

EMBRYOLOGICAL ANALYSIS OF *CONSOLIDA REGALIS* L. (RANUNCULACEAE)

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In *Consolida regalis* L. the development of the embryo sac conforms to the Polygonum type. In the mature embryo sac, the egg apparatus and secondary nucleus are of normal appearance. The synergids are ephemeral and disappear soon after fertilization. The antipodal cells are large and persistent but remain uninucleate and show rhythmical growth. The volumes of antipodal nuclei correspond with ploidy levels from n to $256n$. Cytochemical tests indicate the presence of high amounts of proteins, lipids, polysaccharides, RNA and DNA in the antipodals. They degenerate in the cellular endosperm stage. The endosperm is nuclear; centripetal wall formation starts at the micropylar end and progresses towards the chalaza. Embryogeny is of the Solanad type. The mature embryo lacks organs at shedding of seeds.

Key words: *Consolida regalis*, Ranunculaceae, embryo sac, antipodals, endoreduplication, embryo, endosperm.

INTRODUCTION

The Ranunculaceae include a remarkable array of primitive and advanced morphological and embryological characters: actinomorphic or zygomorphic, bisexual or unisexual flowers; free or fused carpels; multiovulate follicles or uniovulate achenes; crassinucellate, uni-, bi-, or ategmic ovules; Polygonum or Allium types of embryo sac (ES) development; and ephemeral or persistent antipodals (Vijayaraghavan and Bhandari, 1970; Johri et al., 1992). Polyploidization is frequently seen in ES cell types such as antipodals, synergids, endosperm and suspensor (Kozieradzka-Kiszkurno and Bohdanowicz, 2003; Greilhuber et al., 2005), and there is an apparent correlation between their structure and length of life span, suggesting their nutritive role (Raghavan, 1997). The occurrence of endopolyploid antipodals was noted in members of Ranunculaceae (Turała-Szybowska and Wędzony, 1981; Turała-Szybowska and Wolańska, 1989). In this study we examined the development of ES, embryo and endosperm of *Consolida regalis* L.

MATERIALS AND METHODS

Flower buds originating from plants grown in the meadows of Terkos (Istanbul) were fixed in different

stages of development in acetic alcohol (1:3, v/v). After embedding in paraffin, microtome sections 8–12 μm thick were stained with Regaud's hematoxylin; for cytochemical observations, sections were stained with periodic acid-Schiff (PAS) for insoluble polysaccharides, methyl green-pyronin for DNA and RNA, ninhydrin for proteins, and Sudan III for lipids (Schneider, 1981; Bancroft, 1996).

Nuclear volume was calculated according to the formula $\frac{4}{3} r_1 r_2 r_3 \pi$ (Tschermak-Woess and Hasitschka, 1953). In large high-polyploid nuclei the r_3 dimension represents the sum of values from successive microtome sections, and only nuclei of regular shape were calculated.

The preparations were photographed with an Evolution LC color camera and an Olympus BH-2 microscope, and the images were analyzed with Image-Pro Express Version 6.0 scientific image processing and analysis software

RESULTS

In *Consolida regalis* the mature ovule is anatropous, bitegmic and crassinucellate. The outer integument develops first and then the inner integument appears as a small protuberance at the megaspore mother cell (MMC) stage. A single archesporial cell differentiates as a MMC which undergoes meio-

