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CRITICAL STAGES OF OVULE AND SEED DEVELOPMENT

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A study of *Gentiana cruciata* L. (Gentianaceae), *Gymnadenia conopsea* (L.) R.Br. (Orchidaceae) and *Luzula pedemontana* Boiss. et Reut. (Juncaceae) showed differences in the number and characteristics of critical stages in ovule and seed development. The shared critical stages explain the general direction of the formation of reproductive structures and surrounding tissues. The taxon-specific critical stages may have different implications in a given species: they may (1) verify that the ovule belongs to a specific type, (2) indicate their lability in different taxa with the same ovule type, or (3) coincide in species with various ovule types.

Key words: Ovule, seed, development, histochemistry, critical stages.

INTRODUCTION

Earlier we studied some structural and histochemical aspects of the developing ovule in several species belonging to various families and noted differences in their structure (Shamrov, 1990, 1991, 1996, 1999; Shamrov and Nikiticheva, 1992; Shamrov and Anisimova, 1993a,b). The dynamics of some metabolites (proteins, carbohydrates, including starch and dextrins in the cytoplasm; insoluble polysaccharides and lignin in the cell wall; tannins in the cytoplasm) and the character of metabolism in particular ovular tissues were suggested as indicators of metabolite flow (Shamrov, 2000).

This paper analyzes the interrelations between the character of substance accumulation and the structure of ovular tissues during stages of structural-functional reorganization (critical or crucial stages).

MATERIALS AND METHODS

Three species were studied: *Gentiana cruciata* L. (Gentianaceae), *Gymnadenia conopsea* (L.) R.Br. (Orchidaceae) and *Luzula pedemontana* Boiss. et Reut. (Juncaceae). The material was investigated by traditional histochemical methods (see: Shamrov, 2000).

RESULTS

All species investigated have a paracarpous gynoecium and anatropous ovules. However, they differ in structure, metabolite distribution, and also the number and characteristics of periods and critical stages during development.

In *Gentiana cruciata* the gynoecium consists of two carpels. The ovary is superior and unilocular. The ovular primordia develop on four parietal placentae. The ovule is tenuinucellate, unitegmic, mesochalazal and sessile, with endothelium, ephemeral hypostase and procambial cells in the raphe. The archesporial cell transforms into the megasporocyte without cutting off the parietal cell. The megaspore tetrad is linear. The megagametophyte develops from the chalazal megaspore, following the Polygonum type. The endosperm is nuclear. The embryo develops by the Solanad type, and is well-differentiated into organs in the mature seed. In

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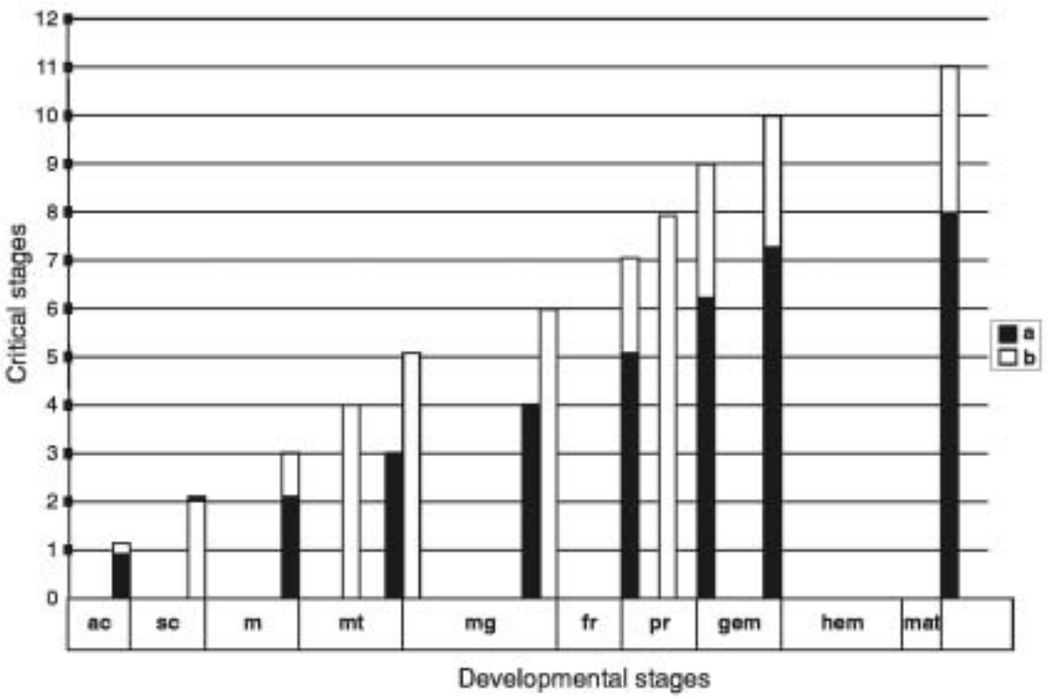

