

Full Length Research Paper

Genetic variability of some cultivars of pistachio tree based in composition of vitamins

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Accepted 6 March, 2007

The identification and the characterisation of some cultivars of pistachio trees of the south of Tunisia were shown to have a remarkable diversity between different cultivars by using molecular markers (ISSR) supported by morphological markers [leaf length, leaf area, fruit length, terminal leaflet shape, length leaflet and chemical composition (content of vitamins)]. The content of vitamin B1 varied from zero in Kermizi, Meknessy 1, Mumtez, Mateur 2 and Mateur 3 varieties to 1.9 mg/100 g in Lybie rouge. For the vitamin B2 content, the value ranged from 0.1 mg/100 g in Mateur 3 variety to 7 mg/100 g in Lybie rouge. Vitamin B6 content varied from 0.16 mg/100 g in Mateur 3 variety to 1.643 mg/100 g in Red Aleppo. Vitamin C vitamin content can reach 9 mg/100 g in Lybie blanc variety.

Key words: Pistachio tree, chemical composition, vitamins, HPLC.

INTRODUCTION

The pistachio tree (*Pistacia vera* L.) is one of the fruit-bearing species known since antiquity. Indeed, the surfaces occupied by the culture of the pistachio tree did not exceed 30 hectares during the 1960s. From the beginning of the 1970s, a project financed by FAO allowed the progressive extension of the surfaces occupied by the culture of pistachio tree. In 2003, the surface occupied by this species became approximately 43054 ha. The main world producers of pistachio nuts are Iran, USA, Turkey and Syria. Commercial exploitation of pistachio commenced in the 1930s in Iran, which still remains the largest producer (Chang, 1990), providing 56.10% of the world's production. The second largest pistachio producer is USA, where kerman is the most commonly grown cultivar. Kerman covers over 90% of the total country production of pistachios. In both Iran and USA, pistachio plantations are irrigated whereas in Turkey there is no irrigation yet in place for this crop.

In Tunisia, *P. vera* is propagated by grafting on seedlings of *P. vera*. This species is adapted to combat climatic and edaphic conditions such as drought, cold, calcareous and rocky soils. Such proprieties have led

farmers to pursue its cultivation in that marginal and arid zones where olive and almond trees cannot grow successfully. The Tunisian cultivars Mateur, Sfax and El Guettar are adapted to low altitudes and can perform well also in temperate zones near the coast.

Currently, there are 44000 ha planted with pistachio, corresponding to about 2730 million trees. Pistachio trees represent about 11% of the country's total area planted with stone fruit trees (excluding date palm and olive trees). Irrigated areas cover about 2000 ha while non-irrigated orchards consist of 42000 ha. The most important pistachio producing zones are Gafsa, Sidi Bouzid, Kasserine, Sfax and Kairouan: they contribute up to 80% of the total national production. The area of Kasserine (the largest concentration of pistachio orchard in the country) contributes 29% in the national pistachio production, while Sidi Bouzid and Gafsa covers 22 and 17%, respectively. A recent increase in interest for this horticulture crop has contributed in the expansion of its cultivated area, which has risen from 9300 ha in 1984 to 44000 ha in 1997. However, production and profitability are still relatively low due to drought, late bearing (7-10 years after establishment) and controlled pollination (Twey, 1998).

In Tunisia, the identification and the characterization of pistachio tree varieties are the main aims of some researchers. The work completed in the areas of the

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Table 1. Designation of the cultivars studied and their place of origin.

Names of varieties	Place of collection	code	origin
Lybie blanc	Chenchou -Gabès	C1	Libye
Kermezi	Chenchou -Gabès	C2	Turquie
Lovy	Chenchou -Gabès	C3	Tunisia
Meknessy1	Chenchou -Gabès	C4	Tunisia
Mumtez	Chenchou -Gabès	C5	Iran
Lybie rouge	Chenchou -Gabès	C6	Libye
El Guettar	Chenchou -Gabès	C7	Tunisia
Meknessy 2	Chenchou -Gabès	C8	Tunisia
Brise vent	Chenchou -Gabès	C9	Turquie
Kerman	Chenchou -Gabès	C10	USA(Iran)
Red Aleppo	Chenchou -Gabès	C11	USA (Syrie)
Mateur 1	Chenchou -Gabès	C12	Tunisia
Mateur 2	Chenchou -Gabès	C13	Tunisia
Mateur 3	Chenchou -Gabès	C14	Tunisia
Mateur 4	Chenchou -Gabès	C15	Tunisia

