting apparatus tolerates sustained alterations and causes down-regulation of the photochemical quenching capacity (PQ) and up-regulation of the non-photochemical quenching capacity (NPQ) in PSII (Porcar-Castell *et al.*, 2014). In the literature, NPQ is considered as a protective mechanism for a plant under stressful conditions (Pilar Cendrero-Mateo *et al.*, 2015). Moreover, electron transport rate ($\text{ETR} = \Phi_{\text{PII}} \times \text{PPFD} \times 0.5 \times 0.84$) is a computational fluorescence variable designed to measure the electron transport of PSII, which is used as an indicator of plant water deficit stress. Likewise, chlorophyll contents are considered as bio-indicators to determine photosynthetic capacity and vegetation health. Several reports have demonstrated that soil water deficit leads to a decrease in chlorophyll content and photosynthesis rate (Roháček, 2002; Ranjbar 2015; Hailemichael *et al.*, 2016; Ranjbar-Fordoie, 2018). Leaf water status depends on type of plant and environmental conditions. Various methods have been practiced to specify plant water status under drought stress conditions. Among them, relative water content and leaf water potential are effective means for characterizing physiological water status of plants (Nayyer *et al.*, 2005). Several studies have confirmed leaf water relation parameters; reduce under drought stress conditions (Jones, 1990; Bayoumi *et al.*, 2008; Fghire *et al.*,

2017, Ranjbar-Fordoie, 2018).

Pistachio (*Pistacia vera* L.) is one of the most important horticultural products which is grown in over vast areas of land in Iran, where water deficit is the main limiting factor for growth, and eventually yield, and nut quality (Kamali and Owji, 2016). Although some studies have been conducted on the response of pistachio to water deficit stress, but investigations on the reaction of photosynthetic apparatus of this species to deficiency of water is limited. The objective of this research was to study the function of photosynthetic apparatus in pistachio trees (*P. vera* cv Akbari) to drought stress. For this purpose, chlorophyll fluorescence variables, leaf water status parameters, and leaf chlorophyll content were examined.

Materials and Methods

Study site, irrigation condition, and plant material

The study area is located in Fakhreh rural area ($34^\circ 14'N$, $51^\circ 29'E$, and 910 m a.s.l.) in the western region of Kashan (Iran). The climate range from arid to hyper arid conditions (<100 mm/year of rainfall, 90% occurs in January and May) with average annual potential evaporation 2700 mm and total radiation 3000 h per year.

This experiment was studied during the growing season of 2017 in a pistachio orchard planted with 12-year-old trees of Akbari cultivar (*P. vera* cv Akbari) and nearly flat. Prior to initiation of the experiment, irrigation water and soil samples were analyzed. Table 1 presents information on physical and chemical properties of the soil of the experimental site. The experimental design was a completely randomized design (RCD) with five replications. Regarding to traditional irrigation of pistachio orchard in the region four irrigation treatments were applied as: irrigation interval of 4 days (T_4) (as control), 12 days (T_{12}), 18 days (T_{18}) and 24 days (T_{24}). The irrigation intervals

corresponding to the soil water content in the treatments (4, 12, 18 and 24 days), are resembled to 20, 15.6, 10.4 and 6 % FC, respectively (Table 2). The volume of water used for all irrigation treatments was the same ($45 \text{ L.m}^{-2} = 45 \text{ mm}$). Irrigation was set

in accordance to the moisture retention curve of the soil, measured using a pressure plate set. A water-flow meter and a potentiometer were used to measure the amount of applied irrigation water and soil water content, respectively (Ranjbar, 2017).

Table 1. Some physicochemical properties of the experimental soil.

Soil depth (cm)	pH	EC dSm ⁻¹	T.N.V. %	Silt %	Clay %	Sand	S.P %	Soil texture
0-30	8.60	5.68	23.11	14.6	15.1	70.4	29.4	SL*
30-60	8.70	5.45	22.36	13.7	14.90	71.10	28.7	SL
60-100	8.70	5.32	23.14	14.7	13.23	72.3	24.4	SL

*sandy loam

Table 2. Soil moisture content based on dry weight for FC and PWP at different soil depth.

