

EMBRYOLOGICAL PROCESSES DURING THE SEED FORMATION IN TWO TRIPLOID SPECIES OF *Taraxacum*

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Abstract. The present paper reports on our observations on embryological processes occurring in the ovules of triploids: *Taraxacum belorussicum* (sect. *Palustris*) and *T. atricapillum* (sect. *Borea*). The reproduction of these dandelions comprises meiotic diplospory, parthenogenesis and autonomous endosperm development. Mature seeds contain viable embryos that provide regular seedlings. At present the importance of cytological and embryological studies of apomicts is specially emphasized because *Taraxacum* is one of the model genus for investigations of apomixis. It forms a polyploid complex within which there is a close relationship between the mode of reproduction and the ploidy level: diploids reproduce sexually, whereas polyploids are apomicts. Apomixis is of a great interest in plant breeding because it allows clonal seed production but asexual seeds formation by apomixis is not found in any crop plants. Unfortunately, to date any attempts at introducing of apomixis into crop species have failed. It is worth mentioning that dandelions are valuable honey plants and numerous species of *Taraxacum* are also used in modern herbal medicine.

Key words: agamic complex, apomixis, dandelion, diplospory, female gametophyte, Nomarski contrast

INTRODUCTION

The *Taraxacum* Wigg. genus belongs to the family Asteraceae, the subfamily Cichorioideae, the tribe Cichorieae and the subtribe Crepidinae [Anderberg *et al.* 2007]. This cosmopolitan genus forms a polyploid complex comprising amphimictic and apomictic taxa. Diploid dandelions reproduce sexually whereas seed formation in polyploid species is realized by gametophytic apomixis, parthenogenesis and autonomous endosperm development [Musiał *et al.* 2013a and references therein]. Common co-occurrence of apomixis and sexual reproduction is one of the reason that cause the large taxonomic complexity of *Taraxacum* [Kirschner *et al.* 2003, Závěská

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Drąbkowá *et al.* 2009]. The genus *Taraxacum* is divided into 60 sections comprising about 2800 species [Kirschner *et al.* 2014].

The Polish dandelion flora is dominated by triploid and tetraploid taxa and is represented by 374 species classified (depending on the classification system) into 12 or 13 sections [Marciniuk *et al.* 2010a, 2012]. Most species belong to the section *Ruderalia* (292), well-represented are also sect. *Erythrosperma* (24), sect. *Palustria* (22) and sect. *Hamata* (11) whereas sections *Celtica*, *Borea*, *Piesis*, *Fontana*, *Alpestris*, *Alpina*, *Naevosa*, *Erythrocarpa* comprise a single dandelion species [Marciniuk *et al.* 2010a, 2012, Marciniuk 2012].

Taraxacum is a very common perennial associated with grassland communities but it is also often found in lawns, roadsides and roadside streets [Sudnik-Wójcikowska 2011]. Although the dandelion is considered to be a burdensome weed, it is also used in many traditional and modern herbal medical systems due to the content of components demonstrating, among others, anti-inflammatory, anti-oxidative and anti-carcinogenic activities [Choi *et al.* 2002, Hu and Kitts 2003, Yarnell and Abascal 2009, Mahesh *et al.* 2010, Michalska *et al.* 2010]. Moreover, dandelion species are valuable honey plants [Weryszko-Chmielewska and Chwil 2006]. Furthermore, it is worth mentioning that *Taraxacum* is a model taxon for the investigation of molecular and genetic background of apomictic processes [Tucker and Koltunow 2009, Barcaccia and Albertini 2013]. Apomixis is of a great interest in plant breeding because this mode of reproduction leads to the formation of populations that are genetically uniform maternal clones and 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 [Barcaccia and Albertini 2013 and reference therein]. Therefore at present, despite the advanced research at the molecular level, the importance of cytological and embryological studies of apomicts is emphasized.

