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Structure, development and histochemistry of embryo and endosperm in *Sesbania speciosa* Taub. Ex Engl.

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Abstract

Sesbania speciosa Taub. ex Engl. is an introduced plant cultivated in India for fibre, green manure and nutraceutical potential. The development and histochemistry of the endosperm and embryo of this plant have not been yet at all reported and the present study was carried out in an effort to bridge that gap in our knowledge. Two-micrometer sections of seeds of *S. speciosa* at various stages of development were cut on a rotary microtome. DNA, ribonucleic acid and insoluble polysaccharides were found to be locally localized. It was observed that the ovule of the *Sesbania speciosa* is camplotropous, bitegmic and crassinucellate and the embryo sac is a 7-celled structure. The synergids possess PAS-positive filiform apparatus. All the cells of the mature embryo sac, before fertilization is bereft of polysaccharide grains. The embryo proper, during early embryogenesis, contains a high concentration of proteins and nucleic acids but lacks polysaccharide grains. At the dicotyledonous embryo stage, the concentration of proteins and nucleic acids declines and is followed by the synthesis of polysaccharide grains. The embryo suspensor is massive. The endosperm development is of the nuclear type. At the late globular pre-embryo stage, the micropylar 1/3 of the endosperm becomes cellular leaving the rest free-nuclear. The endosperm cytoplasm and nuclei aggregate in the micropylar region and are rich in total proteins and nucleic acids. The concentration of these metabolites, however, declines when the endosperm becomes cellular. The aleurone layer, in mature seed, is rich in proteins and nucleic acid compared to the other persisting endosperm layers.

Keywords

Embryo, *Sesbania speciosa*, embryo sac, nuclear endosperm, suspensor, histochemistry

Introduction

The family Fabaceae possess anatropous, bitegmic and crassinucellate ovules (1-2). Occasionally hemianatropous or campylotropous ovules are also reported (3). The embryo sac is 7-celled and eight nucleate consisting of 2 synergids, an egg cell, a central cell and 3 ephemeral antipodal cells. The development of embryo sac in most of the investigated taxa of the family follows the Polygonum type (2, 4) but Rembert reported an *Allium* type of development in *Robinia pseudo-acacia* (5).

The zygote is the starting point of a series of subtle and complex influences in the development of adult organisms. The derivatives of the zygote have particular functions to perform. The basal and terminal cells

differentiate into the embryo suspensor and embryo proper respectively (6). The suspensor cells are replete with wall ingrowths and are involved in the absorption and short distance transport of metabolites during the early stages of embryogenesis. A correlative developmental and histochemical study will throw light in understanding the role of angiosperm suspensor during early embryogenesis (7).

The nuclear endosperm is a characteristic feature of the Fabaceae (earlier Leguminosae) (2). During the later stages of embryo development, the micropylar half to 2/3 of the endosperm becomes cellular and the rest remains free nuclear. The haustorial activity of endosperm is well known (3, 8-13). Endosperm plays an important and unique role in the nutrition and differentiation of the embryo (14-15). It assists in the mobilization of food reserves from the surrounding somatic tissues but the direct evidence is lacking.

Sesbania speciosa is an introduced plant, cultivated in India for fibre, green manure (16) and neutraceutical potential (17). Plant growth and development information is an essential requirement for improving this economically important plant. A study was carried out to bridge the knowledge gap regarding endosperm development and histochemistry in this plant.

Materials and Methods

Seeds of *S. speciosa* at various stages of development were collected from the plants grown in the botanical garden, Department of Botany, University of Delhi, Delhi. The seeds were fixed in precooled 10% aqueous acrolein. Dehydration, infiltration and embedding in glycol methacrylate were done according to Feder and O' Brien (18). Two-micrometer sections were cut on a rotary microtome using glass knives fitted to an indigenously devised glass-knife adaptor. Insoluble polysaccharides were localised with PAS-reaction; total proteins with Coomassie brilliant blue (19); DNA with feulgen reaction (18); and ribonucleic acid with Pyronin Y reaction (20).

Results

Structure and development of the embryo

The ovule is bitegmic and campylotropous. The embryo sac is a 7-celled structure consisting of an egg, 2 synergids, a large central cell and 3 ephemeral antipodal cells. The synergids at the micropylar region possess a filiform apparatus (Fig. 1A). The egg cell and central cell have a large vacuole that occupies most of the cell space surrounded by a thin layer of cytoplasm. A prominent nucleolus is present in the secondary nucleus of the central cell. The pollen tube, after reaching the ovule, travels through the micropyle, and enters the previously degenerated synergid (Fig. 1B) The unpenetrated synergid remains healthy and persists for some time.

The zygote divides transversely resulting in a small apical cell towards the interior of the embryo sac (progenitor of embryo proper) and a large basal cell (progenitor of suspensor) toward the micropyle. Anticlinal and periclinal divisions in these cells result in the formation of a multicellular proembryo (Fig. 1C, D) with small cells containing dense cytoplasm and a well-differentiated, massive multiseriate suspensor. The suspensor cell walls subjacent to the embryo sac wall reveal wall ingrowths (Fig. 1C). The embryo sac wall both at the micropylar and the chalazal ends also shows prominent wall labyrinths (Fig. 2E). An increase in cytoplasmic vacuole formation is observed in the cells of embryo-proper during further development.

The cell divisions accompanied by accelerated cell expansions result in the progression of the preglobular proembryo to the globular stage (Figs 1E, F; 2A, C, D) and then to early heart-shaped embryo with the initiation of cotyledons (Figs. 4; 5A, B, D, E). The suspensor attains maximum size by the early cotyledonary stage (Fig. 3A, D). In the mature seed, the major portion is occupied by an embryo with well-differentiated root and shoots apices, procambium, protoderm and ground meristem (Fig. 5A). A pair of leaf buttress arises below the shoot apex of the young embryo which later differentiates into the leaf primordia (Fig. 5A, C, F). At the late dicotyledonous embryo stage the cells of the hypocotyl and cotyledons are replete with protein bodies (Fig. 5E).

