OBSERVATIONS

I SHOOT APEX

(A) Paspalidium geminatum Stapf

The vegetative apex of *Paspalidium geminatum* is a dome covered by a young leaf sheath. The meristematic cells of vegetative apex are isodiametric, thin walled and unvacuolated. The cytohistological zonation is superimposed upon tunica-corpus organization with a uniseriate tunica bordering a mound of tissue-corpus. Directly below the axial tunica, there is a region with less cell density known as central mother cell zone (Fig. 27,A). These cells show poor affinity towards dyes employed, indicating a low metabolic activity (quiescent) (Figs. 26,B and 27,B).

The vegetative apex comprises of five regions—outer uniseriate tunica (T), central mother cell zone (CM), peripheral zone (pz), leaf primordia (LP) and pith rib meristem (RM) (Fig. 5,1). After receiving floral stimulus the conical vegetative apex peeps out by the activity of intercalary meristem, which grows more in the longitudinal direction. It takes an elongated shape (Fig. 5,2). Due to the differential growth activity, elongated apex attains ridges and furrows like appearance (Fig. 5,3). Ridges showed more synthetic activity for all metabolites than
Fig. 5. Camera lucida drawing of the sequential stages of transformation of the vegetative apex into reproductive state in *Paspalidium*. There are six morphologically distinct stages of development. (1) Dome of the vegetative apex. (2) Apex during evocation. (3) Elongated apex with primary branch primordia (PB). (4) Elongated apex with primary (PB) and secondary (SB) branch primordia. (5) Differentiating floret. (6) Mature floret.

A : anther primordia
C : carpel primordia
F₁ and F₂ : flowers 1 and 2
FR : furrows
G₁ and G₂ : glumes 1 and 2
LM : lemma
LP : leaf primordia
LD : lodicule
P : palea
PB : primary branch primordia
PRM : pith rib meristem
PZ : peripheral zone
RD : ridges
t : tunica
the furrows (Figs. 26, C and 27, L). Thus these ridges grow further into longitudinal rows called primary branch primordia (PB). These primary branch primordia are arranged in an acropetal sequence on the main axis of the differentiating shoot (Fig. 5, 4). Primary branch primordia in turn give rise to secondary branch primordia (SB), from where the floret takes its origin (Fig. 5, 5). These activated sites are highly meristematic and show very high staining intensity (Fig. 7, A, B). Floret primordia differentiate into glumes, palea, lemma and lodicules. Floret contains two flowers, one is bisexual and the other is unisexual (Fig. 5, 6). Thus the differentiating vegetative apex into a reproductive condition is categorized into six morphologically distinct stages and used as a score to determine the quantitative measures of the histochemical preparations (Fig. 5, 1-6).

**RNA:**

Positive staining reaction for RNA occurs in all the regions of the shoot apex with pyronin as well as with gallocyanin. Floral induction is followed by an increase in RNA content and a perturbance in the zonation of the vegetative shoot apex (Fig. 27, B). The two staining reactions, pyronin and gallocyanin employed for the detection of RNA did not coincide with each other. In
Fig. 6. The differentiating apical meristem into inflorescence axis in *Linnophyton*.

1. apical dome seated on the rhizome
2. bifurcating apical meristem to give lateral bud primordia (LBP)
3. male flower
4. bisexual flower
5. floral axis bearing male and bisexual flowers

The black dots indicate the relative size and sites of the distribution of starch granules. The axis bearing bisexual flower is loaded with more starch than the axis of the male flower(5).

- AP : anther primordia
- C : carpel primordia
- CR : corpus
- LBP : lateral bud primordia
- L2 : leaf primordia
- T : tunica
vegetative shoot apex, gallocyanin reaction showed highest extinction values for RNA in the central mother cell zone. But, pyronin reaction registered peak extinction for RNA in the tunica cells of the vegetative apex (Fig. 7,A). Both the techniques manifest that different zones of vegetative apex have different rates of synthetic activity of RNA and exhibit a wide divergence in their extinction values. During floral evocation there is a rise in the extinction values of RNA in the central mother cell zone, but a decrease in RNA extinction values in tunica and leaf primordia. At the third stage of floral differentiation, the cells of ridges showed higher extinction values for RNA than the furrows. At floret differentiation during the sequential stages of shoot apex transformation into reproductive condition both gallocyanin and pyronin techniques showed highest extinction for RNA followed by a decrease at mature floret condition (Fig. 7,A).

Cyto-hotometric analysis of RNA content (pyronin) per cell in different zones of the vegetative shoot apex did not display differences, except in the cells of leaf primordia. These cells showed relatively lower content when compared to the cells of the other zones of the shoot apex (Fig. 8,A). During floral evocation, RNA content in the cells of leaf primordia was further reduced to minimal value and different zones showed different levels
of RNA content. The cells of central mother cell zone showed a little drop from its original content because of a decrease in the cell size (Table 4). But the cells of pith rib meristem showed a little increase in RNA content during floral induction. Further stages of floral differentiation, RNA content per cell increases enormously up to floret differentiation followed by a decline at the mature flower (Fig. 8A).

Total proteins:

Like RNA, total protein extinction values also showed wide variations in different zones of the vegetative shoot apex. The cells of leaf primordia and pith rib meristem showed two fold lower extinction values than the other zones of the vegetative shoot apex (Fig. 7B). The extinction values of central mother cell zone did not show much differences from their neighbouring cells. After floral evocation, there is no significant increase or decrease in the extinction values in the central mother cell zone. But, the cells of tunica and peripheral zone showed an increasing extinction values for total proteins. There is a gradual increase in the extinction values with the advancement of the floral differentiation. Highest extinction for total proteins during stages of transformation was observed at floret differentiation (Fig. 7B).
Fig. 7. Cytophometric analysis of the relative changes in RNA, total proteins and SH protein during transitional stages of vegetative apex of Paspalidium into reproductive stage.

(A) The relative changes in the extinction values of galloycyanin and pyronin stained RNA. During evocation there is a marked increase in RNA extinction values in central mother cell zone (CM) as evidenced by both the techniques (pyronin and galloycyanin).

(B) The relative changes in the extinction values of total proteins and SH group containing proteins. There is a redistribution of the sites of synthesis for different metabolites in different zones. Stages 1 to 6 correspond to the drawings depicted in the figure 5.

CM : central mother cell zone
FL : floret
FK : furrows
LP : leaf primordia
MF : mature flower
PB : primary branch primordia
PM : pith rib meristem
PZ : peripheral zone
RD : ridges
SB : secondary branch primordia
T : tunica
TRANITIONAL STAGES OF VEGETATIVE Apex INTO FLOWERING

FIG. 7
Unlike RNA content, protein content per cell in different zones of vegetative shoot apex showed wide range of fluctuations. The cells of leaf primordia recorded least protein content. The cells of tunica, central mother cell zone and peripheral zone registered two fold higher protein content than the cells of leaf primordia (Fig. 8, B). During floral evocation, the protein content in the cells of leaf primordia and pith rib meristem remained unchanged but a decrease in protein content in central mother cell zone and peripheral zone was noted. During elongation stage of the shoot apex highest protein accumulation was recorded in the ridges while furrows showed relatively low content (Fig. 8, B).

**SH protein:**

Like protein and RNA, SH group proteins also showed differential distribution of extinction values in different regions of the vegetative apex (Fig. 7, B). The cells of leaf primordia registered highest extinction for SH group proteins and the cells of pith rib meristem showed the least extinction. During floral evocation there is an increase in SH extinction values in all regions, except in the cells of leaf primordia. These cells showed little decrease in extinction than the original values (Fig. 7, B). During elongation of the shoot apex there was a two fold increase in the extinction values. During 4th stage of the
sequential transformation of the vegetative apex there is a fall in the extinction both in the cells of primary and secondary branch primordia and followed a steep increase during floret differentiation (Fig. 7, B).

Cytophotometric estimations for SH proteins per cell showed differential content in different zones of the vegetative apex. The cells of leaf primordia and peripheral zone showed relatively higher content than other zones of the apical dome. After floral induction, there is no much significant differences in the total cellular content of the apex. During third stage of sequential transformation of the vegetative apex there is a 2 fold increase in SH proteins. Ridges showed higher content than the furrows of the elongated apex. At this stage of transformation there is a peak accumulation of SH proteins in the transforming apex. Again, second peak of -SH protein accumulation was observed during floret differentiation (Fig. 8, C).

DNA:

Gallocyanin stained DNA (after RNase treatment) behaved differentially in their extinction values in different zones of the vegetative shoot apex. The nuclei of central mother cell zone during vegetative condition showed least extinction for DNA among all other zones (Fig. 9, A). But, a pronounced increase in DNA extinction in these nuclei was recorded after receiving floral stimulus. These cells are
TRADITIONAL STAGES OF VEGETATIVE APEX INTO FLOWERING

FIG. 8
actively engaged in DNA synthesis. After floral induction, there is an immediate rise in DNA synthesis in all zones of the shoot apex except pith rib meristem. At this stage, maximum synthesis of DNA takes place. During fourth and fifth stages of transition of vegetative apex peak extinction values for DNA were recorded (Fig. 9, A).

