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Structural differentiation of the ovule and seed and its importance for reproduction in angiosperms

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Summary: Gaining knowledge about the peculiarities of seed formation is impossible without detailed embryological information about the initial stages of ovule formation. The general pattern of ovular primordium structure is the presence of five zones: peripheral, apical, basal, lateral and transitional. Each zone gives rise to a particular ovular region, the degree of development of which varies among taxa, and results in a variety of ovule types. On the basis of typification principles developed, the revision of existing classifications by nucellus, integument, chalaza and funiculus is performed. A system of various structures and specialized tissues creates a targeted transport of metabolites in the ovule – into embryo sac from vascular bundle through chalaza and then via hypostase, podium, postament, integumentary tapetum, nucellar epiderm and parietal cells. A number of such structures of the ovule and seed (funiculus, obturator, micropyle, micropylar collar, operculum, nucellar cap, aril) play an important role in the process of pollination and fertilization as well as in the dissemination and germination of seeds. In multi-seeded fruits, aberrant seeds revealing various morphogenetic deviations are often found. In such seeds, disturbances in the metabolite transport were detected. The occurrence of aberrant seeds in the fruits results in seed productivity decrease.

Keywords: ovule, seed, ovular primordium, nucellus, integument, chalaza, funiculus, obturator, angiosperms

The rise of the seeds provides the propagation and spreading of plants and the maintenance of species in general. Seed is the general result of yesterday's harvest and the promise of tomorrow's. It is a very complicated and still unstudied in many respects of its biological system. Seed includes three genetically distinct compartments: an embryo (sporophyte of new generation), nutritive storage tissue (endosperm, perisperm) surrounded by the seed coat (Boesewinkel & Bouman 1984; Teryokhin 1996; Marzinek & Mourão 2003; Consonni et al. 2005; Shamrov 2008; Kamelina 2009, 2011; Morozowska et al. 2011; Geisler et al. 2017; Rutishauser 2020).

First works considering ovule and seed have appeared already in the 17th century, but it still keeps a lot of undiscovered mysteries. The fundamental investigations of its genesis could undoubtedly contribute significantly to problem-solving in theoretical botany, morphology and embryology. The peculiarities of ovule and seed structure as well as a number of another embryological characteristics (the number of cotyledons in embryo, types of embryo and endosperm development, types of microsporangium wall formation, pollen grain and tapetum structure etc.) are used as relevant criteria for clarification of disputable questions in systematics, phylogeny and evolution of flowering plants (Davis 1966; Dahlgren 1991; Endress & Igersheim 1997; Takhtajan 1997, 2009; Endress 2003, 2011, 2015; Matthews & Endress 2005; Kamelina 2009, 2011; Vinogradova 2017; Titova et al. 2018; and others).

The elaboration of theoretical bases of plant seed propagation and the revealing of reproductive system plasticity are the guarantee of successful use of new non-standard technologies in genetics, breeding and seed-farming. In view of the practical need of introduction of a number of plants

into the new habitats, it is extremely important to reveal the reasons causing disturbances in course of reproductive processes and leading to a decrease of real seed productivity. One of the reasons is the action of the aggressive environmental factors (heavy metal salts, SO_2, NO_2, HF, O_3 etc.). The reaction of plants to these actions is mostly expressed in the critical periods of ontogenesis. Analysis of the aberrant ovules and seeds is of special interest for appreciation of ovule fertility and sterility. The high-yielding forms of plants could be selected by the quality of seeds they form. It is especially relevant upon the distant hybridization, when the obtaining of viable hybrids could be quite difficult.

At the present time, a lot of facts have been collected, requiring the systematization and comprehension. The diversity of ovule structures often does not fit into the frameworks of existing classifications. In this connection, the problems of searching and identification of new embryological features and characterizing the ovule morphogenesis arise again. A number of theoretical works available today is helpful in solving this problem. The system approach (Vasilyeva et al. 1987; Grefen & Harter 2004; Shamrov 2008, 2018) and its modification known as the integral approach (Wojtaszek 2000) are widely used in biological researches. This approach directs to the revealing of integral properties of the objects and the mechanisms providing them, to the establishing of diverse connections of a complex biosystem and to the working out of an efficient strategy of studying it.

Organization and typification of the ovules and seeds. The ovule of angiosperms is an organ comprising the nucellus, integuments, chalaza and funiculus. The events of archesporium differentiation, megasporogenesis and embryo sac formation take place in it. After fertilization both the embryo and endosperm arise. The complex transformations of the embryo, endosperm and surrounding tissues developing in conjunction with them result in the seed formation. The ovules and seeds are characterized by a considerable diversity in shape, degree of development and structure. They are used to be analyzed from the viewpoint of different features such as the morphological types. The ideas of researchers on the possible morphological types of ovule are based on various criteria. Initially, orthotropous, anatropous, campylotropous and amphitropous types were identified on the basis of the general criterion of the external structure: the shape of morphological axis and the position of the micropyle and the nucellus toward the placenta and funiculus (Mirbel 1829). With the accumulation of knowledge on the diversity of ovules, the classification was improved, with some types being excluded, but at the same time new variants were introduced. Thus, the amphitropous ovule was excluded first, but then this type was restored (Goebel 1933), and the classification was supplemented with a hemitropous ovule. To date, many authors emphasize 5 morphological types (orthotropous, anatropous, hemitropous, campylotropous and amphitropous), not counting variants of the ovule position in the ovary (Goebel 1933; Maheshwari 1950; Savchenko 1973; and others). Based on these features of the ovule morphogenesis, we have suggested a new classification of morphological variants of the ovules and seeds, which includes 4 types and 4 subtypes: orthotropous, anatropous, hemitropous (hemi-anatropous and hemi-orthotropous subtypes), campylotropous (ortho-campylotropous and hemi-campylotropous subtypes) (Shamrov 2018).

The ovular primordium is the earliest stage in the development of the ovule, prior to differentiation of its structures. Three modes of the ovular primordium initiation have been reported: 1) by periclinal divisions of cells of the subepidermis, 2) from the third placental layer

or 3) simultaneous divisions of cells of the subepidermal and third placental layers (Warming 1878). The ovular primordium structure was described in terms of the histogene hypothesis (Schmitz 1872) and the tunica-corpus theory (Satina 1945; Guttenberg 1960; Brunkener 1977). Based on the tunica-corpus concept, Kordyum (1967) distinguishes two groups of ovular primordia. In group A, the ovular primordium comprises a two-layered tunica and corpus cells; in group B, it is composed of the cells of a one-layered tunica and a corpus.

Other authors (Bouman 1978, 1984; Boesewinkel 1984, 1990) suggest that the ovular primordia can be subdivided into three- and two-zonate types, which corresponds to the ovular primordia of groups A and B in Kordyum's (1967) classification. In doing so, they draw on Warming's (1878) assumptions and on Satina's (1945) evidence for the presence of three independent (autonomous) zones (layers) in the ovular primordium of *Datura* chimeras. Applying the basic assumptions of the tunica-corpus theory appears to be unreasonable since it is difficult to follow the fate of tunica and corpus layers in transition from the vegetative apex to the floral primordium, and then to the formation of the placenta and ovule. It is worth noting that the authors accept the parallel use of the terms tunica (dermal and subdermal layers of the ovular primordium) and corpus (its central core).

Analysis of available data revealed that during the formation of ovules lacking the chalazal nucellar zone (Orobanchaceae – Nikiticheva & Teryokhin 1976; Gentianaceae – Shamrov 1990, 1991), periclinal divisions of cells occur in the third placental layer (Fig. 1A–D). In cases like these, the dermal and subdermal layers are the same in the placenta and in the ovular primordium. In many other taxa, the ovular primordium is formed by periclinal divisions of both subdermal and subjacent placental cells, resulting in a 'shift' of the ovular primordium layers with respect to the initial placental layers (Alliaceae, Ceratophyllaceae, Hemerocallidaceae, Grossulariaceae, Juncaceae, Liliaceae, Nymphaeaceae, Paeoniaceae, Santalaceae – Figs 1E–H; 2A–F; Shamrov 2008). In a number of highly specialized taxa (Campanulaceae, Orchidaceae – Shamrov & Nikiticheva 1992; Shamrov & Zhinkina 1994), periclinal divisions are restricted to the subdermal cells of the placenta which becomes two-layered. Whatever the mode of derivation, the ovular primordia comprise the dermal and subdermal layers and axial rows of cells.

As shown earlier for the shoot apex, the role of the meristem in the differentiation of tissues and organs becomes clear in the light of the cyto-histological zonation concept (Foster 1938; Popham & Chan 1952; Gifford & Corson 1971; Mignotte et al. 1989; Hata & Kyozuka 2021; Romanova et al. 2022). Different aspects of the functioning of apical meristem cells including hormonal, genetic and epigenetic control are being studied (Sussex & Kerk 2001; Barton 2010; Shi &Vernoux 2019; Hata & Kyozuka 2021).

Each zone of the ovular primordium gives rise to a particular ovular region, the degree of development of which varies among taxa, and results in a variety of ovule types. The peripheral zone gives rise to the epidermis of the nucellus, chalaza, funiculus, and often the entire integument. A megaspore mother cell (megasporocyte) or a megaspore mother cell and a parietal tissue are formed from the apical zone. The lateral zone gives rise to the lateral nucellar region and the outer region of chalaza and funiculus, whereas the basal zone gives rise to the basal (axial) nucellar region and procambial strands in the chalaza and funiculus. The transitional zone forms the ovular hypostase proper (Shamrov 2008). Genes that influence ovule development are being studied. Developmental-genetic studies of *Arabidopsis* and other eudicots (e.g. *Impatiens*, *Prunus*,

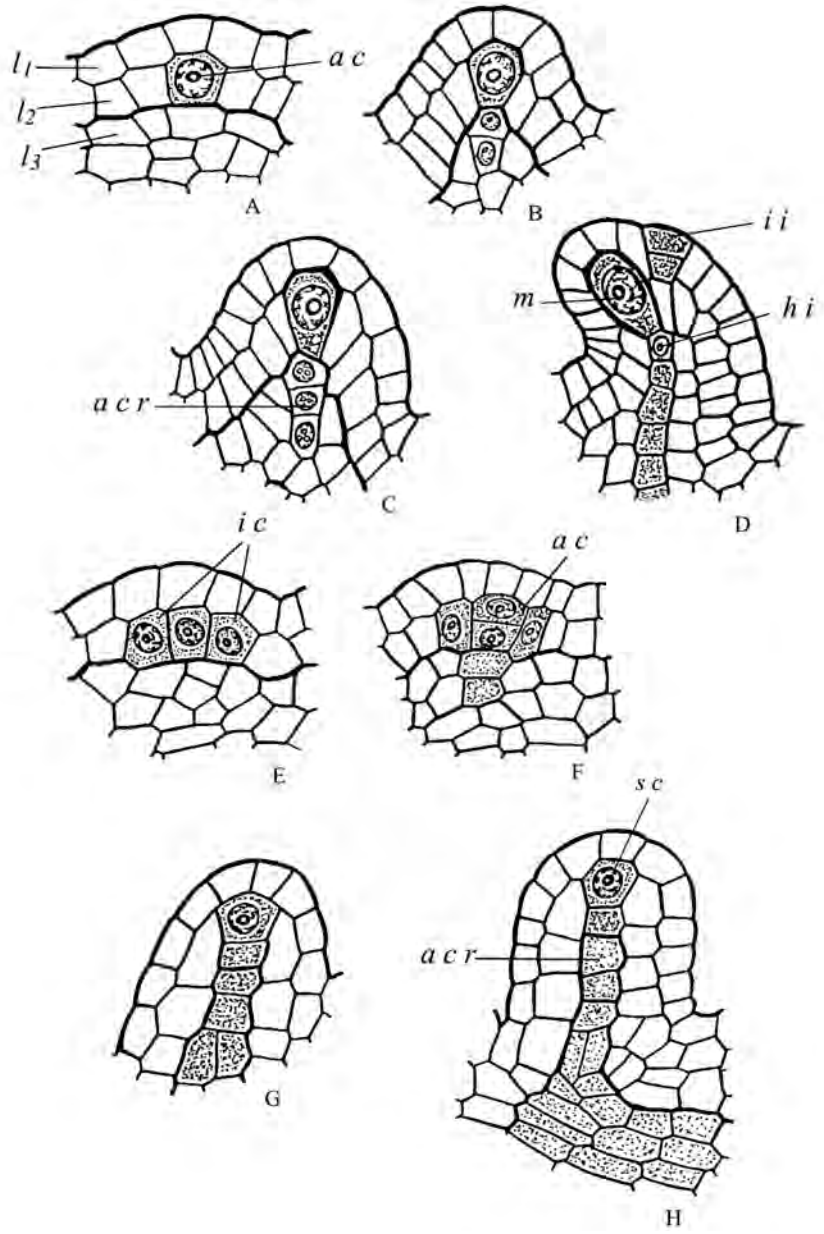

Figure 1. Formation of ovular primordium and differentiation of a one-celled archesporium. A–D in *Gentiana cruciata*, E–H in *Capsella bursa-pastoris*: ovular primordium is formed due to periclinal cell divisions, mainly of the third (A–C) or subepidermal (E–H) layers of the placenta. *a c* – archesporial cell, *a c r* – axial cell row, *h i* – hypostase initial, *i c* – initial cells in the placenta, *i i* – integument initials, *m* – megasporocyte, *s c* – sporogenous cell, *l1, l2, l3* – epidermal, subepidermal and third layers of the placenta, respectively.