Structure and development of the endosperm

The endosperm development is of the nuclear type. In the central cell, free-nuclear divisions of the primary endosperm nucleus give rise to numerous nuclei in the peripheral cytoplasm lining the central vacuole (Fig. 1E). An aggregation of the endosperm cytoplasm and nuclei at the micropylar end of the embryo sac is observed (Fig. 1D). The endosperm nuclei at this end continue to increase in number till the formation of the globular proembryo. Later, wall formation initiates at the micropylar region and about 1/3 of the endosperm becomes cellularized. This portion of the cellular endosperm constitutes the endosperm proper (Fig. 2A).

The rest of the endosperm remains nuclear and its cytoplasm along with nuclei is restricted to the periphery of the embryo sac wall. The endosperm in this region of the embryo sac shows the presence of hypertrophied nuclei, dense cytoplasm and wall-ingrowths (Fig. 2B) and resembles a haustorium. The extreme chalazal end of the endosperm wall shows undulations (Fig. 2B). The cells of the endosperm-proper divide and occupy a large portion of the embryo sac. The endosperm becomes completely cellular at the early cotyledonary embryo stage (Fig. 4). The cells in the central region of the cellular endosperm are large, vacuolate and thin walled whereas the endosperm epidermal cells that are adjoining the embryo sac wall are small and possess dense cytoplasm (Figs 3C, D, E; 4). This small, dense layer is the presumptive aleurone layer. The rapidly growing embryo, gradually digests, the surrounding endosperm tissue (Fig. 5A). Only a few layers of endosperm tissue are left. In the mature seed, on the raphe side and in the region adjacent to the shoot-tip,

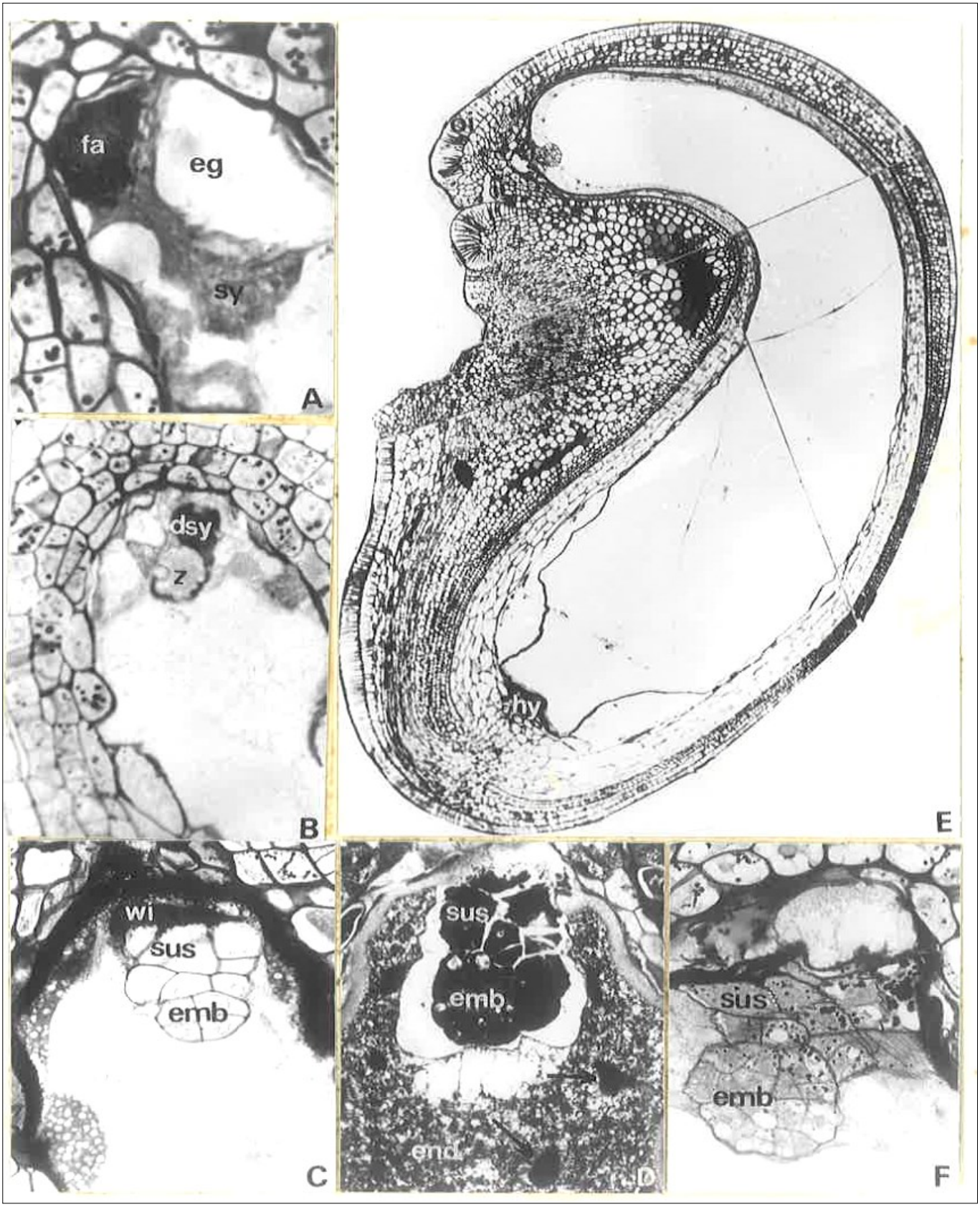

Fig. 1. dsy – degenerated synergid; **eg**, egg ; **emb**, embryo; **end**, endosperm; **fa**, filiform apparatus; **hy**, hypostase; **ii**, inner integument; **oi**, outer integument; **sus**, suspensor; **Z**, zygote.