The relative changes in DNA content per nucleus showed wide disparities among different regions of the vegetative shoot apex. The nuclear DNA content in pith rib meristem cells are highest among other regions of the vegetative shoot apex and the cells of central mother cell zone recorded four times lower DNA content than the pith rib meristem (Fig. 10, A). After floral induction, DNA content in the pith rib meristem cells is reduced to half and reveal an increasing trend in DNA content in other regions of vegetative apex except leaf primordia. There is a 2 fold more higher DNA content in the ridges than the furrows of the elongated shoot apex. During further stages of floral differentiation there is a gradual fall in nuclear DNA content (Fig. 19, A).

Histones:

Different zones of vegetative apex showed different levels of extinction values for ammoniacal silver stained histones. The nuclei of tunica and pith rib meristem are two
extreme ends in maintaining highest and lowest extinction values for histones respectively (Fig. 9,B). The nuclei of leaf primordia and peripheral zone showed no change in extinction values for histones. During floral evocation a decrease in histone extinction values was observed in the nuclei of central mother cell zone, but other zones of the shoot apex showed no remarkable differences (Fig. 9,B). During the fourth stage of floral differentiation, histone extinction values were drastically reduced followed by an elevation during floret differentiation.

The nuclear histone content in different regions of the vegetative apex showed a similar pattern of distribution with that of DNA, by recording highest histone content in pith rib meristem and least in central mother cell zone (Fig. 10,B). During evocation, there is a redistribution of synthetic sites of histones in different regions of the apex. After floral induction, higher histone content per nucleus was recorded in the cells of tunica, central mother cell zone and peripheral regions of vegetative apex. During further stages, the nuclear histone content gradually decreases reaching a least value at mature floret condition (Fig. 10,B).

Non histone chromosomal proteins (NHCP): The zone wise variations in NHCP extinction values in the vegetative shoot apex are not pronounced. The extinction
TRASITIONAL STAGES OF VEGETATIVE APEX INTO FLOWERING

FIG. 9
values for NHCP in different zones ranged between 0.015 to 0.025 only (Fig. 9,C). The nuclei of peripheral zone recorded highest extinction and least value was confined to the nuclei of pith rib meristem. During floral evocation, NHCP extinction values increased in the nuclei of central mother cell zone. Other zones did not bring about marked differences after induction. During shoot apex elongation (stage 3), there is a manifold increase in the NHCP extinction values in the ridges and furrows of the apex (Fig. 9,C). During fourth stage, NHCP extinction values are drastically reduced, which followed by a peak value at the floret differentiation.

During floral evocation, greater accumulation of MHC proteins occurred in central mother cell region and peripheral region of the transforming apex (Fig. 27,D). But, the levels of NHCP in other regions of the shoot apex remain unchanged. Elongated apex of the shoot showed 3 fold higher content of MHC proteins and during further stages of differentiation there is a gradual decrease in the extinction values (Fig. 10,C). Thus MHC proteins are actively synthesized during elongating stage of the shoot apex.

Insoluble sugars (PAS):

PAS reaction is associated with cell walls only. A little diffusible PAS positive reaction is appeared in the
TRASITIONAL STAGES OF VEGETATIVE APEX IN TO FLOWERING

FIG. 10
cytoplasm. Highly meristematic regions, showed less affinity with the PAS reaction (Fig. 26, A). The dome shaped vegetative apex is completely devoid of starch. Starch appears in the basal region below the pith rib meristem. At nodal region there is a widespread distribution of starch granules. After getting floral stimulus in central mother cell zone there is a little more PAS positive tinge and at the peripheral region of the shoot apex the PAS positive reaction becomes faint (Fig. 26, A). During elongation of the shoot apex, the bases of bulging primary branch primordia accumulate starch granules. Abundant starch granules are quite apparent in the differentiating floret (Fig. 26, B).

(B) *Limnohyton obtusifolium* (L.) Miq.

The shoot apex of *Limnohyton obtusifolium* on the rhizotome is dome shaped and encircled by leaves in a spiral phyllotaxy. The dome disappears by a little upright growth (Fig. 6, A). There is a single distinct tunica layer covering a mound of tissue (corpus). There is no cytohistological zonation in the apex. The cells are completely occupied by nucleus. The first sign of the transformation of the shoot apex into inflorescence is the broadening of the apex in a plane oblique to the main axis. This broadening is due to active divisions of cells in the corpus.
region. As a result of differential meristematic activity two growth regions can be distinguished (Fig. 6,B). One is lateral developing into floral bud and the other is apical which continues its growth by giving floral buds in whorls. Lower whorls contain bisexual flowers and unisexual flowers (Pure female flowers are exclusively absent) are in the upper whorls. The sequential stages of the shoot apex transformation into an inflorescence axis is characterised into the following 3 distinct stages.

Stage 1: elongated apex, a mound of highly meristematic tissue bordered by a uniseriate tunica (Fig. 6,A-E). Stage 2: intermediate apex, broader with two bulges, one lateral and the other apical growing region. Stage 3: inflorescence axis, bearing bisexual and unisexual flowers.

Elongated apex showed differential staining for all the metabolites. The cells of tunica showed higher extinction values for RNA, total proteins and SH protein than the cells of corpus. The peripheral region is distinctly marked by a high accumulation of all metabolites (Fig. 28,A-C). During the second stage, the lateral primordia showed higher extinction values for all metabolites than the apical meristem (Table 4). The cells of anther primordia did not significantly differ in their staining characteristics from the carpel primordia. The extinction values for DNA, histone and NHCP also followed a similar fashion with that of RNA,
total proteins, and SH protein. The extinction values for
MHc proteins in all regions always found higher than the
histone extinction (Table 4). Shoot apex during elongation
is completely devoid of starch granules. PAS reaction is
associated with cell wall only (Fig. 29, A). High content
of starch granules are distributed at the basal part of the
apex, and between the bulges arise from the main axis.
There exists an inverse correlation between the presence
of starch granules and meristematic activity of the
differentiating shoot apex (Fig. 29, A). A remarkable
difference in the number, size and pattern of distribution
of starch granules is noted between the bisexual and
unisexual flowers. Bisexual flowers store more starch than
unisexual ones at their base. The relative size, dispersal
and sites of distribution of starch granules in the apical
meristem of Limnophyton obtusifolium during inflorescence
formation are given in the figure 6, 1 to 5.

II ANther

Limnophyton obtusifolium (L.) Miq.

The anther is tetrasporangiate and its wall development
conforms to the monocotyledonous type (Jobri, 1935). The
differentiated anther contains an epidermis, two middle
layers and the tapetum. The middle layers are ephemeral
and they disappear at the time of anther dehiscence.
Endothecium develops fibrous thickenings, distinctly visible
Table 4: Cytophotometric analysis of the relative changes (extinction and content) in RNA, total protein, SH-protein, DNA, histone (FG 8.1) and NRCP (FG 5.0) in different regions of the shoot apex of Limnophyton obtusifolium (L.) Miq. during transformation. The corresponding cellular area and nuclear volume are also depicted in the table.

