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ARAŞTIRMA MAKALESİ /**RESEARCH ARTICLE**

THE ANATOMICAL AND PALYNOLOGICAL PROPERTIES OF *AJUGA REPTANS* L. (LAMIACEAE) AT RISK

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ABSTRACT

In this study, the detailed anatomical properties and morphological structure of the pollen of the Turkish taxon *Ajuga reptans* L. was observed by light microscope for the first time. The plant has a compact root anatomy with a full xylem component in pith. Lamiaceae demonstrated a typical four corners in the stem, and under a single sequenced epidermis is to be found 3-5 sequenced layers of the collenchyma cell, while at the innermost we can see a compact parenchyma. In the leaf mesophyll, the palisade and spongy parenchyma cells are of a similar shape. The leaf is amphistomatic. The plant has an amaryllis, a mesomorphic, and an anisocytic type stomata.

The results of the light microscope investigation revealed the suboblata-subprolata and tricolpatae in the pollen of *Ajuga reptans* taxon. Exine ornamentation was also determined tectatae-granulatae.

Keywords: *Ajuga reptans* L., Anatomy, Pollen morphology, Light microscope, Turkey.

RİSK ALTINDAKİ *AJUGA REPTANS* L. (LAMIACEAE)'NİN ANATOMİK VE PALİNOLOJİK ÖZELLİKLERİ

ÖZ

Bu çalışmada, Türk taksonu *Ajuga reptans* L.'ın ayrıntılı anatomik özellikleri ve pollen morfolojik yapısı ışık mikroskobuyla ilk kez incelenmiştir. Bitki öz bölgesinde tamamen ksilem elemanıyla dolu sıkı bir kök anatomisine sahiptir. Lamiaceae gövdede tipik dört köşeli bir yapı göstermektedir ve tek tabakalı epidermisin altında 3-5 sıralı kollenkima tabakası bulunmaktadır, en içte sıkı bir parankima görülmektedir. Yaprak mezofilinde, palizat ve sünger parankima hücreleri benzer şekillidir. Yaprak amfistomatiktir. Bitki, amaryllis, mezomorfik ve anizositik tip stomaya sahiptir.

Ajuga reptans taksonunun poleninde ışık mikroskobu incelemelerinin sonuçları suboblat-subprolat ve trikolpat yapıyı ortaya koymuştur. Ekzin yapısı da tektat-granülat olarak belirlenmiştir.

Anahtar Kelimeler: *Ajuga reptans* L., Anatomi, Polen morfolojisi, Işık mikroskobu, Türkiye.

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1. INTRODUCTION

The Lamiaceae is a large and showing natural distribution family. Most of species belonging to this family are shrubby and herbaceus, trees extremely rare (Heywood, 1978). The species of this family are cosmopolitans showing worldwide distribution. This family have great importance due to its economical value and being rich in species number.

The genus *Ajuga* L. shows a large distribution in the most of Europe, including Britain, to S.W. Asia and N. Africa. It is represented in Turkey by 13 species and 22 taxa, six species and one subspecies being endemic. (Davis et al., 1982-1988)**.** According to risk assessment results, it is at high risk. (Pacific Islands Ecosistem at Risk, 2005 PIER) The plant is aromatic, astringent, bitter and homeopathic. *Ajuga* has a long history of use as a wound herb and, although little used today, it has known in Anatolia as Mayasil otu. It is still considered very useful in arresting haemorrhages and is also used in the treatment of coughs and spitting of blood in incipient consumption (Grieve, 1984; Chevallier, 1996; Stuart 1979). The plant contains digitalis-like substances (these are commonly found in *Digitalis* species and are used in treating heart complaints) and is thought to possess heart tonic properties (Stuart, 1979). It has also been considered good for the treatment of excessive alcohol intake (Grieve, 1984). The whole plant is aromatic, astringent and bitter (Grieve, 1984; Werker, 1985; Chiej, 1984; Launert, 1981). The plant is usually applied externally (Chiej, 1984). It is harvested as it comes into flower in late spring and dried for later use (Grieve, 1984; Chiej, 1984). It is also commonly used fresh in ointments and medicated oils (Bown, 1995). A homeopathic remedy is made from the whole plant. It is widely used in various preparations against throat irritations and especially in the treatment of mouth ulcers (Chiej, 1984).

