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Genetic diversity of pistachio in Tunisia

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Abstract. A research program is focus on protecting local genetic resources of pistachio and almond. The main traditional zones of pistachio crop in the centre and the south of Tunisia were prospected. Many specimens of *Pistacia vera* and *Pistacia atlantica* were investigated. Subsequently, local and foreign cultivars from collection were studied. Flowering time, nut futures and kernel quality were determined. Results obtained revealed interesting ecotypes behaviours and performances. For all the characteristics, great variability between ecotypes was observed. Differences were particularly important when flowering time and maturity period were considered. Nut futures revealed significant differences in *P. vera* nut weight which varied from 0.48 to 1.03 g. Blank and split rates were ranged respectively from 2 to 60% and from 14 to 95%. *P. atlantica* fruits fresh weights varied between 7.9 and 20 g for 100 fruits. Fat content of *P. vera* and *P. atlantica* ecotypes ranged between 56 and 78% DW and 40.45 and 48.2% DW respectively. Palmitic acid was the main saturated fatty acid, its content varied between 7.1 and 12.9% in *P. vera* and between 16.3 and 24.8% in *P. atlantica*. Oleic acid content ranged from 56.1 to 80.7% in *P. vera* with a significant difference over different ecotypes. For *P. atlantica* genotypes this latter fatty acid rates were ranged between 47.3 and 61% DW. Linoleic acid rate, varied between 8.2 and 29.7% DW in *P. vera* and was ranged between 17 and 19% DW in *P. atlantica*. Great variability was detected in *P. atlantica* germination capacity with extremes germination rates of 5 and 100%.

Keywords. *Pistacia vera* – *P. atlantica* – Characterization – Flowering – Fat content – Fatty acids.

Diversité génétique des pistachiers en Tunisie

Résumé. *La richesse en écotypes locaux de pistachier a été étudiée dans un but de préservation des ressources génétiques locales. Un travail de prospection et de caractérisation a été mené dans les zones traditionnelles de production. Plusieurs spécimens de* Pistacia vera *et* Pistacia atlantica *ont été répertoriés. Ainsi une caractérisation phénologique et pomologique a été établie. Les résultats obtenus ont révélé un potentiel intéressant avec une grande variabilité pour les paramètres analysés. Le poids moyen d'un fruit a varié entre 0,48 to 1,03 g et 0,079 et 0,2 g respectivement pour les écotypes des deux espèces* P. vera *et* P. atlantica*. Les taux de fruits vides et déhiscents pour les spécimens cultivés ont été compris respectivement entre 2 et 60% et entre 14 et 95%. Ces écotypes locaux ont montré une richesse en huile des amandes dont la teneur a été de l'ordre de 56 à 78% et de 40,45 à 48,2% par rapport au poids sec. La composition acidique des huiles a révélé trois principaux acides : l'acide palmitique avec des teneurs comprises entre 7,1% et 12,9% pour* P. vera *et 16,3 et 24,8% pour* P. atlantica*, l'acide oléique de 56,1% à 80,7% pour* P. vera *et 47,3 à 61% pour* P. atlantica *et l'acide linoléique d'une teneur variant de 8,2% à 29,7% pour* P. vera *et 17 à 19% pour* P. atlantica*. De même une grande variabilité a été observée pour la capacité germinative des fruits des spécimens de* P. atlantica*.*

Mots-clés. Pistacia vera – P. atlantica – *Caractérisation – Floraison – Matière grasse – Acides gras.*

I – Introduction

Pistachio is an important nut crop well extended in marginal lands with poor soil (Jacquy, 1973; Ferguson *et al.*, 2005). It was used to develop and valorise arid and semi arid regions subjected

to drought in Tunisia. For this reason, governmental encouragements and FAO projects during 1964-1972 period supported plantation in theses areas (Mlika, 1980). Consequently, pistachio plantation increased spectacularly from 30 ha in 1970, to 4400 ha in 1980 and more than 43,000 ha currently.