A. Micropylar region of the ovule stained for insoluble polysaccharides to show the synergid with deeply stained filiform apparatus. The egg cell is bereft of polysaccharide grains. X1750, **B**. Portion to show the degenerating synergid and a well-developed zygote X1560, **C.** Portion of longisection of seed at preglobular proembryo stage stained for insoluble polysaccharides to show the well-stained embryo sac wall projections. The embryo-suspensor and embryo sac interface, towards the micropyle possess well developed wall-labyrinths. X 1560, **D.** Longisection of developing seed at preglobular proembryo stage, stained for total proteins to show in embryo-proper and embryo-suspensor, a high concentration of cytoplasmic proteins. The endosperm nuclei (arrows) are large and protein rich X1560, **E**. Photomontage of developing seed at globular proembryo stage stained for insoluble polysaccharides. The Campylotropous ovule has a minute proembryo at the micropylar region. The outer integument is five layered whereas the inner integument is two layered. The cell walls of outer and inner integuments are well defined. The hypostase cells show deeply stained and thick cell walls. X200, **F**. Preglobular proembryo enlarged to show prominent embryo sac wall projections. Both the suspensor and organogenic parts of the embryo contain PAS positive grains. X1560.

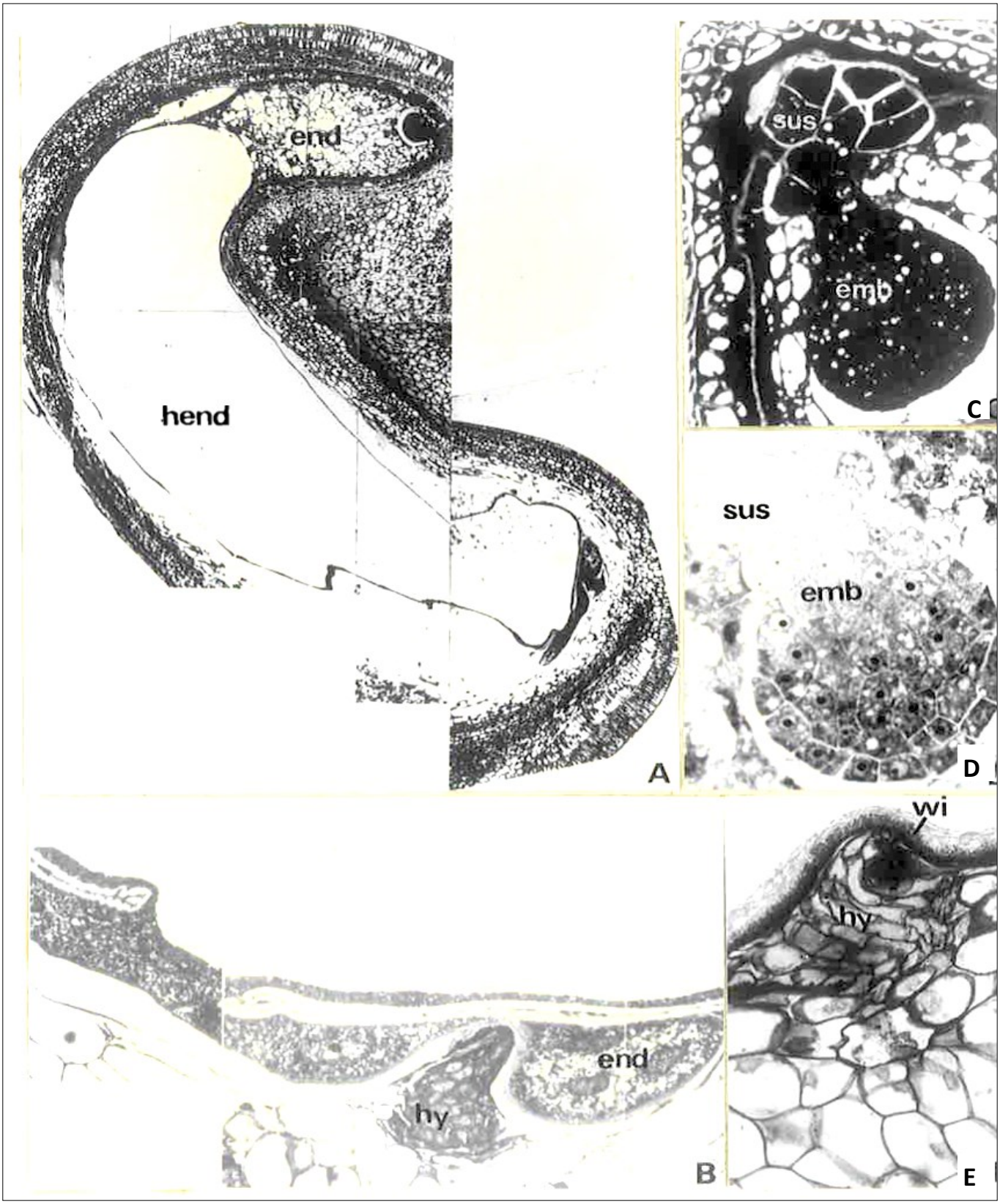

Fig. 2. **emb**, embryo; **end**, endosperm, **hend**, endosperm haustorium, **hy**, hypostase; **sus**, suspensor; **wi** wall ingrowths.

A. Longisection of a developing seed at globular proembryo stage stained for total proteins. The endosperm haustorium is undulate. At the chalazal end the haustorium hypostase interface is noteworthy . Both the embryo-proper and embryo suspensor cells are rich in cytoplasmic and nuclear proteins (photomontage) X200, **B**. Chalazal portion of the endosperm haustorium to show the protein-rich finger-like wall labyrinths and hypertrophied endosperm nuclei X1560, **C.** Globular proembryo stained for total proteins and showing protein-rich embryo and suspensor proper cells. X1560, **D.** Globular proembryo stained for ribonucleic acid. The embryo proper cells show pyroninophilic cytoplasm and nucleoli, while the suspensor cells are feebly stained. X1560, **E.** Chalazal portion of the developing seed stained for insoluble polysaccharides. The well-stained embryo sac wall ingrowths above the hypostase is noteworthy. X1560.

some endosperm tissue persists (Fig. 5A).

