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RESEARCH ARTICLE

Oleaster (var. *sylvestris*) and subsp. *cuspidata* are suitable genetic resources for improvement of the olive (*Olea europaea* subsp. *europaea* var. *europaea*)

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Abstract The olive belongs to *Olea*, a complex genus and to a species with six subspecies. Subsp. *europaea* includes both the cultivated olive and the oleaster, the wild ancestor of the olive. Little is known on the phenotype of fruits from subsp. *cuspidata*. We aimed to compare *europaea* and *cuspidata* trees for oil content and composition and to verify whether natural hybrids may exist between the two subspecies. Specimens were from Kenya and putative natural hybrids between *cuspidata* and *europaea* were from Stellenbosch (South Africa). Cultivar and oleaster trees were sampled in France (continental and Corsica), Italy (Continental and Sardinia) and Tunisia. We have examined the fruit (drupe) structure between subsp. *cuspidata* and *europaea* and extracted oil from the pulp, seed and total drupe. Comparison for oil content was made between the cultivated olive and the oleaster and some *cuspidata* trees from Kenya. A few of *cuspidata* individuals from Botanical gardens do not enable phenotyping for oil content and composition. Oil composition analyses were performed for the main fatty acids to compare the three taxa. We used microsatellite markers at 11 loci to compute genetic distances between *cuspidata*, oleaster and cultivar trees and to reveal eventual hybrids. The SSR polymorphisms were huge between the two subspecies and they revealed that putative hybrids were true hybrids sampled around olive orchards. The whole comparison of oil content shows that olive cultivars display higher oil content than the subsp. *cuspidata* and that the oleaster trees are intermediate, whereas for oil composition of the drupe, *cuspidata* from Kenya shows less oleic acid than *europaea*. However, the *cuspidata* trees show seed oil composition similarities with the other two taxa. The discussion deals with possible reasons to explain the differences and of

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the possible uses of these taxa for breeding both the olive and brown olive.

Keywords Fruit size · Genetic resources · Olive; oil content; oil composition · *Olea europaea*

Introduction

The olive belongs to *Olea europaea* L. (Green 2002). This species is divided into six subsp. that thrive in Africa, Asia, in the Islands of the Indian Ocean (Mauritius, Madagascar), and in Europe. Subsp. *europaea* is unique to the Mediterranean basin but its natural habitat is difficult to reconstitute because of the cultivated olive area is wider than the one where the genuine oleaster spread. Subsp. *europaea* and *maroccana* (Greuter et Burdet) P. Vargas et al., are naturally sympatric in South Morocco (Vargas et al. 2001). The other subsp. are geographically separated (Green 2002). Subsp. *cuspidata* (Wall ex G. Don) Cif. is widespread and is found in China, India, and at higher elevations in North, East and South Africa. Subsp. *laperrinei* (Batt. et Trab.) Cif. is found in the Aïr and Hoggar Mountains (Baali-Cherif and Besnard 2005), and subsp. *cerasiformis* (Webb et Berth.) G. Kundel et Sunding and *guanchica* P. Vargas et al. are found in the Madeira and 'Gran Canaria' Islands, respectively (Brito et al. 2008; Hess et al. 2000).

The subspecies *europaea* spreads in the Mediterranean basin where it is indigenous and in other regions with a Mediterranean climate where it has been introduced, such as in South Africa around Stellenbosch (Costa 1998). Drupes are used as canned (spice) or pressed to produce oil. The olive oil is mostly virgin (natural) and cold pressed. Other grades exist, but they have no such a high reputation (Breton et al. 2004). About 3,000 olive cultivars are named and only a few are now spread world wide (Bartolini et al. 1998). Most are local and display a range of morphological traits of fruits and organoleptic criteria for oil. Many regions in several Mediterranean countries have geographic labels for the oil or fruit quality (Gemas et al. 2004).

Subsp. *cuspidata* known as the Brown or African olive, Indian Olive or Zambujeiro da India is exploited for its wood. The Brown olive is an indigenous tree growing in the dry mountain forests of northern, eastern and southern Africa. Subsp. *cuspidata* is a plastic species adapted to semi-arid to meso-humid climates. It is used as graft stock to the olive to provide vigour and possible resistance to olive fungal diseases. However, grafting is tedious and expensive and has been abandoned (Costa 1998), thus we would explore the opportunity to cross the two subspecies if natural hybrids exist (Besnard et al. 2001). However, in this respect we first examine the oil content and the oil composition of subsp. *cuspidata* and *europaea* to determine whether there are suitable to improve each other as they may also represent potentially new sources of oil.