South of the country (Chenchou and Sfax) revealed the presence of several cultivars with specific morphological, agronomic and ecological characteristics (Zribi, 2004). However, the morphological characters are fluctuating criteria that depend on ecological conditions resulting in considerable differences (colour, size of the fruits). Under these conditions, the exact description of the cultivars of the pistachio tree becomes very difficult and the problem of varieties identification becomes complicated. Thus, other techniques must be used to establish more precise characterization and classification of pistachio cultivars. The aim of this work is to study the composition of vitamins of different pistachio cultivars in the south of Tunisia by using liquid chromatography.

MATERIALS AND METHODS

Samples

Fifteen cultivars pistachio fruit were collected from Chenchou – Gabes (South Tunisian) and were subject of this study (Table 1).

Reagents

The thiamin hydrochloride was purchased from Carlo Erba (Val de Reuil, France). Takadiastase was from Fluka (Buchs, Switzerland). Methanol and acetic acid glacial grade were purchased from Panreac Quimica SA (Barcelona, Spain). Sodium acetate trihydrate was from J.T. Baker (Holland). L-Ascorbic acid standard was reagent grade and was obtained from MP Biomedicals (Eschwege, Germany). Orthophosphoric acid 85% was from Merck (France). The water used in HPLC and sampling was prepared with Millipore Simplicity (Millipore S.A.S, Molsheim, France).

Analytical methods

Vitamin B1, B2, B6 and vitamin C contents determination was done

with Liquid Chromatography method.

Determination of B1, B2 and Vitamin B6s

Twenty five milliliters of 0.1 M sulfuric acid were added to the sample (5 g of pistachio kernel) in a 125 ml conical flask. The solution was placed in the autoclave at 121°C for 30 min. After being allowed to cool it was adjusted to pH 4.5 with 2.5 M sodium acetate. Takadiastase (0.1 g) was then added. The solution was incubated for overnight at 37°C, then diluted to 50 ml with ultra pure water and filtered through a filter paper. The filtrate obtained after a second filtration through a cellulose acetate filter (0.45 µm) was used for chromatography determination of vitamins (Arella et al., 1996).

Liquid chromatography separation was carried out at room temperature on Eurospher C18 column, 100 Å pore size, 18.5 µm particle size, 250 mm x 4.6 mm I.D (Knauer, Germany). The mobile phase used was a solution of ultra pure water, acetic acid glacial and methanol in ration (6:2:1). Prior to use, solvents were filtered over a 0.45 µm membrane filter and sonicated for 15 min in an Ultrasonic Cleaner Model SM 25E-MT (Branson Ultrasonics Corporation, Dambury, USA). The separation was performed at programming of flow rate; 0.5 ml/min for 0-5 min, 1 ml/min for 5-10 min, 1.5 ml/min for 10-15 min, and 2.1 ml/min for 15-30 min. The U.V detector operates at wavelength of 254 nm. Quantification was carried out from integrated pick areas of the sample against the corresponding standard graph.

Ascorbic acid contents

To 3 g of almond, 1 ml of 2% orthophosphoric acid was added, vortexed and the volume was adjusted to 5 ml by adding water ultra-pure. The mixture was centrifuged at 5000 rpm for 8 min at 4°C. The supernatant was filtered and vitamin C level was determined by HPLC, utilizing a column (250 mm x 4.6 mm i.d.) packed with Eurospher C18 reversed-phase materiel (18.5 µm particle size) with mobile phase (water, pH 2.2) at 1 ml/min flow-rate.

