Extrafloral Nectaries in *Cucurbita maxima* Sub. *andreana* (Naudin) Filov

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Introduction: Nectaries, from a functional point of view, are easily defined as plant secreting structures that produce nectar (6). They can be situated in vegetative (extrafloral nectaries) or reproductive organs (floral nectaries), and may have different morphologies and anatomical origins (1). Extrafloral nectaries (*efns* hereafter) are usually small protuberances, which may be covered by protecting non secretory hairs (2, 11). Regardless of their position or origin, the function is to reward animals that provide the mobility that plants lack, that is: vector for pollen dispersal and physical defense (8). Structural nectaries were distinguished from non structural ones, (i.e. without any special differentiated nectariferous tissue) (12), which are more frequent among *efns* (1). Some *efns* are also devoid of vascularization and lack the anatomical organization typical of nectaries, while the most frequent vascular bundles may consist of phloem and xylem or phloem only (1). A continuous thick cuticle covers the epidermal cells of *efns* and nectar release generally takes place through cuticle rupture.

Based on ecological and morphological evidence (4, 5 and 9) and on mitochondrial gene single-base substitutions (10), *Cucurbita maxima* subsp. *andreana* has been recognized as the wild (agrestis) form of domesticated *C. maxima* Duch. However suggestions have been presented to maintain the wild taxon at the specific level, supported by the fact that *efns*, which are present in the abaxial side of leaves of domesticated forms of *Cucurbita*, were absent in subsp. *andreana*; and this overlooked feature should have taxonomic and evolutionary importance (3). These findings were based on herbaria specimens collected in the Córdoba province of Argentina. Due to the viny and vigorous growing habit of subsp *andreana*, it is probably that each exsiccatum was taken from only one or few plants in each provenance. In order to confirm Hunziker and Subilis (3) findings we extended the study to accessions from all the provinces reported by these authors to conform the natural distribution area (Buenos Aires, Entre Ríos, Santa Fe, Córdoba and San Luis), plus Santiago del Estero province; and considered at least five living plants from each provenance.

Materials and Methods: Twelve accessions of *C. maxima* subsp. *andreana* and two cultivated forms of *C. maxima* subsp. *maxima* were grown for assessing the presence or absence of *efns* (Table 1). Plants were seed planted in the spring of 2005 at the Experimental Field of the Agronomy Faculty, Rosario´s National University, located at Zavalla (33º 01' S; 60º 53' W), Santa Fe. The plantation grid was of 1.4 m and 0.8 m between rows and hills in the row respectively. Each accession was set in a non-replicated single row plot of eight hills. After emergence each hill was thinned to two plants. Due to a severe wind storm right after emergence, some plants were cut-off, leaving many plots with less than the optimal sixteen plants. During plant growth leaves were cut (between the fifth and the tenth node), and observed under stereomicroscope in order to determine the presence or absence of *efns*. Each plant was assessed in at least three leaves. In the accessions where *efns* were present, samples were collected, fixed in FAA, conserved in ethanol 70, and further dehydrated in an ethanol series and embebbed in Tecnovit 7100 (Heraeus

Kulzer GmbH). Semi-thin sections (0.5-1μm) were obtained using glass knives and stained with toluidine blue as general stain (7).

Results and Discussion: Ten out of the 12 surveyed accessions of *C. maxima* subsp *andreana* presented plants showing *efns* (Table 1). The two accessions where *efns* were completely absent in all assessed plants were from Córdoba and Santa Fe provinces. The two accessions from Santiago del Estero showed *efns* in all plants. The rest of the provenances of subsp *andreana* presented both plants showing and not showing *efns*. As expected, all the plants of the two cultivated accessions presented *efns*. Moreover, during the 2006 season, the search for *efns* was extended to a set of 72 domesticated *C. maxima* accessions (six plants per entry, data not shown), and the glands were present in all instances.