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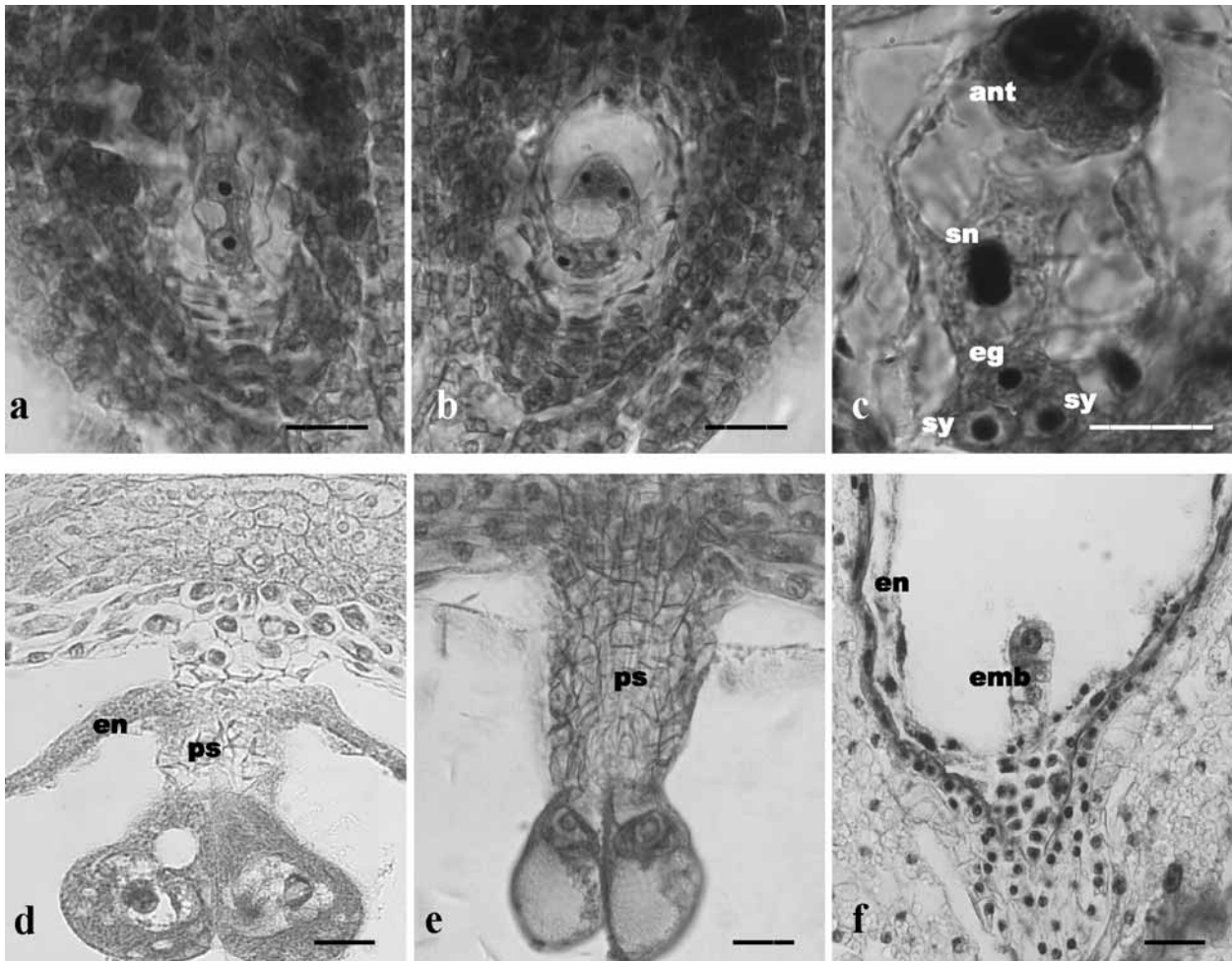


Fig. 1. Development of embryo sac, endosperm and embryo in *Consolida regalis* L. (Ranunculaceae). (a) 2-nucleate embryo sac, (b) 4-nucleate embryo sac, (c) Organized embryo sac with egg apparatus, secondary nucleus and two antipodals, (d,e) Antipodals on the postament, (f) Linear proembryo and nuclear endosperm. (a-c, f) stained with hematoxylin, (d) stained with methyl green-pyronin, (e) stained with ninhydrin. ant – antipodals; eg – egg cell; sn – secondary nucleus; sy – synergid; en – endosperm; ps – postament; emb – embryo. Bars = 10 µm.

sis to form a dyad and then a linear tetrad. At about the functional megaspore stage the ovule is completely anatropous. Mitotic divisions in the functional megaspore result in 2-, 4- and 8- nucleate ES (Fig. 1a-c). The development of the ES conforms to the Polygonum type. In a mature ES the egg apparatus consists of one egg cell and two synergids. The synergids lying above the egg cell are ephemeral, and degenerate simultaneously soon after fertilization. Polar nuclei lie somewhat below the egg apparatus, and they soon fuse to form the secondary nucleus (Fig. 1c). The secondary nucleus is close to the antipodals (Fig. 2c). Two of the three antipodal cells are persistent, but the third one disappears before endoreduplication starts.

The development of the antipodals was followed from the organized 8-nucleate ES to the cellular

endosperm stage when the antipodals degenerate (Fig. 2a-i). The antipodals are located on the postament consisting of parenchymatic cells at the chalaza. Later the postament becomes prominent and pushes the antipodals towards the center of the ES (Fig. 1d,e).