Fig. 1. Critical stages in ovule and seed development of *Gentiana cruciata* (**a**) and *Luzula pedemontana* (**b**). ac – archesporial cell; fr – fertilization; gem – globular embryo; hem – heart-formed embryo; m – megasporocyte; mat – maturation; mg – megagametophyte; mt – megaspore tetrad; pr – proembryo; sc – sporogenous cell.

ovule and seed development we could distinguish the following periods: (1) ovule initiation on the placenta; (2) differentiation of the ovular primordium with the megasporocyte; (3) formation of the main ovule structures and a linear tetrad of megaspores; (4) total destruction of the nucellar cells and formation of the endothelium; (5) fertilization, the first nuclear divisions in the endosperm and the beginning of cell destruction in the endothelium; (6) onset of cellularization in the endosperm and almost total destruction of the endothelium and hypostase; (7) completion of endosperm cellularization and the beginning of cell destruction in the integumental parenchyma; and (8) almost total cell destruction in the integumental parenchyma and chalaza. Corresponding to the periods, the critical stages, which are characterized by structural-functional reorganization in the development of the megagametophyte, embryo and surrounding structures of the ovule, seed and fruit, are as follows: (1) archesporial cell, (2) megasporocyte before meiosis, (3) megaspore tetrad**, (**4) developing megagametophyte, (5) zygote, (6) early globular embryo, (7) late globular embryo, and (8) mature embryo (Fig. 1a).

In *Gymnadenia conopsea* the gynoecium consists of three carpels. The ovary is inferior and unilocular. The ovular primordia grow on the three parietal placenta ridges. The ovule is medionucellate (according to Shamrov, 1998), bitegmic, leptochalazal and funicular, with the postament and ephemeral hypostase, and no vascular tissue. The archesporial cell does not divide and transforms into the megasporocyte. Megasporogenesis ends with the formation of a linear or T-formed megaspore tetrad. The megagametophyte develops from the chalazal megaspore according to the modified Polygonum type (egg cell, two synergids, central cell with two polar nuclei, and one antipodal cell). After fertilization the nucleus of the primary endosperm cell does not divide. The embryo develops according to the Onagrad type, is globular, and is not differentiated into organs in the mature seed. The ovule and seed are characterized by a more dynamic system of developmental structures, and substance accumulation fluctuates in them; as a result they have more critical stages, making it very difficult to distinguish the periods of development. Structural and histochemical investigations revealed eleven critical

