EMBRYO
Very little information is currently available on the histochemistry of embryo development.

**POLYSACCHARIDES**

Embryo: Pritchard (1964) has assessed in considerable detail the distribution of polysaccharides in the embryo and endosperm of *Stellaria media*. According to him, in the 2-celled proembryo protuberance of the primary suspensor cell into the starch-rich nucellus has intensively stained PAS-positive membrane. In his opinion there is a probability of procurement of this carbohydrate fuel required for the extensive protein synthesis in the primary suspensor cell. Similarly, Alvarez and Sagawa (1965) in entire 4-celled proembryo of *Vanda* have reported low polysaccharide tinge in the cytoplasm and in addition, a thick cell wall separating the suspensor from the embryo proper.

Raghavan (1966), and Patricia and Jensen (1969) have reported in *Capsella*, in addition to rich presence of starch grains in the torpedo-shaped embryo, lipid bodies also in the suspensor cells. Diboll (1968) has recorded both lipids and starch in the zygote of *Zea mays*. Schulz and Jensen (1968) in *Capsella*, and Jensen (1968) in cotton, have reported starch even in the zygote.
Panchaksharappa and Rudramuniyappa (1972) have observed the persistence of rich storage starch from the zygote extending into the basal cell tiers of the globular and older embryos in millets. They further state that in the latter stages meristematic regions are characterised by mere PAS-positive tinge in the cytoplasm and thin cell walls, whereas the starch is utilized by the proembryo during its growth. However, reappearance of starch has been reported in the coleoptile, coleorhiza and old leaf primordia of the mature embryo. Similar storage rich quantities of lipid bodies in the globular embryos, and root cap and root cortex of the older embryos of Pennisetum typhoideum. Starch storage is also present in the root cap and cotyledons of Raphanus sativus (Panchaksharappa and Koppar, 1977). Reducing sugars, localized low in the proembryo increases in older one and the endosperm of Pennisetum typhoideum, but not in suspensor (Panchaksharappa and Rudramuniyappa, 1972). Recently, emphasizing on the importance of the formative globular phase in the embryo development of some members of leguminocae, Panchaksharappa and Hegde (1977) are of the opinion that the storage and thick PAS-positive cell walls of the suspensor and integumentary tissues play a prominent nutritional role.

**ENZYMES**

A few reports are available on the fresh material studies, concerning enzyme localization in seeds. Podubnaya
Arnoldi and Zinger (1961) recorded interesting findings on the localization of various enzymes namely peroxidase, cytochrome oxidase, dehydrogenase, catalase and phosphatase in certain orchids and members of Compositae. Forman and Jensen (1965) have correlated the distribution of respiratory enzyme—succinate-dehydrogenase with the areas of active growth and differentiation in *Capsella*. In the globular embryo of this plant, while the enzyme activity was evenly distributed in the entire region of embryo proper, the suspensor showed high concentration. Later, the activity was shifted to the developing cotyledons, and less so in the apical meristem, initially. These results and those of Raghavan (1966) are similar.

Iunyae *et al.* (1970) tested the ovules and seeds of F1 hybrids of *Nicotiana alata* and *N. glauca* for various enzymes, and found that there was an increase in the activity of various enzymes in the beginning of ovule growth, which decreased after fertilization.

Price and By (1970) studied the localization of 3'- and 5'-nucleotidases, phosphatase, lipase and esterase in the dry wheat seeds. All the enzymes showed similar distribution in the entire seed except coleorhiza which is the prominent site of activity.

*Endosperm*: Macleod (1969) has given an account on
histochemistry of endosperm in barley in which the cell walls lack true cellulose in the center of the tissues, as they are essentially hemicellulosic in nature, whereas their nuclei disorganize with the accumulation of starch in this area. Panchaksharappa and Rudramuniyappa (1972), in some young seeds of millets, have observed similarity of the endosperm to the embryo regarding cytoplasmic polysaccharides, storage starch being present only in the older seed.

Lamba (1976) has studied the role of endosperm in nitrogen metabolism in some Oleiferous crucifer seeds. He has reported that the outermost endosperm layer which contains starch grains, is metamorphosed into the characteristic aleurone layer in the mature seeds suggesting that the starch acts as a precursor of aleurone grains and oil globules.

La Berge et al. (1971) and MacGregor et al. (1971) in barley have recorded changes in α-amylase activities in the development of kernel in barley. Here, the enzyme activity, in the presence of starch granules in the latter, increase immediately after anthesis, but decreases to a low level after 10 days in the kernel during its formation. Using biochemical and cytochemical methods, Ga-bara et al. (1972) also studied the changes in the activities of some hydrolytic enzymes during the early stages of endosperm development in Iris, which decline as the seed reached maturity.
PROTEINS

Embryo: The earliest report is by Riestsema and Blondel (1959) on protein metabolism in the growing embryos at torpedo and older stages in Datura, based on the storage of aleurone grains.

Pritchard (1964) by using histochemical technique, has assessed in considerable detail the distribution of protein in the embryo and endosperm of Stellaria media. Accordingly, the rich quantity of protein in the zygote is maintained equally distributed in equal quantities in the terminal and basal cells of the dyad proembryo in contrast to that of RNA. In the later stages of embryo growth protein is present as discrete bodies or plastids in the large primary suspensor cell, suggesting the latter's role as a source for proteinaceous material of the developing embryo. In the region of embryo proper during embryogenesis, protein level, however, rises high. Basic proteins tested for histones, were also localized in the cytoplasm of the young embryo. This situation became reversed as the embryo reached maturity in which basic protein content was more nuclear than cytoplasmic. In the mature one high level of proteins were observed in apical meristematic cells. Cytoplasmic and nuclear basic proteins were markedly reduced in these apical meristematic cells. Protein in cortical cells and procambial strands were high. Alvarez and Sagawa (1965) have reported
in *Vanda* rich content in the embryo proper at 4-celled stage. But, in older ones the protein bodies are reported to be uniformly distributed in all the tiers.