<table>
<thead>
<tr>
<th>REGIONS AT DIFFERENT STAGES</th>
<th>CELL AREA in μ²</th>
<th>RNA</th>
<th>Total protein</th>
<th>SH-protein</th>
<th>RNA</th>
<th>Total protein</th>
<th>SH-protein</th>
</tr>
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<tbody>
<tr>
<td>I Tunica (T)</td>
<td>161</td>
<td>0.121</td>
<td>0.204</td>
<td>0.084</td>
<td>19.43</td>
<td>32.88</td>
<td>13.46</td>
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<tr>
<td>Corpus (CR)</td>
<td>190</td>
<td>0.097</td>
<td>0.107</td>
<td>0.057</td>
<td>21.00</td>
<td>35.55</td>
<td>14.65</td>
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<td>Leaf primordia (LP)</td>
<td>152</td>
<td>0.132</td>
<td>0.222</td>
<td>0.097</td>
<td>20.11</td>
<td>33.73</td>
<td>14.74</td>
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<tr>
<td>Peripheral region (PH)</td>
<td>130</td>
<td>0.114</td>
<td>0.204</td>
<td>0.098</td>
<td>20.45</td>
<td>36.76</td>
<td>17.57</td>
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<tr>
<td>II Tunica (T)</td>
<td>165</td>
<td>0.118</td>
<td>0.204</td>
<td>0.084</td>
<td>19.44</td>
<td>33.70</td>
<td>19.44</td>
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<tr>
<td>Corpus (CR)</td>
<td>190</td>
<td>0.111</td>
<td>0.187</td>
<td>0.080</td>
<td>20.03</td>
<td>35.55</td>
<td>21.03</td>
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<td>Lateral bud primordia (LBP)</td>
<td>150</td>
<td>0.125</td>
<td>0.222</td>
<td>0.111</td>
<td>18.75</td>
<td>33.29</td>
<td>18.75</td>
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<td>III Anther primordia (AP)</td>
<td>93</td>
<td>0.132</td>
<td>0.204</td>
<td>0.111</td>
<td>12.30</td>
<td>19.00</td>
<td>10.30</td>
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<td>Carpel primordia (C)</td>
<td>120</td>
<td>0.140</td>
<td>0.204</td>
<td>0.115</td>
<td>16.76</td>
<td>24.50</td>
<td>24.50</td>
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<tr>
<td>REGIONS AT DIFFERENT STATES</td>
<td>NUCLEAR VOLUME in $u^3$</td>
<td>EXTINCTION VALUES DNA</td>
<td>Histone</td>
<td>NHCP</td>
<td>CONTENT/NUCLEUS in Au DNA</td>
<td>Histone</td>
<td>NHCP</td>
</tr>
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<td>-----------------------------</td>
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<td>I Tunica (T)</td>
<td>1083</td>
<td>0.084</td>
<td>0.111</td>
<td>0.138</td>
<td>90.54</td>
<td>119.9</td>
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<td>Corpus (CR)</td>
<td>1499</td>
<td>0.078</td>
<td>0.104</td>
<td>0.125</td>
<td>117.52</td>
<td>155.6</td>
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<td>Leaf primordia (LP)</td>
<td>767</td>
<td>0.084</td>
<td>0.098</td>
<td>0.132</td>
<td>64.12</td>
<td>74.9</td>
<td>101.5</td>
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<td>Peripheral region (PR)</td>
<td>1100</td>
<td>0.081</td>
<td>0.096</td>
<td>0.128</td>
<td>89.10</td>
<td>105.6</td>
<td>140.8</td>
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<td>II Tunica (T)</td>
<td>1093</td>
<td>0.071</td>
<td>0.111</td>
<td>0.163</td>
<td>77.28</td>
<td>121.0</td>
<td>177.9</td>
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<tr>
<td>Corpus (CR)</td>
<td>1304</td>
<td>0.058</td>
<td>0.104</td>
<td>0.171</td>
<td>75.63</td>
<td>135.6</td>
<td>223.0</td>
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<tr>
<td>Lateral bud primordia(LBP)</td>
<td>1093</td>
<td>0.084</td>
<td>0.111</td>
<td>0.147</td>
<td>91.37</td>
<td>120.0</td>
<td>160.0</td>
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<tr>
<td>III Anther primordia(AP)</td>
<td>607</td>
<td>0.064</td>
<td>0.111</td>
<td>0.171</td>
<td>39.00</td>
<td>67.0</td>
<td>103.0</td>
</tr>
<tr>
<td>Carpel primordia(C)</td>
<td>557</td>
<td>0.084</td>
<td>0.104</td>
<td>0.140</td>
<td>46.56</td>
<td>57.8</td>
<td>77.8</td>
</tr>
</tbody>
</table>
after degeneration of the tapetal tissue. The tapetum is amoeboid and its protoplasts move into the anther locule to invest the MMCs. At the time of tetrad formation, the tapetal nuclei are often found attenuated with deep staining (Fig. 36, D). Microspore tetrads are isobilateral and they were insulated with callose as evidenced by PAS test. The developmental stages of microspore and gamete formation are highly synchronous. The tapetal cells are uninucleate but show high degree of polyploidy because of endoreduplication. The tapetal cells are filled with big nuclei with little cytoplasm. The formation of microspores is closely related with the severance of tapetal mass. After the separation of spores from tetrads, the tapetal tissue completely disappeared. The first division of microspore is asymmetric leaving a big vegetative nucleus and a relatively small generative nucleus (Fig. 36, C, D). The generative nucleus is pushed to the peripheral region of the microspore and elongates enormously, undergoes a mitotic division resulting into two male gametes. The developmental sequence of anther in Limnophyton is characterized by the following stages, and the cytophotometric analysis of various cell constituents were centered on these stages only. The fate of the tapetal cells is referred to with these stages of microspore formation. The developmental stages are as follows.
CONTENT/CELL AREA
TOTAL PROTEIN
---TAPETUM

EXTINCTION VALUES
TOTAL PROTEIN
---TAPETUM

SH PROTEIN

RNA

FIG. II
1. Sporogenous tissue - These cells are compactly arranged with prominent cell walls. The sectional surface area of these cells are relatively bigger than MMCs. 2. Microspore mother cells (MMC) - Elliptical, spheroidal cells with callose border. 3. Dyad - The products of first meiotic division, strongly insulated with callose. 4. Tetrads - four cells in one group completely encircled by callose layer. 5. Uninucleate pollen - spheroidal cells with dense cytoplasm. 6. Binucleate pollen - at this stage the size of the pollen is bigger than the microspore and contains two nuclei of differential volumes. a. vegetative nucleus, b. generative nucleus. 7. Trinucleate pollen - pollens at the time of anther dehiscence. a. vegetative nucleus, b. two gamete nuclei.

RNA:

There is a gradual increase in RNA extinction values from sporogenous stage to microspore formation. Tetrads after separating from its counterpart showed two fold higher extinction for RNA (0.17 to 0.35). During first mitotic division of pollen there is a declining trend in the RNA extinction values. Mature pollen showed two fold decrease (0.35 to 0.16) when compared to uninucleate pollen. Tapetal cells showed higher extinction than the meioocytes and a decline in extinction values during active periods of meiosis (Fig. 11,5). But the relative content of RNA
FIG. 12
per cell in the tapetal tissue is always lesser than the sporocytes. With the progress of microsporogenesis there is a gradual depletion in RNA content in the tapetal cells. During premeiotic stage, there was a little accumulation of RNA in the cells but, during meiosis a decrease in RNA content per cell was recorded. After the completion of meiosis, RNA content was restored in the cells and reached its peak synthetic period at binucleate pollen (Fig. 11,E).

Sulfhydryl group containing proteins:

Like RNA, the SH proteins also showed a linear increase in its extinction values with the advancement of microsporogenesis and touched its peak value during tetrad stage (Fig. 11,D). After meiotic divisions, there was a steep fall in the extinction values which reached a minimum during the mature condition of pollen. Just before the onset of meiosis, the tapetal cells showed higher extinction than the meiocytes, but during the periods of meiosis the extinction values for SH proteins were dramatically reduced.

The relative changes in the SH protein content per cell during microsporogenesis and pollen development showed wide fluctuations. Just before cell divisions (either meiotic or mitotic) SH protein content accumulates in the cells and prepares for divisions. Highest SH protein content was recorded in dyad cells (Fig. 11,C). There is a
FIG. 13
vast difference in SH protein content per cell between dyad and tetrad cells. The tetrads again start synthesizing SH proteins just before pollen mitosis. Mature pollen showed relatively higher SH protein than the young pollen at binucleate condition (Fig. 11,C). SH protein content in the tapetal cells is relatively low when compared to sporocytes during microsporogenesis. At dyad stage, highest accumulation in SH content in the tapetal cells was noticed.

Total proteins:

There is a sudden decline in the extinction values for total proteins during 1st meiotic period followed by an increase at tetrad stage (Fig. 11,B). During pollen development, the extinction values slowly decrease and reach to minimal values. The changes in the extinction values for total proteins at different periods of microsporogenesis showed the similar trend with that of RNA and SH proteins but the rate of loss of protein extinction values in tapetal tissue relatively passive or nil at all. During microsporogenesis total protein content per cell reduces from sporogenous stage to tetrad one and thereafter an increasing trend was noted. Tapetal tissue also showed a gradual loss of protein content with the development of the spores (Fig. 11,A).
DNA:

Feulgen cytophotometry showed a decreasing trend in the extinction values from sporogenous stage to dyad state of differentiation. After first meiotic division, Feulgen extinction values increase up to single nucleate pollen development. After first mitotic division of pollen, the vegetative nucleus showed relatively less extinction than the generative one (Figs. 12 and 36,C). In the generative nucleus the chromatin matrix is highly condensed whereas in vegetative one chromatin material was sparsely distributed (Fig. 36,C,D). The Feulgen extinction values in the vegetative nucleus decreases with the maturation of the pollen and in generative nucleus a reverse trend in extinction values was observed (Fig. 12). At trinucleate condition the nucleolus of vegetative nucleus showed very faint staining for DNA. Feulgen positive granules moved a little towards the periphery of the nucleolar membrane. During stages of pollen development, gamete nuclei of the trinucleate pollen showed highest Feulgen absorbance and its extinction value is 3 fold higher than vegetative nucleus. Tapetal nuclei during all stages of microsporogogenesis showed manifold higher extinction values than the meiocytes, but these values showed a declining trend with the advancement of the microsporogenesis.
Feulgen cytophotometry showed an active period of DNA synthesis before pollen mitosis. Premeiotic nuclei showed relatively higher DNA content than MMCs. The nuclear DNA content of MMCs was reduced to minimal value after meiosis. Generative nucleus showed a little higher DNA content than generative nucleus. At trinucleate condition vegetative nucleus recorded little higher DNA content than at binucleate condition. Thus the feulgen extinction varies in the vegetative nucleus too. The nuclear DNA content in tapetal cells has been amplified many times because of endoreduplication. But after dyad stage of microsporogenesis the nuclear DNA content in the tapetal nuclei was dramatically reduced.