Nicotiana, *Solanum*) have identified several interacting genes that influence ovule and integument development (Skinner et al. 2016; Yamada et al. 2019; Rudall 2021).

The formation of the ovular primordium is directly related to the differentiation of archesporial cells. According to the number of cells that make up archesporium, two types are distinguished: multicellular (Casuarinales, Myricales, Paeoniales, etc.; Fig. 1A, F) and unicellular (Campanulales, Ericales, Gentianales, Orchidales, Scrophulariales, etc.; Fig. 2E, F). As in the case of multicellular

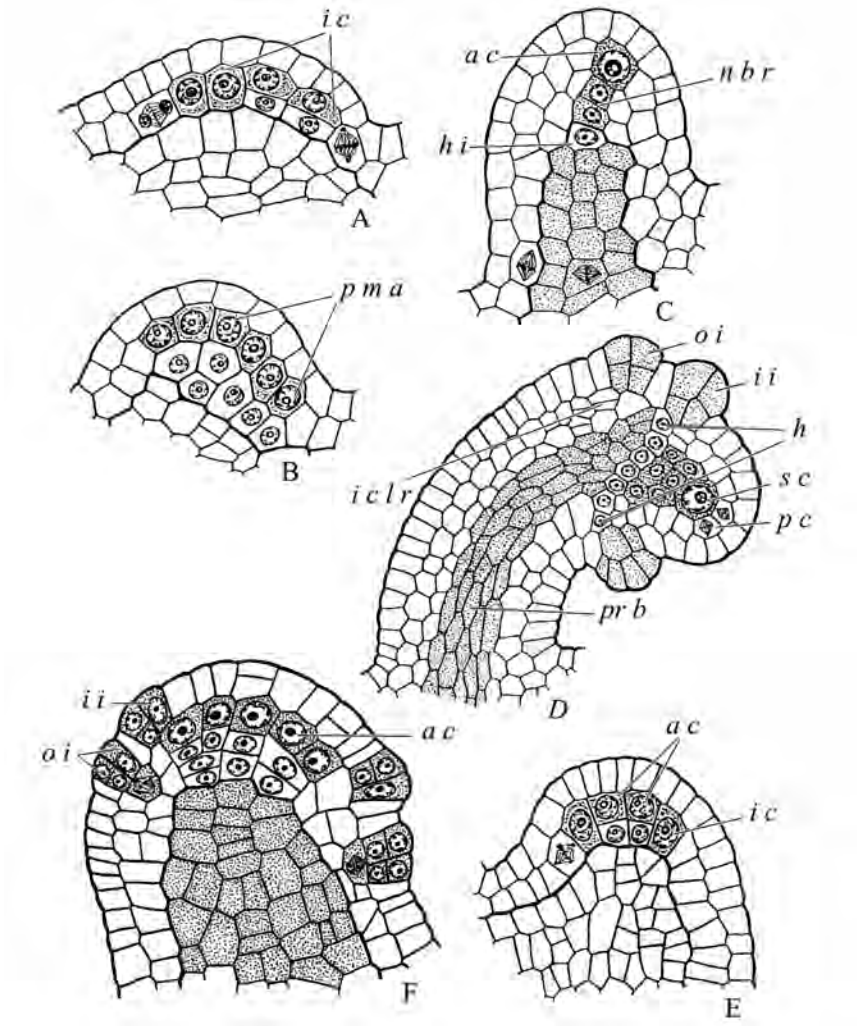

Figure 2. Formation of ovular primordium and differentiation of a many-celled archesporium. A–D in *Nymphaea gigantea*, E–F in *Paeonia lactiflora*: ovular primordium is formed due to periclinal cell divisions, mainly of the subepidermal layers of the placenta. *ac* – archesporial cell, *h* – hypostase, *hi* – hypostase initial, *ic* – initial cells in the placenta, *iclr* – initials of the chalaza lateral region, *ii* – inner integument, *nbr* – nucellus basal region, *oi* – outer integument, *pc* – parietal cell, *pma* – potentially many-celled archesporium, *prb* – procambial bundle, *sc* – sporogenous cell.

and unicellular archesporia, its main property is the ability to differentiate divisions into parietal and sporogenous cells. There is probably another type of archesporium – intermediate or potentially multicellular. In this case, several subepidermal initial cells begin to differentiate according to the archesporium-like type, but later only one megasporocyte is formed (Fig. 2A–D) and, thus, the archesporium becomes actually unicellular (Nymphaeaceae, Poaceae, Santalaceae, etc.) (Shamrov 2008). This is evidenced by the study of the ovule in *Zea mays* (Poaceae). Normally, a unicellular archesporium is formed. However, in *mac1* and *pam1* mutants, it becomes multicellular with the subsequent formation of several tetrads of megaspores and embryo sacs (Sheridan et al. 1996; Voronova et al. 2003). But mechanisms of differentiation of the multiple archesporium have not been studied in detail (Vinogradova & Zhinkina 2020). Some authors explored how organ growth contributes to archesporial cell and then megasporocyte differentiation. They generated

92 annotated 3D images at cellular resolution in *Arabidopsis thaliana*. Their analysis revealed that spore mother cell characteristics first arise in more than one cell, but megasporocyte fate becomes progressively restricted to a single cell during organ growth. Altered primordium geometry coincided with a delay in the fate restriction process in *katanin* mutants. In these plants, frequent divisions are observed in L_1 apical cells that support the expansion of the epidermis, while inner tissues develop. This working model paves the way to explore the role of epidermal geometry in controlling regulators of the cell cycle and megasporocyte fate in L_2 apical cells. The domains of auxin response are restricted to the L_1 dome and cytokinin signaling is localized in a region basal to the megasporocyte (Hernandez-Lagana et al. 2021). As we have shown, the apical and lateral zones in an ovular primordium are derived from the subepidermal layer of the placenta: the apical zone – the zone of differentiating archesporial cells in the subepidermis, the lateral zone – the subdermal cells below the level of archesporial cells (Shamrov 2002a, 2008). The study suggests that at the site of initiation of the ovule in the subepidermal layer, three or more large cells differentiate, which, like the underlying cells of the third layer of the placenta, divide periclinally. The upper derivatives of the central cells can become archesporial cells (Shamrov 1997a, 2002a, 2008). However, in many plants with a small number of cell layers in the apical zone (*Kalanchoe tubiflora*, *K. laxiflora* – Anisimova & Shamrov 2018) or completely devoid of the apical zone (*Arabidopsis thaliana*, *Capsella bursa-pastoris* – Shamrov 2002b, 2007), all remaining initial cells from the sides of the archesporial cells, arising in the subepidermal layer of the placenta, become the initials of the lateral region of the nucellus.

The nucellus is the major part of the ovule, the homologue of the megasporangium of higher plants. The term was proposed by Mirbel (1829). In the nucellus the reproductive cells arise passing the way from the archesporium to the megasporocyte and further to the embryo sac. Based on the nucellus development and structure the crassinucellate (Latin *crassus* for thick) and tenuinucellate (Latin *tenuis* for thin) ovules are usually distinguished. The archesporial cell in the crassinucellate ovule transforms into megasporocyte through division separating the parietal cell; the nucellus is multi-layered and persists for a long time after fertilization. In the tenuinucellate ovule, the archesporial cell turns into the megasporocyte immediately; the nucellus is represented as the epidermal layer and gets disintegrated during megasporogenesis (Van Tieghem 1901, 1903; Asplund 1920; Schnarf 1929; Masheshwari 1950; Davis 1966; Young & Watson 1970; Dahlgren 1980; Kapil & Bhatnagar 1991). The researchers often show simplistic approaches to the treatment of the ovule types from the viewpoint of nucellus genesis and consider the presence (crassinucellate ovules) or absence (tenuinucellate ones) of the parietal tissue as the main criterion for distinguishing the ovule types. It was shown in numerous research works that the massiveness of the nucellus does not always coincide with the presence of the parietal tissue. It was suggested that an intermediate type of ovules exists. Thus, Dahlgren (1927) had distinguished the syndermal ovules, in which the parietal cells are absent, and the apodermal ones, where they are present. Davis (1966), as an independent type, considers the pseudocrassinucellate ovule, in which parietal tissue is absent, but a multi-layered nucellar cap forms. A special epicrassinucellate ovule with a multi-layered nucellar epiderm and a multi-layered parietal tissue is also distinguished (Teryokhin 1996). There are other variants of ovules as well: incompletely tenuinucellate, reduced tenuinucellate, weakly crassinucellate (Endress 2011).

We propose the following ovule typology. Based on the developmental pattern and structure of the nucellus, it includes three main types: **crassinucellate**, **tenuinucellate** and **medionucellate**

(Shamrov 2002a, 2008). These types are distinguished on the basis of the crassinucellate and tenuinucellate condition. The crassinucellate condition criterion: 1) all the nucellar regions (apical, basal and lateral) are topographically well defined and comprise one or more layers, 2) the nucellus, particularly its major region – the lateral region – gets crushed after fertilization. The criterion of the tenuinucellate condition: 1) the nucellar regions are not topographically well defined, the nucellus comprising a single dermal layer, 2) the nucellus normally degenerates prior to fertilization. The presence or absence of an integumentary tapetum is an additional indication of the tenui- and crassinucellate condition. We proposed a number of general approaches and terms to be used in classifying the microsporangium wall types (Teryokhin et al. 2002; Shamrov et al. 2020).

In the crassinucellate ovules, the least variable nucellar region is the lateral region of nucellus. Specific features of its development are used for distinguishing variations of the ovular types: 1) the number of the initial layers of the lateral nucellar region which are differentiated within the ovular primordium, 2) presence or absence of periclinal divisions in the lateral nucellar region. The three variations are: the **complicated variation** – the cells of two initial layers of the lateral nucellar region divide periclinally from the earliest stages of development, so the lateral nucellar region becomes multi-layered (Ceratophyllaceae, presumably Nelumbonaceae, Trapaceae; Fig. 3A–C); the **typical variation** – the cells of a single initial layer divide periclinally and the lateral nucellar region becomes multi-layered (many taxa of di- and monocotyledons: Cabombaceae, Juncaceae, Lauraceae, Magnoliaceae, Nymphaeaceae, Winteraceae; Fig. 3D–F); the **reduced variation** – the cells of a single initial layer do not undergo periclinal divisions and the lateral region remains one-layered (some members of Alliaceae: *Agapanthus umbellatus*, *Brodiaea coronaria*, *B. elegans*, *Hesperocallis undulata*; and Hemerocallidaceae: *Leucocrinum montanum*; Fig. 3G–I).

In tenuinucellate ovules, the nucellus is mainly represented by a single dermal layer. Two variations are recognized: the **typical** or sympetalous variation – the dermal layer completely encloses the developing megasporocyte; the nucellar cells collapse before fertilization, which is frequently correlated with the differentiation of an integumentary tapetum (predominantly in sympetalous dicotyledons: Asteraceae, Gentianaceae, Gesneriaceae, Lamiaceae; Fig. 4A–C); the **reduced** variation – the nucellus comprises a few dermal cells above the apical portion of the megasporocyte only and becomes crushed prior to fertilization; the integumentary tapetum is not differentiated (Asclepiadaceae, Rubiaceae, Theligoniaceae, some members of the Olacaceae, Opiliaceae, Santalaceae; Fig. 4D–F).

