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Development of Seed in *Psoralea corylifolia*

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Fertilization, endosperm, embryo and seed coat of *Psoralea corylifolia* are described. Fertilization is porogamous. Endosperm follows nuclear type of development. Embryogeny falls under the asterad type and according to Sou-èges system it correspond to Period I, series A or B and megarchetype II. A rare case of synergids forming embryos has been noticed. Seed coat is derived from the outer integument alone and its structure is like that of other papilionaceous taxa with characteristic palisade layer coming from outer epidermis of outer integument and the hypodermal layer being formed of hourglass cells.

Key Words - Fertilization Embryogeny Endosperm Seed coat.

Embryological work on Papilionaceae is impressive (Schnarf 1931, Davis 1966). These indicate the existance of significant variation in the type of ovule, megaspore tetrad patterns (Rembert, 1967, 1969; Oomman, 1975; Deshpande, 1977), structure and nature of endosperm haustoria (Rau, 1951. a,b,d,f, 1953; Johri & Garg, 1959; Oomman, 1975; Deshpande, 1977) and embryogeny (Johansen, 1950, Rau, 1950 a,b, 1951c, e, 1954; Crété, 1963; Goursat, 1969). Corner (1951) studied in detail seed-coat structure of Fabaceae and concluded that the Papilionaceous seeds are highly advanced and specialized. The family is manifestly very heterogenous and unique and in this respect differs not only from related taxa but among the angiosperms as a whole.

The present account deals with fertilization, embryo; endoperm and seed coat of *Psoralea corylifolia*, a common winter annual of wasteland and barren fields. The gametophytes and morphology of gynoecium were described by Joshi (1938). Souèges (1950) investigated the embryogeny of *P. bitumiosa* which corresponds to Period I and megarchetype II.

MATERIAL & METHODS - The material was collected locally and fixed in 70% FAA. Open flowers and developing seeds were processed for dehydration, embedding and clearing. The material was sectioned at 8 to 14 m. The slides were stained in Heidenhain's iron-alum haematoxylin, destained in a saturated solution of picric acid and counter stained with light green (Johansen, 1940).

OBSERVATIONS-Fertilization - Fertilization is prorgamous (Fig 1). A single pollen tube enters the embryo sac. Triple fusion precedes syngamy. In a few cases, the synergids remain intact. The embryo sac shows starch grains during fertilization (Fig. 2).

Embryo - The zygote enlarges considerably (Fig. 2) and divides transversely to form terminal cell *ca* and basal cell *cb* (Fig. 3). The division in *ca* is invariably oblique resulting in unequal cells *a* and *b* (Figs. 4,6,8) while that in *cb* may be vertical (Figs. 4,7) or vertically oblique (Fig. 8). The proembryonic tetrad is thus of the B 1 category and this is the common condition. In a few instances, however, the tetrad may be of B2 category where during the division of *cb* orientation

of wall separating cells *m* and *ci* may be variable (Figs. 6,9).

During quadrant formation, the larger daughter cell, *a*, of *ca* divides obliquely and cuts off an epiphysis initial *e* (Figs. 6-9). The sister cell *b* divides vertically (Figs. 6-9). The derivatives of *cb* are referred to here as *m* and *ci*, the latter is invariably slightly larger and, it divides transversely into cells *n* and *n'* (Figs. 7,8). Cell *m* may divide transversely (Fig, 7) or vertically (Fig. 6). At the close of third cell generation, the proembryo has 8 cells. During further development, the epiphysis initial divides vertically, while the elements of *ca* lateral to it, segment in the same way (Figs. 7, 8). The epiphysis initial develops into stem tip while the lateral elements form the cotyledons (*pco*) (Figs. 12-16). Thus, the tier *ca* gives rise to stem tip and cotyledons only. By now cells of *m*, *n* and *n'* also divide, in different planes (Figs. 10, 11). During further development, the adjacent cells derived from *m* and *n* form a composite zone from which are later derived the hypocotyledonary region and the radicle (Figs. 12-16) Elements of *n'* which lie farthest, develop into suspensor. Thus, from the cell *cb*, are derived the hypocotyledonary region (*phy*), initials of central cylinder of the root (*icc*), reticular portion including root cap (*iec* and *co*) and suspensor. This is indicated by the following formula:

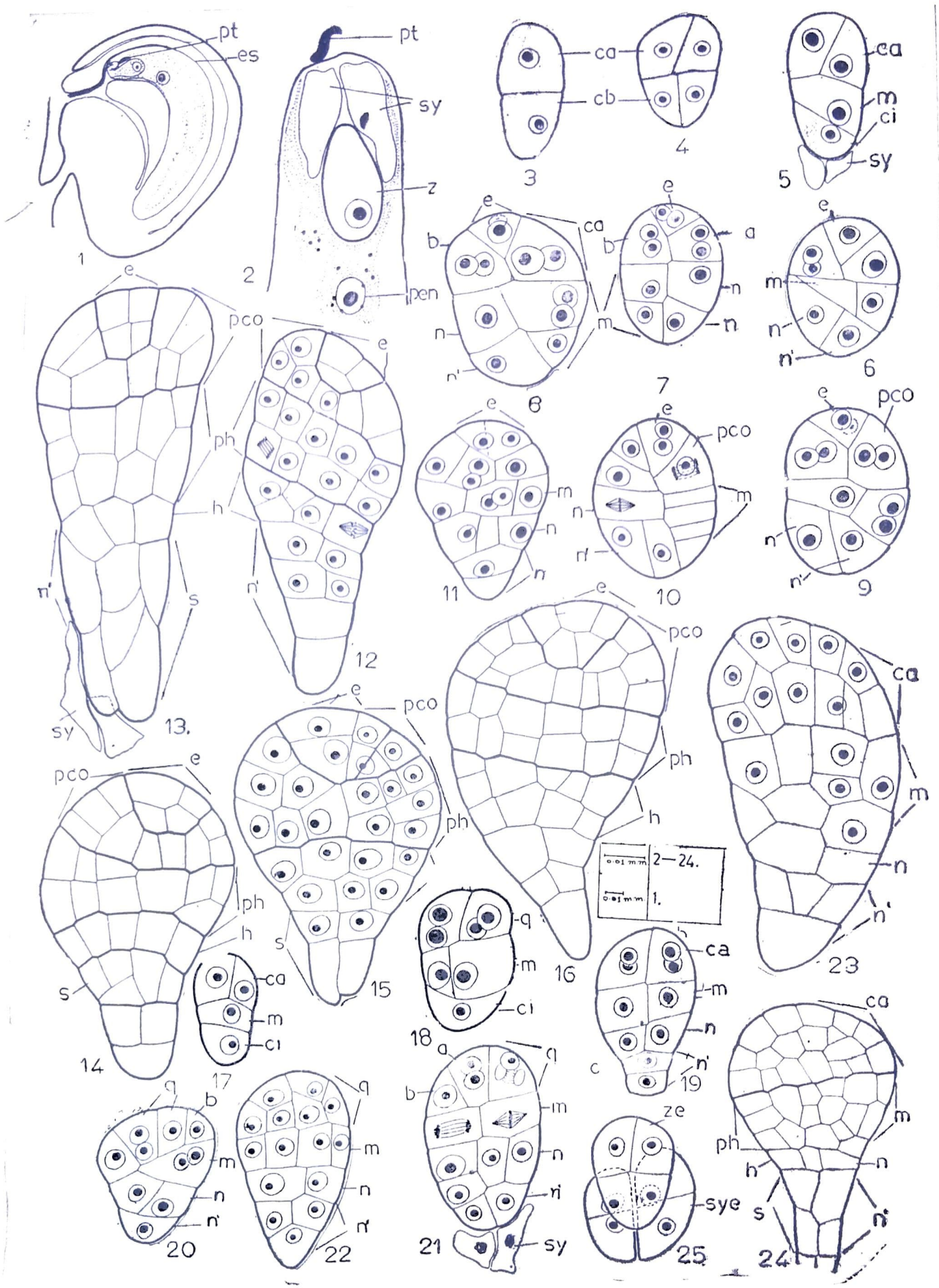
$$ca = pco + pvt;$$
$$cb = phy + icc + iec + co + s$$

Embryogeny in *P. corylifolia*, thus corresponds to Period I, series B1 and megarchetype II as in *P, bituminosa* (Souèges, 1950).

