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ORIGINAL PAPER

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The use of floral characters in *Capparis* sect. *Capparis* to determine the botanical and geographical origin of capers

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Abstract Capers of commerce are flower buds of *Capparis* species, most of them presumably of the species *C. spinosa* and they are considered as such in international food legislation. However, in the Mediterranean countries of Europe, Asia, and North Africa, that are the main producers of capers, four different species have been detected in the commercial product: *C. spinosa*, *C. sicula*, *C. orientalis* and *C. aegyptia*. In this paper the study of morphological characters, easily detectable under optical microscope (number of stamens, anther shape, and morphology of nectary) has proven to be a simple and cheap technique to recognize the presence of the above-mentioned species in commercial capers and the relative percentages of each, this being a good indicator of the most likely geographical origin.

Keywords Quality control · Nectaries · Anthers · Scanning electron microscopy · Optical microscopy

Introduction

The flower buds, the fruits and occasionally the shoots of *Capparis spinosa* L., *C. sicula* Veill., *C. orientalis* Veill., and *C. aegyptia* Lam., have been used as a condiment in the Mediterranean region (countries surrounding the Mediterranean Sea) and neighboring countries since ancient times; those products have been greatly appreciated for their pungent and bitter flavor. Generally the pickled flower buds are the most appreciated product; those buds, when still tightly closed are harvested and then brined and packed in vinegar (capers). These buds are selected by size, the smaller ones being the most greatly appreciated on the market.

Capers are usually commercialized in several Mediterranean countries, the most important being Turkey, Greece, Italy, France, Spain, and Morocco, and they are exported mainly to central European countries, the USA, and the UK as a delicatessen product.

The existing national and international food legislation [1, 2, 3] states that capers are the buds of just *Capparis spinosa*, but there is evidence that other species are also used, so techniques for identification of the species used commercially could be very interesting for the quality control of the product.

With the purpose of determining the botanical and geographical origins of capers, the flavonoid contents were investigated [4] in fresh and commercialized brined material. The study revealed that capers are a great source of flavonoids, very interesting as antioxidant agents, but flavonoids displayed great variability unrelated with botanical or geographical origin and were shown not to be helpful for this particular purpose. *Capparis* DNA research have also been developed [5] and have been very useful in discriminating the species; nevertheless such molecular techniques are quite expensive and very time consuming, and as such are not suitable for quality control. On the other hand, the macromorphological study of living material in *Capparis* showed a high diversity in different characteristics, especially anthers and nectaries, references to which in previous literature are lacking [5]; as a result, the use of this diversity for determining the origin of commercial capers seemed promising.

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Materials and methods

The flower receptacle, having been termed torus [6], is more or less conical (5–6 mm in diameter), has a small, obscurely bilobed adaxial disk, which may attain 0.5–2 mm, but is often hard to trace in dried material. The number of stamens is a characteristic of some importance, being surprisingly constant in the flowers of

Table 1 Field sites where bud and flower samples were collected for SEM study and character analysis

Localities	Anthers data	Nectary data	Identification
Baza, Granada (Spain)	Sharp	Star-like	*C. sicula*
Albudeite, Murcia (Spain)	Sharp	Star-like	*C. sicula*
Castillo Santa Barbara, Alicante (Spain)	Rounded	Triangular-collapsed	*C. orientalis*
Benifayet, Lérida (Spain)	Rounded	Triangular-collapsed	*C. orientalis*
Serós, Lérida (Spain)	Rounded	Triangular-collapsed	*C. orientalis*
Aldover, Tarragona (Spain)	Rounded	Triangular-collapsed	*C. orientalis*
Palma, Balearic Islands (Spain)	Rounded	Triangular-collapsed	*C. orientalis*
Llano del Beal, Murcia (Spain)	Rounded	Pyramidal	*C. aegyptia*
Andraitx, Balearic Islands (Spain)	Sharp	Triangular	*C. spinosa*
Llubí, Balearic Islands (Spain)	Sharp	Triangular	*C. spinosa*

Table 2 Character analysis in processed capers. Numbers of capers with each type of anther and nectary within each sample of 35 capers

