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Wild Tunisian *Capparis spinosa* L.: Subspecies and Seed Fatty Acids

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KEYWORDS

Capparis spinosa; subspecies; seeds; fatty acid; variability, Tunisia

A B S T R A C T

The caper (*Capparis spinosa* L.) is represented in Tunisia by two subspecies: *Capparis spinosa* subsp. *spinosa* in the north of the country and subsp. *rupestris* (Sibth. & Sm.) Nyman which exists from the North to the South. Fatty acids profiles from nine wild populations belonging to both subspecies were identified using GC-MS. The three major fatty acids were oleic, linoleic and palmitic acids. However, a high variation in the composition of fatty acids was observed among the populations and subspecies. The percentages of stearic, palmitoleic, behenic and arachidic acids were high in *C. spinosa* subsp. *rupestris*. Oleic and linoleic acids were more represented in *C. spinosa* subsp. *spinosa*. The only representative population of the south, Chenini Tataouine, showed fatty acids composition different from all other populations. The study proves the geography and the subspecies effects in fatty acids composition of *Capparis spinosa* seeds.

Introduction

Capparis L. (Capparaceae) comprises about 250 species, distributed in the tropical and subtropical regions of both Old and New World (Jacobs, 1965, Fici, 1993). In Tunisia, the caper is a wild plant, known by the Arabic name "Kabbar" or "Jabbar". It includes only one species: *Capparis spinosa* L. (Saadaoui et al., 2011). This species is characterized by wide genetic variability and taxonomic ambiguity (Ghorbel et al., 2001), and represented by two subspecies, *C. spinosa* subsp. *spinosa* and *C. spinosa* subsp. *rupestris*. The two subspecies are allopatric; the subspecies *rupestris* extends

from the north to the south of the country, while the subspecies *spinosa* is limited to several northern regions. Thus, the sites of these two subspecies are characterized by different ecological conditions (Saadaoui et al., 2011). The *C. spinosa* fruit is a berry with a long gynophores (35–70 mm), it's oblong to somewhat pyriform. Seeds (2-4 mm) are reniform (Tutin et al, 1993). Morphological parameters seeds show a difference between the two subspecies: *C. spinosa* subsp. *spinosa* and *C. spinosa* subsp. *rupestris*; the 1000 seed weight is 10,1 and 11,5 g for respectively for subsp. *rupestris* and subsp. *spinosa* (Tlili et al., 2001b).

Caper reproduces by seeds and stem cuttings. The germination percentage under natural conditions is very low (5%) and physical or chemical treatments are required to increase the germination percentage of caper (Orphanos, 1983; Sozzi, 2001; Al-Safadi and Elias, 2011).

The caper is used essentially for flower buds (Fici and Gianguzzi, 1997). Also, flower buds, root bark, and fruits are used in folk medicine due to their analgesic, wound healing, cell regeneration, tonic, and diuretic effects (Arslan et al., 2010). In Greek popular medicine, an herbal tea made of caper root and young shoots is considered to be beneficial against rheumatism (Yang et al., 2010). Recently, the pharmacology and chemistry of this plant have been extensively studied. Biological studies have revealed significant anti-diabetic, antisclerosis, antimicrobial, anti-oxidative, anti-inflammatory, immunomodulatory and antiviral activities providing a support to the ancient uses (Tlili et al., 2011a). Different parts of this plant exhibit biological activity, which anti-hyperglycemic, hypolipidemic (Eddouks et al., 2004) and antihepatotoxic activities (Gadgoli and Mishra**,** 1999). Other activities included antifungal (Ali-Shtayeh and Abu Ghdeib, 1999), antiproliferative and HIV-1 reverse transcriptase inhibitory activities (Lam and Ng, 2009). *C. spinosa* seed produces a lectin and a non-hemagglutinating protein. Both proteins inhibit proliferation of breast cancer MCF-7 cells and hepatoma HepG2 cells. Caper seeds contain also ferulic acid and sinapic acid which contribute to its medicinal value (Tzi-Bun and Sze-Kwan, 2011).

