SUMMARY AND CONCLUSIONS
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Localization of developmental changes in the concentrations and distribution of total polysaccharides and proteins, nucleic acids (DNA and RNA) and ascorbic acid in the successive differentiation of tissues at successive growth phases, have been assessed in tissue-sections of anthers, ovule, seed and seedlings of two members of solanaceae namely Capsicum annuum and Nicotiana tabaccum by employing standardised and confirmed histochemical tests for these substances. An attempt has been made by using present histochemical data — and also the available reports on histochemistry in addition to those from the allied relevant technical approaches namely ultra structural and autoradiographic studies — to assess the possible role of these substances and the tissues that contribute to the development of the said reproductive structures. The following is the summary of the outstanding observational facts obtained in the present studies and described area — and substance-wise, followed by some conclusions inferred based on evaluation made in the discussion.

ANTHER

Polysaccharides: The archesporium and primary sporogenous tissue in the anther primordium show low PAS-positive tinge in the cytoplasm and thin cell walls. The
stainability in these areas increases in the early sporo-
genous tissue, even at its premeiotic stage in N. tabacum. However, the intensity decreases in the early meiotic divisions followed by a rise again in the tetrads of spores. The rich stainability of the cytoplasm is maintained in the young spores with the formation of PAS-positive intine. However, during their growth a fall in polysaccharide occurs followed by a final rise to a very high concentration in the form of cytoplasmic tinge in the shedding pollen grains.

Deposition of additional PAS-positive cell wall-
-thickening around the meiocytes at the preleptotene stage itself is an variable feature. But, the thickening persists during the period of meiosis and disintegrates before the liberation of spores from tetrads.

Excepting tapetum which remains thin walled and low in cytoplasmic PAS-positive tinge during the course of its functional existence, the other anther wall layers and the connective show persistence of PAS-positive thick cell walls and storage starch from the period of meiosis until the pollen shedding.

During the pollen formation and before the anther dehiscence, PAS-positive endothelial thickenings are differentiated using starch from the wall layers and connective, as indicated by the degradation of the storage, subsequently.
Proteins and RNA: These substances persist rich in the sporogenous tissue during the ontogeny of young anther, while they are at low concentration in other tissue layers of anther, as the latter reaches maturity. Both proteins and RNA remain rich in the meiocytes during meiotic and post-meiotic stages, becoming very rich further in the shedding pollen.

Although tapetum shows low content of these substances at the premeiotic stage, their concentration increase during meiosis, as in *Nicotiana*, remaining unchanged during later stages of spore formation until its degeneration. The pollen exine and endothelial thickenings appear faint green with Azure B and pink with acid phloroglucinol indicating lignin constituents in them.

DNA: DNA stainability of archesporium and sporogenous tissue is rich. The meiocytes maintain the same intensity during meiosis-I. The intensity of DNA, however, decreases in tetrads. The spores, although regain the rich DNA content during post-meiotic stages of their growth, the generative cell of the 2-celled pollen differentiates with very rich DNA content which is not comparable to that of the vegetative cell in concentration. The tapetum, on the contrary maintains constantly rich DNA content.

Ascorbic acid: While the archesporium in *Capsicum*
**CONCLUSIONS**

Histochemical organization of reproductive structures when compared to that of vegetative structures, is more complex in tissue - organization. Biochemical assessment on anther development leading to pollen formation presented here, in this regard unravel some causal factors underlying such an organization, as the composition and differentiation of anther tissues go invariably with the histochemical constituents which are synthesized in them and also conveyed into them from the neighbouring tissue, during their ontogeny through chemical correlation.
Growth of anther primordium begins in the floral apex with the differentiation of hypodermal archesporium rich in RNA, DNA, and proteins, and a few ascorbic acid grains which are localized along the cell walls, as in *N. tabaccum*. Early sporogenous tissue and PMCs which are derived from the archesporium, become very rich in these substances. The meiocytes, preparatory to meiosis, are further enriched by the synthesis of ascorbic acid. Polysaccharide content of this tissue is however, low indicating low requirement of carbohydrates and rich synthesis of proteins, RNA and AA for differentiation of meiocyte, the source of energy which appears to have been derived from the neighbouring starch-rich storage tissues. DNA, RNA, proteins and ascorbic acid persist rich in meiotic derivatives, with a parallel increase in polysaccharides during pollen formation, the latter carbohydrate synthesis being provision, as energy source during pollen germination.