The present paper documents megasporogenesis and female gametophyte formation in *Taraxacum belorussicum* Val. N. Tikhom. (sect. *Palustria*) as well as seed formation in *T. atricapillum* Sonck (sect. *Borea*). Previous cytological analysis showed that these species have a triploid number of chromosomes ($2n = 3x = 24$) [Marciniuk *et al.* 2010b, Musiał *et al.* 2015]. Those two species are poorly known, moreover, *T. belorussicum* is one of the endangered taxa because of human activity. Therefore the knowledge of reproductive biology is fundamental for an effective protection of this species.

MATERIAL AND METHODS

Mature seeds of *Taraxacum belorussicum* and *T. atricapillum* were sampled from plants within a natural population, respectively in Mścichy (53°25' N; 22°29' E) and in Żabokliki (52°11' N; 22°19' E) by dr. Jolanta Marciniuk. Then, plants obtained from seeds of *T. belorussicum* were cultivated in an experimental field. From the cultured specimens, whole inflorescences at various developmental stages were collected and fixed in acetic acid: 96% ethanol (1:3, v/v) for at least 24 h. Fixed plant material was stored in 70% ethanol. Then, isolated individual flowers, ovaries or ovules were cleared in methyl salicylate according to a procedure described by Mól [1988] and Musiał *et al.* [2012, 2013b]. Samples were dehydrated in a graded ethanol series and then infiltrated with methyl salicylate. Cleared samples mounted on a Raj slide in a drop of clearing fluid [Herr 2000] were examined with Nomarski interference contrast (DIC optics).

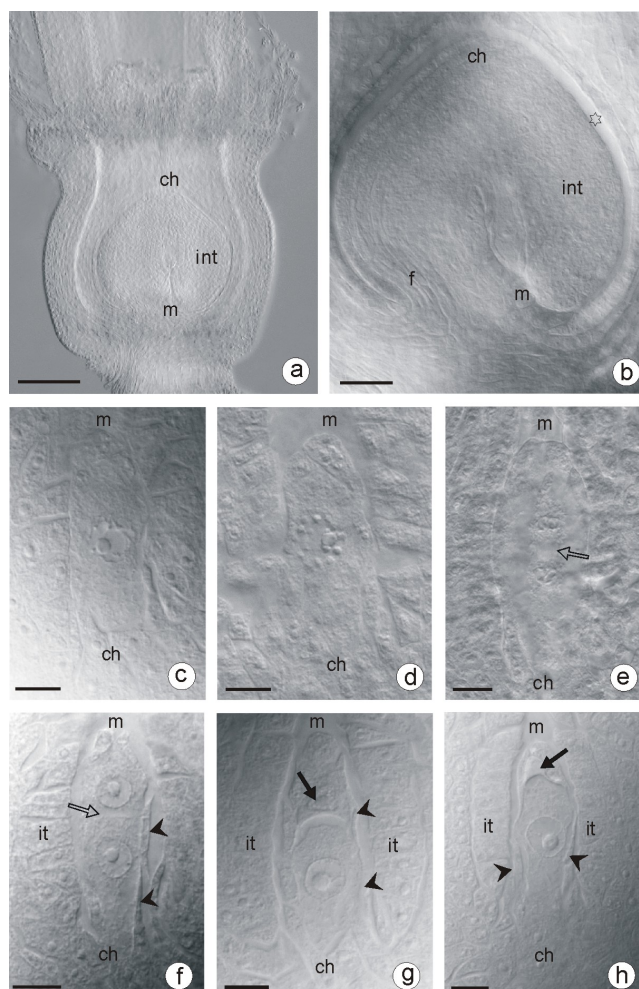
Embryos of *T. atricapillum* were isolated from mature seeds and tetrazolium test was used to estimate embryo viability [Siuta *et al.* 2005].

