

## OVULES ANATOMY OF SELECTED APOMICTIC TAXA FROM ASTERACEAE FAMILY

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**Abstract.** The present paper reports on our observations on the ovule structure of autonomous obligatory apomicts. We analyzed two triploid species of *Taraxacum*: *T. udum*, *T. alatum* and two triploid species of *Chondrilla*: *Ch. juncea*, *Ch. brevirostris*. The ovules of all studied species show a structure typical for the members of Asteraceae. One basal ovule develops into an inferior and unilocular ovary. The ovule is anatropous, tenuinucellate and unitegmic. Structural changes were observed in the ovule at the time of the embryo sac maturation. The innermost layer of integument develops into an endothelium surrounding the female gametophyte. Moreover, considerable modifications occurred in the integumentary cell layers adjacent to the endothelium. These cells show signs of programmed cell death and their walls begin to thicken. Histological analysis revealed that the prominent thick cell walls were rich in pectins. Layers of thick-walled cells formed a special storage tissue which, most likely, is an additional source of nutrients necessary for the proper nourishment of a female gametophyte and then of a proembryo.

**Key words:** *Taraxacum*, *Chondrilla*, Asteraceae, apomixis, ovule

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### Introduction

Apomixis occurs in many wild species but is not found in any food crop plants. Apomictic reproduction leads to the formation of populations that are genetically uniform maternal clones, and due to this fact apomixis is of a great interest in plant breeding. The introduction of apomixis into agriculturally important sexual genotypes could allow for the fixation of heterozygosity and hybrid vigour which may result in tremendous benefits to agriculture and seed production (BHAT *et al.* 2005; BICKNELL & BICKNELL 1999; BICKNELL & KOLTUNOW 2004; CARNEIRO *et al.* 2006; NOYES 2008; VAN BAARLEN *et al.* 1999). Apomictic mode of reproduction was found in over 40 plant families but it is especially prevalent within the three families, i.e. Poaceae, Rosaceae, and Asteraceae (ASKER & JERLING 1992; BICKNELL & KOLTUNOW 2004). Apomixis in the Asteraceae is frequently autonomous and apomicts are almost always polyploids (NOYES 2007). It is worth mentioning that some of the genera representing Asteraceae family, e.g.

*Taraxacum* and *Hieracium*, are used as model systems to investigate the genetic and molecular bases, and evolution of apomixis (VAN DIJK 2003; KOLTUNOW 2000).

*Taraxacum udum* Jord., *T. alatum* H. Lindb., *Chondrilla juncea* L., *Ch. brevirostris* Fisch. & C.A. Mey. are species belonging to the subfamily Cichorioideae, the tribe Cichorieae and the subtribe Crepidinae (ADENBERG *et al.* 2007). They have been previously studied cariologically and embryologically (KOŚCIŃSKA-PAJĄK 2006; KOŚCIŃSKA & BEDNARA 2006; MARCINIUK *et al.* 2010). Caryological analysis showed that these species are triploids and embryological investigations were focused on the observations of micro- and megasporogenesis, on embryo and endosperm development. It was shown that these species are obligatory apomicts with diplosporous embryo sacs of the *Taraxacum*-type in which the embryo and endosperm develop autonomously without fertilization. In previous studies, the ovule anatomy has not received sufficient attention. Therefore this research was focused on the description of the ovule structure.

## Material and methods

Inflorescences of studied species were fixed in FAA and stored in 70% ethanol. Dehydrated material was embedded in paraffin or in LR White acrylic resin. Paraffin sections 10 µm thick were stained by Heidenhain's hematoxylin with alcian blue. Semithin sections were stained with toluidine blue. The periodic acid-Schiff (PAS) reaction was used for the localization of insoluble polysaccharides. Additionally, some samples were cleared in methyl salicylate. Paraffin and semithin sections were examined with a Nikon 400 Eclipse microscope. For analysis of cleared ovaries Nikon Eclipse 80i microscope equipped with Nomarski interference contrast optics was used.

