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RESEARCH ARTICLE

GEOGRAPHICAL VARIATION OF *AJUGA ORIENTALIS* L. TRICHOME, NUTLET AND POLLEN

Ayla KAYA *

Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey

ABSTRACT

In this study, the main features of the trichomes, nutlet, and pollen of *A. orientalis*, which growing in four different provinces of Turkey, were examined as detail and the results were illustrated. *A. orientalis* carries both peltate and capitate trichomes, as well as non-glandular ones. However, the subtypes of capitate glandular and non-glandular trichomes were recognized. The shape of nutlets is obovate, oblong-obovate and the nutlet surface sculpturing is alveolate. The shape of pollen grains is euprolate (P/E 1.59-1.7). The basic exine ornamentation in pollen grains is regulated, regulate-perforated.

Key words: *Ajuga orientalis*, Lamiaceae, Pollen, Nutlet, Trichome

1. INTRODUCTION

The Lamiaceae is a large family and has great importance due to its economic value. The genus *Ajuga* L., is one of the important genera of Lamiaceae (Ajugoideae tribe), known as bugle or bugleweed and it is represented by 301 species all over the world (in Europe, Asia, Africa, Australia and North America) and 23 taxa including 13 species in Turkey. Endemism ratio is 46% on species basis; 30% on taxon basis [1].

Ajuga genus contains many important bioactive compounds like anthocyanins, diterpenoids, sterols, ionones, iridoids, phenyl ethanol and flavonoid glycosides [2-4]. *Ajuga* species has been used in Turkey traditional medicine for centuries such as diuretic, antipyretic, tonic, diaphoretic, emmenagogue agent, astringent and wound-healing [5]. The members of *Ajuga* are also used in traditional medicine all over the world against some illnesses such as gout, rheumatism, malaria, asthma and gastrointestinal diseases and have antibacterial, antitumor [6], neuroprotective effects and antioxidant activities among others biological effects [7].

The plants of genus *Ajuga* are evergreen, clump-forming rhizomatous perennial or annual herbaceous flowering species. *A. orientalis* is also shortly rhizomatous perennial herb. Stems are erect or ascending, greyish lanate-villous, 6-30 cm. Corolla is 10-13 mm and limb resupinate [8]. The plant is locally known as “dağmayasılı” in the regions where they grow [1].

The taxonomic significations of trichome, nutlet and pollen structures are well known in Lamiaceae and related families. In this study, trichome, nutlet and pollen morphology of *A. orientalis* growing in four different regions (Erzurum, Samsun, Antalya and Balıkesir) of Turkey are reported for the first time.

2. PREPARATION OF MANUSCRIPT

A. orientalis was collected during the flowering period from Erzurum-Palandöken (1.7.2006, ESSE 14461), Samsun-Ladik (31.5.1992, ESSE 10209), Antalya-Beycik (18.4.1991, ESSE 9369) and

*Corresponding Author: aykaya@anadolu.edu.tr
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Balıkesir-Madra Dağı (27.7.2005, ESSE 14462) provinces of Turkey Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskişehir, Turkey (Map). For scanning electron microscopy (SEM), stem, leaves, calyces, corolla, nutlet and pollen were mounted on stubs with double-sided adhesive tape and coated with gold. Photographs were taken with a Tabletop Microscope (TM3030 Plus-Hitachi). Measurements and optical observations of nutlets were carried out under a stereomicroscope Wild M5.

3. RESULTS

The main features of the trichomes (on the stem, leaf, calyx and corolla), nutlet and pollen *A. orientalis*, which growing in four different regions of Turkey, are examined in detail and the results are illustrated in Figures 1-3 and summarized in Table 1-2.

3.1. Trichome Features

A. orientalis bears numerous glandular and non-glandular trichomes. These are non-glandular (A type) and glandular trichomes which are divided into two types: peltate (B type) and capitate (C type). Moreover, the subtypes of capitate glandular and non-glandular trichomes have been also identified. Types of trichomes and their distribution are given Figure 1 and Table 1.