The seeds are rich in protein, oil, and fiber; they could be an alternative source of edible proteins (26%) and oil (30%). Protein and oil seeds can be utilized in several forms for food, feed and industry. Tunisian *C. spinosa* seeds are found to be rich in lipids with oil (23 to 33%). The oils had a high content of oleic and linoleic acids. But, the fatty acids composition varies between regions (Tlili et al., 2009). Indeed, the fatty acids profile of the seed oil has great systematic value in the plant kingdom, and there are many studies reporting phylogenetic relationships paralleled by differences in seed fatty acids profiles (Pujadas Salvà and Velasco, 2000). Also, the chemotaxonomic importance and potential of fatty acids was confirmed; in Apiaceae, the infrageneric and intraspecific variation observed in the fatty acids distribution of some Apiaceae genera patterns showed uniformity regarding the distribution of the fatty acids among the species within the corresponding genara (Bagci, 2007). In addition, the biochemical study of seeds fatty acids of six subspecies of *Vernonia galamensis* L. revealed a large subspecific difference (Yuldasheva et al., 2008). Moreover, genetic diversity of Mesoamerican populations of *Jatropha curcas* L. was studied by fatty acids as chemical markers (Ovando-Medina, 2011); they showed that these markers are valid for estimating the genetic diversity of this species.

This study aims to analyze the effect of population and subspecies on fatty acids composition in nine Tunisian wild *C. spinosa* populations.

Material and methods

Plant material

The seeds were collected from nine natural Tunisian populations belong to *Capparis spinosa* subsp. *rupestris* and *C. spinosa* subsp. *spinosa* in July 2006. Main ecological traits of habitats and location were reported in table 1. The subspecies *Capparis spinosa* subsp. *rupestris* is represented by five populations: Chenini Tataouine (IP1), Dahmani (IP2), Houmana (IP3), Haouaria (IP4) and Ghar El Melh (IP5). The subspecies *C. spinosa* subsp. *spinosa* includes four populations, Chouigui (TP1), Mateur (TP2), Lafareg (TP3) and Chemtou (TP4) (figure 1). From each population, seeds were collected from 3 individuals analyzed separately.

Experimental protocol

Oil extraction

For each individual in each population, fatty acids were extracted from 5 g of seeds using the continuous Soxhlet extraction technique with petroleum ether for 3 h. Extracts were filtered and concentrated under reduced pressure and temperature (40°C).

Saponification of the Lipids

FAMEs (Fatty acid methyl esters) were prepared according to Lechevallier (1966). In a methylation tube, 0,2 ml of the concentrated extract was saponified with 4 ml of a methanolic sodium hydroxide solution (0,5 M) for 15 min in a boiling water bath at 65 °C. As for transmethylation, the mixture was homogenized with 3 ml of a methanolic solution of BF3 (14%) and the reaction was allowed to proceed for 5 min. Subsequently 10 ml of water were added to the mixture, and FAMEs were extracted twice with 10 ml of petroleum ether.

Identification of FAMEs (fatty acid methyl esters)

GC-MS was used for identification of FAMEs. Fatty acids methylation was performed by the saponification and etherification procedure described by Metcalfe et al. (1966).

The dosage of methyl esters was achieved using gas chromatography coupled to the spectrometry of mass (GC/MS) equipped with a capillary column of type HP5 MS, 30 m of length and 250 µM of internal diameter; the thickness of the film was of 0.250 µM. The temperature of the injector was 250°C. The vector gas is helium, well stocked to a debit of 0.8 ml / min, the fashion of the injection is the fashion Split 50: 1 and the program of temperature is of 150°C (0 min) to 5°C / 240°C. Quantification of fatty acid methyl esters, expressed as percentages, was obtained directly from the GC-MS peak area integration.

Statistical analysis

The software XLSTAT 2010.3.03 (AddinSoft, USA) was used to compare average percentage within and among subspecies with Student Newman Keuls test at $P < 0,05$. The divergence among all populations from the two subspecies was studied by a Principal Component Analysis (PCA) performed in all identified fatty acids. Also, the correlation between fatty acids was determined.