RESULTS AND DISCUSSION

The seed is one of the fundamental factors of crop productivity. Therefore, knowledge of the essential mechanisms of seed formation is crucial for the progress of agricultural production. Among flowering plants there are two pathways of reproduction through seeds. Most angiosperms produce genetically variable seeds by a fusion of meiotically reduced egg cells and sperm cells. However, some flowering plants have evolved an alternate form of reproduction termed apomixis, often referred to as agamospermy. The apomictic mode of reproduction omits crucial processes occurring in the sexual pathway and apomictic plants produce progeny that is an exact genetic replica of the mother plant. Meiotic reduction is bypassed prior to female gametophyte formation (apomeiosis), the egg cell forms an embryo autonomously (parthenogenesis) and endosperm development is autonomous or may require central cell fertilization (pseudogamy) [Nogler 1984, Asker and Jerling 1992]. As apomictic offspring carries the full genetic maternal constitution, apomixis is a highly desirable trait in plant breeding and seed production. Both in sexual and apomictic mode of reproduction the same generative structures are involved in the developmental trace.

Flowers of the investigated triploid *Taraxacum belorussicum* possess an inferior, unilocular ovary containing one basal, anatropous, tenuinucellate and unitegmic ovule (Fig. 1a, b). Similar structure of ovary and ovule is typical for the members of the Asteraceae family [Johri *et al.* 1992] and has been also described in other dandelion species [Musiał *et al.* 2013a, b, Musiał and Kościńska-Pająk 2013b]. In young ovules of *T. belorussicum*, a single hypodermal archesporial cell differentiates and develops directly into a megaspore mother cell (MMC) which elongates in the micropylar-chalazal axis before the beginning of meiotic division (Fig. 1c). Then MMC enters into the first meiotic prophase (Fig. 1c, d) but because of asynapsis there is a strongly reduced chromosome pairing. The univalents remain scattered over the whole spindle of metaphase I and the restitution nucleus is formed after the first meiotic division, instead of two reduced nuclei. The second meiotic division proceeds without disturbances and leads to the formation of two unreduced megaspores i.e. diplodyad (Fig. 1e, f). Such irregular meiosis is distinctive for meiotic diplospory which was observed previously [Janas *et al.* 2014]. It was also found in other polyploid taxa of *Taraxacum* as well as in species of *Chondrilla*, *Erigeron*, and *Ixeris* [Nogler 1984 and references therein, Kościńska-Pająk 2006, Kościńska-Pająk and Bednara 2006, Musiał *et al.* 2013a]. On the other hand, a regular course of meiosis was documented in the recent embryological examination of a diploid dandelion *Taraxacum linearisquameum* [Musiał *et al.* 2015]. It should also be emphasized that in the analysed ovules of *T. belorussicum* an early formation of integumentary tapetum was observed (Fig. 1f-h). Such a process was also reported in some other examined diplosporous apomicts [Kościńska-Pająk 2006, Musiał *et al.* 2013a, 2015]. It seems that it may be associated with the later autonomous embryo and endosperm development. In the course of further development of *T. belorussicum*, the micropylar cell of diplodyad gradually degenerates whereas the chalazal one acts as a functional megaspore (Fig. 1g, h).

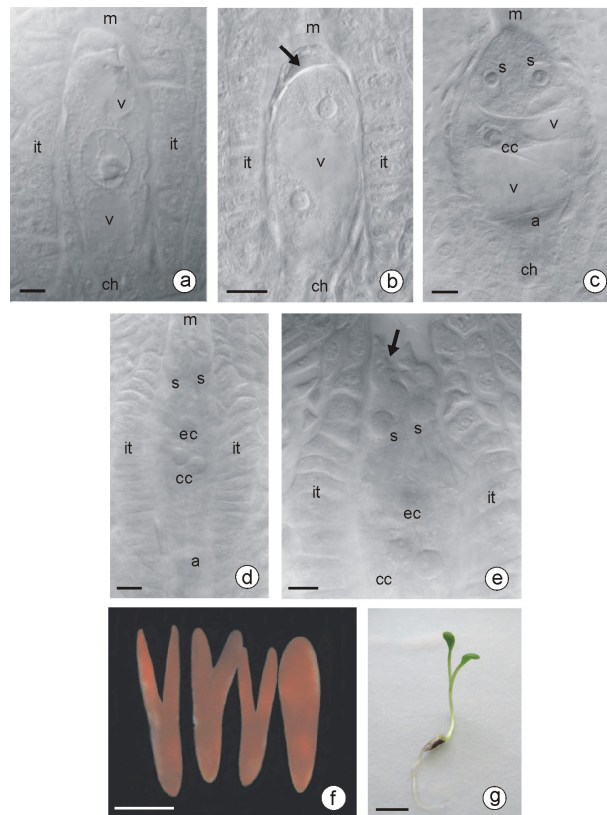
The unreduced female gametophyte (embryo sac) develops from the functional megaspore after three successive mitotic divisions (Fig. 2a-c).