Crosses between the subspecies *europaea* and *cuspidata* are documented (Besnard et al. 2001). The only hybrid individuals between subspecies *cuspidata* and *europaea*, verified with molecular markers, have been obtained 30 years ago by P. Villemur (Besnard et al. 2001). They still grow in the INRA orchards near Montpellier (France). However, the literature lacks of data on the crosses between the other subspecies. Hybrids between subsp. *maroccana* and *europaea* have not been found in the zone where the two subspecies overlapped and Breton et al. (2005a, b, c) suggested that the flowering times of the two subspecies do not coincide.

In this paper, comparisons are made between the morphology of the drupes, olive oil content and composition between some European, Tunisian cultivars, the oleaster, and subsp. *cuspidata* specimens. Data were collected from fruits harvested from trees grown in natural environments and compared to other data in literature. The oleaster and subsp. *cuspidata* have a different drupe structure, oil content, and composition, but not different seed oil composition. Molecular diversity between the two subsp. was examined as well as putative hybrid trees. Results confirm that the two subsp. are crossable and that *cuspidata* can be used as genetic resources for the olive and vice versa.

Materials and methods

Subsp. *europaea*

Cultivars from continental France (Salonenque, Tanche, Picholine, Olivière, Lucques), and Tunisia (Chemlali, Gerboui and Chetoui) were chosen as dominant around Montpellier and in the north of Tunisia. Those from Italy: Ascolana-Tenera, Frantoio, Bosana-Sardinia, were chosen for comparison due to existing data on oil composition in the literature.

Wild olive trees carrying fruits were chosen around Montpellier-France (Massif de la Gardiole), Corsica-France (Lévi), Italy (Sardinia), and from natural ecosystems in Tunisia. Because some trees grow on cliffs or rocky soils, which are unfavourable environmental conditions, they do not produce fruits every year. All oleaster from Tunisia and cultivar trees (European and Tunisian) have already been studied and their morphological comparison has been described in Hannachi (2007) and Pinatel et al. (2006).

The drupes for this study were harvested on oleaster and cultivar trees in the north of Tunisia (Fig. 1), around Montpellier, in Corsica and in Sardinia. The Tunisian oleaster trees were coded O1 (Slouguia), O2 (Testour), O3 (Téboursouk), O4, O5, O6 (Ichkeul), O7, O8, O9 (Tunis), O10 (Medjez El Bab), O11 (Dougga) and O12 (Séliana).

Subsp. *cuspidata*

Fruit samples were taken from wild trees in the Mt. Kenya forests near Naro Moru/Nanyuki Kenya, elevation 2,000 m; latitude 37°E and 0°10′S and were studied for oil content and oil composition. Leaf from *cuspidata* sampled at Naro Moru were also examined and compared to the *europaea* cultivars and oleasters (Fig. 1). Putative hybrid trees naturally produced between *europaea*—introduced and constituting orchards—and *cuspidata*—native form around orchards and used as stocks to graft *europaea*—were sampled around Stellenbosch (elevation 500 m; latitude 19°E and 34°S, South Africa).

Morphology and morphometry of drupes

Classical key criteria based on drupe morphology, pulp and pit (composed of shell + seed) analyses were used. They are list in the International Olive Oil Council recommendations (Khadari et al. 2003). The detailed analysis is given in Hannachi (2007).

Lipid extraction and studies

Drupes (5 g) were fixed with 20 ml of boiling distilled water to neutralise lipase activities between the field and the laboratory. The drupes were crushed in a mortar with a pestle in the presence of chloroform and methanol (1:1, v:v). Oil extraction was performed according to protocol from Allen and Good (1971). To the chloroform–methanol homogenate we added salt water (final concentration 1% NaCl) and then it was centrifuged at 3,000×*g*. The chloroform phase containing the total lipids was retained. After evaporation of chloroform in a rotary evaporator at 50°C, the total lipid fraction was conserved in (1:4, v:v) toluene and ethanol at −20°C (Vorbeck and Marinetti 1965).

Oil content determination

The oil yield is expressed as fatty acid weight/100 g defatted dry matter of total fruit weight (Pinatel et al. 2004).

Fatty acid composition

The fatty acid profiles were done on the whole drupes of green nearly ripe fruit, the pulp only of ripe drupes, the pit (shell + seed) of ripe drupes and the seed only of ripe drupes. The saponification process was performed in a tube of methylation (0.5 ml of total lipids) with methalonic soda at 65°C for 15 min. The transmethylation was performed with boron trifluorure at 65°C for 5 min. Margaric acid ($C_{17:0}$) was used as internal standard. The fatty acid methyl-ester phase was extracted twice with petroleum ether.