A type: The non-glandular trichome is composed of a basal epidermal cell. It is uni-multicellular, uniseriate, unbranched. These trichomes might be subdivided into two subtypes. A type-1; uni-multicellular (of up to one to seven cells) acicular trichomes in a single order. Especially, they are thick-walled and densely covered by micro-papillae. These trichomes are curved or straight at the tip (Figures 1 $a_{3,}$ c_3). A type-2; large, thin-walled, multicellular (of up to twelve cells) trichomes with an acute apical cell, with ridges and marked internodes (Figures. $1a_{1-2,}$ b, $c_{1-2,}$ e_{1-2}).

B type: Peltate glandular trichome consists of a basal epidermal cell, one neck cell and a broad, round multicellular secretory consisting of four cells (rarely eight cells) (Figures 1 $a_{2-3,}$ d).

C type: These trichomes might be subdivided into three subtypes. C type-1; a cup-shaped unicellular head and 2 to 12-celled with ridges and marked internodes (Figure 1d). C type-2; a globose-shaped unicellular head and a unicellular short or long stalked. This type is observed only on nutlets (Balıkesir) (Figure 2d). C type-3; a cup-shaped unicellular head and unicellular stalk (Figure $1e_3$). This type is recognized only inside of the corolla surface.

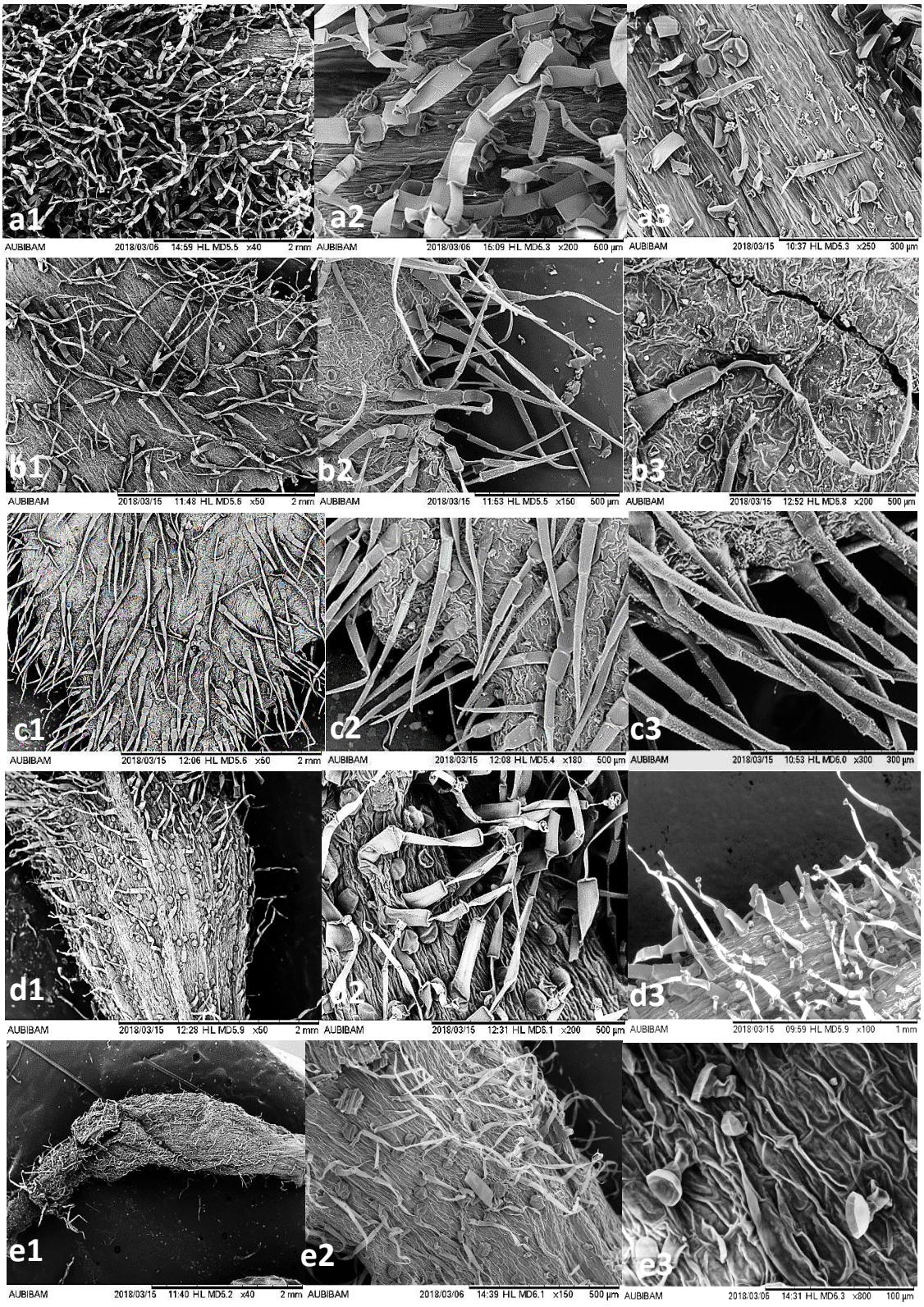

Figure 1. Scanning electron micrographs of trichomes in *A. orientalis*; a1-a2- a3-stem; b1-b2- b3-leaf adaxial surface; c1-c2- c3-leaf abaxial surface; d1-d2-calyx; e1-e2- e-3-corolla.

