SCANNING ELECTRON MICROSCOPY INVESTIGATIONS REGARDING *Adonis vernalis* L. FLOWER MORPHOLOGY

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Abstract: The floral morphology of *Adonis vernalis* L. was observed with a scanning electron microscope (SEM). The investigations are important to clarify some taxonomical problems and also could provide useful diagnostic elements for the identification of this medicinal plant in powdered materials. All floral organs are initiated spirally and centripetally and develop centripetally. The petals (8-12) are shorter than the sepals (5-6) in early developmental stages. The petals are disposed on spiral (with 3-4 whorls). The stamens (numerous) are unbranched and reach maturity centripetally; they are free of the perianth. The anther walls consisting of a single layer epidermis in the anther wall surrounding the sporagenous tissue, one row of endothecium, two to four rows of middle layer and one row of tapetum layer. In the anther walls, the tapetal cells, by glandular type, persist later in ontogenesis. Pollen grains are tricolpate with echinate surface. The gynoecium is multiple, apocarpous with distinct carpels. The carpels are ascidiate from the beginning. At the base of each carpel, numerousness short, unicellular, trichomes are present. The stigma differentiates as two crests along the ventral slit of the ovary. Each carpel contains a single ovule inside the ovary cavity. The mature ovule is anatropous, with two integuments. It is almost parallel to the funicle.

Keywords: anther,flower, pollen, ovary, ovule, tapetum.

INTRODUCTION

Species have wider or narrower ranges of distribution depending on habitat requirements. *Adonis vernalis* L. is distributed in grassland communities in the south-east Europe steppe zone and is characteristic for different xerotermic swards of continental-submediterranean type, belonging to the order *Festucetalia valesiaceae* [8].

Adonis vernalis is a pontic element [1]. Its main distribution area ranges from the eastern part of middle Europe through east and southeast Europe, western Siberia to eastern Siberia reaching the Jenissei region [1]. In middle and southwest Europe the area is disjunct with some isolated growth places in mainly azonal habitats scattered from southeast Sweden to southeast Spain.

For medicinal purposes, mainly the aerial plant parts (stems, leaves, flowers, and fruits) of *A. vernalis* are used. The plant material is either used dried as in phytotherapy or fresh as in homeopathy. The plant contains cardiac glycosides similar to those found in the *Digitalis purpurea* [7]. It also has a sedative action; the herb is cardiotonic, diuretic, sedative and vasoconstrictor. Micromorphological investigations offer useful diagnostic elements for the identification of this medicinal plant in powdered materials.

Because of the increasing focus of molecular developmental studies on flowers of *Ranunculales* better comparative knowledge and understanding of the floral development of *Ranunculaceae* is necessary [15]. Although there is extensive literature on aspects of floral structure and embryology in *Ranunculaceae*, the distribution of developmental studies in the family is unequal [15]. They are many studies on some genera (*Aquilegia, Ranunculus* and *Anemone*), regarding various species and others genera are less extensively investigated or even unstudied.

The aim of this paper is to extend floral morphological studies to uninvestigated and rare species – *Adonis vernalis* and some particular aspects of floral structure.

MATERIALS AND METHODS

Scanning electron microscopy (SEM) investigations – Floral buds in different developmental stages and mature flowers were collected from Valea lui David reservation during April and May 2009, fixed in FEA (formol: ethylic alcohol 70%: acetic acid –5:90:5) for 48 hours, stored in 70% ethanol [4]. After dehydration in a graded ethanol series, the material was dissected and critical point dried with CO_2 (using a EMS 850 Critical Point Dryer), sputter-coated with a thin layer of gold (30 nm) (using a EMS 550X Sputter Coater) and, finally, examined in a scanning electron microscopy (Tescan Vega II SBH) at an acceleration voltage of 27.88 kV.

RESULTS

Flowers are solitary, terminal and have 2–3 cm in diameter. The five to six sepals are green and obovate lanceolate. The 8–12 petals are yellow, narrow obovate and about double the length of the sepals. Nectarines are absent. The stamens and carpels are numerousness; all floral organs are free. The carpels develop into nutlets.

The floral organs are initiated in spiral sequence and the flowers have spiral phyllotaxis (Fig. 1A). The sepal primordia are broad, crescent-shaped, and truncate, but those of petals, stamens, and carpels are rather hemispherical.