The medionucellate ovules (Latin *medius* for middle) are typified by the combination of crassinucellate and tenuinucellate characteristics. Three variations are distinguished. The **apodermal** (Greek *apo* for from and *derma* for skin; the term was proposed by Dahlgren 1927) or crassinucellate variation – all the nucellar regions are topographically well defined, being composed of two to three layers; the bulk of the nucellus degenerates before fertilization, which is correlated with the formation of an integumentary tapetum (Alangiaceae, Davidiaceae, some members of the Araliaceae, Arecaceae, Celastraceae, Rhizophoraceae; Fig. 5A–C). The **syndermal** variation (Greek *syn* for together and *derma* for skin; the term was proposed by Dahlgren 1927) – an ovule in whose nucellus only the lateral and basal regions are distinguishable, resulting in the megasporocyte being located immediately below the epidermis. Two subvariations are

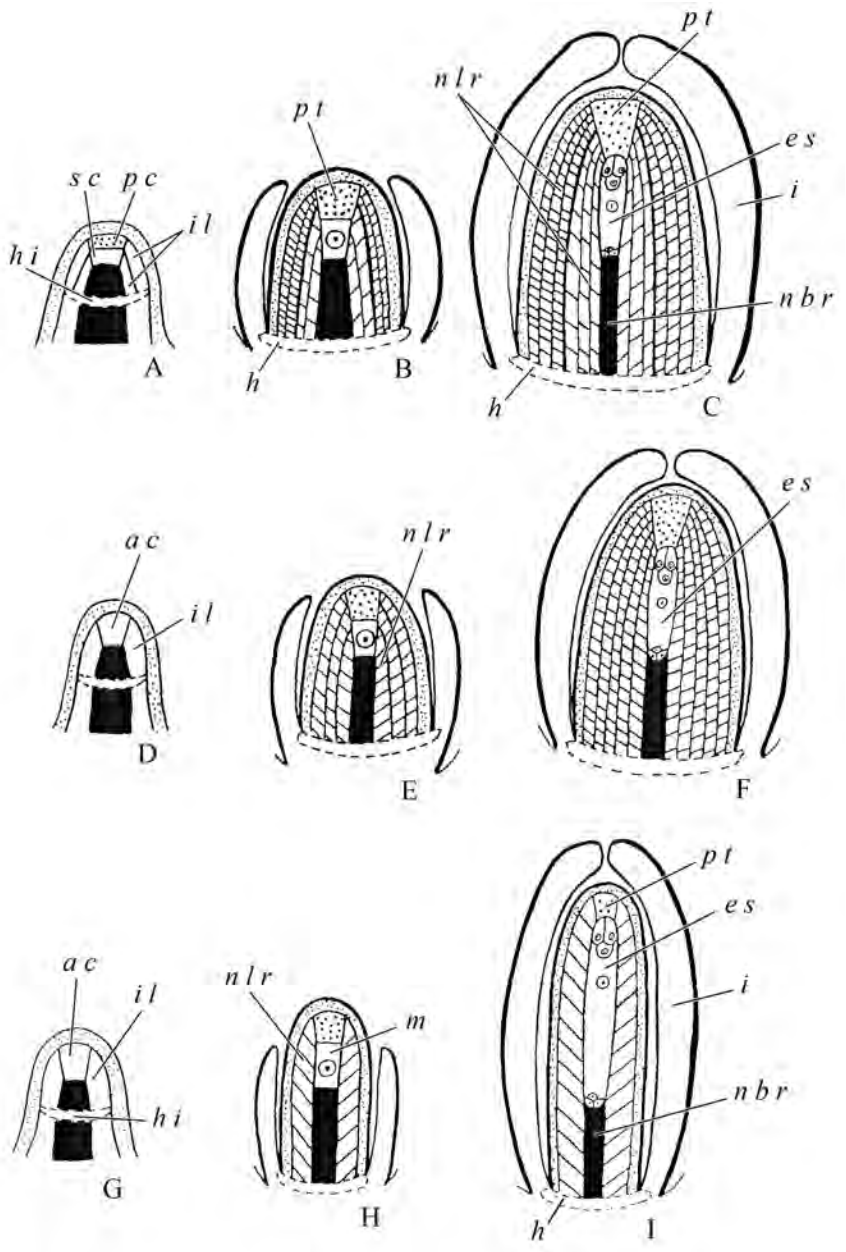

Figure 3. Classification of ovule types based on development and structure of the nucellus, crassinucellate type and its variations. For simplicity reasons only one integument is drawn. A–C – complicated variation, D–F – typical variation, G–I – reduced variation. *a c* – archesporial cell, *e s* – embryo sac, *h* – hypostase, *h i* – hypostase initials, *i* – integument, *i l* – initial layers of the lateral nucellar region, *m* – megasporocyte, *n b r* – nucellus basal region, *n l r* – nucellus lateral region, *p c* – parietal cell, *p t* – parietal tissue, *s c* – sporogenous cell.

recognized: multi-layered – the lateral and basal regions comprise two layers and more; no integumentary tapetum is normally differentiated (predominantly monocotyledons – Liliaceae, Poaceae, Zosteraceae, some Alliaceae, Hemerocallidaceae; Fig. 5D–F) and one-layered – the lateral and basal regions are represented by a single layer; an integumentary tapetum is differentiated (Arecaceae, Balsaminaceae, Campanulaceae, Lecythidaceae; Velloziaceae; Fig. 5G–I). The **permanent** (Latin *permanens* for remaining) or tenuinucellate variation – in addition to the dermal

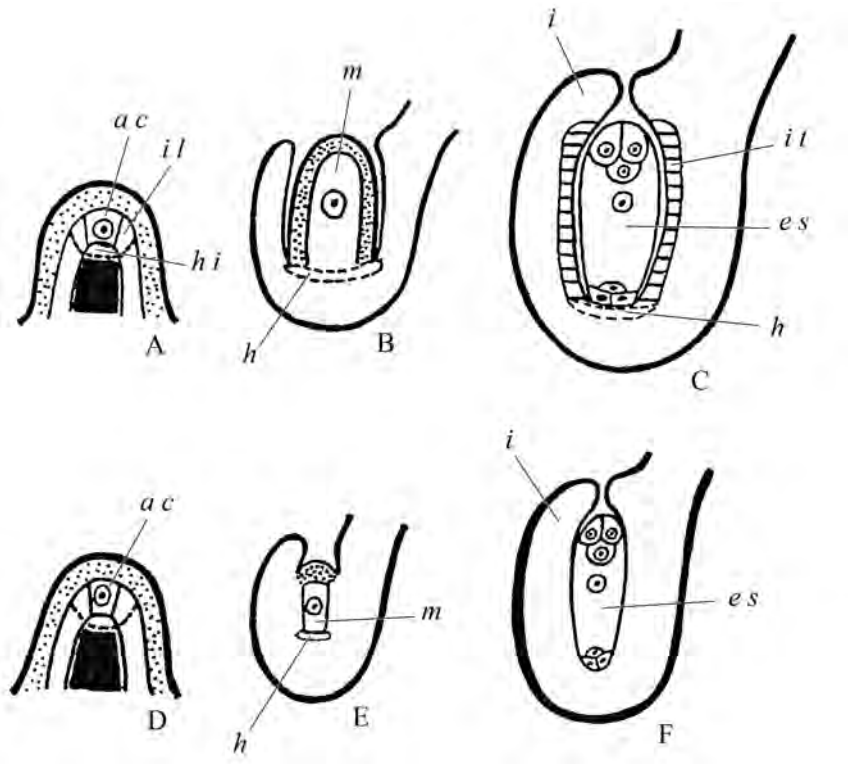

Figure 4. Classification of ovule types based on development and structure of the nucellus, tenuinucellate type and its variations. A–C – typical variation, D–F – reduced variation. *a c* – archesporial cell, *es* – embry sac, *h* – hypostase, *h i* – hypostase initials, *i* – integument, *i l* – initial layers of the lateral nucellar region, *i t* – integumentary tapetum, *m* – megasporocyte.

layer, the poorly developed, one-layered lateral and basal nucellar regions are distinguishable; most of the nucellus gets crushed prior to fertilization; the integumentary tapetum is not invariably present. Depending on the nucellar region which is present in the ovule, two subvariations can be distinguished: basal (Burmaniaceae, many members of the Orchidaceae, some Linaceae: *Radiola*; Fig. 5J–L) and lateral (Dipsacaceae, Scrophulariaceae, some Orchidaceae: *Listera*; Fig. 5M–O).

The integument (Latin *integumentum* for coat, envelope) is an ovular structure surrounding the nucellus (Grew 1672; Malpighi 1675). Closer examination of the ovular structure (Brown 1826) revealed the presence of two 'membranes' or 'envelopes' in it. It was suggested that the inner membrane is the true one. The outer membrane is auxiliary and 'covers', as it were, the developing ovule. This latter membrane was termed 'integument'. Subsequently, the term integument was used to denote both ovular membranes, inner and outer integument respectively (Schleiden 1839).

The ovules of flowering plants are divided according to the number of integuments into **bitegmic** (with two integuments), **unitegmic** (with single integument) and **ategmic** (without integuments). The number of integuments has been useful for taxonomic considerations. Among flowering plants, bitegmic ovules have been reported in 237 families and unitegmic ones in 114 (Kamelina 1991). Bitegmic ovules are characteristic of nearly all monocotyledons and many dicotyledons. Unitegmic ovules have been described predominantly in sympetalous taxa of dicotyledons and some monocotyledons (*Thalassia*, Hydrocharitaceae – Tomlinson 1969; *Gymnostachys* and

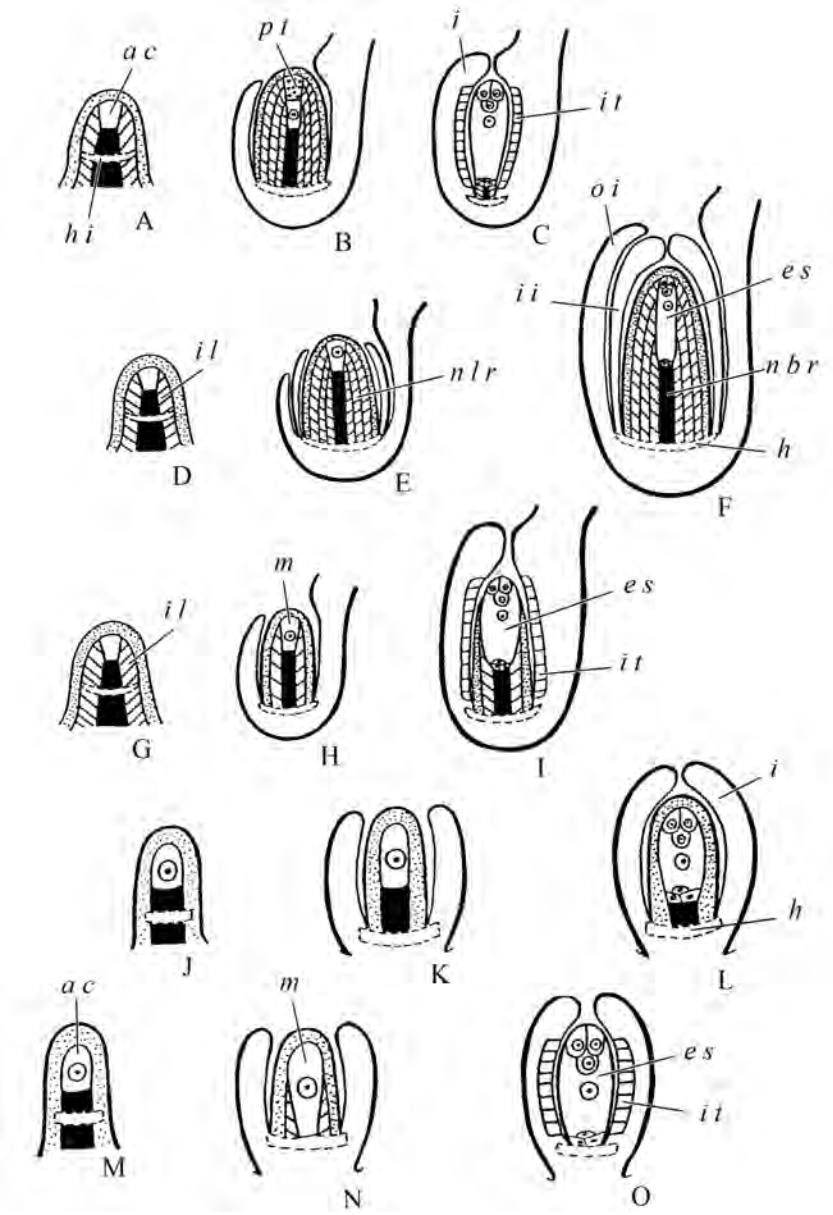

Figure 5. Classification of ovule types based on development and structure of the nucellus, medionucellate type and its variations and subvariations. For simplicity reasons only one integument is drawn in permanent variation. A–C – apodermal variation, D–I – syndermal variation with D–F – many-layered subvariation, G–I – one-layered subvariation; J–O – permanent variation with J–L – basal subvariation, M–O – lateral subvariation. *ac* – archesporial cell, *es* – embryo sac, *h* – hypostase, *hi* – hypostase initials, *i* – integument, *ii* – inner integument, *il* – initial layers of the lateral nucellar region, *it* – integumentary tapetum, *m* – megasporocyte, *nbr* – nucellus basal region, *nlr* – nucellus lateral region, *oi* – outer integument, *pt* – parietal tissue.