Result and Discussion

Review of literature: Fatty acids composition of *Capparis sp.*

The most similar species to *Capparis spinosa* is *Capparis ovata*, or, the two species are synonyms (Jacobs, 2005; Fici, 2001). The two species have wide natural distribution in the Mediterranean region; their oils of the seed were studied, essentially in Tunisia and Turkey. *Table 2* presents the oil content and the fatty acids composition of *C. spinosa* in Tunisia, Turkey and Yzbekistan. The same parameters were studied for *C. ovata* in Turkey. We noticed that the oil content is not different, but, the composition in fatty acids is different. *C. spinosa* is rich in unsaturated fatty acids, contrary to *C. ovata*, which is rich in saturated fatty acids.

Fatty acids of the populations and the subspecies of Tunisian *C. spinosa*

The percentages of fatty acids for the nine populations of the two subspecies were reported in table 3. Nine fatty acids have been identified. The major fatty acids were oleic (46,21%), linoleic (21,79%) and palmitic acids (16,43%). The proportion of unsaturated fatty acids varied between 66,5 to 79,55 % respectively for Chenini Tataouine (IP1) and Lafareg (TP3). The correlation between fatty acids is low; negative correlations were obtained between linoleic and stearic acids (-0,503), palmitic and linoleic acids (-0,474) and palmitic and arachidic acids (-0,461).

A significant variation of fatty acids was observed among populations and subspecies. The most significant difference between populations concerned stearic and myristic acids ($P < 0,05$). The significant difference between subspecies concerned stearic, palmitoleic, behenic and oleic acids ($P < 0,05$). The percentages of stearic, palmitoleic, behenic and arachidic acids are high in subsp. *rupestris*, while, oleic, linoleic and palmitic acids are high in subsp. *spinosa*. Indeed, the unsaturated fatty acids are high in subsp. *spinosa* (73%) and low in subsp. *rupestris* (70%) (table 3).

PCA analysis

The first two axes explained 68,65% of the total variance. The first axis accounted for 33,23% of the total variation. The highest loading parameters were linoleic, behenic and palmitoleic acids. This axis is also negatively correlated to palmitic and stearic acids. The second axis explained 18,74% of total variance and is related to myristic and oleic acids with positive sign; this component is also loaded with negative sign to palmitoleic acid.

A high dispersion of individuals and segregation between the two caper types along the first axis were revealed. Indeed, the thorny individuals (subsp. *spinosa*) are generally poor in stearic, arcahidic and behenic acids, and rich in oleic acid (figure 2). In subsp. *rupestris*, the individuals of Chenini Tataouine population (IP11, IP12 and IP13), located in the south of Tunisia, are separated from the other individuals; they are rich in stearic, behenic and arachidic acids and an important poverty in oleic and linoleic acids (figure 2)

Divergence between populations

The first three axes explained 81,53% of the total variance. The first axis (30,86%) was defined by stearic, behenic, arcahidic and oleic acids. It also signaled that this axis is positively correlated to stearic, behenic and arcahidic acids and negatively correlated to oleic acid.

Table.1 Main characteristics of studied populations

Populations	Population codes	Subspecies	Latitude and longitude	Bioclimates	Regions	Soil texture	1000 seeds weight (g)
Chenini Tataouine	IP1	*C. spinosa* subsp. *rupestris* (inerm caper)	33°42'N 9°23'E	Saharan	Southern	rocky outcrops	7,16
Dahmani	IP2		35°57'N 8°48'E	Semi-arid	Northern West	rocky outcrops	7,15
Houmana	IP3		36°40'N 9°08'E	Sub-humid	Northern West	rocky outcrops	10,19
Haouaria	IP4		37°02'N 10°59'E	Sub-humid	Northern East	old walls	8,45
Ghar El Melh	IP5		37°10'N 10°11'E	Sub-humid	Northern East	rocky outcrops	7.15
Chouigui	TP1	*C. spinosa* subsp. *spinosa* (thorny caper)	36°53'N 9°46'E	Semi-arid	Northern East	rocky outcrops	11,48
Mateur	TP2		37°42'N 9°38'E	Sub-humid	Northern East	silty	12,23
Lafareg	TP3		36°28'N 8°33'E	Sub-humid	Northern West	silt-sandy	14.85
Chemtou	TP4		36°29' 8°44'	Sub-humid	Northern West	silt-sandy	12.41