However, this spreading in cultivated area was not accompanied by an investigation of local genetic diversity. Little is known about variability of this genetic resources and local germoplasm is far from being adequately studied. Very few works were done in this domain.

Actually pistachio was propagated by grafting on *Pistacia vera* seedling. This technique leads to more sensitivity to biotic and abiotic stresses because of low rootstock diversity. Meanwhile, research effort is being focused mainly towards overlooking the security of diversity and the assessment of genetic variability.

Serious genetic erosion and losses of local ecotypes particularly adapted are faced. For this reason, prospecting traditional zones has been carried out in the centre and the south of Tunisia. This paper describes the diversity observed in local pistachio germoplasm based on morphological and lipidic characteristics as well as seed germination capacity.

II – Material and methods

1. Prospected areas

Field *P. vera* prospecting was achieved during two years (2004-2005) in main traditional production zones. It was carried out on the old sites in Sfax and El Guetar regions located respectively in the centre east and west south of Tunisia. These regions represent the famous historical production areas of pistachio. *Pistacia atlantica* field prospecting has been started since 2001 but data presented in this paper were the results of works made from 2004 to 2007 at Sidi Bouzid and Meknassy areas situated in the centre west of Tunisia. In these areas, *P. atlantica* is present as old aged isolated trees that are well adapted to arid climate conditions and poor soils. Morphological and physical characterisation was realized according to the IPGRI descriptors for *Pistacia* spp. excluding *Pistacia vera* L. (IPGR, 1998)

2. Morphological characterization

The most aged trees outside of introductions made through development projects were surveyed and characterized. Important morphological and kernel lipidic traits were analyzed (Table 1). It concerned tree characteristics (flowering date, maturity period and tree habit), morphological description of leaf (color, length, width and l/w ratio), and leaflet (number, terminal leaflet length, width and l/w ratio).

Table 1. Morphological and pomological traits

	Parameters
Tree	Flowering date, Tree shape
Leaf	Leaf color, Leaf length, Leaf width, Leaf ratio (length/width)
Leaflet	Leaflet number, Terminal leaflet length, Terminal leaflet width, Terminal leaflet ratio (l/w)
Nut	Nut weight, Nut length, Nut ratio (length/width), Nut ratio (length/thickness), Maturity (%), Blank rate (%), Split rate (%), Hull color, Kernel color, Fat content, Palmitic acid, Stearic acid, Oleic acid, Linoleic acid

At harvest, the maturity percentage, the split and blank rates were computed. Hull and kernel colors and nut characters (weight, length and length/width and length/thickness ratios) were achieved.

To assess local genetic diversity, Mateur, the major variety grown in Tunisia, was harvested from each location.

3. Lipidic characterization

Mature nuts were harvested and Kernels were removed. Kernel samples were used to determine fat content and fatty acid composition. Total fat content of pistachio kernels was determined using Nuclear Magnetic Resonance (NMR) Spectroscopy. Fatty acids composition was determined by gas chromatography (CPG) of ATI type UNICAM 610. The preparation of methyl esters of oil samples was performed in accordance to the standard method established by IUPAC (1979).

4. Data analysis

Dendrograms were constructed using Unweighted Pair Group Method with Arithmetic averages (UPGMA) and the computation for multivariate analysis was performed with computer "NTSYSpc2.1" program (Rohlf, 1998).

III – Results and discussion

1. Genetic diversity of *Pistacia vera*

After the field prospecting, 89 female specimens (64 from El Guetar and 25 from Sfax) and Mateur variety were characterized basing on morphological and kernel lipidic features. This revealed high variations for all the parameters studied. Local specimens of pistachio were characterized by a wide blooming period, spreading between the last weeks of March to the third week of April. Some of them were more precocious and others were later than Mateur variety. A significant difference in the percentage of fruit maturity was observed through the different ecotypes. Indeed, it varied from 19% for ATK to 92% for Loby7 specimens. Mateur presented a high percentage of fruit maturity reaching 85%. This indicated a large period of fruit maturity for the different ecotypes prospected.

