DOI: 10.1007/s11099-011-0064-5 PHOTOSYNTHETICA 49 (4): 531-538, 2011

Effect of mycorrhizal inoculation on ecophysiological responses of pistachio plants grown under different water regimes

V. BAGHERI[*], M.H. SHAMSHIRI[*,+], H. SHIRANI[**], and H.R. ROOSTA[*]

Department of Horticulture, Agricollege, Vali-e-Asr University of Rafsanjan, 7718897111, Rafsanjan, Iran[*]
Department of Soil Science, Agricollege, Vali-e-Asr University of Rafsanjan, 7718897111, Rafsanjan, Iran[**]

Abstract

In a greenhouse experiment, the influence of arbuscular mycorrhizal fungi (*Glomus mosseae* and *Glomus intraradices*) and water stress [100% field capacity (FC), 75% FC, 50% FC and 25% FC] on maximal quantum yield of photosystem II (PSII) photochemistry (F_v/F_m) and some other ecophysiological characteristics of two pistachio cultivar (*Pistacia vera cv*. Badami-Riz-Zarand and *Pistacia vera cv*. Qazvini) were investigated.

No difference was found in colonization rate between the two arbuscular mycorrhizal fungi (AMF) applied. Water stress reduced the mycorrhizal colonization in both cultivars at the same rate but the difference was significant just with severe water stress level (25% FC). The F_v/F_m was also adversely affected by water stress from 75% FC downwards in Qazvini cultivar while in Badami, increase in water-stress intensity had no significant effect on this parameter. Gas-exchange parameters were decreased with increasing stress intensity and chlorophyll (Chl) pigments were increased with mild water stress (75% FC) compared with control (100% FC) and then decreased with increasing stress intensity. The carotenoids (Car) content increased significantly in the stressed leaves in all water-stress levels irrespective of AMF treatment and cultivar type.

The adverse effects of water stress were significantly reduced by AM inoculation and in the most of measured parameters, both AMF had an equal influence except with the intercellular CO_2 concentration (C_i), where *G. intraradices* was superior. Results obtained from Chl fluorescence probe indicated that inoculated AMF enhanced photochemical efficiency of light reactions of the PSII in intact pistachio leaf tissues both under irrigation and water-stress conditions. Under mild and moderate water stress, mycorrhizal pistachio plants had higher relative Chl and Car content and higher gas-exchange capacity (increased photosynthesis and transpiration rate) but under severe water-stress condition (25% FC), the effects of mycorrhizal treatments were not noticeable. Data obtained in present study emphasized that Qazvini is more tolerant to water stress than Badami because photosynthesis activity in Qazvini was more efficiently protected than in the Badami, as indicated by related parameters.

Additional key words: chlorophyll fluorescence; Mycorrhizae; photosynthesis; pistachio; water stress.

Introduction

Pistachio is a subtropical fruit and has become one of the dominant crops in south-east regions of Iran especially in Kerman province. It is mainly produced in a variety of infertile, salty and alkaline soils and in environments subjected to varying periods of drought stress (Sheibani 1994). Pistachio is commonly known to possess a good capacity to withstand drought stress due to its deep root system and leaf structure. It has been claimed that the performance of pistachio trees under water stress surpasses that of all other fruit tree species (Spiegel-Roy *et al.* 1977). These authors stated that pistachio roots exploit soil moisture effectively at highly negative soil

Received 9 August 2010, *accepted* 14 July 2011.
[+]Corresponding author; tel: +98 391 3202013, fax: +98 391 3202042, e-mail: shamshiri88@gmail.com; shamshiri@vru.ac.ir
Abbreviations: AMF – arbuscular mycorrhizal fungi; Badami – Badami-Riz-Zarand; C_a – atmospheric CO_2 concentration; C_i – intercellular CO_2 concentration; Car – carotenoids; Chl – chlorophyll; E – transpiration rate; F_m – maximal fluorescence of dark-adapted state; F_v (= F_m – F_0) – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; F_0 – minimal fluorescence of dark-adapted state; FC – field capacity; FM – fresh mass; +M – mycorrhizal; –M – nonmycorrhizal; PI – performance index; PSII – photosystem II; P_N – net photosynthetic rate.
Acknowledgements: Here we would like to thank Vali-e-Asr University of Rafsanjan for financial support of the research and Pistachio Research Inst. of Iran (PRI) for providing the pistachio seeds. The results presented in this paper are a part of M.Sc studies of the first author.

water potentials. Although pistachio is reputed for its drought tolerance, limited information is available on its mechanisms of drought tolerance associated with photosynthesis (Vemmos 1994, Novello and de Palma 1995) especially in combination with arbuscular mycorrhizal fungi (AMF) symbiosis.

About the ability of pistachio for establishing symbiotic relations with AMF, Ferguson *et al.* (1998) reported that inoculation of three pistachio rootstocks (*P. atlantica*, *P. integerrima*, and UCBI) at 4–5-leaf stage resulted in colonization percentage ranged from 39 to 80% and there were no significant differences in colonization extent between rootstocks. Kafkas and Ortas (2009) inoculated two genotypes from each of *P. vera* (cvs.'Siirt' and 'Kirmizi'), *P. eurycarpa*, *P. atlantica*, and *P. terebinthus* with ten different mycorrhizal species. They found significant differences among *Pistacia* species in growth, nutrient uptake, and the percentage of mycorrhizal infection. Mycorrhizal infection was 70–95% in *P.vera*, 56.7–95% in *P. eurycarpa*, 53.3–95% in *P. atlantica,* and 51.7–91.7% in *P. terebinthus*.

