

Morphological investigation of genetic diversity of pistachio (*Pistacia vera*) germplasm in arid land of Tunisia

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Background and aims – Pistachio (*Pistacia vera* L.) is an interesting crop for arid areas, well adapted to marginal lands and to drought conditions. Traditional production areas of Tunisia harbour an interesting diversity of pistachio germplasm. In order to identify and describe this diversity, a field study was conducted in the traditional pistachio production areas of El-Guetar and Sfax in 2004 and 2005.

Material and Methods – A total of 256 female and male pistachio specimens were prospected and compared to the main commercial cultivar 'Mateur'. Flowering, tree habit, nut and seed characteristics were determined for 64 pistachio female land races from the El-Guetar oasis and 25 female land races from Sfax.

Key results – Differences were observed among all the land races. Flowering and ripening time were particularly variable. Significant variation of nut weight from 0.48 to 1.03g was also observed. Some land races presented nut weight as much as 10% higher than 'Mateur' i.e. Fourati1 and Fourati10 from the region of Sfax and MTSG10, AMHA5 and EPE3 from the El-Guetar oasis. Blank production and split nuts ratio ranged respectively from 2 to 60% and from 14 to 95%. Furthermore, 20 land races had a blank production below 10% while 14 local land races had the split nuts ratio superior to 81% recorded for 'Mateur'. The phenotypic data were evaluated using cluster analysis. Parameters related to leaf and nut size and fruit quality had high discriminating values. Different groups of land races were identified with similar flowering periods, nut and seed characters for each cluster.

Conclusion – The evaluation of germplasm in Tunisia revealed promising land races. Additional biochemical and molecular studies will provide the necessary complementary information that could result in potentially valuable land race selection.

Key words – *Pistacia vera*, germplasm, characterization, drought conditions.

INTRODUCTION

Pistachio (*Pistacia vera* L.) is considered as one of the more important fruit nuts in the Mediterranean basin and even in the world. It is cultivated in arid areas and is well adapted to marginal lands (Jacquy 1973). This crop is now cultivated all over the world often using innovative cultivation techniques resulting in increasing yields and high economic returns. Relatively few cultivars are currently used in the large-scaled pistachio production (Tous & Ferguson 1996). Land races are often replaced by a few improved cultivars and the destruction of natural habitats is responsible for the loss of wild material and land races. Consequently genetic diversity of pistachio is in risk. Up until now, little scientific attention is given to the genetic variability of the species and the conservation thereof.

The few studies that focused on the variability of pistachio deal with the description of male and female specimens (Oukabli 1995, Vargas et al. 1995). A descriptor list has been developed for the species (IPGRI 1997). However, in Tunisia, where pistachio is cultivated since Carthaginians time (2800 years ago) little is known about its morphological and genetic variability. Jacquy (1973) collected different specimens from traditional production regions of the Centre and the South of Tunisia such as Sfax, El-Guetar and Meknassy. In the North of Tunisia, 'Mateur' cultivar and two main male genotypes precocious 25A and late 40A were selected. Unfortunately germplasm conservation was suspended later on (Ghorbel et al. 2001).

The cultivar 'Mateur' is the predominant cultivar in Tunisia, while land races are often abandoned and risk vanishing

in the near future. This would lead to serious genetic erosion. The preservation of these land races that often show specific adaptations (e.g. to drought conditions, saline soils and high solar radiation) is increasingly important as pistachios are the ideal crops for marginal soils and areas with limited water supply. Therefore, an assessment of the existing cultivar collection has been made (Ghrab et al. 2002, 2005). Traditional zones of pistachio cultivation have been prospected in the Centre and the South of Tunisia respectively in Sfax and El-Guetaar regions in 2004 and 2005. This paper describes the diversity observed in local pistachio germplasm based on morphological features.

MATERIAL AND METHODS

Prospected areas

Surveys of local pistachio germplasm were undertaken for two years (2004–2005) in the regions of Sfax and El-Guetaar (fig. 1). These regions are well-known historical pistachio production areas and are characterized by harsh climatic conditions. The region of Sfax is characterized by a coastal Mediterranean climate with dry and hot summers and wet winters. The climate is arid with annual rainfall of 200 mm. El-Guetaar has a continental Mediterranean climate. In this region annual rainfall is c. 140 mm with high temperature amplitudes between day and night and between seasons. Both regions studied are characterized by a long dry period of four (May–August) to six months (April–September) and high evaporative demand ($ETo > 1400$ mm).

For our survey, only the oldest trees, excluding the introductions made through development projects, were studied.

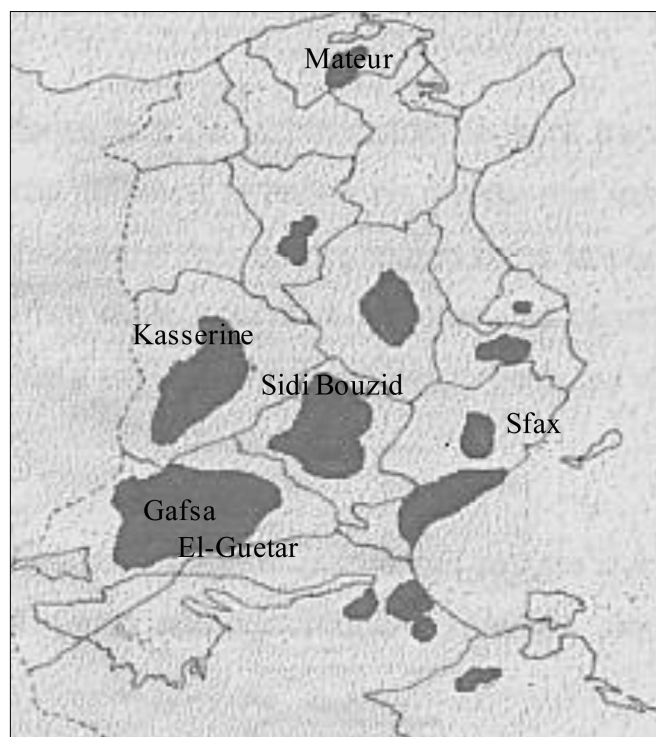


Figure 1 – Geographical distribution of Tunisian pistachio growing area.