During the later stages of seed maturation, the presumptive aleurone layer differentiates into the aleurone layer whose cells become thick-walled, possesses prominent hypertrophied nuclei, dense cytoplasm and protein bodies.

Histochemistry

Insoluble polysaccharides

The embryo sac wall shows PAS-positive wall projections that persist up to the heart-shaped embryo stage (Figs. 1C, 2E).

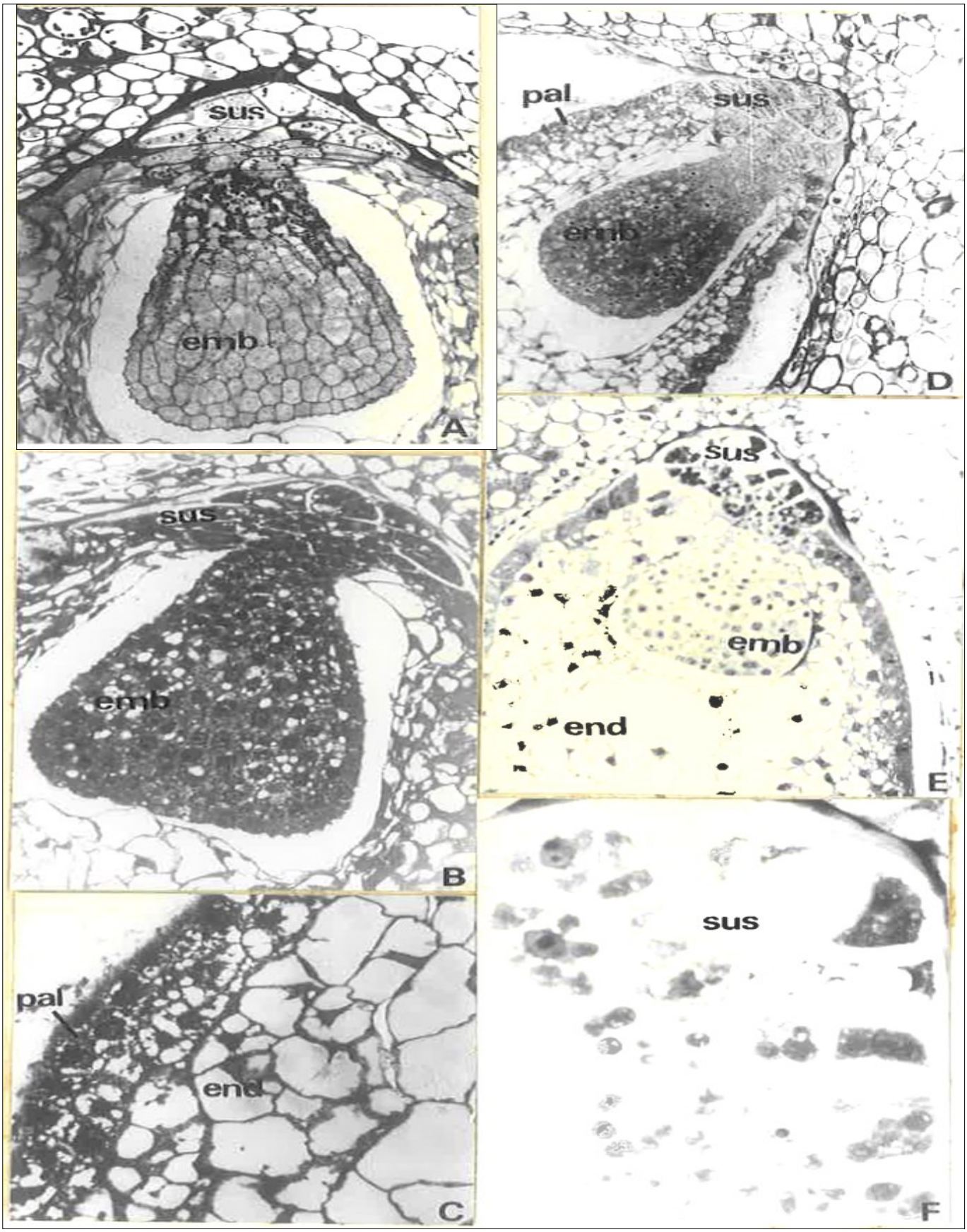

Fig. 3. **emb**, embryo; **end**, endosperm; **pal**, presumptive aleurone layer; **sus**, suspensor;

A. Heart-shaped embryo, stained for insoluble polysaccharides, to show the PAS positive grains in the embryo suspensor and the subjacent embryo cells. X1000, **B.** Same as A, stained for total proteins. The endosperm tissue surrounds the embryo. The embryo proper and embryo suspensor cells are rich in total proteins X1000, **C.** A portion of endosperm at heart-shaped embryo stage stained for total proteins to show the presumptive aleurone layer whose cells have a high concentration of total proteins. X1560, **D.** Longisection of seed at early heart-shaped embryo stage stained for ribonucleic acid. The embryo-proper cells show pyroninophilic cytoplasm and nucleoli and the suspensor cells are feebly stained. The presumptive aleurone layer cells show higher concentration of cytoplasmic RNA than the subjacent endosperm cells. X1000, **E.** Late globular-shaped embryo stained for deoxyribonucleic acid to show the intensely stained nuclei of the embryo-proper, suspensor and endosperm with the presumptive aleurone layer cells. X 620, **F.** Embryo suspensor showing multinucleate cells whose nuclei are well stained X1000.