PSII is well known for its sensitivity to abiotic stresses and hence it is a good choice to study response and adaptation to stress by plants (Strasser *et al.* 2000). F_v/F_m, a parameter commonly known as maximum quantum yield of primary photochemistry or maximal relative electron transport rate (ETR) of PSII reflects the potential quantum efficiency of PSII and is used as a sensitive indicator of plant photosynthetic performance, with optimal values of around 0.83 measured for most plant species (Maxwell and Johnson 2000). Values lower than this will be seen when the plant has been exposed to stress. Per definition, the performance index (PI) accounts for functionality of both photosystems I and II and provides a general quantitative value of the actual status of plant vitality by combining three independent parameters: (*1*) density of fully active reaction centers per Chl (= TR/ABS),where TR is the energy flux for trapping and ABS is absorption; (*2*) efficiency with which a trapped exciton moves an electron into the electron transport chain further than Q_A (= ET/TR), where ET is the energy flux for electron transport; and (*3*) the probability that an absorbed photon will be trapped by the reaction centre RC (= ET/ABS) (Strasser *et al.* 2000).

The objective of this study was hence to determine the mycorrhizal infection of two pistachio cultivars under different water-stress levels and to study the influence of AMF inoculation and water stress on the photochemistry of 'Badami-Riz-Zarand' (Badami) and 'Qazvini', the most popular rootstocks of pistachio in south-east of Iran, under greenhouse conditions.

Materials and methods

Experimental site: A greenhouse experiment was conducted in 2009 at the Agricollege of Vali-e-Asr university of Rafsanjan (30°23′06″N, 55°55′30″E), at 1,523 m a.s.l.

Preparation of AMF inocula: The AMF used in this study was originally recovered from pistachio (*Pistacia vera* L.) orchards grown in different sites of Rafsanjan, Kerman, Iran. These native isolates [*Glomus mosseae* (Nicolson and Gredemann) and *G. intraradices* (Schenck and Smith)] were maintained and propagated in wheat (*Triticum spp.*) pot cultures using autoclaved soil (as it is described below) in a greenhouse (T_{max}: 30 ± 3°C; T_{min}: 22 ± 2°C; RH: 58 ± 3%) and adequate amount of sterilized water was supplied every day during the period from February to May 2009. At maturity, the shoots of the wheat plants were removed and substrate was allowed to dry for a week at 30 ± 5°C. The roots were finely chopped and the dried root/soil mixture was thoroughly mixed to obtain a homogenous inoculum.

Soil preparation and seed sowing: A sandy loam soil was sterilized by autoclaving (121°C, 2 h) on 3 consecutive days in order to eliminate the indigenous endophytes. The major characteristics of the soil were as follows: sand 72.2%, silt 14.2%, clay 13.6%, pH 7.6, P 16 mg kg^{-1} soil, K 76 mg kg^{-1} soil, Fe 0.1 µg g^{-1}, Zn 1.29 µg $g^{-1,}$ Mn 1.3 µg g^{-1} , Cu 1.35 µg g^{-1}, and cation exchange capacity 2.65 dS m^{-1}.

Seeds of two pistachio rootstocks were incubated at 30°C on sterile moist cloth for one week. Five germinated seeds were sown into plastic pots (25 ×35 cm) containing 5 kg of autoclaved soil. The number of seedlings per pot was reduced to 3 within 21 days of germination.

Mycorrhizal inoculation: The inocula consisted of soil, wheat chopped root fragments, spores and hyphae colonized with one of each of the above fungi. 100 g of fresh mass (FM) of inoculum having on average of 75% of infected roots was placed on the soil surface and after placing the 5 germinated seeds on it, seeds were covered with sterilized sand. Control plants received the same amount of an autoclaved mixture of both inocula. Before starting the irrigation treatments, the seedlings were watered every day up to FC level with distilled water during 120 days.

Irrigation treatments: The four water regimes were 100% field capacity (FC) as control, 75% FC (mild stress), 50% FC (moderate stress), and 25% FC (severe stress). For determination of soil moisture content in FC, pots were saturated and kept for 48 h to let the gravimetric water be drained and then pots were weighed. The difference between pot mass after 48 h with initial pot mass (before saturation) was considered as soil water content in FC. In 100% FC treatment, individual pots were weighed, water added to bring the soil to the FC.

For 75, 50, and 25% FC treatments, pots received 75, 50, and 25% of water added to the 100% FC treatment, respectively. Thereafter, for 50 days (from 1 July to 20 August 2009), soil water contents were determined by weighing the pots daily and water was added following at the time of weighing to maintain the predetermined water content in each pot. During the experiment, the average PAR measured at noon ranged from 916 to 1,760 μmol m^{-2} s^{-1} (without additional artificial lightening), the maximum temperature was 35 ± 4°C, the minimum temperature was 21 ± 3°C and the relative humidity was 55 ± 5%.

Root sampling and assessment of arbuscular mycorrhizal colonization: Whole root system with soil was excavated from each plant and carefully rinsed with running tap water then the roots of 3 plants in each pot were mixed and cut into 1 cm long segments. Samples for mycorrhizal assessment were prepared according to method of Phillips and Hayman (1970). Roots were boiled for 1 h in 10% KOH and then washed with tap water. Staining was performed in 0.05% trypan blue for 5 min followed by washing with tap water. Samples were stored in lactoglycerol [mixture of lactic acid, glycerol, and water 1:1:1 (v/v/v)]. Root segments were mounted on glass slides and examined under a compound microscope (*CHS, Olympus Optical Co., Ltd.,* Japan). Mycorrhizal colonization (abundance of hyphae, vesicles, and arbuscules) was estimated according to Giovannetti and Mosse (1980) at 100 × magnification using 40 root segments of each sample.