## Results and discussion

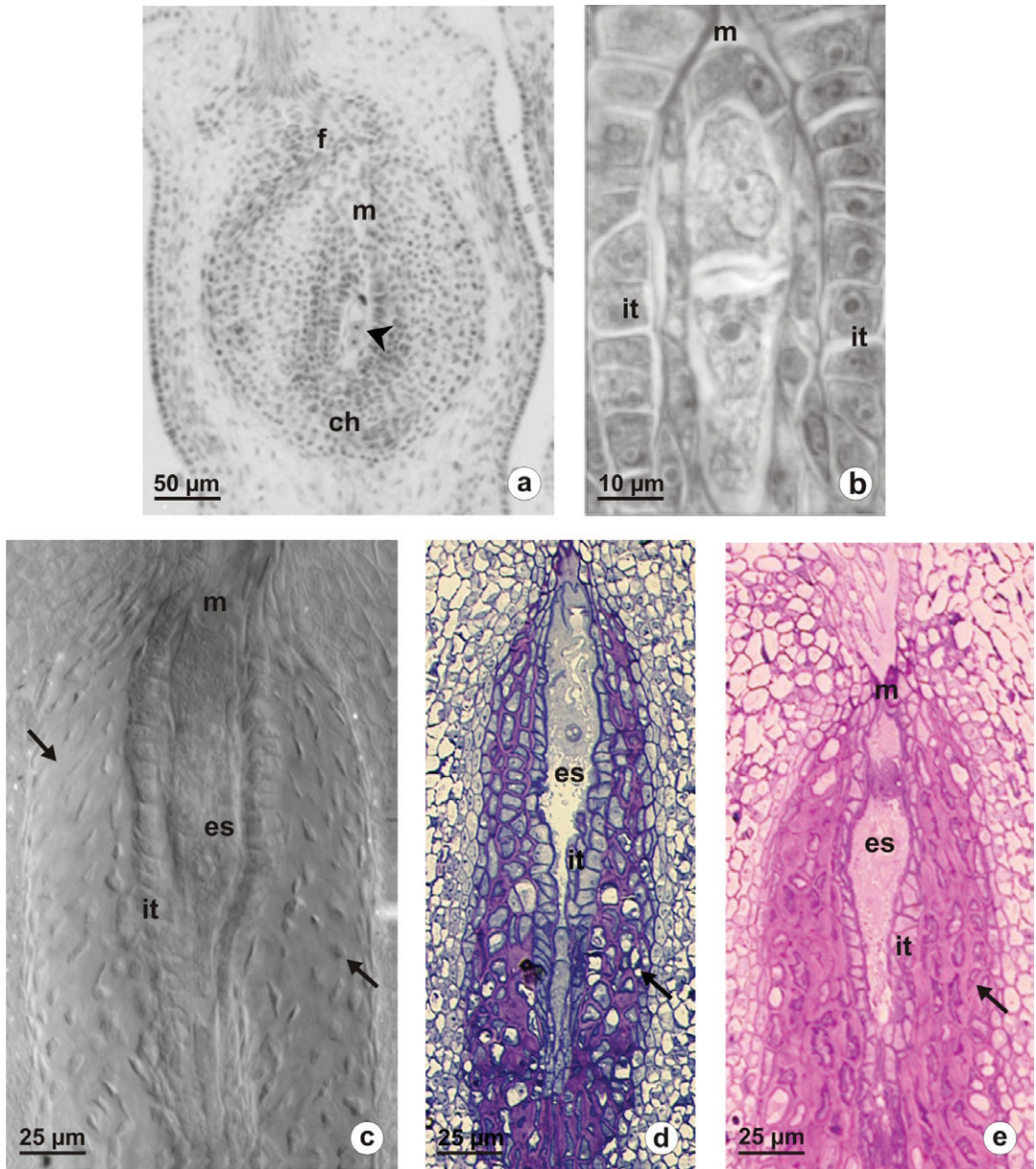
The *Taraxacum* and *Chondrilla* species under investigation have an inferior and unilocular ovary in which one anatropous, tenuinucellate and unitegimic ovule develops (Fig. 1a). Similar structure of ovary and ovule was also described in the other members of Asteraceae (JOHRI *et al.* 1992). In the ovules of the investigated species the restitution nucleus was formed after a disturbed first meiotic division. The second division led to the formation of two unreduced megaspores, i.e. diplodyad. At this developmental stage we observed the formation of uniseriate integumentary tapetum (endothelium) which originated from the innermost layer of the massive integument (Fig. 1b). The endothelium cells were radially elongated with dense cytoplasm surrounding the prominent nucleus. This layer is considered to provide nutrition for the female gametophyte and embryo (KAPIL & TIWARI 1978).

In older ovules, considerable changes were observed in the integumental cell layers adjacent to the endothelium. At the stage of four-nucleate embryo sac, the walls of those cells started to thicken and finally mature female gametophytes were surrounded by cells with extremely thick walls (Fig. 1c, d). The lumen of the cells was considerably reduced, most likely the cells showed signs of programmed cell death.

Histological analysis revealed that the thick cell walls were PAS positive (Fig. 1e) which indicates that the material deposited in the walls is rich in water insoluble polysaccharides. Similarly, PAS positive thick-walled layers were observed in the ovules of *T. linearisquameum* Soest and *T. gentile* G.E.Haglund & Rail. (MUSIAŁ *et al.* 2012). It should also be noted that KOLTUNOW *et al.* (1998) observed intensive liquefaction of the integument cells surrounding the embryo sac in the ovules of apomictic *Hieracium*. The authors emphasized that this special zone probably plays a nutritive role and may have facilitated the evolution of the apomictic trait in this genus. The tissue observed around the female gametophyte of the investigated *Taraxacum* and *Chondrilla* species seems to be an additional source of nutrients for the embryo and presumably reduces the importance of role played by endosperm. It is presumed that the occurrence of somatic nutritive tissue could explain why most of the apomicts in the Asteraceae show autonomous embryo development (VAN BAARLEN *et al.* 1999).