Morphologically, *efns* were observed as small protuberances with a columnar body and a head with a secreting surface (Figure 1). They showed a great variation in size (from 0.4 to 1 mm in length), and were more or less conical or cylindrical; those growing on higher order veins were more or less flattened. The column of long nectaries was covered by hairs, while shorter ones were deprived. This feature (hairy nectaries column) was not depicted by (3) in the cultivated forms of *Cucurbita*. In relation to the anatomical organization, sectioned material also showed a wide variation in size and complexity. The main structure was: in the column, complete vascular bundles with phloem and xilem, cells hold big vacuoles and were photosynthetic, the head contains the secretory tissue, typically constituted of medium sized cells with large nuclei. There is an evident layer of one or two cells dividing the column and the head, where the cell walls and maybe the intercellular space take the coloration of the non living cells of the xylem. No stomata, neither homologous way for nectar release were found. We propose that the mechanism should be holocrine secretion as expected for this kind of nectaries. No insect were observed visiting the *efns* when leaves were surveyed for their presence.

It is evident that the presence of *efns* is a feature of the domesticated forms of *C. maxima*, but not necessarily of the wild ancestral subsp. *andreana*. Some provenances completely lack these glands, while others (especially those from Santiago del Estero), showed, as the cultivated forms, *efns* in all surveyed plants. Two possibilities can explain their presence in cultivated forms, one is that domestication was conducted from populations not segregating for their presence; or, that the attribute of showing *efns* in originally domesticated *C. maxima* was fixed by chance. Inferred from the low percentage of plants with *efns* in some provinces, apparently, their presence is not an ecological important attribute for the survival of subsp. *andreana* in the actual area of distribution, and may be a relic structure from a primitive ancestor. Moreover, in comparison with the great amount of allocations devoted to vegetative and reproductive organs, the reduced resources that these glands involve does not seem to confer a disadvantage.

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Table 1. Number of plants with extrafloral nectaries by accession, and percentage of presence by provenance.

Accesion	Subspecies	Provenance[a]	Source [b]	Number of plants		Presence %
				Presence	Absence	by Provenance
UNR-132	*andreana*	Córdoba	UNR	0	15	
UNR-134	" "	Córdoba	UNR	2	3	12
UNR-133	" "	Córdoba	UNR	1	6	
UNR-138	" "	Santa Fe	UNR	0	13	
UNR-135	" "	Santa Fe	UNR	3	4	15
MAX-81	" "	Entre Ríos	IPK	3	2	60
UNR-141	" "	Santiago del Estero	UNR	11	0	
UNR-140	" "	Santiago del Estero	UNR	5	0	100
UNR-137	" "	San Luis	UNR	3	3	
MAX-66	" "	San Luis	IPK	4	1	64
UNR-139	" "	Buenos Aires	UNR	10	3	
PI 458659	" "	Buenos Aires	NE-9	1	6	55
PI 244702	*maxima*	Brasil	NE-9	6	0	
Zapallito	" "	Argentina	Ferry Morse	8	0	100

[a] For subsp *andrean*a Argentinian provinces as provenances are considered, otherwise country of origin is detailed.

[b] UNR, Rosario´s National University; IPK, Leibniz-Institute of Plant Genetics and Crop Plant Research, Germany ; NE-9, USDA-ARS, Northeast Regional PI Station, Cornell University, Geneva, USA; Ferry Morse Seed Company.