Table 1. Trichome distribution on different parts of *A. orientalis*

		1	2	3	4	5	6
Erzurum	A type-1	-	-	-	±	++	±
	A type-2	++	++	++	+	-	-
	B type	++	+	+	+	+	-
	C type-1	±	-	-	++	-	-
	C type-2	-	-	-	-	-	-
	C type-3	-	-	-	-	+	-
Samsun	A type-1	-	±	-	±	++	±
	A type-2	+	±	++	+	-	-
	B type	±	±	±	±	+	-
	C type-1	±	-	-	++	-	-
	C type-2	-	-	-	-	-	±
	C type-3	-	-	-	-	±	-
Antalya	A type-1	±	+	±	±	++	±
	A type-2	±	±	±	±	-	-
	B type	+	+	++	++	++	-
	C type-1	++	-	-	++	-	-
	C type-2	-	-	-	-	-	±
	C type-3	-	-	-	-	+	-
Balıkesir	A type-1	±	-	-	±	±	±
	A type-2	±	±	±	±	-	-
	B type	+	±	+	+	++	-
	C type-1	++	-	-	++	-	-
	C type-2	-	-	-	-	-	±
	C type-3	-	-	-	-	+	-

1-stem, 2-leaf adaxial surface, 3-leaf abaxial surface, 4-calyx, 5-corolla, 6-nutlet;
(-) absent, (±) rare, (+) dense, (++) very dense

3.2. Nutlet Features

Nutlets of *A. orientalis* are yellowish-brown or dark greenish-yellow in colour. Their size is 2-3.5 x 1-2.5 mm (Table 2), obovate, oblong-obovate, trigonous and apically obtuse, with widely bilobed areole (Figures 2a,b). They are tiny, capitate (stalked) glandular and eglandular haired which are found mainly on the apex of the nutlet and the median edge. The nutlet surface sculpturing is alveolate with deep or shallow spherical-oval, polygonal regular or irregular pits and high prominent or low ridges (Figures 2c-e).