Syamasundar and Panchaksharappa (1976) observed rich distribution of proteins and RNA in the terminal and middle tiers of proembryo which contribute to the embryo proper, but low in the basal tier from which the suspensor and radicle differentiate, indicating the influence of differential intensity of these on the morphogenetic diversity of the areas. Similarly, Panchaksharappa and Koppar (1977) in *Raphanus*, reported rich proteins in the terminal tier and low in the basal tier.

**Endosperm**: Based on earlier reports, Werker and Vaughan (1974) have classified in the mature seeds of *Cruciferae*, three types of aleurone grains which include many small globoids and myrosin grains (Heinricher, 1884; Rest and Vaughan, 1972), and also oil bodies in the space between these grains (Brimsley, 1968; Rest and Vaughan, 1972). According to Rest and Vaughan (1972) myrosin grains stain more intensely with mercuric bromophenol blue than the aleurone grains indicating that the substance is similar to total proteins. Intense staining with millions reagent shows higher proportion of threonine and/or tryptophan and/or phenolic compounds in the grains. Pritchard (1964) has reported rich protein content in the endosperm of *Stellaria*
According to Macleode (1969) the protein content of the epithelial layer in barley is digested with the elongation of its cells presenting larger surface area for the absorption of degradation products derived from the endosperm reserves.

In the early periods of endosperm development, at fienuclear stage, the protein content was reported to be rich, but increased as the tissue became cellular (Syamsundar and Panchaksharappa 1976; Panchaksharappa and Koppar 1977). However, storage of protein was observed in the older seeds of Allium cepa (Syamsundar, and Panchaksharappa, 1975).

RNA

Embryo: The earliest reports on the role of RNA was that of Rondet (1961, 1962) who has studied the embryo development in Myosurus and Alyssium. Accordingly, the zygote is strongly basophilic to RNA, both the cells of 2-celled proembryo show uniform distribution. On further growth, rich content is confined to the embryo proper. In the older embryo during the tissue and area differentiation, the substance is rich in the cotyledons and hypocotyle, but not the suspensor area. As it reaches maturity only procambial strands show further increase in RNA content. The shoot apex is weakly stained, whereas the subapical cell regions are rich in RNA. Rich RNA synthesis was also recorded in
the young endosperm during the early period of embryo growth.

The reports of Pritchard (1964) on *Stellaria media* and Alvarez and Sagawa (1965) on *Vanda*, differ from those of Rondet (1961, 1962) regarding the RNA concentration of the early proembryo. In 2-celled proembryo of *Stellaria media* the suspensor is richer in RNA than the terminal one. However, in older proembryos the quantity diminishes in the former followed by the shrinkage of nucleolus, whereas the terminal area remain rich in the substance. In the older embryo only the procambial strands and cotyledonary tips are rich in concentration. At maturity RNA concentration is confined to apical meristems and procambial strands in which it persists rich. The light and electron microscopic studies by Schulz and Jensen (1968) on *Capsella* reveal that the basal cell of 2-celled proembryo has more RNA than the terminal cell, although the degree of aggregation of ribosome density appear to be same in both the cells. This differential RNA content of the 2-celled proembryo is short lived and is reversed at the next division of the basal cell. Thus, in 3-celled embryo, the terminal cell which has more ribosomes, shows an increase in RNA than the suspensor or basal cells. Further, this difference in terminal and suspensor cells becomes more pronounced during the further development of the embryo. Patricia and Jensen (1969) have noticed a gradual decrease in the stainability for nucleic acids and
also proteins in the suspensor cells of the heart-shaped embryo. From the two celled to heart-shaped stages of embryo growth of several species examined, show no general characteristic histochemical changes in the distribution of RNA that the onset of functional differentiation (Rondet, 1961, 1962; Schulz and Jensen, 1968; Norreel, 1972).

Syamsundar (1974) has observed rich RNA in zygote, persisting until the embryo reached elongation stage. On the contrary, in Dipcadi montanum, low RNA was recorded in the early stages of embryo development. However, in all the instances rich RNA was observed in the procambial strands, invariably in mature embryo.

Endosperm: Rondet (1961, 1962) observed rich RNA synthesis in the endosperm of Myosurus and Alyssum in the proembryo growth. In Stellaria media (Pritchard, 1964) it is rich in quantity. Immolation of the endosperm is reported to have been initiated by the developing embryo, as indicated by reduction in the size of nucleoli and RNA synthesis in the former.

Rich RNA presence in the early periods of endosperm development, at free-nuclear stage followed by gradual decrease in the tissue was also recorded (Syamsundar and Panchaksharappa, 1976; Panchaksharappa and Koppar, 1977).
Embryo: Pritchard (1964) has assessed the distribution of DNA in the embryo of *Stellaria media*. After the first division of zygote DNA reappears followed by its increase becoming richer in the large primary suspensor cell than those of embryo proper. He reports the occurrence of some cytoplasmic DNA occurring as Feulgen granules in the apical meristematic cells. In *Allium*, Syamsundar and Panchaksharappa (1976) observed low DNA stainability in the nucleus of zygote increase in the nuclei of the enlarging embryo, followed by a decline in the mature embryo.

Endosperm: In *Allium cepa*, Syamsundar and Panchaksharappa (1976) have observed low DNA stainability in the triploid free nuclei of endosperm. However, as the embryo grew older, endosperm nuclei at the chalazal end of the embryosac fuse among themselves resulting in hypertrophied nuclei with richer DNA stainability than earlier.
OBSERVATIONS
CAPSICUM ANNUUM L.
EMBRYO : ZYGOTE AND DYAD
POLYSACCHARIDES

The zygote is larger than the egg and shows low cytoplasmic polysaccharide content. Its cell wall is PAS-positive in contrast to those of the nucellar epidermis whose tangential and nearby cell walls of epistase become very thickly stained for the substance during this period (Figs.1,2,3). In dyad proembryo, in addition, small PAS-positive grains are sparcely distributed in both the cells (Fig.4).