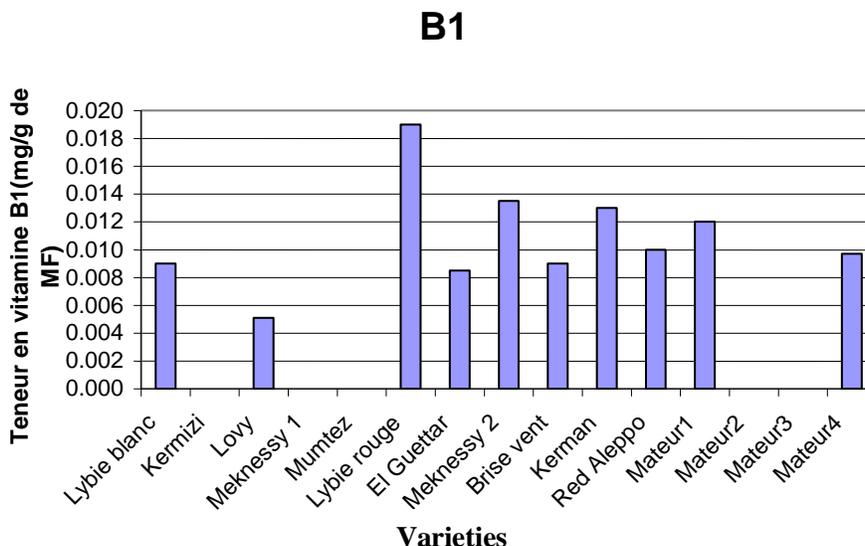


Figure 1. Content of Vitamin B1 of several varieties of pistachio.

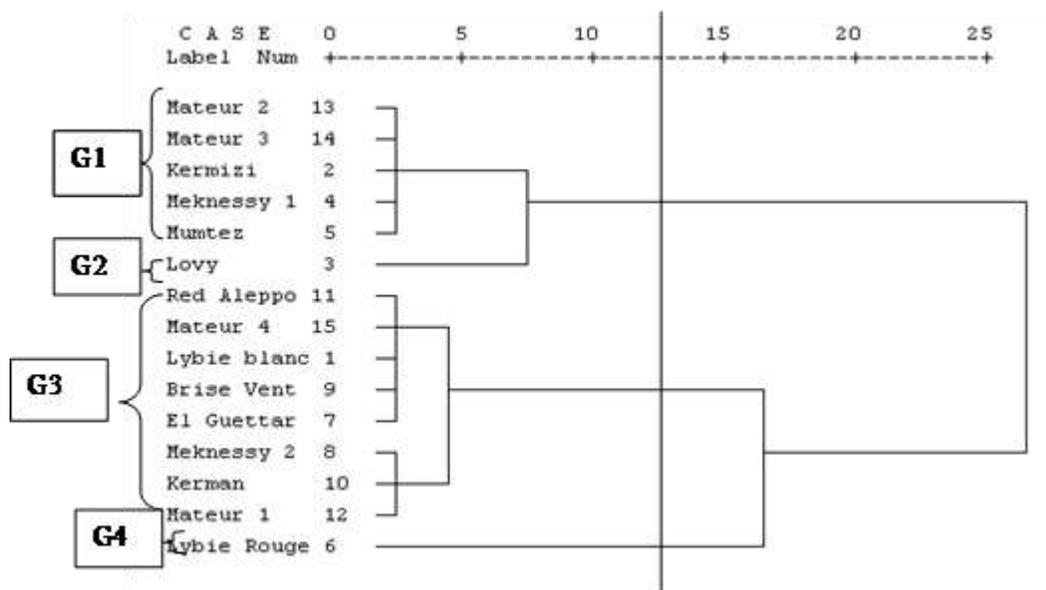


Figure 2. Classification of the varieties of pistachio tree according to the content of Vitamin B1 (software SPSS, UPGMA).

Data analysis

The statistical analyses (hierarchical classification by using UPGMA [(Unweighted Method Par-Group, of Arithmetic Means)] were carried out by software MVSPW and SPSS version 12.0.

RESULTS AND DISCUSSION

Variability of Vitamin B1 content

The values of Vitamin B1 content are illustrated in Figure

1. The content of vitamin B1 (mg of vitamin per g of fresh matter) is very variable from 0 to 1 mg/100 g in Kermizi, Meknessy 1, Mumtez, Mateur 2 and Mateur 3 varieties and 1.9 mg/100 g in Lybie rouge. Analysis of this dendrogram (Figure 2) made it possible to classify the varieties in four groups:

Group 1 (G1): Varieties with equal Vitamin B1 content and Mateur 2, Mateur 3, Kermizi, Meknessy 1, Mumtez with 0 mg/100g content.

Group 2 (G2): One variety, Lovy.