Montrichardia, Araceae – Buzgo 1999). In members of nearly 80 families, a vascular bundle is differentiated within the outer integument (Bouman 1984). This may be simple (unbranched) (Hemerocallidaceae, Magnoliaceae) or branched (Connaraceae, Euphorbiaceae, Meliaceae) which extends up to the level of micropyle. In the single integument of some plants, a vascular bundle can also appear (Campanulaceae, Convolvulaceae, Dipsacaceae, Valerianaceae).

The integument(s) can show varying degrees of development. The highest degree of its reduction to initial cells is characteristic of parasitic members – Gentianaceae (Bouman & Louis 1989), Santalaceae (Shamrov et al. 2001). A total absence of integument has only been observed in undifferentiated ovules (some species of Loranthaceae, Opiliaceae, Santalaceae – Agarwal 1961). However, the absence of integuments is also observed in autotrophic species. These plants include species that are characterized by the so-called naked ovules: *Crinum* (Amaryllidaceae) (Dutt 1957, 1959; Kamelina 2011; Shamrov et al. 2020), *Melocanna bambusoides* (Poaceae) (Petrova 1965), *Cardiopteris quinqueloba* (Cardiopteridaceae) (Tobe 2016). The study of ovules in *Crinum* showed that they stopped developing at the primordium stage (Shamrov et al. 2020). This conclusion was confirmed by molecular genetic studies. Based on the results of comparing the expression of the *ANT* and *BEL1* genes in *Arabidopsis thaliana* (Brassicaceae) and representatives of Santalales order, Brown et al. (2010) made a conclusion, which, from our point of view, allows us to explain the structure of ategmic ovules in 2 variants: 1) ovules with reduced organs (like *Santalum* – ovules probably arose during the fusion of integuments with nucellus) and 2) ovules are not differentiated into structures (*Crinum*, *Cardiopteris*, *Melocanna* – nucellus acquired properties that began to restrict the development of integuments in ancestral species).

In the course of seed development, integuments transform into seed coat – **testa** (outer and single integuments) and **tegmen** (inner integument). During ripening, a protective (mechanical) layer of sclerified cells forms in the seed coat. Depending on the part of the external (single in the unitegmic ovules) or internal integument, where the mechanical layer differentiates, there are exo-, meso- and endotestal as well as exo-, meso- and endotegminal seeds (Corner 1976). For example, in *Capsella bursa-pastoris* (Brassicaceae) (Shamrov 2002b), the seed is endotestal-endotegminal. The seed coat in this species is formed by derivatives of both integuments: the outer one is the outer epidermis of slime cells and the inner epidermis of cells with thickening (endotesta), the inner integument is the endothelium (endotegmen), the operculum with thickened cell walls and the remnants of the middle layer. The seed coat of *Luzula pedemontana* (Juncaceae) has a similar structure (Shamrov & Anisimova 1993). It is also formed on the basis of both integuments and consists of 5–6 layers. Exotesta cells are characterized by lignified outer tangential walls covered with cuticle and wax layers, and they secrete a polysaccharide mucus. Mesotesta (2–3 layers) and endotesta consist of thin-walled cells. Tegmen is represented only by cells of the inner epidermis, the outer and inner tangential walls of which are lignified. There is a cuticular film between the tegmen and the endotesta, and layers of cuticle and wax are between the tegmen and the remaining epidermis of the nucellus. Unlike the two previous species, in *Vaccinium myrtillus* (Ericaceae) (Anisimova et al. 2005), the ovule is unitegmic. The seed coat is represented mainly by the cells of the outer epidermis of the integument (exotestal seeds). Cell walls of exotesta have a different thickness. The cells of the integumental parenchyma are usually obliterated during the development of the embryo and endosperm.

The seed coat of *Aristolochia* s.l., *Asarum*, *Saruma* and some *Thottea* species (Aristolochiaceae) consists primarily of a two-layered testa. In some species the cells of the inner layer of the testa have crystals (González & Rudall 2003). The term 'peritesta' is suggested for the peripheral, band-like integumentary part of the seed coat in *Mangifera indica* (Anacardiaceae). In the mango, the integument (ovule is unitegmic) expands peripherally after fertilization through intercalary meristematic activity. Anticlinal divisions occur and the integumentary part gradually expands and eventually virtually surrounds the chalazal part as a narrow band (Von Teichman et al.

1988). After fertilization, this feature of the integument structure resembles an aril. Arils have different origins. They are often formed by cells of the funiculus, but they can also be from the integument in the antiraphal part, especially in the micropyle region (caruncle) or along the raphe (strophiole) (Corner 1976; Boesewinkel & Boumann 1984; Trusov 2021). *M. indica* is likely to develop a caruncle. The special structure of the exotesta is described in *Tiquilia plicata* and *T. dichotoma* (Ehretiaceae). It developed from small, undifferentiated cells into specialized cells with wall ingrowths on the anticlinal and the proximal periclinal cell walls – transfer cells (Gottschling et al. 2014).

The initiation and early histogeny of the integuments have been studied inadequately. In bitegmic ovules, the first integument to develop is normally the inner one, less frequently the outer integument, or both integuments may differentiate simultaneously. The integuments are formed as ring-shaped ridges and develop from the epidermal and subepidermal initials (Warming 1878; Bouman 1971a, b, 1984). In the apical part of the ovular primordium of *Arabidopsis thaliana*, even before the differentiation of the structures, the expression of the *WUS* gene is detected, and the synthesized protein is localized, where the initiation of the integument begins (Gross-Hardt et al. 2002). Our studies, carried out earlier on plants with different types of ovules, are consistent with the data of molecular genetic analysis. They indicate that such an integument formation is preceded by the accumulation of various metabolites (RNA, proteins, dextrines) and intensive anticlinal division of epidermal cells from the dorsal side at the level of the ovular primordium transition zone. Radially elongated cells appear, some of which become initials, while their size, including nuclei, increases. In the case of epidermal origin, they divide periclinal or oblique cell walls, separating 'terminal initials' (the term was introduced by Roth 1957). In integuments of subepidermal origin, periclinal divisions are observed only in the cells of the subepidermis, whereas in the case of dual origin (due to epidermal and subepidermal initials simultaneously), these divisions occur in the cells of both layers. The position of the integument initials in a layer of the ovular primordium was the basis for the classification of the ways of the integument formation (Shamrov 1997a, b, 2000, 2008). It includes three types: dermal, subdermal and dermal-subdermal.

In the **dermal** type, variations can be distinguished based on the following criteria: 1) the number of epidermal initials, 2) the sequence of their divisions and the pattern of the cell walls' formation at the first stages of development, 3) the participation of initials in the formation of various layers. Variation I: initials in 4 layers; the first to divide periclinally are the cells of the two middle layers, separating the terminal initials. Derivatives of the lateral initials form the epidermal layers of the basal part of the integument and of the middle layers – two central layers in the basal part and the epidermal layers in the apical part (internal integument of *Paeonia lactiflora*; Fig. 6A–E). Variation II: the initials in two layers are divided by periclinal cell walls, and then, through multiple transverse divisions, a two-layered integument is formed (Datiscaceae, Menispermaceae, Polygalaceae, Rosaceae, Solanaceae, etc.; Fig. 6F–N). An increase in the number of layers and the formation of a multi-layered integument can occur due to periclinal divisions of epidermal cells. Variation III: initials in one layer; each cell divides periclinally, separating the terminal initial; longitudinal division occurs in the lower cell and the integument becomes two-layered at the base; then the terminal initial is divided many times obliquely (*Gagea stipitata*, Liliaceae; Fig. 6O–S). In some plants, the first periclinal divisions of the initials are differentiating: the outer cells become the initials of the integument, and the inner ones become the lateral initials of the

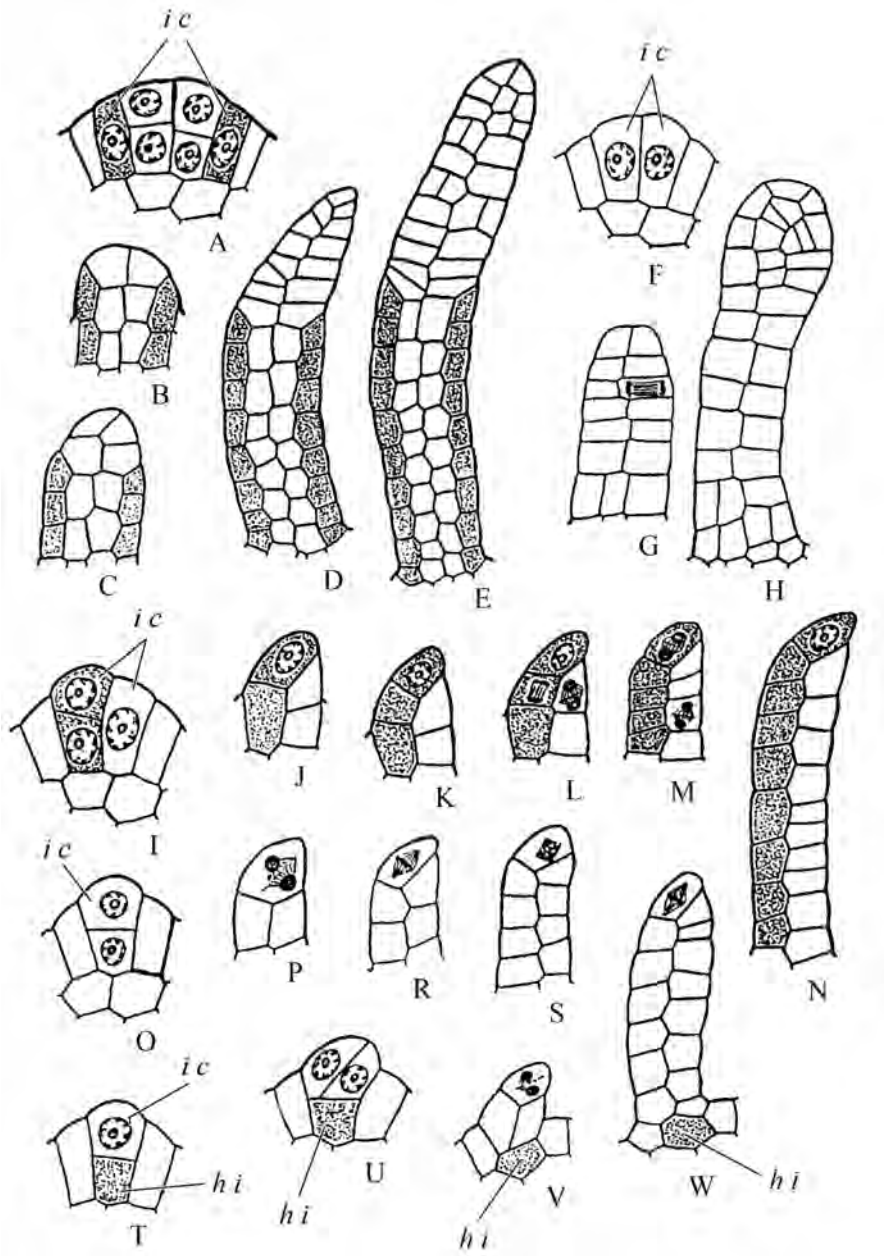

Figure 6. Classification of types and variations of the integument formation, the dermal type and its variations. A–W – integuments are formed due to epidermal initials. A–E – variation I in *Paeonia lactiflora* (inner integument); F–N – variation II in *Nymphaea gigantea* (F–H – both integuments) and in *Juncus filiformis* (I–N – inner integument); O–W – variation III in *Gagea stipitata* (O–S – both integuments) and in *Gymnadenia conopsea* (T–W – both integuments). *hi* – hypostase initial, *ic* – initial cells of integument.

hypostase; each initial integument separates a terminal initial with an oblique cell wall, which divides many times (*Lilium tigrinum*, Liliaceae – Bouman 1971a; *Gymnadenia conopsea*, *Listera ovata*, Orchidaceae – Shamrov & Nikiticheva 1992; Shamrov 2001; Fig. 6T–W).

