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EFFECTS OF TWO ROOTSTOCKS (PISTACIA VERA L. AND PISTACIA ATLANTICA DESF.) ON THE YIELD, MORPHOLOGY, CHEMICAL COMPOSITION, AND FUNCTIONAL PROPERTIES OF TWO PISTACHIO VARIETIES ("MATEUR" AND "ACHOURY")

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ABSTRACT

The low diversification of rootstocks can be a problem in reducing the adaptation of pistachio to the Tunisian climatic and soil conditions. Nowadays, the most used rootstock is *Pistacia vera*; however, the autochthonous *Pistacia atlantica* could be a good alternative. The aim of this work was to study the effect of two different rootstocks *Pistacia vera* and *Pistacia atlantica* on the quality and functionality of two pistachio cultivars (the local variety "Mateur" and the Syrian one "Achoury"). The different parameters studied were: morphological (size, weight), chemical (minerals and fatty acids) and functional (total phenolic content, and antioxidant activity: $ABTS^+$, DPPH$^•$, and FRAP). The pistachios are a good source of minerals (especially Ca and K 19.9 and 9.8 g kg^{-1}, respectively, and Fe 54.5 mg kg^{-1}), polyunsaturated fatty acids (oleic and linoleic acids), and phenolic compounds (16.0 g GAE kg^{-1}). The combination *P. atlantica* and variety "Mateur" led to proper values of tree yield, nut dehiscence, morphology (length and width), and oleic acid content. Thus, it can be concluded that the use of the local rootstock *P. atlantica* led to promising results and can be a good option due to its higher adaptation to the local climatic and soil conditions.

Keywords: total polyphenol content, fatty acids, fruit morphology, mineral content, antioxidant activity

INTRODUCTION

Pistachio (*Pistacia vera* L.) fruit are one of the most liked nut worldwide. Pistachio species are drought and salt tolerant and that is why they are widely cultivated in saline, dry, and hot areas of the Middle East, Mediterranean countries and United States **(Demiral *et al.*, 2009)**. Now, pistachio is among the most spread nut tree crop in Tunisia occupying an overall area of 44000 ha, mainly concentrated in the south and center regions of the country **(Sarra *et al.*, 2015)**. The high nut quality, price, demand, and tolerance to abiotic stresses have encouraged its plantation in large scale in semi-arid areas during the last four decades, including Tunisia **(Chelli Chaabouni *et al.*, 2014; Ghrab and Gouta, 2005)**. Pistachio is mainly propagated by grafting, and many wild *Pistacia* species (*Pistacia integerrima* L., *P. atlantica* Desf., *P. terebinthus* L., *P. vera* L…) are used as rootstocks for *Pistacia vera*, which is the only cultivated species for industrial use. Pistachio industry in Tunisia is based on a main cultivar *Mateur*, with *P. vera* being its rootstock. Thus, the low diversification of pistachio rootstocks in Tunisian orchards and the low adaptation of some cultivars are among the major problems affecting crop sustainability.

It has been clearly demonstrated that the rootstock will drastically affect the three vegetative growth. The use of different rootstock or hybrid seedlings have been shown to significantly influence some key parameters of the tree growth, such as nutrient contents, yield, alternate bearing, resistance to cold and salt stress, and shell splitting **(Ashworth, 1985; Walker *et al.*, 1987)**, and also to key parameters of the nut quality, including weight, size, color, and mineral composition **(Carbonell-Barrachina *et al.*, 2015)**. Their effects were also seen on plant blooming, vegetative growth, fruit quality and total production **(Giorgi *et al.*, 2005; Weibel *et al.*, 2003; Young and Houser, 1980; Zarrouk *et al.*, 2005)**. All these reasons suggest that grafting is a relevant agronomic practice which requires further attention.

Halvorsen *et al.* (2006) determined the antioxidant concentrations of 1113 food products and ranked pistachio nuts among the first 50 items with the highest antioxidant capacity; this is probably due to the fact that pistachio nuts are a rich source of phenolic compounds **(Rodríguez-Bencomo *et al.*, 2015)**. Moreover, the pigments responsible for pistachio purple and green kernel color have been attributed to lutein derivatives and anthocyanins **(Dreher, 2012)**. Pistachio kernels are a good source of fat (50–60%) and contain high quantity of unsaturated fatty acids (linoleic, linolenic and oleic acids), essential for human diet **(Carbonell-Barrachina *et al.*, 2015; Maskan and Karataş, 1998)**.