Very few anatomical studies is exist on *Ajuga reptans* L. in Turkey (Akçin et al., 2006). But no detailed palynological studies had been carried out earlier. We began this research after perceiving the need for biological characteristics of plants to be supported by anatomical and palynological research in order to shed more light on evolutionary and systematic relationships.

Therefore, our purpose was to determine the anatomical and palynological characteristics of *Ajuga reptans* taxon collecting from Geyve (Adapazari) and its environs.

2. MATERIALS AND METHODS

The species *Ajuga reptans* was collected from Geyve (Adapazari) and its environs for this study. A3 Sakarya: Melekşeoruç Village, Melekşeoruç-Kırca Plataeu road, old cemetery, road sides, 40°34'30.9" N-030°13'42.7" E, 475m, 09.08.2003, OUFE: 12609. In order to ensure a systematic study of the material obtained, herbarium samples were prepared and these samples were protected as herbarium samples at the Eskişehir Osmangazi University Herbarium (OUFE 12609). For the anatomical study; the root, stem and leaves were fixed in 70% alcohol and then kept in the same solution until the acquisition of cross-sections from the fresh materials. All the cross sections were stained with Sartur (60 ml lactic acid, 45 ml Sudan III (Merck), 2 gr aniline, 0.2 gr I, 1 gr KI, 10 ml alcohol (95 %), 80 ml distilled water) and were thus made permanent preparates by means of glycerine gelatine. From the Herbarium sample, the species' detailed morphological characteristics were established and pollen preparation arrangements were used for the designated species. For the anatomical investigations, samples were taken from the alcohol by hand and scalpel. The Prior marker was investigated under light microscope and microscope photographs were taken with a Spot In-SIGHT Colour Digital camera and an Olympus type microscope. A variety of foundation anatomical books and conducted studies were used as sources for identification of the plant (Metcalfe and Chalk, 1972; Esau, 1967; Fahn, 1967; Özörgücü et al., 1991; Özörgücü, 1993; Yentür, 1995; Akçin et al., 2006).

The pollen samples were obtained from dried plants at the Osmangazi University Science and Art Faculty Department of Biology Herbarium (OUFE 12609).

The pollen morphology of the taxon in the study was investigated through light microscope. Faegri and Iversen's terminology for the names of the exine layers were used. (Faegri and Iversen, 1975) In the light microscope investigations, the pollen acquired from the samples were obtained set by the method of preparation described by Wodehouse (1935) and Erdtman (1969). Identifications and counts a x 10 ocullar, and x 10 and x 40 plan objectives were used; for the purpose of identification a x 100 plan oil-immersion objective was used. Pollen identifications and counts were obtained by Prior binocular microscope. The spacing between each ocular micrometer was 0.98 μm. When prepared according Wodehouse's (1935) and Erdtman's (1969) methods, the exine and intine thickness

pertaining to taxon is to be measured a minimum of 20 and a maximum of 50 times. From these obtained measurements, a natural mathematical mean is calculated. Microphotographs were taken at the Osmangazi University Science and Art Faculty, Department of Biology by Spot In-SIGHT Color Digital camera and an Olympus type microscope. The photograph dimensions were 10 µm, 25 µm and 100 µm. A variety of foundation palynological books and studies conducted were drawn from for identification of the pollen (Wodehouse, 1935; Erdtman et al., 1954; Pokrovskaia, 1958; Kuprianova, 1965; Erdtman, 1966; Erdtman, 1969; Kapp, 1968; Aytuğ et al., 1971; Charpin et al., 1974; Faegri and Iversen, 1975; Moore et al., 1991; Pehlivan, 1995).