Table 2 Fatty acids composition of *Capparis spinosa L. and C. ovata Desf.*

Authors	Tlili *et al*, (2009)	Yuldasheva *et al*, (2008)	Matthäus and Özcan (2005)	Akgül and Özcan (1999)	Argentieri et al, (2012)	Matthäus and Özcan (2005)	Akgül and Özcan (1999)
Species	*Capparis spinosa*				*C. spinosa* subsp. *rupestris*	*Capparis ovata*	
Localities	Tunisia	Yzbekistan	Turkey		Italy	Turkey	
Oil content (%)	27,7	27,5	32,2	35,2	17,2	28,3	36,7
Myristic acid 14 :0	0,72	0,5	0,53	-	0,68	0,2	-
Palmitic acid 16 :0	15,9	4,9	11,7	13,2	14,46	7,7	11,3
Palmitoleic acid 16 :1	4,5	1,2	-	4,6	4,23	1,9	1,8
Stearic acid 18 :0	4,1	2,0	3,3	3,2	9.31	2,4	2,7
Oleic acid 18 :1	45,8	28,9	45,4	49,87	38,39	37,3	34,6
Linoleic acid 18 :2	25,3	59,3	31,42	25,2	28,02	46,8	24,5
Linolenic acid 18 :3	1,1	-	0,97	1,0	0.84	1,1	0,3
Saturated	22,7	7,4	16,25	16,4	24,8	10,3	14
Unsaturated	77,3	89,4	81,8	80,7	72%	87,1	61,6

Table.3 Average of fatty acids for the populations of the two subspecies

Population codes	Myristic acid C14:0	Palmitic acid C16:0	Palmitoleic acid C16:1	Stearic acid C18:0	Oleic acid C18:1Δ^{9}	Linoleic acid C18:2$\Delta^{9/12}$	Arachidic acid C20:0	Behenic acid C22:0	Lauric acid C24:0
TP2	1,02 a	18,74 a	2,91d	4,51 b,c,d	48,23 a,b	19,77 a,b	0,71 a	0,19 b	0,42 a
TP1	0,19 b	16,24 a	3,60 b,c,d	3,43 d	47,29 a,b,c	22,01 a,b	0,53 a	0,33 b	0,00 a
TP4	0,18 b	15,38 a	3,78 a,b,c,d	4,16 c,d	45,40 a,b,c	24,38 a,b	0,92 a	0,83 a,b	0,55 a
TP3	0,17 b	14,62 a	3,19 c,d	4,66 b,c,	49,74 a	22,62 a,b	0,70 a	0,49 a,b	0,16 a
Subsp. *spinosa*	0,39 ± 0,45	16,24 ± 2,81	3,37 ± 0,73	4,19 ± 0,7	47,66 ± 2,56	22,19 ± 3,27	0,71 ± 0,25	0,46 ± 0,42	0,28 ± 0,57
IP5	0,21 b	17,86 a	4,75 a	4,51 b,c,d	45,66 a,b,c	22,66 a,b	0,89 a	0,79 a,b	0,12 a
IP2	0,28 b	16,81 a	4,54 a,b	4,83 b,c,d	44,41 b,c	24,69 a	0,67 a	1,27 a	0,23 a
IP4	0,72 a,b	16,81 a	3,31 c,d	6,02 a,b	46,48 a,b,c	19,27 a,b	1,09 a	0,96 a,b	0,00 a
IP3	0,56 a,b	16,70 a	3,77 a,b,c,d	5,38 a,b,c	45,99 a,b,c	22,01 a,b	1,12 a	0,98 a,b	0,49 a
IP1	0,43 b	14,74 a	4,08 a,b,c	6,78 a	42,72 c	18,70 b	0,71 a	1,02 a,b	0,46 a
Subsp. r*upestris*	0,44 ± 0,34	16,58 ± 2,75	4,09 ± 0,64	5 ,5 ± 1,15	45,05 ± 2,72	21,46 ± 3,17	0,99 ± 0,44	1 ± 0,42	0,26 ± 0,32