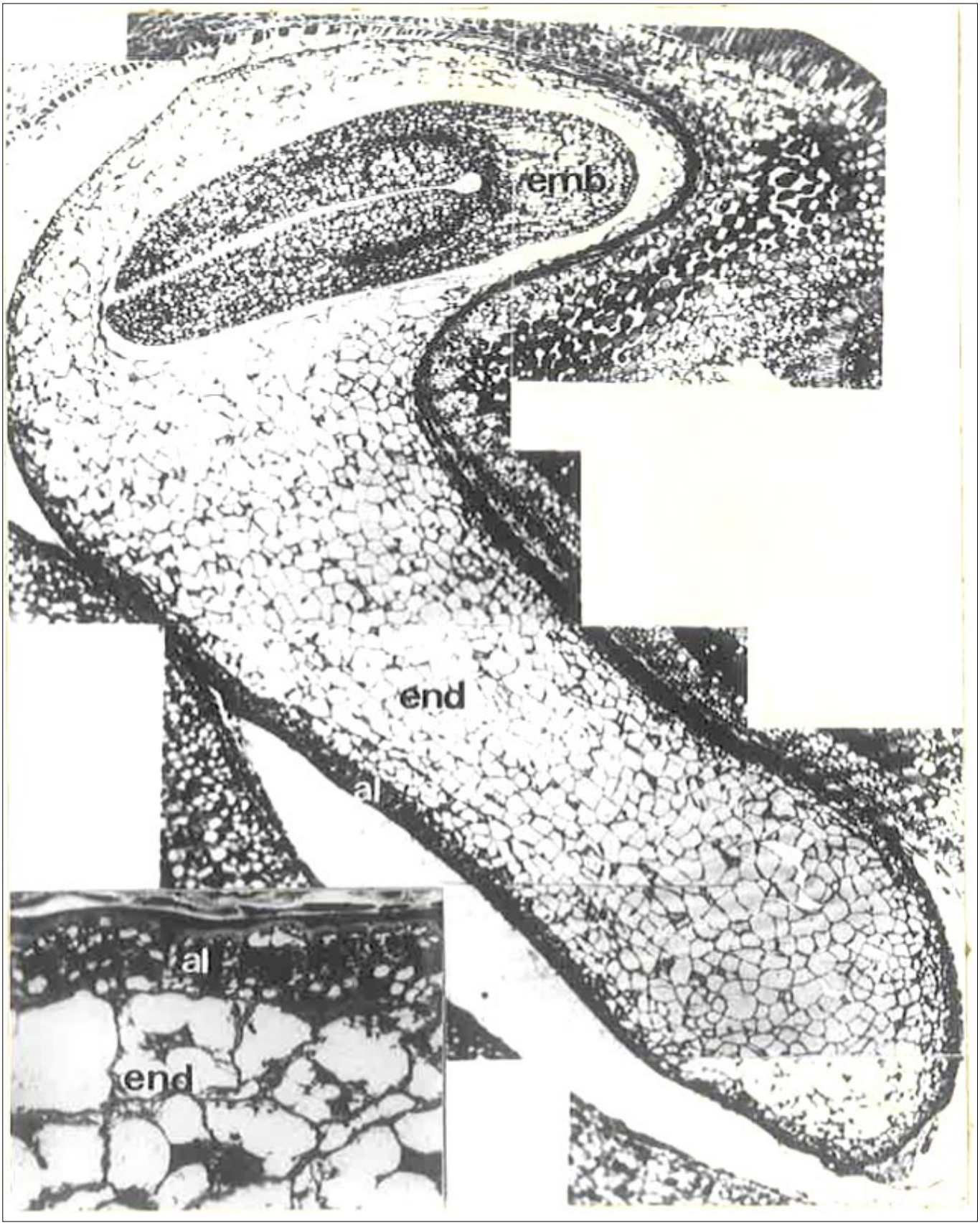

Fig. 4. **al**, aluerone layer; **emb**, embryo; **end** endosperm, Longisection of developing seed at early dicotyledonous embryo stage, stained for total proteins. Dicotyledonous embryo reveals a low level of cytoplasmic proteins in the ground meristem region. The epicotyl and cotyledonary procambium are rich in total proteins. The endosperm is completely cellular. The aleurone layer shows a high concentration of total proteins when compared to the subjacent endosperm tissue (inset) X200.

The cytoplasm of the egg cell, synergids and central cell lacks polysaccharide grains. The cell walls of synergids and egg cells are moderately stained whereas that of the central cell is darkly stained. The filiform apparatus is intensely PAS positive (Fig. 1A). Zygote (Fig. 1B) and the early proembryo (Fig. 1C) are bereft of polysaccharide grains but possess well-developed walls. The suspensor cells towards the micropylar end and juxtaposed to the embryo sac wall, possess well developed and intensely stained wall labyrinths (Fig. 1C). At the preglobular proembryo stage, globular proembryo stage, and heart-shaped embryo stage both