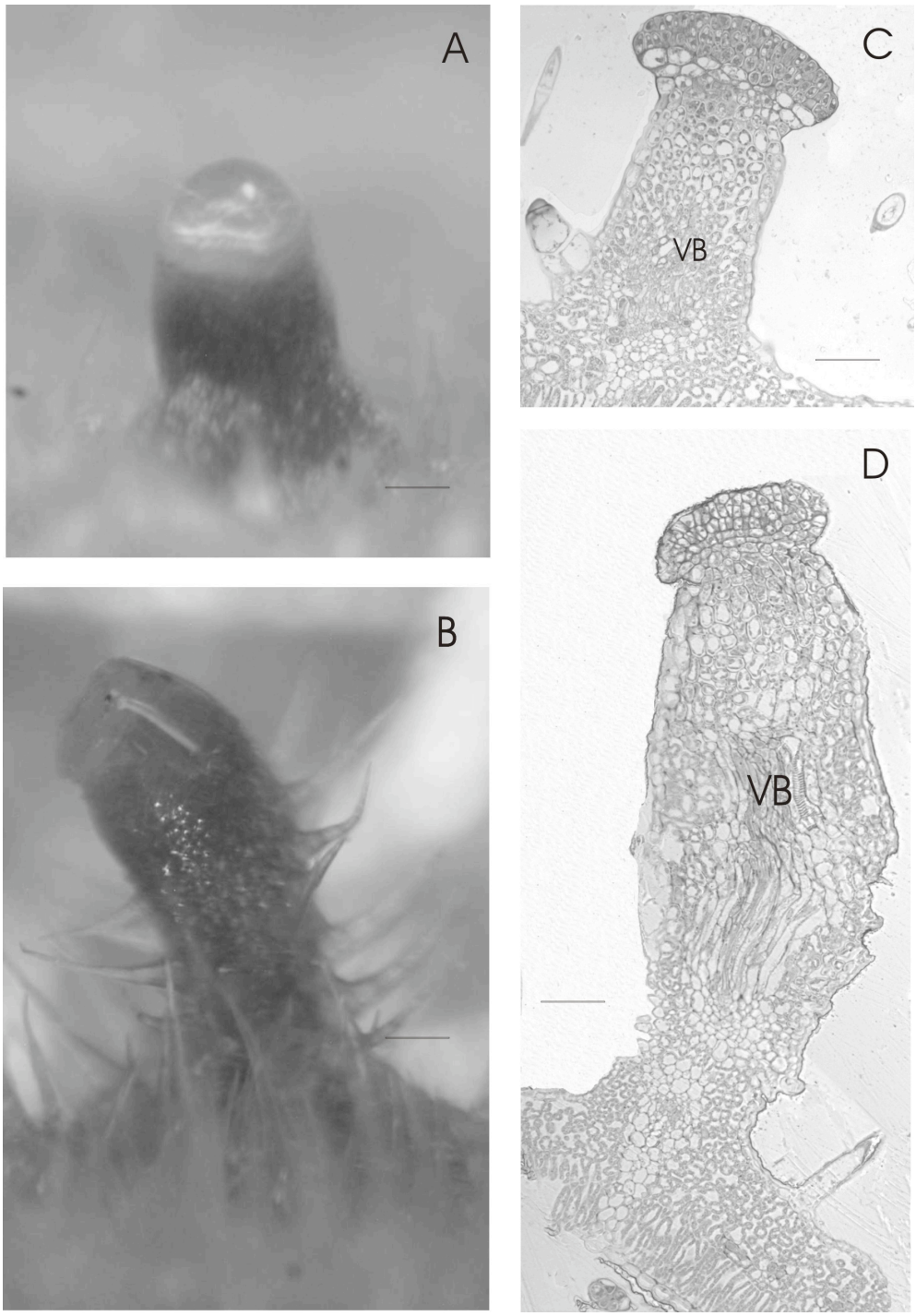

Figure 1. Extrafloral nectaries in *C. maxima* subsp. *andreana*. Bars = 100 µm. (A) Stereomicroscope photograph showing a short nectary with a secreted droplet visible on the head. (B) Stereomicroscope photograph showing a long hairy nectary, the secreted droplet is poured along the column. (C) and (D) Longitudinal sections showing the secreting head and column. In the column a vascular bundle is evident (VB), photosynthetic cells holding big vacuoles are present. The head contains the secretory tissue, typically constituted of medium sized cells with large nuclei.