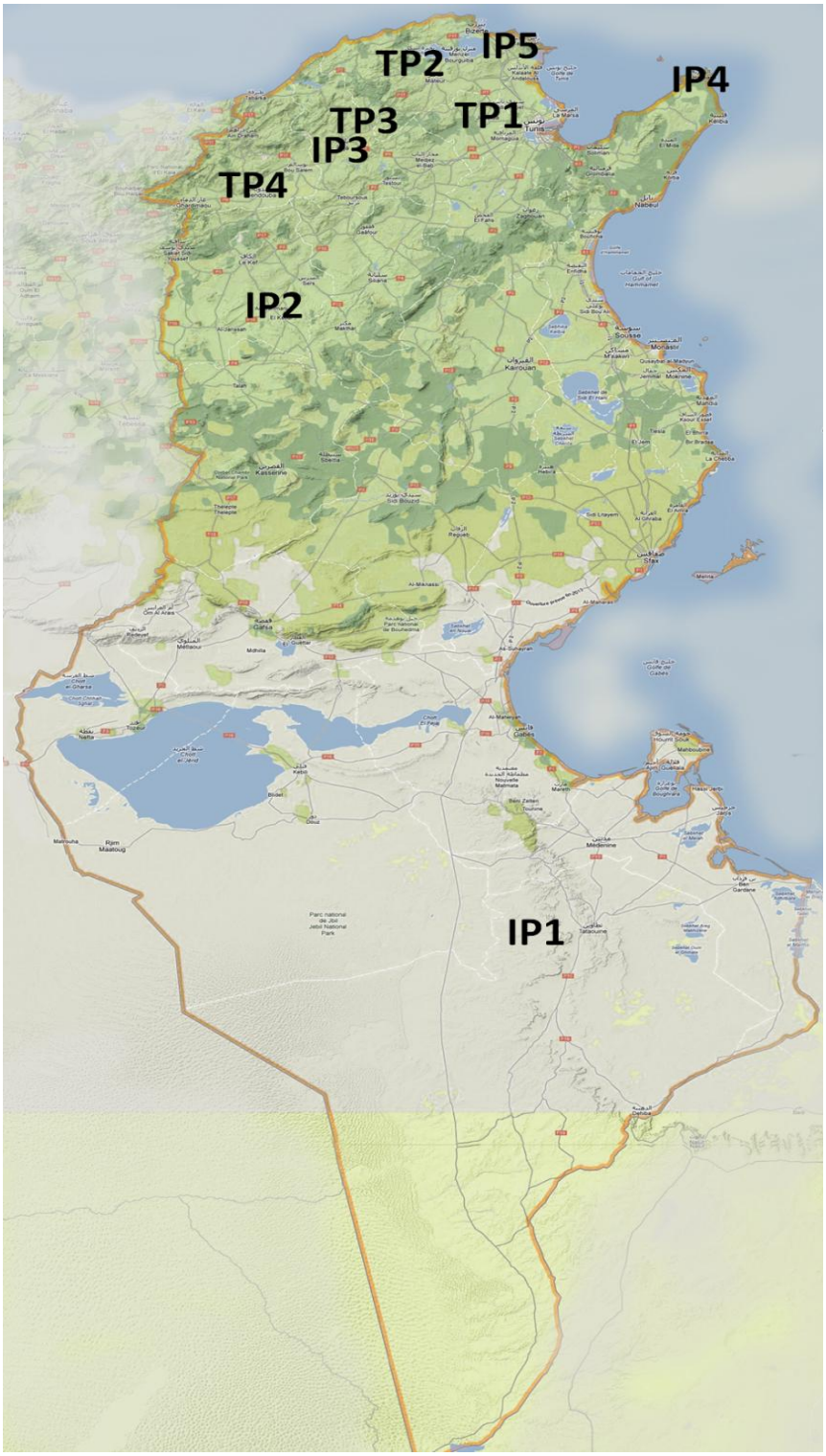

Figure.1 Map of Tunisia: Geographical distribution of the studied populations

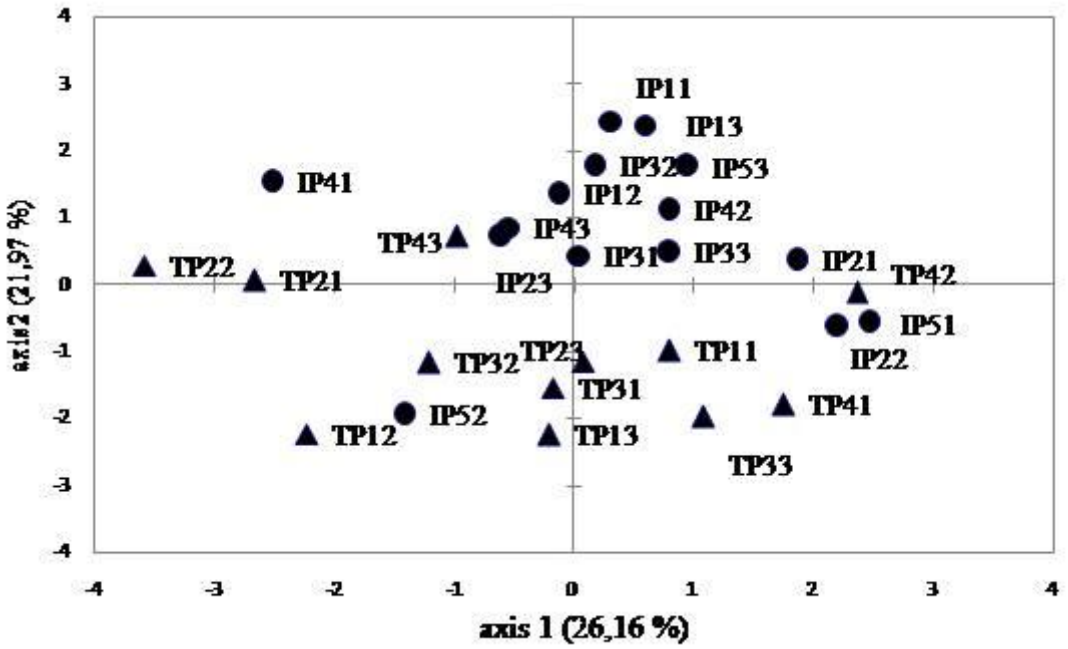

Figure.2 PCA plot of *C. spinosa* individuals according to axes 1-2
▲: *C. spinosa* subsp. *spinosa* ●: *C. spinosa* subsp. *rupestris*