The sepals are the first initiated in floral morphogenesis. In early stages, they are longer than petals. In cross sections they appear thin, with 2-3 mesophyll layers (Fig. 1B). On the lower epidermis, numerousness long, unicellular tector hairs are presents (especially at the sepal's basis).

The petals develop faster and are always larger than the stamens. However, they remain shorter than the sepals up to shortly before anthesis. They are thicker than the sepals in cross section, with 4-5 layers of isodiametric cells in the mesophyll (Fig. 1C). Epidermis consists of single layered polygonal cells on both surfaces and lack papilla. Vascular bundles are reduced.

The stamens are initiated in normal centripetal sequence (Fig. 1A & 1D). The morphogenesis of the anther and different stages in pollen grains development was underlined. The anthers are H-shaped, with 4 locules (tetrasporangiate) (Fig. 3D & 3E). The anther locules, in which pollen develops, appear as convex protrusions on the adaxial surface (facing the carpels). During this stage, the sporogenous cells, which give rise to pollen, are visible within locules of sectioned anthers (Fig. 3A).

The anther walls consist on epidermis, endothecium, middle layer and tapetum (Fig. 3B). The tapetal cells remains viable later during ontogenesis; they are visible even in the anther with mature pollen grains (which shows normally developed exine) (Fig. 3C). These cells degenerate before the anther dehiscence (Fig. 3F & 3I). Pollen grains are tricolpate with echinate surface (Fig. 3F & 3H).