Histone:

The relative changes in the extinction values for histones (Fe.pH 8.0) followed a similar trend with that of DNA. The extinction values for histones decrease from sporogenous stage to dyad stage followed by a sudden upsurge at tetrad stage. Tetrad after separation from each other showed lowest extinction for histones (Fe.pH 8.0). During the first mitotic division of pollen there is a huge difference in the histone extinction values between vegetative and generative nuclei (Fig. 12). Vegetative nucleus showed higher extinction than generative one. At trinucleate condition the histone extinction in the
vegetative nucleus was very much reduced, but the gametic nuclei had 4 fold higher extinction than the vegetative nucleus. The histone extinction values in the tapetal nuclei remained at higher levels than the sporocyte nuclei. At dyad stage, histone extinction in tapetal nuclei is five times higher than dyad nuclei. There is no decreasing trend in the extinction values for histones in the tapetal nuclei.

The relative changes in histone content sharply reflect the DNA changes during microsporogenesis. From sporogenous to meiotic stages the nuclear histone content was dramatically reduced to its lowest ebb. After tetrad stage, the spores showed a little increasing trend in its nuclear histone content (Fig. 13). The two nuclei in binucleate pollen showed antagonistic trend of distribution with that of DNA. Vegetative nucleus showed relatively higher histone content than the generative nucleus of the binucleate pollen. At trinucleate condition, the nuclear histone content of vegetative nucleus decreases and the gametic nuclei showed relatively less histone content than the generative nucleus of binucleate pollen (Fig. 13).
Non-histone chromosomal proteins (NHCP):

The relative changes in the extinction values for fast green (FG, pH 5.0) stained non-histone chromosomal proteins (NHCP) showed a different trend line from that of basic proteins (FG, pH 8.0). During metamorphosis of sporogenous cells into MHCs NHCP extinction values in the nuclei of meiocytes is reduced to half of its initial values (Fig. 12). During meiotic divisions the extinction values increased up to tetrad stage. The tetrads after separating from each other recorded a decreasing trend in the extinction values for NHCP proteins. After first pollen mitosis, generative nucleus showed relatively higher extinction than the vegetative. The extinction values in vegetative nucleus increase with the increase in the period of maturation of the pollen. At trinucleate condition, the gametes recorded higher NHCP extinction value than the vegetative nucleus (Fig. 12). One more striking point is that NHCP extinction values during gemetogenesis are relatively less when compared to the sporogenesis, which is a character different from that of histone and DNA extinction values. There is a sudden increase of NHCP extinction values during the period of meiosis. Like histones, NHCP extinction values in tapetal nuclei also did not show declining trend with the progress of meiosis (Fig. 12).

During stages of microsporogenesis the NHCP content
is gradually reduced and reached to least value at tetrad stage (Fig. 13). Tetrad nuclei after separating from each other showed an active period of NHCP synthesis, but during pollen mitosis a drop in its content was noticed. Binucleate pollen showed higher NHCP content in vegetative nucleus than in the generative. Gametic NHCP content is relatively less than generative nucleus. Tapetal nuclei showed manifold higher NHCP content than sporocytes. At dyad stage of microsporogenesis the tapetal tissue showed highest NHCP content in their nuclei. During second meiotic division the tapetal nuclei showed least value for NHCP content. The changes in histone (FG. pH 8.0) over NHCP (FG. pH 5.0) during stages of pollen development were expressed in the Fig. 16,A. From binucleate pollen to trinucleate condition the ratio of NHCP found increased upto 95% which is the highest percent ratio during pollen development. First meiotic stage also recorded higher ratio of NHCP over histone. Gametic nuclei showed least ratio value for NHCP (Fig. 16,A).

Fistones by ammoniacal silver nitrate method:

Histones are visualised as yellowish brown to blackish colour by ammoniacal silver nitrate method of Black and Ansley (1964). Localization was confined to nucleus only and no positive reaction was observed in the cytoplasm.
But mature pollen grains showed a faint positive reaction in the cytoplasm indicating the presence of cytoplasmic histones. Fast green (pH 8.0) reaction also found positive in the cytoplasm of mature pollen grains. Ammoniacal silver stained histones behaved differently from that of fast green histones, but the trend line for the relative changes in histone during microsporogenesis remain the same. There is no change in the extinction of AS stained histones from sporogenous stage to MMC. But, fast green stained histones showed a decrease between these two stages (Fig. 12). In both the reactions (AS and FG 8.0) histone extinction reached a peak value during tetrad stage followed by a decrease. There are qualitative changes in the AS staining characteristics between generative and vegetative nuclei. Generative nucleus stained as a black silver colloid with no yellow tinge and showed higher extinction value than the vegetative nucleus. Vegetative nucleus stained yellowish with little blackish bodies. These tinctorial changes in the staining character can be attributed to the qualitative changes in the histone composition. In FG stained histones these qualitative changes in staining reaction are not detectable except a difference in the stain intensity. One more difference between AS and fast green staining appeared in the tapetal nuclei. FG stain did not show a declining trend in
the extinction values in the tapetal nuclei during microsporogenesis. But AS reaction showed a gradual decline in the extinction values with the progress of meiosis.

The relative changes in the total AS bound histone content per nucleus showed a parallel trend with that of the changes in fast green (FG, pH 8.0) stained histones except in vegetative nucleus. From binucleate to trinucleate condition AS stained histones found increasing but FG (pH 8.0) stained histones showed a declining trend. Like FG reaction, AS reaction also showed a gradual loss in nuclear histone content during microsporogenesis.

Statistical analysis showed that the relative changes in RNA content during microsporogenesis and pollen growth are positively correlated with the changes of DNA:histone ratio \(r = +0.96\) and a negative correlation with DNA:NHCP ratio \(r = -0.87\). RNA changes also showed a significant relation with histone:NHCP at a significant level \(r = +0.7\). The magnitude of correlation of RNA synthesis with nuclear volume is of the tune of \(r = +0.50\). The relative changes in RNA synthesis are highly coordinated with the changes in NHCP during microsporogenesis.

Increase of NHCP ratio over histone influenced the synthesis of RNA. The relative changes in RNA content during microsporogenesis and pollen development does not
**Fig. 14.** Nuclear and nucleolar volume changes during microsporogenesis (A,B) and megasporogenesis (C,D) in _Limnophyton_. Note the parallel changes in nuclear and nucleolar volumes up to uninucleate pollen development (A,B). But during megasporogenesis the nucleolar growth is not coordinated with the nuclear growth (C,D). Egg nucleus showed highest nuclear volume among all other elements of mature embryosac (C). Highest nucleolar volume is noted in secondary nucleus (D).

**Abbreviations:**
- **ANT**: antipodal
- **EG**: egg
- **GEN**: generative nucleus
- **SNC**: secondary nucleus
- **SG**: synergid
- **VNC**: vegetative nucleus
FIG. 14
show any significant correlation with the changes in total protein content \(r = +0.3\), but showed a little significant negative relation with the changes in SI proteins \(r = +0.62\).

Cellular, nuclear and nucleolar volume changes:

During microsporogenesis the three variables viz. nuclear, nucleolar and cellular volumes displayed a wide spread of values. The mean values of 30 observations (3 slides 10 from each) have been depicted in Table 5. Mean cell volume decreased linearly up to tetrad stage of microsporogenesis. But the tetrads after separation are restored to the original volume. These changes in cellular volume followed the changes of nuclear and nucleolar volume. Significant correlations in the changes of volumes between cell versus nucleus, nucleus versus nucleolus were obtained throughout the stages of microsporogenesis (Table 6). The regression coefficients between nuclear and nucleolar volume changes during microsporogenesis were of the tune of \(r = 0.95\). The regression coefficient between cell volume and nuclear volume changes is relatively less \(r = +0.54\). At each stage, the data of these three variables showed wide fluctuations. Among the three parameters measured, nuclear volume indicated a huge range of values and nucleolar and cellular volumes showed a narrow spread.
The values of correlation coefficients (r) among different parameters during microsporogenesis and pollen development in *Limnophyton*

<table>
<thead>
<tr>
<th></th>
<th>DNA NHCP</th>
<th>DNA histone</th>
<th>histone NHCP</th>
<th>SH-proteins</th>
<th>Nucleolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. RNA</td>
<td>-0.87**</td>
<td>+0.96*</td>
<td>+0.70**</td>
<td>+0.60***</td>
<td>+0.50***</td>
</tr>
<tr>
<td>II. Nuclear volume</td>
<td>+0.99*</td>
<td>+0.85**</td>
<td>+0.1***</td>
<td>+0.80**</td>
<td></td>
</tr>
</tbody>
</table>

III. Nuclear versus nucleolar volume | +0.95**  
Nucleolus versus cell volume | +0.35***  
Nuclear versus cell volume | +0.54***  

* .... Significant at 1% level
** .... Significant at 5% level
*** .... not significant either at 1% or 5% level
Fig. 15. Frequency histograms of percent of cellular, nuclear and nucleolar volumes in a population (at microspore mother cell stage during the anther development in Limnoiphyton). Note the heterogeneity of the distribution of cellular volumes. The distribution of cellular volume is not coinciding either with the nucleolar or with the nuclear volume distribution. But the pattern of nuclear volume is reflecting the nucleolar volume distribution.
FIG. 15

NUCLEOLUS

percent of nuclei

NUCLEUS

percent of nuclei

CELL

percent of cells

VOLUME IN μ₃ (x1000)
Nucleolar changes:

The changes in number, size and texture of nucleoli varies with the progress of microsporogenesis. Sporogenous tissue showed 2-3 nucleoli with fibrillar texture (Fig. 30, A). The total volume of individual nucleoli of sporogenous nuclei showed a highest value (Fig. 14, B). MMC (during leptotene stage of meiotic prophase) contained only one nucleolus having spheroidal structure. During meiotic divisions the nucleolar volume was very much reduced. After pollen mitosis vegetative nucleus contained two nucleoli of relatively higher value than the generative nucleus (Fig. 14, A). Sometimes in generative nucleus the nucleolus is not discernible because of highly condensed chromatin. The changes in the nucleolar volume during microsporogenesis are significantly \((r = +0.95)\) correlated with the growth of the nucleus. The correlation is highly significant during microsporogenesis, but during gametogenesis the correlation is less convincing. The relative changes in nuclear DNA content during microsporogenesis and pollen development strongly reflect the changes in nuclear volume. Thus, nuclear volume is a sensitive parameter to the changes of DNA content.