Table 1 – Code of morphological characters used in this study

	Parameter	Code
Tree	1. Flowering date (day after Mateur)	FD
	2. Tree shape	TSh
	3. Leaf colour	LC
Leaf	4. Leaf length (cm)	LI
	5. Leaf width (cm)	Lw
	6. Leaf ratio (length/width)	LR
	7. Leaflet number	NL
Leaflet	8. Terminal leaflet length (cm)	TLI
	9. Terminal leaflet width (cm)	TLw
	10. Terminal leaflet ratio (length/width)	TLR
	11. Nut weight (g)	NW
Nut	12. Nut length (mm)	NI
	13. Nut ratio (length/width)	NR1
	14. Nut ratio (length/thickness)	NR2
	15. Maturity (%)	M
	16. Blank production (%)	BP
	17. Split nuts (%)	SN
	18. Hull colour	HC
	19. Kernel colour	KC

For every land race we associate the locality name followed by a number of orders. During visits to the El-Guetaar oasis, several male and female specimens were discerned. This oasis is known to house old specimens of pistachio (Jacquy 1973). Old sites of pistachio production in the Sfax region were also prospected, i.e. old farms of Chaal and some private orchards of Thyna, Mahres, Gargour and Hajeb.

Land races characterization

Important morphological traits were identified using to the pistachio descriptor list (IPGRI 1997). For the present study, nineteen characters were retained which represent most of the morphological and production-related variability observed within the pistachio germplasm (Table 1). The flowering date (FD) of the trees was described in terms of days after flowering of the ‘Mateur’ cultivar. Tree shape (TSh) was determined using the following descriptive terms: erect (1), semi erect (2), spreading (3), drooping (4). The morphological description of the leaves was based on leaf colour (LC), leaf length (LI), leaf width (Lw), leaf ratio (LR), leaflet number (NL), terminal leaflet length (TLI), terminal leaflet width (TLw) and terminal leaflet ratio (TLR). Leaf colour was determined using the following scale: clear green (1), green (2) dark green (3). The following nut characters were described: weight (NW), length (NI), length/width ratio (NR1) and length/thickness ratio (NR2). At harvest, ten grapes were collected to determine the mature fruits percentage (M). The blank production (BP) and split nuts (SN) were computed on five samples of 100 nuts per tree. Blank production is the percentage of unfilled nuts which are aborted nuts remain on the tree and grow to about the same size and shape as nuts containing fully developed embryos. Split nuts ratio is the proportion of opened nuts from the total nuts sample. The Hull colour (HC) and kernel colour (KC) were also registered. For hull colour the following descriptive terms were used: yellow (1), purple yellow (2), purple (3), rose (4), clear

Table 2 – Mean values of tree, leaf and nut characters of pistachio germplasm from Sfax region.

FD: flowering date after 'Mateur', TSh: tree shape, LC: leaf colour, LI: leaf length, LW: leaf width, LR: leaf rate, NL: leaflets number, TLI: terminal leaflet length, TLw: terminal leaflet width, TLR: terminal leaflet rate, NW: nut weight, NR1: nut length, NR2: nut rate 2, M: mature fruits percentage, BR: bank rate, SR: split rate, HC: hull colour, KC: kernel colour.

	FD	TSh	LC	LI	LW	LR	NL	TLI	TLw	TLR	NW	NL	NR1	NR2	M	BR	SR	HC	KC
'Mateur'	-	3	3	13.75	11.58	1.19	5	7.68	5.33	1.44	0.93±0.03	19.75	1.71	1.91	88.5±1.5	6±0.2	81±7.5	2	1
Fourati10	5	2	1	11.0	11.0	1.00	5	6.5	3.5	1.86	0.97±0.01	19.19	1.70	1.88	82.9±3.7	3±0.9	69±3.4	5	4
Fourati5	5	3	2	14.0	14.0	1.00	5	7.0	5.0	1.40	0.55±0.05	18.92	1.73	1.89	58.5±15.5	16±4.2	52±8.4	1	2
Fourati9	5	1	1	12.5	15.0	0.83	5	8.5	6.0	1.42	0.68±0.01	19.62	1.73	1.90	85.9±4.3	2±0.4	65±5.4	4	3
Fourati1	9	4	3	10.5	11.5	0.91	5	6.0	4.0	1.50	0.67±0.02	18.96	1.81	2.02	62.2±7.6	23±7.1	60±6.1	3	1
Fourati2	9	3	3	12.5	13.5	0.93	5	7.5	5.0	1.50	1.00±0.01	17.62	1.76	1.85	71.6±3.2	10±3.9	68±1.3	1	5
Fourati3	5	2	2	11.5	14.0	0.82	5	7.5	5.5	1.36	0.78±0.04	18.38	1.52	1.84	58.3±3.4	22±4.7	55±0.2	1	1
Fourati4	9	1	2	16.5	16.0	1.03	5	9.5	5.5	1.73	0.80±0.03	18.72	1.54	1.85	77.4±2.5	7±1.9	33±9.1	1	4
Fourati6	5	2	1	16.0	17.5	0.91	5	9.5	5.0	1.90	0.69±0.02	17.84	1.61	1.94	79.2±2.0	12±2.1	41±8.9	2	3
Fourati7	9	1	1	15.5	16.0	0.97	5	8.0	5.0	1.60	0.61±0.01	18.86	1.65	1.87	71.3±3.4	14±1.6	57±1.8		
Fourati12	9	4	3	12.0	10.0	1.20	5	8.0	5.5	1.45	0.81±0.01	19.25	1.63	1.93	67.6±1.9	9±0.8	21±2.3	2	5
Rmili16	8	4	3	14.0	17.0	0.82	5	8.0	5.0	1.60	0.89±0.01	18.19	1.69	1.98	64.3±14.1	14±0.8	28±4.6	1	3
Ibala3	5	1	1	9.0	10.5	0.86	5	5.5	3.0	1.83	0.68±0.04	17.44	1.70	1.94	62.3±14.3	12±3.0	41±0.6	1	2
Ibala2	2	1	2	10.0	12.5	0.80	5	6.5	5.5	1.18	0.60±0.01	16.47	1.61	1.77	51.0±14.6	23±8.0	36±3.3	1	3
Masmoud1	5	2	2	12.5	12.5	1.00	5	7.0	4.5	1.56	0.53±0.04	15.58	1.58	1.73	76.7±4.0	13±2.2	64±1.1		
Masmoud4	3	1	2	18.0	16.0	1.13	5	9.5	6.0	1.58	0.88±0.04	19.77	1.67	1.73	67.6±4.2	19±5.0	53±6.1		
Sridique1	0	4	1	10.5	11.0	0.95	5	7.0	5.0	1.40	0.70±0.06	19.25	1.72	1.89	66.1±11.3	25±4.7	84±11.4	3	4
Sridique2	0	3	2	10.5	11.0	0.95	5	6.5	4.5	1.44	0.71±0.04	19.78	1.73	1.78	76.8±15.9	18±3.3	87±1.0	2	1
Sridique3	0	4	2	13.5	11.0	1.23	5	8.5	5.5	1.55	0.73±0.01	19.98	1.65	1.92	82.8±16.0	14±5.3	95±1.7	3	2
Loby7	-1	1	3	11.5	17.0	0.68	5	7.0	5.5	1.27	0.74±0.06	16.56	1.64	1.82	91.9±3.1	2±0.1	68±12.5	1	2
LobyB2	0	2	3	14.5	9.5	1.53	5	7.5	7.0	1.07	0.81±0.03	18.79	1.68	1.96	76.5±7.7	2±0.5	39±13.1	2	3
LobyB3	0	2	1	13.5	7.5	1.80	5	7.5	4.5	1.67	0.79±0.04	19.50	1.80	1.95	81.7±1.3	8±3.1	79±0.8	1	4
LobyC2	0	3	1	12.0	11.5	1.04	5	7.0	5.0	1.40	0.77±0.10	18.44	1.65	1.86	62.9±13.4	15±1.5	70±9.1	1	4
LobyC3	-3	4	2	14.5	11.5	1.26	5	8.5	5.5	1.55	0.80±0.04	18.86	1.72	2.01	75.5±14.5	22±5.8	47±8.8	3	1
LobyB8	3	4	2	12.5	10.0	1.25	5	7.5	5.0	1.50	0.86±0.01	17.29	1.56	1.92	77.8±8.8	10±0.3	63±17.7	1	3
LobyC5	-1	3	2	13.0	8.5	1.53	5	7.5	5.0	1.50	0.86±0.03	19.54	1.67	1.90	56.0±5.4	17±4.2	94±3.0	2	3