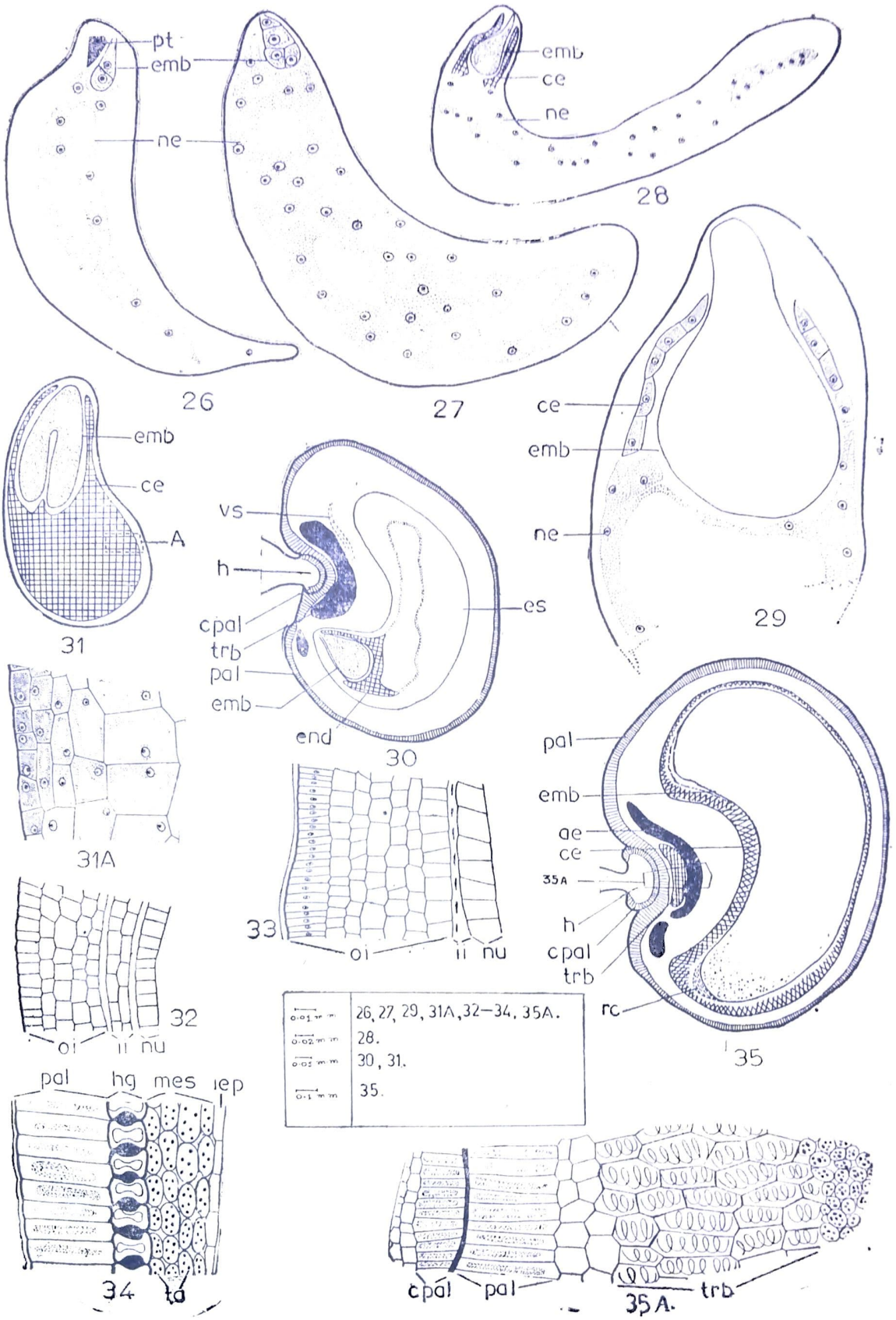
In a few ovules, the first tetrad is of B2 category with well defined tiers of *m* and *ci* (Figs. 6,9). These tiers are clear cut at the 16 celled stage of the proembryo (Fig. 9). Figure 11 represents a later stage of embryo where tiers *m*, *n* and *n'* are well marked. In this embryonic type an epiphyseal cell (*e*) is delimited at the quadrant stage (Fig. 6). Embryogeny here, too, follows megarchetype II under Period I or the Asterad type of Johansen (1950).