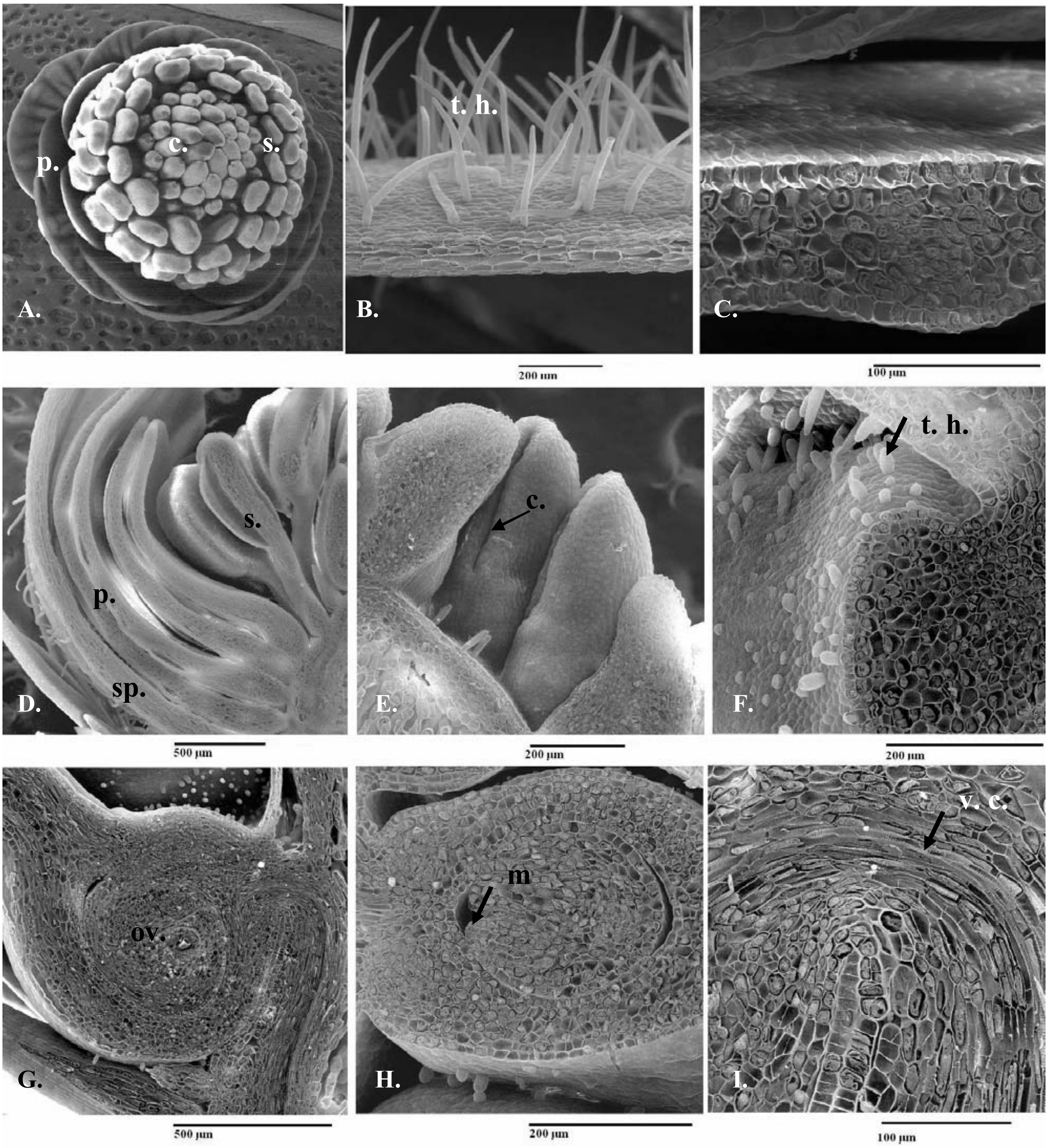

Figure 1. Field emission scanning electron micrographs of the floral organs in *Adonis vernalis*: A – general aspect of the floral bud; B - section through the sepal – unicellular, tector hairs are visible on the lower epidermis; C – section through the petal – a undifferentiated vascular bundle could be observed in the central part; D – longitudinal section through young flower – from the external to the internal part sepals (2) petals (3) and stamens (2) could be observed; E – early stages in the carpels development - they are open at their superior part; F – the external epidermis of the carpel – short unicellular hairs are visible; G – longitudinal section through the ovary – inside is a single anatropous bitegmic ovule; H – longitudinal section through an young ovule; I – longitudinal section through mature ovule – vascular connexions through the funicle with spiralate xylem vessels could be observed (abbreviations: c – carpels, m – micropyle, ov – ovule – anatropous type, p – petal, s – stamen, sp – sepal, t. h. – tector hairs, v. c. – vascular connection) (original).

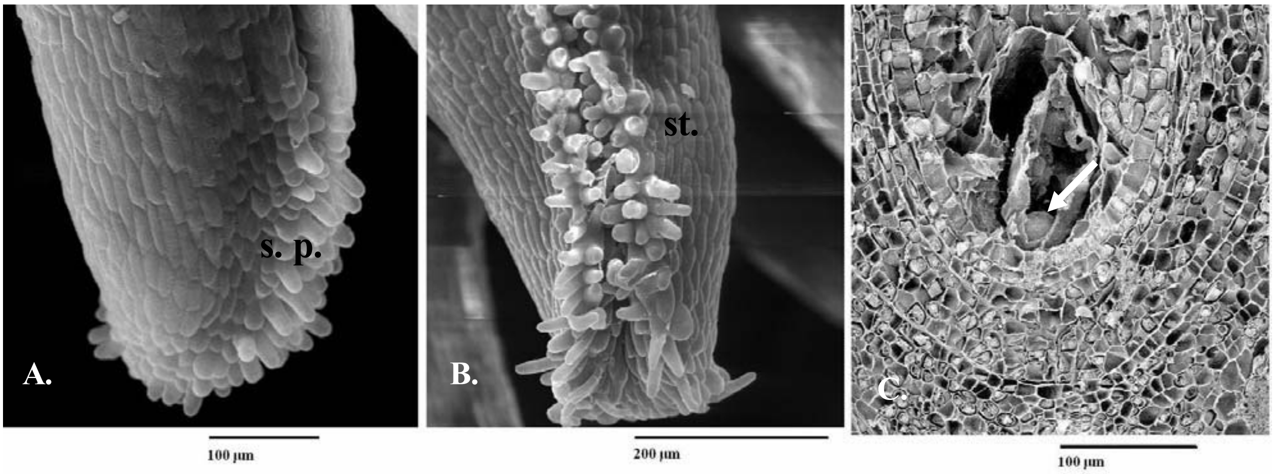

Figure 2. Field emission scanning electron micrographs of the carpels in *Adonis vernalis*: A, B – mature carpels – papilate stigma and style; C – longitudinal section through mature ovule – embryo sac with a large oosphere is visible (white arrow) (abbreviations: s. p – stigma papillae, st – style) (original).