Gas chromatography

The mixture of methylic esters was run on a Gas Chromatograph (Girdel, 300c) with capillary column (30 m × 0.53 mm; the film thickness 1 μm) operated at 200°C. The temperature of the injector was maintained at 230°C and the flame ionisation detector was maintained at 250°C. The carrier gas was nitrogen at 0.4 Pa. However, data on subsp. *cuspidata* were compiled from several measurements using Soxhlet, chromatography or RMN.

Molecular markers

We examined polymorphisms at nuclear microsatellite loci using six ssrOeUA-DCA- (-01, -03, -05, -09, -15, -18) (Sefc et al. 2000, Breton et al. 2006), three

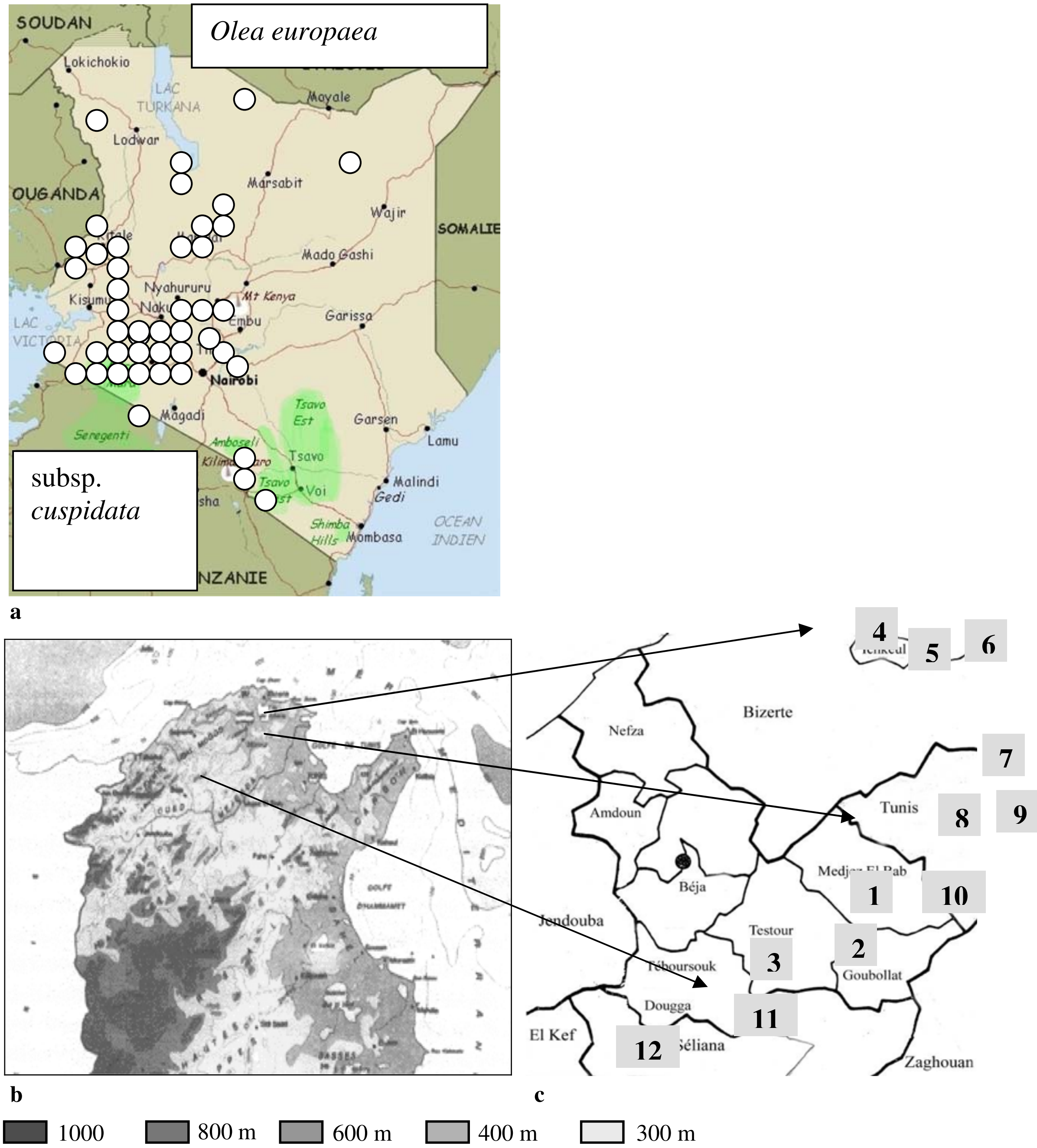

Fig. 1 (**a**) Distribution of *Olea europaea* subsp. *cuspidata* in Kenya. Open circles indicate observed in natural sites. (**b**) Map of North Tunisia and (**c**) the oleasters locations details from 1 to 12. The cultivars Chetoui, Chemlali and Gerboui were cultivated in all these locations

ssrOe-GAPU (71A, 89, 101, Carriero et al. 2002) and two ssrOe-*UDO99*- (-012, -024, Cipriani et al. 2002) to compare oleaster, olive, subsp. *cuspidata* and putative '*europaea* × *cuspidata*' hybrid trees.

