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EMBRYOLOGICAL STUDIES IN THE FAMILY RUTACEAE I. A HISTOCHEMICAL STUDY OF EMBRYO DEVELOPMENT IN *TODDALIA ASIATICA* LAMK.

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This investigation is undertaken to determine qualitatively the changes in concentration and distribution of polysaccharides, nucleic acid and proteins in the developing embryos of *Toddalia asiatica* and to understand their role in the growth and differentiation of embryo.

Keywords : *Toddalia asiatica*; Polysaccharides; Proteins, Nucleic acid.

Introduction

Plant embryos are complex and dynamic system and an integrated approach involving the mutual interest between developmental anatomy and histochemistry will unearth the complexity and dynamism of this system. A knowledge of chemical composition of the embryo at different stages of development and distribution of various compounds in its cells and tissues are often useful to interpret the relationship in cell specialization and embryonic growth. Histochemical data accounting for the ontogenetic differentiation of embryonal organs are limited. The present work is an attempt to study the distribution of these metabolites at different stages of embryo development.

Material and Methods

The seed at successive stages of development were fixed in Carnoy's fluid. Following conventional methods, paraffin blocks were prepared and sectioned at 8–10 micron thickness. Proteins and nucleic acids were tested with mercuric Bromophenol Blue (Mazia *et al.*, 1953) and Azure B (Jensen, 1962) methods, respectively. Periodic Acid Schiff's (PAS) procedure was used to localise insoluble polysaccharides (Jensen, 1962). Necessary control tests were also conducted. The assessment is mainly qualitative.

Observations

The embryogeny conforms to the Soland type of Johansen's sytem.

Polysaccharides— The wall of the zygote is PAS +ve and the cytoplasmic polysaccharides are confined to the area around the nucleus (Fig. 1.) In the early stages of the proembryo the cells derived from *ca* and the cell *cb* maintain the same intensity in the cytoplasm. In the early globular proembryo cytoplasmic polysaccharide is reduced in the suspensor cells. Minute polysaccharide granules make their appearance at the late globular stage of the embryo in the region of suspensor. At the heart-shaped stage, the grains appear to be distributed in the root apex and in the cortical cells. The procambium is rich in cytoplasmic polysaccharide when compared with other regions. In the mature embryo, these grains are distributed in the cortical cells of protoderm (Fig. 6). However the cotyledons and shoot apex are devoid of these grains (Fig. 7).

Nucleic acid—The zygote exhibits a strong reaction for nuclear DNA and cytoplasmic RNA. In the 4-celled proembryo cytoplasmic RNA is more in the embryonal cells than in the suspensor cells. In the early globular proembryo the amount of cytoplamic RNA gets reduced in the suspensor cells whereas the embryo maintains rich quantity of cytoplasmic RNA and nuclear DNA (Fig. 3). When the embryo attains heartshaped stage nucleic acids are concentrated more at the tips of cotyledons and in the procambial strands (Fig. 4). Cytoplasmic RNA gradually gets reduced at the late heartshaped stage in the suspensor cells. In the mature embryo the apices of root, shoot and procambial cells show more cytoplasmic RNA. The cotyledons are feebly stained. There is a decline in the level of cytoplasmic RNA at the later stages of embryo development.

Proteins—The zygote stains feebly for cytoplasmic protein which is concentrated mainly around the nucleus. Nuclear protein is slightly more in the zygote. In the 3-celled proembryo the derivatives of terminal cell *ca* stain intensely for cytoplasmic protein than basal suspensor cells (Fig. 2). The amount of nuclear protein also increases in the embryonal cells. In the young globular embryo protein content is low in suspensor cells and high in the embryo proper. At the late globular stage there is a slight decrease in the level of cytoplasmic protein and with the initiation of cotyledons, tip of the cotyledons and procambial cells show rich quantity of cytoplasmic protein. However, the suspensor cells retain the same quantity of cytoplasmic protein. There is gradual decline in the level of cytoplasmic protein at the late heartshaped stage except in the procambial strands (Fig. 5).

