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# Investigations into Trichomes and Anatomy of *Saccocalyx satureioides*: A Xeromorphic Shrub Endemic to the Arid Zones of Algeria

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**Abstract:** *Saccocalyx satureioides* is an endemic sub-shrub that grows in the wild in a restricted area in Algeria, where it is protected under national law. It is largely used in folk medicine and has been the subject of extensive investigation leading to a comprehensive understanding of its phytochemical traits. However, its morphological and cytological features remain poorly understood. The main objective of this work was to study the morphological, anatomical and karyological characteristics of *S. satureioides* and compare them with other features of the members of Menthinae sub-tribe. All parts of *S. satureioides* showed an important indumentum on young stems, leaves and calyx. Two types of glandular trichomes were found, the capitate ones and peltate ones, with one and 12 secretory cells respectively. Additionally, three types of non-glandular trichomes were observed: unicellular, bicellular, and pluricellular. Trichome distribution and density displayed variations among different plant organs. As well as three types of non-glandular trichomes: unicellular, bicellular and pluricellular. Trichomes distribution and density showed variability among organs. Furthermore, the investigated species showed some adaptive traits to cope with unpredictive rainfall and the characteristic drought conditions of its habitat in this region. These traits include the shape of calyx, which facilitate wind-dispersal of the accrescent fruit, leaves with a thick cuticle, and myxocarpy of nutlets. In the course of our research, mitotic metaphase photomicrographs revealed the presence of 2n=30 chromosomes. To the best of our knowledge, this chromosome count is reported here for the first time.

**Key words**: *Saccocalyx satureioides*, chromosomes, trichomes, Lamiaceae, Algeria, xeromorphic

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While the Lamiaceae family is well represented in Algeria with 183 taxa, of which 19 are endemic (Dobignard and Chatelain, 2012), only few taxa of this family occur in the Presaharian region: *Mentha longifolia* (L.) L., *Salvia verbenaca* L. and *S. Chudaei* Batt. & Trab. (Ozenda, 1983). Additionally,

there is the monotypic genus *Saccocalyx* Coss. & Durieu, which belongs to the Lamiaceae family, subfamily Nepetoideae, tribe Mentheae and subtribe Menthinae. This genus comprises the species *Saccocalyx satureioides* Coss. & Durieu described in 1853 (Cosson and Durieu, 1853).

*S. satureioides* is a strongly aromatic woody sub-shrub propagating by underground stems. The flowering stalks bear whitish flowers, four-lobed, with three equal lobes and the fourth one is largest and bifid, with four sub equal stamens, included within corolla, glabrous, very short, thecae weakly divergent, separate, connective small. The accrescent calyx bears 13 ribs, is bag shaped and flatted (Fig. 1).

From an ecological perspective, this plant is of significant interest due to its role in stabilizing sand dunes through its extensive root system. It is also widely used in in the region of Djelfa, not only as a condiment but also in folk medicine as stomachic, digestive, antidiarrheal, vermifuge and for gastro-intestinal disorders (Fourment and Roques, 1941; Beloued, 2005; Yabrir *et al*., 2018) and respiratory system diseases (Belmekki and Bendimerad, 2012, Adli *et al*., 2021). It is comparable to oregano (*Origanum vulgare* L.) and thyme (*Thymus numidicus* Poir.) because of its fragrance due to the presence of thymol (Mohamadia *et al*., 2015). Locally, it is known as "Zaatar ermel" or "Azir el Ibil" and is commonly referred to as the "oregano of the sands."

## *Systematic position and nomenclature*

The systematic position of *S. satureioides* is yet not clear but it is supposed to be close to the genus Thymus due to its oils composition and morphological features. According to Bräuchler *et al*. (2010), *S. satureioides* belongs to the Micromeria-group including representatives of *Micromeria*, *Origanum*, *Pentapleura*, two *Satureja* species, *Thymbra*, *Thymus*, and *Zataria*. The only cited synonym for this species is *Faustia satureioides* (Coss. & Durand) Font Quer & Rothm. 1940, which is now considered obsolete name.