Regression analysis demonstrated a significant correlation coefficient \((r = +0.99)\) between these two parameters. The relative changes in NHCP content also act as a covariable
Table - 5

Mean cell, nuclear and nucleolar volume ($\mu^3$) changes at different stages during microsporogenesis and pollen development in *Limonophyton*

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cell</th>
<th>Nucleus</th>
<th>Nucleolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microspore mother cell</td>
<td>8797</td>
<td>350</td>
<td>27.6</td>
</tr>
<tr>
<td>2. dyad</td>
<td>5967</td>
<td>150</td>
<td>8.19</td>
</tr>
<tr>
<td>3. tetrad</td>
<td>1643</td>
<td>102</td>
<td>3.5</td>
</tr>
<tr>
<td>4. uninucleate pollen</td>
<td>5115</td>
<td>221</td>
<td>8.19</td>
</tr>
<tr>
<td>5. binucleate pollen</td>
<td>23939</td>
<td>391^x</td>
<td>28.00^xx</td>
</tr>
</tbody>
</table>

^x - total volume of generative and vegetative nuclei

^xx - total volume of generative and vegetative nucleoli
Fig. 16. The percent ratio changes between histone and nonhistone chromosomal proteins during the stages of pollen (A) and embryosac (B) development in Limnophyton.

(A) The stages of pollen development are
1. sporogenous cells
2. microspore mother cells
3. dyad
4. tetrad
5. uninucleate pollen
6. vegetative nucleus in binucleate pollen
7. generative nucleus in binucleate pollen
8. vegetative nucleus in trinucleate pollen
9. gamete nucleus

(B) The stages of embryosac development are as follows:
1. archesporial cell
2. megaspore mother cell
3. degenerating nucleus
4. micropylar nucleus
5. chalazal nucleus
6. embryosac at four nucleate condition
7. egg nucleus
8. synergid nucleus
9. secondary nucleus
10. antipodal nucleus
11. integument nucleus
to influence the changes in nuclear volume but, the extent of correlation between NHCP and nuclear volume was relatively less ($r = +0.85$) when compared to DNA. Furthermore, nuclear volume has no significant correlation with the changes in histone content (FG 8.0) (See table 5).

Variations in nucleolar, nuclear and cellular growth in a population of MMC stage have been depicted in a frequency diagram (Fig. 15). The nuclear volume varied from $3.5 \mu^3$ to $65.5 \mu^3$ and the values having $25 \mu^3$ mid point showed highest frequency ($44\%$). The distribution diagram for nuclear volume also showed more or less similar pattern of distribution with that of nucleolus. Nucleolus showed wide spread of values and the ratio between highest and lowest nuclear volume is 21.6 but in nuclear population, this value is 4.3 and in case of cellular volume 5.9 is the ratio value between the highest and lowest cellular volumes. In nuclear population 52\% of total population contained nuclear volume $395 \mu^3$. But the frequency distribution of cellular volume displayed altogether a different model (Fig. 15).

Polysaccharides:

During anther differentiation there is a strong association of PAS positive tinge with the cell wall and a diffusable staining in the cytoplasm. Afterwards, the sporogenous cells showed PAS negativity in their cytoplasm.
and cell wall staining is dominating. Nucleoli of sporogenous cells showed PAS negativity (Fig. 29,B). Amazingly, tapetal tissue is devoid of PAS substances either in cell wall or in cytoplasm. But the wall layers of anther are loaded with starch grains. Inner wall contained more granules than the outer wall. Just before the separation or metamorphosis of sporogenous cells into MMC the nucleolar PAS reaction is restricted to the centre of the nucleolus (karyosome) but not to the entire nucleolus. During meiotic stages, cell wall and nucleus are stained equally with PAS reaction. The callose which surrounds the meiocytes also showed PAS +ve reaction. During gametogenesis, cell wall staining is dominating, nucleus and nucleolus showed relatively less staining with PAS reaction.

III OVULE

Archespordial cell is polygonal and directly functions as a megaspore mother cell (MMC) (Fig. 28,3). The MMC undergoes a long period of prophase with a wide dispersal of its chromatin in the nucleus. First division of meiosis is asymmetric giving an outer crescent shaped smaller cell and a bigger cell inside. The crescent shaped cell showed intensive nuclear staining for DNA, histone and MNCP. Outer cell showed no part in the further development of embryosac and eventually degenerates (Fig.28,F). The functional cell undergoes one more asymmetric division
Changes in the extinction values of total protein, SH-protein and RNA during embryosac development in *Linnoplyton*. There is no marked differences in the extinction values of RNA in different components of embryosac. But total protein as well as SH protein extinction values showed wide disparities. Antipodal cell showed highest value for total protein. Stages of development are as follows.

1. archesporial cell
2. megaspore mother cell
3. degenerating nucleus
4. micropylar nucleus
5. chalazal nucleus
6. embryosac at 4 nucleate condition
7. egg
8. synergids
9. secondary nucleus
10. antipodals
11. integuments
leaving a bigger micropylar nucleus \(864 \mu^3\) and a smaller chalazal nucleus \(514 \mu^3\). Each of these two nuclei undergoes one more division resulting in four nucleate condition. During this period, embryosac elongates enormously and takes a horse shoe shaped appearance. Because of this bending, there is a disturbance in the situation of the components of the embryosac. Thus, it renders difficult to detect all the components of the embryosac. But, it is a 6 nucleate embryosac with \(3 + 1 + 2\) arrangement. The stages of embryosac development starting from archesporial cell to mature embryosac are arbitrarily divided into following 11 stages for cytophotometric estimations.

1. archesporial cell, 2. megaspore mother cell, 3. degenerating cell, 4. micropylar cell, 5. chalazal cell, 6. 4 nucleate embryosac, 7. egg, 8. synergid, 9. polar nucleus, 10. antipodal, 11. integuments.

Polysaccharides:

A single archesporial cell was identified by a thick PAS positive reaction associated with the wall. Megaspore mother cell showed starch granules in its cytoplasm. After 1st meiotic division, the functional megaspore was loaded with starch granules towards poles. The number of starch granules at the micropylar region is more than at the
chalazal end (Fig. 29, C). The starch granules at the chalazal end gets depleted but the micropylar end maintains its higher counts. The callose which encircles the meiocytes also showed a positive reaction with PAS test. The presence of callose in other stained preparations is also distinctly visible because of its high refractivity (Figs. 2, B and 36, B). The cross walls separating the dividing cells show PAS positive reaction. Integuments are completely devoid of starch granules, but its cell walls showed a faint positive reaction, and cytoplasm showed a very little PAS +ve tinge (Fig. 29, C). The embryosac showed starch granules at the micropylar region. The filiform apparatus showed a strong PAS positive reaction (Fig. 29, D). Egg cell wall is distinctly stained and there are amorphous PAS positive granules dispersed in the cytoplasm. Polar cells also showed a distinct cell wall staining with PAS but the cytoplasm showed negative reaction. Polysaccharides are intensely localized in the antipodal cells.

RNA:

Archesporial cell showed a strong pyroninophilia in the nucleoli and cytoplasm (Fig. 28, E). The micropylar nucleus showed more staining intensity than the chalazal one. During this stage of ovule development there is a strong staining reaction with the cells of the integuments.
Cytophotometric analysis revealed a very significant distribution of RNA from sporogenesis to gametogenesis. The extinction values during sporogenesis are 2 fold higher than the elements of mature embryosac (Fig. 17). There are no wide variations in the extinction values among the components of the embryosac. The extinction values range between 0.073 to 0.81 (Fig. 17). The relative content of RNA per cell showed a reverse trend to that of extinction values. During sporophytic development archesporial cell showed highest content of RNA (Fig. 18). During premeiotic stages there is a sudden drop in RNA content. Among all the components of embryosac, egg cell recorded highest content and antipodal cells stand in the second order. At mature condition, the cells of integuments registered lowest amount of RNA.