The additional PAS-positive wall thickening around meiocytes (PMCs) occurs concomitantly with the onset of meiosis. It extends around spores even, but disappears after the completion of meiosis, as indicated by the separation of spores from a tetrad. Disintegration of the transitory additional wall thickening before freeing of spores, is a reasonable evidence to support that it plays some specific role in causing meiosis. Extension of their
thickening around the spores in a tetrad shows that the thickening plays its due role in the formation of spores, and also intine and initiating exine.

Tapetum differentiates parietally at the beginning of meiocytes formation at interphase of PMCs, and functions with rich histochemical constituents namely DNA, RNA and proteins, but also ascorbic acid in *C. annuum*, keeping the tissue biochemically active during meiosis. This transitory tissue is not rich in polysaccharides. The tissue perhaps contributes necessary biochemical precursors to meiocytes by way of chemical correlation. The reported plasmodesmata connections between tapetum and parietal tissues are severed before meiosis, with the differentiation of additional wall thickening—the selective molecular filter of callose nature (Rodkiewicz; 1970, Schwab, 1971) around the PMCs and spores. Its occurrence before meiosis and disintegration subsequent to the latter adds ample proof to speak of its role in chemical correlation, in bringing into meiocytes some basic nutrients which perhaps cause formation of haploid spores through meiosis.

Storage of starch and AA grains in the anther wall layers as carbohydrate source of energy and its conversion through AA induced enzyme activity, is of specific importance in the supply of energy for tissue differentiation leading to pollen formation in meiocytes through tapetum, and also
in the differentiation of endothecial thickenings for anther dehiscence, as evidenced by the degradation of these storage substances subsequent to the differentiation of pollen and endothecial thickenings.

OVULE

Polysaccharides: Localization of these carbohydrates is observed as PAS-positive cell walls, but low cytoplasmic tinge in the archesporium, MMC, dyads, triads, tetrads and two- to eight-nucleate stages of embryosac. However, in N. tabaccum dyad, triad and tetrad show a rise in the cytoplasmic polysaccharides, as well as PAS-positive cell walls around the spores in both the plants. In the organized embryosac central cell is rich in storage starch, the grains being bigger and around the polar nuclei. In Capsicum annuum, the cells of epistase and hypostase in continuity with embryosac wall show thick PAS-positive wall thickening. Two or three layers of the nucellus and integument generally store starch around the embryosac which is the source of storage in the female gametophyte. Epistase showed exceptionally very rich cytoplasmic polysaccharides followed by thick PAS-positive cell wall thickening.

Proteins and RNA: While the archesporium is rich in both RNA and proteins, its derivative cells namely, MMC, dyads, triads and tetrads; and even two- to eight-nucleate
stages of embryosac show variation in the intensity of these substances between these plants. However, generally, whole premeiotic stages show rich localization of proteins and RNA in the cytoplasm. The substances show greater intensity around the nuclear area of the embryosacs. In the organized embryosac egg, synergids, antipodals and central cell—all show invariably rich content of these excepting in C. annuum in which the central cell shows low tinge of RNA.

The epistase and hypostase are not rich in these. The nucellus and integument during their early growth phases are rich in these substances, but show gradual decline, as the embryosac grows older.

DNA : Rich DNA content of MMC rises during the interphase, as seen in C. annuum followed by a marked fall showing low tinge in spores during meiosis and the female gametophyte. However, fairly rich stainability persists in the nucellus during the ovule development. Hypostase is also rich in DNA content.