Data analyses

Principal component analyses were computed on the data using the software Statistica (Sta Soft Inc., Johannesburg, ZA). We first look for a global structure using PCA and then we compute correlation between parameters. The principle of PCA is to compute parameters as components of axes that are independent. For molecular diversity we computed polymorphisms using Arlequin software (Excoffier et al. 2005) through factorial correspondence analyses.

Results

Morphology

The upper surface of the leaves of *cuspidata* is less varnished than *europaea* and the lower face is a golden green (some times reddish) and not silvered as for *europaea*. Whole fruit description in the three taxa is given. Subsp. *cuspidata* from Kenya displays drupe size between 0.5 and 1.0 cm. The drupes are not fleshy and the pulp is thin, watery, bitter to taste and is not used for culinary purposes. Subsp. *europaea* displays drupe size between 0.8–1.5 and 1.2–3.0 cm for oleaster and cultivars, respectively. The drupes are fleshy and the pulp is oily. Consequently, fruit size is not discriminant between the taxa but pulp morphology is.

Oil content

Subsp. cuspidata

In *cuspidata* from Kenya, the whole fruit oil content is 5.0–7.7%; the pit (shell + seed) oil content is 4.8% and the pulp oil content is 2.9%, with a total of 7.7% oil (Table 1). The pulp oil content is insignificant; the oil is contained in the seed. The proportions of the drupe parts are as follows: the pulp is 36% of the drupe, consisting of mainly water and ca. 2.9% oil. The pit shell is 56% of the drupe and is woody with no oil. The seed is 8% of the drupe with 36% oil.

The proportion of seed and consequently oil was compared to fruit size in the Kenyan *cuspidata* in an experiment on 40 pits taken from 4 different areas (10 from each provenance). The average pit weight ranged from 0.067 to 0.143 g. and the average seed weight ranged from 0.01 to 0.015 g. Hence there is a wide variation in pit size according to provenances. The total oil content, which is contained mostly in the seed, would be the same for each pit but if one measured the % oil content per weight, it would be lower for large pits and higher for small pits.

Table 1 Oil content and fatty acid composition (% of fat) for 12 oleaster trees and the cultivars Chetoui, Gerboui and Chemlali

	% oil	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	UFA	MUFA	PUFA	SFA
Norm	In fruits	7.5–20	0.3–3.5	0.5–5	55.83	3.5–21	<0.9	≥0.6				
Chetoui	27.04	16.657	0.606	3.768	57.198	20.082	0.65	0.675	78.536	57.8	20.73	21.1
Chemlali	17.26	12.825	0.336	4.604	64.889	15.955	0.847	0.544	82.03	65.22	16.8	19.97
Gerboui	21.43	16.744	1.232	2.756	58.635	18.986	0.887	0.759	79.74	59.87	19.87	20.26
O1	9.44	12.919	1.694	2.583	68.905	12.521	0.861	0.519	83.981	70.6	13.38	16.021
O2	10.99	16.513	0.818	2.797	57.812	19.834	1.659	0.567	80.123	58.63	21.49	19.877
O3	15.06	13.69	0.98	2.21	65.72	14.69	1.29	0.41	82.68	66.7	15.98	16.31
O4	7.59	13.09	0.27	2.01	47.03	33.56	3.7	0.32	84.56	47.3	37.26	15.42
O5	8.35	12.11	0.62	1.7	71.55	12.52	1.34	0.13	86.03	72.17	13.86	13.94
O6	8.02	13.99	0.16	2.21	51.52	28.95	2.8	0.34	83.43	51.68	31.75	16.54
O7	10.83	14.23	0.7	2.63	63.74	16.7	1.53	0.44	82.67	64.44	18.23	17.3
O8	9.11	16.2	1.31	1.82	66.96	12.46	0.9	0.29	81.63	68.27	13.36	18.31
O9	8.81	15.14	1.22	2.56	67.03	12.9	0.83	0.3	81.98	68.25	13.73	18
O10	10.96	14.32	1.32	2.63	67.89	12.22	1.25	0.32	82.68	69.21	13.47	17.27
O11	13.43	18.676	2.589	4.356	59.492	13.655	0.64	0.592	76.376	62.08	14.3	23.624
O12	12.83	11.79	1.29	1.98	65.02	15.48	1.52	0.41	83.31	66.31	17	14.18

