

Comparative Study on the Effect of Water Stress and Rootstock on Photosynthetic Function in Pistachio (*Pistacia vera* L.) Trees

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Received: 8 February 2017

Accepted: 9 July 2017

Abstract

The aim of this study is to evaluate the effects of water deficit stress on chlorophyll fluorescence (CF) characteristics of photosystem II (PSII) and pigment contents in two rootstock seedlings (*Pistacia atlantica* L. and *P. khinjuk* L.). Three levels of soil water potential (Ψ_s) was used, including WWD (-0.05 MPa), MWD (-0.7 MPa) and SWD (-1.5 MPa). It was found that water stress increased the minimal fluorescence (F_0), quantum yield baseline (F_0/F_m) and decreased the maximal fluorescence (F_m) and maximum quantum yield of PSII photochemistry (F_v/F_m) parameters in dark adapted leaves. In light adapted leaves, a significant increase in non-photochemical quenching (NPQ) and thermal dissipation of light energy to heat (D) and a decrease in electron transport rate (ETR) and photochemical efficiency of photosystem II (Φ_{PSII}) occurred. The results demonstrated a decline in photosynthetic pigments (Chl*a*), (Chl*b*) and carotenoids (Car) content with increasing water stress, whereas there was no significant effect on Chl (a/b) and Car/(a+b) ratios. Our data revealed there was no different in terms of performance between the two rootstocks in the alteration rate of pigment contents and photosynthetic features against soil water deficit conditions.

Keywords: Minimal fluorescence electron transport, Photosystem, Pigments, Quantum yield, Water deficit.

Introduction

The pistachio tree (*Pistacia vera*) is a native of western Asia and Asia minor, where it can still be found growing wild (Crane and Iwakiri, 1981). It has traditionally been cultivated in semi-arid areas of the Middle East and the Mediterranean basin. Pistachio is the largest orchard product in Iran in terms of area under cultivation and foreign exchange revenues. It accounts for 60% of all agricultural exports. During the last decade, a prolonged drought climate has severely decreased the availability and quality of water. These severe conditions in pistachio-producing areas have resulted in different problems such as low productivity and slender trees (Azizi *et al.*, 2013). Rootstock selection is important in any pistachio orchard and must

be attended at the time of the establishment of the orchard. Selection of the most suitable and compatible rootstock and scion to obtain the highest yield and quality as well as the resistance to biotic and abiotic all have considerable economic importance (Fotouhi Ghazvini *et al.*, 2007). Pistachio seedling rootstocks from different species or hybrids have been reported to significantly affect the vigour and nutrient status of the tree, early nut production, alternate bearing, soil-borne diseases, cold, drought, and salt tolerance (Piccioni and Miyamota, 1990; Ranjbar Fordoei *et al.*, 2001; Karimi *et al.*, 2009; Esmaeilpour *et al.*, 2015). Photosynthesis is a core function of a plant, which captures light energy to produce both chemical energy and reduce equivalents

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via photochemical processes (Zhang *et al.*, 2011). Plants in a field are exposed to a wide variety of abiotic stresses including excessive light, extreme temperatures, water stress, and atmospheric pollutants, which can directly or indirectly affect the photosynthetic function. Water stress is one of the most important limitations leading to a significant inhibition of photosynthesis. Hence, the ability to maintain the functionality of the photosynthetic machinery under water stress is of major importance in drought tolerance (Zlatev, 2009). For most plants, water deficit leads to stomatal closure and reduces photosynthesis. Several studies showed that PSII is highly drought-resistant (Yordanov *et al.*, 2003). However, under conditions of water stress, the photosynthetic electron transport through PSII is repressed (Chakir and Jensen, 1999). Many studies suggested that such water deficit results in damages to the oxygen-evolving complex of PSII (Huseynova, 2012) and to the PSII reaction centres associated with the degradation of D1 protein (Zlatev and Yordanov, 2004). Non-photochemical quenching (NPQ) reflects the thermal dissipation of excessive excitation energy in the chloroplasts and has a strong link with water status (Liu *et al.*, 2012). A sustained decrease in dark-adapted maximum quantum yield of PSII photochemistry (F_v/F_m) and an increase in minimal fluorescence yield (F_0) indicates damage to photosystem II, which reduces the efficiency of absorbed light energy transfer from the light-harvesting complex. These alterations may result from water deficit (Maxwell and Johnson, 2000). Souza *et al.* (2004) observed a tendency for

