Trichomes micromorphology and cytological investigation on *Acinos alpinus* subsp. *meridionalis* in Algeria

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Abstract

Anatomical and karyological features were investigated in four populations of *Acinos alpinus* subsp. *meridionalis* (Nyman) P. Ball., an Ibero-Maghreban species. Plant material was collected in the field during flowering stage, in contrasting bioclimatic conditions. Fresh material was used for analysis of trichomes morphology with light microscopy. Young meristems and flower buds were fixed for karyological analysis. Two types of capitate glandular, one type of peltate glandular, and four types of non-glandular trichomes were observed on different parts of the plants, including the stems, leaves and flowers. Trichomes distribution and density showed variability among organs, but no intraspecific variability was found. The chromosome number $2n = 2x = 18$ is reported for the first time for Algerian populations. Furthermore, aberrant meiotic behavior in the form of cytomixis was observed in one population of *A. alpinus* subsp. *meridionalis* – cytoplasmic strand was formed between more than two cells at different stages of meiosis. Other types of abnormalities such as lagging chromosomes, and formation of triads with microcytes were also discovered. Results are discussed in comparison with literature.

Keywords: *Acinos alpinus* subsp. *meridionalis*, Lamiaceae, chromosomes, trichomes, cytomixis, Algeria

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AI</td>
<td>Anaphase I</td>
</tr>
<tr>
<td>MI</td>
<td>Metaphase I</td>
</tr>
<tr>
<td>MII</td>
<td>Metaphase II</td>
</tr>
<tr>
<td>PI</td>
<td>Prophase I</td>
</tr>
<tr>
<td>PMCs</td>
<td>Pollen mother cells</td>
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The Mentha subtribe, within the Lamiaceae family, is the most important among the Endl. subtribes. Many studies have been conducted on the taxonomic systematics of this group using trichome micromorphology due to its important taxonomic value (Cantino 1990; Navarro & El Oualidi 2000; Harley et al. 2004; Moon et al. 2009, 2010; Salmaki et al. 2009). Chromosome number, variation, and behavior have also been investigated in several groups of Lamiaceae (Morales 1980, 1986, 1990; Mártonfi & Mártonfiová 1996; Kandemir 2003). Although the Lamiaceae of Algeria are widely investigated and well known in the aspects of phytochemistry and molecular bioactivity (Khaled-Khodja et al. 2014), there is a lack of micromorphological studies of the genus Acinos Mill.

The small genus Acinos was described by Miller (1754) and known because of its taxonomic controversy (Ferandes 1959; Ball 1972). Nowadays, Acinos taxa are usually assigned to the genus Clinopodium L. (Govaerts 1999; Harley et al. 2004), however Dobignard & Chatelain (2012) recognize it as an independent genus. There are two Acinos taxa represented in Algeria – A. alpinus Moench subsp. meridionalis (Nyman) P.W. Ball (syn. Clinopodium alpinum (L.) Kuntze. subsp. meridionale (Nyman) Govaerts) and A. rotundifolius Pers. (syn. Clinopodium graveolens (M. Bieb.) Kuntze subsp. rotundifolium (Pers.) Govaerts). Acinos alpinus subsp. meridionalis has several other synonyms including Satureja alpina Scheele subsp. meridionalis (Nyman) Greuter & Burdet, Calamintha alpina Lam. subsp. meridionalis Nyman and C. granatensis Boissier & Reuter (Dobignard & Chatelain 2012), as well as Satureja granatensis (Boiss. & Reut.) Sennen & Mauricio (Morales et al. 2010).

Acinos alpinus subsp. meridionalis is an Ibero-Maghreban subspecies distributed up to 1500 m of elevation in Central and Eastern Europe and Northern Africa (Algeria, Morocco), where it is often found in a wide range of habitats like mountain lawns, oak and cedar forests etc. (Quezel & Santa 1963). It is a perennial herb with flexuous stems. The leaves are elliptical to ovate with toothed margin (2–3 teeth). The inflorescence has 6 pedicellate zygomorphic flowers per verticillastre. Calyx of 13 nerves, 6–8 mm long, gibbous at the base and two-lipped. Upper lip has three small triangular teeth, lower lip with two long triangular teeth, all curved up and ciliated. The corolla is purpurin, of 10–15 mm long with a long exerted tube consisting of 5 merged petals. Flowers with four fertile didynamous stamens, two of which are exserte; anthers have divaricated theca. Gynoecium consists of two carpels; stigma is bifid. The fruit is schizocarp splitting into four ovoid mericarps (nutlets).

**Material and methods**

Plant material was collected during the spring of 2016 from four populations of A. alpinus subsp. meridionalis, with more than 10 individuals per population (Tab. 1). Plant identification was performed using the main Algerian and European floras (Battandier & Trabut 1902; Quezel & Santa 1963; Morales et al. 2010). The adopted nomenclature and taxonomy follows Dobignard & Chatelain (2012).