The thickening of the endothecial cell walls occurs before the anthers opening and is necessary for this process.

The endothecium cells acquire distinct anticlinal ribs or bars which are differentiated on the lateral walls of the cell (Fig. 3F & 3G). These thicknesses appear in parallel with the tapetal cells degeneration.

The gynoecium is multiple, with distinct carpels; it is apocarpous. In the early stages of the development, the carpels are plicate; they are open at their superior part (style and stigma).

During the ontogenesis, the partial fusion of the carpel margins, running along its adaxial surface could be observed (Fig. 1E). At the base of each carpel, numerousness unicellular, trichomes are present (Fig. 1F). Stomata are present in the carpel epidermis in this region.

The mature ovule is anatropous and bitegmic. The ovule body is almost parallel to the funicle and almost completely fuses with it (Fig. 1G, 1H & 1I). The micropyle is formed by the inner integument. Placentation is laterally.

In the developmental process of the carpels, the stigmatic tissue appears from the apex of the style and is decurrent along the ventral suture. There is no obvious difference between style and stigma, both bearing papillae (Fig. 2A & 2B). The embryo sac is by *Polygonum* type (Fig. 2C). In the observed flowers, we found only one ovule in each carpel.

DISCUSSIONS

The spiral initiation sequence is a pattern in *Ranunculaceae* family [15] and shows a low systematic level [9]. According to Ro et al. (1997) [16] and Wang et al. (2009) [18] the *Adonis* genus is closely related to *Trollius*. Jensen et al. (1995) [12] placed *Adonis* in its own subtribe *Adonidinae*, which was included in *Adonideae* together with the unigeneric *Trolliinae* [15]. Ren and their collaborators describe some morphogenetical aspects of floral morphogenesis in *Adonis sutchuenensis* and *Trollius farreri*. *Adonis vernalis* have similar floral development with *A. sutchuenensis*. In *A. vernalis* the tector hairs from the ovary are shorter than in *A. sutchuenensis*, and the stigma papillae are visible along almost style longer.

The petals are retarded in early developmental stages in *Adonis vernalis*, but this feature is very common in *Ranunculaceae* [2, 9]. The petals are thicker than the sepals; the last one shows numerousness tector hairs on the lower epidermis.

Maturation of the stamens in *Adonis* is centripetal in accordance with the initiation sequence of the organs [14]; in the endothecium cells lignified thickness appear before opening. The exact function of these thickening is ambiguous but most probably they act by creating tension in the maturing anthers and help in pollen dispersal [11]. Numerous investigations indicated that tapetum through modified physical and physiological factors initiates the process of polen abortion acting at the meiotic stage [3]. Furthermore, persistent tapetum blocks nutrient transportation, which is necessary for the development of microspores into viable polen grains [5]

A comparative study on gynoecium structure in basal angiosperms revealed new features relevant to systematics [10]. The carpel development is similar in *A. vernalis* and *A. sutchuenensis* [16]. Both presents tector hairs especially on the basal part and the stigma is unicellular papillate and is slightly decurrent along the ventral slit [15]. In *A. vernalis* the tector hairs from the ovary are shorter than in *A. sutchuenensis*, and the stigma papillae are visible along almost style longer. A similar development of the carpels was observed in *Kingdonia* [14] and *Anemone rivularis* var. *flore-minore* [6]. But, unlike Kingdonia flowers, in *A. vernalis* no sterile stamen was observed. In *Trollius* the carpells are pluriovulate, initially open but their closure appears to be earlier than in *Adonis*. These observations do not support the approach between *Adonis* and *Trolius* genus. The ovule is unique in each carpel, bitegmic, crassinucellate. In *Ranunculaceae*, most of the genera have bitegmic ovules, with the exception of *Helleborus*, *Anemone, Clematis, Oxygraphis* and *Ranunculus* [17]. It has been considered that the bitegmic ovules clearly represent a more primitive