higher minimal fluorescence yield values and lower maximal fluorescence yield (F_m) in water-stressed versus non-stressed plants. Alves *et al.* (2013) demonstrated that Φ PSII, ETR and qP decreased significantly in water stressed in comparison to irrigated plants. One of the plant responses to drought stress is change in photosynthetic pigment content. 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). Drought stress decreases the content of chlorophyll *a* and *b* binding proteins, leading to a reduction of the light-harvesting pigment protein associated with photosystem II (Sayed, 2003). The aim of this study was to determine the effects of drought stress on chlorophyll fluorescence parameters in the leaves of two pistachio rootstocks (*P. atlantica sub sp. Mutica* and *P. khinjuk*).

Materials and Methods

This experiment was carried out during the growing seasons of 2015–2016 for two years in a pistachio orchard, in a sandy loam soil, located in Kashan, Iran. The prevailing climate there is of the arid type. Average annual temperature is about 19°C. Total annual precipitation amounts to about 110 mm, with an uneven distribution throughout the year, while evaporation is 2500–2700 mm annually without summer rainfall. Total radiation is 2900–3100 h per year (Arbaby, 2010). Soil physical and chemical properties of the experiment field are presented in Table 1.

Table 1. Soil chemical and physical properties of the experiment field

| Clay (%) | Silt (%) | Sand (%) | pH | Active lime (%) | N (%) | P (ppm) | K (ppm) |
|----------|----------|----------|------|-----------------|-------|---------|---------|
| 14.9 | 14.7 | 70.4 | 7.81 | 23.11 | 0.002 | 2.1 | 146 |

The experimental design was a factorial arrangement (3×2) in randomized blocks (RCBD) with five replicates. Three water treatments were used for the main plots, which comprised subplots with two rootstocks (*P. atlantica sub sp. Mutica* and *P. khinjuk*)

grafted in pistachio nut (*P. vera Cv. Ohadi*), as the following: irrigation to achieve soil water potential of -0.05MPa (Ψ_s = -0.05 MPa), -0.7 MPa (Ψ_s = -0.7 MPa), and -1.5 MPa (Ψ_s = -1.5 MPa) without water deficit (WWD), moderate water deficit (MWD), and severe

water deficit (SWD) treatments, respectively. Irrigation was set in accordance to the moisture retention curve of the soil. A water-flow meter and a potentiometer were used to measure the amount of applied irrigation water and soil water content, respectively. Conventional cultural practices were implemented, including fertilizer application and weeds control.

Measurements

Chlorophyll fluorescence analyses

Chlorophyll fluorescence parameters were measured using a pulse amplitude modulation chlorophyll fluorometer *PAM-2500* (H. Walz, Effeltrich, Germany). Measurements were made on the uppermost fully expanded leaves. Before measuring chlorophyll fluorescence yields (CFYs), the leaves were put in dark adapted state (DAS) for at least 30 minutes (Genty *et al.*, 1989) using light exclusion clips. During DAS, all reaction centers and electron carriers of the PSII were reoxidized, which is essential for rapid fluorescence induction kinetics and for recording chlorophyll fluorescence parameters.

The following CFYs were measured: minimal fluorescence yield in the dark and light-adapted states (F_0 and F_0'), maximal CFY in the dark and light-adapted states (F_m and F_m') and steady state fluorescence yield in light adapted state (F_s) (Zhang *et al.*, 2011).

Some basic, mutually independent, chlorophyll fluorescence parameters (FPs), such as maximal variable fluorescence ($F_v = F_m - F_0$), maximum quantum yield of PSII photochemistry (F_v/F_m), photochemical efficiency of photosystem II (Φ_{PSII}), non-photochemical quenching ($NPQ = NF/F_m$), electron transport rate (ETR) and thermal dissipation of light energy to heat ($D = 1 - F_v'/F_m'$) parameters can be calculated from CFYs. This gives insight into the

photosynthetic processes in chloroplasts and can be used effectively in photosynthesis research (Lichtenthaler *et al.*, 2005; Ranjbarfordoei *et al.*, 2006; Zelatev, 2009).