The aim of this study was to investigate the effects of rootstock on fruit production and quality parameters (minerals, fatty acids, and total phenolic content) and antioxidant capacity of two pistachio cultivars (Mateur and Achouri), grown under rainfed conditions in the northeast of Tunisia.

MATERIAL AND METHODS

Plant material, growing conditions and experimental design

This study was carried out at the INRAT Unity of Agricultural Experimentation of Mornag (Tunisia), which has semi-arid climatic conditions, with a mean temperature range of 10-27 °C, and 450 mm of annual precipitation. 22 year-old trees were cultivated under rainfed conditions. Two pistachio cultivars were considered in this study (i) the Tunisian "Mateur" and the Syrian "Achoury", and both were grafted on: (i) *Pistacia atlantica* or (ii) *Pistacia vera* rootstocks. Monitoring was performed on 5 trees of comparable size and vigor, chosen from each treatment (rootstocks × cultivars combination). Fruits were harvested approximately at the end of August, at their commercial maturity, and the yield per tree was measured at two consecutive seasons, 2014 and 2015.

Pomological parameters

The in-hull and in-shell fruit fresh weights, in-shell dry weight and nut dehiscence were measured for 3 replicates of 100 fruits (3 × 100) per treatment. The nut and kernel sizes were measured with a digital caliper for 25 fruits per treatment following **IPGRI International Plant Genetic Resources Institute (1997)** descriptors.

Mineral analysis

Mineral content was quantified according to **Carbonell-Barrachina *et al.* (2002)**. Approximately 0.5 g ground pistachios were digested and to assess precision and accuracy, the protocol and equipment were validated by using in each batch: (i) the GBW07603 (bush, branches and leaves; Institute of Geophysical and Geochemical Exploration of China) certified reference material, 1 blank, and 1 spiked-sample in each batch. Calibration curves were used for the quantification of minerals and showed good linearity ($R^2 \geq 0.998$). This analysis was run in triplicate.

Fatty acids profile

Fatty acid methyl esters (FAMEs) were prepared according to the method described by **Carbonell-Barrachina *et al.* (2015)** and identical chromatographic set-up and conditions were used. 50 mg of extracted oil (using sonication) were used and identification of FAMEs was made by comparison with authentic standards from Sigma-Aldrich. This analysis was run in triplicate, and results were expressed as % of the total area.

Total polyphenols content (TPC)

The TPC was measured using the Folin–Ciocalteu colorimetric method as previously described by **Gao *et al.* (2000)**, and using an extract obtained after using ~1 g of grinded pistachio and a solution of 80 % aqueous methanol (MeOH) and 1 % HCl. Quantification was conducted by using a gallic acid calibration curve, and results were expressed as mg of gallic acid equivalents (GAE) *per* 100 g of dry mass. This analysis was run in triplicate.

Antioxidant activity

The same extract used for the TPC analysis was also used for the analysis of the free scavenging activity $DPPH^{\bullet}$ method as described by **Brand-Williams *et al.* (1995)**, with a modification in the reaction time (10 min were used in the current study). This analysis was run in five replications, and results were expressed as mmol trolox kg^{-1} dry matter, dm. The $ABTS^{+}$ radical cation and FRAP methods were also used as described by **Re *et al.* (1999)** and **Benzie and Strain (1996)**, respectively. Calibration curves were used for quantification of the three methods of antioxidant activity and showed good linearity ($R^2 \geq 0.998$). The analyses were run in five replications, and results were expressed as mmol Trolox kg^{-1} dm.

Statistical analyses

The data was subjected to one-way analysis of variance (ANOVA) and later to Tukey's multiple-range test to compare the means. Differences were considered statistically significant at $p<0.05$. All statistical analyses were done using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD).

RESULTS AND DISCUSSION

Yield production

The yield per tree of both "Mateur" and "Achoury" cultivars during 2014 and 2015 seasons is shown in the Table 1. Trees exhibited significantly higher yields in 2014 than in 2015, which was indicative of an alternate bearing phenomenon. During the 2015 season, the "Mateur" variety grafted on *P. atlantica* rootstock had a significantly higher fruit yield than the "Achoury" scion/rootstock combinations; however, no significant effects of the rootstock on the yield were observed in the 2014 season.

