HISTOCHEMICAL CHANGES DURING THE DEVELOPMENT STAGES OF *CHARA VULGARIS* LINN

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HISTOCHEMISTRY of developmental stages of certain flowering plants has been reported[1–4]. Studies on plants where apical cell continues meristematic activity and culminating in differentiation of reproductive organs without reduction division are scanty. *Chara vulgaris* L is one such plant and its thallus organization[5] cytology of apical cell[6] and differentiation of antheridial cells into sperms[7] have been studied. The present study relates to the histochemical analysis of developmental stages in *Chara*.

The material was collected from the Botanical Garden of Gujarat University, Ahmedabad. The apical region, globule and nucule were fixed at their different stages of development in FAA/Carnoy's fluid for 1 hr and transferred to 70% ethanol. Dehydration and infiltration were done using TBA series. The paraffin mounted material was sectioned at 12 μm with Leitz Wetzlar minot microtome. Standard staining schedule was followed to localise DNA[1], RNA[8], total proteins[9] and -SH proteins[3] in different regions of the thallus. Control tests were performed using specific enzymes. The absorbance of monochromatic light by the chromophore was recorded with cytophotometer[10] and absorption values were recorded using narrow band filters[11], ranging in wavelength from 500–690 μm. Cytophotometric data were expressed in three parameters, *viz* extinction value (log blank – log stain of transmitted light), content (extinction value × sectional surface area of cell or nucleus) and concentration per unit area (extinction value/area of cell or nucleus), in terms of arbitrary units.

The present observation showed the highest absorbance for all the metabolites studied in the apical cell. The high content of DNA (1.88), RNA (18.09), total protein (8.3) and -SH proteins (20.22) help the apical cell to maintain its meristematic activity. This agrees with the studies of Barlow[12], especially with regard to the relationship between DNA content and meristematic activity in cells. The highest content of -SH proteins (47.8) in the internode facilitates cytoplasmic streaming and ion transport[4, 13].

During globule development (figure 1), extinction values for total proteins are positively correlated with that of DNA and RNA, except manumbrium, where the extinction values for -SH proteins diminished to the minimal level, from 0.03 to 0.01.

Higher concentration of DNA during globule development was observed in primary capitulum (0.6) and antheridial filaments (0.8). RNA concentration showed differential distribution ranging from 0.02 to 0.44 and total proteins indicated three peaks (table 1). -SH proteins showed high concentration of 0.69 in

Table 1 *Extinction value, content and concentration of DNA, RNA, total proteins and -SH protein at different thallus regions of Chara*

	Extinction value				Content				Concentration			
Thallus regions	DNA	RNA	Total proteins	-SH proteins	DNA	RNA	Total proteins	-SH proteins	DNA	RNA	Total proteins	-SH proteins
Apical cell	0.03	0.04	0.05	0.06	1.88	18.09	8.30	20.22	0.48	0.09	0.25	0.19
Nodal initial	0.02	0.05	0.03	0.05	1.11	9.32	10.50	9.45	0.20	0.18	0.09	0.22
Internodal initial	0.02	0.02	0.02	0.03	0.92	5.18	6.34	7.13	0.24	0.08	0.03	0.13
Node	0.02	0.07	0.02	0.04	0.96	54.68	4.46	10.23	0.47	0.10	0.11	0.14
Internode	0.02	0.07	0.02	0.05	0.83	66.54	3.95	47.80	0.26	0.05	0.06	0.44
Antheridial initial	0.03	0.04	0.03	0.07	1.88	11.32	3.78	11.25	0.48	0.18	0.38	0.44
Primary capitulum	0.03	0.04	0.02	0.05	1.50	7.25	2.23	5 15	0.60	0 24	0.22	0.41
Secondary capitulum	0.03	0.04	0.02	0.04	1.50	6.60	1.95	2.36	0 60	0.26	0.26	0 61
Antheridial filament	0.03	0.04	0 03	0.03	1.13	3 88	3 00	1.31	0 80	0 44	0 30	0 69
Oogonium	0.04	0 04	0.05	0.06	1.72	21.02	8 30	16 33	0 68	0 01	0.09	0 03
Tube cell	0.02	0.04	0.02	0 03	1.53	7.38	7.26	13 69	0.32	0 23	0 03	0 06
Corona	0 04	0 04	0 02	0.04	3.72	10 61	4 41	8.30	0 56	0 16	0 05	0 25

