SUMMARY

In the light of the mass of evidence accumulated on the role of ascorbic acid in most of the vital metabolic activities during the life history of plants, a histochemical study was undertaken to understand its involvement in the nucleic acids metabolism, and its relationship with basic proteins and sulfhydryl groups. The plant selected for the above study was Cyperus rotundus L. This histochemical study was carried out on some of the stages of (i) Reproductive differentiation of the shoot apex, spikes and florets; (ii) Anther development and microsporogenesis; (iii) Carpel and ovule development including megasporogenesis and (iv) Seed development and embryogenesis.

Attempts were made to study the following aspects:-

1. Changes in the ascorbic acid concentration in the cells and tissues involved in the above mentioned stages;

2. Corresponding changes in the nucleic acids (DNA and RNA);

3. Changes in the basic proteins;

4. Changes in the content of sulfhydryl group; and

5. Changes in the cellular, nuclear and nucleolar sizes in the cells and their relationships.
Ascorbic acid was localized by using the acidified, alcoholic silver nitrate method. DNA was localized by means of Feulgen technique. DNA and RNA together were localized by methyl green-pyronin technique and RNA alone by staining with pyronin at pH 4.4 in acetate buffer. Alkaline Fast green method was used for the localization of basic proteins. Sulfhydryl content was determined by employing DDD method. Cross sectional area of the cells, nuclear and nucleolar diameters were measured and from that nuclear and nucleolar volumes were calculated. Ratios of nuclear/cell area as well as nucleolar/nuclear vol. were calculated. All these are given in tables.

The intensity of stain reaction for each histochemical determination was measured by a cytophotoelectrometer devised in this laboratory. The difference between transmission values for cells of control and stained tissue sections gave extinction values (e.value). The content of per cell and the content per unit area of the cell were obtained by multiplying the e.values by the corresponding cell area and by dividing the e.values by the corresponding cell area respectively. All these are presented in graphs for different stages of development.

In order to further elucidate the relationship between the metabolites studied linear regressions of DNA on AA, RNA on AA, basic protein on AA, -SH on AA, RNA on DNA,
Major findings are as follows:

1. **Shoot apex**

   a) There is an increase of AA content in the apical cells and organogenetic centres of apices throughout the successive stages studied.

   b) DNA, both nuclear and cytoplasmic, increases in the primordial inflorescence. Even though there is a general decrease of staining intensity during further stages, comparatively greater staining persists at the apex and peripheral organogenetic regions.

   c) There is an increasing trend in RNA content reaching a peak at the spike formation stage. There is a general increase in cytoplasmic and nucleolar RNA. The increase is particularly noticeable at the apex and organogenetic centres and differentiating primordia.

   d) Intensity of staining for basic proteins also increases. Increased cytoplasmic and nucleolar...
staining corresponds with a decrease in staining of chromatin region in the peripheral cells of the inflorescence axis prior to spike development.

e) Sulfhydryl groups also increase.

f) There is an increase in nuclear and nucleolar volumes as well as in the ratio of nucleolar/nuclear size at the time of spike formation.

2. Floret.

a) There is a higher intensity of stain for all the metabolites studied in the cells of the initials and differentiating organs of the floret.

b) Cytoplasmic staining for basic proteins increases in intensity in the cells destined to develop into florets and organs of the floret.

c) There is a general parallelism between AA, DNA, RNA, basic protein and sulfhydryl groups in the differentiating sites.

3. Anther development and Microsporogenesis.

a) High AA concentration in cells of stamen primordium and sporogenous cells is correlated with the shifting of staining for basic proteins from chromatin to cytoplasm and nucleolus. This is also directly correlated with RNA concentration.

b) There is a high cytoplasmic and nuclear staining for
DNA in microspore mother cells and meiocytes.

c) Staining pattern for sulphydryl groups also corresponds with that of DNA.

d) General parallelism has been observed in the total content of all metabolites per cell as well as content per unit area of the cell studied in the differentiating tissues of anther.

e) There is a rise in nuclear volume in sporogenous cells and in microspore mother cells. The increase is fourfold.

f) Nucleolus attains its maximum volume in sporogenous cells and microspore mother cells and the highest value for the ratio of nucleolar/nuclear size is observed in sporogenous cells.