ch – chalaza, f – funicle – sznureczek, int – integument, it – integumentary tapetum – tapetum integumentalne, m – micropylar canal – kanał mikropylarny, arrowheads – degenerated cells of nucellus – groty strzałek – zdegenerowane komórki ośrodka
scale bars – podziałki: (a) 100 μ m, (b) 20 μ m, (c-h) 10 μ m

Fig. 1. Ovary, ovule and megasporogenesis in *Taraxacum belorussicum*: a – young, inferior and unilocular ovary; b – anatropous, tenuinucellate ovule with one integument, star shows ovary chamber; c – early prophase I in MMC; d – diakinesis in MMC; e – telophase II, arrow indicates phragmoplast; f – young diplodyad, arrow points to the forming cell plate; g, h – subsequent stages of functional megaspore differentiation, arrows show degenerating micropylar cell of diplodyad

Rys. 1. Zalążnia, zalążek i megasporogeneza u *Taraxacum belorussicum*: a – młoda, jednokomorowa zalążnia w słupku dolnym; b – anatropowy, tenuinucellarny zalążek z jednym integumentem, gwiazdka wskazuje komorę zalążni; c – wczesna profaza I w KMM; d – diakineza w KMM; e – telofaza II, fragmoplast oznaczony strzałką; f – wczesne stadium diplodiady z tworzącą się przegrodą pierwotną (strzałka); g, h – kolejne stadia różnicowania się megaspory funkcjonalnej, strzałka wskazuje degenerującą mikropylarną komórkę diplodiady



a – antipodal cell – antypoda, cc – central cell – komórka centralna, ch – chalaza, ec – egg cell – komórka jajowa, it – integumentary tapetum – tapetum integralne, m – micropylar canal – kanał mikropylarny, s – synergid – synergida, v – vacuole – wakuola
scale bars – podziałki: (a-e) 10 μ m, (f) 1 mm, (g) 5 mm

Fig. 2. Female gametophyte formation in *Taraxacum belorussicum* (a-e), embryos and seedlings of *T. atricapillum* (f-g): a – one-nucleate embryo sac; b – two-nucleate embryo sac, arrow indicates degenerated micropylar cell of diplodyad; c, d – seven-celled embryo sac; e – magnification of micropylar part of embryo sac visible on d showing synergids elongated towards micropylar canal, arrow indicates irregular and thickened synergids wall; f – isolated embryos after tetrazolium test; g – young, viable and properly formed seedling

Rys. 2. Formowanie gametofitu żeńskiego u *Taraxacum belorussicum* (a-e), zarodki i siewki *T. atricapillum* (f-g): a – 1-jądrowy woreczek zalążkowy; b – 2-jądrowy woreczek zalążkowy, strzałka wskazuje zdegenerowaną, mikropylarną komórkę diplodiady; c, d – 7-komórkowy woreczek zalążkowy; e – powiększony fragment mikropylarnej części woreczka zalążkowego z fot. d, widoczne wydłużone w kierunku kanału mikropylarnego synergidy, strzałka wskazuje nieregularną, zgrubiałą ścianę synergid; f – wypreparowane z nasion zarodki po teście tetrazolinowym; g – młoda, prawidłowo wykształcona siewka