Although the data was not always statistically significant, it is implied that the local "Mateur" variety was slightly more productive than the Syrian "Achoury" cultivar, and seemed to be better grafted on *P. atlantica* rootstock. These results agreed with the findings reported by **Carbonell-Barrachina *et al.* (2015)**. These authors reported a significant effect of rootstock on the tree yield with higher yield being recorded for the "Kerman" cultivar grafted on *P. atlantica* than for *P. integerrima* and *P. terebinthus*. They also found a significantly higher tree yield in 2012 season (40.1 kg) compared to that registered in 2013 season (12.0 kg), clearly due to an alternate bearing, a characteristic phenomenon of this type of crop. **Ghrab and Gouta (2005)** reported a great variation of "Mateur" and "Ohadi" pistachio cultivars tree yield over fifteen year of study (1983-1997), and they established a link between the production and the annual rainfall. Moreover, **Johnson and Weinbaum (1987)** found that production can vary 3 to 5 times between "off" and "on" years.

It is difficult to establish a clear behavior of the nut splitting as affected by the rootstock, because the rate of nut dehiscence changed from year to year for both varieties under study (Table 1). "Achoury" exhibited a significantly higher nut dehiscence (76-84%) than "Mateur" (30-33%) in 2014; however, no effect of the rootstock (*P. vera* and *P. atlantica*) was noticed for this parameter for both varieties under study. On the other hand, in the following season (2015), a significantly higher nut dehiscence (89%) was recorded for the "Mateur" pistachios grafted on *P. atlantica*. Besides, it seemed that the rootstock had no effect on the dehiscence of the "Achoury" nuts. Current results supported those by **Loudyi (2001)**, who reported greater variation of "Mateur" nut splitting from year to year. The difference between both varieties can be explained by their maturity degree and adaptation to climatic and drought conditions. The level of cold temperature during the vegetative dormancy of the pistachio trees seems to have an effect on the nut dehiscence of "Mateur" trees **(Oukabli, 1998)**. It is well known that shell splitting can be enhanced by management practices, especially by properly managing the irrigation water **(Goldhamer *et al.*, 1987)**.

Table 1 Average yield (kg/tree) and nut dehiscence rate (%) as affected by pistachio variety and rootstock, on seasons 2014 and 2015.

Variety	Rootstock	Yield (kg)		Nut dehiscence rate (%)	
		2014	2015	2014	2015
Mateur	*P. vera*	8.41	3.29 ab†	32.52 b	60.12 b
	P. atlantica	9.14	4.44 a	30.97 b	89.01 a
Achouri	*P. vera*	7.45	0.78 b	83.84 a	67.06 b
	P. atlantica	7.16	1.05 b	76.54 a	68.33 b
ANOVA test‡		NS	**	**	*

† Values (mean of 5 replications) followed by the same letter, within the same column, were not significantly different (p < 0.05), Tukey's least significant difference test. ‡NS = not significant at p< 0.05; *, and **, significant at p< 0.05 and 0.01 respectively.

Morphological parameters

Data presented in Table 2 indicated that whole and de-hulled fruit size parameters were significantly different for each variety, with "Mateur" pistachios being bigger that those from the "Achoury" variety. Fruits from both varieties under study, showed values of length/with (l/w) higher than 1.80. A previous study **(Caruso *et al.*, 1998)** claimed that in Italian, Greek, and Tunisian pistachio varieties, the nuts were elongated (l/w > 1.80), but the Iranian, Turkish and, to a minor extent, Syrian ones were ovoid (l/w = 1.50–1.80). However, it is difficult to establish these trends because the physical characteristics can be influenced by location and the particular year of alternate bearing **(Seferoglu *et al.*, 2006)**, or even by the pollinator **(Ak, 1998)**.

