

四数獐牙菜的胚胎学及其系统学意义^{*}

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Embryology of *Swertia tetraptera* Maxim. (Gentianaceae) and its systematic implication

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Abstract The embryology of *Swertia tetraptera* is reported for the first time. The anther is tetrasporangiate and its wall, of which the development conforms to the dicotyledonous type, is composed of epidermis, endothecium, middle layer and tapetum at the mature stage. The tapetum has dual origin and is similar to the glandular type. Cytokinesis is of the simultaneous type and microspore tetrads are mostly tetrahedral. Pollen grains are mostly 3-celled when shed. The ovary is bicarpellate and unilocular with four series of ovules. The fused margins of two carpels intensively protrude into the locule. The ovule is unitegmic, tenuinucellate and orthotropous. The development of embryo sac is of the Polygonum type. The development of endosperm conforms to the nuclear type and the embryogeny corresponds with the Physalis variation of Solanad type. Three antipodal cells persist at the mature embryo sac. The embryo is at the globular stage when seeds are released from the capsule. Compared with the other studied species in *Swertia*, *S. tetraptera* shows differences from them in three distinctive embryological traits: enlargement and protrusion of the fused margins of two carpels into the ovary locule; orthotropous ovules and a developed hypostase. These three characters were previously only found in the genus *Halenia* of the tribe Gentianinae in the Gentianaceae, and thus suggest that *S. tetraptera* should be related to *Halenia*. The results partly correspond with the molecular evidence (Yuan & Kupfer, 1995). The monophyletic *Halenia* clade in Yuan & Kupfer's molecular cladogram includes *Halenia*, *S. tetraptera* and *Frasera* (section *Frasera* in *Swertia* s.l.); nevertheless, up to now, no embryological study on *Frasera* has been reported. The embryological results imply that the taxonomic status of *S. tetraptera* needs to be reevaluated and the genus *Swertia* s.l. may not be a monophyletic group.

Key words *Swertia tetraptera*; Embryology; Systematics

The section *Heteranthos* T. N. Ho & S. W. Liu of *Swertia* (s.l.) includes one species, *S. tetraptera* Maxim. (Ho, 1988) or two vicarious species, *S. tetraptera* and *S. tetrapetala* (Ho *et al.*, 1994) distributed in NW and NE China. This entity was thought as an independent genus, *Anagalidium* Grisebach, separated from the genus *Swertia* (Ho & Shih, 1976; Grisebach, 1845). The ITS analysis indicates that *S. tetraptera* forms a monophyletic clade with *Halenia* and *Frasera* (section *Frasera* in *Swertia* s.l.), while a

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more typical representative of *Swertia* s. s. , *S. franchetiana* nests with *Gentianella-Cormastoma* branch (Yuan & Kupfer, 1995). The molecular information implies that it may be more reasonable to exclude *S. tetraptera* and the section *Frasera* from the genus *Swertia* s. l. . The present paper reports the embryology of *S. tetraptera* for the first time to provide some information for a better taxonomic treatment and for clarifying its systematic relationship.

1 Materials and methods

Buds, flowers and fruits at different stages of development were collected from Menyuan County of Qinghai Province, China. Materials were fixed in the formalin-acetic-alcohol (FAA), stained with Ehrlich's hematoxylin diluted solution for three days, and dehydrated and embedded in usual way. Sections were cut at 6~10 μm . Photographs were taken under Olympus BH2 microscope.

2 Observation and result

2.1 Microsporangium, microsporogenesis and male gametophyte

The anther was tetrasporangiate. The archesporium was hypodermic and underwent a periclinal division, resulting in a primary parietal layer and a primary sporogenous layer (Plate : 1). The former divided periclinally to form two layers (Plate : 2), of which the inner one functioned as the main origin of the tapetum, while the outer underwent another periclinal division, resulting in an outer endothecium and a middle layer. The wall formation conformed to the dicotyledonous type (Davis, 1966). The anther wall at the mature stage was made up of four layers: epidermis, endothecium, middle layer and tapetum (Plate : 3). The epidermis persisted at maturity and the endothecium developed fibrous thickenings (Plate : 9). The tapetum was of dual origin, mainly coming from the primary parietal layer, and partly from the ground tissue near the connective tissue (Plate : 3). Some tapetal cells were binucleate or trinucleate and showed radial elongation and protruded into the anther locule at the tetrahedral stage (Plate : 4). The tapetal cells degenerated at their original sites following microsporogenesis (Plate : 7). Therefore, the tapetum was approximately of the glandular type.

Microsporocytes (Plate : 3) originating from the primary sporogenous layer and the secondary sporogenous cells (Plate : 1~2) underwent meiosis resulting in tetrahedral microspore tetrads by centripetal furrowing (Plate : 4~6). The microspore divided to form a large vegetative cell and a smaller generative cell (Plate : 8), and the generative cell underwent a further division resulting in two sperms (Plate : 10). Pollen grains were shed at this stage. At this time, most ovules were at the 4-nucleate embryo sac stage; flowers were protandrous.