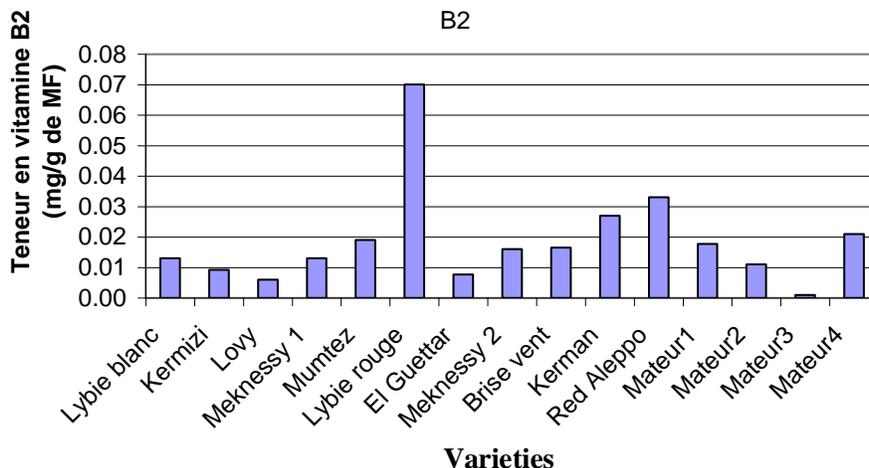


Figure 3. Vitamin B2 of several varieties of pistachio.

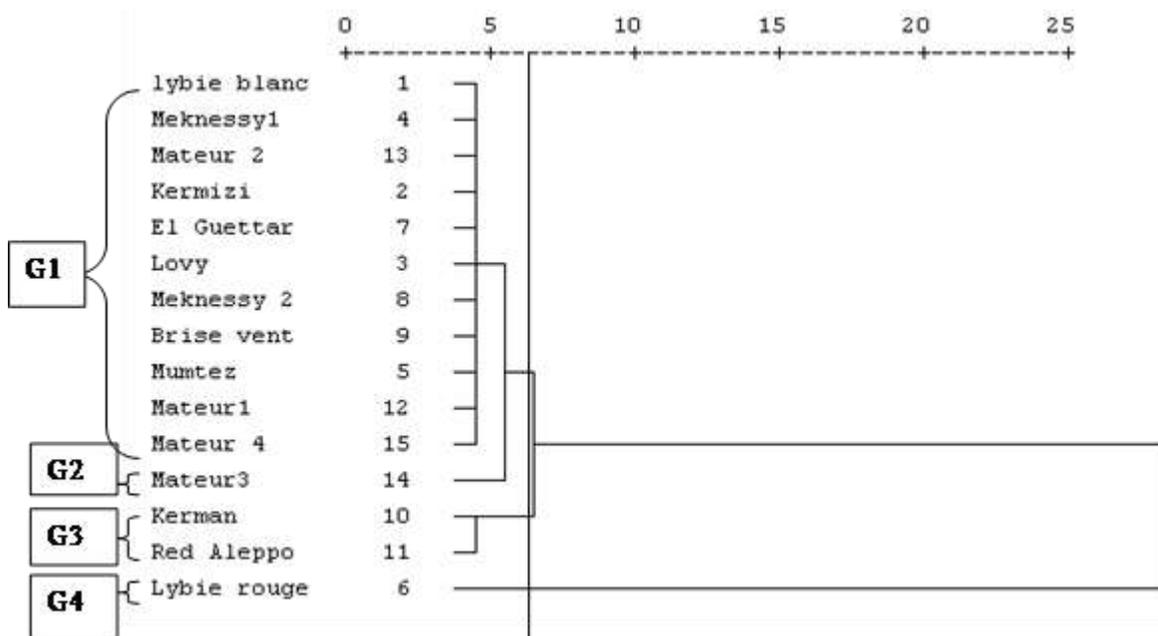


Figure 4. Classification of the varieties of pistachio tree according to Vitamin B2 content (software SPSS, UPGMA).

Group 3 (G3): The majority of the varieties are included in this group and they are characterized by Vitamin B1 content between 0.9 mg/100g and 1.3 mg/100g .This group contains 8 varieties; Red Aleppo, Mateur 4, Lybie blanc, Brise vent, El Guettar, Meknessy2, Kerman and Mateur 1.

Group 4 (G4): One variety, Lybie rouge.

Variability of Vitamin B2 content

The values of Vitamin B2 content are shown in Figure 3.