The epiphyseal cells next segment vertically in a plane at right angles to the earlier one and form 4 cells (Fig. 11). Dermatogen initials are demarcated later (Figs. 12, 13). Elements on either side of epiphysis (*pco*) then divide in different planes (Figs. 12,13) and soon dermatogen initials are cut off (Fig. 15). The region immediately below *ca*, soon becomes resolved into two tiers with well marked dermatogen (Figs. 12-15). This is the zone that later forms the hypocotyledonary axis of the embryo (*ph*). The hypophyseal region (*h*) in single layer initially (Figs. 12-14) but it later becomes two layered (Fig. 16). At the globular stage of embryo, the different regions are clearly set apart. (Fig. 16). The suspensor cells derived from *n'* do not show any regular sequence of development. These are comparatively larger and highly vacuolated. During later stages of development they elongate (Fig. 13). The suspensor persists till early dicot stage of embryo.

In several ovules, the proembryonic tetrad, however, belongs to series A, category A2 (Fig. 17) At the quadrant stage, epiphysis initial is not delimited (Figs. 18, 19). During octant formation, cells of the tier *q* divide by vertically-oblique walls to delimit 4 circumaxial cells, *a*, from the peripheral cells *b* (Figs. 20,21) as in *Urtica pilulfera* (Souèges, 1921). Later on dermatogen is cut of here (Fig. 22). Cells of *m* divide vertically first (Fig. 21) and dermatogen differentation takes place (Fig. 22). Tiers *n* and *n'* are clear at this stage. The tier *n* serves as the hypophyseal region during subsequent development (Fig. 24). The embryo development here follows the Asterad type and keys out to Erodium variation, since tier *n'* only forms suspensor. According to Souèges (1951) system, embryogeny in such cases follows Period I series A and megar



1. : L.S. ovule showing path of the pollen tube in the embryo sac. 2. : L.S. micrapylar part of embryo sac, showing synergids, fertilized egg, and primary endosperm nucleus; note pollen tube and starch grains inside the embryo sac. 3-24 : Various stages in the development of embryo; note the formation of epiphysis in figs. 6-16 and their absence in figs. 18-24. 25. : Zygotic proembryo, with 2 synergid embryos. Note persisting synergids in figs. 5,13 and 21.



26,27 : Nuclear endosperm at 2-celled and 4-celled embryo. 28, 29 : Cellularization of nuclear endosperm at micropylar end at the globular stage of embryo. 30 : L.S. developing seed showing cellular endosperm around globular embryo and free-nuclear endosperm. 31. : Discot embryo and completely cellular endosperm. 31A. : Part of cellular endosperm marked in fig. 31 magnified to show details. 32-34 : Various stages in the development of seed coat; note degeneration of inner integument in Fig. 33 and the conspicuous palisade and hour glass cells of the seed-coat. 35. : L.S. maturing seed (diagrammatic) showing dicot embryo with a thin layer of endosperm, palisade layer of seed coat; note the counter palisade tracheidal bar and stellate parenchyma in the subhilar region. 35A. Part of seed coat from subhilar region, from fig. 35, enlarged to show details.

chetype II. Later stages of maturing embryo are depicted in Figures 28-31. The mature embryo has two massive cotyledons that enclose the plumule and almost straight radicle with prominent root cap (Fig. 35). It shows distinct vasculature and practically occupies the entire seed.

It is apparent that in *P. corylifolia*, the first tetrad may belong to either series A or B. Moreover, embryo development follows megarchetype II, a pattern rather in the family.

A rare case of synergids developing into embryo has been noticed. Both synergid embryos are 2-celled while the zygotic embryo is 4-celled (Fig. 25). The persisting nature of synergids is evident from Figures 13 and 21.

Endosperm The primary endosperm nucleus divides earlier than zygote. The initial divisions are free nuclear. There are 8 endosperm nuclei in the embryo sac at the 2-celled stage of embryo (Fig. 26) and around 32 at the tetrad stage (Fig. 27). These are arranged peripherally around a large central vacuole. By further divisions, a large number of nuclei are organized in the enlarging embryo sac that gradually extend towards the chalaza (Figs. 28, 29). The chalaza part of endosperm remains free nuclear for quite some time (Fig. 30) and when the cotyledons are well formed, the endosperm becomes completely cellular (Fig. 31). The endosperm tissue has a superficial layer of tubular cells around the bulk of polygonal cells. The cells in the central part are largest and highly vacuolated (Fig. 31A). Endosperm development is thus of the nuclear type. The enlarging embryo consumes the entire endosperm tissue. Mature seeds thus are non-endospermic.