SH - protein:

Sulphydryl group containing proteins are sparsely distributed in the meiocytes during sporogenesis. But megaspore mother cell showed intensive reaction with DDD for sulphhydryl group containing proteins. Cytophotometric analysis of DDD stained chromophore showed higher extinction values during gametogenesis than the sporogenesis, a case reverse to that of the extinction values of RNA (Figs. 17 and 18). In the mature embryosac synergid cells showed highest extinction values amongst its counterparts, and the
antipodal cells stood in the second order. During sporogenesis MMC showed highest content of SH proteins. After second meiotic division the micropylar cell is loaded with two fold more content of SH than the chalazal cell. During gametogenesis mature egg showed highest content of SH proteins and antipodal cells stand in the second order.

Total proteins:

The distribution of total proteins sharply reflects the RNA changes during megasporeogenesis. The relative absorbance values for bromphenol blue stained proteins during megasporeogenesis are more or less the same, except an increase in the degenerating cell (Fig. 17). Micropyler cell showed higher extinction values than the chalazal cell. In the mature embryosac antipodal cell recorded the highest absorbance value, which is two fold higher than the egg cell and 3 times higher than polar cell. The cells of integuments also showed higher extinction values than egg, synergid and polar cells. Archesporial cell showed highest accumulation of proteins. The cell towards the micropyler end contained two fold more content of protein than chalazal one. Among the elements of mature embryosac, antipodal cells registered highest total protein content. Next comes egg cell and polar cell showed least amount as evidenced by our cytophotometric estimations.
Fig. 19. The changes in DNA, histone and NHCP extinction values during stages of embryosac development in *Limnothyton*. In the mature embryosac antipodal nucleus showed highest extinction values. The extinction values for histone and NHCP in antipodal nucleus are equal to the extinction values of the integumentary nucleus. Egg and polar nuclei showed least extinction for Feulgen DNA. The details of the eleven stages are as follows.

1. archesporial cell
2. megaspore mother cell
3. degenerating nucleus
4. micropylar nucleus
5. chalazal nucleus
6. embryosac at four nucleate condition
7. egg nucleus
8. synergid nucleus
9. secondary nucleus
10. antipodal nucleus
11. integument nucleus
EXTINCTION VALUES

NHCP 0-05
(F G pH S o )
3-22r
HISTONE
(F G pH 8-0 )
DNA
( FEULGEN )

FIG. 19
The relative changes in protein content during megasporogenesis and in the components of embryosac fairly coordinate with the changes in RNA content (Fig. 18).

DNA:

Feulgen reaction was strictly confined to the nucleus only; no cytoplasmic stain was observed. Feulgen cytophotometry showed a gradual fall in DNA extinction values from the archesporial nucleus to 4 nucleate embryosac. During megasporogenesis, two spores, micropylar and chalazal did not show any difference in their extinction values for DNA (Fig. 19). The elements of the mature embryosac differed from each other by the changes in DNA extinction values. Egg cell showed lowest Feulgen extinction, polar nucleus also showed similar value, synergid nuclei recorded two fold more extinction value than the egg nucleus. Furthermore, antipodal nuclei showed many fold higher extinction than egg nucleus. Integument nuclei showed the 2C DNA value for its absorbance (Fig. 19). Feulgen cytophotometry showed highest content of DNA in MMC. After meiotic divisions, there is a half reduction in its nuclear DNA content. Micropylar nucleus showed more content than chalzal nucleus, though the extinction values are same (Fig. 20). Among all the components of the embryosac, egg cell showed highest DNA content. Antipodal nuclei showed very dense chromatin and contained relatively higher content of DNA.
than polar and synergid nuclei.

Feulgen reaction in the egg cell showed wide variations with the maturation of the embryosac. In the early stages of embryosac, the egg nucleus is spherical and it is in the center of the egg cell. At this condition, feulgen reaction is intensive and homogenous in distribution. With the maturation of the egg cell, nuclear volume increases and attains an elliptical shape and it was pushed towards the peripheral side of the egg cell (Fig. 35A). The staining pattern changes to a heterogenous distribution and it was granular in appearance. Feulgen positivity is relatively more around the nucleolus and towards the nuclear membrane. These qualitative changes in feulgen reaction augur the readiness of the egg cell to participate in the act of fertilization. Thus with the increase of nuclear volume there is a decrease in the feulgen extinction values. To find the age related changes in the DNA content of the egg nucleus, a survey of 50 egg nuclei of different maturity was performed and the results thus obtained are depicted in the form of histograms, which show the frequency distribution of different parameters viz. extinction value, nuclear volume and DNA content (Fig. 21). The extinction values of egg nuclei showed wide deviations ranging from 0.001 to 0.67. The distribution of these values is highly heterogenous and did not follow any
symmetric pattern. Highest percent of frequency (39\%) ranges between 0.027 to 0.031. The frequency of second order (36\%) fluctuated between 0.001 to 0.015. The remaining values are distributed below 4\% under different class intervals (Fig. 21). The changes in nuclear volume of egg cell also showed heterogeneous distribution like that of its extinction values. The lowest nuclear volume of egg cell is 734\(\mu^3\) and highest value is 3334\(\mu^3\) which is 4\(\times\) times higher value than the least nuclear volume. 44\% of egg nuclei showed highest frequency fluctuating between 2534 to 2734\(\mu^3\). Next order (20\%) comes to the class interval 1934-2134\(\mu^3\). The third parameter DNA content (Ex. value \times Nuclear volume) of egg nucleus also followed the distribution pattern of the extinction and nuclear volume. In our study of 50 egg nuclei, the DNA content ranged between 28 to 69\(\text{AU}\). The class interval 34 to 37 showed highest frequency (32\%) of DNA content and 24\% of nuclei fall between 43-46 nuclear DNA content. There is a big gap of distribution in the DNA content between 49 to 60\(\text{AU}\). No DNA content of egg nucleus falls under this range. Thus the values for feulgen extinction, nuclear volume and DNA content of egg cell are highly heterogeneous in distribution.

Histones:

The localization pattern of histones with alkaline
Fast green (pH 8.0) were found similar to the Feulgen reaction. Cytophotometric analysis showed no wide variation in extinction values between MMC to 4 nucleate condition. The extinction values fluctuated between a narrow range of 0.03 to 0.06. Like Feulgen extinction values, there is no difference between the micropylar and chalazal nuclei, the same extinction value is maintained even up to 4 nucleate condition. Different components of mature embryo sac showed differential extinction values for histones. Antipodal cell again recorded the highest extinction values (Fig. 19) for histones too. There was no difference in the extinction values between egg and synergid nucleus. Polar nucleus showed the lowest extinction value. Cytophotometric study of the changes in histone content per nucleus strictly followed the trend of DNA content. When there is an increase in histone content there is a corresponding increase in DNA content too (Fig. 20). Egg nucleus showed highest content of histones. There is no much difference in the nuclear content among the components of embryo sac other than egg nucleus.

Non-histone chromosomal proteins (NHCP):

The stain intensity for NHCP is higher than the histone stain, indicating higher content of NHCP than histone. The extinction values of NHCP of a stage is always found higher than the histone values. The extinction values
Fig. 21. Histograms represent the frequency distribution of the percent of Feulgen extinction, DNA content and nuclear volume of egg nucleus in Limnophyton. The data represents a study on fifty egg nuclei at different periods of maturity. These three parameters are highly heterogeneous in distribution. But the distribution pattern of nuclear volume somewhat follows the frequency distribution of the DNA content/nucleus.
Fig. 21

- Extinction $(10^{-2})$
- Nuclear Volume
- DNA/Nucleus in A.U.
of histones during megasporogenesis and gametogenesis varied between 0.03 to 0.17, whereas the extinction values for NHCP for the similar stages of development fluctuated between 0.04 to 0.21. The changes in extinction values of NHCP during megasporogenesis and gametogenesis showed a parallel trend with that of Feulgen extinction values, showing a gradual decline from archesporial nucleus to 4 nucleate embryo sac (Fig. 19). In the mature embryo sac antipodal nuclei showed highest extinction which is equal to the nuclei of integuments. Polar nucleus showed least extinction value. Cytophotometric estimations revealed the highest content of NHC proteins in egg nucleus. Egg nucleus contains 86% NHC proteins, remaining percent is attributed to histones (Fig. 16,B). The relative changes in NHC proteins run parallel with the changes in DNA during stages of embryo sac development (Fig. 20). The changes in NHCP during megaspore and gametogenesis are always maintained higher percent than histone content. During megasporogenesis only two stages of nuclei (degenerating and micropylar) showed identical percent ratios of NHCP and histone contents. In all stages of development more than 50% of total nuclear protein content is occupied by NHC proteins. Synergid nuclei showed 64% and antipodal nuclei contained 56% of NHCP and remaining part is histone (Fig. 16,B).
Nuclear and Nucleolar growth:

The differentiation of archesporial cell into megaspore mother cell is followed by an enormous increase in nuclear volume (323 µ³ to 768 µ³) of the former (Fig. 14, C). The second meiotic division during measporogenesis resulted into two nuclei of differential volume. Micropylar nucleus attained highest nuclear volume (864 µ³). In the mature embryosac egg nucleus showed highest nuclear volume among other components of embryosac. Antipodal cells contain nuclei of smallest size with dense chromatin. Measurements of nuclear and nucleolar volume changes displayed the lack of coordination between these two parameters during sporogenesis and gametogenesis, thus the growth of nucleus and nucleolus are independent from one another. During transition of archesporial cell into MMC there is no marked changes in nucleolar volume. After second meiotic division there is a drop in the nucleolar volume (Fig. 14, D). In the female gametophyte secondary nucleus contained a nucleolus having highest volume (514 µ³). The nucleoli of egg and synergid showed five times lower volume than the nucleolar volume of secondary nucleus.
IV    EMBRYO

The first division of the fertilized egg is transverse and asymmetric leaving a bigger basal cell and a relatively small terminal cell (Figs. 32,A and 33,C). The basal cell does not undergo further divisions but enlarges enormously (Figs. 32,D and 33,D). The maximum sectional surface area of the suspensor cell observed was 8581 μ². The terminal cell forms an embryo, which passes through globular, elongated and horse shoe shaped (mature embryo) stages. The seed contains an incumbent embryo with a large cotyledon. The cotyledon is terminal having only a small aperture, where edges of the sheath overlap a plumule (Fig. 33,F). A procambial trace links cotyledon to radicle, while a branch of this from below the level of the apex supplies the leaf primordia. The embryonal shoot apex contains a single layer tunica surrounding a mound of corpus region (Fig. 33,F).