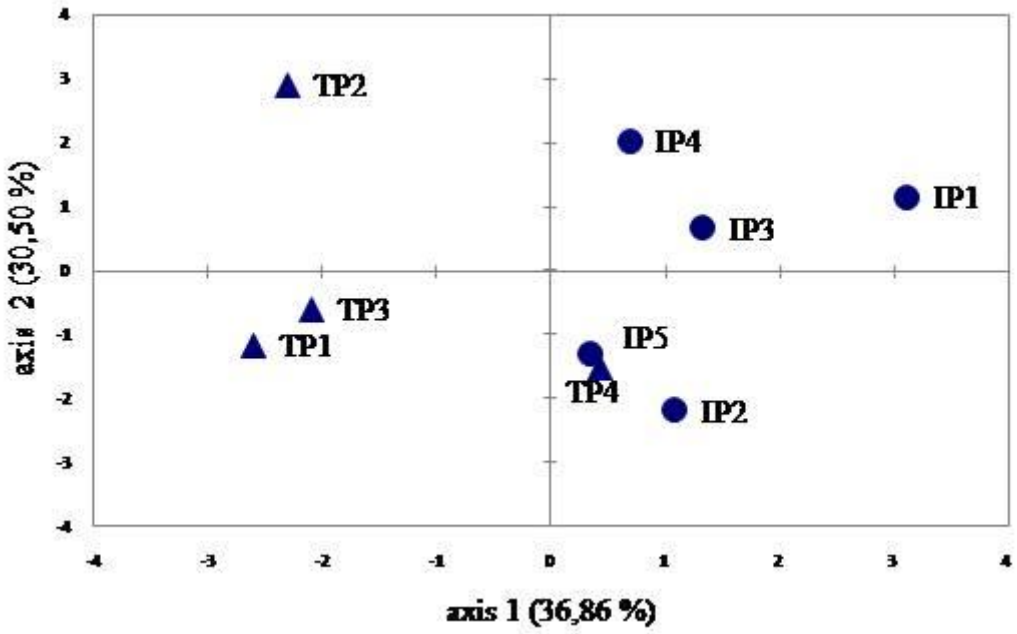

Figure.3 PCA plot of *C. spinosa* populations according to axes 1-2
▲: *C. spinosa* subsp. *spinosa* ●: *C. spinosa* subsp. *rupestris*

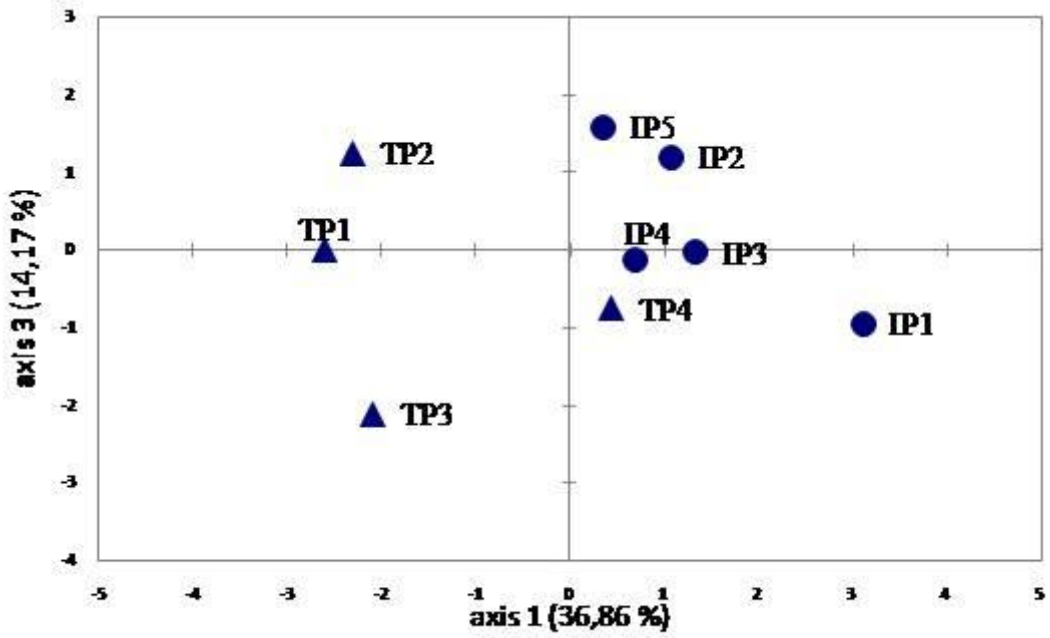

Figure.4 PCA plot of *C. spinosa* populations according to axes 1-3
▲: *C. spinosa* subsp. *spinosa* ●: *C. spinosa* subsp. *rupestris*

The second axis explained 30,50% of total variance and is related to myristic (+), linoleic (-) and palmitoleic (-) acids. The third axis accounted for 14,17% of the total variation. The highest loading parameter was palmitic acid, with a positive correlation.

Segregation between the two subspecies was observed (fig. 3). *C. spinosa* subsp. *spinosa* showed a high rate of oleic and linoleic acids, while *C. spinosa* subsp. *rupestris* is characterized by a high percentage of stearic, behenic and arachidic acids. The population of Chenini Tataouine (IP1) of subsp. *rupestris* showed the highest content of stearic (6,78%) and arachidic acids (1,19%) and the lowest amounts of oleic (42,72%) and linoleic acids (18,7%). This population exists in Saharan bioclimate which annual average of pluviometry is low to 100 mm.