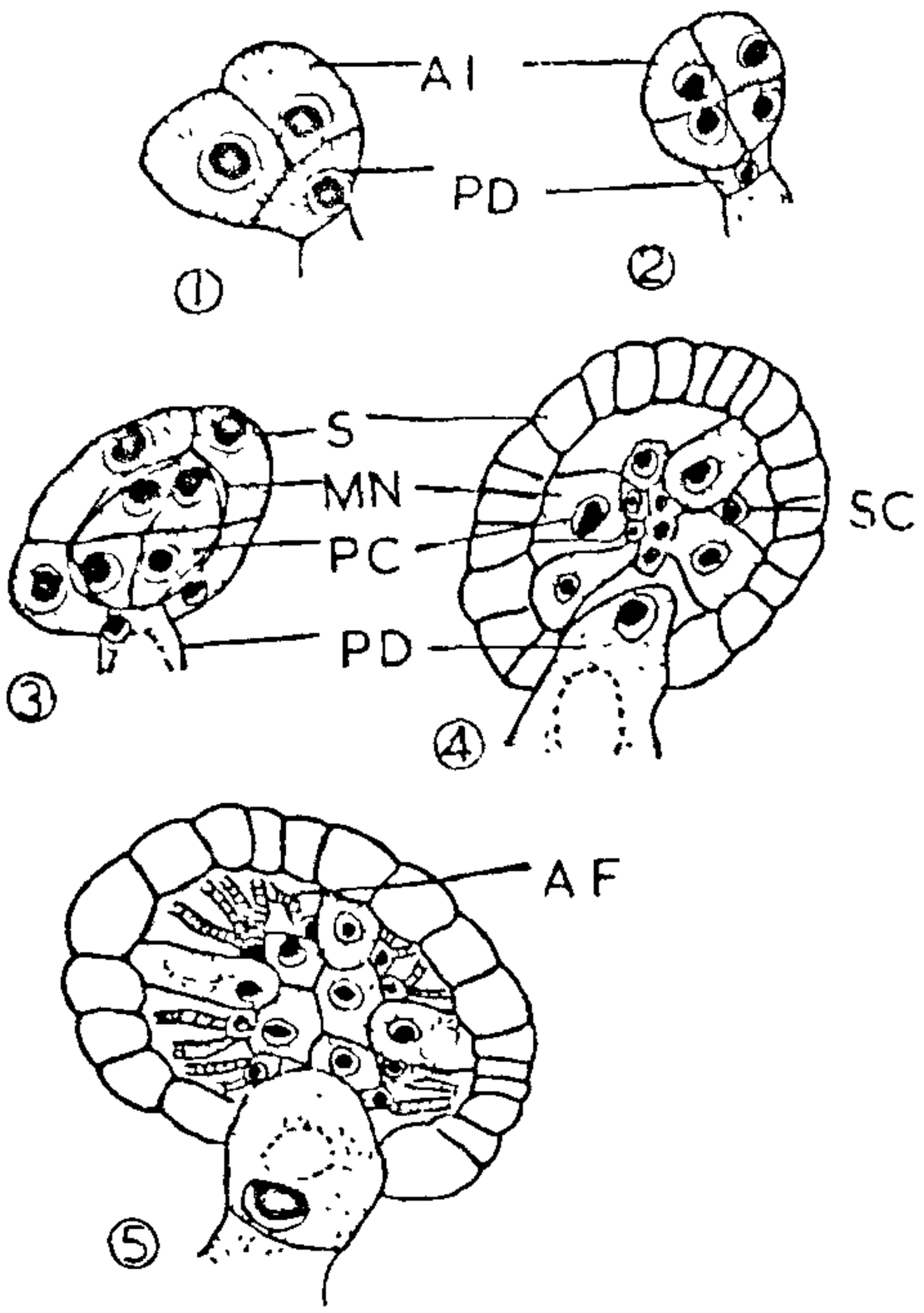

Figure 1. 1–5 stages of globule development. AI-Antheridial initial, PD-Pedicel, S-Shield cell, MN-Manumbrium, PC-Primary capitulum, SC-Secondary Capitulum, AF-Antheridial filament.