3. RESULTS

3.1 Anatomical Features

For the anatomical investigations, sections were taken from the plants root, stem, and leaves.

3.1.1 Root

The plant has an external periderm (Figure 1), followed by 2-3 layers of flat and wide shaped cells, after which the phelloderm can be found. The cortex covers a very small area, and underneath is the endodermis, which is composed of a ring-shaped dense cell wall. Beneath this is situated the pericycle, which is composed of a much thinner cell wall. After the pericycle 2-3 layers constitute the dense cell wall, from which we can locate the ring-shaped sclerenchyma. Following this, the bundle sheath is situated from the xylem to the root and is filled with root xylem components. On the outside of the xylem is an even thinner cell wall which is made up of small, internal denser wall and larger xylem components. Scattered between the sclerenchyma rings and the xylem, we can indistinctly observe the phloem constituted by 2-3 layer cells.

3.1.2 Stem

The plant is of the typical four corner type, with a thick cuticle with small cell epidermis beginning on the outside. A large amount of blanketing and secreting down erupt from the epidermis. Following the epidermis, the 5-6 cells that make up the collenchyma can be seen in line leading up to the cortex, and immediately under the 2-3 layer of the rigid cell wall is the chloranchyma. After the chloranchyma, bulk cells and the ring-shaped starch sheaves are observable this stem plate we encounter the endodermis. Schlerancymatic cell clusters are situated in patches beneath the starch sheath, and between these schlerancyma cells and the xylem, covering a small area, are 1-2 layers of phloem. Following this, there is a wide space covered by trache, tracheid, and xylem parenchyma and between these, in the interior, is the xylem scleranchyma. In the pith, there is a covering with wide space parencymatic cells (Figure 2).

3.1.3 Leaf

The most outward dense cuticle; a range of epidermis can be found on the underside (Figure 3). Epidermis cells can be observed at different sizes. The contour of the palisade and spongy parenchyma cells cannot be easily distinguished in the mesophyll tissue. The underside epidermis cells generally more fully demonstrate the leaf's bifacial characteristic. Both the upper surface of leaves and lower surface of leaves are covered by copious amounts of blanketing and secreting down (Figures 3-5). The leaf is amphistomatic.The plant has an amaryllis, a mesomorphic and and a diasitic and rarely anisositic type stomata.. The leaf's central vascular transporter is composed of the phloem bundles and the xylem.A typical parenchymatic bundle is surrounded by the bundle sheath cells.

3.2 Pollen Morphology Studies

Ajuga reptans pollens are suboblata-subprolata and tricolpatae, P/E= 0,99 (N), 0.89 (A). Ornamentation is tectatae-granulatae. Exine 1.23 µ (N), 1.46 µ (A), Tectum granule (Figure 6a-d , Table 1).

4. DISCUSSION

During anatomic observations of transverse cross-sections of the upper root, it can be seen from the composition of the cortex structure and periderm that secondary growth is a result of the plant's long-existence. This point has been stressed in literature related to the subject (Metcalfe and Chalk, 1972; Esau, 1967; Fahn, 1967; Özörgücü et al., 1991; Özörgücü, 1993; Yentür, 1995; Akçin et al., 2006). The plant profits in terms of protection, durability, and resistance against external effects from the ring-shaped vascular bundles of the surrounding scleranchyma (Özörgücü et al., 1991).

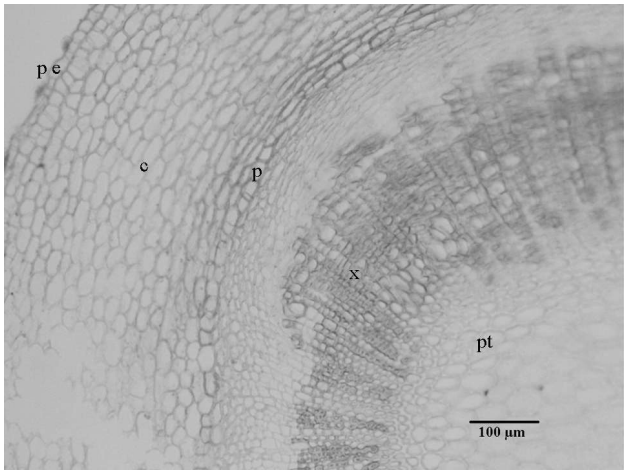

Figure 1. Cross-section of the root of *Ajuga reptans* L. Key: **pe:** peridermis, **c:** cortex, **x:** xylem, **p:** phloem, **pt:** pith.