UFA unsaturated fatty acids, *MUFA* mono-unsaturated fatty acids, *PUFA* poly-unsaturated fatty acids, *SFA* saturated fatty acids

Table 2 Fatty acid (% of fat) composition of various parts of drupe of *Olea europaea* subsp. *cuspidata*

Subsp. *cuspidata* seed origins	Maro Moru Church			Gathiuru Forest		
Fatty acid	Whole green drupe	Ripe pulp	Ripe pit (shell + seed)	Seed sample 1	Seed sample 2	Seed sample 3
16.0	12.1	20.0	13.08	7.43	9.1	8.3
16.1	0.1	0.1	0.1	0.07	0.2	0.1
18.0	3.6	3.2	4.1	4.37	4.2	4.0
18.1	44.3	21.4	39.9	60.93	54.5	59.6
18.2	33.3	36.0	33.0	24.68	30.2	25.4
18.3	1.3	3.2	1.4	0.5	0.6	0.5
20.0	0.9	0.6	0.9	0.9	0.4	0.8
22.0	0.6	0.4	0.6	0.6	0.5	0.5

Subsp. europaea

Oleaster oil content studies are less documented. The subsp. *europaea* displays oil content between 8–15 and 18–28% for oleaster and cultivar, respectively (Table 1). In Italy and France, the oleaster fruit oil content is in the range 8–15%. Some oleaster trees bear fruits that show a fiber-rich pulp with little oil whereas others display as much oil as some cultivars (Table 2). We did not examine fruits from natural *cuspidata* × *europaea* hybrids indigenous to South Africa.

Oil composition

Subsp. cuspidata

Fatty acid data were compiled (1991–1993) for subsp. *cuspidata* Kenya (Table 3). From the scarce data we have, we can see that the oleic acid content was lower in the pulp oil than in the seed oil.

Subsp. europaea

In the 12 oleasters fatty acids are mainly unsaturated, from 76.4 to 86% of total and a little, saturated acids from 14 to 24% (Table 1, Fig. 2). Unsaturated fatty acids included the mono-unsaturated (from 47.30 to 72%) and poly-unsaturated (from 14 to 37%) fatty acids. Another oleaster oil sample received from (Lévi, Corsica-France, F. de Lanfranchi) displayed 81% OAC, (Table 2). The oleic acid is the major fatty acid in the olive oil (Tables 2, 4), it is also the major fatty acid in the oleaster oil (Table 1, Fig. 2). The oleaster O5 had a higher rate of oleic acid (72%) than the cultivars Chetoui (57%), Chemlali (65%) and Gerboui (58%).

Microsatellite diversity

Microsatellite diversity was examined at six DCA, three GAPU and two UDO loci. Subsp. *europaea* displays less alleles ($n = 59$) than *cuspidata* ($n = 103$), although the transfer of primers to *cuspidata* may bias the result. In the AFC, all *cuspidata* individuals cluster far from *europaea* while putative hybrids sampled by H. Sommerlatte by 2003 around olive orchards in Stellenbosch (area of the Cape, South Africa) breakdown close to the *cuspidata* extreme cloud (Fig. 3). Studies by Rallo et al. (2003) using IAS markers and Besnard et al. (2007) confirm the differences between the two subsp.

Discussion

Genuine and feral oleasters trees were chosen in Tunisia where oleaster was established before the Phoenician period and during the Roman period. Oleaster was found in the Djerba Island and in the west hills of the country (L'olivier 1953). Subsp. *cuspidata* trees are indigenous and grow wild in Kenya and Pakistan. There is no ambiguity between the two subsp. except that in the oleaster some hybrids (olive × oleaster) may have escaped to the natural site and they display a more or less intermediate phenotype.