From this figure we can deduce that the vitamin B2 content was varied from 0.1 mg/100 g in Mateur 3 variety to 7 mg/100 in Lybie rouge variety. The dendrogram (Figure 4) showed four groups of varieties:

Group 1(G1): The majority of the varieties are included in this group whose Vitamin B2 content oscillated between 1.6 mg/100g and 2.1 mg/100g. This group contains 11 varieties, Lybie blanc, Meknessy 1, Mateur 2, Meknessy2, Kermizi, El Guettar, Lovy, Brise vent, Mumtez, Mateur 1 and Mateur 4.

Group 2 (G2): One variety, Mateur 3.

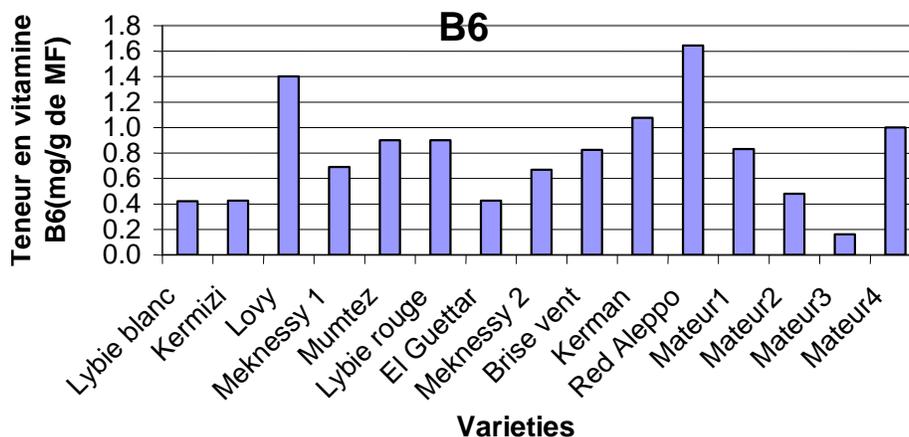


Figure 5. Content of vitamin B6 of the various varieties of pistachio.

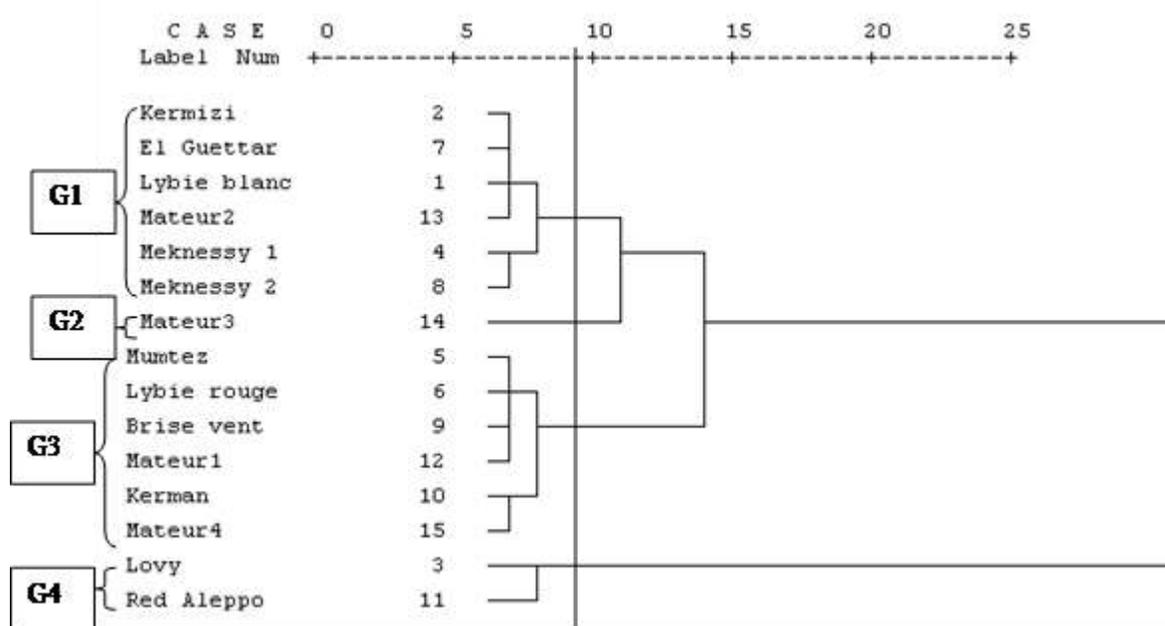


Figure 6. Classification of the varieties of Pistachio tree according to the content of vitamin B6 (software SPSS, UPGMA).