Soil depth (cm)	FC (%W)	0.96 FC	0.75 FC	0.50 FC	0.30 FC	PWP (%W)
0-30	22.15	21.26	16.61	11.07	6.37	8.02
30-60	21.22	20.37	15.91	10.61	6.11	7.50
60-100	19.04	18.27	14.28	9.52	5.48	7.00
average	20.80	20	15.6	10.4	6	7.50

Measurement and calculation of chlorophyll

fluorescence parameters

Measurement of chlorophyll fluorescent variables was carried out using a pulse amplitude modulation fluorometer (PAM-2500, Walz, Effeltrich, Germany). F_0 and F_m were measured in 30 min dark-adapted leaves. Thereafter, the same leaves were light saturated and F_s and maximum fluorescence were

measured. Based on the determined fluorescent parameters, some basic fluorescence variables like F_v , F_v/F_m , Φ_{PII} , F_s/F_0 , NPQ, and ETR can be calculated. Photo-inhibition (PIHN) was calculated as described by Dodd *et al.* (1998) (Table 3).

Table 3. Quantifications of PAM chlorophyll fluorescence parameters applied in the present study.

Parameter	Definition	Formulation	Physiological relationship
F_0, F_m	Base and maximum fluorescence parameters from dark adapted leaves	measured parameters	Level of fluorescence when Q_a is maximally oxidized and reduced, respectively (Roháček, 2000)
F'_0, F'_m, F_s	Base, maximum and steady state parameters from light adapted leaves, respectively.	measured parameters	The parameters provide information on photosynthetic performance. Generally, $F'_0 < F_s < F'_m$ (Roháček, 2000)
F_v/F_m	Maximal quantum yield of PSII at dark saturated state	$(F_m - F_0)/F_m$	Maximum efficiency at which light absorbed by PSII. In healthy plants, this ratio changes in a range between 0.75 and 0.80 (Bolhar Nordenkampf and Oquist, 1993).
F'_v/F'_m	Maximum efficiency of PSII at light saturated state	Genty parameter	Conversion efficiency of the energy of electrons by open reaction centers of PSII into chemical energy (Schreiber <i>et al.</i> , 1994)
F_0/F_m	Quantum yield baseline	calculated parameter	The ratio of base fluorescent to minimal fluorescence in dark-adapted leaves (F_0/F_m) is a useful parameter in detection of stress effect on the activity of PSI (Roháček, 2000)

Table 3. Continued

Φ_{PII}	Effective quantum yield of PSII	$(F'_m - F_s) / F'_m$	Fraction of photons absorbed in PSII photochemistry (Roháček, 2000)
ETR	Electron transport rate	$(\Phi_{PII} \times \text{PPFD} \times 0.5 \times 0.84)$	The parameter is an agent for monitoring of gross photosynthesis, and strongly correlates with leaf CO_2 exchange rates (Flexas <i>et al.</i> , 2014)
qP	Photochemical quenching	$(F'_m / F_s) / F'_v$	Relates PSII maximum efficiency to operating efficiency. Non-linearly related to proportion of PSII centers that are open. In total, $0 < q_p < 1$ (Roháček, 2000)
NPQ	Non-photochemical quenching or relative rate constant of regulated thermal dissipation	$(F_m / F'_m) - 1$	Monitors the rate constant for heat loss from PSII (Roháček, 2000)
PIHN	photoinhibition	$\text{PIHN (\%)} = 100 - [(F_v / F_m \text{ noon}) / (F_v / F_m \text{ predawn})] \times 100]$	The parameter is characterized by a sustain depression of F_v / F_m (Dodd <i>et al.</i> , 1998)

Measurement of photosynthetic pigments and leaf water status indices

Foliar photosynthetic pigment contents including chlorophyll a (Chl. *a*), Chlorophyll b (Chl. *b*), total chlorophyll content (Chl. *a+b*), and carotenoids (*Car*) were measured during the experiment course. The foliar analysis was carried out on the samples collected from the same leaves used for measuring the basic chlorophyll fluorescence variables. Pigments were extracted from fresh leaf samples following the methodology of Arnold (1949). Aliquots of the extracts were read at 645, 663, and 480 nm using a spectrophotometer (U-2001-Hitachi) against 80% acetone as a blank cuvette. The *Car* concentration was estimated using the formula of Kirk and Allen (1965). Pigment contents were calculated and expressed in milligram per gram fresh mass (mg g^{-1}). Total chlorophyll degradation (TCD) was calculated using method presented by Sivakumar *et al.*, 2017.