Table 3 Comparison of oil content and oil composition (% of fat) between some cultivars and European oleasters data from Hannachi et al. (2007) and Ollivier et al. (unpublished)

Subsp. var.	Country-location	Cultivars	Oil content (%)	Palmitic acid content	Stearic acid content	Oleic acid content	References
europaea	France	Salonenque	21.6–26.3	14.47	23	55	Pinatel et al. (2006)
		Tanche	22.2–26.5	8.49	22,5	66	
		Picholine	17.8–21	10.47	19	61	
		Olivière	11.4–14.9	12.31	9	42	
	Italy	Lucques	11.9–18.1	11.81	17	54	
		Aglandau	12.8–34.1	12.29	1.5	72	
		Ascolana-Tenera	18%	12.3	1.5	75.1	
		Frantoio	19%	13.7	1.5	70.2	
	It (Sardinia)	Bosana	18%	12	2.5	65	
	Tunisia	Chetoui	27.04	16.657	3.8	57.198	2004
		Chemlali	17.26	12.825	4.6	64.889	2004
		Gerboui	21.43	16.744	2.8	58.635	2004
sylvestris	Spain		ND	10.5%	3%	76.7%	[a]
	Tunisia		8–15%	11.8–18.7	1.7–4.3	47–71.5	2004
	Italia (Sardinia)		Estimation 8–15%	12–30	1–3	58–70	M. Mulas (personal communication)
	Fr (Corsica) Lévi		Estimation 8–15%	ND	ND	81%	2006
	Fr Hérault La Gardiole		8, 9%	ND	ND	ND	2007

[a] http://www.essentialoil.in/olive_oil.html

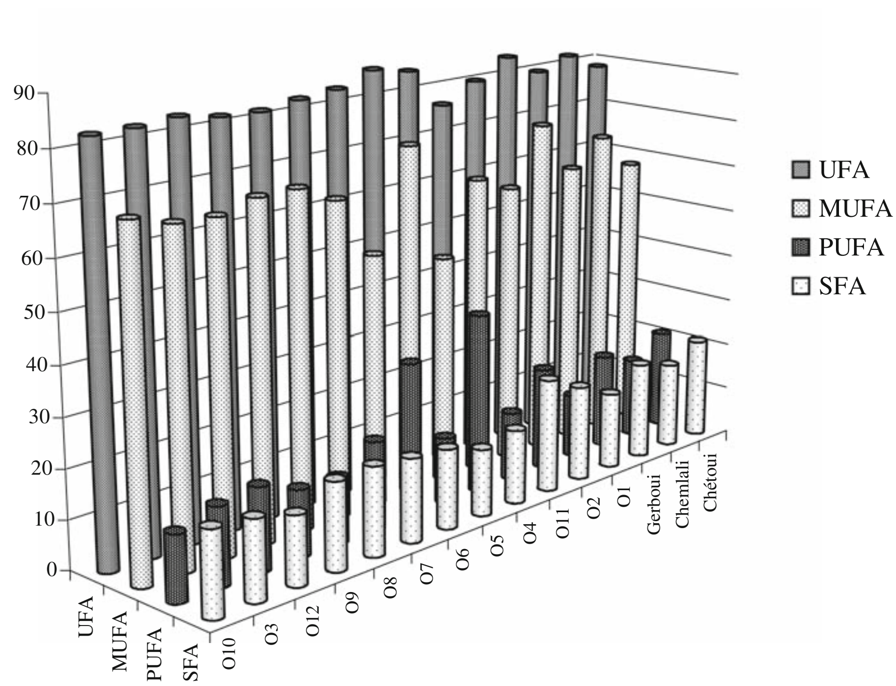

Fig. 2 Unsaturated (UFA), saturated (SFA), mono (MUFA) and polyunsaturated (PUFA) fatty acids from Oleaster and cultivar oil samples from Tunisia

Table 4 Comparison of oil content and fatty acid composition between mesocarp and kernel in three cultivars according to Thakur and Chadha (1991)

Cultivars	Aglandau		Ascolana-Tenera		Frantoio	
Tissue	Mesocarp	Kernel	Mesocarp	Kernel	Mesocarp	Kernel
Oil percentage	28%	5%	26%	4%	29%	6%
16.0	15.4	11.2	12.3	8.0	13.7	11.4
16.1	2.2	1.0	1.2	0.2	1.0	1.1
18.0	1.5	2.5	1.5	3.0	1.5	2.3
18.1	72.0	68.0	75.1	70.4	70.2	67.6
18.2	7.5	15.6	8.6	16.4	12.2	15.9
18.3	0.8	0.5	0.7	0.4	0.8	0.5
20.0	0.3	0.5	0.3	0.6	0.3	0.4
22.0	–	0.3	–	0.4	–	0.4

