FEMALE GAMETOPHYTE DEVELOPMENT AND EMBRYOGENESIS IN *TAXUS BACCATA* L.

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ABSTRACT

Crassinucellate ovules are initiated in *Taxus*, directly from the shoot apex. The rudimentary pollen chamber is formed in the nucellus. A linear tetrad of megaspores with a functional chalazal megaspore is formed. A free-nuclear stage is characteristic at the beginning of megagametophyte development. Archegonia without ventral canal cell are solitary or in complexes. The embryo has a very long suspensor even after maturation. Two types of polyembryony have been revealed: i) embryogenic redifferentiation of suspensor cells and ii) cleavage of embryonic region in the early embryo. In the northern temperate climate of St. Petersburg one month delay in development of reproductive structures has been noted.

KEY WORDS: *Taxus baccata* L., archegonium, megagametophyte, embryogenesis, suspensor.

INTRODUCTION

Conifers are a phylogenetically old group of plants. The genus *Taxus* is of special interest since the taxonomic position of the *Taxaceae* is widely debated. Some scientists strongly advocate excluding *Taxaceae* from the *Coniferales* order and changing its taxonomic status to a new order or even class, because of absence of seed cones (Chaw et al., 1993). In this connection, the study of embryological features, as the most conservative, is of importance. There are several reports concerning embryology of yew (Jäger 1899, Sterling 1948a, b, Favre-Duchartre 1958, Zenkteler, Guzowska 1970, Pennel, Bell 1987). Nevertheless, investigations of reproductive organs in yew, presented here, can not fail to be of interest since they concern embryological features attributed to individuals introduced in the northern, temperate climate of St. Petersburg.

MATERIAL AND METHODS

Ovules and seeds of *Taxus baccata* L. were collected in the Botanical Garden of the Russian Academy of Sciences in St. Petersburg during April-October, 1992-1995. The material was fixed in 7:7:100 (by vol.) formalin-acetic acid-ethanol mixture and in Carnoy's fluid, dehydrated in graded ethanol series, embedded in paraffin. Sections (10-12 µm) were stained by Feulgen reaction followed by counterstaining with

alcian blue. Staining was also performed with safranin in 50% ethanol and with alcian blue.

RESULTS

Ovules of *T. baccata* are formed at the base of shoot apex and take up a terminal position. An orthotropous crassinucellate ovule has a single integument (Fig. 1), the base of which develops later into an aril. Cells of the chalazal half of nucellus are more densely stained and have a smaller size than the cells of micropylar half. In the latter part of April, – beginning of May a small concentric area of sporogenous cells is differentiated in the chalazal part (Fig. 1). Cells of this area are deep-stained and have a high nuclear/cytoplasmic ratio with nucleus diameter 8-11 µm. One or more cells of the sporogenous complex may further become the megaspore mother cell(s).

After meiotic divisions megaspore mother cell transforms into a linear tetrad of the megaspores. In nucellus a few sporogenous cells enlarge, but only one tetrad develops. One, usually chalazal megaspore is functional (Fig. 2). However, Dupler (1917) and Sterling (1948a) reported that two or more megaspores may begin to germinate in *T. canadensis* and *T. cuspidata*, respectively. The functional megaspore enlarges, small vacuoles in its cytoplasm fuse into one large vacuole which occupies the central part of the cell pressing the nucleus against the cell membrane (Fig. 2). Soon after central vacuole is formed, the nucleus proceeds to synchronous mitotic

Fig. 1. Crassinucellate ovule during formation of sporogenous cells. x66.

Fig. 2. Concentric sporogenous complex in the center of nucellus. x264.

Fig. 3. Cellular megagametophyte with mature archegonia. Note free archegonia and archegonia in the complex surrounded by jacket cells. x66.

Fig. 4. The same that in Fig. 3; other section. x106.

Fig. 5. Fusion of sperm nucleus and egg nucleus in the center of the egg cell. x264.

Fig. 6. Zygote nucleus with large nucleolus in the bottom of the cell. x264.

Fig. 7. Four-nucleate proembryo inside an archegonium. x264.

Fig. 8. Three layers of deeply-stained embryonic cells dividing by anticlines. Secondary suspensor cells are large and lightly-stained. x264.