PROTEINS

Rich quantity of proteins, visible in stainability, is present greatly confined to the terminal nuclear area of the large zygote (Fig.5). The substance is enriched and distributed unequally rich in the dyad cells of the pro-embryo. Nucleoli are proportionately stained very rich in these (Fig.6).

RNA

The zygote is RNA rich similar to its protein content, confined to the terminal nuclear area of the cell (Fig.7). However, the substance, contrary to the state in
protein localization is reduced in the dyad cells, in the nucleoli also (Fig. 8).

ASCORBIC ACID

The ascorbic acid content of zygote resembles that of polysaccharides being low in the zygote (Fig. 9). The quantity, during cell division, slightly increases in the dyad cells in the form of small AA grains (Figs. 10, 11). No ascorbic acid content could be discerned at the area of phragmoplast in the dividing zygote (Fig. 11). At the latter stage the surrounding nucellus shows fairly rich synthesis of AA grains in the neighbouring epistase and integument.

LINEAR PROEMBRYO

POLYSACCHARIDES

Although the cytoplasm of the 3-celled proembryo shows low PAS-positive tinge, surrounding area of the centrally placed large nuclei of the embryonal cells is rich in starch grains, more so in basal tiers. The cell walls of these are, however, show very low stain (Fig. 12). However, these grains are scattered in the cytoplasm of the 4-celled embryo (Fig. 13) followed by aggregation of the same around the nuclei, at the subsequent enlargement of the linear embryo (Fig. 14). The latter feature in polysaccharide constitution of proembryo-growth persists with the accumulation being more towards the suspensor (Fig. 15). The
PAS-positive thickenings of epistase during this period of proembryo growth are enhanced.

**PROTEINS**

Rich protein content of dyad cells is retained during the growth, but uniformly distributed, as in 3- and 4-celled proembryos, the nucleoli being richly stained (Figs. 16, 17). Similar state could be seen in the neighbouring epistase also.

**RNA**

The rich RNA distribution in the dyad, showing richly stained clumps, increases with the growth of the linear proembryo (Figs. 18, 19). The intensity of RNA is in contrast to that of proteins at this stage, as it is very high, more so in the RNA rich bodies which are perhaps clumps of ribosomal areas, as seen in the 3-celled proembryo (Fig. 20).

**ASCORBIC ACID**

Low cytoplasmic content of dyad cells rises gradually, but richly confined to the large nuclei of 3-celled embryo. The area of telophase chromosomes show rich lining of ascorbic acid (Fig. 21). This state is followed by a diffuse dispersal of AA grains in the cytoplasm of 4- and 5-celled linear proembryos being dense in the basal tiers.
(Figs. 22, 23). The young transverse walls and nuclear areas (Telophasic) also show rich ascorbic acid lining (Fig. 22). Nucellar epidermis and epistase are invariably very rich in ascorbic acid, reaching a peak in its synthesis.

**QUADRANT EMBRYO**

**POLYSACCHARIDES**

The quadrant stage is characterised by the synthesis of polysaccharide tinge in the cytoplasm—perhaps by the metabolism of starch of preceeding growth phase—in all the tiers of the proembryo including the suspensor. However, here sparcely distributed starch grains could be seen diminishing in their sizes. Cell wall stainability could also be noticed. The cell walls of epistase persists invariably thick being richly PAS-positive (Fig. 24).

**PROTEINS**

Marginal difference in protein concentration between the rich region of embryo proper and the low stained suspensor-area could be discerned at the quadrant embryo, but not much in the micropylar area of the seed (Fig. 25).

**RNA**

RNA distribution more or less resembles that of proteins.
ASCORBIC ACID

Ascorbic acid constitution of this growth phase of the proembryo do not differ much from the preceding one. AA content is fairly rich in the areas of embryo proper and suspensor. However, the intensity is very high in epistase, specially along the cytoplasm of PAS-positive wall thickening in the area (Fig. 26).

OCTANT AND GLOBULAR EMBRYO

POLYSACCHARIDES

The low cytoplasmic polysaccharide content of the embryo proper at quadrant and its starch storage in suspensor cells are differentially stained in octant proembryo. These two areas show utter contrast in Fig. 27. An increase in the cytoplasmic polysaccharides in the cells of embryo proper and starch storage in the basal cell tiers is noticed as the embryo grows older (Figs. 29, 30). However, a considerable reduction in cytoplasmic stainability in the former takes place in the early globular stage of the embryo, with a proportionate reduction in the starch storage of the suspensor area also (Figs. 31, 32). The fully grown globular embryo, however, shows rich distribution of cytoplasmic polysaccharides in the entire embryo, evenly (Fig. 33).
PROTEINS

Protein concentration of the early globular embryo, rich in the region of embryo proper, is uniformly enriched in the cytoplasm and nuclei, as it grew older (Fig. 34). However, the globular embryo on its full growth shows considerable reduction in the substance in all the areas perhaps because of multiplicity of the cells.

RNA

In the region of embryo proper rich RNA concentration of the quadrant which increased successively at the octant, is continued to be synthesized in the early and old globular embryos (Figs. 36-38). The suspensor, however, maintains comparatively low RNA tinge in cytoplasm and the cellular endosperm during the process showed a fall in RNA synthesis at early globular stage (Fig. 30).

ASCORBIC ACID (AA)

In contrast to its persistent rich protein and RNA content, globular embryo (Fig. 39), shows gradual increase in ascorbic acid synthesis, followed by marked reduction on reaching full growth (Figs. 40-42). In all the stages of the developing embryos no marked difference could be seen between the suspensor and the embryo proper in this regard (Figs. 40-42). The endosperm and other maternal sporophytic
tissues also show parallel reduction in AA content.

