Impact of osmotic drought stress on carbon isotope discrimination and growth parameters in three pistachio rootstocks (Pistacia spp., Anacardiaceae)

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Impact of osmotic drought stress on carbon isotope discrimination and growth parameters in three pistachio rootstocks (*Pistacia* spp., Anacardiaceae)

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Abstract. Pistachio (*Pistacia vera* L., Anacardiaceae) is one of the major horticultural crops in Iran with high plantation surface areas, and production and export rates. However, growing water deficiency has become a main limiting factor for growth and development, and especially for maintaining yield quality and volume of this crop. In this study, the responses of carbon isotope discrimination ($\Delta^{13}C$) and some growth parameters of three pistachio rootstocks (*P. vera* cv. Badami, *P. vera* cv. Sarakhs and *P. terebinthus*) to different osmotic drought stress levels (-0.1, -0.50, -1.0 and -1.5 MPa) were investigated in a greenhouse experiment carried out at the Faculty of Bio-science Engineering, Ghent University, Belgium. The impact of different rootstocks on leaf $\Delta^{13}C$ varied significantly. Results show that *P. vera* cv. Sarakhs had the highest discrimination for carbon isotopes, whereas *P. vera* cv. Badami had the middle and *P. terebinthus* the minimum discrimination values, respectively. An increase in drought stress intensity caused a decrease in leaf carbon isotope discrimination values in all three rootstocks, although these differences were not significant. Drought stress significantly decreased both plant fresh and dry weight, shoot dry weight, root dry weight, leaf area and stem elongation. There also was a significant rootstock effect of treatments on these growth parameters. Under osmotic stress treatments, root/shoot ratio increased significantly. Control plants showed normal elongation growth, but growth was stopped for all drought stress treatments, and differences were significant for all three rootstocks. There were no significant differences between pistachio rootstocks and drought stress treatments for leaf shedding.

Keywords. Biomass – Water deficiency – Plant growth parameters.

Impact du stress osmotique dû à la sécheresse sur la discrimination isotopique du carbone et les paramètres de croissance chez trois porte-greffes de pistachier* (Pistacia *spp., Anacardiaceae)

Résumé. *Le pistachier (*Pistacia vera *L., Anacardiaceae) est une des espèces majeures de l'horticulture en Iran avec de vastes surfaces plantées, et de forts taux de production et d'exportation. Toutefois, le manque d'eau croissant est devenu un principal facteur limitant pour la croissance et le développement, et en particulier pour le maintien de la qualité et du volume de production. Dans cette étude, les réponses à la discrimination isotopique du carbone ($\Delta^{13}C$) ainsi que certains paramètres de croissance de trois porte-greffes de pistachiier (*P. vera *cv. Badami,* P. vera *cv. Sarakhs et* P. terebinthus*) à différents niveaux de stress osmotique dus à la sécheresse (-0,1, -0,50, -1,0 et -1.5 MPa) ont été testés dans une expérimentation en serre menée en Belgique, Faculty of Bio-science Engineering, Ghent University. L'impact des différents porte-greffes sur $\Delta^{13}C$ des feuilles a varié significativement. Les résultats montrent que, respectivement,* P. vera *cv. Sarakhs a la plus forte valeur de discrimination isotopique du carbone, tandis que* P. vera *cv. Badami a la valeur moyenne et* P. terebinthus *a la plus petite valeur. Une augmentation de l'intensité du stress de sécheresse a causé une baisse des valeurs de discrimination isotopique du carbone des feuilles chez les trois porte-greffes, bien que ces différences ne soient pas significtives. Le stress de sécheresse a fait baisser significativement le poids frais et poids sec des plantes, le poids sec des pousses, le poids sec des racines, la surface foliaire et l'élongation des tiges. Il y a eu également un effet significatif des traitements des porte-greffes sur ces paramètres de croissance. Sous traitements de stress osmotique, le ratio racines/pousses a augmenté significativement. Les plantes témoins ont montré une croissance d'élongation normale, mais la croissance a été stoppée pour tous*

les traitements de stress de sécheresse, et les différences ont été significatives pour les trois porte-greffes. Il n'y a pas eu de différences significatives entre porte-greffes de pistachiers et traitements de stress de sécheresse pour la chute des feuilles.

Mots-clés. *Biomasse – Manque d'eau – Paramètres de croissance de la plante*.