The trichomes distribution and morphology were examined using binocular loupe and light microscope. To prepare
material for light microscopy, the stems, leaves and calyces were sectioned using hand microtome, and then cross sections were treated with Mirande’s reagent (Deysson 1954; Mondolot et al. 2001). The chromosome numbers were calculated by analyzing mitotic metaphases plates in young root tips obtained from seedlings. Preparations were 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, ethanol:acetic acid) for 72 h and then stained using acetocarmine solution. The chromosome number analysis was carried out on 100 cells during the metaphasis and anaphasis. The best metaphasic plaques were captured using a Carl Zeiss light microscope.

For analysis of meiotic behavior in pollen mother cells, flower buds were collected before anthesis from plants growing under natural conditions and fixed in Carnoy’s fixative for 72 h, and conserved at 4°C for at least 48 h. Anthers were squashed and stained with 2% acetocarmine. About 20 slides were prepared from different anthers/flowers, more than 50 PMCs were observed, in each case. The best cells for chromosome analysis were photographed.

Results

Trichomes

All organs bear abundant glandular and non-glandular trichomes of different morphological types. The stem and leaf cross-sections give more details on their structure (Tab. 2). Three different types of glandular trichomes were observed on stems, leaves and calyces and were classified as follows. Type I – small capitates glandular trichomes with unicellular head, and a bicellular stalk (Fig. 1 A, B). Type II – capitulate glandular trichomes with oblong unicellular head and a stalk of two cells, one large and one thin. In the subcuticular space a bulk of the secretion was observed (Fig. 1 C, E). Type III – peltate trichomes comprising a basal cell, a short stalk cell and 12 secretory cells arranged in two concentric layers (the outer layer bearing eight cells and the inner layer consisting from four cells) (Fig. 1 A, D).

We noticed homogeneity in the repartition of the glandular trichomes on surface of leaves, but they were more frequent on the abaxial side with a maximum density near the nerves. Trichomes of type I and II are more abundant than type III on all organs of A. alpinus subsp. meridionalis.

Non-glandular trichomes are present in all organs but are more frequent in
the stems and leaves. On the leaves, they occur mostly along the midribs and lateral veins, on both adaxial and abaxial surfaces. Unicellular and multicellular non-glandular trichomes are more or less densely distributed along the veins, but absent on other parts of calyx. In general, three types of non-glandular, simple, uniseriate and unbranched trichomes were observed (Fig. 1 E–I): unicellular, bicellular, and multicellular very long trichomes abundant on the leaves edge and on the apical region of calyx. Unicellular trichomes occurred in two subtypes – straight and curved.

The four studied populations do not exhibited a difference on types and density of trichomes.

**Chromosome number and meiotic behavior**

In mitotic cells, we counted $2n=18$ for all populations of *Acinos alpinus* subsp. *meridionalis*. This number is reported here for the first time for Algerian populations. The chromosomes are rather small.

The chromosome number was confirmed from the presence of nine bivalents in the PMCs at MI (Fig. 2 A). These bivalents showed regular segregation during AI, in three populations (Djebel Boutaleb, Djebel Tafat, and Djurdjura) further meiotic course was also regular resulting in normal tetrad formation.

One population of *A. alpinus* subsp. *meridionalis* growing on the mountain Megriss at 1677 m a.s.l. showed abnormal meiotic behavior in form of cytomixis, which involves transfer of chromatin material among different PMCs along cytoplasmic strands at various stages of meiosis. This phenomenon was observed at all stages of meiosis I but was more frequent in the PI, MI (Fig. 2 B, C) and AI (Fig. 2 E).

In some cases, more than three PMCs at the same stages were involved in cytomixis (Fig. 2 B–D), through narrow and broad cytomictic channel (Fig. 2 F). We sometimes observed total chromatin migration from one meiocyte into another, thereby creating completely empty meiocytes (Fig. 2 G).

The phenomenon of cytomixis induced various meiotic irregularities in the Megriss population, such as lagging chromosomes (Fig. 2 H, I), formation of PMCs with

<table>
<thead>
<tr>
<th>Surface</th>
<th>Capitate glandular</th>
<th>Peltate glandular</th>
<th>Non-glandular</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Type I</td>
<td>Type II</td>
<td>Type III</td>
</tr>
<tr>
<td>Abaxial surface,</td>
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<td>3</td>
<td>1</td>
</tr>
<tr>
<td>leaf blade</td>
<td></td>
<td></td>
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<tr>
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<td>1</td>
<td>0</td>
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<tr>
<td>leaf midrib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adaxial surface,</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>leaf blade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
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<tr>
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<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Stem</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Trichomes distribution on the leaves, calyx and stems of *Acinos alpinus* subsp. *meridionalis*. Trichomes density: 0 – inexistent; 1 – very sparse; 2 – sparse; 3 – dense; 4 – very dense.
Figure 1. Trichomes of *Acinos alpinus* subsp. *meridionalis*: A – cross section of the stem with type III (arrow) and type I (arrowhead) glandular trichomes; B – details of type I glandular trichome observed on the abaxial leaf surface; C – details of type II glandular trichomes observed on the abaxial leaf surface; D – front view of type III glandular trichome on the calyx; E – type II glandular trichome with secretion (arrowhead) and non-glandular trichome (arrow) observed on the stem cross section; F – non-glandular trichome of bicellular type on the adaxial leaf surface; G – unicellular non-glandular trichome on the adaxial leaf surface (arrow); H – multicellular non-glandular trichome (arrow) and bicellular trichome (arrowhead) on the midnerve on abaxial leaf surface; I – cross section of the stem with unicellular non-glandular trichome (arrow). ac – apical cell; bc – basal cell; c – cuticle; nc – neck cell; s – secretion; stc – stalk cell. Magnifications: A, G–I – × 400; B–F – × 1000. Locations: A–C, E, F, I – Megri; D, G, H – Djurdjura.