2.2 Megasporangium and female gametophyte

The ovary was superior and bicarpellary, syncarpous, and the fused margins of two carpels enlarged and intensively protruded into the ovary locule with four lines of orthotropous ovules (Plate : 8). Ovules were tenuinucellate (Plate : 1), unitegmic and orthotropous (Plate : 10). A single hypodermic archesporial cell was differentiated in the young nucellus and functioned directly as the megaspore mother cell (Plate : 1). We did not find the megaspore tetrad, but at the 2-nucleate embryo sac stage, three degenerating megaspores were detected at the micropyle end (Plate : 2). The two daughter nuclei of the functional megaspore underwent two successive mitotic divisions resulting in the formation of 4-nucleate (Plate : 3~4) and 8-nucleate embryo sac (Plate : 5~7). The egg apparatus

consisted of two synergids and an egg. Two polar nuclei fused into the secondary nucleus before fertilization (Plate : 8 ~ 9). The development of the embryo sac followed the Polygonum type.

Unlike the case in most angiosperms, the three antipodal cells here observed were not ephemeral at the 8-nucleate embryo sac stage (Plate : 8 ~ 9). Free nuclear divisions took place in each cell before fertilization. These antipodal cells enlarged considerably and each of them had a prominent nucleus and dense cytoplasm (Plate : 7). Even at the four-celled proembryo stage, we still found some traces of the antipodal cells (Plate : 5). Most antipodal cells began to degenerate at the stage when zygote came into division and disappeared completely after the wall formation took place among free endosperm nuclei at the polycellular proembryo stage.

A hypostase appeared at the late stage of 4-nucleate embryo sac and disappeared at about polycellular proembryo stage. It contained a group of special cells with dense cytoplasm and especially with very thickened walls and bordered directly on the persistent antipodal cells after the gametophyte was mature (Plate : 7). The hypostase began to degenerate after all antipodal cells were absorbed.

2.3 Endosperm and embryo

The development of the endosperm is of the nuclear type (Plate : 3, 5). The zygote divided transversely to form a terminal cell and a basal cell (Plate : 2). Both the terminal and the basal cell underwent further divisions giving rise to a linear proembryonal tetrad (Plate : 3). The eight-celled proembryo (Plate : 4) consisted of a linear row of eight cells from the third transverse division of the tetrad. Four cells from the basal cell underwent a vertical division to form a two-seriate suspensor, and four cells from the terminal cell contributed to the development of the entire embryo (Plate : 6). Therefore, the embryogeny conformed to the Solanad type (Hu, 1982). The third generation of the zygote consisted of a linear row of eight cells, and the cells from the top cell of the tetrad at last contributed to the formation of the stem tip and cotyledons. Therefore, the variation type was similar to the Physalis variation of Solanad type (Johanson, 1950).

Most seeds were shed from the capsule at the globular embryo stage (Plate : 6). The time's span between flowering to shedding seeds amounted to about 20 days. Before germinating, seeds must undergo a post maturing. The seed contained abundant endosperm. The epidermis of the integument remained to form the seed coat while the inner layers were crushed and absorbed with the development of the embryogeny.

3 Discussion

As regards the embryological features of *Swertia* s.l., there are four species (*S. carolinensis*, *S. corymbosa*, *S. angustifolia*, *S. minor*) concerning to earlier reports (Rao & Nagara, 1982; Maheshwari & Laksjminarayana, 1977; Rao, 1975). The present study and our recent work on the embryology of *S. franchetiana* and *S. erythrosticta* (to be published) show that these species of *Swertia* s.l. share the following embryological characters: tetrasporangiate anthers, dicotyledonous type of anther wall development, glandular tapetum, simultaneous cytokinesis in the microsporocytes, unitegmic, tenuinucellate ovules, Polygonum type of embryo sac development, nuclear endosperm, Solanad type of embryogeny, two or four seriate-celled suspensor and endosperm seed. The above-mentioned features have also been found in other genera (Liu & Ho, 1997, 1996a, b; Li & Wang, 1994) and conform to the embryological framework of the tribe Gentianinae in the Gentianaceae (Rao & Nagara, 1982).

However, *S. tetraptera* displays three traits of its own distinctive from the other

species of *Swertia* s.l. , which are rarely found even in the whole Gentianinae and are thus of systematic significance. Firstly, the fused margins of two carpels enlarge and intensively protrude into the ovary locule. Second, all ovules are orthotropous and thus different from the anatropous ovules reported in the other taxa. Third, a hypostase tissue exists from the two-nucleate embryo sac to polycellular proembryo stage. These characters suggest that it should be better to exclude *S. tetraptera* and its allied species *S. tetrapetala* from the genus *Swertia* s.l. , and bestow them a generic rank, because all these characters are usually used to circumscribe taxa above the generic rank (Töbe, 1989). Up to now, besides in the species under question, these three characters have only been found in the genus *Halenia* of Gentianinae (Xue *et al.*, to be published).