Group 3 (G3): It was characterized by a content from mg/100g to 3.3 mg/100 g. This group contained 2 varieties, Kerman and Red Aleppo.

Group 4 (G4): One variety, Lybie rouge.

Variability of the content of Vitamin B6

The values of Vitamin B6 content are illustrated in the Figure 5. From this figure we can deduce that the vitamin B6 content varied from 0.16 mg/100 g in the variety Mateur 3 to 1.643 mg /100g in Red Aleppo. The use of software SPSS for the classification of varieties made it

possible to establish the dendrogram. This dendrogram (Figure 6) puts forward four groups of the varieties:

Group 1 (G1): The varieties which are included in this group have the content of vitamin B6 between 0.42 mg/100 g and 0.69 mg/100 g. This group contained 6 varieties; Kermizi, El Guettar, Meknessy1, Mateur 2, Meknessy2 and Lybie blanc.

Group 2 (G2): Only one variety, Mateur 3.

Group 3 (G3): It was characterized by a high content of Vitamin B6 (0.82 - 1.075 mg/100). This group contained 6 varieties; Mumtez, Lybie rouge, Brise vent, Mateur 1, Kerman, and Mateur 4.

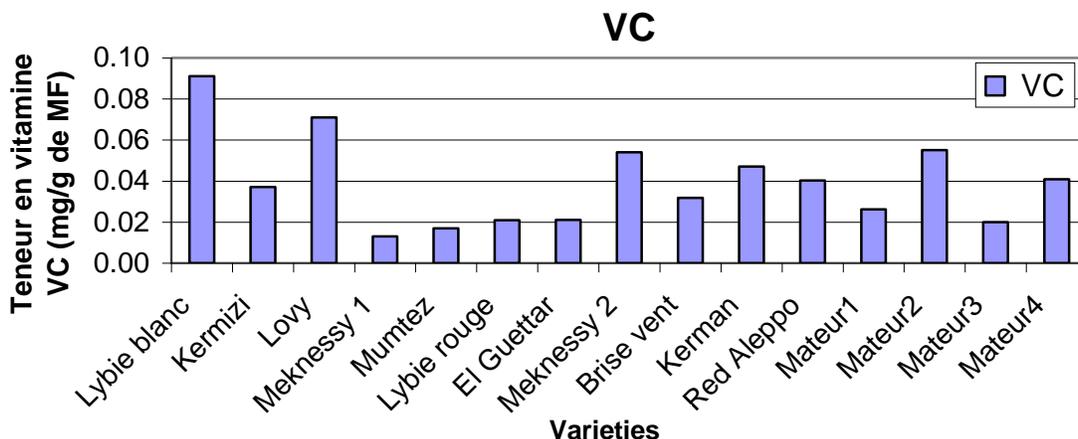


Figure 7. Content of vitamin vitamin C of the various varieties of pistachio.