HEART-SHAPED AND DIFFERENTIATING EMBRYOS

POLYSACCHARIDES

Tissue and organ differentiation in the embryo development begins with the heart-shaped embryo which shows low PAS-positive tinge in the cytoplasm, but thin PAS-positive cells walls. The cell walls of the epidermis are however, thick and PAS-positive (Fig. 43). The suspensor is contrastingly very rich in cytoplasmic polysaccharides (Fig. 43), which perhaps persists in its nutritional correlation for metabolic supply to the embryo. As the embryo grows older, the procambial strands, cotyledons, and shoot-root areas are differentiated, all showing characteristically low stainability for polysaccharides during the growth period (Fig. 44). However, low content of cytoplasmic polysaccharides in the cells of future root apices, the cell walls specially at the root cap region, stains rich for polysaccharides (Figs. 45, 46).

PROTEINS

Increase in cytoplasmic proteins takes place during differentiation at the growing embryonic tissue areas, as observed in the cotyledonary initials, shoot and root apices, and procambial strands (Fig. 47).
RNA

Although early heart-shaped embryo shows low stainability for RNA in the embryo proper (Fig. 48), in the older heart-shaped embryo RNA synthesis increases (Figs. 49, 50), being rich in the cotyledonary tips, procambial strands and root-shoot apices, lesser in the latter. In remaining areas of embryo RNA concentration is low (Figs. 51-53).

ASCORBIC ACID

Distribution of ascorbic acid grains which was at the lowest ebb in the globular embryo, is pronounced in the heart-shaped embryo during the period of differentiation. AA grains are rich in the cytoplasm of the embryonic cell walls (Fig. 54). The grains are rich in cotyledonary tips and shoot, and along the expanding cell walls of the procambial strands and peripheral layers of the embryo.

MATURE EMBRYO

POLYSACCHARIDES

Shoot: As the embryo grows older causing apical curvature, the shoot apex becomes flanked by the cotyledons (Figs. 55). The cytoplasmic polysaccharides of the shoot apex and cotyledonary areas show low tinge (Figs. 55, 56). But, it soon increases in the latter leading to starch storage. Procambial strands however, differentiate with low PAS-
positive tinge, but rich in wall materials (Fig. 55).

Root: The root epidermis at the tip and procambial strands show contrastingly thick walls very rich in polysaccharides (Fig. 56), but not comparable to cortical cells in this regard.

PROTEINS

Shoot: The well differentiated shoot terminal area of the embryo shows rich protein content, in the cytoplasm, richer in the cotyledons and cortical areas of the hypocotyl. However, procambial strands and shoot apex are not rich in the substance (Figs. 57, 58).

Root: Protein content of the root tissues rises the tissues during the growth of embryo. While the root-tip and cortex show rich stainability (Fig. 60), the older procambial strands show comparatively low tinge (Figs. 59, 60).

RNA

Shoot: In the mature embryo shoot apex shows rich cytoplasmic RNA. Similarly, young cotyledonary cells and the procambial cells show rich RNA content (Fig. 61).

Root: The root tissues are clearly differentiated in the mature embryo. The root-tip cells towards the apical point show intense stainability indicating very rich content
of RNA in the cytoplasm, extending the same stainability to the epidermis and procambial strands. Comparatively, the remaining areas of the embryo including cortical cells show less rich tinge (Fig. 62).

**ASCORBIC ACID**

**Shoot**: Conspicuous reduction in the ascorbic acid content was observed in the shoot apex area of the mature embryo. Shoot - apical dome shows low content of ascorbic acid, whereas cotyledonary cells contain numerous grains. Even procambial strands remain very low in AA distribution.

**ENDOSPERM**

**POLYSACCHARIDES**

Endosperm in the early stages of its differentiation shows low PAS-positive tinge in the cytoplasm (Fig.3). This low stainability is replaced by starch storage at octant stage (Figs. 29,30), which persists at the globular embryo (Figs. 31,32). With the inception of cellular endosperm-tissue at the heart-shaped stage, low cytoplasmic tinge persists, even after the embryo is well organised (Figs.44, 45).

**PROTEINS**

During the early stages of embryo growth, endosperm is rich in proteins (Figs. 6,17). The substance remains
rich even after the tissue is formed in it, but confined greatly to nuclei and young wall area at the micropylar end during the growth of the globular embryo (Figs. 34, 35). At mature embryo the endosperm tissue is very rich in protein content (Figs. 57, 59).

RNA

RNA content of the endosperm is rich at all the early stages of embryo growth (Figs. 8, 18, 20). The substance decreases at the heart-shaped embryo stage (Figs. 36, 37, 38, 48) but, concentration rises in the tissue at the mature embryo (Fig. 62).

ASCORBIC ACID

Ascorbic acid is rich in the endosperm during the early stages of embryo growth (Figs. 11, 21, 22, 23, 26). The quantity increases specially in the nuclear area of the cytoplasm in the tissue at the early globular stage (Figs. 40, 41), followed by a gradual fall during subsequent stages of seed growth (Fig. 42).

SEED COAT

POLYSACCHARIDES

The young seed coat has thick PAS-positive cell walls and low cytoplasmic tinge of polysaccharides. No starch
deposition was noticed in it at any growth phase, although, on the contrary, the suspensor of embryo and the endosperm showed rich starch storage. However, the innermost epidermal layer of seed coat closer to the embryosac wall, becomes conspicuous by its thick PAS-positive cell walls at the early stages of seed growth (Figs. 1-4, 13-15).

PROTEINS

During the early stages of embryo growth, seed coat shows rich protein content which gradually diminishes during later stages. However, fairly rich tinge persists in the innermost epidermal layer (Figs. 5,6).

RNA

Micropylar half of the young seed shows low RNA tinge (Figs. 7-9), whereas the chalazal area is rich in the substance. In the old seed, as at the octant, proembryo and onwards, RNA content is not traceable (Fig. 36).