Ascorbic acid (AA) : Ovule primordium at MMC is rich in ascorbic acid which is reduced in the latter, but are confined to the nuclear area. During meiosis AA grains much seen reduced, but considerably along the cell walls and around the nucleus in the functional megaspore. These grains were however, richly localized in the cytoplasm of the female
gametophyte followed by further increase in the older embryo sac, and also in 2 or 3 layers of nucellus surrounding the latter. Epistase show exceptionally very rich content of ascorbic acid.

CONCLUSIONS

The female gametophyte—the embryo sac, differentiates in the ovule due to cumulative biochemical formative changes which constitute a basis for its organization in the ontogeny of ovule. Its development from the functional megaspore is governed not only by the differential histochemical constitution of its constituent tissues, but also by the chemical correlations influenced by the metabolites and other biochemical constituents of surrounding maternal tissues. The latter influence begins on MMC itself to cause meiosis in the latter.

Histochemical composition of megaspore mother cell and of its meiotic derivatives is by the rich nuclear DNA, and cytoplasmic RNA, less rich proteins and ascorbic acid. Chalazal tissue and integument which influence nucellar growth, are also rich in these substances. Occurrence of thick PAS-positive cell wall around dyads and megaspores, as observed in Capsicum annum, during meiosis appears to be the requirement for histochemical correlation in causing selective permeability of some histochemical substances.
to effect meiosis. The nature of the specific wall thickening is said to be callose material (Schwab, 1971; Rodkiewiez, 1970). The histochemical feature is comparable to similar happening in PMCs of anther (Southworth, 1971).

During its differentiation, embryosac is rich in proteins and RNA around the nuclei at polar ends. Its biochemical environment in the young ovular tissues namely, nucellus and integument, is denoted by rich synthesis of cytoplasmic polysaccharides, RNA, DNA, proteins and ascorbic acid. Epistase and hypostase in C. annuum are equipped exclusively with thick PAS-positive cell walls, rich ascorbic acid in the former and rich DNA in the latter. These specialized tissues act as biochemical apparatuses in the synthesis of some substances required to influence the differentiation of embryosac, the female gametophyte in its organization.

In the organized embryosac egg, although shows low cytoplasmic polysaccharides, is rich in cytoplasmic and nucleolar RNA, proteins and ascorbic acid suggesting its metabolic activity. Synergids which are very rich in cytoplasmic polysaccharides, RNA and proteins are biochemically active and nutritionally very rich. Antipodals resemble synergids in having rich RNA, DNA, proteins and ascorbic acid, but differ in being low in polysaccharides. Therefore,
presence of rich metabolites suggest that antipodals are metabolically rich and physiologically active, which play an important contributory role in nutritional mechanism and thereby, organization of the embryosac. The central cell is rich exclusively in the storage of big starch grains and provides nutritional and biochemical environment to the egg apparatus, during preparatory to fertilization and also for zygote the immediate future progeny. Comparatively rich cytoplasmic RNA and AA around polars, and fairly rich DNA containing polar nuclei contribute to its physiologically active state.

EMBRYO

As examined in anther and ovule, biochemical constitution of the tissues in embryo-formation and the underlying causes for their differentiation was assessed in the developing seed.

Polysaccharides: While an increases in cytoplasmic polysaccharides begin in the dyads of *Nicotiana*, storage of starch grains is observed in the proembryos of *C. annuum*. The latter grow large and rich in quantity of storage extending into the suspensor cells in the older proembryo followed by gradual reduction in both the areas namely suspensor and embryo proper regions in number and size of grains as the embryo reaches globular stage. The disappearance of starch in these regions results in subsequent increase of cytoplasmic
polysaccharides, as observed in the globular embryo. Pro-
cambial strands in the cotyledons and the root are charact­
erised by less-rich PAS-positive cell walls and cytoplasm
in contrast to very low content of cytoplasmic tinge in the
areas of shoot and root apices except in the root cap region
the cytoplasm of which stains rich for polysaccharides.