Young antipodal cells are bigger than the egg and synergids. They have dense, nonvacuolated cytoplasm and spherical or ellipsoidal nuclei. The number of antipodal cells and the number of their nuclei do not increase during development, but they enlarge considerably. Two of these cells persist to the formation of endosperm and show substantial enlargement. One antipodal disappears without leaving any remnants. The enlargement of persistent antipodals is a result of vacuolation of the cytoplasm, together with an increase in the dimensions

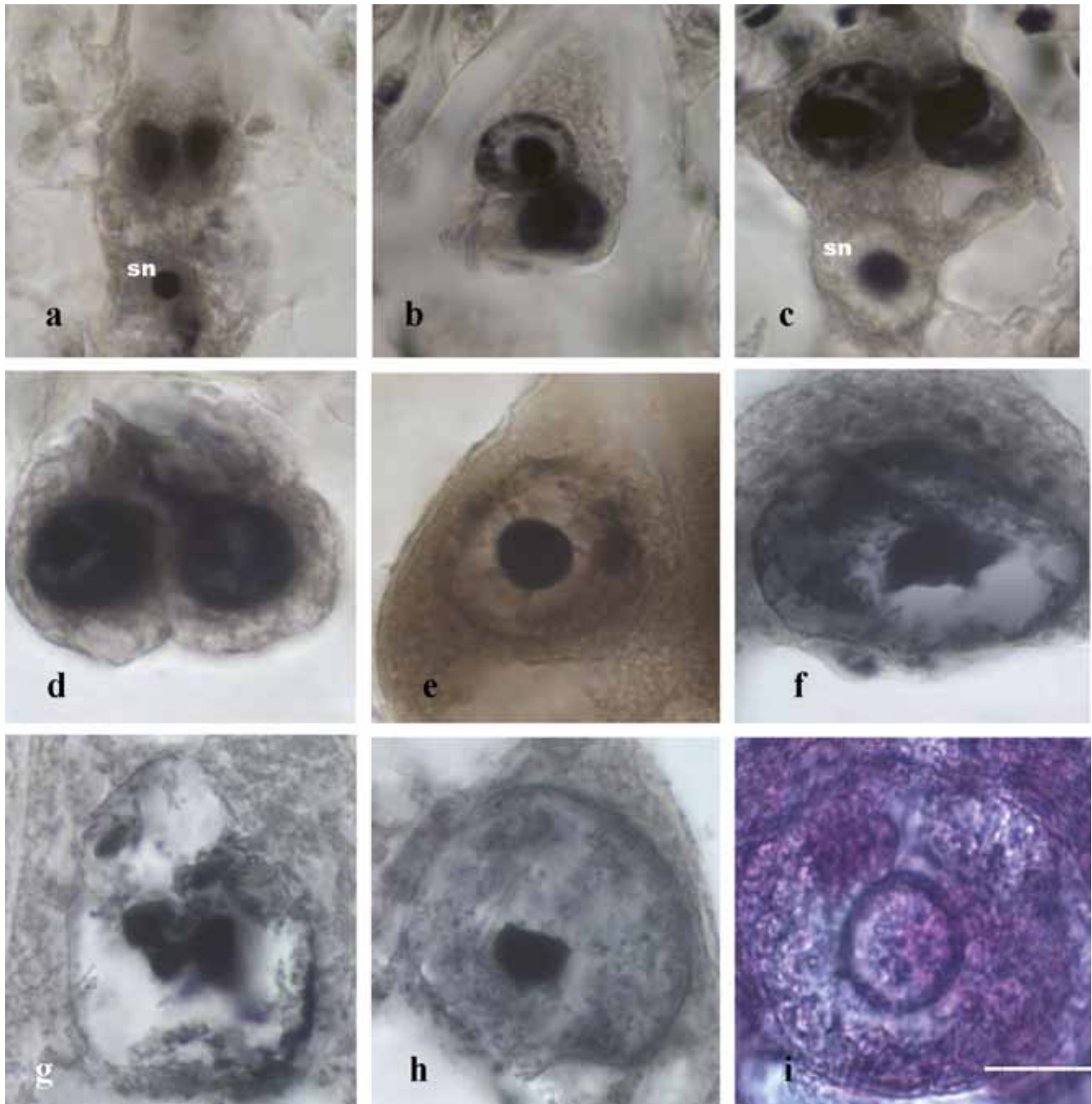


Fig. 2. Antipodals with polyplid nuclei. (a) n , (b) $2n$, (c) $4n$, (d) $8n$, (e) $16n$, (f) $32n$, (g) $64n$, (h) $128n$, (i) $256n$. (a–h) stained with hematoxylin, (i) stained with ninhydrin. sn – secondary nucleus. Bar in (i) = $10\ \mu\text{m}$ and also applies to (a–h).