I – Introduction

Pistachio belongs to the Anacardiaceae family. Only *P. vera* L., i. e. cultivated pistachio, has sizeable economic importance. Iran, as the region of origin of pistachio has always had the largest cultivation area in the world. In Iran, pistachios are usually cultivated under dry and saline conditions (Sheibani, 1995), as they have a high tolerance to drought and soil and water salinity. Still, water deficit and salinity can cause a reduction in plant growth, and eventually yield and nut quality.

Drought stress adversely affects growth, dry mass accumulation, and productivity of plants (Anjum *et al*., 2011) and causes a higher rate of impairment than any other environmental factor (Shao *et al*., 2009). Higher drought tolerance of wild pistachio species could be related to a deep taproot, high water conservation ability by stomatal adjustment, stomatal features, leaf characteristics, and leaf shedding. Therefore, these wild species are very often used as rootstock. It's in this context that drought stress was evaluated for *P. vera* Kerman grafted onto three different pistachio rootstocks. Grafting onto hybrid rootstock (UCB#1) and *P. terebinthus* resulted in a higher growth reduction compared with *P. atlantica* under drought stress (Gijón *et al*., 2010).

Carbon isotope discrimination (δ) decreases with increasing salinity in leaves, stems and roots of pistachio seedlings. However, there were no significant difference in carbon isotope discrimination between three *P. vera* (Sarakhs, Badami-zarand, and Ghazvini) rootstocks was evidenced by Hokmabadi *et al*. (2005).

There are three pistachio species in Iran (Esmaeilpour and Khezri, 2006) which are grown under different environmental conditions (altitude 900-2000 m; latitude 24-37 N; temperatures ranging between -10°C in winter and 48°C in summer, low to moderate humidity, and long, hot summers). Responses of two of the three species have been investigated to osmotic drought stress treatments by Fardooei (2001). *Pistacia vera* L. is the country's most common rootstock. Yet, physiological responses of this pistachio rootstock to drought stress and comparison to other recommended rootstocks have not been studied enough. The aim of this study was to evaluate the effects of osmotic drought stress on carbon isotope discrimination and plant biomass, leaf area, elongation rate and root/shoot ratio of *P. vera* cv. Badami, *P. vera* cv.Sarakhs (native), and *P. terebinthus rootstocks (used in Turkey)*.

II – Materials and methods

This study was carried out in a greenhouse at Ghent University (51°3' N, 3°42' E). Certified seeds of two pistachio rootstocks, *P. vera* L. cv. Badami (Badami) and *P. vera* L. cv. Sarakhs (Sarakhs) were collected from Rafsanjan, Iran and *P. terebinthus* (Terbinthus) seeds were obtained from the pistachio production area in Turkey. Transplanted 1-year-old seedlings were grown hydroponically in a glasshouse environment using Hoagland solution (Picchioni *et al*., 1991). Osmotic drought treatments were control (Ψ_s = -0.10) and three drought stress levels (Ψ_s = _0. 5; Ψ_s = _ 1.0, Ψ_s = _ 1.5 MPa) using PEG 6000. Stress levels were maintained for two weeks; then all solutions were replaced by the control treatment, and this level was maintained for two recovery weeks.

Dried leaf samples were ground by a grinder. Five mg subsamples of ground plant material were packed in tin capsules and analyzed for natural abundance of C ^{13} by combustion to CO_2 in the present of O_2 by an elemental analyzer (EA) coupled to an isotope ratio mass spectrometer (IRMS) to measure $\delta\,^{13}C$ (Staelens *et al*., 2012).

At the end of the second recovery week, seedlings were harvested. Plant fresh weight (PFW) of leaves, shoots, and roots was determined. Plant dry weight (PDW) of the respective plant fractions was determined after drying at 85°C for 72 hours. Plant height was measured with a ruler (± 0.1 cm) at the beginning and end of the experiment. The experiment was designed as a randomized complete design (RCD). Each experimental unit contained 5 plants and a total of 20 plants were used for four treatments in every rootstock. A two-way analysis of variance was used to test for effects of drought treatments and rootstocks. Means were compared using Tukey's HSD test (P = 0.05). All analyses were performed in SPSS 22.