As regards to commercial features, nut weight was ranged between 0.48 to 1.03 g. Mateur had a nut weight of 0.93 g. Some local ecotypes had similar or more interesting nut weight.

Results showed that the majority of ecotypes (50) have ovoid shape ($1.5 < Nl/Nw < 1.7$), whereas 37 ecotypes have elongated shape ($Nl/Nw > 1.7$).

The blank rate, considered as disadvantage criteria, showed an important variation between the local ecotypes and ranging between 2 and 60%. Thirty ecotypes have a blank rate superior than 20%. Twenty ecotypes have a blank rate below 10%. Mateur presented a blank rate of 6%. However, blank fruit was attributed to pollen availability and their viability.

Split rate was an interesting parameter for cultivars selection. Results obtained showed an important variation of split rate within local specimens. It ranged between 14 and 95%. This nut character was submitted to considerably yearly fluctuation.

Basing on the interesting characters some ecotypes were distinguished for their better nut quality. Sridique3 and LobyC5 were identified in the region of Sfax for their interesting split rate and kernel color which are more appreciate by the consummators and manufactures. In the oasis ecosystem, the variability was more apparent and ecotypes regrouped interesting and disadvantages qualities.

Oil content ranged between 56 and 78% of the kernel dry mass. Mateur variety presented an oil content of 64% and 70.4% for Sfax and El Guetar locations respectively. Local ecotypes appeared to be rich in fat, some of them with oil content more than 70%. These results were

similar to the range for other pistachio cultivars (Ferguson *et al.*, 2005; Tous and Ferguson, 1996) and other nut crop as almond reported a range of fat content of 51-55% for seven pistachio cultivars. Oil content of Mateur variety varied significantly from different localities. This result revealed that environmental conditions also appear to be important determinants.

Oleic acid content ranged from 56.1 to 80.7% with a significant difference over different ecotypes. The weak and the high oleic acid content were observed respectively for local specimens Fourati1 and Sridique1. Mateur presented little variation of oleic acid content with localities (71.6 and 72.0% respectively at Sfax and El Guetar). Fourati10, Fourati9, Ibala2, Ibala3, Sridique1, Sridique3, LobyB3, LobyB8, LobyC5 from the region of Sfax and AMMS4, TTL8, MTSG7, MTSG10, BMS3, ABT2, MTS1, EPE2 and HAB3 from El Guetar oasis were very interesting ecotypes with important oleic acid content reaching more than 75%.

The selection of pistachio phenotypes containing higher amounts of oleic acid and lower content of linoleic acid would create varieties with more nutritious seeds and nuts.

Previous reports showed that pistachio nut development and quality could be affected by the pollinating tree (Crane and Iwakiri, 1981). In fact, the male genotype can influence some important quality characteristics such as production of blank fruits.

Results of Mateur cultivar characteristics were consistent with those described in previous studies (Vargas *et al.*, 1998; Ghrab *et al.*, 2005). Mateur presented an elongated shape (IPGRI, 1997) with a nut ratio (Nl/Nw) of 1.7. However, for split rate, data obtained was different from those recorded in previous years (Vargas *et al.*, 1997; Zakynthinos and Rouskas, 1998; Rouskas, 2002; Ghrab *et al.*, 2002). This nut character was suggested to be affected by rootstocks (Crane and Iwakiri, 1986), and may change from year to year and when irrigation is available (Spiegel-Rov *et al.*, 1977).

Hierarchical clustering was carried out to classify the ecotypes based on all the measured characters using UPGMA method. The classification showed that ecotypes were classified into 5 and 9 distinct groups respectively for Sfax and El Guetar localities (Figs 1 and 2).