Table 2 Fruit morphology (mm) as affected by pistachio variety and rootstock

Variety	Rootstock	Whole fruit (mm)			De-hulled fruit (mm)			Edible kernel (mm)		
		Length	Width	Thickness	Length	Width	Thickness	Length	Width	Thickness
Mateur	*P. vera*	25.44 a†	13.44 b	12.28 a	21.18 a	11.73 a	10.51 a	16.64 b	8.21 b	7.96 ab
	P. atlantica	25.35 a	13.84 a	12.40 a	20.98 a	11.89 a	10.44 a	16.94 a	8.83 a	8.04 a
Achouri	*P. vera*	23.43 b	12.31 c	11.26 b	19.80 c	10.89 b	9.84 b	15.61 c	8.36 ab	7.64 c
	P. atlantica	23.68 b	12.24 c	11.31 b	20.12 b	10.98 b	9.92 b	16.38 b	8.06 a	7.75 bc
ANOVA test‡		**	**	**	***	**	***	*	*	**

† Values (mean of 5 replications) followed by the same letter, within the same column, were not significantly different (p < 0.05), Tukey's least significant difference test. ‡NS = not significant at p< 0.05; *, **, and **, significant at p< 0.05, 0.01 and 0.001 respectively.

Mineral content

The mineral contents of pistachio kernels are shown in Table 3. Values recorded in ''Achoury'' kernels were, in general, significantly higher than those of the "Mateur"; the lowest values were found for the "Mateur" fruits grafted on *P. vera*. No statistical significant differences among samples were recorded for the Mg content. The most important conclusion of this section is that the worst combination rootstock and variety was *P. vera* and "Mateur", which led to the lowest contents of Ca, K, Fe, Cu, Mn, and Zn. There seemed to be a trend to be demonstrated in future studies with more replications that the use of *P. atlantica* increased the mineral contents, although no statistically significant differences were found in the current study. It is difficult to clearly establish which were the effects of the pistachio cultivar/variety and the rootstock on the mineral contents, and in this way **Küçüköner and Yurt (2003)** found no significant differences in Turkish pistachio nuts for Cu and Mg. While, **Carbonell-Barrachina *et al.* (2015)** only found *P. atlantica* nuts contained higher amounts of Fe, Cu and Zn, than the other rootstocks under analysis (*P. integerrima* and *P. terebinthus*).

Table 3 Mineral content in pistachio samples as affected by variety and rootstock

Variety	Rootstock	Macro-elements (g kg^{-1})			Micro-elements (mg kg^{-1})			
		Ca	Mg	K	Fe	Cu	Mn	Zn
Mateur	*P. vera*	18.0 b[†]	4.8	9.3 b	51.4 b	13.7 b	7.4 b	34.8 b
	P. atlantica	19.7 ab	4.9	9.7 ab	52.6 ab	15.3 ab	8.9 ab	35.3 b
Achouri	*P. vera*	19.8 ab	4.9	10.1 a	55.6 ab	15.8 a	9.4 a	40.5 a
	P. atlantica	22.1 a	5.0	10.2 a	58.5 a	16.1 a	9.9 a	37.6 ab
ANOVA test[‡]		*	NS	**	**	**	***	**

[†] Values (mean of 5 replications) followed by the same letter, within the same column, were not significantly different ($p < 0.05$), Tukey's least significant difference test. [‡]NS = not significant at $p< 0.05$; *, **, and **, significant at $p< 0.05$, 0.01 and 0.001 respectively.

Fatty acid profile

Five fatty acids [palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2)] were detected in all pistachio samples (Table 4). Palmitic acid and palmitoleic acid contents were similar in all samples, with no significant differences among samples, and with C16:0 being higher (mean of 10.37 % for all samples) than C16:1 (mean of 0.85 %). Data showed no statistically significant differences for palmitic, palmitoleic, and stearic acids; whereas, significant differences between varieties were found for oleic and linoleic acids. The oleic acid was the predominant compound in both studied varieties, reaching a mean value for all studied samples of 67.63 %. This finding agreed with previous studies **(Chahed *et al.*, 2008; Satil *et al.*, 2003)**. The highest and lowest content of oleic acid were found in "Achoury" variety grafted on *P. atlantica* (69.58 %) and "Mateur" variety grafted on *P. vera* (65.58 %), respectively. These results agreed with those of **Chahed *et al.* (2008)**, who reported that the oleic acid ranged from 54.2 to 76.8 % in "Mateur" kernels cultivated in four areas with different climatic conditions; the lowest amount of oleic acid (54.2 %) was found in the area with semi-humid climate compared to the other three areas with semi-arid to arid climates.