III – Results and discussion

1. Carbon isotope discrimination

The impact of drought stress treatments on $\Delta^{13}C$ varied significantly in the different rootstocks. Leaf carbon isotope discrimination was highest in Sarakhs (23.00 ‰), average in Badami (22.19 ‰) and lowest in Terebinthus (22.07 ‰) rootstocks. Increasing drought stress intensity decreased the values of leaf carbon isotope discrimination, although differences were not significant (Fig. 1 A and B). Findings of this study on leaf $\delta^{13}C$ for drought stress treatments are in agreement with the results of Hokmabadi *et al*. (2005). However, latter authors reported there was no significant difference in carbon isotope discrimination among pistachio rootstocks, whereas in this experiment we observed significant differences among evaluated rootstocks.

There were negative significant relationships between carbon isotope discrimination ($\delta^{13}C$) with PFW and PDW values (data not shown). Based on the correlations, we evidenced carbon isotope discrimination would seem to be a good indicator of drought stress effects that significantly influence biomass factors of pistachio plants in water deficit conditions.

2. Biomass characterization

Drought stress treatments significantly decreased PFW, PDW, shoot dry weight (SDW) and leaf dry weight (LDW) compared with control plants. Although root dry weight was not affected by the osmotic stress treatments, root/shoot ratio increased significantly (Table 1, figure 1). Plant leaf area was not affected by treatments, although overall control plants had a higher leaf area than drought-exposed plants. Control plants showed elongation growth during the experimental period, but a growth arrest was noted under drought stress treatments. There were no significant differences among drought treatments in shedding rates, although the latter increased with drought stress intensity (Table 1). Effects of rootstock were significant on all growth parameters. Terbinthus had the highest values for biomass parameters, leaf area and stem elongation, while Badami had the highest value for leaf shedding compared to the other rootstocks (Table 1).

Dry biomass is considered as an appropriate parameter for stress tolerance evaluation in many crops (Munns, 2002). In our experiment, when plants were subjected to osmotic drought stress treatments, we observed dry biomass reduction values were found by more than 30.7%, 42.8% and 42.3% in mild (-0.5 MPa), moderate (-1.0 MPa) and severe (-1.5 MPa) drought stress levels compared to control respectively. The significant decline in total plant biomass of pistachio root-