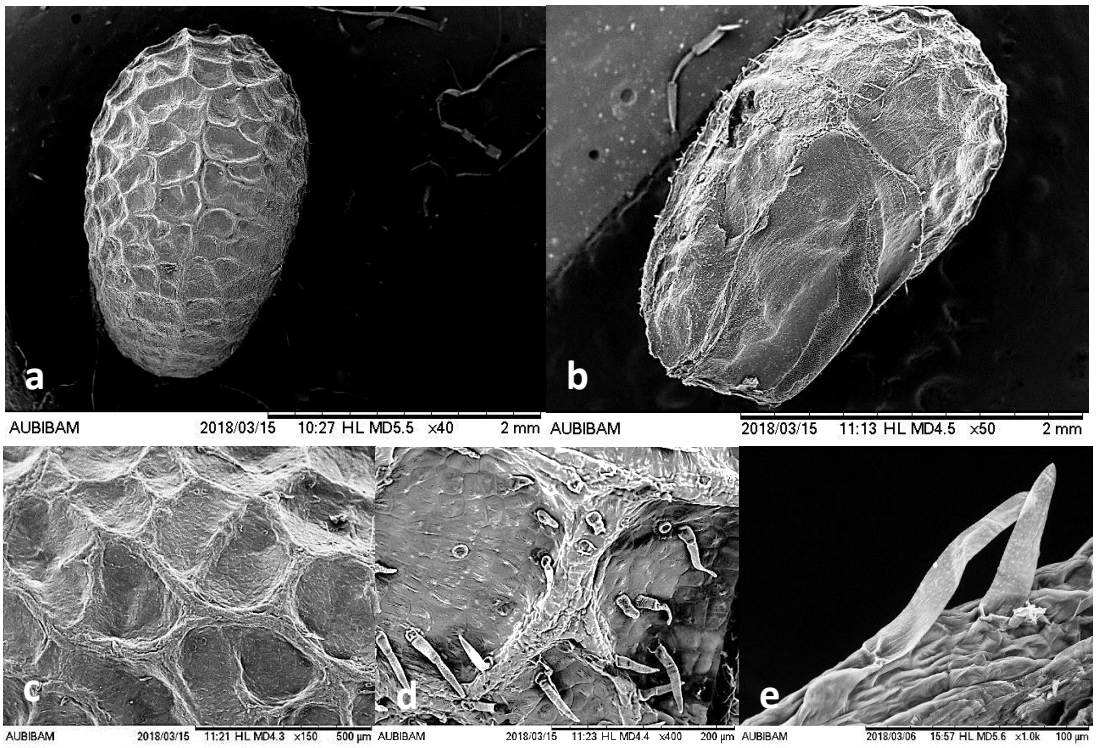

Figure 2. Scanning electron micrographs of nutlet in *A. orientalis*; a- nutlet dorsal, b-nutlet ventral, c-nutlet surface, d-nutlet trichome

Table 2. Nutlet and pollen features of *A. orientalis*

Locality	Nutlet (mm)	Pollen (µm)				
		P	E	P/E	Cl	Clg
Erzurum	2.2-3.4 x 1-1.8	26-32.4	14.5-19.6	1.6-1.7	22.1-30	1-2.6
Antalya	3.2-3.5 x 1.8-2.5	27.6-33.5	17.4-21	1.59	21-31.5	1.7-3
Samsun	2.5-2.8 x 1.5-1.8	29-35.4	17.6-21.3	1.6	22-29	1-2.4
Balıkesir	2-3 x 1.5-1.8	28.5-35.2	17.3-20.3	1.6-1.7	23.5-32.3	1-1.6

3.3. Pollen Features

Pollen grains are shed as monads, isopolar and tricolpate. The dimension ranges are: 26.0-35.4 µm in polar length (P), 14.5-21.3 µm in equatorial width (E), 21.0-32.3 µm in colpus length (Cl) and 1-3 µm in colpus width (Clg), (Table 2, Figures 3). The shape of pollen grains is euprolate (P/E 1.59-1.7). The basic exine ornamentation is regulated, regulate-perforated. The outline in the polar view is elliptic.

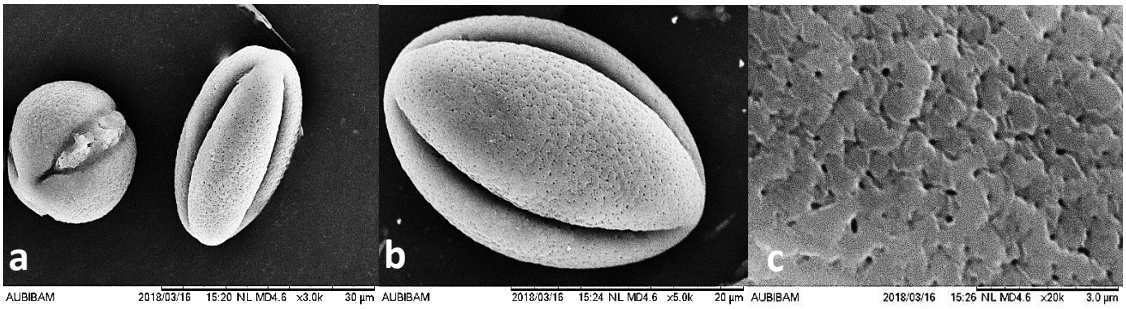

Figure 3. Scanning electron micrographs of pollens in *A. orientalis*; a-b-pollen c-pollen surface

4. DISCUSSION

The present study sought to provide useful information on the trichome, nutlet morphology, and palynology of *A. orientalis* growing different four provincens of Turkey. Trichomes are widely distributed over the aerial reproductive and vegetative parts of plants of Lamiaceae and are ordinarily distinguished as glandular and non-glandular trichomes. The systematic value of trichome types in the Lamiaceae family was demonstrated by most the systematics [9-12].