The starch storage occurs in the endosperm when it
becomes fully cellular, and also in the seed coat, ..
chalazal and funicular areas of N. tabaccum. The cell walls
and cytoplasm of epistase are, however, are richly PAS-
positive at the basal end of the developing proembryo.

RNA and proteins : These substances are rich in the
region of embryo proper of the proembryo and become confined
greatly to the areas of procambial strands and the growing
regions of the older embryo undergoing differentiation. In
the mature embryo, RNA and proteins are confined to the
shoot and root apices and the procambial strands. However,
rich storage of protein bodies occurs in the cotyledons, and
subdistal area of the shoot \( \) of the mature embryo in N.
tabaccum.

These substances are rich in free-nuclear endosperm
mainly confined to nuclear area and in the cellular tissue
during the early stages of its differentiation. In the
endosperm tissue these are reduced gradually. However,
storage of protein bodies persists in the endosperm of *N. tabacum*, as the embryo reaches maturity.

**Ascorbic acid**: While the zygote embryo shows low ascorbic acid content, it increases in 2-celled embryo. As smaller grains appear in four- and five-celled proembryos, the nuclei become dense with AA grains in the latter. Synthesis of the substance extends to the cytoplasm in all the cell-tiers, followed by a marked reduction in the octant-embryo. In the heart-shaped stage small AA grains are localized to the cell wall areas of the growing tissues. Cytoplasm of root and cotyledonary apices show rich accumulation of AA grains in the differentiating embryo. The mature embryo, however, shows low AA content in all the tissue areas.

Endosperm is rich in AA at the early stages of embryo growth. The substance declines in the older tissue. Similar state is seen in the differentiation of seed coat layers, although they are rich in early stages of seed growth.

**CONCLUSIONS**

The histochemical constitution of the proembryo is rich and comprises of nuclear AA, cytoplasmic RNA and proteins in terminal part, but sparcely distributed small starch grains - rich and large in suspensor in *C. annuum*. In the
older proembryo rich concentrations of cytoplasmic polysaccharides, RNA, proteins and ascorbic acid are confined to the terminal regions. These cause differentiation of the terminal tiers of the proembryo to contribute to the embryo proper which in turn develops into the areas of cotyledons, procambial strands, and shoot and root apices on further differentiation into an organized embryo. Suspensor differs from the rest of the embryo in having low content of RNA and proteins, but rich in starch storage during early periods of embryo growth - the latter being rich source of energy for growth activities in differentiation. Rich quantity of AA observed in the nuclei appears to enhance the synthesis of nucleic acids for the latter purpose. Later localization of small AA grains along the cell walls of the enlarging cells in procambial and fundamental storage tissue - areas suggests that the growth activities leading to the differentiation of the areas of mature embryo - including procambial strands, shoot and root apices; and preparation of cotyledons for storage - are brought about by the regulatory role played by ascorbic acid in the metabolism of the embryo. This has been experimentally shown by Tonzig and Marre (1961), Chinoy et al. (1969). The synthesis of these histochemical substances that cause tissue and organ differentiation in the organization of the embryo, is from the maternal tissues of the seed. To a certain extent free - nuclear endosperm which is rich in metabolites namely polysaccharides and
proteins confined to nuclear area and in the form of protein bodies in *Nicotiana* during later stage, contribute to the nutritional requirements of the proembryo. However, much of it is the primary need for its own growth to become storage tissue for seedling growth. Rich starch storage of the seed coat is a reservoir which is drawn by degrading it through the hydrolytic enzymes to be used as a source of energy for the developing embryo. The pathway for entry of these metabolites into the embryo is the functional epi-stase rich in polysaccharides and ascorbic acid, which appears to act as a source of energy-rich materials to the embryo development from the micropylar end.