Chl and Car contents were determined according to Lichtenthaler (1987). At the end of the experiment, 3 fully extended leaves from each pot (one leaf from each plant) were collected and wrapped in aluminium foil to avoid degradation of pigments by light. The extract was prepared from fresh leaves (1 g) by grinding in a cold mortar and pestle together with 10 ml of 80% aqueous acetone. After filtering, absorbance of centrifuged extracts was measured at 663, 645, and 470 nm using a spectrophotometer (*U-2000,Hitachi Instruments,* Tokyo, Japan).

Gas-exchange parameters and Chl fluorescence: Gas-exchange traits [net CO_2 assimilation rate (P_N), transpiration rate (E) and intercellular CO_2 concentration (C_i)] were measured between 10:00–12:00 h at the end of the experiment using a portable photosynthetic system (*LI-6200, LI-COR Inc.*, Lincoln, NE, USA). Top fully expanded leaf was clamped to the leaf chamber and the observations were recorded when RH and atmospheric CO_2 concentration (C_a) reached a stable value. Photosynthetically active radiation, air temperature, relative humidity and CO_2 concentration inside the sensor head were set at 1,300 ± 100 μmol m^{-2} s^{-1}, 32 ± 2°C, 60% and 335–340 μmol mol^{-1}, respectively, when measuring P_N. At the same time, Chl fluorescence parameters were measured with a portable handy *Plant Efficiency Analyzer* (*PEA, Hansatech Instruments Ltd*., UK). Three leaves were selected from each pot and pre-adapted to dark period for 20 min by fixing special tags on each leaf blade before measurements were taken. During dark adaptation, all the reaction centers are fully oxidized and available for photochemistry and any fluorescence yield is quenched. After 20 min of dark adaptation, the sensor cup was fitted on the leaf for measurement. The vitality state of the pistachio plants was characterized with the performance index PI (Strasser *et al.* 2000).

Experimental design: A completely randomized design method was adopted in the experiment with three mycorrhizal treatments, four irrigation regimes and two pistachio cultivars in five replications. The data were statistically analyzed by three-way analysis of variance using *MSTATC* software (Michigan State University, USA) and the means were separated by *Duncan*'s multiple range test (P<0.05). Correlation coefficients analysis was performed using *Minitab* software, version *11*.

Results

All water-stress levels and AMF treatments had significant effects on almost all of photosynthetic and Chl fluorescence parameters and pigments contents. These parameters were significantly different between two cultivars except the mycorrhizal infection percentage and total Chl. The interaction effects of applied treatments (M×S, M×C, and S×C) were significant for leaf pigments content (Table 1). Only severe water stress reduced the mycorrhizal colonization in both cultivars. Non-mycorrhizal (–M) plants showed no AMF colonization (Fig. 1).

Fig. 2*A* shows the maximum quantum yield of PSII photochemistry (F_v/F_m) of pistachio seedlings. Values of F_v/F_m below 0.800 were recorded for the plants of all treatments. The F_v/F_m was adversely affected by water stress from 75% FC downwards in Qazvini cultivar while in Badami the increase in water-stress intensity had no significant effect on this parameter. Surprisingly, well watered pistachio plants showed the lowest values in nearly all treatments. Mycorrhizal treatments improved F_v/F_m and PI significantly as compared with –M plants and the best results were obtained with Badami cultivar (Fig. 2*A*). Well watered plants in all treatments showed the lowest values of PI and the effect of water stress led to reduced PI values from 75% FC downwards for Qazvini cultivar whereas Badami maintained higher PI levels. In most of the cases PI values were decreased with increasing water-stress intensity except with –M Badami

Table 1. *ANOVA* results for mycorrhizal infection percentage (MI), chlorophyll fluorescence and photosynthesis parameters and pigments content of Badami and Qazvini pistachio cultivars (C) exposed to varying intensities of water stress (S) and different AMF (M) treatments. F_v/F_m – maximal quantum yield of PSII photochemistry, PI – performance index, P_N – net photosynthetic rate, E – transpiration rate, C_i – intercellular CO_2 concentration, Chl *a* – chlorophyll *a*, Chl *b* – chlorophyll *b*, Chl (*a*+*b*) – total chlorophyll, Car – carotenoids. *** – significant (P<0.001); ** – significant (P<0.01), * – significant (P<0.05); ns – not significant.

Parameters	M	S	C	M×S	M×C	S×C	M×S×C
MI%	***	***	ns	***	ns	ns	ns
F_v/F_m	*	*	***	ns	ns	ns	ns
PI	ns	***	***	ns	ns	*	ns
P_N	***	*	**	ns	*	ns	**
C_i	***	ns	***	***	***	ns	***
E	***	***	***	ns	ns	ns	*
Chl *a*	***	***	*	*	***	***	ns
Chl *b*	***	***	*	*	**	*	ns
Chl (*a*+*b*)	***	***	ns	*	**	**	ns
Car	***	***	**	***	***	*	ns

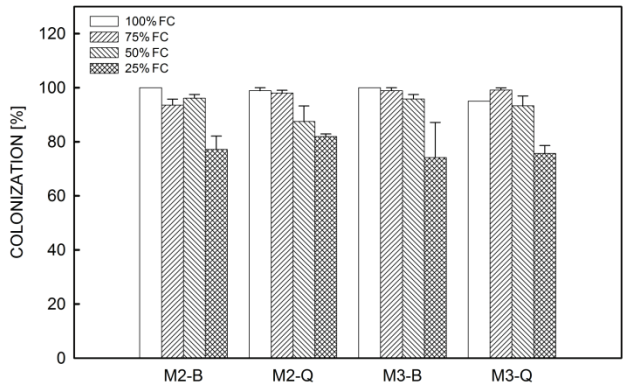

Fig. 1. Effect of different water-stress levels on mycorrhizal infection percentage (MI) in 'Badami' (B) and 'Qazvini' (Q). Values are means (n = 5) and the *vertical bars* indicate standard error. M2 – *G. mosseae*; M3 – *G. intraradices*.

plants or inoculated with *G. intraradices* (Fig. 2*B*, Table 1).