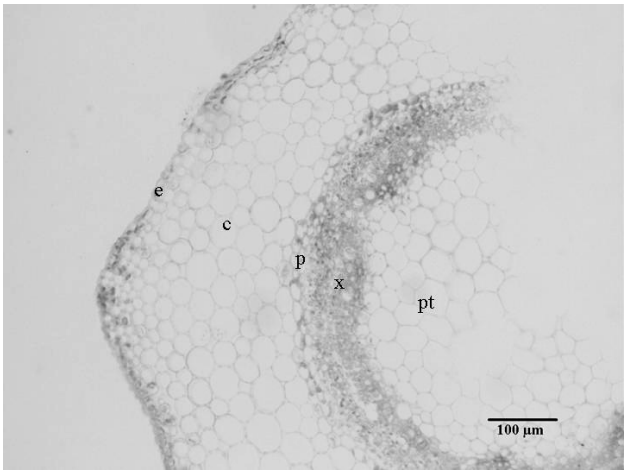

Figure 2. Cross-section of the stem of *Ajuga reptans* L. Key: **e:** epidermis, **c:** cortex, **x:** xylem, **p:** phloem, **pt:** pith.

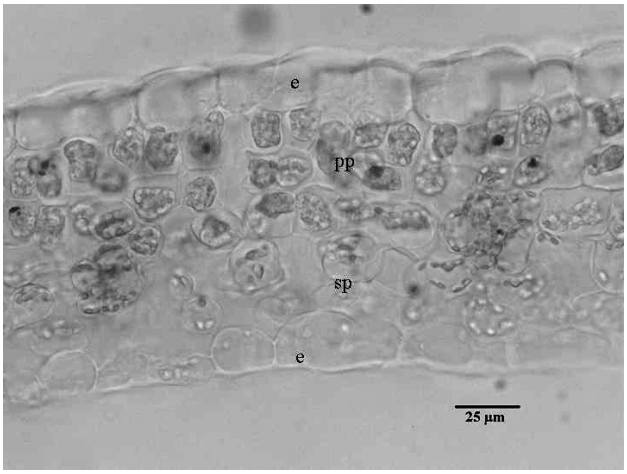

Figure 3. Cross-section of leaf of *Ajuga reptans* L. Key: **e:** epidermis, **pp:** palisade parenchyma, **sp:** spongy parenchyma.

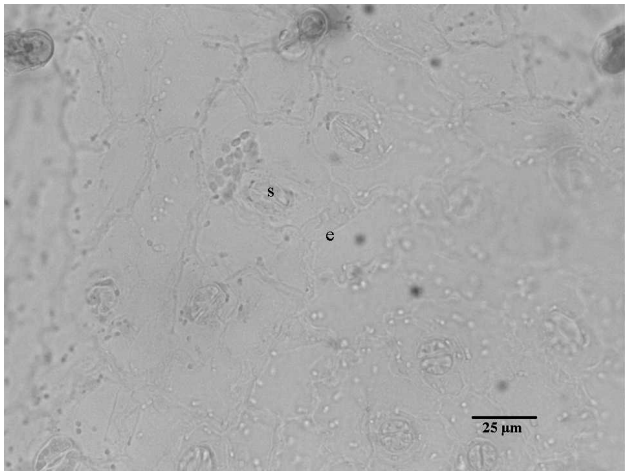

Figure 4. Upper surface section of leaf of *Ajuga reptans* L. Key: **e:** epidermis, **s:** stoma.