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irregular chromosome number, microcytes formation, as well as production of triads and monads (Fig. 3 B–D). However, normal tetrad formation also appeared (Fig. 3 A).

**Discussion**

The glandular trichomes are very frequent in all Lamiaceae and are known as the sites of essential oil secretion (Fahn 1988; Antunes & Sevinate-Pinto 1991; Werker et al. 1993; Antunes et al. 1997; Bezić et al. 2001; Naidoo et al. 2013). They differ in size, shape and number of stalk and basal cells, as well as they vary in number of secretory cells. Glandular trichomes are surmounted by a sub-cuticular space formed by separation of the cuticle from the cells. This sub-cuticular space is filled with the product of secretion.

Trichomes of type I and type II are described as capitate glandular trichomes and have a relatively smaller head consisting of one or two cells and an obvious stalk. In general, capitate glandular trichomes have only limited storage capacity, and there is some evidence to suggest that their secretion consists mainly from a complex...
mixture of carbohydrates, lipids, and proteins (Werker et al. 1993; Ascensão & Pais 1998). They appear to have less taxonomic importance at the generic level (Cantino 1990; Harley et al. 2004), but demonstrated a great value for investigations on specific level (Öztürk Çali 2017). For the genus Acinos from Turkey, Kaya (2016) described four types of capitate trichomes distributed on the stems: type 1 – with unicellular head and sessile; type 2 – with unicellular head and unicellular stalk; type 3 – with unicellular head and two-cellular short or long stalk; type 4 – unicellular head and three-cellular stalk. In our study we have found only trichomes of type 2 and type 3, and we have not observed type 1 and type 4.

In this study, trichomes of type III belong to the so-called subsessile glandular (Abu-Assab & Cantino 1989) and also known as peltate (Marin et al. 2008). These trichomes are present in all Lamiaceae and they show a great variation in the structure. 11 subtypes of such trichomes were described for Lamiaceae by Cantino (1990). They are considered as being taxonomically significant (Cantino 1990; Harley et al. 2004). The peltate trichomes described by Kaya (2016) on the stem of Acinos from Turkey bears four secretory cells, while that found by Kaya & Koca (2004) on the leaves have 12 secretory cells and corresponds to the trichomes we observed in the current study.

The unicellular non-glandular trichomes are widespread in Lamiaceae and are nearly always accompanied by multicellular hairs (Harley et al. 2004). They can play a role in defense of the epidermis against drought.

The diploid cytotype reported for A. alpinus subsp. meridionalis \(2n = 2x = 18\) in Algeria seems to be very stable among the studied populations. This count was also reported for different populations in Morocco (Galland 1988) and in Mediterranean regions (Ubera 1979; Strid 1983; de Montmollin 1986; Ruíz de Clavijo 1993).

Despite on stability in chromosome number, one of the four investigated populations exhibited an abnormal meiotic behavior in form of cytomixis. No cytomixis phenomenon was reported...
for *A. alpinus* subsp. *meridionalis* before. However, it has been reported for some other members of Lamiaceae, such as *Ocimum basilicum* L. (Datta et al. 2005) and *Salvia miltiorrhiza* Bunge (Song & Li 2009), and interpreted as a natural cytological event under direct genetic control and physiological factors.

Although the cytomixis is studied in many plants, it seems to be a general lack of detailed knowledge about this phenomenon, which is considered versatile in flowering taxa (Bellucci et al. 2003; Mandal et al. 2013). Very little is known about cytomixis and different explanations are given in literature. In regards to the rate of cytomixis it could have an influence on the formation and viability of pollen grains. Thus, further more detailed investigations on the sexual system of *A. alpinus* subsp. *meridionalis* and meiotic behavior could provide more clarifications.

**Conclusions**

No differences on the structure and density of both glandular and non-glandular trichomes were noticed among the four studied populations. Therefore it seems that there is no relation between the bioclimatic conditions and the trichomes structure and distribution in *A. alpinus* subsp. *meridionalis* from Algeria. The cytomixis and other abnormalities such as lagging chromosomes and formation of triads with microcytes were observed.

**References**


Miller P. 1754. The gardeners dictionary: Containing the methods of cultivating and improving all sorts of trees, plants, and flowers, for the kitchen, fruit, and pleasure gardens. John and James Rivington, London.