SEEDLING

Localization and distribution of polysaccharides, nucleic acids, proteins and ascorbic acid was studied in the root and shoot apices, hypocotyl and cotyledons of one- to six-days old seedlings at these successive stages of seedling growth to assess the contributory role of these substances in the differentiation of latter tissue-areas. The purpose was to verify the impact of environmental factors and histochemical substances on tissue differentiation in the juvenile plant.

*Polysaccharides:* Shoot apex of one- and two-day old seedling shows low cytoplasmic tinge, whereas it is markedly rich in procambial strands. The concentrations
persist in these areas in later stages. The stability of cytoplasmic polysaccharides becomes very rich in the young shoot and root apices, richer in the former at the region of initiation of leaf primordia, as in Capsicum. Parallaly with this, starch grains accumulate in cotyledon and hypocotyl-don regions of the plant indicating constant source of energy maintained for the embryonic apices which undergo regeneration.

Proteins and RNA: The areas of shoot apex, cotyledons and hypocotyl of one-, two- and three- days old seedlings in Nicotiana show rich accumulation of big protein bodies which are degraded for source of energy during the later stages of seedling growth, as comparable to a similar state in Capsicum in which starch is the source of energy for these stages of seedling growth. Shoot and root apices are rich in cytoplasmic proteins, but intense in flank zones and procambial strands, which indicate their primary role as precursors in bringing growth and differentiation of these specialized tissues, namely xylem and phloem and in causing are institution of meristem in the leaf primordia for the future tissue differentiation in the leaves.

DNA: Rich DNA content persists in the shoot and root apices of two-, three-, and four- days old seedlings. Flank zone - areas where leaf primordia originate, are invariably rich in DNA.
Ascorbic acid: While the cotyledons and procambial strands of two- and three-day old seedlings show low AA content, the substance increases in the shoot apex of four-day old seedling and the grains appear as smaller clumps in cytoplasm of cotyledons, but greatly confined to the elongating cell wall - areas of the embryonic tissues including procambial strands.

CONCLUSIONS

The seed germination and seedling growth brings in marked changes in the morphogenetic behaviour of organized embryo in adopting itself to the physical environment on germination for regeneration activities resulting in organic pattern. The study of insoluble macromolecular substances assessed here recitals interestingly their primary role in tissue - differentiation at the embryonic shoot and root apices from which the entire organism emerges through developmental processes. It is greatly contributory to the better understanding of the plant organization. The events are more complex than what is apparent. These are caused by biochemical developmental changes in succession during the seedling growth.

Similar to starch storage, protein storage also acts as reserve source of energy in the early stages of seedling growth, as recorded here in the fundamental storage areas of root and shoot apices, cotyledons and also in the seed
coats at the early periods of germination in C. annuum and N. tabacum, respectively. This indicates nutritional autonomy of the seedling for self-regulatory processes. Utilization of these reserves is indicated by their gradual disappearance and reoccurrence for cytoplasmic and cell wall materials specific to the tissue-area during the inception, differentiation and development of the leaf primordia and procambial strands at the shoot and root apices. Differential distribution of cytoplasmic RNA and proteins in the embryonic areas of tissue and organ differentiation, is indicative of their need for histochemical differentiation. These are reduced in the fully differentiated tissues followed by the rich localization of polysaccharides cell walls, and rich cytoplasmic content appears to be connected with wall material synthesis. This is evidenced by the rich localization of AA grains in the cytoplasm along the expanding cell wall-areas in hypocotyl and cotyledons. The continued presence of rich quantities of (1) RNA and proteins in the root apex, and flank zones of shoot apex young leaf primordia and the (ii) subtending provascular strands suggest that the growth substances are primary requirements in the differentiation of these areas. The storage substances namely starch and protein bodies in the areas subtending these apices and in older tissues are obviously the sources of energy for the synthesis of varied histochemical substances specific to the fundamental and vascular tissues their in the vegetative structures of the developing seedlings.