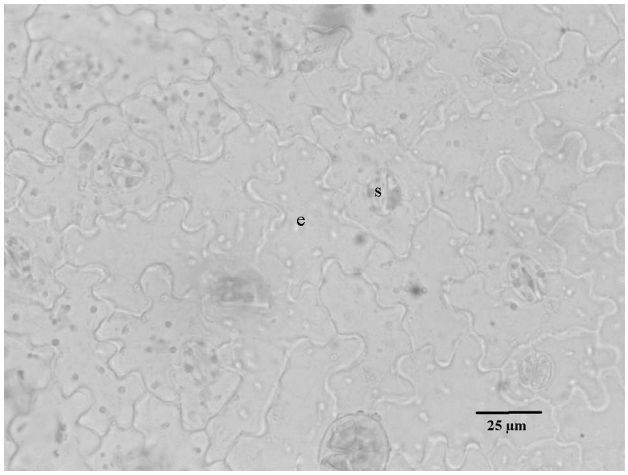

Figure 5. Lower surface section of leaf of *Ajuga reptans* L. Key: **e:** epidermis, **s:** stoma.

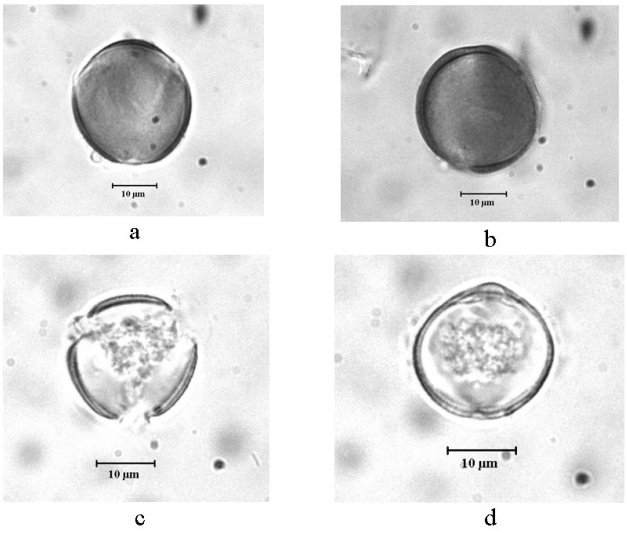

Figure 6. a-d. Pollen microphotography of *Ajuga reptans* L., a) Polar view of a non acetolysed pollen in Light microscope, b) Equatorial view of a non acetolysed pollen in Light microscope, c) Polar view of an acetolysed pollen in Light microscope, d) Equatorial view of an acetolysed pollen in Light microscope.

Table 1. Morphometrical Parameters of *Ajuga reptans* L.

TAXON	P			E			P/E	L			clg			clt			t			Ex			I		
	M	S	Var.	M	S	Var.		M	S	Var.	M	S	Var.	M	S	Var	M	S	Var.	M	S	Var	M	S	Var.
Ajuga reptans L. **(N)**	25,57	1,91	30-21	25,71	2,11	31-19	0,99	26,85	1,46	30-21,5	21,8	3,10	28-18	8,32	4,42	16-2,5	10,41	5,04	17-3	1,23	0,29	2-1	0,63	0,26	1-0,25
Ajuga reptans L. **(A)**	19,02	2,15	24-15	21,32	2,12	26-18	0,89	23,15	2,64	30-20	13,62	1,53	17-10	6,1	1,94	9-2	6,32	2,83	12-2	1,46	0,42	2-1	-	-	-

ABBREVIATIONS: N: Non acetolysed pollen (LM), A: Acetolysed pollen (LM), P: Polar axis, E: Equatorial axis, L:Equatorial countour diameter, t : Apocolpium, clg: Length of the colpus, clt: Width of the colpus, M: Mean, S: Standard deviations, Var: Variation.

