Comparative Morphoanatomical Study of Three Species Pertaining to the *Crocus* Genus: *C. nevadensis, C. nudiflorus* and *C. sativus*. Differentiating Characteristics

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Abstract

A morphoanatomical study of three *Crocus* species has been carried out. Two of these species, *C. nevadensis* and *C. nudiflorus*, which are wildflowers found in mountain meadows, were picked, respectively, on March 12, 2003 and November 5, 2002 in the Alcaraz mountain range (Albacete Province). The third species, *C. sativus*, is a cultivated species that was picked on November 5, 2002 in Santa Ana, Albacete. The study was done in order to establish the morphoanatomical parameters, which differentiate wild and cultivated species. With this in mind, three specimens from each species were picked. The first one was processed in the laboratory using conventional techniques for inclusion in paraffin. Microphotographs were then taken with an optical OLYMPUS BH2. The other two specimens from each of the three species were filed in the herbarium in the Department of Plant Biology at the E.T.S.I.A. Both common and differentiating characteristics were found among the three species:

- ✓**Root: number of vascular strands, layers of storage tissue in the cortex and in the outer form of the perimeter**
- ✓**Stem: perimeter morphology.**
- ✓**Leaves: the shape is quite different in transverse sections, as is the number and position of the vascular strands.**
- ✓**Stigmas: three stigmas with a large glandular surface covered by secreting epithelium and lysogenic cavities.**

INTRODUCTION

The name Crocus finds its origins in the Greek word *krokos*, saffron, which in turn derives from the Semitic word *karkom*, one of the oldest names for this plant (The Royal Horticultural Society, 2003).

The *Crocus* L. genus (Mathew, 1980; Sánchez-Monge, 1991) is known mainly for the cultivated species *Crocus sativus* L, which is of prime economic importance. However, there are also other species belonging to this genus, which are highly prized for their colorful flowers, and thus used extensively in specialized gardening.

With the exception of *C. sativus*, the species found in the Province of Albacete are wild and flower at different times of the year on grassy hills and mountains, in meadows and on river banks, giving a touch of color to these areas. Two of these wild species are *C. nevadensis* Amo and Campo, and *C. nudiflorus* Smith.

It's of interest to note that these wild species may participate in the first phases of post-fire plant recolonization since their bulbs are buried a few centimeters below the surface, a depth which protects them from destruction by fire (De las Heras Ibáñez and Martínez Sánchez, 1991).

Their shallow fasciculated root systems, which cling to the topsoil, also make these species ideal for preventing erosion (Raven et al., 1992).

Due to its wide range of applications, the *Crocus* genus is used extensively in different industries (e.g. cosmetic, food, textile, dyeing, pharmaceutical, etc.). Villar et al.

(2001) also mentions the possible inclusion of *Crocus* in the diets of wild animals that eat roots, tubercles and bulbs, since species of this genus are found in meadows and forest clearings where wild boars root.

No anatomical studies have been carried out on *Crocus* species, in spite of their singular importance. The main purpose of this study is therefore to establish the morphoanatomical similarities and differences between the cultivated *C. sativus* species and the wild *C. nevadensis* and *C. nudiflorus.*

MATERIALS AND METHODS

Materials

C. nevadensis (white saffron, wild saffron) is native to southern Spain and Northern Africa (Herranz Sanz and Gómez Campo, 1986; Azafrán de Sierra Nevada, 2003), appearing in rocky areas and in alpine meadows. Some forms are extremely attractive, although their cultivation is considered difficult by even expert growers (Autumn *Crocus*, 2003).

After fire, this species can increase its percentage of ground cover with respect to other species due to increased sunlight and the high temperatures the bulbs are exposed to. These factors may also promote flowering (De las Heras Ibáñez and Martínez Sánchez, 1991).

C. nudiflorus (crazy, brave or mountain saffron) is found in the north, east and central regions of the Iberian Peninsula, the southwest of France and in Great Britain, growing on grassy hills and mountains (Polunin and Smythies, 1981). Its attractive flowers make it highly prized in gardening, while the easy cultivation of its tuber-bulbs allows for widespread commercialization by specialized distributors.

Due to the wide variety of therapeutic properties attributed to *C. nudiflorus*, it is also considered a medicinal plant, although its current use is limited to homeopathic cures for cough, loss of appetite, digestive difficulties and smallpox (Azafrán, 2002). The flower is the organ of the plant which is used for these treatments since it contains various essential oils and volatile aromas, plus chemical components such as carotenoids (Nuestro Entorno, 2003).

C. sativus (saffron) possesses three bright red, hanging stigmas between 25 and 30 mm long (Azafrán, 2002). These stigmas are not branched, although their tips are wide and dentated. The dried stigma gives off an intense fragrance, constituting what is commonly termed as “saffron” (El cultivo del azafrán, 2003).