Fig. 9. Embryonic apex. Note visible spindle of anticlinal division in the outer layer and periclinal division in the inner layer. x264.

Fig. 10. Group of embryonic cells connected with long suspensor. Note the lateral group of embryonic cells (on the left) formed from suspensor. x106.

Fig. 11. Multicellular embryo apex with long secondary suspensor before promeristem formation and differentiation of cotyledons. x106.

Fig. 12. The same that on Fig. 13. x264.

Fig. 13. Dycotyledonous mature embryo with procambial strands. x66.

Fig. 14. Start of a cleavage process in an embryonic region. x264.

Fig. 15. Embryogenic redifferentiation of suspensor cells resulting in formation of two additional embryonic groups. x106.

Fig. 16. Polinucleate cells of a megagametophyte in the mature seed. x264.

Fig. 17. Large starch grains in the cells of megagametophyte in a mature seed. x264.

Fig. 18. More numerous starch grains in megagametophyte. x264.

divisions (Fig. 3). Nuclei in the free-nuclear gametophyte line up along the cell wall. A thin cytoplasmic layer occupies the peripheral region. Most of the nuclei in free-nuclear stage are situated in the chalazal part of the megagametophyte. After 7-8 synchronous divisions the megagametophyte becomes multicellular (diam. 250-280 μm).

Primary cells of the megagametophyte usually contain lightly stained cytoplasm and large vacuoles. Subsequent cell divisions give rise to the cells of meristematic appearance; such cells are seen in the whole gametophyte, but most frequently in the micropylar part. These cells divide periclinally and some become archegonial initials with large vacuoles.

An archegonial initial divides further forming a neck cell and a central cell. The latter transforms into the egg cell. The neck cell divides once or twice. The ventral canal cell, which is typical for conifers, was not found. The initial cells and archegonia are surrounded by small cells of the jacket (Figs 3, 4). There were 3-7 archegonia in the ovule. Archegonia matured in the end of June. Frequently, archegonia are joined in complexes (Figs 3, 4). The nucleus of the central cell is lightly stained, and cytoplasm is concentrated like a mantle around the nucleus. By this time, the pear-shaped gametophyte occupies a large part of nucellus, and is surrounded by 10-12 layers of gradually resorbed nucellar cells.

Pollination occurs in the end of April, fertilization takes place from the end of June till the middle of July. The rudimentary pollen chamber originates as a small hollow in nucellar tissue due to degradation of some cells (Fig. 1). Pollen tubes may germinate into nucellus at early stages of megagametophyte development in May until the appearance of proembryo in July. Frequently, several tubes can penetrate into nucellus.

After the pollen tube has met the egg cell, its content enters the egg cytoplasm. Sperm nucleus and egg nucleus fuse in the center of the egg cell (Fig. 5). Then fused nuclei with distinct boundary migrate toward the base of the cell (Fig. 6). The similar pattern of fertilization in *Taxus* was described by Sterling (1948a, b).

The zygote (diam. up to 80 μm) has a large nucleus with a well-defined nucleolus. The divisions of zygotic nucleus give rise to free-nuclear proembryo (Fig. 7). The following divisions result in enlargement of polynucleate proembryo accompanied by decrease of the size of the nuclei. Nuclei move to the base of the cell and are arranged in two tiers. Cell wall formation follows 4-5 synchronous divisions of the zygote. Konar and Oberoi (1969) describe wall formation in 8-16-nucleate proembryos in *Taxus*, although Jäger 1899, Sterling 1948b, Chowdhury 1963, reported 32 free nucleate proembryos.

After proembryo cell wall formation, the cells of the lower tier divide forming the third tier. The uppermost tier cells usually opened toward the micropyle, later degenerate. The middle tier gives rise to a long suspensor (Figs 10, 11). Cells of the lower tier divide twice forming two embryonic layers and a secondary suspensor (Fig. 8).

Embryonic cells are of meristematic appearance with considerably more dense cytoplasm than the secondary suspensor cells. At first, cells of the embryonic group divide mostly by the anticlinal walls (Fig. 8) extending surface of the embryonic apex. Then anticlinal and periclinal divisions alternate (Figs 9, 10), and the embryonic group increases. By this time the proembryo grows through the base of the archegonium base and between the megagametophyte cells, some of them with 2-4 small nuclei. These events may be considered as the end of the proembryo stage in *Taxus* (Sterling 1948b).