## *Distribution area and ecology*

*S. satureioides* has restricted distribution area limited to the Presaharian regions of Algeria and Morocco. It grows in the wild in discontinuous zone close to salt lakes along the sand cord of a semi-arid region (Battandier and Trabut, 1902; Quezel and Santa, 1963; Ozenda,


*Fig. 1. Saccocalyx satureioides in Bousaada-Algeria. a and b: General shape of plants on a fixed dune; c and d: flowering stems.*

*Table 1. Characteristics of the sampling sites*

| Location | Latitude | Longitude. | Altitude | Bioclimatic | Drought duration in months (Bagnouls and Gaussen, 1953) | Formation |
|---|---|---|---|---|---|---|
| Sand cord Boussaâda | 35°14′44.229″N | 4°12′18.062″E | 475 m | cold semi-arid | 4 months | Sand |
| El Mesrane Djelfa | 35°0′43.207″N | 3°17′15.571″E | 860 m | cold semi-arid | 6 months | Sand |

1983). This species has also been reported from the region of the West South part of Algeria in Saïda, Naâma, Ain Sefra at the Algerian-Moroccan borders (Jahandiez and Maire, 1934; Djebaili, 1984; Aidoud-Lounis, 1984; Fennane and IbnTattou, 1998; Gordo and Hadjadj-Aoul, 2019).

*S. satureioides* is a psammophilous species, that thrives in depressions, and the sandy veils of the dunes. It can resist long periods of drought and presents typical xeromorphic adaptations like small leaves with revolute margins bearing thick cuticle. The developed roots form a horizontal network covering the surface of the soil. In this way it can use all the rain water. However, this plant suffers from grazing pressure due to the abundant livestock in the region.

The micromorphological traits are well known for their taxonomic value in Lamiaceae and have been used in systematics and phylogenetics by several authors (Abu-Assab and Cantino, 1987; Ascensão *et al.*, 1999; Moon *et al.*, 2009, Moon *et al.*, 2010), and at generic level by many others (Navarro, 1995; Navarro and El Oualidi, 2000; Salmaki *et al.*, 2009; Marin *et al.*, 2012; Naidoo *et al.*, 2013; Saouli *et al.*, 2018). The primary objective of this paper is to provide a comprehensive description of cytology and leaf micromorphology of *S. satureioides* using both light and scanning electron microscopy (SEM). These characteristics are discussed with respect to their relation with other taxa of the Mentheae tribe.

## Materials and Methods

### *Plant material*

Plant material was collected from two populations of *S. satureioides* during the flowering stage from two locations (Table 1). Plant identification was performed using the main Algerian floras (Battandier and Trabut, 1902; Quezel and Santa, 1963; Ozenda, 1983). Seeds were collected by the first author during several field trips and preserved in dry conditions until use. A specimen from Paris herbarium (P04366131) was used for the SEM analyses.

### *Trichomes analysis*

To prepare material for light microscopy, stems and leaves were sectioned using hand microtome, and then cross sections were treated with Mirande's reagent. For scanning electron microscopy (SEM), live material was mounted onto aluminium stubs using double-sided carbon tape, and coated with 20 nm gold for 60 seconds at the 30 mA/pa pressure three times (Jeol FJC-1200 Fine coater). Samples were investigated using the Hitachi SU3500 Scanning Electron Microscope. The main traits studied were shape, density and size of trichomes. The classification of glandular and non-glandular trichomes adopted in this work is that of Fahn (1988).

### *Karyological analysis*

The chromosome number was determined by analysing mitotic metaphases of young root tips for both populations. Preparation was made using the squash technique modified from Jahier *et al.* (1992). Root tips were treated with cold water for 12 h, fixed in Carnoy's solution (3:1 of ethanol: acetic acid) for 72 h and then stained using acetocarmine solution. The chromosome number analysis was carried out on 100 cells during the metaphase and anaphase. The best metaphasic plates were photographed using a Carl Zeiss Light microscope.

## Results and Discussion

### *Glandular and non-glandular trichomes*

All aerial organs of the plant bear abundant glandular and non-glandular trichomes of different morphological types. The stems and leaves cross-section give more details about their structure. Types of trichomes observed

*Table 2. Trichomes distribution on the aerial part of Saccocalyx satureioides*

| Trichome's types / Organs | Glandular trichomes | | Non glandular trichomes | | |
|---|---|---|---|---|---|
| | Type I | Type II | Unicellular trichomes | Bicelullar trichomes | Pluricellular trichomes with papillae |
| Young stems | + | +++ | ++ | ++ | +++ |
| Abaxial leaf blade | | ++++ | - | - | - |
| Adaxial leaf blade | ++ | +++ | - | - | - |
| leaf margin | | | | | ++ |
| Calyx blade | ++ | ++++ | ++ | ++ | ++++ |
| Calyx nerves | ++ | ++++ | ++ | ++ | ++++ |

Trichomes density: -: Non-existent, +: Very sparse, ++: Sparse; +++: Dense, ++++: Very dense.

and their distribution are provided in Table 2. They differ in size, shape and contents of the head cell, as well as in the number of stalk and basal cells.