The main features and distributions of the trichomes (on the stem, leaf, calyx and corolla), summarized in Table 1. The distributions of the trichomes are usually similar according to the table. However, there are some differences. For example, A type-1trichomes in the Antalya region (stem and leaves) are denser than the other localities. A type-2 and B type trichomes are present, except corolla and nutlet, in all localities. C type-2 is usually absent except Balıkesir, Antalya and Samsun nutlets. C type-3 is the only present in corolla surfaces in all localities.

Like other members of the Lamiaceae, *A. orientalis* carries both peltate and capitate trichomes, as well as non-glandular ones. It was also determined that reproductive organs have numerous glandular trichomes whereas vegetative organs possess abundantly non-glandular trichomes. Peltate trichomes comprise a broad head of several secretory cells (four, rarely eight) a wide short stalk and a basal epidermal cell. Similar peltate trichomes were recognized by *Ajuga orientalis*, growing Amasya provience of Turkey [13], *Teucrium* L. species [14] and *Salvia divinorum* Epling & Játiva [15]. Like the peltate trichomes, the capitate ones are very common and constitute a significant taxonomic character in many Lamiaceae species [12], [16]. Multicellular trichomes with ridges and marked internodes are recorded by some of the genus of Lamiaceae. The present study showed that *A. orientalis* has the capitate trichomes (C type-1) in the agreement with previous studies [17-18]. The non-glandular trichomes of *A. orientalis* displayed some variation in morphology. Therefore they were divided into two subtypes. A type-1 is very common in the Lamiaceae species studied previously [14]. A type-2 was described in *Teucrium* species [19] and *Ajuga orientalis* [13].

Nutlet morphology has important taxonomical significance at the sectional level [20-21]. In examined samples, the biggest (3.2-3.5 x 1.8-2.5 mm) and dark greenish-yellow nutlets are belonged to Antalya locality. The capitate (stalked) glandular and non-glandular trichomes are found on the nutlet surfaces, however tiny capitate type trichomes (C type-2) are observed only in Balıkesir samples. The surface sculpturing of nutlets is alveolate with deep or shallow spherical-oval, polygonal pits and high prominent or low ridges. However, there are some differences in depth among localities. The alveoli are deep in Antalya and Samsun nutlet samples however the alveoli are shallow in the other localities. The alveolate type sculpturing and glandular and non-glandular trichomes were also recognized on nutlet of some

Teucrium species by Ecevit Genç et al. [17-18] and Eshratifar et al. [19]. Their study is confirmed by this study.

Pollen morphology has been proved to be useful in the systematics of the Lamiaceae [22]. Erdman [23] classified the family Lamiaceae into two subfamilies (Lamioideae and Nepetoideae) based on palynological characteristics. Later, Cantino et al. [24] revised the classifications of all genera in the Lamiaceae and placed it within the subfamily Lamioideae since the genus *Ajuga* has tricolpate pollen grains. According to our finding, The longest (29-35.4 μm) and largest (17.6-21.3 μm) dimensions of pollen grains were recorded by Samsun examples. The basic exine ornamentation is regulated, regulate-perforated. Kose et al.[25] reported that the pollen morphology of eight Turkish species of *Ajuga*, including *A. orientalis* and the exine sculpture in the pollen of *A. orientalis*, which collected from Bursa locality, was recorded as granulate. Their result was different from ours.

Combining all data, the macro-micromorphology of *Ajuga orientalis* has high systematic value and strongly gives significant information within this species based on morphological data.

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CONFLICT OF INTEREST

The author stated that there are no conflicts of interest regarding the publication of this article.

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