Pigment extraction and quantification

The measurements of pigment contents in the leaves of the studied plants were performed during the experimental period. Analyses were accomplished in samples collected from the same leaves upon which the FPs were determined. Chlorophyll concentrations were determined following the methodology of Arnon (1949). Half-a-gram of fresh leaf material was ground with 10 ml of 80% acetone at 4°C and centrifuged at 2500xg for 10 minutes at 4°C. This procedure was repeated until the residue became colourless. The extract was transferred to a graduated tube and made up to 10 ml with 80% acetone and immediately assayed.

Three ml of 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. The carotenoid content 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} FM).

Results

Table 2 shows a significantly negative effect of water deficiency stress on the values of chlorophyll fluorescence parameters in the assimilatory organs of the studied rootstock plants (*P. atlantica* and *P. khinjuk*). The species showed increases in F_0 with increasing water deficiency ($p < 0.01$). In contrast, the F_m value decreased with progressing water deficit ($p < 0.01$). *Pistacia atlantica* showed higher F_m at all water treatments, while *P. khinjuk* had lower mean values of F_m (Table 2).

Table 2. Effects of water deficit treatments on the values of FPs in *P. atlantica* and *P. khinjuk* plants during the experimental period

| Treatments | F _m | F ₀ | F _v /F _m | F ₀ /F _m | NPQ | ΦPSII | D | ETR |
|---------------------|-------------------------|------------------------|--------------------------------|--------------------------------|------------------------|------------------------|-------------------------|----------------------|
| <i>P. atlantica</i> | | | | | | | | |
| WWD | 1220±37 ^a | 244±12 ^a | 0.80±0.02 ^a | 0.20±0.02 ^a | 0.68±0.07 ^a | 0.50±0.01 ^a | 0.40±0.005 ^a | 49±4 ^a |
| MWD | 1143±14 ^b | 268±8 ^b | 0.76±0.01 ^a | 0.23±0.01 ^b | 0.71±0.03 ^b | 0.47±0.04 ^b | 0.48±0.01 ^b | 47±6 ^b |
| SWD | 965±20 ^c | 337±9 ^c | 0.65±0.03 ^b | 0.35±0.03 ^c | 0.85±0.06 ^c | 0.35±0.01 ^c | 0.59±0.01 ^c | 28±4 ^c |
| <i>P. khinjuk</i> | | | | | | | | |
| WWD | 966±18.7 ^a | 197±12.25 ^a | 0.82±0.01 ^a | 0.20±0.01 ^a | 0.71±0.03 ^a | 0.50±0.01 ^a | 0.42±0.01 ^a | 57±7 ^a |
| MWD | 927±9.16 ^b | 217±15.94 ^b | 0.77±0.01 ^b | 0.23±0.02 ^b | 0.80±0.05 ^b | 0.46±0.01 ^b | 0.41±0.03 ^b | 52±4 ^b |
| SWD | 771±14.52 ^c | 268±10.23 ^c | 0.66±0.02 ^c | 0.35±0.02 ^c | 0.96±0.04 ^c | 0.40±0.08 ^c | 0.52±0.03 ^c | 38±5 ^c |
| ANOVA | | | | | | | | |
| WT | 167905.54 ^{**} | 10286.52 ^{**} | 0.123 [*] | 0.0587 ^{**} | 0.070 ^{**} | 0.025 [*] | 0.034 [*] | 697.81 ^{**} |
| PR | 66978.28 ^{**} | 17112.61 ^{**} | 0.013 ^{**} | 0.0098 ^{**} | 0.029 ^{**} | 0.000 ^{**} | 0.008 ^{**} | 257.43 ^{**} |
| WT × PR | 25335.36 ^{**} | 242 ^{ns} | 0.006 ^{**} | 0.0035 ^{**} | 0.003 ^{ns} | 0.002 ^{**} | 0.004 ^{**} | 8.29 ^{ns} |

Means followed by the same letter for each tested parameter are not significant by Duncan's test ($P < 0.01$) and presented by \pm SD.