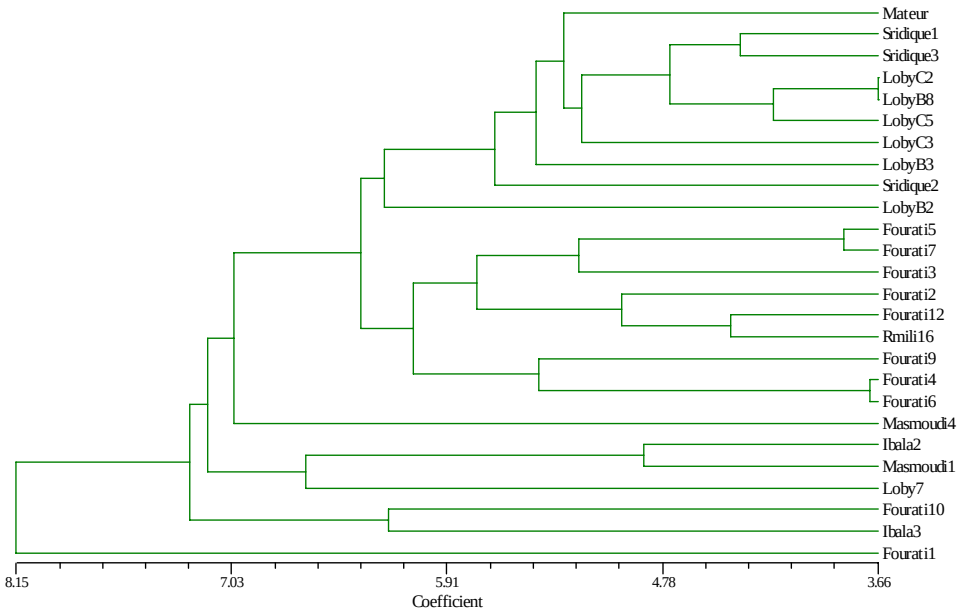

Fig. 1. Cluster dendrogram of pistachio ecotypes from Sfax region using UPGMA method.