Table 3 – Mean values of tree, leaf and nut characters of pistachio germplasm from El Guetar oasis.

FD: flowering date after 'Mateur', TSh: tree shape, LC: leaf colour, LI: leaf length, Lw: leaf width, LR: leaf rate, NL: leaflets number, TLI: terminal leaflet length, TLw: terminal leaflet width, TLR: terminal leaflet rate, NW: nut weight, NR1: nut length, NR2: nut rate 1, NR2: nut rate 2, M: mature fruits percentage, BR: bank rate, SR: split rate, HC: hull colour, KC: kernel colour.

	FD	TSh	LC	LI	Lw	LR	NL	TLI	TLw	TLR	NW	NL	NR1	NR2	M	BR	SR	HC	KC
'Mateur'	-	3	3	13.75	11.58	1.19	5	7.68	5.33	1.44	0.94±0.03	20.78	1.72	1.90	85.0±1.9	10±2.2	78±10.5	2	1
AMMS1	10	2	3	16.0	15.0	1.07	5	9.0	5.0	1.80	0.64±0.02	17.98	1.75	1.82	42.3±16.4	14±1.4	53±3.0	3	3
AMMS2	-6	2	2	15.5	13.0	1.19	5	8.5	4.5	1.89	0.75±0.24	17.62	1.65	1.80	64.5±6.1	23±5.2	40±1.3	1	2
AMMS3	10	1	3	14.5	12.0	1.21	5	7.5	4.0	1.88	0.81±0.10	18.12	1.63	1.81	47.8±16.3	12±3.0	35±3.5	3	3
AMMS4	-6	1	3	9.5	10.0	0.95	5	7.0	3.5	2.00	0.77±0.17	19.77	1.60	2.00	73.2±5.6	17±4.4	61±1.5	1	2
YAG1	5	2	3	12.0	9.0	1.33	5	7.5	4.0	1.88	0.82±0.04	19.58	1.56	1.86	53.8±13.2	18±0.6	34±5.4	1	3
YAG2	5	2	3	13.5	12.0	1.13	5	7.5	3.5	2.14	0.69±0.05	18.82	1.65	1.74	68.9±2.6	21±1.0	68±5.8	5	1
TTL1	5	2	2	14.5	13.5	1.07	5	7.5	4.0	1.88	0.88±0.06	18.99	1.69	1.79	63.3±17.4	15±1.6	85±13.5	1	5
TTL2	-1	2	2	15.0	17.0	0.88	5	10.0	6.0	1.67	0.48±0.02	18.76	1.69	2.00	50.9±8.4	9±2.3	58±6.2	3	1
TTL3	5	1	2	15.5	14.5	1.07	5	9.0	5.0	1.80	0.74±0.01	19.81	1.59	1.92	74.2±5.8	16±4.2	49±11.1	1	4
TTL4	10	1	2	10.0	9.5	1.05	5	5.5	3.5	1.57	0.76±0.05	17.50	1.77	1.72	52.6±3.3	10±1.8	52±5.7	3	5
TTL5	-1	1	2	13.0	12.5	1.04	5	7.0	3.5	2.00	0.81±0.02	17.17	1.52	1.72	51.3±9.4	24±5.9	28±8.6	5	5
TTL8	10	1	2	16.0	13.0	1.23	5	9.0	5.0	1.80	0.68±0.03	20.02	1.62	1.84	78.7±2.2	35±5.6	82±4.1	1	3
TTL10	-1	1	1	10.0	11.5	0.87	3	6.0	4.0	1.50	0.71±0.07	18.38	1.65	1.88	56.6±6.1	26±3.0	35±0.9	1	4
TTL11	-1	1	3	10.0	8.0	1.25	3	6.0	3.0	2.00	0.72±0.12	17.99	1.70	1.76	44.2±7.8	42±12.2	26±0.9	2	3
ASM	12	1	1	10.5	12.5	0.84	5	7.0	4.0	1.75	0.73±0.15	19.77	1.75	1.92	65.6±2.2	29±6.1	75±3.6	1	1
MTSG1	5	4	1	8.5	8.0	1.06	5	4.5	4.0	1.13	0.80±0.01	19.27	1.57	1.77	36.3±1.8	18±3.2	59±7.6	1	3
MTSG2	-6	3	2	14.0	12.0	1.17	5	8.0	3.0	2.67	0.70±0.01	19.84	1.63	1.96	40.6±18.3	7±2.1	79±4.1	1	2
MTSG6	5	2	2	13.0	13.0	1.00	5	7.5	4.0	1.88	0.64±0.04	19.56	1.64	1.96	59.4±11.9	13±2.1	73±3.1	1	3
MTSG7	12	2	2	10.0	9.0	1.11	5	6.0	5.0	1.20	0.81±0.01	18.92	1.66	1.89	61.5±17.6	9±1.3	54±6.2	1	3
MTSG8	-6	2	2	12.0	10.5	1.14	5	6.0	4.5	1.33	0.74±0.03	18.65	1.71	1.81	55.3±16.0	13±4.0	40±12.4	1	3
MTSG9	-6	2	2	9.0	9.0	1.00	5	5.0	3.0	1.67	0.87±0.01	19.00	1.63	1.90	74.5±19.2	22±3.6	28±2.5	3	5
MTSG10	5	3	2	13.5	13.0	1.04	5	7.0	4.5	1.56	1.03±0.04	19.17	1.46	1.49	46.2±11.0	13±3.6	35±2.8	1	6
MTSG11	5	2	3	12.0	10.5	1.14	5	7.0	6.0	1.17	0.77±0.03	19.25	1.73	1.93	20.5±13.4	9±1.9	53±2.3	1	3
ADJ1	9	4	2	12.5	12.0	1.04	5	6.5	4.5	1.44	0.62±0.08	20.19	1.58	2.24	61.7±8.9	7±2.7	67±0.8	1	3
MASS1	-1	3	2	15.5	13.5	1.15	5	9.5	5.5	1.73	0.76±0.20	20.45	1.64	2.09	50.3±13.1	6±2.0	51±2.6	2	2
MASS3	-6	3	2	15.0	12.0	1.25	5	9.0	5.5	1.64	0.66±0.08	15.58	1.64	1.71	72.1±18.6	15±1.9	30±2.6	1	5
MASS4	-1	2	1	12.0	12.0	1.00	5	7.0	4.5	1.56	0.66±0.06	17.20	1.66	1.62	73.0±1.5	14±4.9	64±1.2	1	1
MASS6	-1	1	1	14.0	13.5	1.04	5	9.0	5.5	1.64	0.81±0.01	16.56	1.70	1.66	49.2±0.3	13±3.7	36±2.8	5	5
MASS7	-6	1	1	11.5	10.5	1.10	5	6.5	4.5	1.44	0.74±0.02	18.72	1.80	1.80	48.3±15.2	18±3.3	28±3.3	3	3
BASCHI	-6	2	2	10.5	11.0	0.95	5	5.0	3.5	1.43	0.84±0.07	20.79	1.67	1.82	54.8±18.6	21±8.5	70±0.8	5	2
BMS1	5	1	3	13.0	13.0	1.00	5	6.5	6.0	1.08	0.75±0.01	19.87	1.73	1.79	56.7±8.2	27±8.0	77±2.3	3	3
BMS2	-6	1	3	13.0	10.5	1.24	5	5.0	4.0	1.25	0.73±0.03	19.77	1.80	1.98	50.5±8.1	14±0.8	60±1.0	1	4
BMS3	-9	1	3	13.0	12.0	1.08	5	7.5	5.0	1.50	0.71±0.00	19.78	1.65	1.90	68.0±17.7	13±3.6	52±3.9	1	3