Localities	Anthers		Nectaries			
	Sharp	Rounded	Triangular	Star-like	Triangular-collapsed	Pyramidal
Morocco 1	3	32	1	2	0	32
Morocco 2	15	20	2	14	0	19
Morocco 3	35	0	0	35	0	0
Morocco 4	35	0	20	15	0	0
Morocco 5	31	4	16	19	0	0
Turkey 1	35	0	35	0	0	0
Turkey 2	34	1	4	31	0	0
Turkey 3	34	1	19	16	0	0
Turkey 4	3	32	2	0	0	33
Iberian Peninsula 1	35	0	0	35	0	0
Balearic Island 1	35	0	35	0	0	0
Balearic Island 2	35	0	0	35	0	0
Balearic Island 3	35	0	35	0	0	0
Balearic Island 4	31	4	16	3	16	0
Italy 1	0	35	0	0	35	0
Greece 1	35	0	12	23	0	0

each plant species but more variable among species. Floral characteristics which have most frequently been considered in the literature are the length and concavity of the largest and most saccate of the four sepals and also some measurements of the ovary [6, 7]. Anther shape has received scarce attention although, as we will show, is worthy of study. Similar scarce attention has been paid to nectary morphology.

Flower buds. Flower buds were collected from ten field sites, including wild and cultivated populations on the Iberian Peninsula and Balearic Islands (care was taken not to collect perforated buds or those damaged by insects) (Table 1). Voucher specimens have been deposited in herbarium MUB (University of Murcia, Spain). Flower buds were fixed for 4–5 h, kept at 4–5 °C using melting ice, with 4% glutaraldehyde in a 0.1 mol/l solution of cacodilate buffer (pH=7.2–7.4), and post-fixed for 1 h in a 1% solution of osmium tetroxide. From each sample 30 buds were studied, the type of anthers and nectaries were annotated using an Olympus SZ11 binocular microscope, and 5 buds were processed for SEM microscopy. These data were confirmed with optical microscopy analysis of further samples from Morocco, Syria, Lebanon, Greece, Italy, France, and Turkey. These latter were processed in the same way as the botanical herbarium specimens, but still retained the anther and nectary morphology.

Capers. Sixteen commercial samples processed in brines were studied. They were produced in different Mediterranean countries. Samples from Spain (5; 1 from the Iberian Peninsula and 4 from the Balearic Islands), Turkey (4), Morocco (5), Italy (1), and Greece (1) were studied (Table 2). All samples were preserved in brines, with the exception of the sample from Italy that was preserved in salt. From each sample 35 buds were studied; the type of anthers and nectaries were annotated using an Olympus SZ11 binocular microscope (Table 2).

SEM study. Samples were dehydrated with critical point drying that causes almost no deformation of specimens, which retain their original shapes. For coating, the vacuum evaporation method and the sputtering method were used. Substances used were C and Au, suitable for the observation magnification. The observation was made using a Jeol 6.100 SEM with a microanalysis system at 10 kV.

Results

Scanning electron microscope study of anthers in natural unprocessed buds

Stamens have typical anthers provided with a pair of tecae, each one containing two pollen bags. Dehiscence is through longitudinal fissures. Anthers are oblong in outline. Anthers vary in size with the flower but are otherwise much the same, excepting the anther apex types. Two main apex types were found: sharp-pointed and rounded. These types may be observed using a simple binocular microscope, being clearly shown under SEM. The anther apex type is detected even in flower buds and thus the interest in trying with pickled buds.

The sharp pointed anthers are characteristic of *Capparis spinosa* and *Capparis sicula* (Fig. 1). This anther type, although almost oblong in outline, has a rounded

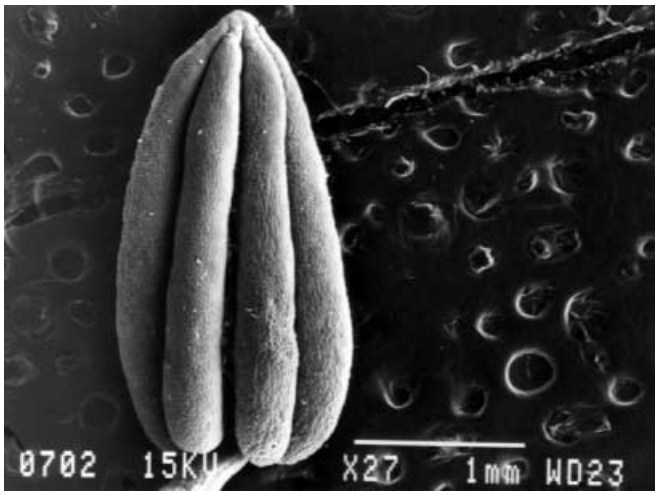

Fig. 1 Scanning electron microscope image of sharp anthers (*C. spinosa*, *C. sicula*)