Two types of glandular trichomes were observed on the young stems, leaves and calyx and were classified as follows:

Type I: small glandular trichomes with elongated unicellular head, and a bicellular stalk (Fig. 2a, Fig. 3a).

Type II: glandular trichomes, which comprise a basal cell, a short stalk cell and 12 secretory cells arranged in two layers. The outer layer contains eight cells and the internal layer contains four cells (Fig. 2b, c; Fig. 3b, c, d).

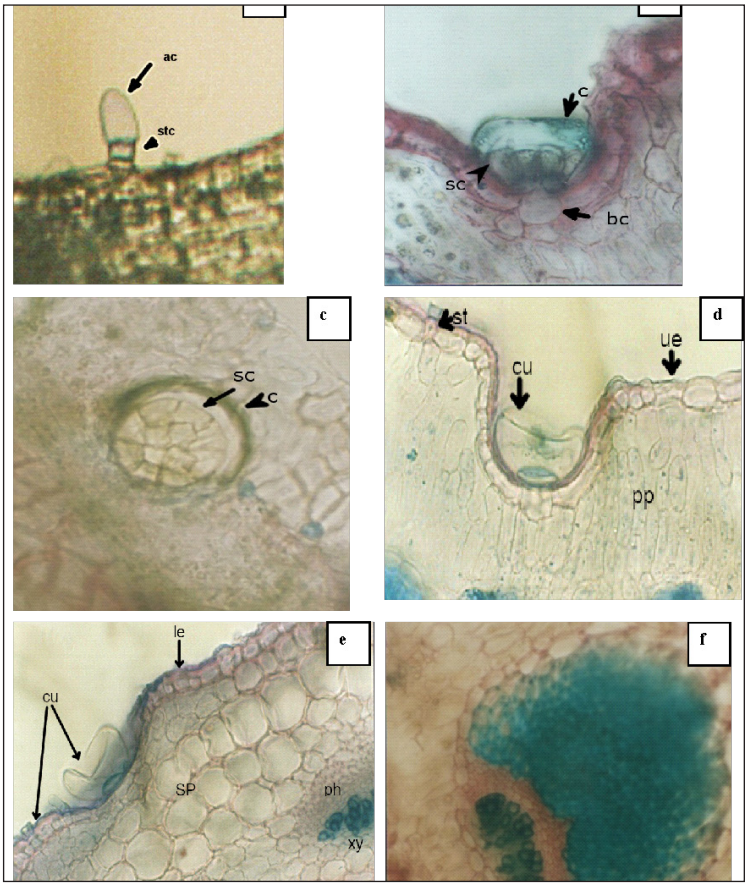


*Fig. 2. Hand cross section of fresh leaves of S. satureioides with glandular trichomes (a): type I glandular trichomes; (b): front view of type II glandular trichomes; (c): side view of type II glandular trichomes; (d): Leaf cross-sections showing an empty glandular trichomes in adaxial face (e): Leaf cross-sections showing an empty glandular trichomes in abaxial face.*
*ac: Apical cell, stc: Stalk cell, sc: Secretory cell, bc: Basal cell, cu: Cuticle, ue : Upper epidermis, st: stomata, le: lower epidermis, pp: palisade parenchyma, sp: spongious parenchyma, ph: phloem, Xy: Xylem.*