Table 3 (continued) – Mean values of tree, leaf and nut characters of pistachio germplasm from El Guetar oasis.

FD: flowering date after 'Mateur', TSh: tree shape, Lw: leaf length, Lr: leaf colour, Lf: leaflet number, TLl: terminal leaflet length, TLw: terminal leaflet width, TLR: terminal leaflet rate, NW: nut weight, NL: nut length, NR1: nut rate1, NR2: nut rate2, M: mature fruits percentage, BR: bank rate, SR: split rate, HC: hull colour, KC: kernel colour.

	FD	TSh	LC	LI	Lw	LR	NL	TLl	TLw	TLR	NW	NL	NR1	NR2	M	BR	SR	HC	KC
BMS4	-9	1	1	11.0	8.5	1.29	5	6.0	5.0	1.20	0.74±0.08	19.50	1.61	1.81	57.7±3.9	7±1.8	84±6.9	5	5
BMS6	-6	2	3	13.5	12.0	1.13	5	7.0	4.0	1.75	0.86±0.09	19.98	1.76	1.96	46.3±2.4	13±4.3	34±4.5	1	3
BMS7	-6	1	3	14.0	12.0	1.17	5	5.5	4.0	1.38	0.77±0.01	18.78	1.79	1.94	76.4±17.5	6±1.7	67±7.5	1	4
BMS9	-6	1	3	10.5	11.0	0.95	5	6.0	3.5	1.71	0.73±0.04	19.49	1.65	1.93	43.4±18.3	40±12.3	46±3.8	2	3
AMHA2	5	1	2	11.5	9.0	1.28	5	4.5	4.0	1.13	0.68±0.02	18.99	1.56	2.11	84.4±4.0	14±3.5	33±12.8	2	3
AMHA1	5	4	2	12.5	12.0	1.04	5	6.0	5.0	1.20	0.91±0.03	18.86	1.77	1.96	49.2±2.8	24±4.2	34±4.1	3	1
AMHA5	5	3	2	14.5	14.5	1.00	5	7.5	5.5	1.36	1.00±0.02	17.29	1.84	1.84	58.7±2.2	13±4.0	36±4.6	2	4
AMHA7	5	4	2	13.0	13.5	0.96	5	7.0	5.0	1.40	0.69±0.07	18.65	1.63	1.86	58.6±2.0	45±6.4	14±4.9	6	1
ABT2	2	2	3	12.5	11.5	1.09	5	6.5	5.0	1.30	0.73±0.04	19.44	1.61	2.11	59.6±17.5	12±2.5	70±8.5	5	5
TMM1	9	2	1	12.5	12.0	1.04	5	8.5	5.5	1.55	0.73±0.02	19.58	1.65	1.98	31.0±13.4	25±7.7	92±5.3	3	3
TMM2	-6	2	2	11.0	11.0	1.00	5	6.0	4.0	1.50	0.80±0.05	17.84	1.60	1.80	75.8±16.7	16±5.8	26±9.7	1	5
TMM3	-6	3	1	8.5	9.0	0.94	5	4.0	3.0	1.33	0.73±0.08	18.44	1.70	1.79	54.0±10.7	27±3.4	26±10.2	1	3
TMM4	9	2	2	14.0	10.5	1.33	5	9.0	5.0	1.80	0.64±0.07	18.82	1.75	1.90	69.9±7.1	14±1.8	53±5.3	5	1
TMM6	-6	1	2	10.0	11.0	0.91	5	5.5	4.5	1.22	0.75±0.04	19.93	1.65	2.03	42.6±2.0	23±4.4	40±13.7	2	1
MTS1	5	1	2	14.0	14.0	1.00	5	8.0	6.0	1.33	0.66±0.01	19.25	1.72	1.89	52.6±3.4	17±0.8	67±0.5	3	3
MTS3	9	2	2	12.5	14.0	0.89	5	9.0	5.0	1.80	0.73±0.03	19.51	1.81	2.10	33.4±15.0	11±1.0	52±10.2	3	3
EPE1	-6	2	2	10.0	11.0	0.91	5	6.0	3.5	1.71	0.93±0.09	20.63	1.79	1.86	63.1±1.5	22±6.1	57±9.5	6	3
EPE2	-11	2	1	12.0	10.5	1.14	5	5.5	3.5	1.57	0.89±0.08	20.53	1.78	2.03	53.2±14.8	41±10.1	50±1.1	1	1
EPE3	-6	3	1	7.5	8.0	0.94	5	4.0	3.0	1.33	0.94±0.09	18.92	1.73	1.89	46.2±14.4	20±7.7	17±9.3	5	5
EPE4	-6	4	2	9.0	8.0	1.13	5	6.0	3.0	2.00	0.89±0.07	19.28	1.69	1.91	64.9±16.2	13±3.3	34±0.1	3	1
EPE5	-6	3	2	7.5	7.0	1.07	5	3.5	2.5	1.40	0.72±0.02	18.87	1.81	2.03	42.6±17.5	21±6.2	91±3.3	2	4
EPE6	-6	3	2	8.5	10.0	0.85	5	4.5	4.0	1.13	0.93±0.02	19.74	1.78	1.99	47.8±14.5	9±2.8	29±2.4	6	5
HAB3	-6	4	2	8.0	10.5	0.76	5	5.5	4.0	1.38	0.69±0.03	20.43	1.69	1.96	59.8±0.4	21±5.0	68±11.7	5	3
HAB4	-6	4	2	10.5	10.5	1.00	5	6.0	3.5	1.71	0.71±0.10	18.96	1.81	2.02	55.5±2.5	11±3.5	84±0.8	1	1
HAB5	-6	1	3	9.5	10.0	0.95	5	5.0	3.0	1.67	0.63±0.09	18.79	1.68	1.96	40.4±3.5	27±6.0	87±2.2	1	2
MTS2	9	2	3	11.0	11.5	0.96	5	5.5	4.5	1.22	0.85±0.03	19.71	1.72	1.91	36.7±3.9	15±4.8	22±5.5	1	4
MTS5	12	2	2	9.5	10.0	0.95	5	5.5	4.0	1.38	0.79±0.01	19.24	1.49	1.54	59.5±2.3	12±2.1	83±4.3	1	3
MSCh	10	3	2	10.5	11.5	0.91	5	5.5	4.5	1.22	0.68±0.01	18.19	1.69	1.98	33.3±5.4	24±5.8	87±1.7	1	3