4. Ovule development and Megasporogenesis.

a) Generally there are high concentrations of AA, DNA, RNA, basic protein and sulphydryl groups in the cells of carpel and ovule primordia and to a certain extent in the young differentiated ovules.

b) Concentration of all the above metabolites decrease in older ovules.

c) There is high cytoplasmic staining for basic proteins during megasporogenesis and a decrease of staining in chromatin region of the nucleus in megaspore mother cell.

d) A general parallelism exists between all the metabolites studied during the development of ovule.
5. Embryo and Endosperm development:

a) There is an increasing trend in ascorbic content of the embryo during its development.

b) High amount of AA was observed in the organogenetic region of the globular embryo (tier 1 and m) and its suspensor cells.

c) There was considerable AA in early endosperm cells.

d) There is a reduction in concentrations of both the nucleic acids during embryogenesis.

e) An increase in RNA and sulfhydryl contents in the free nuclear endosperm coincides with the biggest sizes of nucleus and nucleolus.

f) There is a reduction in the basic protein (nuclear) of the early globular embryo.

6. Ovary wall.

In the ovary wall cells generally there is higher amount of all the metabolites studied in its early developmental stages which decrease as the embryo sac is formed in the ovule. Later on there is an increase of AA, RNA, -SH and to a lesser extent DNA. This conforms to the differentiation and growth pattern of ovary wall.

Conclusions

The data presented in this thesis leads to the following conclusions:

1. The increase of RNA in the transforming shoot apex,
especially in the cells of organogenetic centres eg.,
spike, florets etc., is parallel to the increase of
AA in the same regions. Decrease of stain intensity for
the basic protein in the chromatin region of the nuclei
of cell in the inflorescence prior to spike formation
has led to the assumption that AA releases histones
from DNA which diffuses to the cytoplasm thereby
activating DNA for the synthesis of RNA, perhaps a new
m-RNA which triggers the metabolic activities leading
to the morphological differentiation of inflorescence
and individual spikes in *Cyperus rotundus*. The increase
in sulfhydryl content during the transformation of shoot
apex gives further evidence for the enhanced enzymatic
cell division activities.

2. Anther development and microsporogenesis in *Cyperus
rotundus* is visualized in terms of not only AA
activated DNA and RNA synthesis but also in terms of
their nucleocytoplasmic interactions within the cells
as well as the interrelationships between the cells
undergoing microsporogenesis and tapetum.

3. The synchronous occurrence of high amounts of AA, DNA,
RNA, basic protein and sulfhydryl groups in the
primordia of carpel and ovule in *Cyperus rotundus*
and a general parallelism between the above metabolites
observed in the different tissues of ovule leads to
the conclusion that during differentiation and early developmental stages of carpel and ovule also the same mechanism of AA activated nucleic acid synthesis and the change in the metabolic norm may be working. Considerable weakening of Feulgen staining in both sporophytic and gametophytic cells may be due to a qualitative change in DNA. However, the presence of AA, RNA, basic proteins and -SH in antipodals and synergids, before and after fertilization indicates that these cells in *Cyperus rotundus* also are not physiologically inactive and probably may have a role in supplying nutrients to the cells in the embryo sac.

4. The increase of AA in the embryo during its development and higher concentration in those cells which are destined to become the embryo axis point to the importance of AA in organogenesis. The low nucleic acid content may perhaps be due to the non-viability of seeds.

5. An increase of AA, RNA and -SH in the free nuclear endosperm which coincides with the development of embryo up to the early globular stage is suggestive of some regulatory role of free nuclear endosperm in the development of embryo.

6. Comparatively higher content of all the metabolites studied in the cells of the differentiating ovary wall is further proof for the interrelationship between these
metabolites in the differentiation processes.

7. The findings of the previous workers that the metabolism of ascorbic acid is an important factor in the problems related to growth and development in plants is corroborated.

8. The universally observed correlation between AA on the one hand and DNA, RNA, basic protein and -SH on the other during reproductive differentiation of the shoot apex, and during the development of reproductive organs like anther and ovule shows that the metabolism of these substances is closely interlocked.