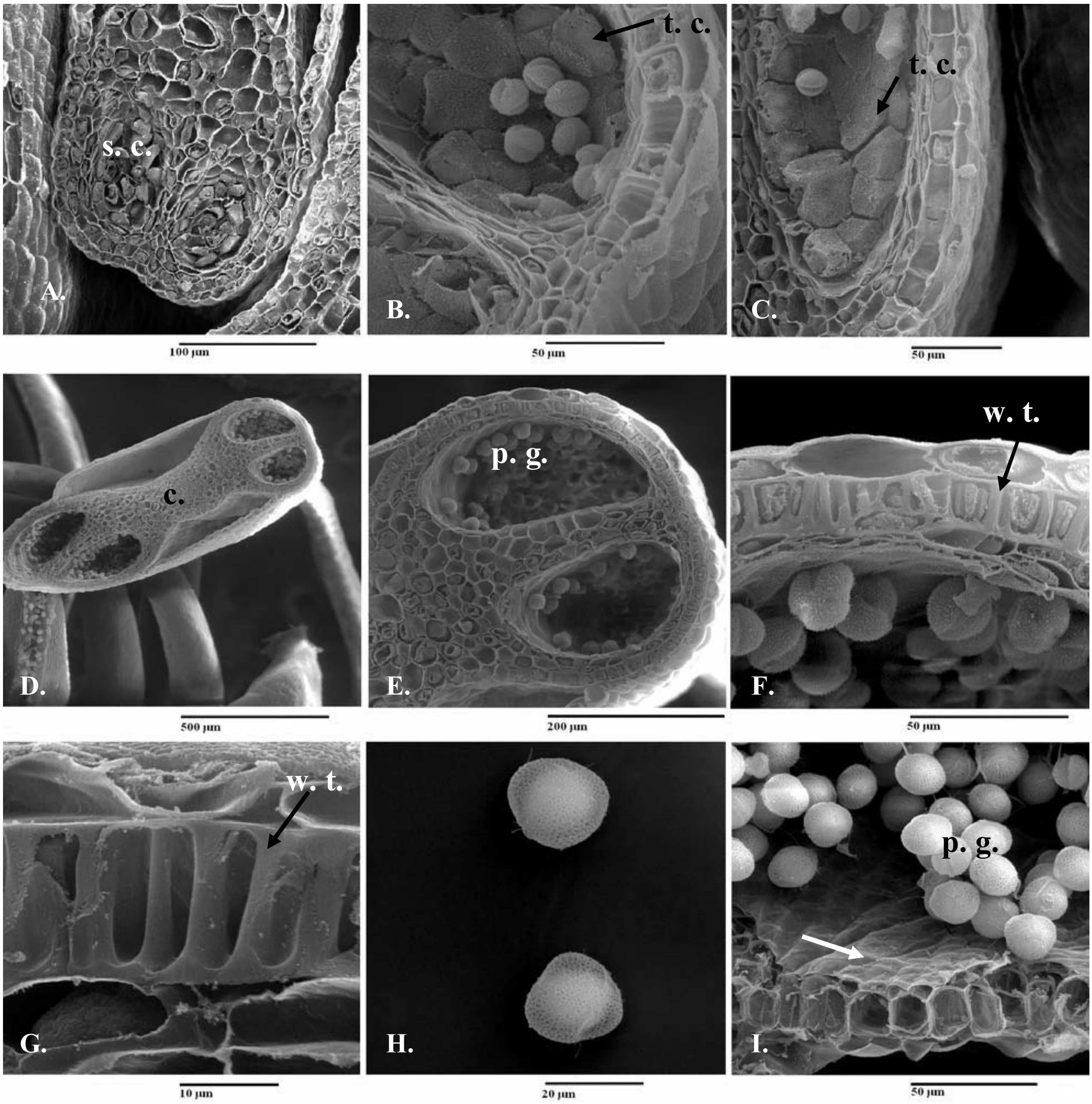

Figure 3. Field emission scanning electron micrographs of the stamen in *Adonis vernalis*: A – cross section through an young anther; B - E – mature anther before dehiscence (I) – tapetal cells are still visible; F, G - mature anther before dehiscence – tapetal cells are degenerescent, thickness in the endotecium cells become visible; H – pollen grains; I – mature anther at dehiscence (abbreviations: c – connective, p. g – pollen grains, s. c. – sporogenous cells, t. c. – tapetal cells, w. t. – wall thickness, (original).

structure than unitegmic ones, and that the unitegmic ovules are derived from bitegmic ones [13, 17]. Crassinucellar ovules are predominant in basal angiosperms [14].

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