On the other hand, the combination of *P. vera* and "Mateur" variety led to the highest content of linoleic acid. Although both unsaturated fatty acids have interesting health effects, a higher linoleic acid content can be responsible for faster oxidative alterations of pistachio oil.

Table 4 Fatty acid profile (%) in pistachio samples as affected by variety and rootstock

Variety	Rootstock	Fatty acids (%)				
		C16:0	C16:1	C18:0	C18:1	C18:2
Mateur	*P. vera*	10.46	0.89	2.03	65.58 b[†]	21.03 a
	P. atlantica	10.23	0.80	2.59	68.70 ab	17.68 b
Achouri	*P. vera*	10.50	0.90	2.54	66.64 ab	19.44 ab
	P. atlantica	10.29	0.80	2.72	69.58 a	16.61 b
ANOVA test[‡]		NS	NS	NS	*	**

[†] Values (mean of 5 replications) followed by the same letter, within the same column, were not significantly different ($p < 0.05$), Tukey's least significant difference test. [‡]NS = not significant at $p< 0.05$; * and **, significant at $p< 0.05$ and 0.01, respectively.

Total polyphenolic content and antioxidant activity

A number of studies have shown that the presence of phenolic compounds in food and especially in fruits can be of particular importance for consumers, because of their beneficial health properties **(Chong *et al.*, 2013)**. Current data showed that total polyphenolic compounds values ranged between 1556 and 1629 mg GAE 100 g^{-1} fresh weight, fw (Table 5). There were no significant differences between the different variety/rootstock combinations.

The antioxidant potential of pistachio fruit can be affected by many factors, including maturity stage. Pistachio nuts are a rich source of phenolic compounds, and have recently been ranked among the first 50 food products highest in antioxidant potential **(Tomaino *et al.*, 2010)**. Antioxidants play a fundamental role in everyday life due to their unquestionable beneficial effects on living organisms that enable them to overcome, for instance, oxidative injuries, modulating biological pathways and membrane functionality, showing anti-inflammatory, anti-infective, antifungal, antiviral, antibacterial and antioxidant activities **(Barreca, Bellocco, Laganà, *et al.*, 2014; Barreca, Bellocco, Leuzzi, *et al.*, 2014; Smeriglio *et al.*, 2014)**.

The only method reporting differences among the studied samples was $ABTS^+$. The $ABTS^+$ activity was significantly higher in the "Achouri" as compared to the "Mateur" ones; however, no statistically significant differences were found for the rootstock (Table 5).

Table 5 Antioxidant activity (mmol Trolox kg^{-1} fresh weight, fw) and total polyphenol content (mg GAE 100^{-1} g fw) in pistachio samples as affected by variety and rootstock

Variety	Rootstock	FRAP	DPPH	ABTS	TPC
		(mmol Trolox kg^{-1} fw)			(mg GAE 100^{-1} g fw)
Mateur	*P. vera*	220	49.9	12.5 b[†]	1581
	P. atlantica	219	49.5	12.2 b	1556
Achouri	*P. vera*	229	52.9	15.6 a	1629
	P. atlantica	223	50.4	15.3 a	1615
ANOVA test[‡]		NS	NS	***	NS

[†] Values (mean of 5 replications) followed by the same letter, within the same column, were not significantly different ($p < 0.05$), Tukey's least significant difference test. [‡]NS = not significant at $p< 0.05$; *** significant at $p< 0.001$.

CONCLUSION

The morphological, chemical, and functional parameters depended more on the pistachio variety (“Mateur” or “Achoury”) than on the rootstock (*Pistacia vera* or *P. atlantica*). The replacement of the most popular *P. vera* by *P. atlantica* in “Mateur” fruits had positive effects on yield, nut dehiscence rate, and oleic acid, while in “Achoury” pistachios showed positive effects on the morphology, and oleic acid. Thus, it can be concluded that the use of the autochthon rootstock, *P. atlantica*, can be a good alternative for Tunisian pistachios orchard, because its use improved or maintained the global quality parameters as compared to the other studied rootstock, *P. vera*.

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