Metcalfe and Chalk (1972) gave information about general anatomical characteristics of Lamiaceae family, pointing out that the family to which the species belongs has a rectangular stem that helps us to recognize that they belong to a different species. They also remarked that there were collenchyma in each corner. The same researchers stated that the rays consist of 2-12 or more lines of cells in this family. In this study it was discovered that these rays consist of 1-7 line cells. Because the number of rays is different in every species, this can be used as a species-distinguishing feature.The same qualities were observed in the root anatomy of *Ajuga reptans* in the other study (Akçin et al., 2006). The character of the element xylem along with their fullness, make up the lignified cell wall. This event is the result of the development of secondary growth. The typical stem is four-cornered. Under the epidermis are 5-6 cortex cells layers, and below these in the 2-3 layers of the dense cell wall are situated in the chloranchyma. It is important for the plant that there is the existence of chloranchyma as a typical response to the photosynthetic property of the stem. In this way, the effect of photosynthesis on the leaf and also the stem is increased (Fahn, 1967). The typical four-cornered stem-type with dense collenchymatic cell walls located in the corners is a distinguishing characteristic of the *Lamiaceae* family (Metcalfe and Chalk, 1972). After the chloranchyma, a layer of bulk cells and ring shaped starch sheath are situated. The root of the starch sheath constructs a homologue endodermis and encompasses the surrounding vascular system like a belt (Fahn, 1967; Özörgücü, 1993). Beneath the starch sheath, schlerancymatic cell clusters can be seen in patches. In the stems of the species studied, mechanisms for resistance and support against external effects are available. Hardly any selective phloem can be found between the schlerancymatic cell clusters and the xylem. Forming the xylem are trache, tracheids, and the parenchyma and between these, there are scattered scleranchyma clusters. Here in the scleranchyma, support against external effects to the stem is supplied. In the pith, covering a wide area, is situated the parencymatic root. The existence of the stem's parencymatic is observable in the literature (Akçin et al., 2006). Few stomas are seen in the stem. In the leaf anatomy, there is an external dense cuticle, and on the underside of this is a layer of epidermis. The dense cuticle of the epidermis is positioned alongside the plant's water loss management and this condition is a characteristic peculiar to xerophytic plants (Yentür, 1995). Epidermis cells can be observed at different sizes with larger epidermis cells occurring on the underside. The contour of the palisade and spongy parenchyma cells cannot easily be distinguished in the mesophyll tissue. The leaves are bifacial. The occurrence of the leaf being bifacial has been reported in the literature (Akçin et al., 2006). Both the upper surface and lower surface of leaves are covered by copious amounts of blanketing and secreting down. There are amaryllis and a diasitic and rarely anisositic type stomas on both surfaces of the leaf. Thus, the leaf is amphystomatic (Akçin et al., 2006). In the transportation bunch of the central vein, 1-2 cell levels occur on the outside of the phloem above the xylem, and the interior of the xylem covers a wider space. 1-2 layers of schlerancymatic cells can be seen in the xylem, as well as under the parencymatic cells. The bundle encloses a control from the parencymatic cells.

The results of the light microscope investigation revealed the suboblata-subprolata and tricolpatae in *Ajuga reptans* taxon. It was also determined upon scrutiny of the exine *Ajuga reptans* is tectatae-granulatae. The essential criteria for the determination of the philogenetic relationship of the characteristics of the aperture and exine function of this species has been reported in the Literature (Kuprinova, 1967; Cronquist, 1968; Walker, 1974a-b; Takhtajan, 1980). In our analysis of this taxon, we observed that determined genetic distinctions encompassed differences in the measurements determined, raising objections to the possession of a morphological characteristic passing to the pollen structure of this species. (Cronquist, 1968).

We believe that we may have distinguished a criterion in the pollen morphology of *Ajuga reptans* taxon' systematic system which characteristizes ancillary sequence. This study at the same time has also shed light on the exposed systematic-philogenetic relationship of investigated taxon. The determination of the taxon' pollen morphological structure has led us to better consider the usefulness of pollen studies in distinguishing the characteristics possessed by taxon.

We believe that the important results concluded from the study of anatomy and pollen morphology will lead to a better understanding of the species and provide a contribution to any future studies.

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