Net photosynthetic rate (P_N) was decreased significantly as the effect of different water deficit levels especially with 25% FC treatment, where a 44% reduction in mean P_N was recorded compared with control (100% FC) (Fig. 3*A*, Table 1). Root colonization by *G. mosseae* and *G. intraradices* enhanced P_N by 191 and 205%, respectively, irrespective of water-stress level and pistachio cultivar. The P_N of Qazvini was significantly higher compared with Badami cultivar (Fig. 3*A*, Table 1). Furthermore, Qazvini obtained the best P_N in combination with *G. intraradices* whereas Badami had the highest photosynthesis with *G. mosseae*. *E* was influenced adversely by water-stress extent where a negative relationship was found between stress intensity

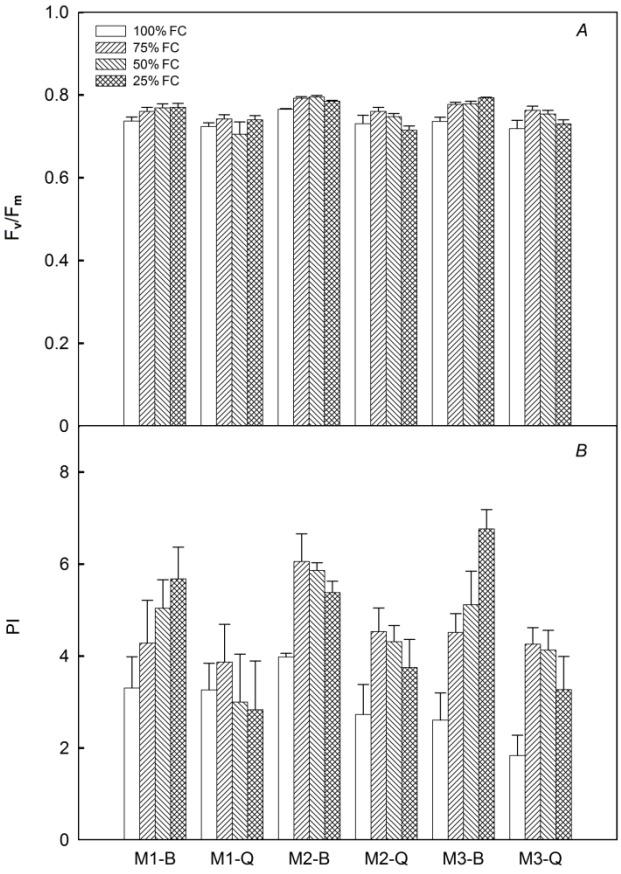

Fig. 2. Effect of mycorrhizae application and different water-stress levels on chlorophyll fluorescence parameters, F_v/F_m (*A*) and PI (*B*) in 'Badami' (B) and 'Qazvini' (Q). Values are means (n = 5) and the *vertical bars* indicate standard error. M1 – not inoculated with AMF; M2 – *G. mosseae*; M3 – *G. intraradices*.

and transpiration (Fig. 3*B*). C_i also decreased slightly with increase in water stress level but the differences were not significant (Fig. 3*C*). Applied mycorrhizal species influenced *E* of treated plants significantly and the highest value was obtained with *G. intraradices* on Qazvini cultivar. The results about C_i followed the same pattern where the highest value was recorded with the same AMF and cultivar. In general, *E* and C_i were significantly higher in Qazvini with respect to Badami regardless of water-stress and AMF treatments (Fig. 3*B*, *C*).

Chl pigments were increased with mild water stress (75% FC) compared with control (100% FC) and decreased with increasing stress intensity (Fig. 4*A–C*), especially with the 25% FC treatment. The Car content increased significantly in the stressed leaves in all water stress levels irrespective of AMF treatments and pistachio cultivar (Fig. 4*D*). Applied AMF caused a significant increase in all the Chl pigments and in the Car content compared with –M plants under different water stress levels (Fig. 4*A–D*). Mean Chl *a*, Chl *b*, and Car content were significantly higher in Badami than in Qazvini. Car/Chl was kept relatively stable in all water-stress

levels except with the severe water-stress (25% FC) treatment where a remarkable increase was observed especially in Qazvini cultivar (data not shown).

Total Chl showed significant correlation with fluorescence indices under severe water stress. The correlation value between Chl and F_v/F_m was 0.005 at 100% FC and 0.731 at 25% FC. Higher correlations were found between PI and total Chl content (Table 2). Gas-exchange parameters did not show significant correlation with fluorescence parameters. A strong correlation was found between Chl and Car content especially under mild and moderate water stress.