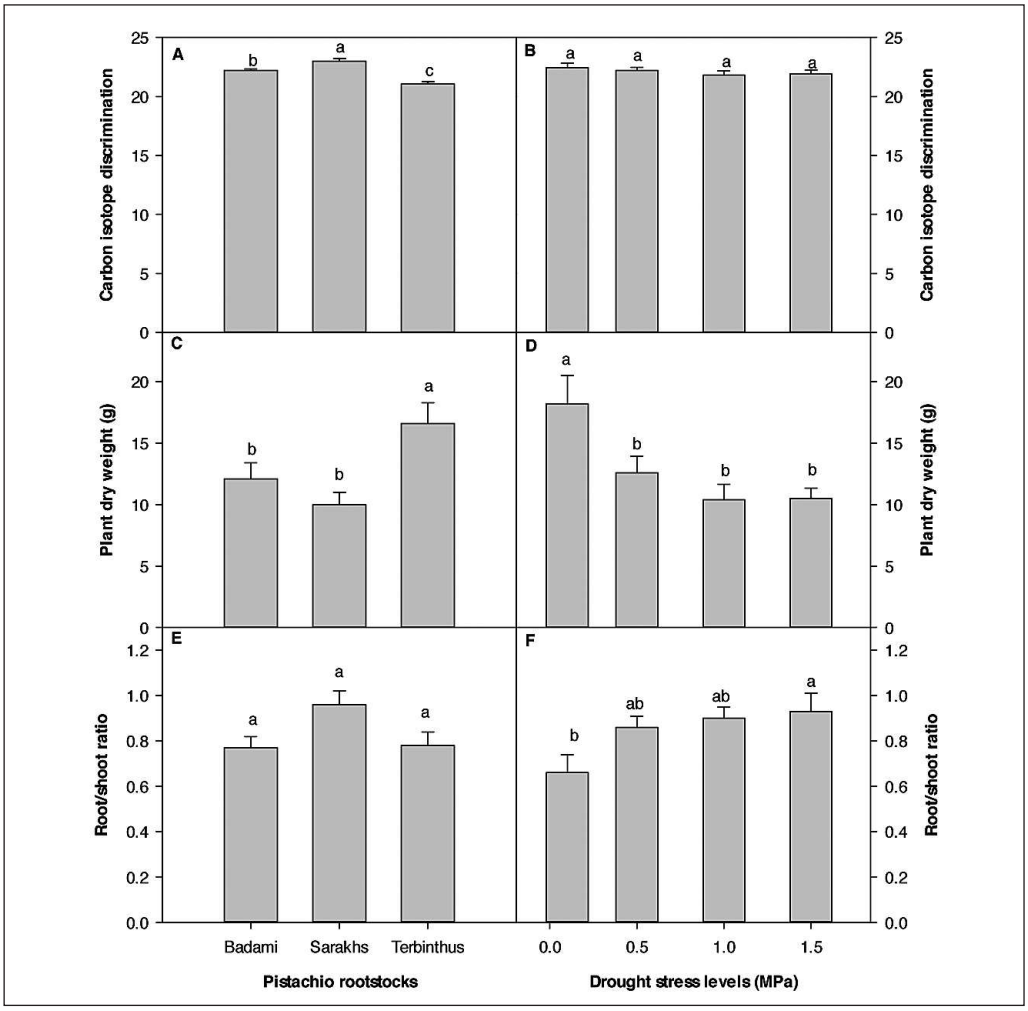

Fig. 1. Changes in carbon isotope discrimination in rootstocks (A) and treatments (B); plant dry weight in rootstocks (C) and treatments (D); and root/ shoot ratio in rootstocks (E) and treatments (F) in three pistachio rootstocks. For each column, different letters indicate significant differences ($P \leq 0.05$) according to Tukey's range test.

stocks with increasing drought is in line with results reported by Abbaspour *et al*. (2012), Habibi and Hajiboland (2013) and Ranjbarfordoei *et al*. (2000) in pistachio species, Rouhi (2007) in almond species, and Maraghni *et al*. (2011) in *Ziziphus lotus* plants.

Osmotic stress resulted in growth arrest for all rootstocks, and no regrowth was observed after 2 weeks of recovery. Root growth is generally less sensitive to drought stress compared to other biomass components (Hsiao and Xu, 2000). An increase in root/shoot ratio has been proposed as one of the mechanisms involved in the adaptation of plants to drought stress (Turner, 1997). In our study, increasing root/shoot ratios under osmotic stress are only related to a decrease in aboveground biomass (Table 1).

Table 1. Effects of osmotic stress treatments (T, MPa) on plant fresh weight (PFW, g), shoot dry weight (SDW, g), leaf dry weight (LDW, g), root dry weight (RDW, g), leaf area (LA, cm), elongation length (EL, cm) and shedding (Shed, g) after two weeks of recovery for pistachio rootstocks (C)

		PFW	SDW	LDW	RDW	LA	EI	Shed
Treatments	-0.1	52.9 a	11.7 a	6.1 a	6.6 a	22.8 a	16.2 a	0.020 a
	-0.5	29.7 b	6.9 b	3.2 b	5.7 a	18.7 a	1.6 b	0.167 a
	-1	25.9 b	5.6 b	2.5 b	4.8 a	17.1 a	0.9 b	0.174 a
	-1.5	24.5 b	5.5 b	2.5 b	5.0 a	16.7 a	2.3 b	0.150 a
Rootstocks	Badami	32.2 b	7.1 b	2.7 b	5.1 ab	12.1 b	5.7 ab	0.27 a
	Sarakhs	22.6 b	5.2 b	2.1 b	4.9 b	12.9 b	2.3 b	0.05 b
	Terbinhus	45.0 a	10.0 a	5.9 a	6.7 a	31.4 a	7.7 a	0.06 b
Anova	Rootstock	**	**	**	*	**	*	**
	Treatment	**	**	**	ns	ns	**	ns
	Rootstock*treatment	ns	ns	ns	ns	ns	**	ns

Within each column in every rootstock, means superscripted with different letters are significantly different [** - (P< 0.0 1), * - (*P< 0.05*) and ns- none significant].

Under control conditions, Terbinthus maintained the highest PDW compared to both other rootstocks (Fig. 1C) but this rootstock showed the largest decrease for this parameter in reaction to severe drought stress. Reduction rates were 42.4%, 33.1% and 47.1% for Badami, Sarakhs and Terbinthus, respectively. Therefore, Sarakhs kept the lowest PDW reduction values among the other rootstocks in this study.

IV – Conclusion

Pistachio trees are considered drought tolerant, yet the applied osmotic drought stress induced significant reductions in leaf carbon isotope discrimination. In conclusion, Sarakhs better tolerated the applied drought stress as shown by the growth reduction performance in the severest drought condition as compared with Badami and Terbinthus. However, further research in field conditions is needed to confirm this survey's research results**.**

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