of the nucleus and nucleolus. The chromatin material appears as granular structures. They are haploid only at a very early stage, and then undergo rhythmical growth. The volume of nuclei was calculated and the results are presented in Table 1. The volume of haploid nuclei ranged from 139 to $37432\ \mu\text{m}^3$. From the calculations, nine classes of nuclei corresponding to ploidy levels n , $2n$, $4n$, $8n$, $16n$, $32n$, $64n$, $128n$ and $256n$ were established.

The presence of spherical polyplid nuclei with enlarged nucleoli, the rhythmical growth of nuclear volume, and the appearance of chromatin suggest repeated chromosome endoreduplication cycles. Since the two antipodal nuclei are almost the same size, polyplidization takes place synchronously and the antipodals have the same degree of ploidy. The giant antipodal cells differ from young cells by their less stained, vacuolated cytoplasm and by their

TABLE 1. Volume and degree of ploidy of the antipodal nuclei in *Consolida regalis*

Degree of ploidy	Extreme values (μm^3)	Average (μm^3)	q	Number of nuclei
n	139.0 – 164.5	153.2	–	4
2n	253.8 – 353.7	297.3	1.94	10
4n	497.4 – 707.6	622.0	2.09	7
8n	1057.7 – 1321.6	1213.0	1.95	7
16n	2147.7 – 2579.6	2404.3	1.98	8
32n	4432.5 – 5211.1	4775.8	1.99	10
64n	8965.6 – 10114.0	9772.0	2.05	4
128n	18593.2 – 19989.4	19148.9	1.96	7
256n	34098.8 – 37432.1	35765.5	1.87	2
Total number of nuclei				59

TABLE 2. Cytochemical analysis of cells of egg apparatus and antipodals

Cytochemical substance	Test	Indication	Egg Cell	Synergids	Antipodals
DNA-RNA	Methyl green-pyronin	DNA – green, RNA – red	weak	weak	strong
Insoluble polysaccharides	PAS	magenta	weak	weak	strong
Proteins	Ninhydrin	pinkish-purple	weak	weak	strong
Lipids	Sudan III	red	weak	weak	strong

thick wall. When the antipodal cells enlarge, the vacuoles fuse to form a large one. The nuclei in these cells become more vacuolated and more irregularly shaped. During cellularization of the endosperm, the giant antipodals are crushed and then degenerate.

Cytochemical analysis showed that all cells of young ES have low amounts of cytoplasmic polysaccharides, RNA and proteins, and their nuclei show less stainability for DNA. When ES matures, the cytoplasm of antipodals shows a stronger PAS-positive reaction than the egg apparatus cells (Tab. 2). The methyl green stainability for DNA and the pyronin stainability for RNA in giant antipodals were comparable to that of the nuclei in somatic cells (Fig. 1d). The nucleolus stained intensely for RNA. The cytoplasm of antipodals showed very strong stainability for RNA and proteins (Fig. 1e). They contained starch grains giving a positive reaction with PAS. At the cellular endosperm stage the giant antipodals shrunk. They lost their RNA and proteins. Their nuclei showed less stainability for DNA.

The endosperm is of Nuclear type (Fig. 1f). Free nuclei remain embedded in a cytoplasmic sheet around the central vacuole. Centripetal wall formation sets in when the proembryo is at 6- to 8-tiered stage. The endosperm becomes completely cellular at the globular embryo stage. The cells of the mature endosperm are mostly uninucleate, rarely binucleate.

The zygote is smaller than the egg cell, and divides by a transverse wall. Embryo development conforms to the Solanad type. The suspensor is initially uniseriate, then becomes biseriata. The embryo is small and reduced, and does not differentiate into organs when the seeds are ready for dispersal.