Discussion

Although mycorrhizal colonization levels have sometimes been reported to be unaffected by water stress (Nelsen and Safir 1982, Allen and Boosalis 1983, Simpson and Daft 1990), these results are in agreement with the work of Osonubi *et al.* (1991) and Busse and Ellis (1985). A reduction in mycorrhizal colonization by water stress is dependent on root exudates (Graham *et al.* 1982, Schwab *et al.* 1983) which under these conditions will be limited due to reduced photosynthesis, as stomata most often remain closed to conserve water. In addition,

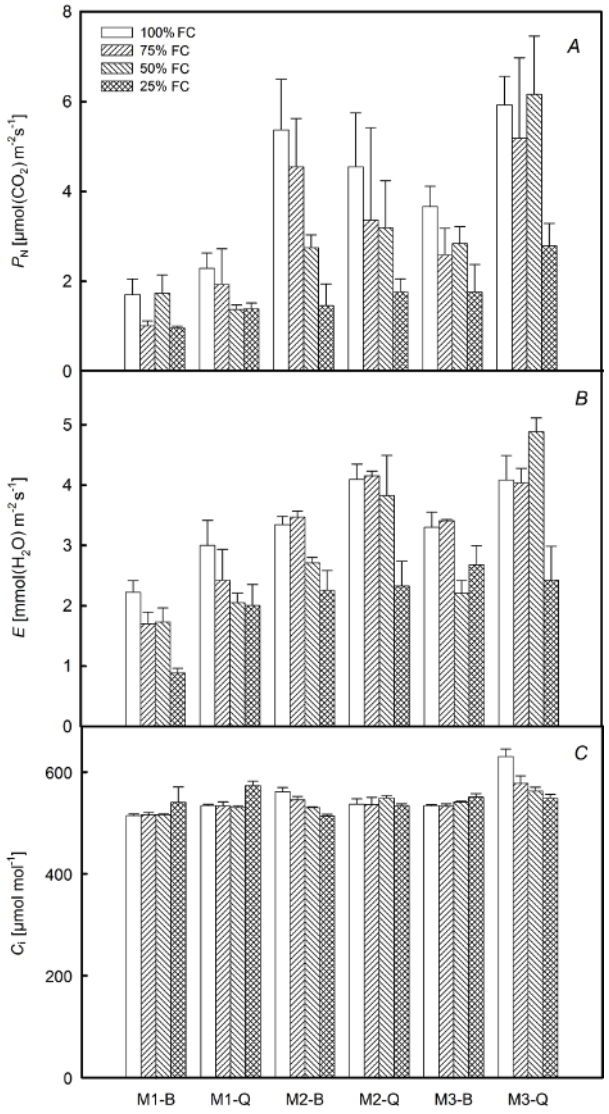

Fig. 3. Effect of mycorrhizae application and different water-stress levels on leaf gas-exchange parameters, Net photosynthetic rate, P_N (*A*), transpiration rate, *E* (*B*) and intercellular CO_2 concentration, C_i (*C*) in 'Badami' (B) and 'Qazvini' (Q). Values are means (n = 5) and the *vertical bars* indicate standard error. M1 – not inoculated with AMF; M2 – *G. mosseae*; M3 – *G. intraradices*.

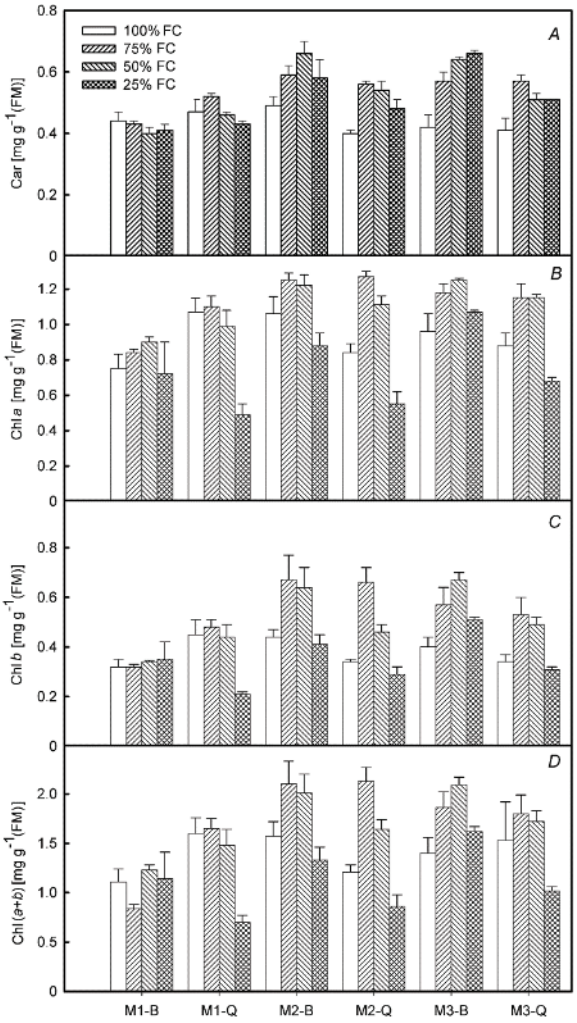

Fig. 4. Effect of mycorrhizae application and different water stress levels onchlorophyll (Chl) *a* (*A*), Chl *b* (*B*), Chl (*a*+*b*) (*C*) and Car content (*D*) [mg g^{-1}(FM)] in 'Badami' (B) and 'Qazvini' (Q). Values are means (n = 5) and the *vertical bars* indicate standard error. M1 – not inoculated with AMF; M2 – *G. mosseae*; M3 – *G. intraradices*.

Table 2. Correlation coefficients analysis in pistachio seedlings between leaf pigments, photosynthesis and chlorophyll fluorescence parameters and mycorrhizal infection percentage (MI). F_v/F_m – maximal quantum yield of PSII photochemistry, PI – performance index, P_N – net photosynthetic rate, E – transpiration rate, C_i – intercellular CO_2 concentration, Chl *a* – chlorophyll *a*, Chl *b* – chlorophyll *b*, Chl (*a*+*b*) – total chlorophyll, Car – carotenoids. *** – $P<0.001$; ** – $P<0.01$; * – $P<0.05$; ns – not significant.