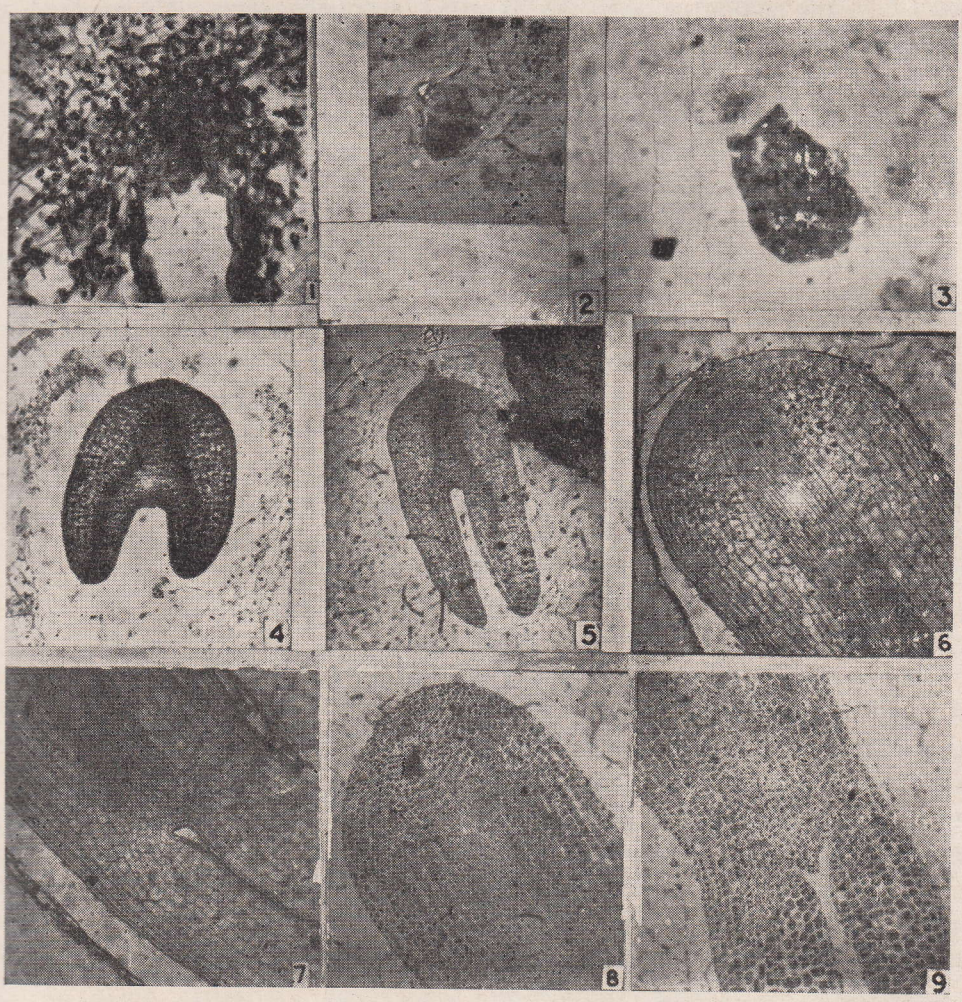

Fig. 1–9.
Fig. 1. Zygote with rich cytoplasmic polysaccharide at the proximal end x 75; Fig. 2. 3-Celled proembryo showing high concentration of cytoplasmic protein in the embryonal cells x 67; Fig. 3 Globular embryo (showing rich nucleic acids in the embryonal cells) x 65; Fig. 4. Heart shaped embryo, tips of the cotyledons and procambium rich in nucleic acids x 35; Fig. 5. Embryo at a later stage—procambial cells showing rich level of proteins x 30; Fig. 6. Mature embryo—Root apex—PAS +ve grains in the cortical cells and protoderm x 35; Fig. 7. Mature embryo shoot apex and cotyledons devoid of PAS +ve grains x 30; Fig. 8 & 9. Root and shoot apex showing low level of cytoplasmic protein in mature embryo, respective x 35, x 40.

In the mature embryo the level of cytoplasmic protein in the root and shoot apices and in the procambial cells is reduced (Fig. 8, 9) whereas, nuclear protein is more in the apices of root and shoot. Protein bodies make their appearance in the mature embryo They are spherical and distributed more in the cotyledonary cells and in the ground tissue of the hypocotyl. The root and shoot apices are devoid of protein bodies.

Discussion

The distribution of polysaccharides, protein and RNA around zygotic nucleus is suggestive of the zone of metabolic activity. Such a polarity in distribution of metabolites is maintained in the subsequent stages of embryo development. Similar demarcation has also been reported in *Capsella bursa-pastories* (Schulz and Jensen, 1968 a, b) and *Panicum miliaceum* (Rudramuniappa and Panchaksharappa, 1979). From the quadrant stage of the proembryo and onwards, the histochemical demarcation is noticed in the terminal region contributing to embryo proper where there is high concentration of the metabolites. The staining intensity reaches the peak in the globular embryo and then gradually declines. The synthesis of these substances in the embryonal region clearly indicates their importance in growth and differentiation of embryonic tissue. The basal suspensor shows low levels of these metabolites. This appears to be the case with most of the angiosperm embryos (Pritchard, 1964; Schulz and Jensen, 1968b). In contrast the PAS +ve grains are localised in this area during the early stages which appear to play an important role in translocating and providing nutrition to the developing embryo. Protein bodies are observed in the cotyledonary cells. Such bodies are observed in in the cotyledonary cells of *Arachis hypogea* (Periaswamy and Sampoornam, 1980) and *Linum usitalissimum* (Vijayaraghavan *et al.*, 1981).

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References

Jensen W A 1962, Botanical Histochemistry. Freeman & Co. San Franscisco

Mazia D, Brewer P A and Alfert M 1953, *Biol. Bull.* **104** 57

Periaswamy K & Sampoornam 1980, *In Histochemistry Developmental and Structural Anatomy of Angiosperm* A symposium K. Periaswamy (ed), P & B Publications Trichy.

Pritchard H N 1964, *Amer. J Boi.* **51** 472

Rudramuniappa C K and Panchaksharappa M G 1979, *Beitr Biol Pflanzen* **54** 165

Schulz R and Jensen W A 1968a, *Amer. J Bot.* **55** 807

Schulz R and Jensen W A 1968b, *J. Ultrast. Res.* **22** 376

Vijayaraghavan M R, Bhat V and Prabhakar K, 1981, All India Symposium on Normal and Pathological Development & Structure of Economic plants, Gujarat, India p. 41-42.