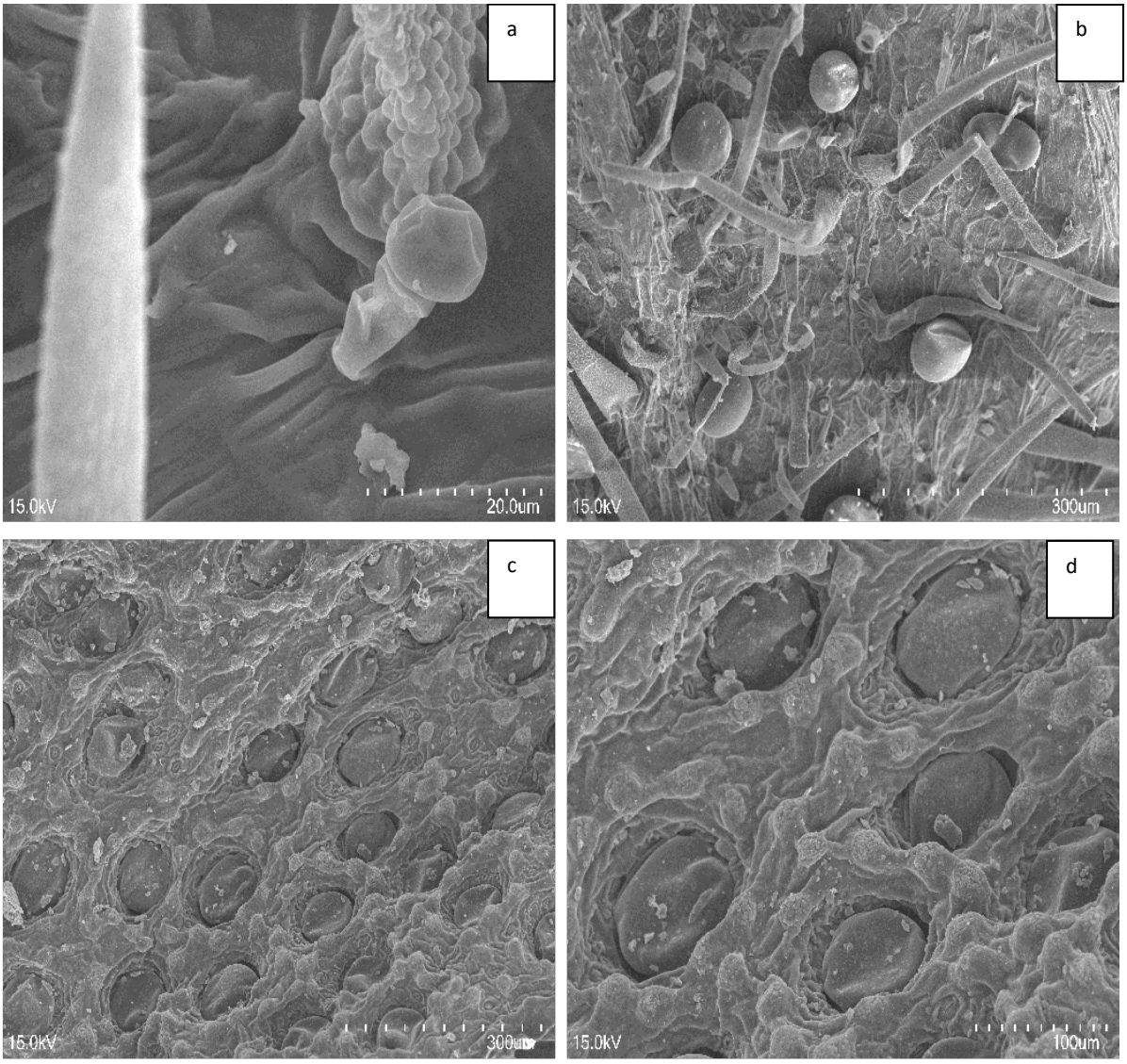


*Fig. 3. Scanning electron micrographs of Saccocalyx satureioides. (a): Type I glandular trichomes on the calyx blade; (b): Calyx with glandular trichomes and non-glandular trichomes, (c) and (d): Glandular trichomes type II on the abaxial side of leaf.*

Glandular trichomes are very frequent in Lamiaceae and have been described by authors as the site of the secretory of essential oils (Fahn, 1988, Antunes *et al.*, 1997). They differ in size, shape and in number of stalk and basal cells as well as in number of secretory cells and are surmounted by a sub-cuticular space formed by separation of the cuticle from the cells that fill the product of secretion.

Type I is described as capitate glandular trichomes and has a relatively smaller head of one or two cells and an obvious stalk. In general, capitate glandular trichomes have only limited storage capacity, and there is some evidence, which suggests that their secretion consists mainly of a complex mixture of carbohydrates, lipids, and proteins (Ascensão and Pais, 1998). They appear to have less taxonomic importance at the generic level (Harley *et al.* 2004; Kaya and Kofa, 2004). While type II is known as peltate trichomes (Fahn, 1988). It belongs to the sub sessile glandular trichomes so-called by Abu Assab and Cantino (1987) and shows great variation in structure. Eleven subtypes were described for the Lamiaceae by Cantino (1990). They are present in all Lamiaceae and vary in structure. Therefore, they seem to be taxonomically significant (Cantino, 1990, Harley *et al.*, 2004).