The increase of water-deficit stages provided the decline in F_v/F_m . All plants in WWD and MWD showed the F_v/F_m ratio within the range of healthy plants (values between 0.76 and 0.82) (Bolhar-Nordenkamp and Oquist, 1993). However, increasing water deficiency stress resulted in a significant reduction in F_v/F_m ratio in SWD.

In response to water deficit, the quantum yield baseline (F_0/F_m) of both rootstocks showed a clear gradient from low values in WWD to high values in SWD. However, the development of F_0/F_m in response to the water deficit was quite similar between the two rootstocks. A drastic increment in NPQ was observed by water deficit treatments. This increment was higher in *P. atlantica* than in *P. khinjuk* (Table 2).

The ΦPSII showed decreases in the increasing level of water deficit stress ($p \leq 0.01$). The quenching of light energy focused on the photosynthetic apparatus to increase heat with increasing water deficit ($p < 0.05$). The increase in the dissipation of light energy in the form of heat (D) reached average values of 34% in the *P. khinjuk* and the *P. atlantica* 24% when exposed in SWD (Table 2). Regarding electron ETR, the plants from the studied rootstocks were significantly affected and presented a reduction of 23.5% (*P. atlantica*) and 21% (*P. khinjuk*).

The results on the effect of water-deficit treatments on the pigment parameters in leaves of *P. atlantica* and *P. khinjuk* are presented in Table 3. On average, Chl *a*, Chl *b*, Chl (*a* + *b*), and Car contents were higher in *P. khinjuk* than in *P. atlantica*.

Table 3. Effects of water deficit treatments on the values of pigment content in *P. atlantica* and *P. khinjuk* plants during the experimental period

| Treatments | Chla (mg g ⁻¹ FM) | Chlb (mg g ⁻¹ FM) | Chl (a + b) (mg g ⁻¹ FM) | Car (mg g ⁻¹ FM) | Chl (a/b) | Car / (a + b) |
|---------------------|---------------------------------|---------------------------------|--|-----------------------------|------------------------|------------------------|
| <i>P. atlantica</i> | | | | | | |
| WWD | 1.050±0.12 ^a | 0.840±0.17 ^a | 1.89±0.02 ^a | 0.96±0.07 ^a | 1.25±0.06 ^a | 0.51±0.09 ^a |
| MWD | 0.840±0.09 ^b | 0.760±0.14 ^b | 1.60±0.01 ^b | 0.81±0.10 ^b | 1.10±0.13 ^a | 0.50±0.10 ^a |
| SWD | 0.690±0.046 ^c | 0.600±0.11 ^c | 1.29±0.03 ^c | 0.70±0.07 ^c | 1.15±0.06 ^a | 0.45±0.02 ^a |
| <i>P. khinjuk</i> | | | | | | |
| WWD | 0.980±0.072 ^a | 0.870±0.06 ^a | 1.85±0.03 ^a | 0.98±0.06 ^a | 1.12±0.11 ^a | 0.53±0.05 ^a |
| MWD | 0.900±0.044 ^a | 0.780±0.09 ^b | 1.68±0.01 ^b | 0.86±0.09 ^b | 1.15±0.03 ^a | 0.51±0.08 ^a |
| SWD | 0.740±0.056 ^c | 0.660±0.017 ^c | 1.40±0.02 ^c | 0.72±0.06 ^c | 1.12±0.17 ^a | 0.51±0.05 ^a |
| ANOVA | | | | | | |
| WT | 0.13 ^{**} | 0.07 ^{**} | 0.41 [*] | 0.01 ^{**} | 0.03 ^{ns} | 0.003 ^{ns} |
| PR | 0.009 ^{ns} | 0.006 ^{ns} | 0.02 ^{ns} | 0.0004 ^{ns} | 0.02 ^{ns} | 0.003 ^{ns} |
| WT × PR | 0.008 ^{ns} | 0.003 ^{ns} | 0.01 ^{ns} | 0.006 ^{ns} | 0.006 ^{ns} | 0.015 ^{ns} |

Means followed by the same letter for each tested parameter are not significant by Duncan's test ($P < 0.05$) and presented by \pm SD.

In all studied seedlings, water deficit induced a dramatic reduction in Car. However, significant differences were found among water-deficit treatments, while the interactive effects of different water-deficit stress levels were not significant on Chl (a/b) and Car / (a + b) ratios (Table 3).