In the **subdermal** type, the subepidermal initials, located in one or two layers, divide periclinally, and the cells of the epiderm – anticlinally (single integument in the unitegmic and external

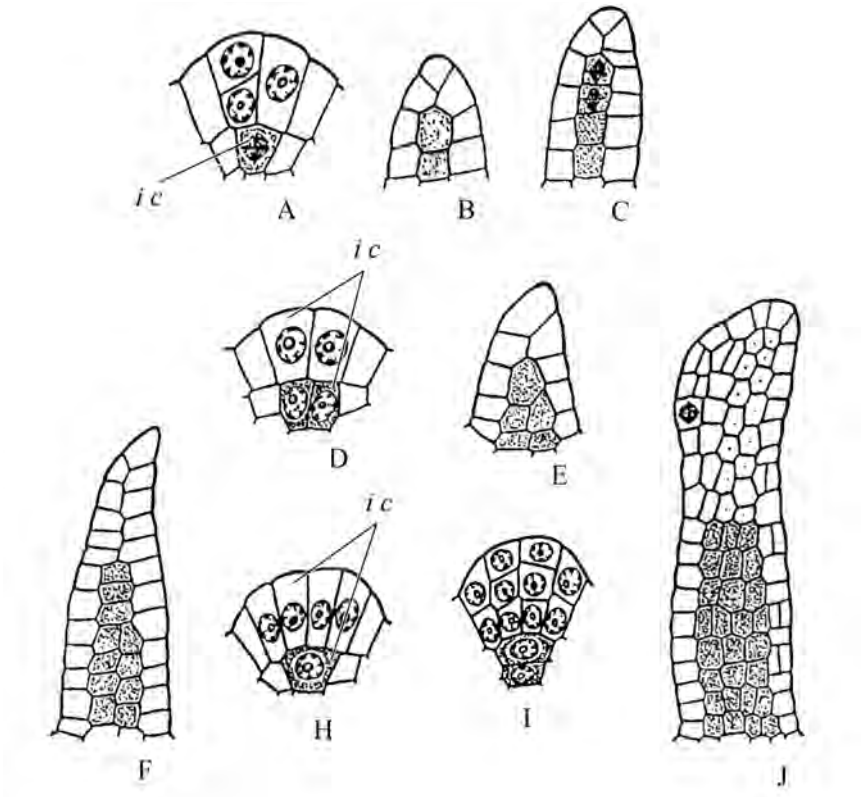

Figure 7. Classification of types of the integument formation, subdermal and dermal-subdermal types. A–C – subdermal type (integuments are formed due to subepidermal initials) in *Juncus filiformis* (outer integument); D–J – dermal-subdermal type (integuments are formed due to epidermal and subepidermal initials) in *Ceratophyllum demersum* (D–F) and *Paeonia lactiflora* (H–J – outer integument). *ic* – initial cells of integument.

integument in the bitegmic ovules; Fig. 7A–C). This pattern of formation is typical of Cucurbitaceae, Magnoliaceae, Ranunculaceae, some Brassicaceae, Juncaceae. The multi-layered integument is created due to periclinal divisions of the middle layer cells being of subdermal origin, less often cells of the outer (Cucurbitaceae) or inner (Magnoliaceae) epidermis.

The **dermal-subdermal** type is characterized by the dual origin of the integument, which is formed by epidermal and sub-epidermal initials. The initials can be located in 2 (*Ceratophyllum demersum*) or 3–4 (*Paeonia lactiflora*) layers (Fig. 7D–I). As a result of periclinal divisions, the subepidermal initials form the parenchyma, and the epidermal (with the separation of terminal initial cells) – the micropylar zone and epidermal layers in the middle and chalazal zones of the integument (Shamrov 1997a, b). In a similar way, probably the single integument is formed in *Phyllostylon rhamnoides*, Ulmaceae (Dottori 1991).

The **chalaza** is a basal region of the ovule, where the bases of the nucellus, integuments and funiculus form a united structure. The term was proposed by Treviranus (1805). Periasamy (1962) distinguishes three types of chalaza: a normal chalaza (constitutes a small portion of the ovule and seed and makes a minor contribution to the seed coat formation), a massive chalaza or pachychalaza in Corner's (1976) terminology (accounts for the bulk of the seed and seed coat) and a perichalaza (according to the definition of Corner 1949) (makes up a significant portion of the seed and seed coat and is formed by intercalary growth in the raphal and antiraphal regions).

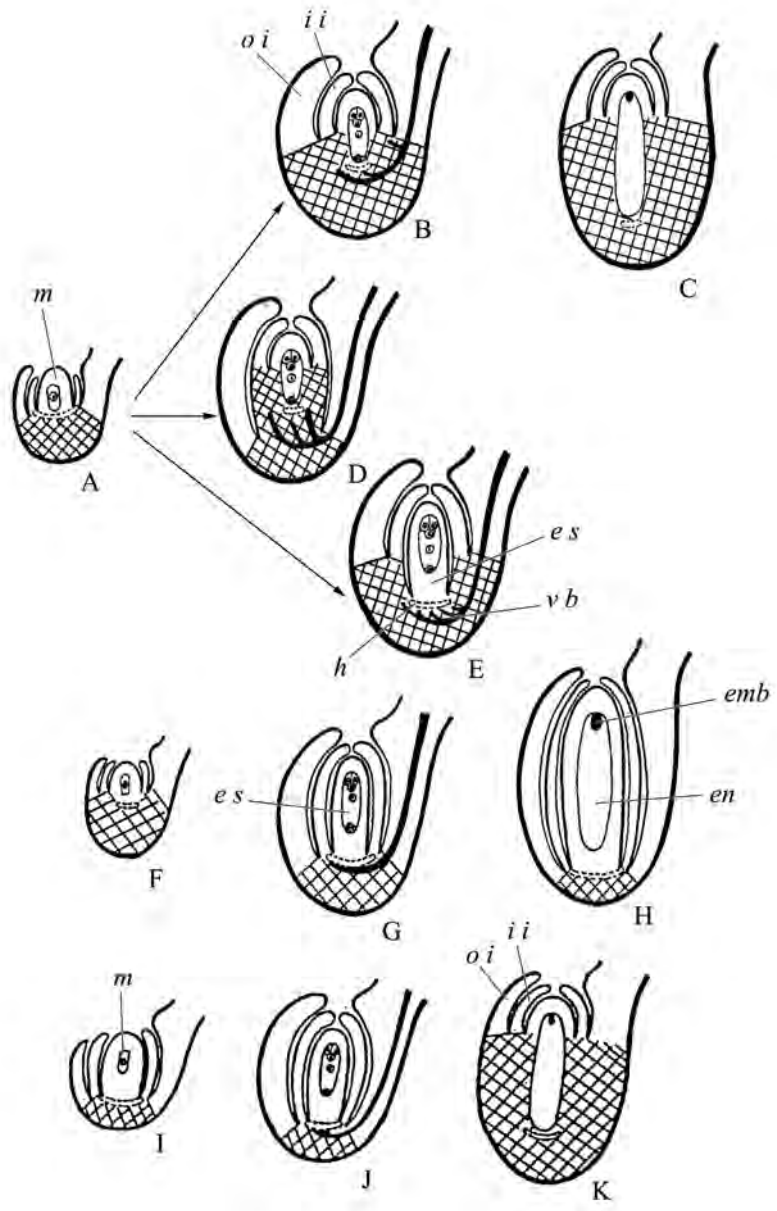

Figure 8. Classification of ovule types based on development and structure of the chalaza, pachychalaza and its variations. A–E – typical variation: A–C – exo-endopachychalaza, A, D – endopachychalaza, A, E – exopachychalaza; F–H – juvenile variation; I–K – mature variation. *en* – endosperm, *emb* – embryo, *es* – embryo sac, *h* – hypostase, *ii* – inner integument, *m* – megasporocyte, *oi* – outer integument, *vb* – vascular bundle.

We propose a different classification of the chalaza types based on the following criteria: 1) the chalaza position in the ovular primordium and its size in relation to other ovular structures, 2) the vascularization pattern and 3) the extent of its contribution to the seed coat formation. The following types of chalaza are distinguished: **pachychalaza** (Greek *pachys* for massive); **mesochalaza** (Greek *mesos* for middle (Shamrov 2004), normal chalaza according to Periasamy (1962)); **perichalaza** (Greek *peri* for around, near); and **leptochalaza** (Greek *leptos* for thin (Shamrov 2004)).

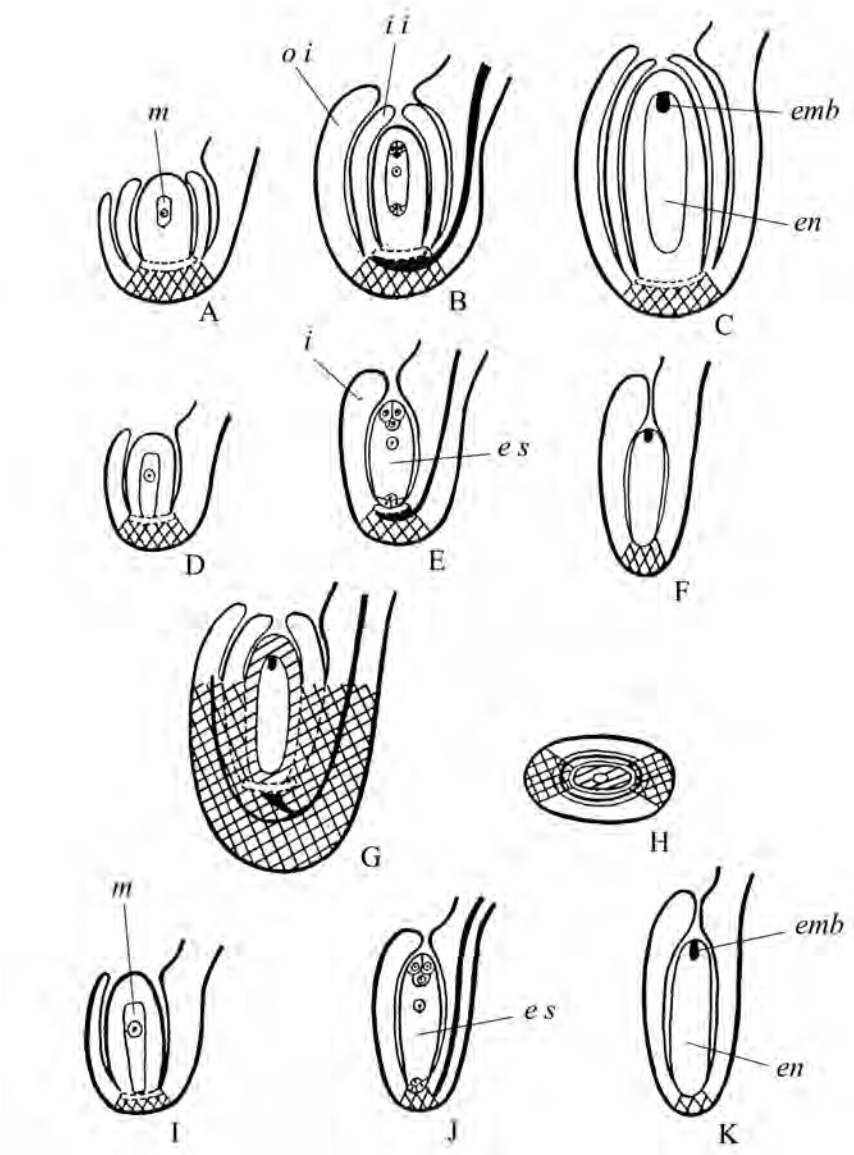

Figure 9. Classification of ovule types based on development and structure of the chalaza, meso-, peri- and leptochalaza. In the mesochalaza the upper row is related to the crassinucellate ovule type and the lower one to the tenuinucellate type of the ovule. A–F – mesochalaza, G, H – perichalaza, I–K – leptochalaza. *en* – endosperm, *emb* – embryo, *es* – embryo sac, *i* – integument, *ii* – inner integument, *m* – megasporocyte, *oi* – outer integument.