Non glandular trichomes developed from the epidermal cells are unicellular or multicellular uniseriate and unbranched and vary in size and

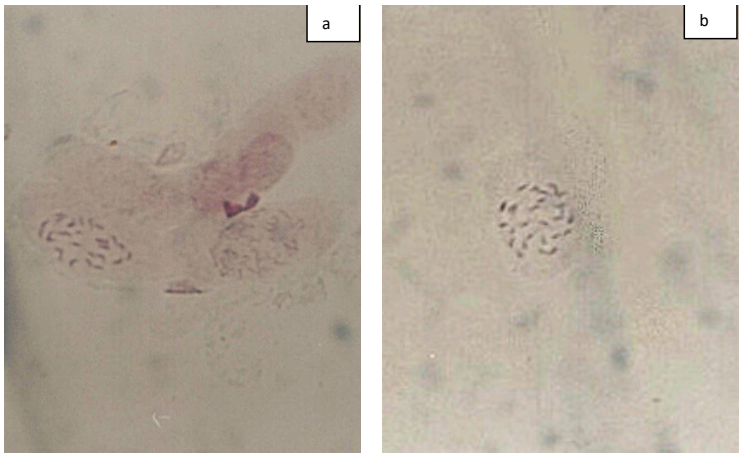


*Fig. 4. Photomicrographs of chromosome spreads prepared using meristematic root tips of Saccocalyx satureioides (X1000). a :population from the locality of Djelfa (Algeria), b: population from Boussaada (Algeria).*

structure (Fig. 3a, b). Non-glandular trichomes are present in all organs (young stems, leaves, and calyx) but are rare in the stems. In the leaves, they are more frequent along the midvein and lateral veins, on both adaxial and abaxial faces. They are more densely present on the vein of calyx. Four types of non-glandular, uniseriate and unbranched trichomes were observed: (i) straight unicellular trichomes, (ii) curved unicellular trichomes, (iii) bicellular trichomes, and (iv) multicellular trichomes with papillae are very long trichomes present at the base and margins of the leaves. They are also densely present in the apical region of calyx.

The non-glandular trichomes are widespread in all the family taxa, and are nearly always accompanied by multicellular hairs (Harley *et al.*, 2004). They must have a role in defence of the epidermis against drought by reflecting off solar radiation from the leaf surface (Liakoura *et al.*, 1999) and prevent heat damage by lowering leaf temperature (Klich, 2000).

### *Anatomy*

The leaves are amphistomatic with a bifacial mesophyll which is limited by uniseriate rectangular epidermis cells covered by a thick cuticle in both adaxial and abaxial side as it appears in the transverse section of the leaves (Fig. 2d, e). The midrib is surrounded by a layer of collenchyma (Fig. 2f.).

The fruits are oblong nutlets and present a myxocarpy when wetted. It should be noted that nearly all the fruits contain one or two viable nutlets while the others do not achieve maturity.

## Chromosome number

The chromosome number of *S. satureioides* 2n=30 chromosomes, is reported here for the first time. Both populations assessed had the same cytotype. The chromosomes are rather small and rod-like (Fig. 4a; b).

## Conclusion

The present study sought to provide useful information on the micromorphology and distribution of trichomes, and karyology of *S. satureioides*, an endemic species with a limited geographical range in the semi-arid region in Algeria and Morocco. This strong aromatic shrub contains a huge number of glandular trichomes, with the identification of two distinct types. The chromosome number 2n=30 is given here for the first time and is the same for the two investigated populations. The small population size and the apparent sterility make the survival of this taxon dependent on its ability for vegetative propagation. Vegetative reproduction is essentially made by stolons, which are several meters long. Notably, despite the presence of large, wind dispersed calyces, direct *in situ* observation has failed to reveal any seedlings. A major part of its habitat faces a significant destruction, because of the high anthropogenic activities, especially in the location of Boussaada. Even though this species is protected and carries a status of endangered species in Algeria, it is in a critical situation suffering serious regeneration problems and factors should have a more valuable status and be added to the IUCN Red List.

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**Abbreviations:** Batt. &Trab.: Battandier and Trabut; Coss.: Ernest Cosson (1819-1889); nm: nanometer; Poir. - Jean-Louis Marie Poiret (1755-1834); Rothm. - Werner Rothmaler (1908-1962); SEM: Scanning electron microscopy

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