C. sativus is believed to have evolved from the ancient *C. cartwrightianus*, a species with long stigmas that flowers in autumn and which grows wild in the eastern Mediterranean region (The Royal Horticultural Society, 2003). Saffron has been cultivated in Greece, Asia Minor and Iran over the last 3,500 years, with Iran currently consider to be the number one world producer.

The Romans introduced saffron into Great Britain, while the Arabs brought saffron to Spain (The Royal Horticultural Society, 2003). The species has adapted well to the Mediterranean habitats, where it grows wild in many areas.

Among saffron’s qualities, it is considered to be tonic, sedative, emmenagogic, antispasmotic, carminative and also an aid for difficult births. High dosages (over 30 g) can be toxic and abortive (Azafrán, 2002).

Saffron also possesses properties which combat tumors, a fact which may, in the future, allow saffron to serve as an alternative to aggressive chemical treatments used to treat certain cancers (El cultivo del azafrán, 2003). The most recent therapeutic application of this species that is currently being developed calls for maceration of stigma and petal extract in ethanol (Hosseinzadeh and Younesi, 2002).

In addition to its medicinal properties, saffron can be useful in other areas. Crocin is employed as a coloring both in the cosmetic and food industries and safranine is used in biochemistry and microbiology, although saffron is no longer used in the textile industry due to its elevated cost (El cultivo del azafrán, 2003). Where saffron is most used

nowadays is in the food industry. The dairy industry continues to color cheeses and butter with saffron; and high-quality bakery goods use it for its color, aroma and flavor. Saffron is extensively used as a condiment to season various dishes (El cultivo del azafrán, 2003) with its strong, bitter and slightly piquant taste (Azafrán, 2002).

The best specimens of *C. nevadensis* and *C. nudiflorus* in the Province of Albacete are found in the area around the town of Paterna del Madera, between 1300 and 1500 meters above sea level in the Sierra del Agua. These specimens are found growing on lime substrates in *Pinus nigra* pine groves with abundant rainfall. *C. nevadensis* starts to blossom when show melts, as early as February; and *C. nudiflorus* flowers between September and November (Herranz Sanz and Gómez-Campo, 1986; Plantas medicinales, 2002). Harvesting of these species took place in the "Puerto del Barrancazo" (1430m), an area near the tree line with a tree cover of less than 50%, with forest clearings being occupied by bush. *C. nevadensis* was harvested on March 12, 2003 and *C. nudiflorus* on November 5, 2002.

The bioclimatology in this area is inferior supramediterranean with an inferior subhumid ombroclimate (Orozco Bayo, 1991).

C. sativus can be found in the Alcaraz Region in autumn, being extensively cultivated on a wide, mainly flat plain. The material used for this study was harvested in a family-owned and cultivated saffron field in the central region, near the town of Santa Ana (Nuestro Entorno, 2003) on November 5, 2002. The natural vegetation in the area has been reduced greatly over the centuries due to agricultural activity. The bioclimatology in the area (Orozco Bayo, 1991) is defined as superior bioclimatic mesomediterranean with a dry inferior ombroclimate.

Methodology

The laboratory technique used for the histological study was designed by Gattuso and Gattuso (2002) and calls for inclusion in paraffin, cutting and pasting.

Conservation in paraffin was considered necessary since the plant material under study was too fragile to be cut and manipulated otherwise. The paraffin allowed for the preservation of the plant material over a longer period of time.

Microphotographs were then taken with an optical OLYMPUS BH2.

RESULTS

Root

1. *C. nevadensis*. The endodermis is corky in the IV phase of differentiation. The cortical cellular stratum closest to the endodermis shows thickened anti-clinal walls while only the interior periclinal wall is thickened. The cortex possesses very few cellular strata, between 5 and 6, with an average width of 0.02 mm. A central tracheid is present in the xylem of the vascular cylinder (Figure 1).

2. *C. nudiflorus*. The endodermis is on occasion bistratified and corky in phase IV, accompanied by a cortical cellular stratum with thickened walls. The cortex possesses from 7 and 8 cellular strata, and each stratum has an average width of 0.03 mm. Tetrach roots.

3. *C. sativus*. The endodermis is monostratified in phase IV with a cortical cellular stratum with thickened walls. The cortex possesses an elevated number of cellular strata, from 11 to 12, and each statum has an average width of 0.04 mm. Polyarch roots.

Scape

1. *C. nevadensis*. The scape of this species is circular, with an average diameter of 1.20 mm.

2. *C. nudiflorus*. The scape is pentagonal in shape. Average diameter: 1.50 mm (Figure 2).

3. *C. sativus*. This can be considered a typical stem. Its contour is irregular. Average diameter : 2.5 mm.