The pachychalaza has been reported in a number of taxa from different levels of the phylogenetic system of flowering plants. We suggest that three pachychalaza variations can be recognized on the basis of spatio-temporal development of ovular structures. The **typical variation** – from the very beginning, the chalaza occupies the major portion of the ovule and is completely formed in the mature seed (Cannaceae, Rhizophoraceae, Tropaeolaceae). The chalaza may arise below the site of attachment only of the inner integument (tegminal pachychalaza or endopachychalaza in Rhizophoraceae – the term proposed by Boesewinkel & Bouman 1984; Alliaceae, Euphorbiaceae – Vinogradova 2013, 2017; Titova et al. 2018), or of the outer integument (exopachychalaza

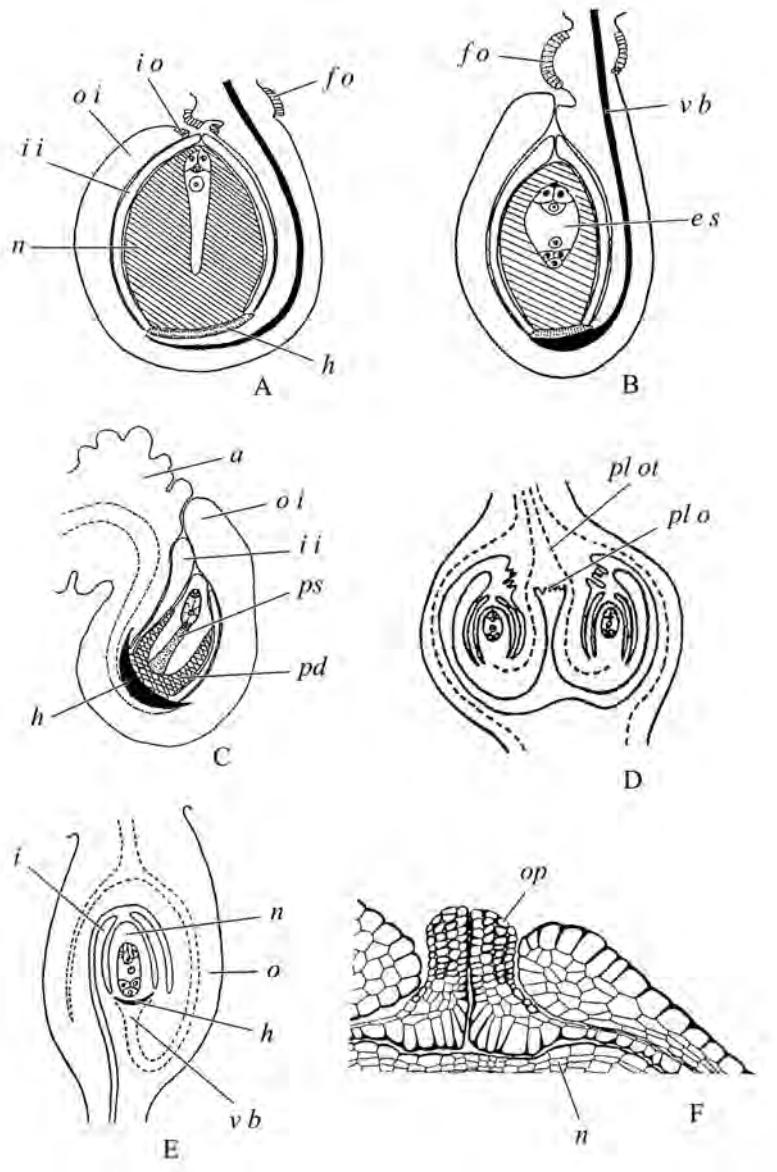

Figure 10. Special tissues of ovule. A–C – bitegmic, funicular ovules with straight (A, B) and curved (C) micropyle formed by inner integument (endostome) in *Victoria amazonica* (A), and by both integuments (exo- and endostomes) in *Ribes aureum* (B) and *Ungnadia speciosa* (C); funicular obturator (B), funicular and integumentary obturators (A), and aril (C); sessile ovule with placentary obturator in *Luzula pedemontana* (D); afunicular and unitegmic ovule in *Ceratophyllum demersum* (E); operculum formed by inner integument in *Nuphar lutea* (F). *a* – aril, *es* – embryo sac, *fo* – funicular obturator, *h* – hypostase, *i* – integument, *ii* – inner integument, *io* – integumentary obturator, *n* – nucellus, *o* – ovary, *oi* – outer integument, *op* – operculum, *pd* – podium, *plo* – placentary obturator, *plot* – placentary outgrowth, *ps* – postament, *vb* – vascular bundle.

in Nelumbonaceae – the term proposed by Shamrov 2004; Fig. 8A, B, D, E). In many species, along with nucellus, both integuments are involved in chalaza formation (exo-endopachychalaza – Shamrov 2004; Fig. 8C). The **juvenile variation** (Latin *iuvenilis* for young) – the chalaza is initiated as in the typical variation, however, prior to fertilization (Ceratophyllaceae) or immediately after fertilization (Scheuchzeriaceae, some members of the Santalaceae), the micropylar portion of

the seed becomes the more massive part (Fig. 8F–H). The **mature variation** (Latin *maturus* for ripe, mature) – the chalaza becomes massive after fertilization, due to intercalary growth below the site of attachment of the integument(s) (some members of the Lauraceae, Myristicaceae). The funicular vascular supply normally forms a network of chalazal bundles. The pachychalaza derivatives constitute the bulk of the seed coat (Fig. 8I–K).

The mesochalaza has been found in ovules of the majority of flowering plants. The chalaza in this case occupies a small part of the developing ovule and seed and makes a minor contribution to the formation of the seed coat. The vascular bundle terminates, as a rule, in the chalaza (Fig. 9A–F). The perichalaza is characterized by unidirectional growth of the chalaza in the raphal and antiraphal regions. The chalazal vascular bundle ramifies into the integument. The seed coat is formed predominantly from integumentary cells (Annonaceae, Monimiaceae, Vitaceae, some Lauraceae) (Fig. 9G, H). The leptochalaza is typical of the tenui- and medionucellate ovules of some taxa in the upper level of the phylogenetic system of flowering plants (Begoniaceae, Gesneriaceae, Orchidaceae). It is represented by a small group of cells degenerating during seed development. Procambial cells, formed in the funiculus, are lacking in the chalaza or are not differentiated at all (Fig. 9I–K).

The **funiculus** is a structure attaching the ovule to the placenta. The term was proposed by Mirbel (1829). The classification of ovules according to the degree of funiculus development (Shamrov 2004) includes three types: **funicular** (the ovule has a funiculus – Fig. 10A–C) (typical of many taxa), **afunicular** (the funiculus as a structure is lacking – Ceratophyllaceae, Euphorbiaceae, Poaceae; Fig. 10E) and **sessile** (Latin *sessilis* for sessile, assidenous or attached directly by its base); the funiculus is not pronounced, the ovule being attached to the placenta by the basal portion of the raphe – Campanulaceae, Juncaceae, Paeoniaceae; Fig. 10D).

Special complexes of ovule and seed tissues. Various specific structures are formed in the nucellus. In the apical part of the nucellus, the epidermal cells may divide. The nucellar cap is formed. It consists of more than two cell layers (Calycanthaceae, Nymphaeaceae, Polygonaceae, Winteraceae). In some members of Juncaceae, Lemnaceae, Nymphaeaceae and Zingiberaceae, the cell walls of the nucellar cap are lignified and the **epistase** arises (the term was proposed by Van Tieghem 1901) (Fig. 11A–E). Sometimes, an irregularly multi-layered epidermis of nucellus (Marantaceae) can occur, and mucus deposition is observed in its cells (Malvaceae, Resedaceae). Due to the division of the parietal cell, an external derivative of the periclinally dividing archesporial cell, a special parietal tissue is formed. It forms the apical region of the nucellus of the crassinucellate ovule (Fig. 11E).

Some authors do not distinguish between such nucellar structures as the postament and podium. The available evidence suggests that the term hypostase, proposed for describing a tissue composed of cells with often lignified walls and serving as a barrier tissue to the encroachment of the embryo sac on the ovular base (Van Tieghem 1901, 1903), should be regarded as a common term referring to the 'hypostase proper', postament and podium. We propose our own treatment of these structures (Shamrov 2002a, 2008, 2015).

The **postament** is a column-like tissue located below the sporogenous or gametophytic structures. The term was proposed by Westermaier (1890). The postament represents the axial part of the nucellus in crassinucellate and medionucellate types (Figs 12A; 13A–C). It is made up of elongated or flat cells arranged in rows extending from the hypostase up to the embryo sac and often

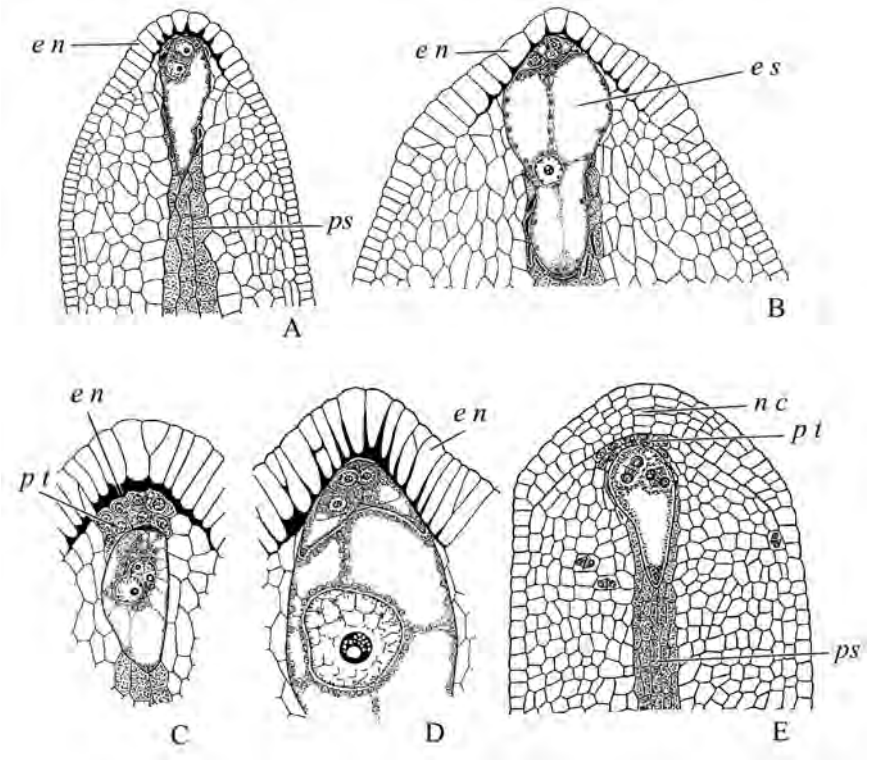

Figure 11. Transformations of the nucellus epidermis in representatives of Nymphaeaceae. A – *Nymphaea alba*, B – *N. gigantea*, C, D – *Victoria cruciana*, E – *Nuphar lutea*. *en* – epiderm of nucellus, *es* – embryo sac, *nc* – nucellar cap, *ps* – postament, *pt* – parietal tissue.

resembling to procambial cells. The postament longevity is correlated with the developmental and functional patterns of other nucellar structures. Most commonly, this column of cells collapses prior to or immediately after fertilization. In some taxa (Fagaceae, Nymphaeaceae, Orchidaceae, Paeoniaceae, Ranunculaceae), the postament represented by thin-walled cells degenerates halfway embryogenesis, in others (Araceae, Costaceae, Geissolomataceae, Grossulariaceae, Zingiberaceae) it is composed of lignified cells and persists in the mature seed.

The **podium** is a cup-shaped structure arising in the chalazal zone of the nucellus. The term was proposed by Dahlgren (1940). The description of the structure was provided later (Bor & Bouman 1974; Bor & Kapil 1974); its origin has not been traced. Our studies suggest that the podium is formed in crassinucellate and medionucellate ovules with a persisting lateral region. It differentiates as a cup-shaped structure in the chalazal nucellar zone early in ovule development. In a mature ovule, its upper boundary lies at the level of the antipodals. The nucellar parenchyma, intervening between the podium and the embryo sac, disintegrates in the course of seed development, and the podium gradually comes to lie in contact with the endosperm (Figs 12A; 13A–C). Variations in the structure and timing of differentiation of the podium are correlated with the massiveness of the crassinucellate ovule (Ceratophyllaceae, Juncaceae). Normally, the podium comprises a few layers of flat cells with dense cytoplasm. Initially, the cells are thin-walled and may show partial degeneration during seed development. Most commonly, they become thick-walled. The podium persists, completely or partially, as a cup-like structure in the mature seed (Ceratophyllaceae, Grossulariaceae, Juncaceae, Nymphaeaceae, etc.; Fig. 13C).