Predawn leaf water potential (Ψ_{pd}) was measured 1 hour before the daybreak. Within this time, leaf water potential is in balance with the water potential of the soil (Williams and Araujo 2002). The Ψ_{pd} was measured with 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

Comparison of mean differences among water deficit treatments were carried out through the analysis of variance (ANOVA) and Duncan's test. Data were analyzed with SPSS 18.0 for windows.

Results

Soil water deficiency altered fluorescence parameters

The effect of various water-regime treatment (T_4 , T_{12} , T_{18} and T_{24}) was statistically significant ($p \leq 0.01$) for all evaluated leaf fluorescence parameters. The results showed that negative of drought stress on fluorescence variables were initiated from T_{18} and continued to T_{24} (Table 4). A significant increase in F_0 was observed at T_{18} and reached the highest value at T_{24} . A notable reduction in F_m was observed with decreasing soil water potential at T_{18} with the lowest value at T_{24} . The similar trend was observed also for F_v and F'_m . The first three watering regimes did not show any significant change in F_v / F_m ratio but a noticeable reduction was observed at T_{24} . The value of F'_v / F'_m also decreased significantly due to water regime treatments. A significant reduction in the parameter was observed at T_{18} and reached the lowest value at T_{24} . However, a similar trend was observed for F_s / F_0 . Results depicted in Table 4 show that Φ_{PII} and *ETR* decreased significantly due to the imposition of water deficit regimes. The highest

reduction in these properties was observed at T₂₄. Water deficit stress severely decreased the value of *qP*, so that the highest and the lowest values in the parameter were observed at T₄ and T₂₄, respectively. On the contrary, NPQ showed an increasing tendency

due to the reduction of soil water potential, the significant increment was observed at T₁₈ and continued to T₂₄. Data presented in Table 4 show an increasing trend in PIHN and a drastic enhancement at T₁₈, which reached the highest value (31%) at T₂₄.

Table 4. The function of chlorophyll fluorescence parameters (CFPs) in pistachio trees (*P. vera* L.) subjected to different water-regime treatments (WRTs).

CFPs WRTs	F _m	F _v	F _v /F _m	F' _v /F' _m	F ₀ /F _m	ΦPII	ETR	<i>qP</i>	NPQ	PIHN (%)
Control (T ₄)	774 ^a	608 ^a	0.78 ^a	0.72 ^a	0.20 ^a	0.55 ^a	82 ^a	0.54 ^a	0.35 ^a	7 ^a
T ₁₂	773 ^a	606 ^a	0.78 ^a	0.73 ^a	0.20 ^a	0.55 ^a	83 ^a	0.54 ^a	0.34 ^a	6 ^a
T ₁₈	760 ^b	580 ^b	0.76 ^a	0.69 ^b	0.24 ^b	0.50 ^b	0.75 ^b	0.43 ^b	0.42 ^b	17 ^b
T ₂₄	743 ^c	542 ^c	0.73 ^{ab}	0.64 ^c	0.27 ^c	0.42 ^c	0.68 ^c	0.38 ^c	0.50 ^c	31 ^c
ANOVA										
<i>F</i>	35.113	142.124	109.133	22.800	51.363	355.127	3.364	1.374	67.08	32.047
<i>MS</i>	870.50	3457.56	0.003	0.018	0.226	16.604	0.037	460.293	0.021	0.036
<i>P</i>	0.00**	0.00**	0.05*	0.00**	0.00**	0.00**	0.006**	0.00**	0.00**	0.00**

Means followed by the same letter for each tested parameter are not significant by Duncan's test ($P < 0.01$)

Reaction of pigment contents to soil water

Deficiency

The results on the effect of water-regime treatments on the pigment parameters in the leaves of *P. vera* are shown in Table 5. A significant reduction in leaf pigment contents was observed in T₁₈ and, continued to the lowest value in T₂₄. No significant

difference was observed among water water-regime treatments with respect to Chl. (*a/b*). (Chl. *a+b*)/*Car* ratio was significantly affected by soil water content (water-regime treatment) at T₁₈ and continued without drastic decreasing to T₂₄ (Table 5).