MKG	10	1	1	11.5	9.5	1.21	5	6.0	3.5	1.71	0.55±0.05	17.44	1.70	1.94	27.5±19.2	28±3.5	73±3.9	1	2
AT	-6	2	2	12.5	12.0	1.04	5	6.0	4.0	1.50	0.62±0.09	16.47	1.61	1.77	32.3±2.6	43±10.0	92±0.6	1	3
AKT	10	2	2	12.0	10.5	1.14	5	5.5	3.5	1.57	0.56±0.04	17.99	1.71	1.89	19.4±11.5	60±14.4	76±1.0	1	5

Table 4 – Eigenvalues, proportion of variation and eigenvectors associated with the axes of the PCA in pistachio germplasm for the localities of Sfax and El Guetar

FD: flowering date after 'Mateur', TSh: tree shape, LC: leaf colour, LI: leaf length, Lw: leaf width, LR: leaf rate, NL: leaflets number, TLI: terminal leaflet length, TLw: terminal leaflet width, TLR: terminal leaflet rate, NW: nut weight, NL: nut length, NR1: nut rate1, NR2: nut rate 2, M: mature fruits percentage, BR: bank rate, SR: split rate, HC: hull colour, KC: kernel colour.

Locality	Sfax region		El Guetar oasis		
PC (axes)	1	2	1	2	3
% variation	70.272	22.480	54.912	26.423	11.455
Characters					
FD	-0.099	0.009	0.063	-0.061	-0.086
TSh	0.011	-0.026	-0.004	-0.008	-0.007
LC	-0.008	0.006	-0.001	0.005	-0.002
LI	-0.019	0.055	0.008	0.032	-0.036
Lw	-0.049	0.052	0.011	0.015	-0.033
LR	0.003	0.002	0.000	0.001	0.000
TLI	-0.008	0.031	0.004	0.014	-0.029
TLw	-0.006	0.014	0.005	0.008	-0.025
TLR	0.000	0.003	0.000	0.000	0.002
NW	0.001	0.003	-0.001	0.001	-0.001
NI	0.018	-0.002	0.009	0.014	-0.008
NR1	0.001	0.000	0.000	-0.001	0.000
NR2	-0.001	0.001	0.001	0.000	-0.001
M	0.193	0.833	-0.130	0.922	0.351
BR	-0.006	-0.520	0.031	-0.354	0.930
SR	0.974	-0.164	0.989	0.136	0.023
HC	0.016	0.019	-0.012	-0.004	0.002
KC	0.001	0.018	-0.000	0.003	-0.013

yellow (5), and reddish yellow (6). The kernel colour was described using the following terms: yellow (1), greenish yellow (2), green sallow (3), light green (4), green (5) and dark green (6).

Data analysis

To determine the relative importance of the individual characters on the variability between the local land races of pistachio, principal component analyses (PCA) were performed using the software Multi Variate Statistical Package (MVSP) Plus version 3.12e (Kovach computer services, Anglesey, UK). Using this information a dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic averages (UPGMA). For the cluster analysis the 'Gower's General Similarity Coefficient' was used. This coefficient allows calculating a similarity measure using at the same time quantitative and qualitative data (Gower 1967).

RESULTS

Characterization of land races

During two years (2004–2005) of field prospecting, a total of 256 male and female specimens were found. However, only 89 female land races (64 from El-Guetar and 25 from Sfax) and 'Mateur' cultivar were characterized based on morphological features (tables 2 & 3).

The pistachio land races were characterized by a long blooming period, from the last week of March to the third week of April. Compared to the 'Mateur' cultivar, some were flowering earlier and some later. LOBY-C3 from the region of Sfax and AMMS2, AMMS4, MTSG2, MTSG8, MTSG9, MASS3, MASS7, BASCH1, BMS2, BMS3, BMS4, BMS6, BMS7, BMS9, TMM2, TMM3, TMM6, EPE1, EPE2, EPE3, EPE4, EPE5, EPE6, HAB3, HAB4, HAB5 and AT from the El-Guetar oasis are all potentially precocious land races.