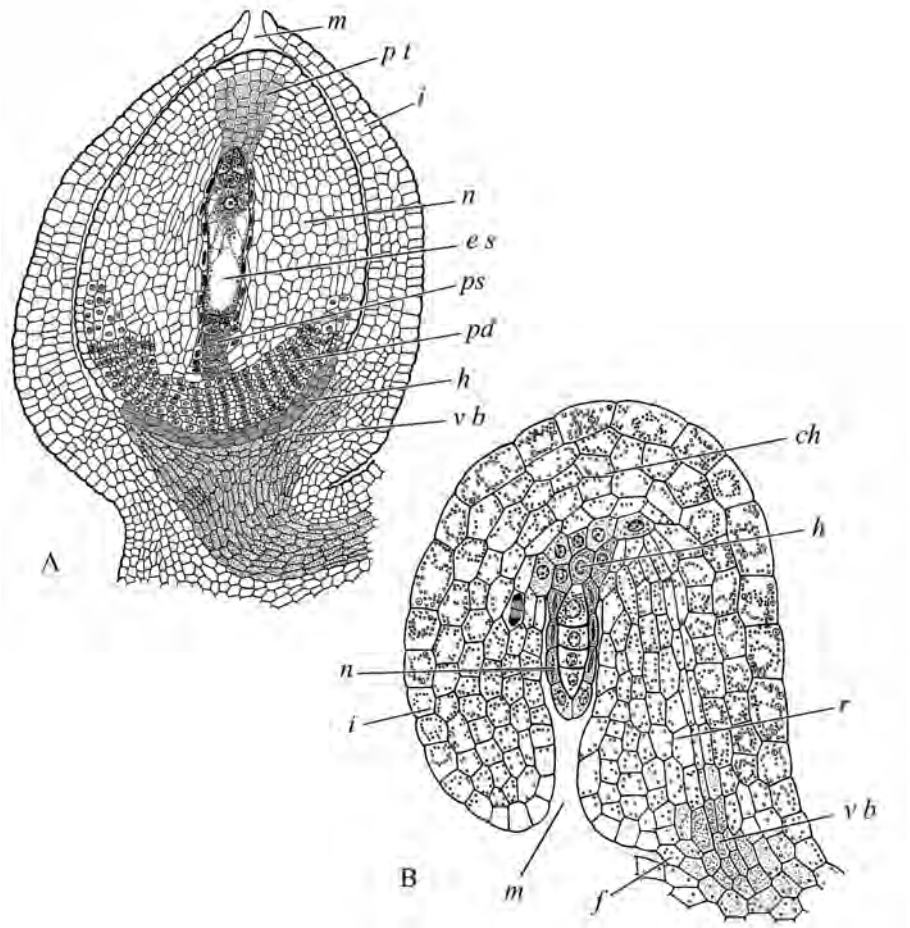

Figure 12. Ovule structure in *Ceratophyllum demersum* (A) and *Gentiana cruciata* (B). A – hemi-orthotropous, crassinucellate, unitegmic, afunicular ovule with postament, podium and hypostase, B – anatropous, tenuinucellate, unitegmic, funicular ovule with only hypostase. *ch* – chalaza, *e s* – embryo sac, *f* – funiculus, *h* – hypostase, *i* – integument, *m* – micropyle, *n* – nucellus, *pd* – podium, *ps* – postament, *p t* – parietal tissue, *r* – raphe, *v b* – vascular bundle.

The **postamento-podium** is a specialized structure formed in the chalazal region of the nucellus and combining the characteristics of the postament and the podium. The formation of this structure is usually preceded by degeneration of cells of the micropylar and middle nucellar zones prior to fertilization, the persisting chalazal zone assuming the shape of a column (Fig. 14A–C, E). In *Allium caspium*, *Azorina vidalii*, *Gagea stipitata*, *Hemerocallis citrina*, the chalazal zone of the nucellus is presented by rows of elongated (nucellar basal region) cells and a one- to two-layered lateral region. The rows of elongated cells potentially can become transformed into a postament and the central portion of a podium. The cells of the lateral region are undergoing one to two periclinal divisions. While showing a tendency to differentiate into a postament and a podium, the chalazal zone of the nucellus behaves as a single structure. In the course of seed formation, it gradually disintegrates, starting at the apical part. No structures like these (postament, podium, postamento-podium) are formed in tenuinucellate ovules.

The **hypostase** is a boundary tissue between the nucellus, integuments and chalaza. It occurs in the ovules of most angiosperms. In tenuinucellate ovules (Gentianaceae, Lamiaceae), the

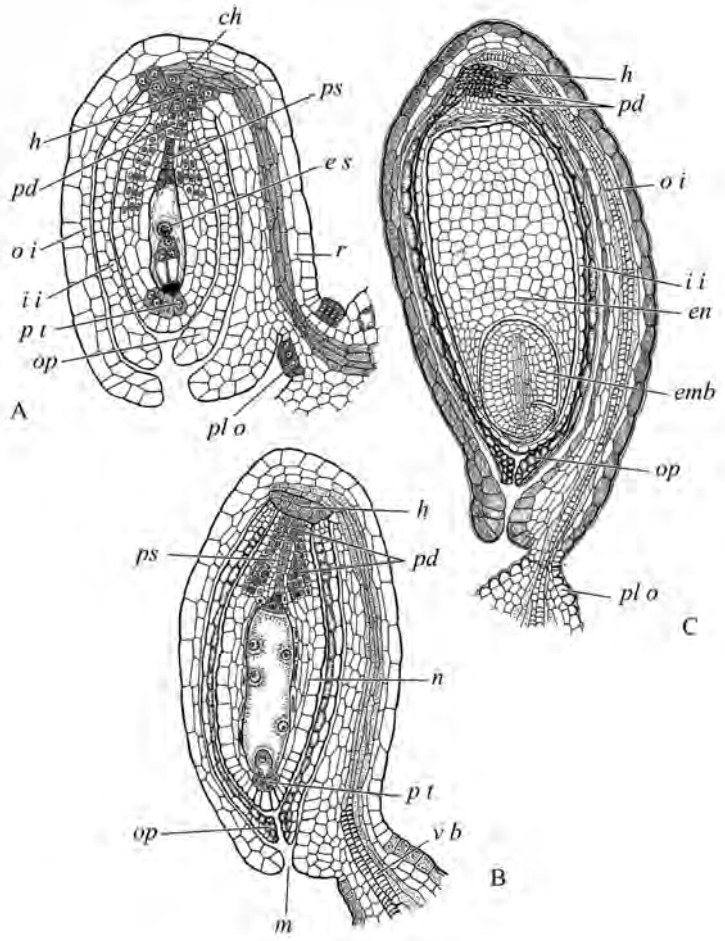

Figure 13. The ovule and seed structure in *Juncus filiformis*. A – ovule before fertilization, B – forming seed in the early stages of development of the helobial endosperm, C – mature seed. *ch* – chalaza, *e s* – embryo sac, *en* – endosperm, *emb* – embryo, *h* – hypostase, *i i* – inner integument, *m* – micropyle, *n* – nucellus, *o i* – outer integument, *op* – operculum, *pd* – podium, *ps* – postament, *pl o* – placentary obturator, *p t* – parietal tissue, *r* – raphe, *v b* – vascular bundle.

hypostase is situated immediately below the embryo sac (Fig. 12B). In crassinucellate ovules (Ceratophyllaceae, Elaeagnaceae, Nymphaeaceae, Resedaceae; Figs 12A; 13A–C) and in many of medionucellate ovules (Alliaceae, Campanulaceae, Liliaceae, Poaceae; Fig. 14A–E) between the hypostase and the embryo sac, there is the nucellar tissue which may break down during seed development, resulting in the hypostase gradually coming to lie under the endosperm. Most commonly, the hypostase is represented by a cluster of cells arranged in the form of a disc or cup. Initially, these cells are isodiametric, thin-walled, but later they become flat and vacuolate. The cell walls may become lignified or suberized, the cell contents showing tannin-like substances (Ceratophyllaceae, Cyperaceae, Grossulariaceae, Nymphaeaceae, Paeoniaceae). The hypostase's longevity is correlated with the development pattern of the nucellus, integuments and embryo sac and tends to be reduced in evolutionary advanced taxa. The major function of all the special structures in question appears to be that of directed translocation of nutrients: the hypostase – to the nucellus and integuments; the podium – to the lateral (lateral transport through integumentary tapetum and central cell) and, presumably, apical nucellar regions (apical transport through synergids and parietal tissue); and the postament – to the basal nucellar region

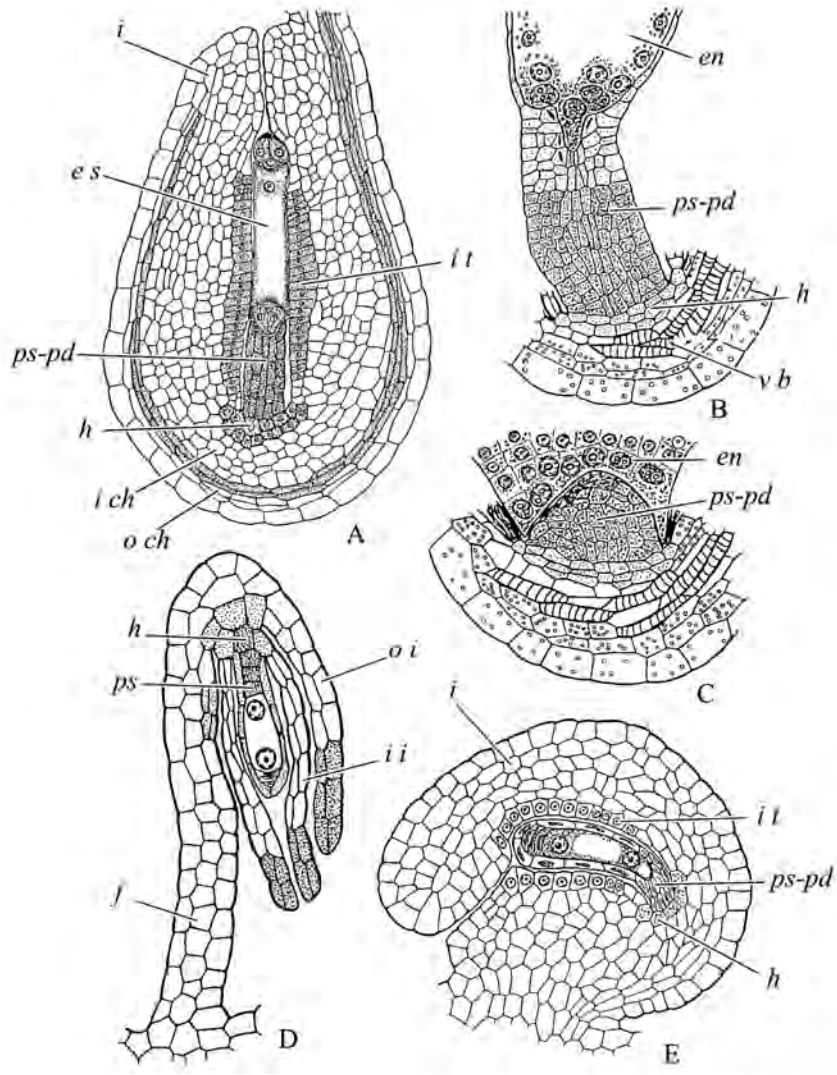

Figure 14. Transformation of the nucellar chalazal zone of the medionucellate ovules into the postamento-podium (A–C, E) and postament (D). A – *Azorina vidalii*; B, C – *Gagea stipitata*; D – *Gymnadenia conopsea*; E – *Vaccinium myrtillus*. *en* – endosperm, *e s* – embryo sac, *f* – funiculus, *h* – hypostase, *i* – integument, *i ch* – inner chalaza, *i i* – inner integument, *i t* – integumentary tapetum, *o ch* – outer chalaza, *o i* – outer integument, *ps* – postament, *ps-pd* – postamento-podium, *v b* – vascular bundle.

(basal transport through antipodals). It should be noted that the interpretation of the concept of 'hypostase' is widely discussed in the literature (Van Tieghem 1903; Maheshwari 1950; Corner 1976; Boesewinkel & Bouman 1984; Teryokhin 1996; Marzinek & Mourão 2003; Shamrov 2008; Rudall 2021). The concept is very close to our understanding in the work devoted to the structure of the seed in *Chorisia speciosa* (Bombacaceae) (Marzinek & Mourão 2003). In the chalazal region, a group of cells with thin cell walls between the integuments occurs. The cells contain phenolic substances and constitute a hypostase.