	Drought level	MI	P_N	E	C_i	Chl (*a*+*b*)	Car	F_v/F_m
PI	100% FC	0.105 ^{ns}	0.031 ^{ns}	0.327 ^{ns}	0.332 ^{ns}	0.003 ^{ns}	0.747 *	0.606 ^{ns}
	75% FC	0.241 ^{ns}	0.240 ^{ns}	0.086 ^{ns}	0.012 ^{ns}	0.205 ^{ns}	0.226 ^{ns}	0.795 *
	50% FC	0.212 ^{ns}	0.004 ^{ns}	0.024 ^{ns}	0.066 ^{ns}	0.218 ^{ns}	0.317 ^{ns}	0.967 ***
	25% FC	0.016 ^{ns}	0.059 ^{ns}	0.006 ^{ns}	0.166 ^{ns}	0.843 **	0.369 ^{ns}	0.767 *
	100% FC	0.076 ^{ns}	0.029 ^{ns}	0.048 ^{ns}	0.062 ^{ns}	0.005 ^{ns}	0.369 ^{ns}	
F_v/F_m	75% FC	0.420 ^{ns}	0.231 ^{ns}	0.124 ^{ns}	0.024 ^{ns}	0.150 ^{ns}	0.263 ^{ns}	
	50% FC	0.310 ^{ns}	0.003 ^{ns}	0.001 ^{ns}	0.012 ^{ns}	0.287 ^{ns}	0.354 ^{ns}	
	25% FC	0.003 ^{ns}	0.003 ^{ns}	0.005 ^{ns}	0.079 ^{ns}	0.731 *	0.335 ^{ns}	
Car	100% FC	0.148 ^{ns}	0.001 ^{ns}	0.289 ^{ns}	0.074 ^{ns}	0.227 ^{ns}		
	75% FC	0.698 *	0.626 ^{ns}	0.714 *	0.379 ^{ns}	0.887 **		
	50% FC	0.662 *	0.022 ^{ns}	0.012 ^{ns}	0.069 ^{ns}	0.948 ***		
	25% FC	0.512 ^{ns}	0.055 ^{ns}	0.530 ^{ns}	0.117 ^{ns}	0.698 *		
Chl (*a*+*b*)	100% FC	0.035 ^{ns}	0.233 ^{ns}	0.086 ^{ns}	0.268 ^{ns}			
	75% FC	0.619 ^{ns}	0.507 ^{ns}	0.752 *	0.233 ^{ns}			
	50% FC	0.704 *	0.045 ^{ns}	0.041 ^{ns}	0.162 ^{ns}			
	25% FC	0.152 ^{ns}	0.004 ^{ns}	0.058 ^{ns}	0.171 ^{ns}			
C_i	100% FC	0.238 ^{ns}	0.739 *	0.431 ^{ns}				
	75% FC	0.342 ^{ns}	0.810 *	0.481 ^{ns}				
	50% FC	0.470 ^{ns}	0.776 *	0.824 *				
	25% FC	0.306 ^{ns}	0.626 ^{ns}	o.oo1 ^{ns}				
E	100% FC	0.618 ^{ns}	0.435 ^{ns}					
	75% FC	0.855 **	0.698 *					
	50% FC	0.384 ^{ns}	0.895 **					
	25% FC	0.606 ^{ns}	0.307 ^{ns}					
P_N	100% FC	0.339 ^{ns}						
	75% FC	0.632 ^{ns}						
	50% FC	0.462 ^{ns}						
	25% FC	0.005 ^{ns}						

water shortage in the soil can reduce and delay AMF spore germination (Tommerup 1984), root growth and thereby subsequent mycorrhiza development.

Values of F_v/F_m below 0.800 were recorded for the plants of all treatments. This suggests that photosynthetic apparatus was not fully developed or slightly injured, which could occur in plants cultivated under greenhouse conditions (Klamkowski *et al.* 2009). The decrease in F_v/F_m is a good indicator of photoinhibitory damage caused by light when plants are subjected to environmental stresses. At low water availability, leaves exhibiting low assimilation rates may be exposed to high irradiance stress, especially at midday (Lootens and Heursel 1998). Under such conditions, the photosynthetic apparatus suffers photodamage as a result of over-excitation of PSII (Powles 1984). Although PSII plays a key role in the response of leaf photosynthesis to environmental perturbation (Baker 1991), in the present study, parallels between P_N and F_v/F_m cannot be drawn and no significant correlation was found between these two parameters (Table 2). The Chl fluorescence traits were less affected by water stress respect to the photosynthesis capacity, however, some studies have shown that the maximal PSII photochemical efficiency is only slightly affected by water stress (Genty *et al.* 1987, Stuhlfauth *et al*. 1990, Cornic and Briantais 1991) and thus, it is still a matter of uncertainty about how water stress affects PSII photochemistry. Our results seem to support the recent view that PSII is highly resistant to water stress (Cornic 1994) although many other studies have shown that water stress decreases PSII function (Masojídek *et al.* 1991, He *et al.* 1995, Giardi *et al.* 1996).

Photochemical quantum yield was enhanced by AMF association and this effect was more pronounced under water-stress condition respect to –M plants (Fig. 2). This indicates that AMF inoculation can enhance drought tolerance in pistachio by ameliorating to some degree of the injury done to the photosystems reaction centers.