Seed Coat The ovules are bitegmic. At fertilization, the inner integument is 2 layered while the outer one shows as many as 5 layers (Fig. 32). During post fertilization development, the inner integument is completely absorbed (Fig. 33) and seed coat is formed by the outer integument alone. At the globular stage of embryo, the number of layers of outer integument increases to 7 (Fig. 33). These layers persist in mature seed. After this stage, morphological and histological changes take place in various layers of outer integument. The cells of outer epidermis enlarge considerably in the radial direction (Fig. 33). These are converted into 'macroscleroids' or 'Malpighian cells' (Fig. 34) forming the characteristic palisade layer of the seed coat. A well defined cuticular layer is present over it. The cells of hypodermal layer after enlargement, become unevenly thickened and organise the conspicuous layer of hour glass cells of the seed coat (Fig. 34). The mesophyll of the seed coat comprises 4 layers of ellipsoid cells below the layer of hour glass cells. These cells enclose tannin. The inner epidermis comprises highly flattened cells.

Seed coat structure in the subhilar region - The Papilionaceous seed is distinctive in possessing a relatively large hilum which may be round, oblong or extended with a clear median groove. The hilar region has micropylar region on one side and the region of raphe on the other. The part of the seed coat lying between the hilum and the embryo is comparatively thick. This is subhilar region. The subhilar region shows a counter palisade, adjacent to palisade,and it is derived from basal cells of hilum (Figs. 35, 35A). Another distinctive feature is the presence of cresent shaped tracheidal bar. It comprises short thick walled cells (Fig. 35A). The part of subhilar region on sides of tracheidal bar shows thick walled aerenchyma with stellate ends. Below the tracheidal bar are present a few rows of rounded cells with tannin deposition (Fig. 35A).

DISCUSSION The endosperm development, *sensu stricto*, follows the nuclear type as in most

other investigated members of the family (Davis, 1966). There are, however, large variations in different taxa in respect of (i) the number of nuclei present at the time of zygotic division, as also at 8 celled stage of embryo, (ii) time and place of initiation of wall formation, (iii) organization of cellular endosperm and (iv) presence or absence of endosperm haustorium, its size and shape. The degree of cellularization of endosperm shows great variation in the family. In *Indigofera* and a few other taxa, the entire endosperm becomes cellular without organising a haustorium. In *Desmodium* (Rau, 1953; Johri & Garg, 1959; Deshpande, 1977) and *Cymopsis psoralioides* (Rau, 1953) the endosperm becomes cellular except at the chalazal tip which remains free nuclear and becomes haustorial. In other taxa the cellular endosperm may be restricted to micropylar half or upper 2/3 part of developing seed, with a prominent chalazal free-nuclear process that later degenerates. Such a situation is reported by Rau (1951, 1953); Johri & Garg (1959); Kapuskar, (1964); Terzijski (1971); Deshpande & Bhasin (1976).

A meagre development of cellular endosperm, restricted to a few layers around embryo, is reported in some members of the *Phaseoleae* (Rau, 1951, 1955) and recently in Lathyrus (Terzijski *et al*, 1974). The extreme condition, "total absence of transition of nuclear endosperm into stable cellular form" first reported by Guignard (1882) in some members of Vicieae, was confirmed by Rau (1953) in *Stylosanthes mucronata*, Danilina (1959) in *Lathyrus annuus* and by Terzijski *et al* (1974) in *L. sylvestris* and *L. tuberosus*. In such species, the endosperm remains free nuclear throughout its development.

The embryogeny corresponds to megarchetype II in First Period of Souèges (1951) system or the Asterad type of Johansen (1950). The proembryonic tetrad falls under both series, A and B, erected by Souèges (1951). This indicates existence of significant variations in respect of embryo development in the family. The works of Rau, (1954), Souèges & Crété (1952), Crété (1963), Goursat (1969), Oomman (1975), Deshpande (1977) have established the presence of great variations of profound magnitude in the development of embryo in the family. These differences are not restricted to the tribal, subtribal or generic level, but extend at the specific level. This is not all, even the same species may exhibit different patterns of embryo development. The present findings testify to this.

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