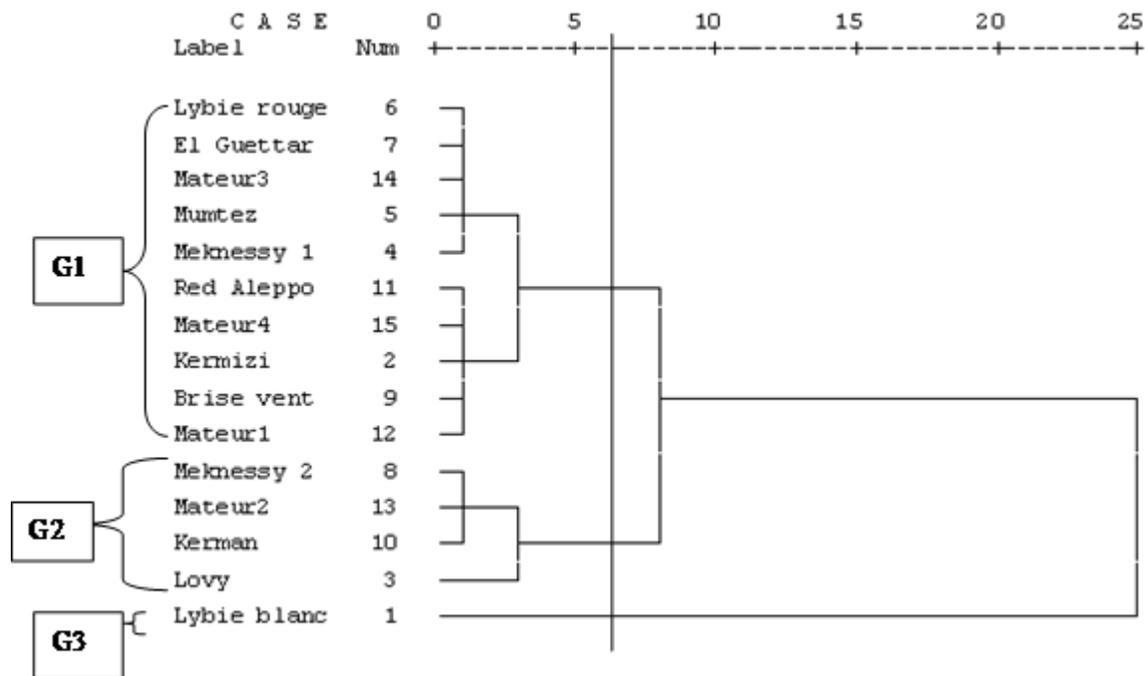


Figure 8. Classification of the varieties of Pistachio tree studied according to the content of vitamin vitamin C (software SPSS, UPG).

Group 4 (G4): Two varieties, Lovy and Red Aleppo.

Variability of the content of vitamin C

The values of the content of vitamin vitamin C of the several varieties are illustrated in Figure 7. From this we can deduce that the content of vitamin C varied from 1.3 mg/100 g in Meknessy 1 to 9 mg/100 g in Lybie blanc. The analysis of this dendrogram (Figure 8) made it

possible to classify the varieties in three groups:

Group 1 (G1): The varieties which are included in this group have vitamin C contents between 1.3 and 9 mg/100 g. This group contained 10 varieties; Kermizi, El Guettar, Meknessy1, Mumtez, Lybie rouge, Brise vent, Mateur 1, Mateur4, Red Aleppo and Mateur 3.

Group 2 (G2): Mateur 2, Meknessy2, Kerman and Lovy.

Group 3 (G3): Contains only one variety which is Lybie blanc.

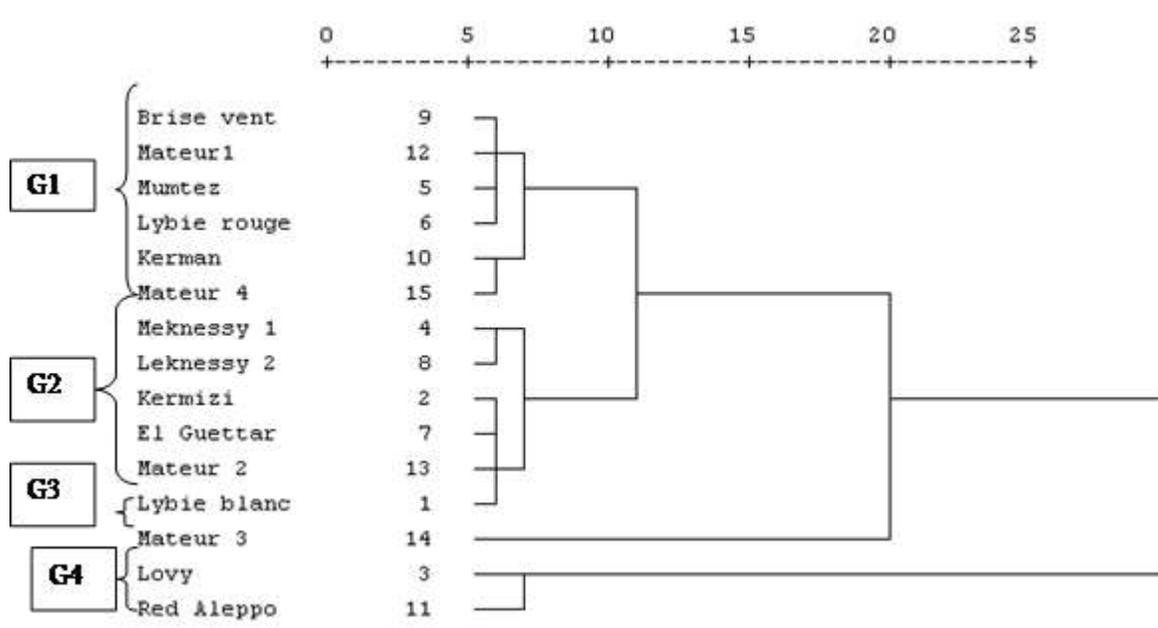


Figure 9. Classification of the varieties of Pistachio tree studied according to the content of vitamins (software SPSS, UPGMA).