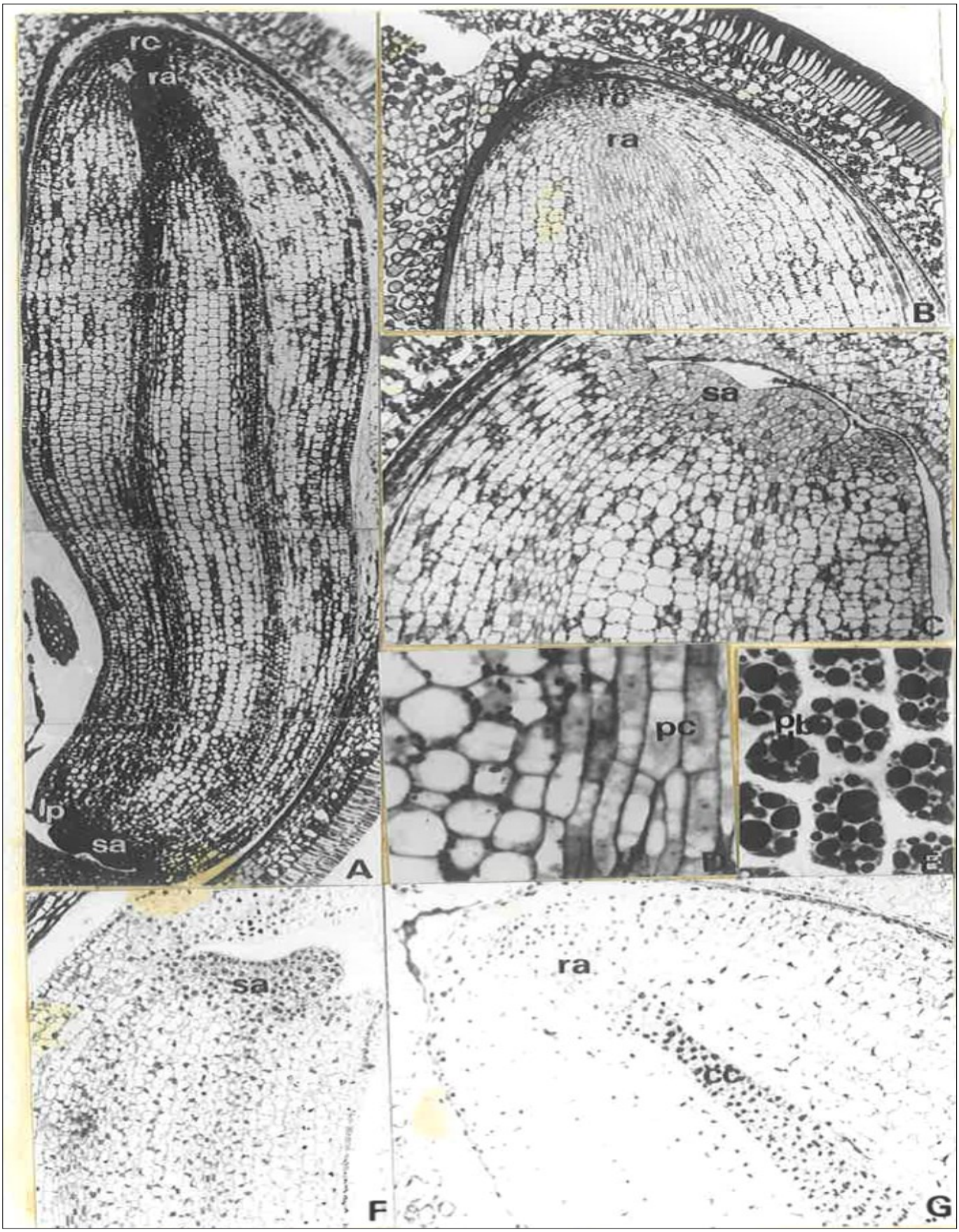

Fig. 5. **(A-G.), CC**, central column; **lp**, leaf primordium; **pc**, procambium, **ra**, root apex; **rc**, root cap; **sa**, shoot apex.

A. Longisection of a seed at late dicotyledonous embryo stage. The root and shoot apices and leaf primoidia stain intensely for total proteins. The procambium and protoderm show a high concentration of cytoplasmic protein as compared to the ground meristem (photomontage). X200, **B.** Same stained for insoluble polysaccharides. The cells of root apex, root cap and ground meristem show accumulation of polysaccharide grains. X 250, **C.** Same, showing shoot apex that possess modicum of polysaccharide grains. The ground meristem and cotyledonary parenchyma cells show prominent polysaccharide grains. X400, **D.** Cotyledonary cells showing accumulation of polysaccharide grains. X 1000, **E.** Cotyledonary cells at late dicotyledonous embryo stage are engorged with protein bodies X1000, **F.** Shoot tip stained for deoxyribonucleic acid, showing intensely stained nuclei of the shoot apex, leaf primordium, cotyledon, ground meristem and procambium cells. X1000, **G.** Radicular end of the late dicotyledonous embryo to show intensely stained nuclei of the root cap, root apex, ground meristem and central column. X1000.

the suspensor and organogenic parts of the embryo contain polysaccharide grains (Fig. 1F, 3A). The embryo proper and suspensor interface region at dicotyledonous and later stages are affluent with polysaccharide grains. The walls of the cells of the embryo are feebly stained. During progressive stages of seed development, the embryo cells reveal a gradual increase in the concentration of polysaccharide grains and staining intensity of the walls. The root apex lacks polysaccharide grains, but the cells of the root cap and the ground meristem are replete with such grains (Fig. 5B, C).

The epicotyl apex reveals only a few small polysaccharide grains. As the embryo matures, a dense accumulation of PAS-positive grains occurs in the root-cap, shoot-apex, hypocotyl and cotyledonary storage parenchyma whereas the leaf primordium has a modicum of grains (Fig. 5 B, C and D). In mature and dehydrated seeds, the cotyledonary cells show intensely stained cell walls.

During early stages of development, the endosperm is nuclear and the coenocytic endosperm cytoplasm is bereft of polysaccharide grains. The endosperm cells possess-feebly stained walls and cytoplasm without polysaccharide grains. At the late dicotyledonous embryo stage, polysaccharide grains appear in the endosperm epidermis which is the presumptive aleurone layer (Fig. 4).

Total Proteins

The nuclei of synergids, the egg cell and the central cell are well stained The synergids show a high concentration of cytoplasmic and nuclear proteins. The filiform apparatus is protein negative. The zygote is rich in both cytoplasmic and nuclear proteins. The suspensor cells and the organogenic part of the embryo are protein-rich. At the globular proembryo stage the concentration of proteins in embryo-proper is high (Fig. 1D, 2 C). The cells of heart-shaped embryo-proper and embryo-suspensor are rich in cytoplasmic and nuclear proteins (Fig. 3B) The concentration of cytoplasmic proteins is, however, less as compared to the previous stage due to the vacuolation in these cells (Fig. 3B).

The early dicotyledonous embryo reveals a low profile of cytoplasmic proteins. The cells of embryo-proper are vacuolate (Fig. 4). The root and epicotyl apices and the procambium are protein-rich (Fig. 5A). At the late dicotyledonous embryo stage, the embryo reveals a high concentration of total proteins mainly due to the formation of protein bodies in the embryo-axis and the cotyledonary parenchyma cells (Fig. 5E). The root and shoot apices and the root-cap reveal well-stained cytoplasm and nuclei but are bereft of protein bodies.