These three embryological characters might be evolutionarily advanced, for they are uncommon in the angiosperms and might have derived from more common states with more special functions (Töbe, 1989). The three synapomorphies of *S. tetraptera* and *Halenia* indicate a close relationship between them. They may represent a small monophyletic branch in Gentianinae and may have come from a common ancestral group. The results partly correspond with ITS sequence phylogeny (Yuan & Kupfer, 1995). In the molecular cladogram, *S. tetraptera*, *H. elliptica* and *Frasera speciosa* form a monophyletic clade while *S. frachetiana*, a more typical species in *Swertia* s.l., nests in *Gentianella-Comastoma* branch. Nevertheless, up to now, no embryological study on *Frasera* has been reported. The embryology of *S. tetraptera* reported here, combined with the molecular data, shows that the genus *Swertia* s.l. may be a paraphyletic group and the taxonomic status of *S. tetraptera* needs to be reevaluated. However, we hesitate to make a final taxonomic decision before new and stronger evidence is obtained and the evolutionary trends of the gross morphology are finally elucidated.

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Explanation of plates

ANT. Antipodal cells; DM. Degenerated megaspores; E. Egg cell; EP. Epidermis; EN. Endothecium; M. Middle layer; Pn. Polar nucleus; Sp. Sperm. Sy. Synergid cell; T. Tapetum

Plate 1. Archesporial cells, primary parietal cells and primary sporogenous cells. 2. The dividing of the primary parietal layer, and of the secondary sporogenous cells. 3. Anther wall, the arrow showing the tapetum originating from the ground tissue near the connective tissue. 4. The arrow indicating the elongating and protruding of the 2 ~ 3-nucleate tapetum cells at the tetrahedral stage. 5. Anaphase of meiosis in a microsporocyte. 6. Tetrahedral microspore tetrad. 7. Showing the degenerating of the tapetum cells at the single-nucleate stage, noting the central tapetum cells (arrow) being from the early differentiation protruding (see Plate : 4). 8. Bicellular pollen grain showing the vegetative and generative cells. 9. The persistent epidermis as well as thickened endothecium. 10. 3-celled pollen grain. (1. $\times 824$; 2 ~ 4. $\times 768$; 7. $\times 652$; 9. $\times 448$; 5 ~ 6, 8, 10. $\times 1920$).

Plate 1. Megaspore mother cell. 2. 2-nucleate embryo sac, showing three degenerated megaspores. 3 ~ 4. Successive sections of a 4-nucleate embryo sac. 5 ~ 7. Successive sections of a 8-nucleate embryo sac. 8 ~ 9. Successive sections of a mature embryo sac. 10. Showing the unitegmic and orthotropous ovule. (1 ~ 9. $\times 768$; 10. $\times 136$).

Plate 1. Zygote. 2. 2-celled proembryo; 3. Linear 4-celled proembryo. 4. Linear 8-celled proembryo. 5. Free endosperm nuclei. 6. Globular embryo when seeds shed. 7. Showing the hypostase and persistent antipodal cells at the mature embryo sac. 11. Showing the intensive protruding of the fused carpels and four lines of ovule. (1 ~ 4. $\times 768$; 5. $\times 270$; 6. $\times 384$; 7. $\times 540$; 8. $\times 86$).

摘要 四数獐牙菜花药四室,药壁发育为双子叶型;绒毡层腺质型。小孢子母细胞减数分裂为同时型,四分体的排列方式为四面体形;成熟花粉为3-细胞。子房2心皮,2心皮连接处强烈内凸,4列胚珠。薄珠心,单珠被,直生胚珠。胚囊发育为蓼型。胚乳发育为核型。胚胎发育为茄型酸浆变型。反足细胞在胚囊成熟时期宿存。果实成熟时,种子只发育至球型胚阶段。承珠盘存在。与已研究的獐牙菜种类的胚胎特征相比较,该种存在三个显著的特点:直生胚珠,心皮连接处强烈内凸,承珠盘存在。这三个特征目前仅在龙胆族中的花锚属中发现。经过性状分析,它们均是演征,此三个共有演征表明四数獐牙菜与花锚属是一单系群,它们的亲缘关系最近。这与 Yun & Kupfer (1995)的分子证据部分吻合,因为分子系统树上的花锚单系支包括花锚、四数獐牙菜和北美的密花属(广义獐牙菜属中的密花组),而后的胚胎学特征未有任何报道。胚胎学研究暗示四数獐牙菜的分类学等级有待重新评价,广义的獐牙菜属有可能不是一单系发生群。

关键词 四数獐牙菜;胚胎学;系统学