Endosperm is free nuclear during the early stages of embryo and becomes cellular from peripheral side of the embryo sac, but does not extend to the centre. Developing embryo is surrounded by nuclear endosperm. The nuclei of endosperm are heterogenous in distribution exhibiting a high degree of polyploidy.

The nuclei showed no much significant differences in the extinction values for different metabolites but the total dye content showed a wide deviation because of large
differences in nuclear volume. Thus the endosperm exhibits a heterogenous distribution for different metabolites. The embryo development was analysed for the following seven stages.

1. zygote, 2. proembryo, 3. 16 celled, 4. 32 celled, 5. globular, 6. elongated, 7. mature (horse shoe shaped embryo).

During these stages of embryo development the changes of metabolites were studied in embryo, endosperm and suspensor cells. Cytophotometric data was collected in endosperm and suspensor only after proembryo development.

RNA:

After fertilization, RNA extinction values decreased in the dividing cells of the embryo upto 16-celled development. At a stage before globular condition the extinction values for RNA reached its peak value followed by decline during further stages (Fig. 22,E). The suspensor cell of the proembryo showed higher extinction values than the cells of the embryo. But, during later stages RNA extinction values in the suspensor cell stood at lower levels than embryonal cells. After proembryo stage the changes in the extinction values for RNA in the suspensor cell increased with the advancement of embryo growth and at elongated condition, suspensor cell showed a highest extinction value for RNA.
Fig. 22. Cytophotometric analysis of the relative changes in extinction and content of total protein, Si protein and RNA during stages of embryo development in Limnophyton. The autolysing endosperm and suspensor showed declining trend in extinction and content for total protein, Si protein and RNA after globular stage of embryo development. There is no significant changes in RNA content throughout all stages of embryo development, but suspensor cell showed manifold higher content than embryonal cells (F). Similar is the case with total protein (D) and Si protein too (D). The stages of development of embryo are as follows.

1. zygote
2. proembryo
3. 16 celled embryo
4. 32 celled embryo
5. globular embryo
6. elongated embryo
FIG. 22
Among all the tissues in the embryosac during embryo development, the cells of endosperm showed strong pyroninophilia. Throughout all stages of embryo growth endosperm cells showed higher extinction for RNA than the cells of embryo and suspensor cell (Fig. 22,E). The endosperm of proembryo recorded two fold higher values than embryonal and suspensor cells. There was a fall in RNA extinction during third and at globular stage. During elongation stage the extinction values for RNA are found little increasing in the endosperm.

Cytophotometric analysis showed no significant changes in the content of RNA per cell in embryonal cells during development (Fig. 22,F). The relative content varies between 20 to 25 AU only. The levels of RNA content per cell in endosperm were found relatively higher than the embryonal cells. During 32 celled stage of embryo development, endosperm recorded highest RNA content per cell. During further stages of embryo growth there was a gradual decline in RNA content in the cells of endosperm (Fig. 22,F). Suspensor cell always accumulated higher RNA than any other tissue in the embryosac. Suspensor cell showed highest synthetic activity for RNA just before globular stage of embryo. During this stage, suspensor cell showed 50 times more RNA content than embryonal cells. After globular stage, there was a gradual diminution in its RNA content.
Total proteins:

Like RNA, the protein extinction values during embryo development also showed a sharp decrease in the early stages of its growth. But, further stages of embryo development is correlated with an increasing trend of extinction values up to globular stage, which records the peak extinction value for proteins (Fig. 22,A). Elongating embryo was marked by a poor extinction value for total protein. Endosperm cells showed little increase in extinction values for total proteins up to globular stage of embryo development. After this stage there was a sudden fall in the extinction values for proteins in all three components. The changes in the extinction values in the suspensor cell with the differentiation of the embryonal cell showed a similar trend of distribution as in endosperm.

Cytophotometric estimation of total proteins content per cell showed no significant changes from proembryo stages up to 4th stage of development. Globular stage showed highest accumulation of total protein content. Afterwards there is a gradual decrease. The cells of endosperm during early stages of embryo showed relatively higher protein content than the cells of the developing embryo (Fig. 22,B). During 32 celled stage the cells of endosperm showed peak synthetic activity for proteins followed by a gradual decline in protein content during further stages. Suspensor
cell also recorded the peak synthetic activity at 32 cell stage of embryo development. The protein content of suspensor cell was manifold higher than any other cell (embryo and endosperm) in the embryosac. There was a little correlation observed \(r = 0.6\) between the relative changes in protein and RNA content during the stages of embryo development. The fluctuations of these two metabolites, (RNA and proteins) in endosperm and suspensor highly coordinate with each other.

**SH protein:**

Cytophotometric analysis showed a little drop in the extinction values for sulphhydryl group proteins during first few divisions of the zygote (Fig. 22,C). Further stages of embryo development showed a steep increase and 32 celled stage of embryo development recorded the highest extinction values and again a drastic fall in the same was observed. Globular stage of embryo development recorded lowest extinction values for sulphhydryl group proteins, again an increasing trend in extinction values during elongation of embryo was observed (Fig. 22,C). Endosperm cells also registered lowest absorbance values at globular stage of embryo, but these values increase during elongative stage of the embryo. Suspensor cell showed highest extinction value during third stage of embryo development. Further stages showed
a gradual decline in extinction values for sulphydryl group containing proteins.

Cytophotometric analysis of sulphydryl group containing protein per cell in the embryonal cells showed a sharp increase during post fertilization period and at the third stage peak accumulation of sulphydryl group containing proteins was observed in the embryonal cells. Further growth of embryo was associated with a lower content of SH proteins and the cells of globular embryo recorded lowest content (Fig. 22,D). With the elongation of the embryo there was a little increase in SH protein content. SH group containing proteins per cell in the endosperm tissue are found increasing in the early stages of embryo development and thereafter, there was a drastic fall in its content. Similar pattern of changes were observed in suspensor cell too. But the SH proteins in the suspensor cell was manifold higher than the embryonal cells and highest content of SH proteins in suspensor cell was observed at 32 celled stage of embryo development (Fig. 22,D).

Feulgen cytophotometry showed a steep increase in extinction values after fertilization. There was a 2.5 fold increase in feulgen extinction at proembryo stage and this increase immediately followed by a sharp decrease at 16 celled stage of development. Further stages of embryo growth
is marked by a gradual loss in the feulgen extinction values (Fig. 23). There were no much changes in the feulgen extinction values in nuclei of endosperm with the advancement of the embryo growth. These values fluctuated only between 0.04 to 0.055. Suspensor nucleus maintained higher values of feulgen extinction than in the nuclei of embryonal cells up to globular stage. Afterwards there was a drastic fall in the extinction values. Relatively, highest DNA content per nucleus was recorded in the nuclei of proembryo. During third stage of embryo development, the nuclear DNA content was reduced to half of its initial value. There was a little stability in the nuclear DNA content during globular stage, but during elongation, there was a gradual decline in DNA content. Though there was no wide variation in the feulgen extinction values in the endosperm nuclei. The DNA content per nucleus showed wide deviations. The nuclear DNA content in the endosperm was found increasing during third stage of embryo growth thereafter the DNA content in the endosperm is depleted. During third and fourth stage of embryo development, the nuclei of endosperm showed 4 times higher DNA content than embryonal cells. Suspensor nucleus showed manifold higher values for DNA than embryonal nuclei throughout all stages of development (Fig. 24). With the development of the embryo the DNA content in the suspensor nucleus is amplified manifold up to globular...
stage. At proembryo stage, the relative DNA content in the suspensor nucleus was 238 AU and at globular stage the DNA content was increased to 2853 AU. After globular stage, the suspensor nucleus showed a drastic fall in its DNA content.