In the integument, the formation of various specialized structures occurs also. One of them is a **micropyle**, a channel formed at the tip of the ovule by one or both integuments for the passage of the pollen tube into the embryo sac. In the bitegmic ovules it can be formed by the single

integument or both ones (in the latter case the outer part, called the exostome, and the inner one, i.e. the endostome, can be distinguished). Depending on the integuments structure the micropyle can be long or short, straight, zigzagged or U-shaped (Fig. 10A–F). The epidermal cells in the apical part of the inner integument can divide periclinally, resulting in an operculum (Haloragaceae, Lemnaceae, Liliaceae, Nymphaeaceae, Zosteraceae; Fig. 10F).

In the tenuinucellate, seldom in crassinucellate (Alangiaceae, Davidiaceae, Griseliniaceae, Linaceae, Paeoniaceae) and medionucellate (Asteraceae, Brassicaceae) ovules, the cells of the internal epidermis of the integument (of the inner integument in the bitegmic ovules) adjacent to the embryo sac transform as a rule into the **integumentary tapetum** or endothelium. In bitegmic orthotropous, some anatropous, hemitropous and campylotropous ovules, the integuments develop as ring-shaped structures around the nucellus and are cup-shaped. In the majority of anatropous, some hemitropous and campylotropous ovules, the outer integument or the single one often is asymmetrical or semi-annulate. In this case the congenital fusion of the part of integument and funiculus occurs at the dorsal part of the ovule in course of development resulting in **raphe** formation (Shamrov 2000, 2008, 2018).

Before fertilization, periclinal cell divisions and a local increase in the number of parenchymal layers occur in the apical part of the external integument of some plants, as a result of which a ring-shaped structure is formed – a **micropylar collar**. The latter was found only in monocotyledonous plants belonging to the Commelinales and Zingiberales orders. In the area of the micropylar collar, the inner integument and nucellus form folds (Grootjen & Bouman 1981; Grootjen 1983). The micropylar collar is retained in the mature seed, while its inner layer can harden. Often developing together with the operculum, it forms a massive mechanical tissue of the seed coat, providing additional protection for the micropylar region of the seed and facilitating the germination process (Boesewinkel & Bouman 1984).

The **obturator** is a tissue of the secretory type composed of elongated epidermal cells of the ovary or ovule, which grows towards the micropyle and often plug the entry into it. The term was proposed by Baillon (1858). Savchenko (1973) recognizes the following types of obturator: funicular, integumentary, placental and carpellary. In addition, Veselova (1991) distinguishes the nucellar and the septal obturators. The term 'carpellary obturator' does not seem to represent the origin of the obturator adequately enough, and, since this arises from epidermal cells of the ovary wall, it should be called 'parietal' (Latin *paries* for wall). We suggest that two major types of obturator be distinguished on the basis of their origin: ovular (Latin *ovulum* for ovule) and ovarian (Latin *ovarium* for ovary). According to the position of obturator within the ovule or ovary, the following variations are recognized: within the ovular type – integumentary (Araceae, Nymphaeaceae; Fig. 10A), funicular (most taxa; Fig. 10A, B) and nucellar (Nyctaginaceae, Polygonaceae, Trapaceae); within the ovarian type – placental (Alliaceae, Juncaceae, Liliaceae; Fig. 10D), parietal (Euphorbiaceae, Rosaceae, Thymelaeaceae) and septal (Caryophyllaceae). It has been found that in funicular ovules the obturator is normally of the ovular type, whereas in sessile and afunicular ovules it is ovarian (Shamrov 2004).

Aberrant ovules and seeds: structure and diagnostics. Aberrant (Latin *aberrans* – deviating) ovules and seeds are characterized by deviations from the norm in shape, structure or functions that result in particular degeneration or their total die off. Synonyms: aborted, abnormal, sterile ovules and seeds.

The ovule and the seed are complex dynamic systems with a pulsating character of the development of structural elements. It is possible to fully assess the significance of internal and external factors in the implementation of the development program only under experimental conditions. The studies carried out have confirmed the presence of critical stages that are sensitive to the effects of certain environmental factors, at which the ovules of the seeds can stop developing (Shamrov & Anisimova 2003).The time of appearance of abnormalities, their character and degree of expression is believed to be taxon-specific; in various plants, different structures are destroyed. Aberrant ovules can differ from fertile ones, for example, by their smaller size (*Oxalis magnifica*, Oxalidaceae – Guth & Weller 1986), by increase in integument sizes and decrease in nucellus size. Aberrant ovules could be diagnosed by the change in their morphological type, occurrence of asymmetrical integument, which encircles the nucellus incompletely, lack of micropyle or formation of an extremely wide micropyle (*Rhododendron nutallii*, Ericaceae – Palser et al. 1990). In the mutant *BEL1* ovule of *Arabidopsis thaliana* (Brassicaceae), a single structure develops instead of two integuments, which could be lobed (Robinson-Beers et al. 1992). Abortion of ovules can be caused by degeneration of the funiculus (*Pistacia vera*, Anacardiaceae – Shuraki & Sedgley 1996) or chalaza (*Persea americana*, Lauraceae – Steyn et al. 1993). So-called ovuloids, constituted only from the cells of outer and inner integuments (*Eucalyptus woodwardii*, Myrtaceae – Sedgley 1989) or integument and chalaza remnants (*Vaccinium*, Ericaceae – Anisimova et al. 2005) were discovered. Aberrant ovules of *Paeonia lactiflora* (Paeoniaceae) are characterized even before fertilization by the whole complex of feature-markers, such as increase of layer number and hypertrophy of cells in integumentary tapetum and apical part of the inner integument, precocious nucellus degeneration in micropylar and middle ovule parts, precocious tannin accumulation in the cells of outer epidermis of the outer integument or the change of cell structure in placental obturator (Shamrov 1997a, 2008). In unfertilized ovules, the features of tissue and cell destruction are observed first in the inner integument and nucellus near the conductive bundle; precocious lignification of cell walls in the hypostase is noted (*Daphne arbuscula*, Thymelaeaceae – Erdelská 1999). Then, this process includes the outer integument.

Not only structural abnormalities, but also the character of metabolism in certain organs present values for diagnostics of aberrant ovules and seeds. In these ovules, even before fertilization, the cells of chalaza, integuments, nucellus and hypostase develop thick callose walls. As a result, the pathways of substance transport in the ovule are changed. The delay of cell lysis in the apical part of the nucellus prevents pollen tubes from penetrating into the embryo sac (Brassicaceae, Fabaceae, Poaceae, Rosaceae, Solanaceae – Bingham & Hawkins-Pfeiffer 1984; Vishnyakova 1991). Different rapid tests for revealing of aberrant ovules just before fertilization were suggested: fluorescence of callose (Vishnyakova 1991), reaction to pectin substances, acid polysaccharides, and acid phosphatase in micropyle region (Chudzik & Śnieżko 1999).

Abnormalities during ovule development appear to be induced by morphological, genetic, physiological, anthecological and ecological causes. One of the important factors is connected with the position of ovules in the ovary. In this case, fertilization of the first ovule and seed development from it in polyspermous fruits results in the redistribution of nutrient supply. As experimental investigation has shown, lack of development of seeds in the lower part of the fruit (in *Pongania pinnata*, Fabaceae – from 2–3-seeded it becomes one-seeded) is connected with inhibiting effect of plant growth hormones after fertilization of upper ovules (Arathi

et al. 1999). In two *Kalanchoe* species, the specific features of the internal structure of ovules were found, located in the upper (*K. tubiflora*) or lower (*K. laxiflora*) parts of the ovary. They are probably due to changes in some functional characteristics during plant development. At the border of chalaza and procambial cell strands in the ovule, a high level of phytohormones (possibly cytokinins – Terceros et al. 2020) appears to be created, which activate cell division and growth. This was reflected in the massiveness of the chalazal region of nucellus, hypostase, the creation of a weakly expressed endopachychalaza and a more massive external integument (Anisimova & Shamrov 2018).

According to Charlesworth (1989), in populations of plants, especially perennial and exclusively cross-pollinating plants, a 'genetic load' (recessive lethal mutation) is accumulated that decreases the viability of the population. Seeds with 'harmful' mutations die off first. Experiments with *Capsicum annuum* (Solanaceae) testified to the influence of anthecological factors on the formation of aberrant ovules (Marcelis & Baan Horman-Eijer 1997). They have shown that with insufficient quantity of pollen during flowering a considerable number of aberrant ovules and seeds was produced in the fruits. With additional pollination the number of fertilized ovules increases, but there is also abortion of the fruits developing after the first pollination. That is why for obtaining a larger number of fruits, a smaller pollen quantity is recommended than needed for fruit setting from ovules available.

The occurrence of aberrant ovules in the ovary results in a decrease in real seed productivity. Ovules with deviations can degenerate completely during the developmental process or be preserved, transformed into seeds that are distinguished from normal ones by shape, size, colour and inner structure. The structural and often functional variations of seeds within the same fruit or the same plant are believed to be the basis of heterospermy. In *Vaccinium myrtillus* (Ericaceae), it was revealed that the morphogenesis of ovules and seeds in one fruit is characterized by asynchrony. In the process of their development, various anomalies were found: destruction of the entire ovule or its individual structures at different stages of formation. In fruits collected during the dissemination, the seeds differ in the degree of formation of the embryo, endosperm and seed coat: large, medium and small fractions. Only the seeds of a large fraction, which have a normally developed embryo and endosperm, are capable to give the seedlings. Medium and small fractions are aberrant seeds at different stages of degeneration (Anisimova et al. 2005). Ovules and seeds develop similarly in *Rhododendron luteum* and *R. schlippenbachii* (Ericaceae). Large seeds often contain large embryo and endosperm with an endospermal cavity. These seeds usually germinate. Medium seeds are smaller, often deformed and flattened. Their endosperm is usually not fully formed, and the embryo may be absent. Small seeds are 'dusty', and their shape corresponds to the ovules that have stopped developing. They are mainly represented by preserved integument and chalaza cells with thickened cell walls, without signs of development of embryonic structures. Seeds of medium and, especially, small fractions of *R. luteum* often lack a wing border, which is present in large seeds of this species. In them as well as in some of the seeds of the large fraction of both species, secretory cells are formed in the base of funicular region of raphe, which, possibly, perform the function of elaiosomes. Among the large and medium seeds, there were seeds whose embryos stopped at the globular and early heart-shaped stages. It is possible that such seeds with underdeveloped embryos form the soil bank of seeds, the germination of which will not occur in the year of maturation, but later (Shamrov et al. 2021).

The phenomenon of aberrant ovules and seeds is widespread in angiosperms and is connected in certain plants with adaptation to dispersal. Such ovules and seeds are observed mainly in polyspermous fruits and often in plants, the fruits of which are dispersed by water, wind or animals. The analysis of aberrant ovules is of particular interest in assessing their fertility and sterility. According to the quality of the formed seeds, it is possible to select productive forms of plants (Shamrov 2008; Bukharov 2020). The appearance of aborted ovules in certain plants, connected with redistribution of nutrients from degenerating ovules to developing ones, is regarded as one of the elements of life strategy in extreme habitat conditions (Erdelská 1999). Investigation and diagnostics of aberrant ovules and seeds are of great importance theoretically and practically. This direction of research is especially relevant in connection with the revealing of mechanisms of unfavourable external factors influencing the reproductive structures and in connection with the preservation of biological diversity. The revealing of feature-markers and further elaboration of rapid methods for appreciation of developing ovules, especially just before fertilization, remains one of the paramount tasks in the investigation of reproductive biology of rare, disappearing and economic plant species.

Conclusion

The central problem of modern plant embryology is the study of the patterns of differentiation of the ovule and seed, which ensure the development of reproductive structures. Studies have shown that the ovule and seed are integrated dynamic systems. Their main elements determine the normal megasporogenesis, embryo sac formation and, after fertilization, the specifics of the embryo and endosperm development. These features ultimately ensure the reproduction and seed productivity of angiosperms.

The development of reproductive structures is influenced by various biogenic and abiogenic factors. They can cause various anomalies in ovule and seed structure and fertility. Aberrant ovules and seeds revealing various morphogenetic deviations are often found: a change in the morphological type of ovule; disturbances of spatial and temporal coordination in the development of nucellus, integument, chalaza and funiculus; premature degeneration, absence or formation of additional structures. Disturbances in the metabolite transport were detected as well. For their identification, rapid tests are proposed. Deviations in the development can lead to a decrease in seed productivity. The information obtained is of particular importance in the study of the reproductive biology of rare, disappearing and economic plant species.

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