The plot of the first and the third components showed the high dispersion of populations. The segregation between the populations is based on the effect subspecies and the geographical effect. Indeed, there is a separation between the north and the south populations of subsp. *rupestris* and a detachment between northeast's and northwest's thorny populations (subsp. s*pinosa*) (figure 4). Moreover, the two northwest's thorny populations (TP3 and TP4) are characterized by the highest 1000 seeds weight (table 2).

Geographical and subspecies effects in fatty acids composition

The variability observed between the nine inerm and thorny caper populations proves the effect of the subspecies in the fatty acids composition. Besides, within every subspecies, there is a geographical variability. Indeed, there is a difference in subsp. r*upestris* between the populations of the south and those of the north. Also, this variability exists between the northeast's and the northwest's populations of subsp. *spinosa*. These results prove the subspecific

and geographical effects in fatty acids composition of *C. spinosa* in Tunisia.

The composition in fatty acids seeds of the Tunisian caper obtained is the similar to that from Turkey, although seeds of the Tunisian *C. spinosa* are relatively richer in stearic and palmitic acids (Akgül and Özcan, 1999, Matthäus and Özcan, 2005, Tlili et al, 2009). The percentage of this acid is the most elevated for *C. spinosa* subsp. *rupestris* in Italy (Argentieri et al, 2012). However, it is different from that obtained in Uzbekistan, where seeds of *Capparis spinosa* are richer in linoleic (59,3%) and oleic acids (28,9%) (Yuldasheva et al, 2008). This difference is due to environmental and genetic factors. Indeed, both environmental and factors play an important role in the biosynthesis of oil and fatty acids contents (Thompson et al, 1994).

The fatty acids composition among the nine studied populations is directly related to the effect of subspecies. *C. spinosa* subsp. *spinosa* is rich in oleic and linoleic acids and *C. spinosa* subsp. *rupestris* is rich stearic, palmitoleic, behenic and arachidic acids. This result joins that obtained by Argentieri et al, (2012) for *C. spinosa* subsp. *rupestris* in Italy. Also, The two subspecies showed morphological differences in the port, presence or absence of thorns and weight of the seeds (Saadaoui et al, 2011) and they have different ecological characteristics such as the bioclimate and the soil (table 2). However, some chemical parameters are similar between the two subspecies, which the contents of protein and oils; they are respectively 26,38 and 30,79% for subsp. *spinosa* and 26,08 and 30,38% for subsp. *rupestris* (Tlili et al, 2011b). Thus, the population of Chenini Tataouine (IP1), belonging to *C. spinosa* subsp. *rupestris*, is separated from others populations by stearic, oleic and linoleic acids.

This population, representative of the ecotype of the south of Tunisia in this study, is characterized by different morphological traits, mainly the presence of hair (Saadaoui et al, 2011). The correlation between phenotypic variability/subspecies and fatty acids composition shows that this last parameter is discriminating for the study of infraspecific variability of *C. spinosa*. Moreover, in subsp. *spinosa*, the population of Chemtou (TP4) showed fatty acids composition close to that of subsp. *rupestris*. This result is understandable by the coexistence of the both subspecies in this site and the possibility of hybridization. Indeed, Intermediate forms between both subspecies are frequent in Chemtou (Saadaoui, 2012).

In addition, the oil content of *C. spinosa* seeds is similar among related species as *C. ovata* and *C. aphylla* (Akgül and Özcan, 1999). However, the interspecific difference exists in fatty acids composition, which differs between *C. spinosa* and *C. ovata*. The variation concerned palmitic and linoleic acids, the percentages are respectively 18,33 and 22,44% for *C. spinosa*, and 11,28 and 34,16% for *C. ovata* (Küsmenoğlu et al, 1997). This difference also exists between *C. spinosa* and *C. aphylla*, the latter is rich in oleic, palmitic and stearic acids and poor in lionoleic acid (Sen Gupta and Chakrabarty, 2006).

These biochemical parameters allowed to separate the two subspecies and they are interesting to support genetic improvement and develop a breeding program based on fatty acids of *C. spinosa* and their interests.

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