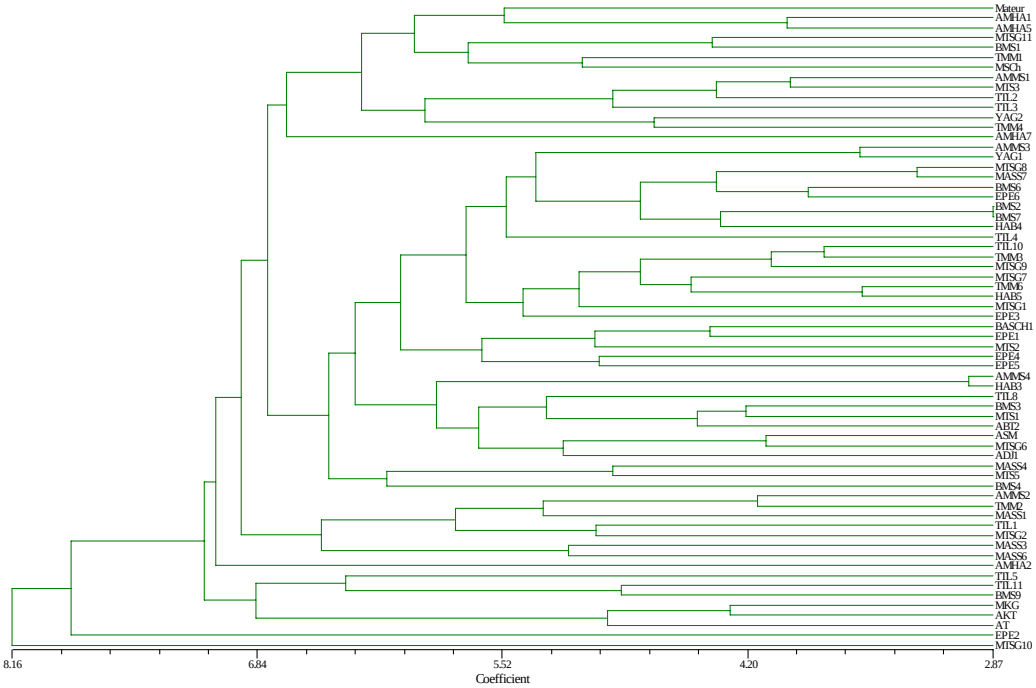

Fig. 2. Cluster dendrogram of pistachio ecotypes from El Guetar region using UPGMA method.

For the region of Sfax, the clusters 1 grouped 19 ecotypes and presented many subgroups. Within these distinct subgroups, high similarity was observed between some ecotypes. These are the cases of LobyC2 and LobyB8, and Fourati5 and Fourati7, and Fourati4 and Fourati6. It is interesting to note that Mateur formed singular a subgroup which integrated cluster 1. This cluster grouped ecotypes having lower Lw, high nut length (Nl) and high split and blank rates.

Clusters 3 and 4 grouped respectively 3 and 2 ecotypes (Fig. 1). Ecotypes from cluster 3 were characterized similar nut size, high fat and oleic acid contents. Fourati10 and Ibala3 integrated cluster 4 and were distinguished as late ecotypes with small terminal leaflet, similar nut size low fat content and high oleic acid content. Masmoudi4 and Fourati1 formed singular clusters 2 and 5. The first ecotype was characterised by high leaf and leaflet sizes, highest nut length and highest linoleic acid content. Fourati1 as late ecotype presented the highest nut ratio (NR1 and NR2) and lowest oleic acid content.

Morphological data assessed using cluster analysis classified pistachio ecotypes from El Guetar oasis on 9 distinct groups (Fig. 2). Three main clusters (6, 7 and 9) were discerned and contained respectively 7, 35 and 13 ecotypes.

MSTG10, EPE2, AMHA2 and AMHA7 ecotypes singular formed clusters 1, 2, 5 and 8. MSTG10 was a late ecotype having the highest nut weight. EPE2 presented the most precocious flowering period, the highest oleic acid content and the lowest linoleic acid content. AMHA2 was distinguished by high maturity rate and the highest fat content. AMHA7 presented low split rate and high blank rate and fat content.

Mateur cultivar integrated cluster 9 and was characterised by high maturity and split rates. Cluster 7 grouped more than 50% of surveyed ecotypes and divided in many subgroups. Within these distinct subgroups, high similarity was observed between some ecotypes. It's the cases of BMS2 and BMS7 and AMMS4 and HAB3.

Genetic diversity in *Pistacia vera* is considered to be very narrow. According to data assessed using cluster analysis, a high degree of variation for prospected ecotypes characteristics is considered. The potential of some proves to be especially interesting. A better resolution can be expected once the set of available descriptors includes all those indicated in the descriptor list. On the other hand, further analysis such as molecular characterization is expected to produce significant data for the characterization of local pistachio germoplasm. The combination of morphological traits and molecular markers increased the level of accuracy of germoplasm identification and further assist in preservation of genetic variability (Barazani *et al.*, 2003).

2. *Pistacia atlantica* genetic diversity

Results obtained in the two areas of Sidi Bouzid and Meknassy revealed relative great intra specific variability in fruit fresh weight (Fig. 3). This parameter varied between 8 and 17.6 g for 100 fruits in the Meknassy area and between 16.2 and 20 g in the Sidi Bouzid one. Fruits have globular or obovoid forms. At complete maturity their colours varied from blue green to dark green. Generally, leaves have terminal leaflet and are 3 to 6 pair of lateral leaflets. Leaf length and width averages are respectively 11.6 and 8 cm for Sidi Bouzid genotypes and 10.4 and 7.4 cm for Meknassy ones.