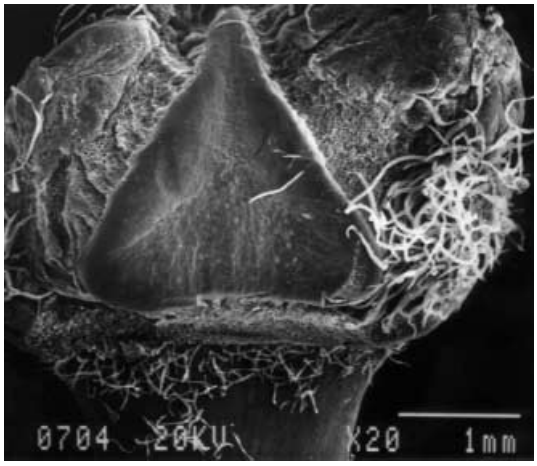

Fig. 3 Scanning electron microscope image of triangular nectary (*C. spinosa*)

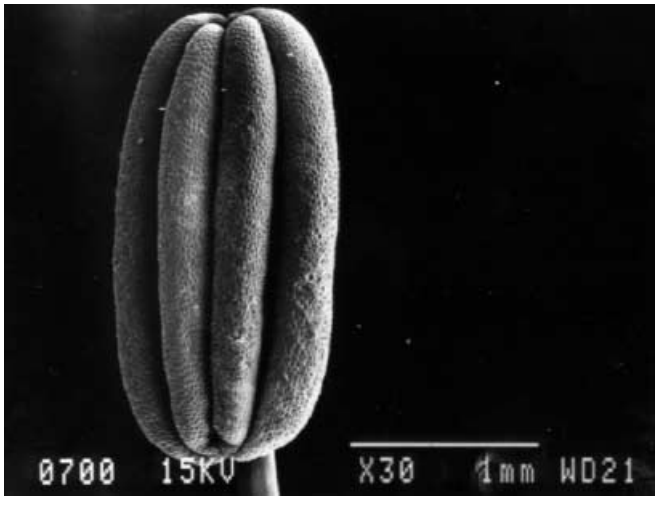

Fig. 2 Scanning electron microscope image of rounded anthers (*C. aegyptia*, *C. orientalis*)

basal part, being gradually attenuated in the apical end. This feature is better detected in fresh material in the form of an acutely sharp anther apex.

Almost oblong anthers are found in *Capparis orientalis* and *Capparis aegyptia* (Fig. 2). The apical end of this anther type is rounded to almost rounded, with the four pollen-bags ending at the same level and not gradually attenuated towards the central apical point. The basal end is somewhat rounded also.

Scanning electron microscope study of Nectaries in natural unprocessed buds

Floral disks are convex to somewhat conical. Depending on the total number of stamens, flowers with 50–75 stamens have an almost rounded floral disk. Flowers with 90–125 or more stamens have a more clearly conical disk. Each floral disk has a single triangular shaped basal-lateral nectary. Nectary morphology is a good character for differentiating the four taxa studied, all belonging to *Capparis* Section *Capparis*. This morphology may be studied using optical and electronic microscopy. Four basic types have been differentiated: triangular, star-like, pyramidal, and collapsed-triangular:

1. Triangular nectaries, almost triangular shaped (base/height=1), are characteristic of *Capparis spinosa*. These nectaries have rounded corners and edges, and almost straight margins. The nectary surface is almost rounded. The nectaries appear slightly raised above the level of the floral disk (Fig. 3).
2. Star-like nectaries are characteristic of *Capparis sicula.* These appear in the form of a three-armed star, with the upper arm displaying a broad and shallow furrow in the middle; the two basal arms are somewhat broader. Altogether the three apices appear markedly rounded and obtuse. It may also be described as a variant of the above triangular type (base/height=1), but with concave margins and broader edges and corners. The nectaries appear markedly raised above the level of the floral disk (Fig. 4).
3. Pyramidal nectaries are characteristic of *Capparis aegyptia.* These appear in the form of a straight triangle, with a relatively large base when compared with the smaller height (base/height≅2). The three arm apices appear rounded, the upper being markedly obtuse. It may also be described as a variant of the above triangular type but with convex margins and broader base. The nectary appears markedly raised above the level of the floral disk (Fig. 5).
4. Collapsed triangular nectaries are typical of *Capparis orientalis*. The upper pair of margins are clearly concave, sharp edged, the three angles are markedly sharp, acute. The surface appears somewhat collapsed, with a basal hole. The lateral margins are somewhat obscure, gradually attaining the level of the floral disk, which is almost rounded (Fig. 6).