The endosperm nuclei and cytoplasm that agglomerate below the proembryo are rich in proteins (Fig. 1D). The endosperm cells which form at the micropylar region during the globular proembryo stage are vacuolated and stain weakly for proteins (Fig. 2A). The remaining free-nuclear endosperm, has wall undulations and continues to act as haustorium; the cytoplasm and hypertrophied nuclei that are engulfed in these labyrinths are protein-rich (Fig. 2B). At the heart-shaped stage too, the proteins in the endosperm are at low ebb mainly due to vacuolation followed by cellularization. The peripheral endosperm layer that forms the aleurone layer, however, is protein-rich (Fig. 3C). At the dicotyledonous embryo stage the endosperm cells adjacent to the aleurone layer remain highly vacuolated and show a low profile for proteins (Fig. 4). At the late dicotyledonous embryo stage, the aleurone cells are gorged with protein bodies.

Deoxyribonucleic acid

The egg, zygote, synergids and central cell nuclei are intensely stained with the Feugel reaction At the early proembryo stage, the nuclei of both the proembryo proper and suspensor cells are identical in size but the nuclei of the former show denser staining. The embryo proper and suspensor cell nuclei at the preglobular, globular and heart-shaped embryo stage are well stained, but the latter are comparatively more intensely stained (Fig. 3E). The suspensor cells are multinucleate and each cell has five to seven nuclei (Fig. 3F). At the young dicotyledonous embryo stage the nuclei of embryo-proper cells are weakly stained. The epicotyl-apex cell nuclei are prominent and show more staining intensity than the nuclei of the ground meristem At the late dicotyledonous embryo stage, the nuclei of root and shoot apices, root-cap, leaf primordium, ground meristem, procambium and cotyledonary parenchyma cells are well-stained (Fig. 5F, G).

The nuclei of endosperm at the free-nuclear as well as late cellular stages are hypertrophied and well-stained.

Ribonucleic acid

The synergids are rich in cytoplasmic and nucleolar RNA but the egg cell shows a low profile for this metabolite. The suspensor and embryo-proper cells of preglobular proembryo are rich in both cytoplasmic and nucleolar RNA. The cells of embryo-proper show a higher profile for both cytoplasmic and nucleolar RNA than the embryo suspensor cells at globular, heart-shaped and dicotyledonous embryo stages (Fig. 2D). The presumptive aleurone layer is rich in cytoplasmic RNA. At the early dicotyledonous embryo stage, the ground meristem, cotyledonary parenchyma cells and root- and shoot-apices have weakly stained cytoplasm but intensely-stained nucleoli.

At late dicotyledonous embryo stage, the ground meristem and cotyledon parenchyma cells show negligible cytoplasmic RNA but pyroninophilic nucleoli. The root and shoot apices, leaf-primordia and procambium cells reveal feebly-stained cytoplasm but well-stained nucleoli (Fig. 3D).

The presumptive aleurone layer during the early dicotyledonous embryo and the aleurone layer during the late dicotyledonous embryo stages reveal a high concentration of cytoplasmic and nucleolar RNA (Fig. 3D).

Discussion

In *Sesbania speciosa* numerous, PAS positive wall-ingrowths are present at the micropylar and the chalazal ends of the embryo sac and also around the embryo. The micropylar wall ingrowths proliferate, increase in size and

number, persist for a long time, and act as conduit for the nucellar lysate to nourish the egg, zygote and the developing proembryo and endosperm. These embryo sac wall ingrowths also enhance metabolite flow from the integuments and the nucellus to the developing endosperm and embryo. Such wall ingrowths are known in *Zea mays* (21), *Capsella bursa-postoris* (22-24), *Pisum sativum* (25) *Helianthus annuus* (26); *Stellaria media* (27); *Jasione montana* (28); *Euphorbia helioscopia* (29); *Iberis amara* and *Alyssum maritimum* (30); *Glycine max* (31) and *Nigella demascena* (32). In *Capsella bursa-postoris* wall ingrowths develop only after fertilization, continue to increase in size and persist up to the heart shaped embryo stage (22-24), the embryo sac wall ingrowths in *Sesbania speciosa* play a dual role. They not only help in absorbing the metabolites but also play an active role in dividing the coenocytic endosperm into compartments. The involvement of central cell wall projections in compartmentalization of the endosperm has been suggested in *Helianthus annuus* (33), *Stellaria media* (27) and *Iberis amara* and *Alysum maritimum* (30).

The most prominent feature of synergies in *Sesbania speciosa* is the presence of well-developed filiform apparatus at the micropylar end. The filiform apparatus is reported in many investigated taxa except *Coronopus didymus, Brassica rapa*, *Farsetia hamiltonii*, *Lepidium sativum*, *Malochima africana* (34) and *Nicotiana rustica* (35). The filiform apparatus consists of a network of finger-like projections which stain intensely for PAS reaction as reported in *Gossypium hirsutum* (36); *Capsella bursa-pastoris* (22); *Aquilegia formosa* (37); *Zephyranthes rosea*, *Lagenaria vulgaris* (38); *Ranunculus sceleratus* (39); *Linaria bipartite* (40); *Argemone mexicana* (41); *Ornithogalum caudatum* (42), *Scilla sibirica* (43); In *Sasbania speciosa,* the synergid cytoplasm is bereft of polysaccharide grains as also observed in *Zea mays* (44); *Argemone mexicana* (41); *Linaria bipartita* (40); *Ornithogalum caudatum* (42); and *Glycine max* (31); Synergids are also rich in total proteins and RNA is *Sesbania speciose* (39, 41). Synergids help in absorption, storage and transportation of nutrients from the surrounding tissues through FA which have transfer cell (44-48).

In *Sesbania speciosa*, the cells of embryo-proper at the pre-globular prombryo stage contain a few polysaccharide grains but at the globular proembryo and heart-shaped embryo stages, the embryo-proper and embryo suspensor interface is replete with such grains. Therefore it supports the contention that the suspensor cells act as transfer cells; absorb nutrition from the adjacent ovular tissues and pass it to the developing embryo. At the late dicotyledonous embryo stage, numerous polysaccharide grains accumulate as reserve metabolite in the cotyledonary, hypocotyledonary and ground meristem cells. The stored polysaccharide grains are utilized during the progressive stages of seed maturation. Accumulation of starch as a reserve metabolite in the embryos has been reported in *Glycine max* (49) and *Lupinus angustifolius* (50).