ASCORBIC ACID

Ascorbic acid is rich in the young seed coat, more so towards the micropylar side (Figs. 10,11,21). But, in older seeds only a few grains could be seen sparcely distributed in the seed coat (Figs. 41,42).
A large zygotic cell shows very low PAS-positive tinge in the cytoplasm, whereas the central cell has PAS-positive grains around the polars as well as the zygote (Fig. 1). Its contiguous epistase shows similar state of polysaccharides in the cytoplasm. However, considerable rise in the cytoplasmic tinge was noticed in dyad with a parallel persistence of starch grains, but degraded in Fig. 2.

PROTEINS AND RNA

The zygote, in contrast to its polysaccharide constitution, shows very rich protein and RNA contents in its cytoplasm and nucleolus, as well in the neighbouring epistase also (Figs. 3, 5). This intensity in these cell-areas is lessened considerably at the dyad proembryo.

LINEAR AND QUADRANT PROEMBRYO

POLYSACCHARIDES

The low tinge of cytoplasmic polysaccharides of dyad cells is further reduced in 3-celled proembryo (Fig. 7). Cytoplasmic polysaccharides as small PAS-positive grains which are sparsely distributed in all the cells (Figs. 7, 8),
Subsequent enlargement of the cells of the quadrant, resulting in the gradual reduction in the intensity of the cytoplasmic tinge in the proembryo (Figs. 8,9). Thickly layered small PAS-positive grains along the newly formed phragmoplast are also lost with the formation of new cell walls (Figs. 8,9). The thick walls of epistase remain PAS-positive during this period of embryogenesis.

PROTEINS AND RNA

Although rich content of proteins of dyad cells remain undiminished in the proembryos at 4-celled and quadrant stages (Figs. 9,10), their nucleoli stain very rich for the substance. No marked difference could be seen in the suspensor in this regard. Epistase area is conspicuous by the absence of proteins.

RNA synthesis shows an increasing trend during this period of proembryo growth, greatly confined to the area of embryo proper (Figs. 11,12). The endosperm nuclei, in particular, are rich in RNA and proteins, whereas the cells of epistase show very low activity for protein and RNA synthesis.

OCTANT AND GLOBULAR EMBRYO

POLYSACCHARIDES

Growth of the octant proembryo shows an increase in the PAS-positive cytoplasmic tinge in the region of
embryo proper and also in the suspensor to a lesser extent (Figs. 13, 14). The intensity of polysaccharides increases to a very rich level in the terminal tiers of octant, cell walls being PAS-positive (Figs. 14, 15). The epistase wall thickenings remain very rich in polysaccharides during this period, whereas the cells of endosperm show low PAS-positive tinge which is in contrast to cytoplasmic and cell wall nature of suspensor as well as the embryo, accounting for polysaccharide based tissue differentiation (Fig. 15).

PROTEINS

At the octant embryo, the region of embryo proper is well marked by an increase in the substance in the embryo-proper, the suspensor showing lesser rate of synthesis (Fig. 16). Subsequently, the intensity becomes almost uniformly rich in concentration in both suspensor and embryo proper regions of the early globular embryo which, however, shows greater nucleolar activity in it before maturity (Fig. 18). Although the endosperm remains rich, nucellus, seed coat and other sporophytic tissue show a gradual decrease in the substance.

RNA

Growing difference in the synthesis of cytoplasmic RNA in suspensor and embryo proper regions in globular embryo is similar to that of proteins (Figs. 19, 20). The reduction
in RNA content in endosperm, nucellus and integument resemues the happenings in the embryo.

HEART-SHAPED AND MATURE EMBRYO

POLYSACCHARIDES

Polysaccharide rich cytoplasm of the globular embryo is reduced with the growth and differentiation of the shoot-root axis and cotyledons through heart-shaped stage (Figs. 21,22), the cell walls remaining PAS-positive. This state is followed by a rise in the stainability of the cytoplasm as the embryo reaches maturity (Fig. 23). The peripheral layers of cotyledons as well as the radicle also show accumulation of starch grains (Fig. 24), the outer cell walls of root-cap becoming thick and PAS-positive.

PROTEINS

Protein rich globular embryo undergoes differentiation based on protein localization during its elongation in the origin of embryo proper (Fig. 25). The cotyledonary initials rich in protein at their tips grow into cotyledons, whereas the axiate cells in the central core differentiate into procambial strands, in continuity with the shoot and root tip areas rich in protein content (Fig. 25). The endosperm at this begins showing protein rich storage bodies (Fig. 26) which persist in mature embryo also (Fig. 28). As
the embryo becomes organized both shoot and root apical cell areas and the cotyledons become studded with very rich deposition of protein bodies (Figs. 27, 28).

RNA

In contrast to protein rich shoot and cotyledonary areas of globular embryo and also endosperm, gradual decline in RNA content was noticed in the heart-shaped embryo, ultimately showing the persistence of the substance greatly in the nucleolar area of the radicle, procambial strands in particular (Fig. 29).
DISCUSSION

EMBRYO

Embryo is the progeny of the plant. It develops from the zygote in a seed through growth and differentiation into its embryonal tissue areas namely shoot and root apices, root-hypocotyl - cotyledonary axis, and cotyledons. In these both fundamental and procambial tissues are differentiated for the purpose. Development of an embryo begins when the biochemical constitution of zygote and its nutritional environment are well equipped and balanced for formative processes which are also basically biochemical and considerably influenced by the biochemical correlation from the endosperm and maternal sporophytic tissues in the seed. Therefore, in the histochemistry of embryo development, biochemical interactions between these areas are vital for assessment in addition to its own histochemical constitution which is changeable at successive growth events, hence the importance of assessment on differential distribution of histochemical constituents and their contributory role.

Although the available data specially on enzyme histochemistry is quite inadequate to draw any broad based generalisations in this regard, an attempt has been made in the following discussion using the results from the present observation and a few available reports on histochemistry.
Zygote: Zygote is a totipotent cell. It gives expression to morphogenetic traits during its ontogeny developing itself into an organised embryo. The process of development requires suitable biochemical and physiological environment to influence these innate abilities. It has been considered by Torrey (1969) that its growth is heterotrophic, as it is dependent completely on the constituents of the central cell, and later, on endosperm of the embryosac and surrounding maternal tissues. This is greatly true. However, zygote has its own innate potency to synthesise its requirements for differentiation, influenced by its physiological environment. In the present investigation the histochemical constitution of zygotes in the cytoplasm is characterised by low PAS-positive tinge and contrasting very rich proteins, RNA and ascorbic acid mainly confined to nuclear area. Richness of the latter is for cell enlargement preparatory to division.