Table 5. Effects of water-regime treatments (WRTs) on the values of foliar pigment contents (FPCs) and total chlorophyll degradation (TCD) in *P. vera* trees during the experimental period.

FPCs WRs	Chl. <i>a</i> (mgg ⁻¹ FW)	Chl. <i>b</i> (mgg ⁻¹ FW)	Chl. <i>a+b</i> (mgg ⁻¹ FW)	<i>Car</i> (mgg ⁻¹ FW)	TCD (%)	Chl. (<i>a+b</i>) / <i>Car</i>
Control (T ₄)	1.01 ^a	0.63 ^a	1.64 ^a	0.75 ^a	0.00	2.19 ^a
T ₁₂	0.98 ^a	0.62 ^a	1.60 ^a	0.74 ^a	2.44 ^a	2.16 ^a
T ₁₈	0.76 ^b	0.52 ^b	1.28 ^b	0.63 ^b	22.00 ^b	2.03 ^b
T ₂₄	0.65 ^c	0.43 ^c	1.08 ^c	0.54 ^c	34.14 ^c	2.00 ^b
ANOVA						
<i>F</i>	236.548	36.511	46.841	23.532	31.172	0.574
<i>MS</i>	0.122	0.035	0.038	0.220	0.047	0.020
<i>P</i>	0.000***	0.000***	0.000***	0.000***	0.000***	0.050*

Means followed by the same letter for each tested parameter are not significant by Duncan's test ($P < 0.01$)

Water-regime treatments induced changes in leaf**water status indices**

The data presented in Table 6 showed that the water-regime treatment induced significant changes

in Ψ_{pl} , Ψ_{pr} , and RWC in leaves of pistachio tree.

Table 6. Effects of water-regime treatments (WRTs) on the values of leaf water status parameters (LWSPs).

LWSPs	Ψ_{pl} (MPa)	Ψ_{pr} (MPa)	RWC (%)
WRTs			
Control (T ₄)	-1.60 ^a	-2.10 ^a	93 ^a
T ₁₂	-1.70 ^a	-2.10 ^a	91 ^a
T ₁₈	-2.00 ^b	-2.60 ^b	86 ^b
T ₂₄	-2.30 ^c	-2.88 ^c	81 ^c
ANOVA			
<i>F</i>	8.5571	15.429	17.895
<i>MS</i>	0.400	0.360	113.333
<i>P</i>	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}

Means followed by the same letter for each tested parameter are not significant by Duncan's test ($P < 0.01$)

The results showed that RWC decreased under soil water deficit, the highest (93%) and the lowest (81%) values were observed in T₄ and T₂₄, respectively. However, highly significant ($P < 0.01$) differences were observed among water-regime treatments with respect to Ψ_{pl} . Similar trend was observed for Ψ_{pr} (Table 6).

The decrease of Ψ s provided the decline in F_v/F_m . Plants in T₄ and T₁₂ showed F_v/F_m ratio within the range of healthy plants (values between 0.750 and 0.840) (Bolhar-Nordenkampf and Oquist, 1993).