In *S. speciosa*, the globular and heart-shaped embryos are rich in proteins and nucleic acids. Such a condition is reported in *Stellaria media* (51), *Vanda cultivars* (52); *Panicum miliaceum* (53) and *Crotalaria retusa* and *C. spectabilis* (54). During later stages of development, only the organogenic part of the embryo shows cytoplasmic proteins and nucleic acids. An increase in RNA level at the time of cotyledon development is reported in *Vicia faba* (55-57) and *Phaseolus vulgaris* (58). Concomitant with the RNA increase, there is an increase in protein synthesis as well.

In *S. speciosa*, the massive suspensor differentiates during the proembryo stage and persists up to the dicotyledonous embryo stage. Many PAS-positive wall ingrowths are present in the basal suspensor cells which are in contact with the adjacent ovular tissue. The wall ingrowths are believed to increase the surface area of the plasma membrane and facilitate the absorption of metabolites from the adjacent ovular tissue. As in the present investigation, wall ingrowths in the suspensor cells are reported in *Capsella bursa-pastoris* (24), *Phaseolus coccineus* (59-61), *Phaseolus vulgaris* (62); *Stellaria media* (63); *Alyssum maritinum* (30); *Medicago sativa* and *M. scutellata* (64) and *Crotalaria retusa* (48). In *C. retusa,* numerous mitochondria are associated with wall ingrowths and this feature is characteristic of transfer cells. The mitochondria supply the needed energy for the active transfer of metabolites. In *S. speciosa,* the close association between the nucellus and the wall ingrowths of suspensor cells suggests that the latter are involved in the translocation of nutrients from the nucellus into the basal region of the suspensor.

In S*esbania speciosa*, the suspensor cells are multinucleate at globular and heart-shaped embryo stages as reported in *Pisum sativum* (65) and four species of *Lathyrus* (66). Endopolyploidy and polyteny have been reported in suspensor cells of *Phaseolus vulgaris* (67), *P. coccineus* (68-69); *Brassica nigra* (70); *Tropaeolum majus* (71) and in many other taxa (see also 72-73). The nuclei in the suspensor cells of *Crotalariaretusa* and *C. spectabilis* are hypertrophied and lobed (48). The lobing of the nuclei results in an increased area and consequently, increased nucleo-cytoplasmic interactions. The presence of nuclear materials in the cytoplasm further supports this view (68, 74-75) and reiterates that the suspensor cells are metabolically very active. Studies are on the suspensors of 120 angiosperms and compared them to the trophoblast of mammals (76). He correlated similarities in development, chromosome behaviour (endomitoses, DNA amplification, formation of polytene chromosomes) and ultrastructural details (wall ingrowths and microvilli) and rightly concluded that both the organs are concerned with the synthesis and transport of specific nutritive substances to the respective sites.

In *S. speciosa*, the endosperm development is of the Nuclear type. During early embryogenesis, the endosperm cytoplasm and the nuclei aggregate at the micropylar part of the embryo sac. Wall formation occurs in the vicinity of the embryo between the aggregated nuclei. The lower 2/3 in the endosperm remains free-nuclear, assumes vesicular contour, contains hypertrophied nuclei and functions as haustorium. This feature appears ubiquitous for all the

legumes (3). The development of endosperm at the micropylar and the chalazal region appears to be induced by the surrounding tissue. The development at the micropylar portion is perhaps controlled by the proximity towards the embryo, the suspensor and the adjoining starch-filled integumentary cells and at the chalazal region is regulated by the adjacent chalazal proliferating tissue.

In *S. speciosa* during the initial free nuclear divisions, the concentration of polysaccharide grains decreases in the endosperm. This decline may be due to the utilization of this metabolite for the early growth and the development of endosperm. Such accumulation of polysaccharide grains in the young endosperm is known in *Stellaria media*, *Capsella bursa-pastoris*, *Gossypium hirsutum*, *Ranunculus sceleratus* and *Alyssum maritimum*. During early embryogenesis when the endosperm nuclei show active mitosis and aggregate at the micropylar end, the endosperm cytoplasm is rich in proteins, DNA and RNA as reported in *Stellaria media* (51) and *Ranunculus sceleratus* (77). These observations indicate that the endosperm is metabolically very active but lacks detectable storage metabolites during early ontogeny (78). The onset of cellularization is met with a strong decline in the levels of macromolecules. It is, thus, postulated that the actively dividing endosperm, during early ontogeny, needs nutrients for its own growth. At this stage the endosperm may not contribute significantly to the nutrition of the proembryo. After cellularization the endosperm acts as a sink and a storehouse of various metabolites. The polysaccharide grains are present in the few peripheral layers of the endosperm but are absent in cells close to the embryo. It is believed that embryo acts as a strong sink for various metabolites and withdraws nutrients and prevents accumulation of reserves in tissues around it (79). In *S. speciosa,* the endosperm around the embryo is gradually lysed. In *Alyssum maritimum* many organelles around the embryo are observed at different stages of degradation (77). A few autophagic vacuoles do occur in the endosperm tissue adjoining the embryo. A clear area around the embryo devoid of any endosperm suggests lysis and utilization of endosperm lysate by the developing embryo. The initiation of endosperm breakdown at the embryo-endosperm interface suggests that the embryo releases factors that promote the lysis.

The endosperm haustorium is metabolically active and may be involved in the nutrition of the developing endosperm. The absorptive role of the haustorium is suggested by studies on *Vaccinium macrocarpum* (80); *Crotalaria retusa* and *C. spectabilis* (54, 81). A similar role of endosperm haustorium in *S. speciosa* is also envisaged since the haustorium, at the chalazal end of embryo sac, becomes undulated to various extents and thus, increases the absorptive surface.

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Authors contributions

NB and JK. conceptualized and conceived the idea. NB, PM, JK, PC, SG, RK compiled and formulated the manuscript. All the authors edited the manuscript to its final form.

Compliance with ethical standards

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