DISCUSSION

In *Consolida regalis* the ovules are anatropous, bitegmic and crassinucellate. Megasporogenesis is regular. The chalazal megaspore gives rise to a monosporic ES of the Polygonum type. Although the family Ranunculaceae were once considered to show uniform development (Polygonum type) of the female gametophyte (Kordyum, 1959), several variations have been reported. *Adonis* shows only the Allium type (Bhandari, 1962; 1963; Uluce and Ünal, 1996) whereas development is bisporic in *Trollius acaulis* and monosporic in *T. europaeus* (Bhandari and Kapil, 1964). In *Thalictrum angustifolium* var. *flavum*, Picci (1964) described tetrasporic, 16-nucleate ES of the Pyrethrum type, but in *T. javanicum* the development of the female gametophyte is monosporic, 8-nucleate, and of the Polygonum type.

The antipodals in *Consolida regalis* were placed at the apex of the postament. In crassinucel-

late ovules the cells of the proximal region develop into the postament, which is a characteristic structure in Ranunculaceae (Batygina and Shamrov, 1999). Among the cells of the ES, the persistent antipodals exhibit the greatest variation (Hasitschka-Jenschke, 1962; Ūnal et al., 1997). In the family Ranunculaceae, two types of polyploidization have been reported in antipodal cells. Polyploidization may result from disturbed mitosis, as, for example, in a few species of *Anemone* (Hasitschka-Jenschke, 1959; Trela, 1963; Ūnal, 1978; 1984), or from endomitosis as in *Ranunculus peltatus* (Turała-Szybowska, 1980) and *Aconitum* (Tschermak-Woess, 1956). Endoreduplication has also been observed in some representatives of the Ranunculaceae: for example, in four species of *Aconitum* (Tschermak-Woess, 1956), in *Helleborus niger* (Hasitschka-Jenschke, 1959), *Ranunculus peltatus* (Turała-Szybowska, 1980) and *R. baudotii* (Wędzony, 1982). The repeated endoreduplication cycles lead to the formation of $1024n$ antipodals in *R. baudotii* and in *Aquilegia vulgaris* (Wędzony, 1982; Turała Szybowska and Wolańska, 1989). The antipodals of *Consolida regalis* showed rhythmical growth, and attained $256n$ as a result of endoreduplication.

The presence of low amounts of cytoplasmic RNA, polysaccharides, proteins and lipids suggests that the cells of the egg apparatus are metabolically inactive in *Consolida regalis*. The cytoplasm of antipodal cells is basophilic, being rich in ribosomal RNA. The very big nucleolus and cytoplasm are stained intensely for RNA. The large nucleus is also densely stained by methyl green for DNA. All these features suggest high metabolic activity in the antipodals.

The endosperm is nuclear as in other Ranunculaceae, and becomes cellular by centripetal wall formation. Chitralakha and Bhandari (1993) saw dictyosomes and their vesicles as well as ER and occasional arrays of microtubules in conjunction with the freely growing anticlinal walls during cellularization.

Embryo development is regular and follows the Solanad type in *Consolida regalis*. The variation in embryogeny found in most representatives of Ranunculaceae was not observed in this species. Reduced embryos without the usual organization into a radicle plumule and cotyledons have also been described in other taxa of Ranunculaceae (Natesh and Rau, 1984).