The conspicuous change, noticed following fertilisation, is the completion of PAS-positive wall around the zygote. The carbohydrate source of it is the storage starch derived from the micropyle, in turn from the integument and funiculus, which is obviously by way of chemical correlation through enzyme activities. Such a carbohydrate nutritional source has been reported earlier in Arachis hypogaea (Panchaksharappa and Hegde, 1977) and Panicum (Panchaksharappa
and Rudramuniyappa, 1972). Ultrastructural studies on zygote have recorded increase in the activities of dictyosomes for the synthesis of wall materials, using energy from storage lipids and plastids containing starch (Møbjergensen, 1973; Schulz and Jensen, 1968), and mitochondria and endoplasmic reticulum (Diboll, 1968) which cause preparatory functions for energy source, all these do indicate growth activity for differentiation. The presence of plasmodesmata connections between zygote and the surrounding nucellar cells in Capsella (Schulz and Jensen, 1968) bring in nutritional correlation to the zygote from the neighbouring nucellar cells. Therefore, zygote with these is biologically prepared for onward growth processes for unravelling embryonal pattern.

**Proembryo**: While the dyad proembryo of Capsicum shows low cytoplasmic polysaccharide tinge, PAS-positive grains from the neighbourhood cause an increase in PAS-positive tinge, as noticed in Nicotiana. Proteins and ascorbic acid also become rich. The rich presence of AA indicates its active participation in the synthesis of both RNA and protein - the former (AA) being a need for the latter - as experimentally shown by Price (1966), Garif (1966, 1967), Chinoy (1967) and Kapoor (1968). Ascorbic acid acts as a regulator of enzymes for the synthesis of these substances. Rich distribution of proteins in Capsella (Schulz and Jensen,
1968) and millets (Rudramuniyappa, 1973) and RNA in Mysorus and Alyssum (Rondet, 1961, 1962) have been reported. However, there are instances showing early histochemical differentiation by the differential distribution of these metabolites among the dyad cells itself. While the terminal cell shows rich RNA and proteins in Vanda (Alvarez and Sagawa, 1965) and Cotton (Jensen, 1968), and Lipids in Capsella (Schulz and Jensen, 1968), the basal cell on the contrary has low tinge of these except in Stellaria media (Pritchard, 1964) in which RNA concentration is more dense in the primary suspensor cell than the embryonal cell. However, basic proteins are uniformly distributed in both cells, perhaps caused by RNA rich suspensor. Aforementioned differential distribution of polysaccharides, RNA, proteins and AA during the ontogeny appears to prepare the proembryo to attain globular phase. In Capsicum starch grains are confined to the basal tier and cytoplasmic proteins, RNA and ascorbic acid to terminal tier whereas in Nicotiana although starch is absent, RNA and proteins are comparatively more in the terminal tiers thus indicating the diversity in histochemical concentrations, bringing about the morphogenetic diversity.

While Capsicum annuum shows starch grains in the linear proembryo and its subsequent quadrant and octant stages, Nicotiana tabaccum has mere PAS-positive tinge in
the cytoplasm. Carbohydrate synthesis for storage starch in *C. annuum* and its degradation to cytoplasmic tinge is as seen in the former, are the successive happenings a need indicative of its use for energy source in its self-regulatory embryo growth. Starch synthesis has also been reported earlier in the proembryo stages of *Capsella* (Raghavan, 1966), and *Panicum* and *Triticum* (Panchaksharappa and Rudramuniyappa, 1972). Although the proembryo has its own energy source, its dependence on the endosperm for nutrition cannot be ruled out, as the latter is in the neighbourhood and rich in starch, proteins, RNA and ascorbic acid. Although plasmodesmata connections have not been reported between endosperm and embryo, the former which normally represents a large pool of reserve materials, appears to meet the demands of embryo in the form of soluble solutes required for the embryo development, in addition to meeting its own needs. Immolation of endosperm cells bordering growing embryo supports such a correlation. This nutritional dependence, however, requires verification from enzyme localisation. In *N. tabacum* carbohydrate source of energy comes from the sporophytic tissues of the seed, neighbouring the embryosac, namely nucellus and integuments which store rich quantity of starch. The storage is nutritional contrivance for the development of proembryo. Thick PAS-positive cell walls in the epistase which is in continuity with the
suspensor of the embryo, appears to act as a liaison tissue histochemically differentiated having rich cytoplasmic polysaccharides and ascorbic acid for the translocation of the metabolites. These evidences indicate prevalence of nutritional correlation from the surrounding sporophytic storage tissues to the developing proembryo.

Electron microscopic studies made by Schulz and Jensen (1968) throw some light in support of this histochemical correlation for the development of proembryos. Earlier to fertilization plasmodesmata connections of the PAS-positive embryosac wall establish cytoplasmic bridging between the zygote and neighbouring nucellar cells. These are severed after the first cell division of the zygote, followed by reoccurrence of fresh plasmodesmata connections among the cells of the 2- and 3-celled proembryos. According to Schulz and Jensen (1968) such reorientation in cell cytoplasmic connections reflect a change in the nutritional source for the proembryo, depending on its physiological requirements. These changes also account for the possibility of the proembryos in attaining their nutritional autonomy. Reported increase in the storage starch and lipid bodies in the basal suspensor cells of the proembryos in Capsella (Schulz and Jensen, 1968) unravel the possible dependance of terminal tiers on nutritionally rich basal suspensor. These features persist even in the older proembryos. Older
embryos in the present studies are rich in cytoplasmic polysaccharides, proteins, RNA and ascorbic acid which supports the above stand. However, severance of plasmodesmata connections between the basal cell and the neighbouring ovular tissues, after the dyad stage (Schulz and Jensen, 1968), does not seem to prevent the proembryo from absorbing the nutrients from the maternal tissues, as there is a gradual loss of storage materials from maternal tissues during the subsequent stages of embryo development as observed in the present investigation and also earlier ones on millets (Panchaksharappa and Rudramuniyappa, 1972), Dipcadi (Syamsundar and Panchaksharappa, 1976), pulses (Panchaksharappa and Hegde, 1977), Raphanus (Panchaksharappa and Koppar, 1977). Possibility of dependence of the embryo on the endosperm and the subtending maternal tissues of the ovule in turn, even for other chemical nutrition other than metabolites, cannot be ruled out. In this regard, fresh material studies on soluble substances and ultrastructural studies are of utmost value in this regard.