One of the first responses of plants to drought is the stomatal closure, which protects plants against excessive water loss, but also restricts the diffusion of CO_2 into the photosynthetic parenchyma (Chaves *et al.* 1991). In our study, the lowest rates of gas exchange were recorded for the stressed –M pistachio plants (Fig. 3), instead the root

colonization by AMF enhanced P_N of the plants, both well watered and water-stressed ones. In inoculated pistachio seedlings, P_N may have been stimulated by the increase of the sink strength arising from the additional carbon requirements of the AMF colonizing the roots (Wright *et al.* 1998).

Chl loss is a negative consequence of stress (Moran *et al.* 1994, Loggini *et al.* 1999), on the other hand, it has also been considered as an adaptive feature in plants grown under extreme climatic conditions, usually exposed to an excess of excitation energy (Maslova and Popova 1993, Kyparissis *et al.* 1995). Chl loss reduces the amount of light intercepted by leaves and at the same time it reduces the possibility of further damage to the photosynthetic machinery by the formation of activated oxygen under high light (Munné-Bosch and Alegre 2000, Kranner *et al.* 2002). In our study, the mild and moderate water stresses resulted in the small increase in total Chl content per unit leaf FM, which in a certain degree might happen due to the decline in relative water content of leaves under drought. Our results indicated that the AMF symbiosis could enhance the Chl concentration of pistachio leaves, which is in agreement with the results of other studies (Giri and Mukerji 2004, Sannazzaro *et al.* 2004, Colla *et al.* 2008). This, together with no significant association between the enhancement of relative Chl concentration and the improvement of gas-exchange parameters *via* AM symbiosis (Table 2), suggests that the mycorrhizae-mediated increase in gas-exchange parameters is not related with mycorrhizae-mediated improvement of Chl concentration.

The Car contents significantly increased as the effect of water stress. In addition, the ratio of Car/Chl significantly increased under severe water stress while no significant change was observed in the other stress levels. Car content and Car/Chl ratio are correlated with the capacity of light-protecting mechanisms. Besides their structural roles, they are well known for their antioxidant activity, inhibiting lipid peroxidation and stabilizing membranes (Demmig-Adams and Adams 1996). They also play a critical role in the assembly of the light-harvesting complex and in the radiationless dissipation of excess energy (Munné-Bosch and Alegre 2000). Therefore, the results of our experiment revealed that +M pistachio plants provided stronger photoprotective system against water stress compared with –M plants by increasing leaf Car.

It could be concluded from this study that there were enhancements of quantum yield of PSII of the used pistachio cultivars (Badami and Qazvini) by *G. intraradices* and *G. mosseae* inoculations. It was established that one of the mechanisms employed by AMF association in bringing about increase in photosynthetic rate during water stress is alleviating the quantum yield of PSII photochemistry, which consequently leads to quantum yield of noncyclic electrons of photosystems (Genty *et al.* 1989, Flagella *et al.* 1995), but a direct relation was not found between these two parameters since despite of predominance of Badami in Chl fluorescence parameters and pigments content, Qazvini had higher photosynthesis and transpiration rate. A comparison between two applied AMF reveals an equal influence of them on measured parameters except with C_i, where *G. intraradices* was superior. Based on our results, in comparison with Badami, Qazvini produced more biomass (data not shown), thus it is evident that photosynthetic rate is more viable that directly contributes to plant productivity.

References

Allen, M.F., Boosalis, M.G.: Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. – New Phytol. **93**: 69-76, 1983.

Baker, N.R.: Possible role of photosystem II in environmental perturbations of photosynthesis. – Physiol. Plant. **81**: 563-570, 1991.

Busse, M.D., Ellis, J.R.: Vesicular-arbuscular mycorrhizal (*Glomus fasciculatum*) influence on soybean drought tolerance in high phosphorus soil. – Can. J. Bot. **63**: 2290-2294, 1985.

Chaves, M.M.: Effects of water deficits on carbon assimilation. – J. Exp. Bot. **42**: 1-16, 1991.

Colla, G., Rouphael, Y., Cardarelli, M., Tullio, M., Rivera, C.M., Rea, E.: Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. – Biol. Fert. Soils **44**: 501-509, 2008.

Cornic, G., Briantais, J.M.: Portioning of photosynthetic electron flow between CO_2 and O_2 reduction in a C_3 leaf (*Phaseolus vulgaris*L.) at different CO_2 concentrations and during drought stress. – Planta **183**: 178-184, 1991.

Cornic, G.: Drought stress and high light effects on leaf photosynthesis. – In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis. Pp. 297-313. Bios Scientific Publishers, Oxford 1994.

Demmig-Adams, B., Adams, W.W.: Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. – Planta **198**: 460-470, 1996.

Ferguson, F., Kaur, S., Epstein, L.: Arbuscular myocrrhizal fungi on pistachio rootstock in California. – Acta Hort. **470**: 211-218, 1998.

Flagella, Z., Pastore, D., Campanile, R.G., Di Fonzo, N.: The quantum yield of photosynthetic electron transport evaluated by chlorophyll fluorescence as an indicator of drought tolerance in durum wheat. – J. Agric. Sci. **125**: 325-329, 1995.

Genty, B., Briantais, J.M., Viera da Silva, J.B.: Effects of drought on primary photosynthetic processes of cotton leaves. – Plant Physiol. **83**: 360-364, 1987.

Genty, B., Briantais, J.M., Baker, N.R.: The relationship between the quantum yield of photosynthesis electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Giardi, M.T., Cona, A., Geiken, B., Kucera, T., Masojidek, J.,

Mattoo, A.K.: Long-term drought stress induces structural and functional reorganization of photosystem II. – Planta **199**: 118-125, 1996.

Giovannetti, M., Mosse, B.: An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. – New Phytol. **84**: 489-500, 1980.

Giri, B., Mukerji, K.: Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. – Mycorrhiza **14**: 307-312, 2004.