The mature female gametophyte contains an egg apparatus at the micropylar pole, highly vacuolated central cell and three antipodal cells at the chalazal pole (Fig. 2c). The central cell occupies the largest part of the mature embryo sac and it initially

contains two polar nuclei (Fig. 2d) or secondary nucleus. Such a structure of unreduced female gametophyte corresponds to a meiotic embryo sac of *Polygonum* type [Johri *et al.* 1992]. It is worth emphasizing that, despite the fact that *T. belorussicum* is a diplosporous apomict in which the embryo and endosperm develop autonomously before flower opening, the egg apparatus shows a typical polarity. Both synergid cells are elongated towards the micropylar canal and the wall between them is irregular and thickened (Fig. 2e). Recent embryological investigations of other dandelion species revealed the similar structure of synergid in amfimictic and apomictic taxa, however differences in cytoskeleton were mentioned [Musiał and Kościńska-Pająk 2013a, Musiał *et al.* 2013a]. It is well known that in sexually reproducing plants, the synergids are involved in the reception of pollen tube and fertilization [Li *et al.* 2009]. However, the role of synergids in obligatory apomicts, in which embryo and endosperm develop independently of any contribution from pollen is not fully elucidated and further embryological studies are required. It is known that in some apomictic dandelions synergids may maintain physiological activity up to the globular embryo stage in [Musiał and Kościńska-Pająk 2013a]. Tetrazolium test performed on a sample of mature seeds of *T. atricapillum* showed that they contain well developed and viable embryos (Fig. 2f). Moreover seedlings developed normally, without malformations and symptoms of necrosis (Fig. 2g).

CONCLUSIONS

Taraxacum belorussicum (sect. *Palustria*) and *T. atricapillum* (sect. *Borea*) are apomictic species. The same embryological structures are engaged in the seed development, similarly as in sexually reproducing plants. The mode of reproduction involved diplospory *Taraxacum* type, unreduced female gametophyte formation from the chalazal cell of diplodyad. The organization of a mature embryo sac corresponds to the *Polygonum* type. Seeds develop without any contribution of pollen and contain viable embryos.

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PROCESY EMBRIOLOGICZNE ZWIĄZANE Z FORMOWANIEM NASION U DWÓCH TRIPLOIDALNYCH GATUNKÓW *Taraxacum*

Streszczenie. Praca dotyczy obserwacji procesów embriologicznych w zalążkach triploidalnych mniszków: *Taraxacum belorussicum* (sect. *Palustris*) and *T. atricapillum* (sect. *Borea*). Rozwój nasion u tych taksonów obejmuje mejotyczną diplosporię, partenogenezę i autonomiczny rozwój endospermy. Z dojrzałych nasion, zawierających żywotne zarodki, rozwijają się prawidłowo wykształcone siewki. Badania cytologiczne i embriologiczne tego poliploidalnego kompleksu mają istotne znaczenie, bowiem *Taraxacum* jest jednym z modelowych rodzajów wykorzystywanych w genetycznych i molekularnych badaniach procesów apomiktycznych. W obrębie *Taraxacum* istnieje ścisła zależność pomiędzy stopniem ploidalności a sposobem rozmnażania. Diploidy rozmnażają się seksualnie, podczas gdy poliploidy są apomiktami. Ze względu na to, że apomiktyczne potomstwo powiela genotyp rośliny matecznej, od wielu lat apomiksja pozostaje w centrum zainteresowania hodowców roślin. Jednak u roślin uprawnych nie stwierdzono apomiktycznego formowania nasion, a dotychczasowe próby wprowadzenia apomiksji do szlaku rozwojowego ważnych gospodarczo roślin nie powiodły się. Należy także zaznaczyć, że mniszki są wartościowymi roślinami miododajnymi, a ponadto liczne gatunki *Taraxacum* znajdują zastosowanie w farmacji.

Słowa kluczowe: apomiksja, diplosporia, gametofit żeński, kompleks agamiczny, kontrast Nomarskiego, mniszek

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