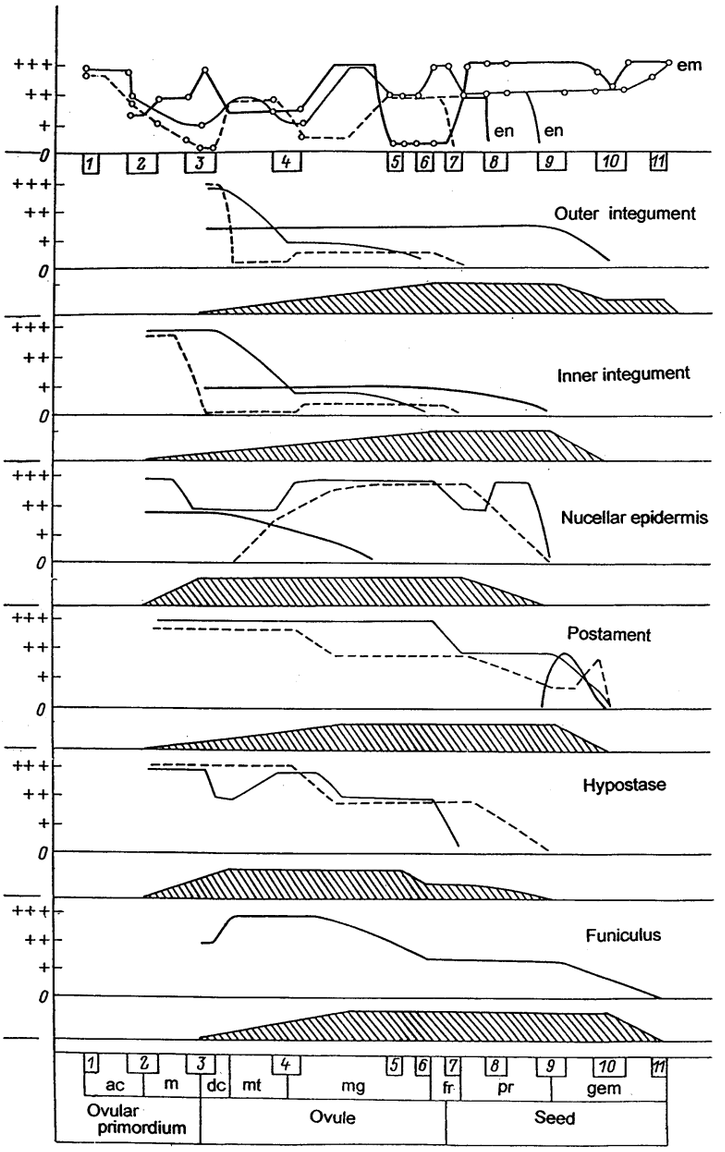

Fig. 2. Critical stages in ovule and seed development of *Gymnadenia conopsea*. Starch shown as thick line, proteins as thin line, dextrins as dashed line; hatched areas show dynamics of developing structures (relative time of formation, development maximum, beginning of degeneration and complete disappearance). ac – archesporial cell; dc – dyad of cells; em – embryo; en – endosperm; fr – fertilization; gem – globular embryo; m – megasporocyte; mg – megagametophyte; mt – megaspore tetrad; pr – proembryo.

stages in development of the ovule and seed of *G. conopsea*: (1) archesporial cell, (2) megasporocyte before meiosis, (3) megasporocyte at beginning of meoisis, (4) functional megaspore, (5) megagametophyte following cellularization, (6) mature megagametophyte, (7) zygote and primary cell of endosperm, (8) two-celled proembryo, (9) octant stage of proembryo, (10) early globular embryo, and (11) late globular **(**mature**)** embryo (Fig. 2**).**

In *Luzula pedemontana* the gynoecium consists of three carpels. The ovary is superior and unilocular. The ovular primordia develop on basal placentae. The ovule is crassinucellate, bitegmic, mesochalazal and sessile, and has a postament, po-

TABLE 1. Differences in dynamics of substance accumulation in *Gentiana cruciata* and *Swertia iberica* during critical stages of developing ovules