Graham, J.H., Leonard, R.T., Menge, J.A.: Membrane mediated decrease in root exudation responsible for phosphorus inhibition of VA mycorrhiza formation. – Plant Physiol. **68**: 548-552, 1982.

He, J.X., Wang, J., Liang, H.G.: Effects of water stress on photochemical function and protein metabolism of photosystem II in wheat leaves. – Physiol. Plant. **93**: 771-777, 1995.

Kafkas, S., Ortas, I.: Various mycorrhizal fungi enhance dry weights, P and Zn uptake of four *Pistacia* species. – J. Plant Nutr. **32**: 146-159, 2009.

Klamkowski, K., Borkowska, B., Treder, W., Tryngiel-Gać, A., Krzewińska, D.: Effect of mycorrhizal inoculation on photosynthetic activity and vegetative growth of cranberry plants grown under different water regimes. – Acta Hort. **838**: 109-113, 2009.

Kranner, I., Beckett, R.P., Wornik, S., Zorn, M., Pfeifhofer, H.W.: Revival of a resurrection plant correlates with its antioxidant status. – Plant J. **31**: 13-24, 2002.

Kyparissis, A., Petropoulou, Y., Manetas, Y.: Summer survival of leaves in a soft-leaved shrub (*Phlomis fructicosa* L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. – J. Exp. Bot. **46**: 1825-1831, 1995.

Lichtenthaler, R. K.: Chlorophylls and carotenoids - pigments of photosynthetic biomembranes. – In: Colowick, S. P., Kaplan, N. O (ed.) Methods in Enzymology. Vol. 148. Pp. 350-382. Academic Press, San Diego – New York – Berkeley – Boston – London – Sydney – Tokyo – Toronto 1987.

Loggini, B., Scartazza, A., Brugnoli, E., Navari-Izzo, F.: Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. – Plant Physiol. **119**: 1091-1099, 1999.

Lootens, P., Heursel, J.: Irradiance, temperature, and carbon dioxide enrichment affect photosynthesis in *Phalaenopsis* hybrids. – HortScience **33**: 1183-1185, 1998.

Maslova, T.G., Popova, I.A.: Adaptive properties of the plant pigment systems. – Photosynthetica **29**: 195-203, 1993.

Masojídek, J., Trivedi, S., Halshaw, L., Alexiou, A., Hall, D. O.: The synergistic effect of drought and light stresses in sorghum and pearl millet. – Plant Physiol. **96**: 198-207, 1991.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence—a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Moran, J.F., Becana, M., Iturbe-Ormaetxe, I., Frechilla, S., Klucas RV, Aparicio-Tejo P.: Drought induces oxidative stress in pea plants. – Planta **194**: 346-352, 1994.

Munn-Bosch, S., Alegre, L.: Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. – Planta **207**: 925-931, 2000.

Nelsen, C.E., Safir, G.R.: Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. – Plant Soil **154**: 407-412, 1982.

Novello, V., de Palma, L.: Observation on the pistachio photosynthetic activity in southern Italy. – Acta Hort. **419**: 97-10, 1995.

Osonubi, O., Mulongoy, K., Awotoye, O.O., Atayese, M.O., Okali, D.U.U.: Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi on drought tolerance of four leguminous woody seedlings. – Plant Soil **136**: 131-143, 1991.

Phillips, J.M., Haymann, D.S.: Improved procedures for cleaning roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. –T. Brit. Mycol. Soc. **55**: 158-161, 1970.

Powles, S.B.: Photoinhibition of photosynthesis induced by visible light. – Annual Rev. Plant Physiol. **35**: 15-44, 1984.

Sannazzaro, A., Ruiz, A., Albertó, E. Menéndez, A.: Presence of different arbuscular mycorrhizal infection patterns in *Lotus glaber* growing in the Salado River Basin. – Mycorrhiza **14**: 139-142, 2004.

Schwab, S.M., Menge, J.A., Leonard, R.T.: Quantitative and qualitative effects of phosphorus on extracts and exudates of sudan grass roots in relation to vesicular-arbuscular mycorrhizal formation. – Plant Physiol. **73**: 761-765, 1983.

Sheibani, A.: Pistachio production in Iran. – First Int. Symp. Pistachio Nut, Adana 1994.

Simpson, D., Daft, M.J.: Interactions between water-stress and different mycorrhiza inocula on plant growth and mycorrhizal development in maize and sorghum. – Plant Soil **121**: 79-186, 1990.

Spiegel-Roy, P., Mazigh, D., Evenari. M.: Responses of pistachio to low soil moisture conditions. – J. Am. Soc. Hortic. Sci. **102**: 470-473, 1977.

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M.: The fluorescence transient as a tool to characterize and screen photosynthetic samples. – In: Yunus, M. (ed.): Probing Photosynthesis: Mechanisms, Regulation and Adaptation. Pp. 445-483. Taylor & Francis, London 2000.

Stuhlfauth, T., Scheuermann, R., Fock, H. P.: Light energy dissipation under water stress conditions. – Plant Physiol. **92**: 1053-1061, 1990.

Tommerup, I.C.: Effect of soil water potential on spore germination by vesicular-arbuscular mycorrhizal fungi. – T. Brit. Mycol. Soc. **83**: 193-202, 1984.

Vemmos, S.N.: Net Photosynthesis, stomatal conductance, chlorophyll content and specific leaf of pistachio tress (cv. Aegenes) as influenced by fruiting. – J. Hort. Sci. **69**: 775-782, 1994.

Wright, D.P., Scholes, J.D., Read, D.J.: Effect of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L. – Plant Cell Environ. **21**: 209-216, 1998.