A significant difference in the percentage of fruit maturity was observed among the different land races. It varied from 19% for AKT to 92% for LOBY7 specimens (tables 2 & 3). 'Mateur' presented a high percentage of fruit maturity reaching 85%. This indicated a large period of fruit maturity for the prospected land races. As regards to commercial features, nut weight was ranged between 0.48 to 1.03g. Some local land races had a similar or more interesting nut weight than 'Mateur' (0.93g).

The blank production, considered as a disadvantage character, showed an important variation between 2 and 60% within local land races. Thirty land races have a blank production over 20% while twenty land races have a blank production below 10%. 'Mateur' presented a blank production of 6% (Sfax) and 10% (El-Guetar).

Split nuts ratio is a relevant parameter for the selection of genotypes. Split nuts ratio ranged between 14 and 95% among local specimens. This character shows large annual fluctuations. Fourteen local land races had higher split nuts ratio than the cultivar 'Mateur' (81% in Sfax and 78% in El-Guetar), whereas, 34 land races had lower split nuts ratio (< 51%). The majority of prospected land races (79) have an ovoid shape ($1.5 < NI/Nw < 1.8$), whereas 8 land races were elongated ($NI/Nw > 1.8$).

Principal component analysis (PCA)

Data analysis using PCA revealed two main principal components (PCs). The sum of these PCs explained respectively 92.60 and 81.48% of the variability observed between specimens from Sfax and El-Guetar. Explicitly the two PCs account for 70.15 and 22.44% of the variability observed for Sfax region whereas for the locality of El-Guetar the two PCs explained 54.61, and 26.86 (table 4).

Split nuts (SN) were positively correlated with PC1 (table 4) in Sfax. According to PC1, land races having medium split nuts ratio (35 to 55%), high nut length, nut (l/w) ratio (> 1.5) and a largest leaf were easily distinguished such as FOURATI4, FOURATI6, FOURATI7 and MASMOUDI4. PC2 integrated characters related to fruit maturity (M) and blank rate (BR). It is positively correlated to fruit maturity. However,

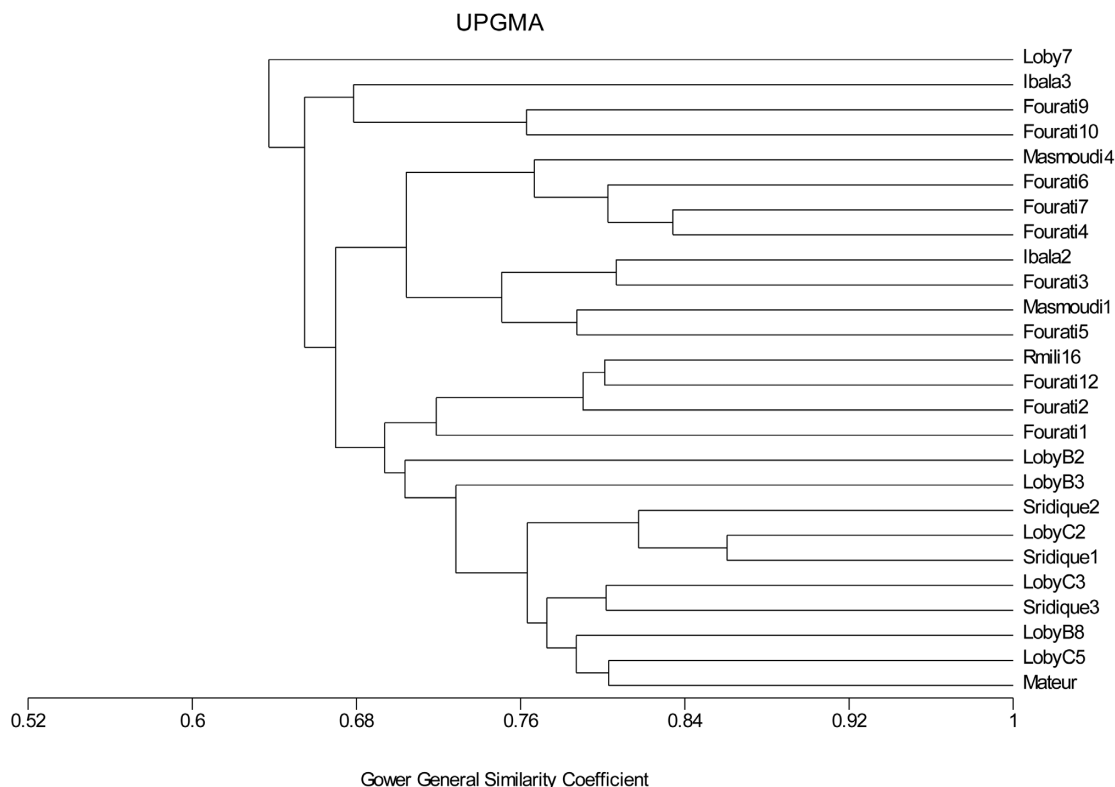


Figure 2 – Cluster dendrogram of pistachio germplasm from Sfax region using UPGMA method.

blank production and split nuts (BP and SN) were negatively correlated.

Principal component analysis was performed in order to identify the main characters responsible of El-Guetar pistachio land races differentiation (table 4). It revealed that PC1 was significantly correlated with split nuts (SN) and negatively with fruit maturity rate (M). PC2 was positively related to fruit maturity (M) and split nuts (SN) and negatively with blank production (BP), whereas PC3 integrated nut characters as maturity (M) and blank production (BP). PCA case scores established revealed that ‘Mateur’ cultivar could be distinguished as having high fruit maturity rate, high nut length and higher split nuts ratio.