Critical stages	Ovule and ovary structures	*Gentiana cruciata*			*Swertia iberica*		
		starch	dextrines	proteins	starch	dextrines	proteins
1.	Archesporial cell	++++	0	0	++	0	++++
	Ovular primordium		0	+++		0	+++
	epidermis	+++					
	subepidermis	+++			++		
	Placenta	+++	0	+++	++	0	++
	Ovary wall	++++	0	+	++++	0	+
2.	Megasporocyte	++	0	+	+	0	++++
	Ovular primordium						
	Sites of initiation						
	Nucellus	0	0	++++	0	0	++++
	Integument	0	0	++++	++	0	++++
	Chalaza	+++	0	+	++	0	0
	Hypostase	+++	++	++	++	0	++++
	Axial cell row	0	+++	++++	0	+	++++
	Placenta	++	0	+	0	0	+
	Ovary wall	+	0	0	+++	0	0
3.	Megaspore tetrad	++	0	+	+	0	+
	Nucellus	0	+++	+	0	+++	+
	Integument	++++	0		+++	0	
	outer epidermis			+			++
	endothelium			++++			++++
	parenchyma			0			0
	Chalaza	++	+	+	+++	0	0
	Hypostase	0	++	++++	+	+	++++
	Raphe	+++	+++	0	++	+	0
	procambial cells	+	+	0	+	+	0
	Placenta	+	0	0	+	0	0
	Ovary wall	0	0	0	++	0	0
4.	Developing megagametophyte	++	0	++	0	0	++
	Mature megagametophyte		0				
	egg cell	+		0	+	0	+
	synergids	0		++++	0	0	+++
	antipodals	0		+	0	0	++++
	central cell	+++		0	++	0	0
	Integument						
	outer epidermis	++++	0	0	+++	0	0
	endothelium	0	+	++	0	++	++
	parenchyma	0	0	0	++	++	0
	Chalaza	+	0	++	++	0	0
	Hypostase	0	+++	0	0	+++	++
	Raphe	++	0	+	+	+	0
	procambial cells	+	0	+	+	+	+
	Placenta	+++	0	+	+	+	0
	Ovary wall	+	0	+	+	0	0

Levels of substance content in the cells – very high (++++), high (+++), average (++), low (+), lacking (0).

dium, nucellar cap, operculum, persistent hypostase and well-differentiated vascular bundle. The archesporium is one-celled, rarely two-celled. It transforms into the megasporocyte with the cutting off of the parietal cell. Meiosis ends in the formation of the linear tetrad. The megagametophyte develops by the Polygonum type. The endosperm is helobial. Embryogenesis conforms to the Onagrad type. The mature embryo is small and well-differentiated into organs. We could distinguish the critical stages as

well as the periods in its development. The latter are as follows: (1) ovular primordium formation; (2) initiation of ovular structures during formation of the sporogenous cell; (3) formation of ovular structures during transformation of the sporogenous cell into the megasporocyte; (4) initiation of the podium and postament in the nucellus at the onset of meiosis; (5) formation of the podium and postament during the completion of megasporogenesis; (6) specialization of different structures (vascular bundle and placental obturator) during the coenocytic phase of megagametophyte development; (7) fertilization and first nuclear divisions in the endosperm; (8) completion of nuclear divisions in the endosperm, destruction of the cells of the outer epidermis of the inner integument, and initial lignification in the hypostase cells; (9) onset of cellularization in the endosperm and destruction of the nucellar cells of the postament and those around the chalazal chamber of the helobial endosperm; (10) completion of cellularization in the endosperm and almost total destruction of the nucellus, except for the epidermal layer, podium and parietal tissue; and (11) maturation of the seed and destruction of the apical cells of the podium. The critical stages in ovule and seed development are these: (1) archesporial cell, (2) sporogenous and parietal cells, (3) megasporocyte before meiosis, (4) megasporocyte at prophase I, (5) functional megaspore, (6) mature megagametophyte, (7) zygote, (8) two-celled proembryo, (9) early globular embryo, (10) late globular embryo, and (11) mature embryo (Fig. 1b).