Table 2. Combination of group of pistachio (B1, B2, B6 or of their combination).

Group	Group according to the content of B1	Group according to the content of B2	Group according to the content of B6	Group according to the content of vitamin C	Group according to the contents of B1, B2, B6, vitamin C
1	Mateur 2, Mateur 3, Kermizi, Meknessy 1, Mumtez	Lybie blanc, Meknessy 1, Mateur 2, Meknessy2, Kermizi, El Guettar, Lovy, Brise vent, Mumtez, Mateur 1, Mateur 4	Kermizi, El Guettar, Meknessy1, Mateur2, Meknessy2, Lybie blanc	Kermizi, El Guettar, Meknessy1, Mateur 3, Mumtez, Lybie rouge, Brise vent, Mateur1, Mateur4, Lovy, Red Aleppo	Brisevent, Mateur1, Kerman, Mateur4 Mumtez, Lybie rouge
2	Lovy	Mateur 3	Mateur 3	Meknessy2, Mateur2, Kerman	Meknessy1, Mateur2, Meknessy2, Kermizi, El Guettar, Lybie blanc
3	Red Aleppo, Mateur 4, Lybie blanc, Brise vent, El Guettar, Meknessy 2, Kerman, Mateur 1	Kerman, Red Aleppo	Mumtez, Lybie rouge, Brise vent, Mateur 1, Kerman, Mateur 4	Lybie blanc	Mateur 3
4	Lybie rouge	Lybie rouge	Lovy, Red Aleppo		Red Aleppo Lovy

Variability according to the combination of various vitamins (B1, B2, B6, vitamin C)

The total vitamins contents are illustrated in Figure 9. Using the combination of the content of vitamin B1, B2,

B6 and vitamin C, the pistachio varieties can be distinguished into 4 groups:

Group 1(G1): Gathered six varieties, Brise vent, Mateur1, Kerman, Mateur4, Mumtez and Lybie rouge

which are characterized by fairly high B1 and B6, and low content of B2 and vitamin C.

Group 2 (G2): The six varieties in this group; Meknessy1, Mateur2, Meknessy2, Kermizi, El Guettar and Lybie blanc had a low content of B2, vitamin C and B6, and fairly high B1.

Group 3 (G3): Contained only one variety Mateur 3 with low content of B1, B2, B6 and vitamin C.

Group 4 (G4): Contained two varieties, Red Aleppo and Lovy which are characterized by low B1 and B2 contents, high percentage of B6 and low content of vitamin C.

The analysis of chemical variability made it possible to distinguish variability in the level of the chemical composition in B1, B2, B6 and vitamin C and to classify the varieties into homogeneous groups (Table 2). The content of vitamins was different into these varieties. The content of Vitamin B1 for El Guettar, Lybie blanc, Brise vent and Mateur 4 varieties was about 0.81 mg/100 g, while it was 1 mg/100 g for Red Aleppo, Mateur1, Kerman, and Meknessy 2. It varied from 0 to 1.9 mg/100 g in Lybie rouge. This value was smaller than the content in rice which ranged from 0 to 5.34 mg/100 g (Voahanginirina, 2001) whereas for the *Prunus dulcis* this content was 0.241 mg/100 g ([http:// tous-les-fruits.com/fruit-364.html](http://tous-les-fruits.com/fruit-364.html)). The content of vitamin B2 for Lybie blanc, Brise vent, Mateur 4, Meknessy 1, Mumtez, Meknessy 2, Mateur 1 and Mateur 2 varieties was equal to 1.60 mg/100 g, and 7 mg/100 g for Lybie rouge. This value was higher than the vitamin B2 content in *Prunus dulcis*, 0.811 mg /100 g and rice (0.02 to 0.04 mg/100 g). For vitamin B6 it oscillated from 0.16 to 1.643 mg/ 100g, which is higher than the value existing in *Prunus dulcis* and rice. The vitamin C for Kermizi, Meknessy 1, Mumtez, Lybie rouge, El Guettar, Brise vent, Mateur 3 and Mateur 1 varieties is 1.3 to 9 mg/100g. In Lybie blanc this values are comparable with those published by De Beer et al. (2003).

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