In vitro seed germination of 29 genotypes was realized in order to select higher germination capacity genotypes that were needed for rootstock production and reforestation. As illustrated in Fig. 4, Meknassy genotypes germination rates were between 5 and 100%. For Sidi Bouzid genotypes, less variation in germination capacity were observed (germination rates were between 36 and 92%).

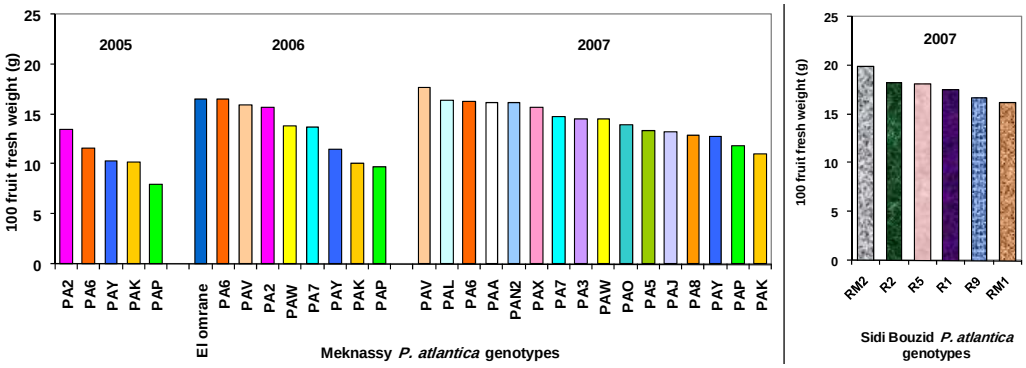

Fig. 3. *P. atlantica* fruit fresh weight variation in the Meknassy and Sidi Bouzid areas (prospecting data of 2005, 2006 and 2007).

For fruit nutritional evaluation, fat content was analysed for 16 genotypes. Results showed that oil content varied between 40.45 and 48.2% DW. Fruit and seed fatty acid analysis revealed dominance of oleic acid (C18:1) at an average rate of 55.9% (47.3 to 61%) linoleic (C18:2) and palmitic (C16:0) acids were present at the respective mean rates of 20 and 21%. These two acids rates varied, respectively, between 17.3 to 25.7% and 16.3 to 24.8%. Seeds oleic acid content was also dominated by oleic acid (50%) followed by linoleic acid (35%) and palmitic acid (10.9%).

P. atlantica Tunisian germplasm prospecting is far from being completed. Great efforts might be done to explore this rich genetic pool that is still underutilised. Numerous potentialities of this species merit valorisation to develop sustainable agriculture in arid and semi-arid areas.

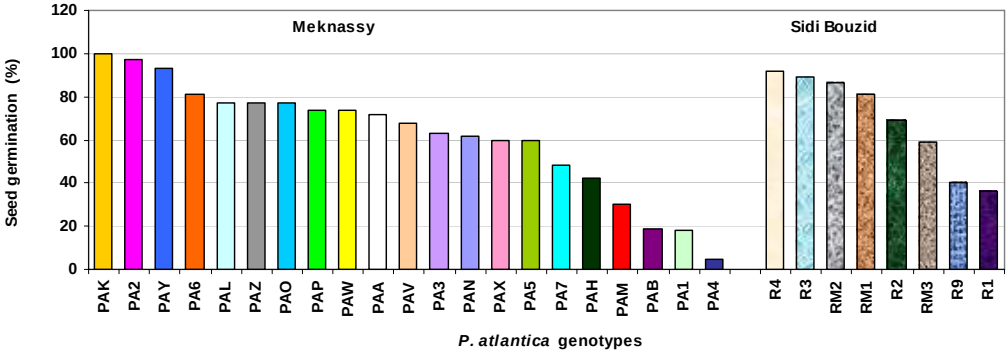

Fig. 4. *P. atlantica in vitro* seed germination of Meknassy and Sidi Bouzid genotypes.

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