## References

- ANDERBERG A.A., BALDWIN B.G., BAYER R.G. *et al.* 2007. Compositae. In: KADEREIT J.W., JEFFREY C. (eds), The families and genera of vascular plants. VIII. Flowering plants, Eudicots, Asterales. 61–588. Springer, Berlin Heidelberg.
- ASKER S.E., JERLING L. 1992. Apomixis in plants. CRC Press, Boca Raton, FL.
- BICKNELL R.A., BICKNELL K.B. 1999. Who will benefit from apomixis? *Biotechnol. Dev. Monit.* 37: 17–20.
- BICKNELL R.A. & KOLTUNOW A.M. 2004. Understanding apomixis: recent advances and remaining conundrums. *Plant Cell* 16: 228–245.
- BHAT V., DWIVEDI K.K., KHURANA J.P., SOPRY S.K. 2005. Apomixis: An enigma with potential applications. *Curr. Sci.* 89: 1879–1893.
- CARNEIRO V.T.C., DUSI D.M.A., ORTIZ J.P.A. 2006. Apomixis: Occurrence, applications and improvements. In: TEIXEIRA DA SILVA J.A. (ed.), Floriculture, ornamental and plant biotechnology: Advances and topical issues (1<sup>st</sup> Ed., Vol. I): 564–571. Global Science Books, Isleworth.
- JOHRI B.M., AMBEGAOKAR K.B., SRIVASTAVA P.S. 1992. Comparative embryology of Angiosperms. Springer-Verlag, Berlin.
- KAPIL R.N., TIWARI S.C. 1978. The integumentary tapetum. *Bot. Rev.* 44: 457–490.



**Fig 1.** Ovule structure of *Taraxacum udum* (a, c) and *Chondrilla juncea* (b, d, e): **a** – longitudinal section of ovary with anatropous, unitegmic ovule, arrowhead indicates two-nucleate embryo sac; **b** – diplocyte surrounded by integumentary tapetum; **c** – ovule at the stage of mature embryo sac, arrows point to a characteristic somatic nutritive zone around the integumentary tapetum, image was obtained from unstained cleared ovule using Nomarski DIC optic; **d** – semithin longitudinal section of ovule, arrow indicates layers of thick-walled cells; **e** – ovule after the periodic acid-Schiff treatment, PAS positive reaction is visible in thick walls of the integumentary cells adjacent to the endothelium (arrow). **Abbreviations:** **ch** – chalazal pole; **es** – embryo sac; **f** – funiculus; **it** – integumentary tapetum; **m** – micropylar pole.

- KOLTUNOV A.M., JOHNSON S.D., BICKNELL R.A. 1998.** Sexual and apomictic development in *Hieracium*. *Sex. Plant Reprod.* **11**: 213–230.
- KOLTUNOW A. 2000.** The genetic and molecular analysis of apomixis in the model plant *Hieracium*. *Acta Biol. Cracov., ser. Bot.* **42**: 61–72.
- KOŚCIŃSKA-PAJĄK M. 2006.** Biologia rozmnażania apomiktycznych gatunków *Chondrilla juncea* L., *Chondrilla brevirostris* L. i *Taraxacum alatum* Lindb. z uwzględnieniem badań ultrastrukturalnych i immunocytochemicznych. 1–104. KonTekst, Kraków. (in Polish).
- KOŚCIŃSKA-PAJĄK M., BEDNARA J. 2006.** Unusual microtubular cytoskeleton of apomictic embryo sac of *Chondrilla juncea* L. *Protoplasma* **227**: 87–93.
- MARCINIUK J., RERAK J., GRABOWSKA-JOACHIMIAK A., JASTRZĄB I., MUSIAŁ K., JOACHIMIAK A. 2010.** Chromosome numbers and stomatal cell length in *Taraxacum* sect. *Palustria* from Poland. *Acta Biol. Cracov., ser. Bot.* **52**: 117–121.
- MUSIAŁ K., PŁACHNO B.J., ŚWIĄTEK P., MARCINIUK J. 2012.** Anatomy of ovary and ovule in dandelions (*Taraxacum*, Asteraceae). *Protoplasma* DOI 10.1007/s00709-012-0455-x.
- NOYES R.D. 2007.** Apomixis in the Asteraceae: diamonds in the rough. *Funct. Plant Sci. Biotechnol.* **1**: 207–222.
- NOYES R.D. 2008.** Sexual devolution in plants: apomixis uncloaked? *Bioessays* **30**: 798–801.
- VAN BAARLEN P., VERDUIJN M., VAN DIJK P.J. 1999.** What can we learn from natural apomicts? *Trends Plant Sci.* **4**: 43–44.
- VAN DIJK P.J. 2003.** Ecological and evolutionary opportunities of apomixis: insights from *Taraxacum* and *Chondrilla*. *Philos. Trans. R. Soc. Lond. B* **358**: 1113–1121.