REFERENCES

- BANCROFT JD. 1996. *Theory and practice of histological techniques*. Churchill Livingstone, New York.
- BATYGINA TB, and SHAMROV II. 1999. New approach to interpreting the ovular basic structures. *Phytomorphology* 49: 223–231.
- BHANDARI NN. 1962. Studies in the family Ranunculaceae. III. Development of the female gametophyte in *Adonis annua* L. *Phytomorphology* 12: 70–74.
- BHANDARI NN. 1963. Studies in the family Ranunculaceae. V. The female gametophyte of *Adonis aestivalis* L. A reinvestigation. *Phytomorphology* 13: 317–320.
- BHANDARI NN, and KAPIL RN. 1964. Studies in the family Ranunculaceae. VII. Two types of embryo sacs in *Trollius* Linn. *Beitrage zur Biologie Pflanzen* 40: 113–120.
- CHITRALEKHA P, and BHANDARI NN. 1993. Cellularization of free-nuclear endosperm in *Ranunculus sceleratus* Linn. *Phytomorphology* 43: 165–183.
- GREILHUBER J, DOLEZEL J, LYSAK MA, and BENNETT MD. 2005. The origin, evolution and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. *Annals of Botany* 95: 255–260.
- HASITSCHKA-JENSCHKE G. 1959. Vergleichende karyologische Untersuchungen an Antipoden. *Chromosoma* 15: 229–267.
- HASITSCHKA-JENSCHKE G. 1962. Notizen über endopolyploide Kerne im Bereich der Samenanlage von Angiospermen. *Österreichische Botanische Zeitschrift* 109: 125–137.
- JOHRI BM, AMBEGAOKAR KB, and SRIVASTAVA PS. 1992. *Comparative embryology of Angiosperms*. Springer Verlag, Berlin.
- KORDYUM EL. 1959. On the modes of reproduction of endosperm nuclei in *Nigella sativa*. *Ukrainskyi Botanichnyi Zhurnal* 14: 40–46.
- KOZIERADZKA-KISZKURNO M, and BOHDANOWICZ J. 2003. *Sedum acre* embryogenesis: Polyploidization in the suspensor. *Acta Biologica Cracoviensia Series Botanica* 45: 153–157.
- NATESH S, and RAU MA. 1984. The embryo. In: Johri BM [ed.], *Embryology of Angiosperms*, 377–434. Springer-Verlag, Berlin.
- PICCI V. 1964. Ricerche embriologiche sul genere *Talictum*. Embryologia di *Thalictum angustifolium* L. v. *flavum* L. *Giardino Botanica Hal* 71: 258–267.
- RAGHAVAN V. 1997. *Molecular embryology of flowering plants*. Cambridge University Press, Cambridge.
- SCHNEIDER H. 1981. Plant anatomy and general botany. In: Clark G [ed.], *Staining procedures*, 315–331. Williams and Wilkins, Baltimore, MD.
- TRELA Z. 1963. Embryological studies in *Anemone nemorosa* L. *Acta Biologica Cracoviensia Series Botanica* 6: 1–14.
- TSCHERMAK-WOESS E, and HASITSCHKA G. 1953. Veränderungen der Kernstruktur Während der Endomitose, rhythmistisches Kernwachstum und verschiedenes Heterochromatin bei Angiospermen. *Chromosoma* 5: 574–614.
- TSCHERMAK-WOESS E. 1956. Notizen über die Riesenkerne und "Riesenchromosomen" in den Antipoden von *Aconitum*. *Chromosoma* 8: 114–134.
- TURALA-SZYBOWSKA K. 1980. Endopolyploidy in the antipodals of *Ranunculus peltatus* Schrank. and *R. penicillatus* (Dumort.) Bab. *Acta Biologica Cracoviensia* 22: 163–173.
- TURALA-SZYBOWSKA K, and WĘDZONY M. 1981. Endopolyploidy in the antipodals of *Ranunculus subgenus Batrachium*. *Acta Societatis Botanicorum Poloniae* 50: 161–164.
- TURALA-SZYBOWSKA K, and WOLAŃSKA J. 1989. Two mechanisms of polyploidization of the antipodals in *Aquilegia*

- vulgaris* L. *Acta Biologica Cracoviensia Series Botanica* 31: 63–74.
- ULUCE N, and ÜNAL M. 1996. Embryological studies in *Adonis microcarpa* DC. *XIII National Biology Congress*, 17–20 September 1991. Istanbul, Turkey.
- ÜNAL M. 1978. Cytological and embryological studies on *Anemone pavonina* Lam. *Istanbul Üniversitesi Fen Fakültesi Mecmuası* 43: 185–208.
- ÜNAL M. 1984. Embryological studies on *Anemone coronaria*. *Istanbul Üniversitesi Fen Fakültesi Mecmuası* 49: 89–97.
- ÜNAL M, YILDIRIM C, and EKAL S. 1997. The development of embryo sac and mechanism of polyploidization in the antipodals of *Epimedium pubigerum* DC. *Bulletin of the Polish Academy of Sciences* 45: 101–105.
- VIJAYARAGHAVAN MR, and BHANDARI NN. 1970. Studies in the family Ranunculaceae: Embryology of *Thalictrum javanicum* Blume. *Flora* 159: 450–458.
- WĘDZONY M. 1982. Endopolyploidy and structure of nuclei in the antipodals and synergids of *Ranunculus baudotii* Godr. *Acta Biologica Cracoviensia Series Botanica* 24: 43–62.