The embryo grows in a slit formed by dissolution of cells in the central and chalazal parts of megagametophyte (Figs 10, 11).

Two types of polyembryony were revealed in the present study: i) cleavage of embryonic region of early embryo (Fig. 14), and ii) embryogenic redifferentiation of suspensor cells (Figs 10, 15). Though several cleaved embryos are frequently found during early embryogenesis, only one embryo is usually contained in the mature seed. In rare cases there are two embryos in the mature seed.

Small embryonic cells have larger nuclei (10-13 μm) than bigger cells of the megagametophyte (6 μm). During the period of suspensor elongation, the nucellus is almost entirely resorbed. Due to apical growth of the embryo, the quantity of cells in embryonic group gradually increases. The length of embryonic group is approximately one fifth of the length of the suspensor (Figs 10, 11).

Cell divisions in the embryonic group result in formation of shoot and root apical meristem and procambial zone. Cotyledonary primordia develop from the subepidermal cells. In developing cotyledons, procambial strands are visible. They are connected with the procambial core in the mature embryo (Fig. 13).

The embryo in the mature seed of *T. baccata* is massive cylindrical dicotyledonous (to 1.5 mm in length). The suspensor is of the same length as the embryo. There is a large number of small starch grains in the embryo cortex, but none in the suspensor.

Cells of megagametophyte in the mature seed contain from 2 to 10 small free nuclei (Fig. 16). This phenomenon has been previously reported in *Taxus* spp. (Sterling 1949, Zenkteler, Guzowska, 1970). The cells of the mature megagametophyte contains numerous starch grains (Fig. 17).

Mature embryos in seeds of *T. baccata* were found in September. The embryo develops for 3-4 months.

DISCUSSION

The data are principally in agreement with those obtained previously on megagametophyte development and embryo formation in *T. baccata*. Some differences are related to the temporal traits of development. On the average, one month delay in development of reproductive structures has been noted. This is attributable to the cold climate of the St. Petersburg region.

An absence of seed cones, solitary situated ovules, formation of rudimentary pollen chamber in the nucellus, absence of ventral canal cell in the archegonia are all the peculiar features of megagametophyte development in *T. baccata*.

It is interesting that archegonia shown in the present study may be either simple or in complexes scattered all over the gametophyte.

A free-nuclear stage (16-32 free nuclei), degenerating relic nuclei and two types of polyembryony are typical in *Taxus*.

These observed facts and the short period of gametophyte and embryo development may be considered as evolutionary advanced features of reproductive processes. However, this is presumably inadequate to exclude *Taxaceae* from the *Coniferales*.

The data on female gametophyte development and embryogeny in *T. baccata* as characteristic of the "zygotic reference model" (Durzan 1988), we consider them in the elaboration of a protocol for in vitro somatic embryogenesis. The embryonic cells of yew give no evidence yet of having embryogenic competence in vitro. Stable-growing embryogenic cultures of *Taxus* might be useful for producing antitumour diterpenoid taxol (Heinstein, Chang 1994).

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ROZWÓJ ŻEŃSKIEGO GAMETOFITU I EMBRIOGENEZA *TAXUS BACCATA* L.

STRESZCZENIE

U *Taxus*, gruboosrodkowe zalążki powstają bezpośrednio na szczycie pędów. W ośrodku tworzy się komora pyłkowa, a po mejozie linearna tetrada megaspor z funkcjonującą megasporą chalazalną.

Rozwój gametofitu żeńskiego zaczyna się od stadium wolnojądrowego, potem w komórkowym stadium powstają rodnie z komórkami kanałowo-brzusznymi leżące pojedynczo lub w grupach. Zarodek ma długi suspensor zachowujący się nawet w stanie dojrzałości. Wyróżniliśmy dwa typy poliembrionii; z odróżnianych komórek suspensora oraz z rozszczepienia młodych zarodków. Zauważyliśmy jednomiesięczne opóźnienie rozwoju. Rozwój organów rozmnażania jest spóźniony około jednego miesiąca w warunkach klimatu St. Petersburga.

SŁOWA KLUCZOWE: *Taxus baccata*, rodnie, gametofit żeński, embriogeneza, wieszadełko.