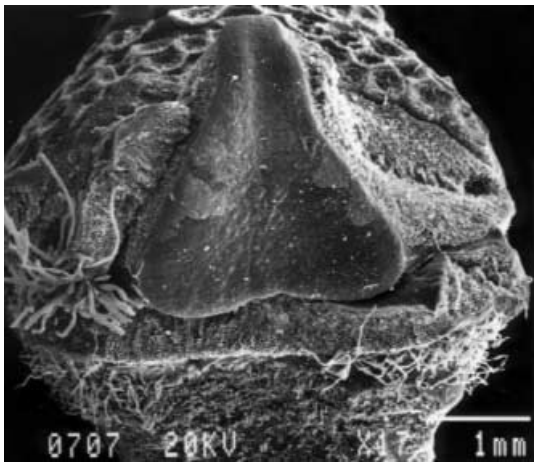

Fig. 4 Scanning electron microscope image of star-like nectary (*C. sicula*)

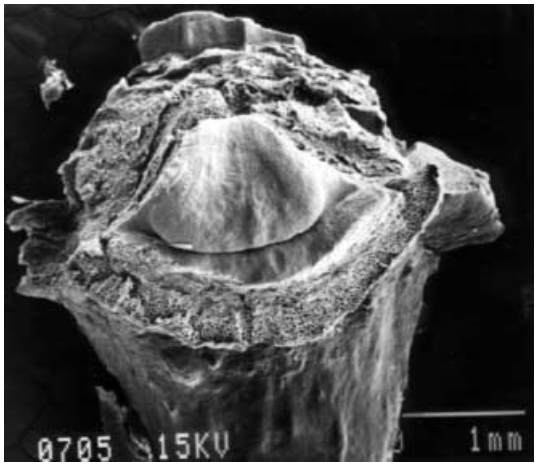

Fig. 5 Scanning electron microscope image of pyramidal nectary (*C. aegyptia*)

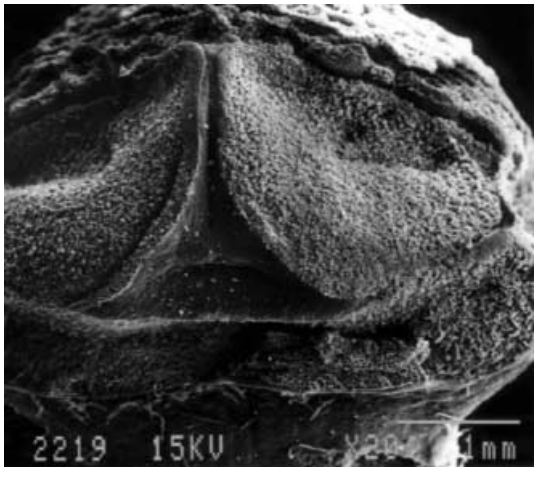

Fig. 6 Scanning electron microscope image of collapsed-triangular nectary (*C. orientalis*)

Optical microscope study of anthers and nectaries in processed brined buds

After the analysis of brined buds (capers) from different localities and countries, the variability of the selected characters was easily detected with the use of binocular microscope. Table 2 summarizes the results of the observations. Anther variability is more easily detected than that of nectaries. As a rule the samples proved homogeneous, except sample 2 from Morocco and sample 4 from the Balearic Islands. Anther and nectary morphologies agreed well with those previously discovered in unprocessed samples (Table 1).

Discussion

The higher reliability of anther shape in relation to nectary shape in processed capers is presumably related to the differences in water availability in the brine, modifying the shape of nectaries more than that of anthers. Notwithstanding, the four types of nectaries allow us to determine more precisely the species of origin. Combining both data the results are optimized and determination is achieved (Table 3).