Stigmas

1. *C. nevadensis.* The stigma has an average length of 4 mm. Its tissue is glandular and the long, papillose epidermic cells are covered by a cuticle with various tricomes. Lysogenic cavities with an average diameter of 537 µ appear among the parenchymatous cells.

2. *C. nudiflorus*. The stigma has an average length of 10 mm. The papillose epidermic cells contain many tiny tricomes but only a few large tricomes. Lysogenic cavities with a medium diameter of 82 µ are present among the parenchymatous cells.

3. *C. sativus*. The average stigma length is 25 mm. The epidermis of the stigma is glandular, with numerous tricomes. Lysogenic cavities are not present among the parenchymatous cells (Figure 3).

Leaf

1. *C. nevadensis*. Stomata in the crypts and de abaxial epidermis. Papillose cells appear in the crypts. The vascular bundles reach the epidermis (Figure 4).

2. *C. nudiflorus*. The stomata appear inside crypts, which have a papillose epidermis. The vascular bundles, which do not reach the epidermis, are surrounded by fibers.

3. *C. sativus*. The stomata are arranged on the same level as the epidermic surface, in the crypts, where large unicellular tricomes are found. The vascular bundles reach the epidermis.

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Figures

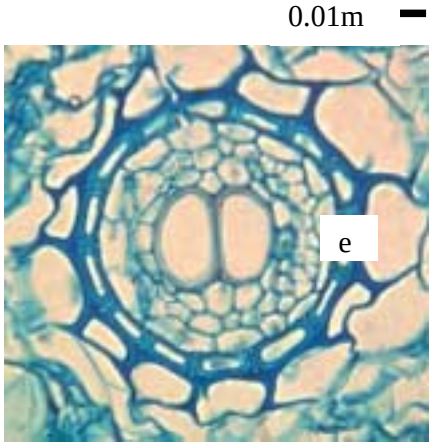

Fig. 1. C.S. Vascular cylinder of *C. nevadensis* root. e: endodermis.

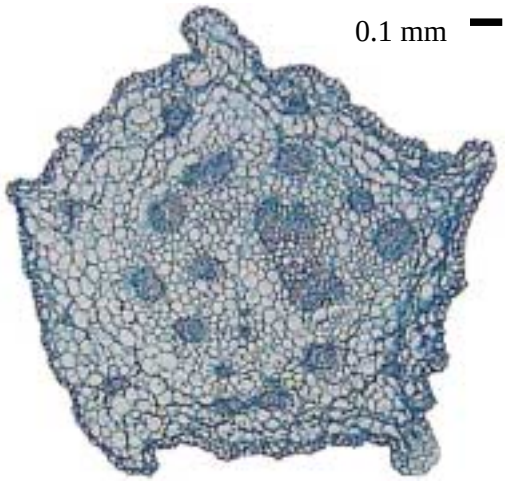

Fig. 2. C. S. *C. nudiflorus* scape.

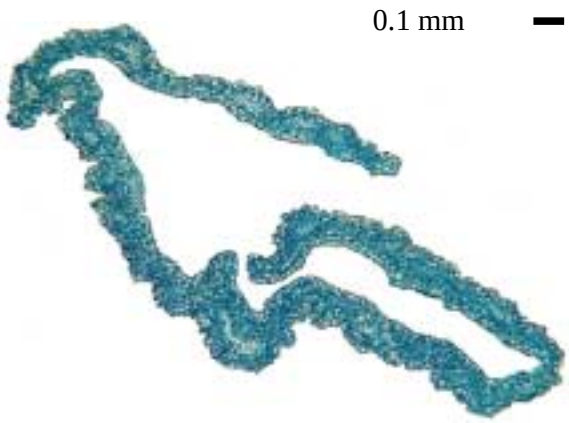

Fig. 3. C.S. *C. sativus* stigma.

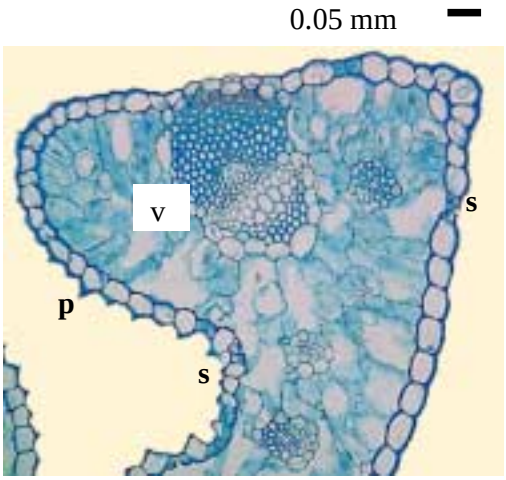

Fig. 4. C.S. *C. nevadensis* leaf. v: vascular bundle; p papillose cell; s: stomata.