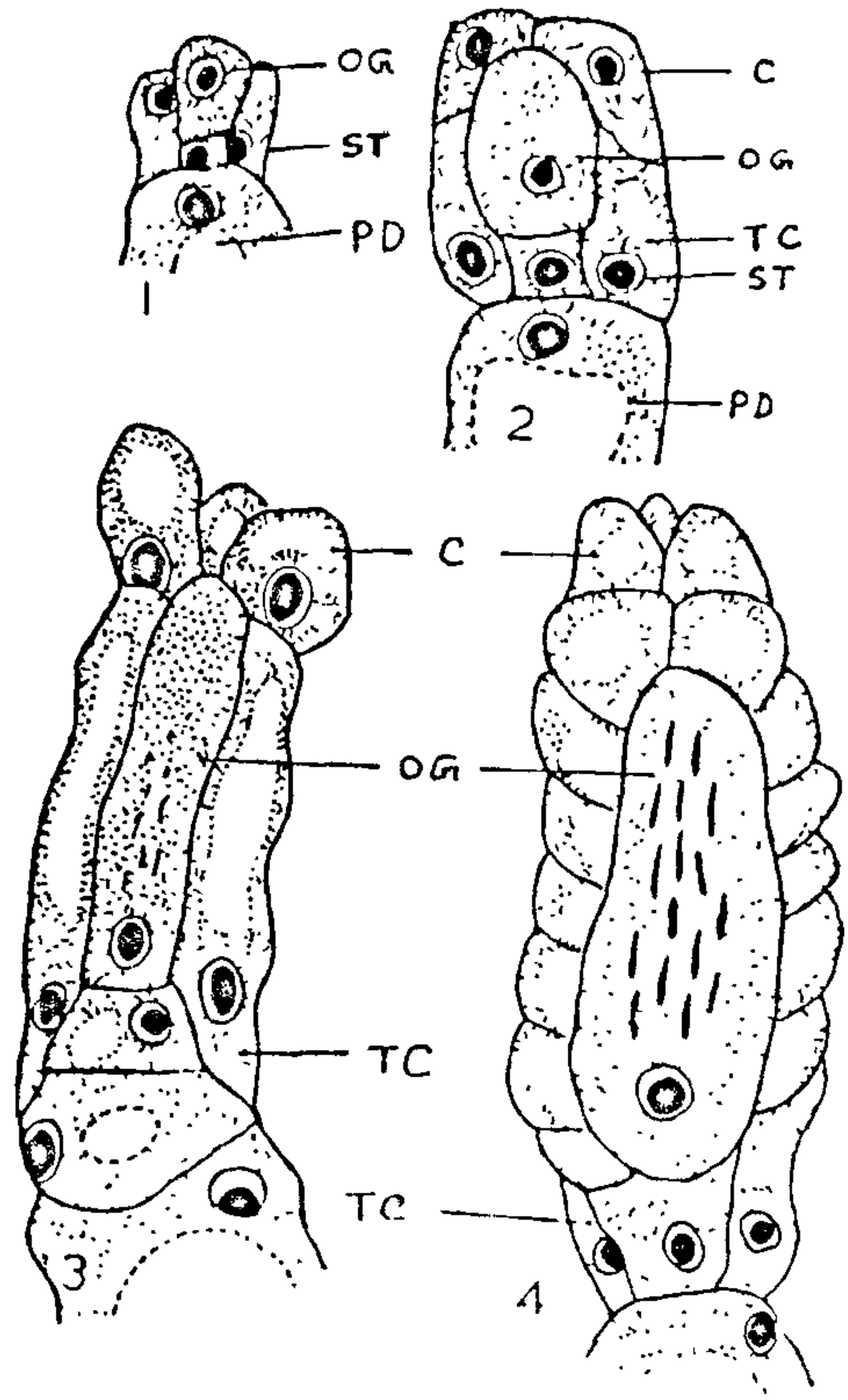

Figure 2. 1–4 stages of nucule development OG-Oogonium, ST-Stalk cell, PD-Pedicel, TC-Tube cell, C-Corona.

antheridial initial, primary and secondary capitula. The cytophotometric analysis, which indicated constancy of metabolites in the thallus cells, showed no meiosis at gamete formation in *Chara* as evident from sustained staining intensity, instead the gametes seem to have formed by direct transformation of the cell content. This corroborates earlier observations[14].

In the case of nucule, just before full maturation, synthesis of DNA and RNA was in active state. In the matured nucule, the concentration of RNA was very low (0.01), but total proteins showed a higher value (0.04), -SH proteins did not show much change. This finds support from the increase in size of the nucule at maturity (figure 2).

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POLYMORPHIC MULTI-MICROCYTES IN *JASMINUM PUBESCENS* WILLD.

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JASMINUM PUBESCENS Willd is a well-known ornamental plant for its fragrant flowers and it is multiplied only by vegetative propagation. One of the major problems in the life cycle of this ornamental plant is the failure of seed-set. This plant species is indigenous to the temperate and paleo-tropical regions and has also been reported to be male sterile[1], although exhibiting normal meiosis. However, during the course of studies on the cytogenetic behaviour, several interesting cytological irregularities were observed during meiosis I and II.

The chromosomes appeared sticky and in an appreciable number of cells, the metaphase commenced with irregularities. The chromosomes in these plates exhibited a disturbed behaviour; a few chromosomes arranged themselves in the vicinity of the equatorial plate. Further, during the chromosome separation to the poles, the amount of movement and the manner of chromosome passing to each pole varied.

Abnormalities were also observed in metaphase II; however, the grouping of the chromosomes at the equator was largely anomalous and they remained scattered all along the spindle. The multi-polar separation of the chromosomes in different phases of meiosis ultimately resulted in an increase in the number of nuclei at telophase II. In addition to the chromosomal aberrations, abnormal cytokinesis was fairly frequent, resulting ultimately in the formation of polymorphic multi-microcytes (figure 1). These microcytes formed were of varied size and ranged in number from 4 to 11 per pollen mother cell.

Figure 1. Polymorphic multi-microcytes in *Jasminum pubescens*.

Moreover, a few microcytes were found to be of special interest in their being vacuolated with scanty cytoplasm, indicating under-development and ultimately leading to a large scale degeneration. The tapetal layer also remained massive throughout meiosis and persistant even during the maturation of the microspores. It disintegrated completely only prior to anthesis. The failure of the tapetal cells to degenerate at the proper stage, therefore, seems to be one of the causes for non-production of the viable microcytes, as reported earlier in *Lilium usitatissimum*[2].

All these factors, *viz* chromosomal aberrations and retention of tapetum, made the genetic complement imbalanced in the microsporocyte which resulted in the expression of polymorphic non-viable pollen grains.

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