Cluster analysis

The land races of each prospected area were classified using hierarchical clustering based on all the measured characters by UPGMA method. These land races were placed into 4 and 11 distinct homogenous groups respectively for Sfax and El-Guetar localities (figs 2 & 3). For Sfax, the clusters grouped respectively 14, 8, 3 and 1 land races (fig. 2). Within these distinct clusters, high similarity was observed between some land races. It is the case of SRIDIQUE1 and LOBY-C2 from cluster 1. It is important to note that ‘Mateur’, LOBY-C5 and LOBY-B8 formed a subgroup which integrated cluster 1. This cluster grouped land races having low Lw, high nut length (NI) and high split and maturity rates. Cluster 2 was divided in two main subgroups. The first subgroup grouped

four land races (FOURATI5, MASMOUDI1, FOURATI3 and IBALA2) characterized by their late flowering, dark green colour of leaf, low nut weight, yellow hull colour and high blank production. The second subgroup containing FOURATI4, FOURATI7, FOURATI6 and MASMOUDI4 with late flowering, erected tree shape, high LI, Lw and TL1 values, and high fruit maturity rate. IBALA3, FOURATI9 and FOURATI10 land races from Cluster 3 were characterized respectively by the high NR1 and NR2 values and lowest BP. IBALA3 was distinguished by small leaf and terminal leaflet with the lowest values of LI, TL1 and TLw. Thereafter, LOBY7 land race formed by itself a distinct cluster 4. This specimen had the lowest LR and BP values and the highest maturity percentage (M).

Morphological data assessed using cluster analysis classified pistachio land races from El-Guetar oasis into 11 distinct groups at the Gower similarity coefficient of 0.75 (fig. 3). Four main clusters (2, 3, 4 and 10) were discerned and contained respectively 13, 20, 7 and 10 land races. Each of ‘Mateur’ cultivar and MTSG1 land race formed respectively cluster 1 and 9. Clusters 5, 6, and 8 involved 2 members per group. Clusters 7 and 11 had respectively 4 and 3 land races each. ‘Mateur’ cultivar as reference was distinguished by the highest fruit maturity rate (85%), the highest nut length (NI) and high split nuts ratio (78%) and a weak blank production (10%). Clusters 2 and 3 were the largest groups including accessions with high degree of similarity. Indeed, BMS2 and BMS7 from cluster 3 had the highest degree of similarity. Then BMS1 and MTS1 from cluster 2 and HAB3 and

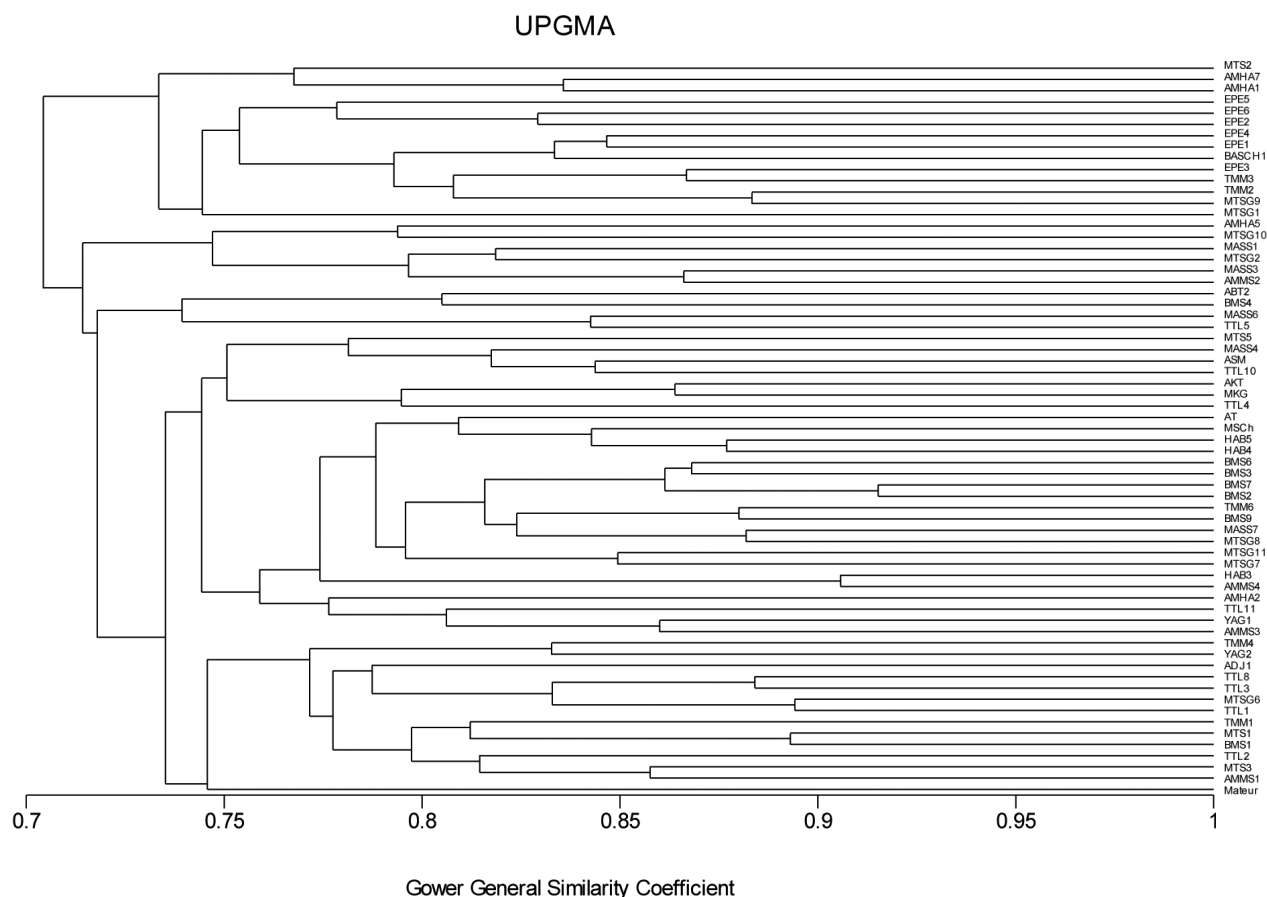


Figure 3 – Cluster dendrogram of pistachio germplasm from El-Guetar oasis using UPGMA method.

AMMS4 from cluster 3 were associated at $d > 0.9$. The first two accessions were late ripening land races with the same values of LR, TLw and NR1. MTSG8 and MASS7 were precocious land races with similar leaf, leaflet and nut characters and equal nut weight. These land races accessions seemed to be identical. Since their origin is oasian ecosystem, part of these similarities can possibly be explained by a common genetic origin.

The land races integrating the second cluster were distinguished by very late flowering, medium nut weight, interesting split nuts ratio, purple hull colour and high leaf length and width values. One land race (AMMS1) presented the highest leaf length and the second (TTL2) was distinguished with the highest values of leaf width and terminal leaflet length and width. TMM1 presented the highest split nuts ratio (92%). Cluster 3 grouped 20 land races divided in subgroups. It integrated land races with similar tree shape (TSch), leaf colour (LC), leaf ratio (LR), fruit maturity rate (M) and kernel colour (KC). HAB3 and AMMS4 formed a subgroup characterized by precocious flowering, similar leaf, leaflet, blank production and split nuts ratio. Cluster 4 was divided into two subgroups containing respectively 3 and 4 land races. The first one grouped very late land races, having similar leaf, leaflet and nuts characters. The second had land races with the same leaf colour (LC), similar leaf and nut

sizes, medium maturity rate, high split nuts ratio and purple hull colour.