Discussion

As shown in Table 3, soil water deficit showed a significant increase in F_0 and a significant decrease in F_m and F_v . A significant increase in F_0 characterizes inactivation of PSII, whereas a decline in F_m and F_v may attribute to the increase in NPQ process (Roháček, 2000). However, during the examination, reduction of nearly 11% was observed in F_v/F_m , which shows the inappropriate functioning of PSII reaction centers due to the destruction of the D1 and

D2 proteins responsible for the transfer of electrons to PSII reaction center (Maria *et al.*, 2006). Soil water deficit led to an increase in F_0/F_m . A significant rise in F_0/F_m depicts that the initial rate of reduction of the Q_a was higher than the rate of Q_b and the activity of PSI when trees were subjected to lower soil water potential (Ranjbar, 2017). Roháček (2002) suggested the increase relation F_0/F_m as stress indicator. While For healthy, non-stressed plants, it is mostly $0.14 \leq F_0/F_m \leq 0.2$.

A statistically significant reduction of Φ PSII and ETR under water-regime treatments (T₁₈ and T₂₄) might be attributed to harm to the photochemical complexes of the thylakoid membranes and consequently reduction of electron transport between photosystems I and II. Our findings confirm previous findings obtained by Hailemichael *et al.* (2016), who demonstrated that Φ PSII, ETR and qP decreased significantly in non-irrigated in comparison to irrigated grapevines. NPQ reflects the thermal loss of excessive excitation energy in the chloroplasts. In the present study, the obtained results showed a drastic

increase in NPQ with a decrease in soil water content. This result is in line with those of Alves *et al.* (2013), Ranjbar (2015), and Ranjbar (2017) who reported a strong increase in NPQ as soil water deficit progressed. Compared to the control, the degree of PIHN in the leaves was 2.42 and 4.43 folds in T₁₈ and T₂₄, respectively, which showed that soil water deficit development is an important factor affecting the level of measured PIHN. Our findings on PIHN are in agreement with the previous findings reported by Pokorska *et al.* (2007).

Chlorophyll indices are greatly used to describe the general function of the photosynthetic apparatus (Zhang *et al.*, 2011). Our results showed that foliar pigment contents (Chl. *a*, Chl. *b*, Car and TCD) were significantly affected by water-regime treatments. Several reports have confirmed the essential role of Car in photoinhibition. The decrease in Chl. (*a+b*)/Car under drought stress shows the important function of Car in photoprotection (Neha *et al.*, 2014). The reduction in photosynthetic pigments combined with significant changes in fluorescent parameters such as reduction of F'_v/F'_m , Φ_{PII} , ETR and qP under soil water deficit stress shows the detrimental effect of drought stress on photosynthetic apparatus of pistachio trees. Such an effect can be ascribed to damage of PSII, which eventually results in photoinhibition. Our results on leaf pigment content were consistent with those of Anjum *et al.* (2011) in the maze, Ranjbar (2015) in *Smirnovia Iranica*, and Hidelblandi *et al.* (2017) in *Atriplex nummularia*.

Results presented in Table 6 show the effect of soil water deficit on leaf water status of the examined trees. By decreasing soil water potential, a significant reduction of Ψ_{pl} , Ψ_{pr} , and RWC was observed at T₁₈ and T₂₄. Several reports showed that the RWC values declined under drought stress (Ranjbarfordoei *et al.*, 2002; Rahbarian *et al.*, 2011; Shekari *et al.*, 2015). Our results confirm previous findings obtained by Li

et al., (2002), who demonstrated that any decrease in soil water content leads to a further decline in Ψ_{pl} and Ψ_{pr} . A reduction in Ψ_{pr} can be attributed to either a decrease in osmotic water fraction or an osmotic adjustment, physiological mechanisms that act to maintain leaf turgor pressure (Zlatev and Lidon, 2012).

Conclusions

From the results of the present study, it can be concluded that exposure of pistachio trees (*P. vera* cv Akbari) to a rate of soil water potential close to T₁₈ may not significantly affected the parameters measured. This means that with an increase in irrigation intervals to 16-days, compared to the traditional interval (varies from 8 to 10-days), soil may be able to provide enough moisture for the pistachio trees to complete metabolic activities. With respect to all the parameters investigated in the current study, it seems that PSII activity in *P. vera* cv Akbari leaves is efficiently protected. Thus, under proper irrigation, pistachio tree could adapt itself to prolong drought stress.

Acknowledgements

This research was supported by the University of Kashan.

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