Thickening of cell wall between the basal and terminal tiers of the 3-celled proembryo is considered to serve as means to increase the efficiency in localized absorption and metabolism for the nutrition of terminal tiers (Schulz and Jensen, 1968). In this regard the belief of Patricia and Jensen (1969) that the basal cells or
suspensor, having lipid storage for longer time, function as an embryonic root in the absorption and translocation of nutrients from the integuments to the embryo proper, is cojent. In this context Mogensen's (1973) view, that the pathway for translocation of food is neither through suspensor cells nor synergids even, requires verification.

Synthesis of rich ascorbic acid (AA) in the early stages of proembryo growth is known to cause rich energy release through coenzymes, as it has been considered as an electron carrier (Saxena et al. 1969 and Chinoy et al. 1969) or regulator of enzymes (Tonzig, 1950; Chinoy, 1962; Tonzig and Marrie, 1961). According to Garag (1966, 1967), Chinoy (1967), Price (1966) and Kapoor (1968), ascorbic acid is also reported to play a vital role in the metabolism of nucleic acids and proteins.

In the present investigation, both ascorbic acid and proteins show high intensity at dyad. In addition to these, rich synthesis of cytoplasmic polysaccharides in the form of PAS-positive tinge and RNA, also takes place in the terminal tier at quadrant and octant stages, suggesting the active participation of AA in the synthesis of all these substances. Synthesis of AA in the linear proembryo begins in the nucleolui extending into extra-nuclear cytoplasmic area, as could be observed in the successive stages of
proembryo growth. Similar parallel increase of cytoplasmic AA in the neighbouring micropylar tissues suggests that the possibility of protein metabolism in the latter which serves as nutritional environment to both ascorbic acid-and protein-synthesis in the proembryo growth.

Chinoy's (1962) hypothesis of "Ascorbic acid-nucleic acid-protein metabolism" appears to hold good here. In accordance with this concept synthesis and utilisation of ascorbic acid stimulates the production of nucleic acids which participate in the protein synthesis. Increased production of proteins brings about quantitative increase in the protoplasm, and thus stimulating metabolism and upsurge in the rate of organizer centres in cells preparatory to cell elongation, division and differentiation.

**Globular embryos**: In the regions of embryo proper and suspensor, globular embryo is rich in PAS-positive tinge. Large starch grains are present in the latter in *Capsicum annuum*. Degradation of starch in the enlarging embryo, in rich PAS-positive tinge in the cytoplasm of the terminal tiers which contribute to cotyledons. Carbohydrate synthesis in these tiers is indicative of successive need of the carbohydrate fuel for the differentiation of embryonal organs-namely, shoot, root and cotyledons having fundamental and procambial tissues. Recorded rich RNA, protein and ascorbic
acid synthesis in this region, as also observed by Pritchard, (1964), is contributory to the process of growth and differ-
entiation of the embryo.

**Suspensor** : The suspensor in C. annuum and N. tabaccum becomes functionally conspicuous at the quadrant or octant stage of the proembryo growth. In N. tabaccum polysaccharide constituents of the suspensor increase at the globular embryo, whereas in C. annuum, the carbohydrate metabolism begins much early at the proembryo very much in the form of PAS-positive tinge as well as starch grains. As the embryo reaches globular stage, the area remains rich in cytoplasmic polysaccharides and also proteins. However, rich ascorbic acid content of the suspensor is lost at the early globular embryo having the area enriched with metabolites.

General source of carbohydrate nutrition for the embryo growth is from the starch storage, mainly from the maternal tissues. Rich storage, present in the seed coats of N. tabaccum appears to be the source during the early stages of embryo growth, as evidenced by its gradual loss from the seed coats in the older seeds. Translocation of this energy source into the embryo is through suspensor, needed in the synthesis of proteins and RNA which persist equally rich in the suspensor and the embryo before tissue differ-
entiation. This feature was also reported in Stellarria media (Pritchard 1964).
Based on the plasmodesmata connections which occur among the suspensor cells - but not between the suspensor and the adjoining tissues, Newcomb (1973) has accounted for the physical translocation of metabolites into the region of embryo proper. However, between the suspensor and its adjacent tissues, translocation of soluble nutrients is through cell walls by absorption. Based on occurrence of wall ingrowth in the basal cell and also the abundant mitochondria and endoplasmic reticulum, in addition to the presence of plasmodesmata connections in the end cell walls of the suspensor, Newcomb and Fowke (1974) have suggested that the suspensor is differentiated exclusively for translocation. Such wall projections are also noticed at the micropylar end of the suspensor cells in Capsella (Schulz and Jensen, 1968). The active involvement of suspensor in the nutrition of the embryo is further justified by the rich presence of polytenic chromosomes in the suspensor of Phaseolus (In Bhojawani and Bhatnagar, 1974), protein bodies and absorption of these by the growing embryo in Stellaria media (Pritchard, 1964) and synthesis of RNA and proteins in Phaseolus coccineus (Sussex et al. 1973 - In Bhojawani and Bhatnagar, 1974).