DISCUSSION

Ovules and seeds are integral dynamic systems. Structural and functional interrelationships between their elements (nucellus, integuments, megagametophyte, embryo and endosperm) define the specific character of embryo development and seed reproduction. To appreciate the concordance of morphogenetic and morphophysiological processes in the development of the embryo and surrounding tissues of the seed, we apply the terms "critical periods," "critical stages" and "critical points" (Vasilyeva et al., 1987; Shamrov, 1990, 1991; Batygina et al., 1992). The term "developmental stage" is most often applied to describe embryological processes. In this connection we use the term "critical stage," as done earlier (Vasilyeva et al., 1987), to mean an interval of time characterized by structural-functional reorganization in ovule and seed development. The more prolonged interval during which the developing structures remain relatively constant in character, is designated by the term "period" (Shamrov and Nikiticheva, 1992; Shamrov and Anisimova, 1993b; Shamrov, 1996).

Existing notions (Batygina et al. 1992) about common and taxon-specific critical periods (stages) are developed further. Our investigation has shown that the species studied differ in both the number and characteristics of the critical stages in ovule and seed development. Above all, development is conditioned by variation in ovule and seed structure, differences in the dynamics of metabolite accumulation, and possibly by peculiarities of substance transport. More complex ovules and seeds, as defined by the number of constituent elements and the massiveness of the structures, are characterized by a large number of critical stages in their development, and by the fluctuation of the transitions from stage to stage. Therefore, based on the data available, we can conclude that the critical stages shared by crassi-, tenui,- and medionucellate ovules (archesporial cell, megasporocyte before meiosis, early and late globular embryos, mature embryo) explain the general direction in the formation of reproductive structures and surrounding tissues.

The implications of taxon-specific critical stages may differ from one species to another. Some of them verify that the ovule belongs to a specific type. In *Luzula pedemontana* the ovule is characterized by very massive structures (it is a crassinicellate, bitegmic and mesochalazal ovule), which as a rule begins to degenerate at the middle stages of embryogenesis. Its specific critical stages are stages 2 and 8. During critical stage 2 the sporogenous and parietal cells are formed. At the time of stage 8 (two-celled proembryo), proteins and dextrins are accumulated in the parietal tissue through which the apical transport route to the developing embryo is established (Shamrov, 2000).

In the medionucellate, bitegmic and leptochalazal ovule of *Gymnadenia conopsea*, processes of destruction of structures occur during megagametophyte maturation. For that reason, one of the specific critical stages is stage 5 (megagametophyte following cellularization), when the outer hypostase cells begin to deteriorate and starch grains nearly disappear from the somatic tissues of the ovule. Another critical stage in the ovule development of *G. conopsea* is stage 8 (two-celled proembryo), which is connected with the degeneration of the nucellar epidermis and inner hypostase cells.

In the tenuinucellate, unitegmic and mesochalazal ovule of *Gentiana cruciata*, processes of transformation of structures take place at a still earlier developmental period. Stage 3 (megaspore tetrad) is considered to be a specific critical stage in this species. The initial degeneration of the nucellus is correlated with the formation of procambial cell rows in the raphe and the subsequent increase in transport of metabolites through the vascular bundle and their accumulation in most cells of the ovule.

On the other hand, species with various ovule types can have the same specific critical stages. The two-celled proembryo provides an example. In *Gymnadenia conopsea*, degeneration of structures (nucellar epidermis and the inner layer of the hypostase) occurs, whereas in *Luzula pedemontana* the structures show no signs of destruction, and metabolism increases in intensity. There are also specific critical stages that show lability in different taxa with the same ovule type. For example, the tenuinucellate, unitegmic and mesochalazal ovules of *Gentiana cruciata* and *Swertia iberica* from the Gentianaceae (Shamrov, 1990, 1991) show various dynamics of substance accumulation during development (Tab. 1). The ovule of *S. iberica* (archesporial cell, megasporocyte and megagametophyte) contains more proteins, while that of *G. cruciata* has more starch. These differences in substance accumulation appear to be connected with metabolite transport. Metabolism in *G. cruciata* relies chiefly on carbohydrates, whereas protein metabolism is predominant in *S. iberica*.

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