Discussion

The F_0 value showed a significant increase upon water deficit treatments. This value may increase if the transfer of excitation energy from the antenna to the reaction centres is impaired (Schreiber *et al.*, 1998). Thus, the increase in F_0 observed in the rootstock plants studied may be associated with damage to the photosynthetic apparatus, such as inactivation reaction centres of PSII (Ranjbar Fordoei *et al.*, 2013). In addition, the observed significant decrease in F_m can be associated with increased non-photochemical dissipation as heat associated with the xanthophyll cycle (Muller *et al.*, 2001).

Our results on F_v/F_m are similar to the results 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) with respect to *Lotus corniculatus*.

The F_0/F_m value was increased by water deficit in the both rootstock plants. The higher F_0/F_m indicates that the initial rate of reduction of the Qa is higher than the rate of Qb and the activity of PSI when plants are exposed to water stress (Cicero *et al.*, 2012). Rohmáček (2002) points to the increases in the ratio of F_0/F_m as stress indicative, suggesting normal values, observed between 0.14 and 0.20, as the standard.

A significant increase in NPQ was observed by water deficit treatments. A clear increase in NPQ indicates that energy cannot be utilized to transport photosynthetic electrons, and it must be dissipated harmlessly as heat energy from the PSII antennae (Kate and Giles, 2000). Our findings on NPQ are consistent with those previously reported by Liu *et al.* (2012) regarding maize cultivars.

The decrease in Φ_{PSII} can be ascribed to the increased rate of dissipation of photon energy to heat in

the PII antenna complexes as well as a decrease in the proportion of photons and excitation of electrons of chlorophyll (Schreiber *et al.*, 1998). The impact of water stress on the decrease of Φ PSII values has been similarly reported for *Prunus dulcis* (Miller) (Ranjbar Fordoei, 2006, *Smirnovia iranica* (Ranjbar, 2015), *Pyrus pyraster* L., and *Sorbus domestica* L. (Šajbidorova *et al.*, 2015). The increases in D reflect the activation of several processes of non-photochemical nature during the light period, mostly leading to non-radiative dissipation of the excitation energy as heat (Oxborough, 2004). Our findings on D are consistent with those previously reported by Ranjbar (2015) regarding *Smirnovia iranica* shrubs.

The ETR value shows the sum 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 decreased ETR for *Smirnovia Iranica* (Ranjbar, 2015) *Mangifera indica* (Cicero *et al.*, 2012), and *Vitis vinifera* (Qing-Ming *et al.*, 2008).

In both rootstock plants, the decreases of Chl ($a+b$) and Car in parallel with the decreases in F_v/F_m , indicate that the pigment breakdown was accompanied by a decrease in the decreasing maximum photochemical efficiency. Other authors explained this phenomenon as a photo-protection mechanism, in which reducing light absorbance results in a decrease in pigment contents (Galmes *et al.*, 2007; Elsheery and Cao, 2008). The decrease in the chlorophyll content under water stress is a commonly observed phenomenon (Reynolds *et al.*, 2005; Shekari *et al.*, 2015). Therefore, our results are in agreement with those of Ranjbar (2015). The decrease in the chlorophyll content under drought stress might be due to the reduced synthesis of the main chlorophyll pigment complexes encoded by the *cab* gene family (Allakhverdiev *et al.*, 2000), or destruction of

chiralmacro-aggregates of light harvesting Chl *a* or *b* pigment protein complexes (CHCIIIs) that protect the photosynthetic apparatus (Shirani Rad and Zandi, 2012), or due to the oxidative damage of chloroplast lipids, pigments and proteins (Mafakheri *et al.*, 2010).

In conclusion, the fluorescence parameters, measured in both light and dark states under water-deficit stress, could be used as a worthy measure to determine stress severity. The results demonstrated a content-dependent decline in photosynthetic pigments Chl a , Chl b , and Car content with increasing water stress, while no significant effect on Chl (a/b) and Car/ $(a+b)$ ratios were observed. Our data revealed that there was no difference in performance between the two rootstocks in terms of alteration the rate of pigment contents and photosynthetic features against soil-water-deficit conditions.

Acknowledgement

The author would like to acknowledge the University of Kashan for its financial support.

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