Degeneration of suspensor cells begins with the differentiation of embryonal organs, which indicates the termination of its function after the embryo becomes self
reliant in its growth requirement, as said by Newcomb (1973) that nutritional role of suspensor is no longer required by the older embryo. The rich histochemical constitution of suspensor and its neighbouring maternal tissues, its ultrastructural composition and the specific duration of functional existance are clearly indicative of positive role the suspensor plays in the differentiation and development of the embryo.

Mature embryo: In the heart-shaped embryo polysaccharides, proteins, RNA and ascorbic acids are rich and uniformly distributed. As the cotyledons and embryonal axis grow, differential distribution of these substances was noticed which suggest their role in the formation of tissue and organs in the mature embryo.

During the early periods of differentiation the entire embryo shows less rich PAS-positive tinge in the cytoplasm and thin cell walls, except at root-cap area in which cell walls are thick and PAS-positive. As the embryo grows older, PAS-positive tinge increases in the cytoplasm of cotyledons followed by starch storage beginning from the peripheral layers. Similar happening takes place in the root apex region of Nicotiana tabaccum, but not in Capsicum annuum. This starch storage provides energy for the growth of seedling. The storage occurs after the degeneration of
suspensor and, therefore, dependance of the embryo on the maternal tissues for energy source ends, perhaps because the embryo has adequate reserve.

RNA and proteins are rich in the shoot and root apices, procambial strands and cotyledonary tips which are embryonic regions undergoing differentiation. These histochemical characteristics are also recorded by Pritchard (1964). In addition to these, reducing sugars are also present in these (Rudramuniyappa, 1973). These areas are also endowed with rich organelles such as: ribosomes, lipid bodies, plastids, mitochondria, endoplasmic reticulum and dictyosomes (Diboll, 1968; Schulz and Jensen, 1968) which are causal in the synthesis of these substances, and tissue and organ differentiation.

Storage of total proteins in the form of protein bodies was observed in the shoot apex and the cotyledons of mature embryo in *Nicotiana tabacum*. These, similar to starch, are the source of reserve energy, and have been reported in *Phaseolus vulgaris* (Opic, 1968). Opic (1968) is of the opinion that in the cotyledons which synthesize varied groups of proteins, storage protein is one of such kinds.
ENDOSPERM

In angiosperms endosperm develops concomitantly with the embryo due to its unique feature of double fertilisation. The tissue is a known source of nutrition to the developing embryo, at least during its earlier growth phases and also a reservoir during seed germination. The tissue can be compared to the tapetum of anther in which the latter differentiates in a similar way, concomitantly with the differentiation of meiocytes causing pollen formation. Although the ontogenetic origin and histochemical compositions of both these tissues are divergent, the function in case of tapetum relates to the dependence of the meiocytes on it which is nutritionally and biochemically correlative, as some specific chemical substances appear to cause meiosis in the former (Helsop-Harrison, 1972; Southworth, 1971, 1973). Similarly, juxtaposed position of the endosperm to the embryo—both have a common origin through double fertilisation and undergo development parallelly—appear to be the need for the latter for its growth and differentiation to become organised, ultimately. The correlation from endosperm to embryo appears to be basically nutritional, specially in the earlier stages of embryo growth as evidenced by immolation of bordering cells of the endosperm. The present histochemical assessment of the endosperm on *Nicotiana* and *Capsicum* supports this general stand, Pritchard (1964)
observes that decrease in RNA content of the endosperm goes with the gradual immolation of the tissue by the developing embryo which is also observed in the present investigation.

The endosperm during its growth, undergoes histochemical changes in a sequence. Initially, the central cell enclosing primary endosperm nucleus with its big nucleoli is rich in proteins and RNA. It also shows rich PAS-positive cytoplasmic tinge and starch grains. Further, during its free nuclear stage, the tissue has rich proteins and RNA and ascorbic acid. Through these histochemical changes, the primary endosperm cell undergoes tissue differentiation for its future storage function, also nourishing the young embryo, perhaps, marginally. During this period of endosperm growth, starch storage of the central cell is utilised to become storage in function again, finally. In Capsicum annuum storage of starch begins at the octant stage, whereas in Nicotiana tabacum it starts only after globular stage. However, carbohydrate storage generally persists at later stages of embryo growth, as reported in millets (Panchaksharappa and Rudramuniyappa, 1972), Dipcadi (Syamsundar and Panchaksharappa, 1976). But in pulses (Panchaksharappa and Hegde, 1977) it begins very early at the young proembryo itself.
Free-nuclear endosperm is rich in RNA and proteins. Rich presence of RNA and proteins is the characteristic feature of the growing endosperm at free nuclear stage. This state is followed by decline in these substances followed by starch storage. Carbohydrate metabolism, therefore, depends on full growth of the endosperm both at its free-nuclear and cellular state. This feature has been observed by Pritchard (1964) and Rondet (1961, 1962). During the organisation of cellular tissue, decline in RNA and protein contents appears to be a pre-requisite of the endosperm, which begins from the micropylar end in the present observation.

Other tissues: In addition to the embryosac, role of: other tissue namely epistase of nucellus and integument, which becomes seed coat of the seed - lies in the ultimate nutrition of the embryo being complementary, as the latter are considerably rich in their histochemical constitution. In solanaceae starch accumulates in the seed coat layers at the micropylar end and also in the chalazal end, funicular area of the seed. Accumulation takes place during the early stages of embryo growth, but towards the chalaza in older seeds. Rich content of proteins, RNA and ascorbic acids of integument, and young seed coat areas - present during the early stages of embryogenesis - diminishes as the proembryo reaches globular stage because starch storage replaces them in these areas.
Therefore, nutritional source of the embryo begins from the funicule and translocates upward towards the micropyle, as also observed by Mongensen (1973).

The cell walls and the cytoplasm of epistase are richly PAS-positive. The tissue is equally rich in ascorbic acid. Although it shows low tinge of RNA and proteins, the tissue appears to act as a source of energy-rich materials to the embryo development from the micropylar end.
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