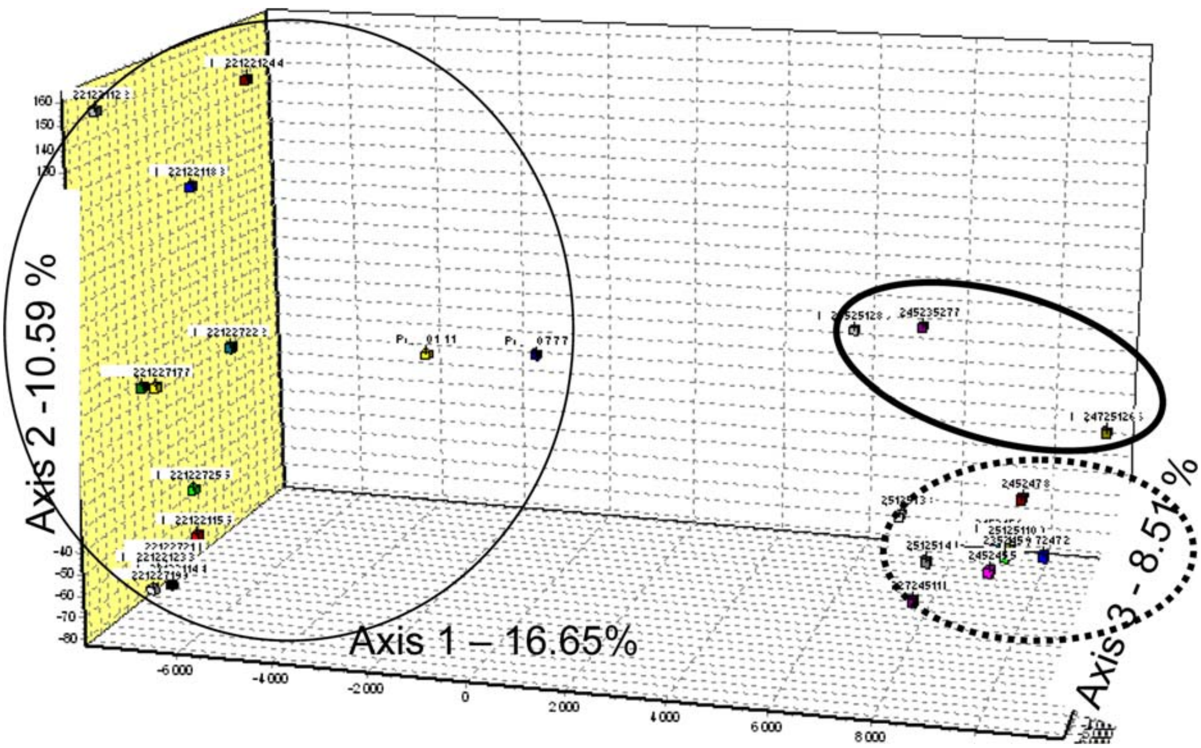

Fig. 3 Factorial correspondence analysis on microsatellite data for some *europaea* cultivars (thin line circle), *cuspidata* Kenya individuals (thick line circle) and putative hybrids (dotted line circle) harvested in fields around olive orchards grafted on *cuspidata* at Stellenbosch (South Africa)

Comparisons based on DNA analyses

Hybridization between subsp. *cuspidata* and the cultivated olive occurs spontaneously around orchards when the cultivated olive has been introduced into the subsp. *cuspidata* area in South Africa and Iran as presumed by Costa (1998) and Omrani-Sabbaghi et al. (2007). Rallo et al. (2003) studied some of these samples with four IAS-oli1, -oli12, -oli17, and -oli22 and showed the high genetic distances between subsp. *cuspidata* and *europaea* samples. Moreover, the two subsp. *cuspidata* and *europaea* have also been introduced into Hawaii and Australia and several reports stated that natural hybrid spread (Besnard et al. 2007). Sedgley (2000) and Breton et al. (2008) did not support this view. Natural hybrids probably exist in the Cape Area (Stellenbosch, Kirstenbosch) and could have been moved to Australia and Hawaii and not obtained in Australia.

Comparison based on morphology

Between *cuspidata* and *europaea* the differences are at the leaf and fruit levels. For the fruits, the structure differs mainly in the pulp. The *europaea* has a fleshy pulp and the Kenyan *cuspidata* a thin and watery pulp and the ratio of pulp to pit is much higher for *europaea*. The morphological distinction between *cuspidata* from Asia and Kenya has not been made due to the lack of data from Pakistan.

Comparison based on oil content

Presented differences between culltivars, oleasters, feral trees and *cuspidata* are mere estimations since all these trees were not grown in the same environment. Moreover, the description of methods to extract and evaluate fatty acids is succinct and they are variable between sources of data. Nevertheless, from the data of European cultivars compiled by Ollivier et al. (2005, 2006), one estimated that the relative genetic variation for the oil content according to years is up to 20% whereas in comparison to absolute oil content subsp. *cuspidata* Kenya displayed 5–7%, oleaster up to 8–10%, and cultivars 11–28%, hence differences are probably meaningful.