Histone:

There were wide variations in the histone extinction values during embryogeny. The extinction values of embryonic nuclei for histones decline during post fertilization period, but 16 celled embryo registered relatively higher extinction followed by a drop at 32 celled stage of development (Fig. 23). Further periods of embryo growth showed an increasing trend of extinction values for histones. Histone extinction values in the endosperm nuclei decrease with the advancement of the embryo growth. At elongated stage, the endosperm nuclei showed lowest extinction values. Similar trend line was observed in the suspensor nucleus too. But, the extinction values in the suspensor nucleus always maintained manifold higher values than the nuclei of endosperm and embryo (Fig. 23).

Cytophotometric analysis of relative changes in nuclear histone content of embryo during its stages of development showed two active periods of synthesis one at zygotic condition and another at globular stage of development. The intermediate stages showed relatively less histone content. After globular stage, the nuclear histone content
Fig. 23. Cytophotometric analysis of the changes in extinction values of lAA, histone and HICP during embryo development in Limnophyton. During early stages of the division of fertilized egg there is a sudden upsurge in lAA and HICP extinction values followed by an immediate decline, but histone extinction values differ significantly. HICP extinction values are always higher than histone extinction. The stages (1-6) are as follows.

1. zygote
2. proembryo
3. 16 celled embryo
4. 32 celled embryo
5. globular embryo
6. elongated embryo
diminishes with the elongation of the embryo. The nuclei of endosperm contained manifold higher histone content than the embryonal nuclei (Fig. 23). At 16 celled stage, endosperm nuclei showed 3 fold higher content than the nuclei of the embryo. During early stages there was a slow increase in histone content in the suspensor but during globular stage the histone content of the suspensor nucleus was many times and reached peak accumulation. After globular stage, there was a drastic fall in the histone content in the suspensor nucleus.

Nonhistone chromosomal proteins (NHCP):

NHCP maintained higher extinction values than histone in the nuclei of endosperm, suspensor and embryo throughout all stages of embryo development. The changes in the extinction values of NHCP in the embryonal nuclei sharply reflect the changes in feulgen extinction. After fertilization there was a steep increase in the extinction values for NHCP (Fig. 23). Further stages showed a declining trend up to globular stage, but after globular stage, these values increase a little. The extinction values for NHCP in the endosperm nuclei showed wide fluctuation during embryogeny. The NHCP extinction values in endosperm nuclei are lower than the nuclei of embry except at globular stage, where endosperm nuclei showed 2 times higher extinction for NHCP.
Fig. 2. Cytophotometric estimation of the relative changes in DNA, histone and HMCP content per nucleus during stages of embryo development in Limnophyton. Suspensor cell recorded many times higher DNA content than embryonal cells. With the advancement of embryo growth there is a gradual decline in DNA content. Both histone and HMCP showed peak synthetic activity in suspensor cell at the globular stage of embryo development. The stages of embryo development are as follows:

1. zygote
2. proembryo
3. 16 celled embryo
4. 32 celled embryo
5. globular embryo
6. elongated embryo
FIG. 24
than embryonal cells. After globular stage the NHCP extinction in the endosperm nuclei is found very much reduced. After proembryo stage the NHCP extinction values were reduced and during further stages of embryo development the rate of decrease in the extinction values for NHCP in the suspensor cell was passive.

The changes in the NHCP content per nucleus showed a declining trend during early stages of development and again the nuclei started accumulating NHCP and reached a peak synthetic period during globular stage of development. Further growth of embryo marked by relatively low content. Endosperm nuclei showed highest NHCP content at 32 celled and during further stages, NHCP content was found decreasing at a faster rate (Fig. 24). Like other nuclear constituents (histone and DNA) NHCP content also found many times higher in suspensor nucleus than embryonal nuclei and they are found increasing upto globular stage followed by a sudden decline.

Mature embryo (horse shoe shaped):

Mature embryo showed segregated stain intensity which follows the production of specialized cells, tissues and organs. In the horse shoe shaped embryo plumular region is very distinct by strong affinity towards the dyes employed (Fig. 33,F). Thus the differences in the cellular activities of the different regions of the embryo were apparent and recognizable after staining reactions.
RNA:

The cells of the tunica layer of the embryonal shoot apex of mature embryo recorded highest extinction values for RNA. The cells of the corpus and leaf primordia showed relatively lower values than the tunica (Figs. 25 and 33). There were no marked differences in the extinction values between the cells of the hypocotyl and cotyledon, but the cells of the radicle showed little higher values than the cotyledon. Cytophotometric estimations revealed highest RNA content in the cells of tunica and the cells of the radicle come in the second order in accumulating RNA content. Remaining regions of the mature embryo did not show marked differences in RNA content per cell (Fig. 25).

The cells of leaf primordia showed highest extinction values for total proteins. The cells of tunica of embryonal shoot apex and radicle showed lesser extinction than the cells of leaf primordia. The cells of corpus were recognized by the lowest extinction values for proteins. There was no difference in the extinction values between the cells of the cotyledon and hypocotyl (Fig. 25). The cells of tunica registered highest content of protein per cell and the cells of leaf primordia lie in the second order. There were no marked differences in the protein content in other regions of the mature embryo. The differences in the extinction values of SH proteins in different regions of the
mature embryo were not pronounced, but the cells of the corpus showed lowest extinction values for SH proteins.

The content of SH group proteins per cell also showed a similar trend of distribution in different regions of the embryo.

Feulgen cytophotometry showed no differences in the extinction values for DNA in different regions of mature embryo. All regions recorded a similar extinction value (0.0836) (Fig. 25). But there existed a little fluctuation in the estimates of relative DNA content per nucleus. The relative DNA values in all regions of the nuclei fall in two groups. The nuclei of leaf primordia, cotyledon and hypocotyl predominantly showed a common relative unit for DNA value (64 AU). While the nuclei of tunica of shoot apex and radicle contained 51 and 47 AU respectively.

Unlike Feulgen extinction, histone extinction values exhibited variations in the different regions of the mature embryo. The nuclei of tunica, shoot apex, leaf primordia and radicle did not differ in their extinction value (0.0706) for histones, but the other regions of mature embryo have showed relatively low extinction (Fig. 25). The relative values in the nuclear histone content found higher in the nuclei of leaf primordia and hypocotyl than other regions of the mature embryo.
Cytophotometric estimation of DNA, RNA protein, total protein, DNA histone and HNCP in different regions viz. tunica-carpus of plumule, leaf primordia (leaf/embryo), cotyledon (cotyledon) hypocotyl and radicle (radicle) of horse shoe shaped embryo of Limnothyron. The region wise distribution in DNA, RNA protein and total protein showed little disparities in their extinction and content. But the main nuclear constituents DNA, histone and HNCP are not significantly differed in different regions of the embryo.
**FIG. 25**

- **EXTINCTION VALUES**
  - RNA
  - SH-PROTEIN
  - TOTAL PROTEIN

- **CONTENT/CENT**
  - DNA
  - NHCP
  - HISTONE

- **CONTENT/NUCLEUS**
  - Tunica
  - Corpus
  - LEAF PR
  - Cot.
  - HYPOGOT.
  - RAD.

**MATURE EMBRYO**
Cytophotometric analysis showed two fold higher extinction values for NHCP (PG pH 5.0) than histones (PG, pH 8.1), in all regions of the mature embryo. The extinction values for NHCP did not show wide variations among different regions of mature embryo. The values ranged between 0.140 to 0.155 only. But, NHCP content per nucleus showed deviations from region to region in the mature embryo. The nuclei of cotyledon registered highest NHCP content, the hypocotyl nuclei came in the second order and the radical pole contained least NHCP content.

**PAS reaction:**

When the egg gets fertilized, a change in the distribution of PAS positive substances was noticed. After fertilization, the cytoplasmic PAS positive tinge of the egg cell disappeared. The cell wall staining was intensive as evidenced by deep purplish red PAS reaction. During early stages of embryogenesis PAS reaction was mostly associated with cell walls and the nuclei (Fig. 29,E). During further stages cytoplasm showed the diffused PAS positive reaction. There was no sign of storage products like starch in the cytoplasm upto globular stage of development. After reaching its mature condition the cells of the embryo started accumulating the starch granules in the cytoplasm and when the seed is completely filled with embryo,
the cells of cotyledon are fully loaded with starch granules. At this stage PAS reaction was confined to cytoplasm only and it was in granular form indicating starch. These grains when tested with potassium iodide confirmed as starch. These granules are perinuclear leaving a big space in the cytoplasm. In the mature embryo, the cells of the cotyledon region are relatively most abundant with starch granules. Plumular region is completely devoid of starch even in dormant condition. The big suspensor cell showed a characteristic PAS positive reaction. There was a PAS positive network in the suspensor cell (Fig. 29, E and F). This network did not show positive reaction with any other dye employed. There also appeared a little amorphous PAS positive reaction in the cytoplasm of suspensor cell. Endosperm showed a very distinct PAS positive reaction throughout all stages of embryo development (Fig. 29, F). PAS reaction was associated with cell wall and with the nucleus. The staining intensity progressively decreased from chalazal to micropylar end. Starch granules are sparsely distributed in the cytoplasm of the endosperm. The nuclei also showed PAS positive reaction. Upto globular stage the intensity of PAS reaction in the endosperm found increased, but during later stages of differentiation the presence of PAS positive reaction found impoverished in the endosperm.