Land races TTL5 and MASS6 which integrated cluster 5, flowered at the same time and had erected tree shape and similar leaf dimensions. These land races presented the same nut weight, and hull and kernel colour. Maturity rate was about 50% with low split nuts ratio. Furthermore, cluster 6 grouped two land races distinguished by high split nuts ratio ($> 70\%$), low blank production ($< 12\%$), clear yellow colour of hull and green sallow colour of kernel. Cluster 7 grouped four precocious land races having similar leaf colour and size and nut characteristics. AMHA5 and MTSG10 formed Cluster 8 as late land races with the highest nut weight ($NW > 1g$), the lowest nut ratios (NR1 and NR2) and similar split nuts ratio and blank production (SN and BP). The land race MTSG11 formed itself cluster 9. This land race was characterized by the highest nut weight (NW), the lowest nut ratios (NR1 and NR2) and low maturity rate, split nuts ratio and blank production (M, SN and BP). Cluster 10 included precocious land races with similar leaf and leaflet characteristics. Moreover, cluster 11 grouped land races having late flowering, green colour of leaf and similar nut characteristics.

DISCUSSION

The major goal of this survey is to characterize the local land races and to identify those with higher yield and better quality, or adaptability to specific conditions. Improved yield and quality can be achieved by high total yield, high percentage of splits and low blank ratio (Chao et al. 1998). Some land races were distinguished by their better nut quality. SRID-IQUE3 and LOBY-C5 were identified in the region of Sfax for their higher split nuts ratio and kernel colour, in comparison to 'Mateur' cultivar, two characters appreciated by both consumers and producers. In the oasian ecosystem, the variability was more apparent and land races regrouped more or less valued qualities. In fact, land races having high split nuts ratio and suitable kernel colour were deprived by the important blank production. In comparison to the 'Mateur' cultivar, TTL1 and TTL8 seem promising with high split nuts ratio and nut weight. However, the medium maturity rate and blank production could be improved. Other land races such MTSG10 and AMHA5 have the highest nut weight (> 1 g) and a blank production of less than 15% but are deprived by low maturity and split nuts ratio.

Some land races have high split nuts ratio ranging from 84 to 92 but have small nuts. Mature fruits percentage presented large variability related to ripening period. Under arid conditions with high evaporative demand, ripening precocity is valued. The El Guetar oasis was rich with precocious specimens which give the market opportunities. Chao et al. (1998) reported that valuable specimens may also be discovered by developing precocious and vigorous growing ones as well as those with either earlier or later ripening.

Using the 'Mateur' cultivar characteristics (as reference), our results were consistent with previous reports (Vargas et al. 1997, Caruso et al. 1998, Zribi et al. 2004, 2006). This cultivar presented an elongated shape (IPGRI 1997) with a nut ratio (Nl/Nw) of 1.7 and high percentage of fruit maturity.

The important variation of blank production seems to be attributed to pollen availability and viability. Previous reports showed that pistachio nut development and quality could be affected by the pollinator (Crane & Iwakiri 1986). In fact, the male genotype can influence some important quality traits such as production of empty fruits (blanks). Due to the wide genetic variation and great differences between male and female specimens, pollen origin and quality have important effect on nut characters. Moreover, this criteria can be influenced by others factors rather than genetic dependent.

Split nuts ratio of 'Mateur' cultivar was different from those recorded in previous years at Taous station near Sfax (Zribi et al. 2004) and from other results obtained in Spain and Greece (Vargas et al. 1997, Rouskas 2002). Some researchers suggested that split nuts may be affected by rootstock (Crane & Iwakiri 1986) and irrigation (Spiegel-Rov et al. 1977).

In conclusion, genetic diversity in *Pistacia vera* is considered to be very narrow. In comparison with other fruit trees having similarly ancient histories, only a very small number (less than 100) pistachio cultivars have been described (Vargas & Romero 1998). The reasons for this pau-

city of cultivars are thought to be the long juvenility of pistachio, the long life duration of the trees and the hybridization phenomenon that is very common among different species within the genus *Pistacia* (Maggs 1973). Furthermore, many causes of genetic erosion such as replacement of land races, monoculture, population pressure and environmental degradation, contribute to the lessening of the cultivar platform (Caruso et al. 1998). Nevertheless, a considerable degree of variation still exists among pistachio germplasm. Over the two traditional production zone of pistachio in Tunisia large morphological diversity was observed.

This survey has identified several local female specimens. While being based on morphological characters a classification of these specimens in groups of similar accessions was established. Similarly, a highly diversified population of cultivars and inter specific hybrids of several *Pistacia* genotypes were revealed using morphological features (Kafkas et al. 2002). Then cluster analysis divided most of these accessions into main genetic groups according to their geographic origin. A threshold of similarity of < 0.5 to among the male and female of pistachio accessions from Mediterranean countries was recognized for both the morphological and molecular traits analysis (Caruso et al. 1998).

In this study and according to cluster analysis, a high degree of variation of prospected female land races characteristics is considered. The potential of some proves to be especially good in reference to 'Mateur' cultivar such as FOURATI10, FOURATI2, LOBY7 and LOBY-C7 from Sfax region and BMS4, TTL1, TTL8 and MTSG10 from El-Guetar region. A better resolution can be expected once the set of available descriptors includes all those indicated in the descriptor list. These descriptors would undoubtedly enhance the accuracy of discrimination. On the other hand, further analysis such as lipid and molecular characterization could also be expected to produce significant data for the characterization of local pistachio germplasm. Recently, lipidic characterization of kernel pistachio revealed more promising local land races with better fatty acid composition and fat content than 'Mateur' cultivar (Ghrab et al. 2010). The combination of morphological traits and molecular markers increased the level of accuracy of germplasm identification and further assist in preservation of genetic variability (Barazani et al. 2003).

ACKNOWLEDGMENTS

The authors wish to thank Mr Naceur Hamda, engineer from CTV El-Guetar Gafsa, for his excellent technical assistance in prospecting the oasis and identification of local land races.

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Manuscript received 30 Nov. 2010; accepted in revised version 4 Jun. 2012.

Communicating Editor: Steven Dessen.