Samples proved relatively homogeneous and thus multi-specific mixtures were relatively rare. Only two samples (Balearic-4 and Morocco-2) may comprise two clearly different species in differing proportions, *C. spinosa+C. orientalis* and *C. sicula+C. aegyptia* respectively. Mixtures of *Capparis sicula* and *C. spinosa* are likely (samples Morocco-4 and 5, Turkey-3, Greece-1) but in this case doubts will arise because the determination of nectary data are somewhat unclear. Thus these mixtures may also correspond with pure samples of *C. spinosa.*

The Balearic sample 2 is more relevant because it corresponds to a pure sample of *C. sicula*. It is an imported material (*C. sicula* is extremely rare on the Islands and not locally gathered for commercial purposes). Since the name “Alcaparras de Mallorca” is internationally protected through formal agreements between Spain and other countries [2], and local Balearic capers are almost exclusively *C. spinosa* and, more rarely, *C. orientalis*, the use of the above name for this sample may be fraudulent. Otherwise, its commercialization simply as “capers” or in Spanish “alcaparras” is not contradictory to the law.

The Moroccan samples can be divided in two groups. Samples 1 and 2 with rounded anthers and nectary type *C. aegyptia* predominant in the samples came presumably from the Safi region (southern Morocco) where *C. aegyptia* is the dominant species and extensively under cultivation. Samples 3, 4, and 5 display sharp anthers and star-like nectaries predominantly; these contain *C. sicula* and came presumably from the Fes region (central Morocco). Turkish material is also diverse, involving three different kinds. Sample 4 that is identified as *C. aegyptia* may have been collected in Southern Turkey or imported from Syria or Lebanon where it is quite a common plant. The

Table 3 Botanical ascription of the samples on the basis of types of anthers and nectaries

Localities	Anthers data	Nectary data	Identification
Morocco 1	*C. aegyptia*/*C. orientalis*	*C. aegyptia*	*C. aegyptia*
Morocco 2	*C. aegyptia*/*C. orientalis*+*C. spinosa*/*C. sicula*	*C. sicula*+*C. aegyptia*	*C. sicula*+*C. aegyptia*
Morocco 3	*C. spinosa*/*C. sicula*	*C. sicula*	*C. sicula*
Morocco 4	*C. spinosa*/*C. sicula*	*C. spinosa*+*C. sicula*?	*C. spinosa*+*C. sicula*
Morocco 5	*C. spinosa*/*C. sicula*	*C. spinosa*+*C. sicula*?	*C. spinosa*+*C. sicula*
Turkey 1	*C. spinosa*/*C. sicula*	*C. spinosa*	*C. spinosa*
Turkey 2	*C. spinosa*/*C. sicula*	*C. sicula*	*C. sicula*
Turkey 3	*C. spinosa*/*C. sicula*	*C. spinosa*+*C. sicula*?	*C. spinosa*+*C. sicula*
Turkey 4	*C. aegyptia*/*C. orientalis*	*C. aegyptia*	*C. aegyptia*
Iberian Peninsula 1	*C. spinosa*/*C. sicula*	*C. sicula*	*C. sicula*
Balearic Island 1	*C. spinosa*/*C. sicula*	*C. spinosa*	*C. spinosa*
Balearic Island 2	*C. spinosa*/*C. sicula*	*C. sicula*	*C. sicula*
Balearic Island 3	*C. spinosa*/*C. sicula*	*C. spinosa*	*C. spinosa*
Balearic Island 4	*C. spinosa*/*C. sicula*+*C. aegyptia*/*C. orientalis*	*C. spinosa*+*C. orientalis*	*C. spinosa*+*C. orientalis*
Italy 1	*C. aegyptia*/*C. orientalis*	*C. orientalis*	*C. orientalis*
Greece 1	*C. spinosa*/*C. sicula*	*C. sicula*+*C. spinosa*?	*C. spinosa*+*C. sicula*

single sample containing exclusively *C. orientalis* is from Italy, where most capers are prepared from flower buds of this species. The sample from Greece is likely a mixture of *Capparis sicula* with (in lesser quantity) *C. spinosa*.

Therefore type of anther tip and nectary morphology can both be successfully used to determine the botanical origin and sometimes, when the distribution of the species is well known, the geographical origin of commercial capers. The morphological screening of crude material or processed (brined buds) using a binocular microscope may become routine in the industral processing of this kind of food, as a supplementary tool for quality control.

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