In a study on the content and composition of oil extracted from wild olive samples of *O. europaea* subsp. *cuspidata* from five different locations in the hilly areas of Pakistan, the oil content of the dried fruits was between 34.11 and 36.69% which based on the fresh weight would be about 10–13% oil (Gulfraz et al. 2006a, b) This may suggest that the subsp. *cuspidata* from Pakistan has a fleshy, oily pulp with an oil content similar to that found in oleasters. An hypothesis is put forward later to explain this anomaly.

Thus in summary (1) subsp. *cuspidata* Kenya has the lowest oil content, and the structure of the pulp is watery; (2) subsp. *cuspidata* Pakistan has a higher oil content than the Kenyan *cuspidata*; (3) Oleaster displays a range of variation, but we cannot determine whether the higher oil content fruit are progenies from feral oleasters or from hybrids between oleasters and cultivars; (4) The olive cultivar on average displays the highest oil content but oil content depends on the cultivar. The lowest oil % values recorded for a cultivar was 11.4 and the highest values 27.04% (Ollivier et al. 2006, Table 4).

Comparison based on oil composition

Looking at comparisons of the oleaster and cultivars: Oleaster O5 displayed less saturated fatty acids than the cultivars Chetoui, Chemlali and Gerboui. For the oleaster the stearic acid content is rather low in comparison to palmitic acid content. Stearic acid variation range is important for cultivars (6–32%), and for palmitic acid content it is relatively less (Pinatel et al. 2004). Some oleasters displayed more oleic acid contents than the cultivars Chetoui, Chemlali and Gerboui. Four oleaster trees showed fatty acids composition in agreement with the International Olive Oil Council (IOOC, Madrid) norms (Table 1). Thus, olive oil composition is qualitatively similar in both botanical varieties of the subsp. *europaea* olive tree but it is variable in composition of fatty acids as already signalled by several authors (Hannachi 2000; Sedgley 2000) (Tables 1–3).

Even though the range of variation for fatty acid composition is wide between the subspecies and between samples within the subspecies we can say there are no distinguishing differences for fatty acid composition between the seed oil of *cuspidata* Kenya and fruit oil of *cuspidata* Pakistan, oleaster, and cultivars (Thakur and Chadha 1991; Gulfraz et al. 2006a).

Hypotheses on differences in pulp oil between the Kenya and the Pakistan *cuspidata*

Kenya is on the equator and therefore experiences no winter. This environmental factor may be a cause of little oil and different fatty acid profile in the pulp of the Kenya *cuspidata*. In India and Pakistan, particularly in the north there is a winter similar to the

Mediterranean which may explain why the Indian *cuspidata* has more pulp oil. This is also true for *Cofea* species that displays a gradient for oil content and composition with geography (Dussert et al. 2000). Hence there is variation in pulp oil content and fatty acid profile which is presumably determined by environmental influences and little variation in seed oil content and fatty acid profile which is determined by the genome. In Kenya, a few cultivars from Europe have been planted but in every case there has been no flower and fruit induction which may indicate that a Mediterranean climate with a mild winter is necessary may be associates with a compatible day-length for olive oil production (H. Sommerlatte, personal communication).

The fatty acid compositions of the two subsp. *europaea* and *cuspidata* from Pakistan or Kenya were similar as the two varieties *europaea* (cultivated olive) and *sylvestris* (oleaster) was qualitatively identical. Crosses therefore between the two varieties of *europeae* will enable us to look for oleasters with low content in saturated fatty acid and high content in poly-unsaturated fatty acids to improve cultivar oil composition. We can also predict that crosses of cultivars with oleasters to the benefit of favourable genes will not drastically change the oil composition of the pulp of the progenies. Phenol composition should be determined on *cuspidata* and oleaster oils.

Conclusion

Surprisingly, evaluation of the oleaster or other related taxa as genetic resources for the olive has not been undertaken. We have shown here the oil content and oil composition of the *cuspidata* and oleaster trees to determine whether olive-oleaster and olive × *cuspidata* progenies can produce oil in the olive oil range of variation.

Olive improvements deal with stress resistance against mostly fungi and many insects that grub into the fruits causing depreciation and oxidation of oil. Such *cuspidata* × *europaea* hybrid trees could bring advantage as stocks for the cultivar grafting to offer vigour and possible resistance to established olive diseases. Subsp. *cuspidata* from Pakistan displays a better oil content than in Kenya and should be preferred to create new hybrids with *europaea*.

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