A Cytochemical Study of Anther Development in *Iphigenia pallida* Bak.

M. G. Panchaksharappa¹ and J. Syamasendar

Regional Research Station, Dharwar-580005, India

Received July 15, 1972

In the past few decades, the morphological aspects of microsporogenesis and development of male gametophyte have been studied in detail in many angiosperms. In the recent past, after realizing the importance of microsporogenesis and the development of the male gametophyte, the techniques such as anther culture, autoradiography, quantitative cytochemistry and microdensitometry have been wisely employed by various researchers to reveal some of the important biochemical and physiological happenings that accompany such developmental stages. The present qualitative cytochemical investigation of insoluble polysaccharides, deoxyribose nucleic acid (DNA), ribose nucleic acid (RNA) and proteins in the anther tissues of *Iphigenia pallida* Bak. during microsporogenesis was taken up with an aim to understand the probable role of these macromolecular substances during such developmental stages.

Material and methods

Iphigenia pallida Bak. is a common bulbous herb flowering in June and July months of the year. The flower buds of different sizes were collected and fixed in Carnoy's fixative (6 parts of absolute alcohol+3 parts of chloroform+1 part of acetic acid) for 1 to 2 hrs. After washing in 80% alcohol the buds were dehydrated using alcohol-butanol series. Later they were embedded in 56°C paraffin. The serial sections of about one cell thickness were mounted using gelatin adhesive (Jensen 1962).

The standard methods employed for the localization of macromolecular substances are listed in the Table 1.

Observations

The anther in *I. pallida* is tetrasporangiate. Two or three middle layers are persistent between the hypodermal endothecium and the tapetum. The nuclei in the glandular tapetum generally enlarge with the beginning of meiosis. Occasionally, the tapetal cells become binucleate. Successive cytokinesis accompanies the meiosis. The tetrads are isobilateral and the shedding pollen grains are 2-celled.

In the anther primordium, the cytoplasmic polysaccharides (Fig. 1) and proteins (Fig. 3) are at a low level in the sporogenous tissue whereas RNA concentration is at a high level (Fig. 2). The large nucleoli are intensely stained with azure B

¹ Reader in Botany, Karnatak University, Dharwar-580003, India.

(Fig. 2). An increase in the cytoplasmic polysaccharides (Fig. 4) and proteins (Fig. 6) is noticed in the differentiating pollen mother cells (PMCs), but RNA content remains at the same rich level (Fig. 5). Much variation is not observed in the concentration of these macromolecular substances in dyads (Figs. 7, 8, 9), but the differentiating spore tetrads and also the microspores have cytoplasm rich

Table 1.

Sl. no.	Cytochemical substance	Tests (from 'Botanical Histochemistry', Jensen 1962)	Indication
1.	DNA and insoluble polysaccharides	Azure A Schiff's and periodic acid Schiff's (Azure A-PAS) combined method (Himes and Moriber 1956)*	DNA—blue polysaccharides—magenta
2.	Starch	Iodine potassium iodide (I_2KI) method (Johansen 1940)	blue
3.	DNA	Feulgen method (Gomori 1952)	deep magenta
4.	RNA	Azure B method (Flax and Himes 1952)	RNA—purple or deep blue DNA—greenish blue
	Control	Differential extraction of DNA and RNA—perchloric acid method (Erickson, Sax and Ogur 1949) and testing with azure B method.	
5.	Proteins	Mercuric bromophenol blue test (Mazia, Brewer, and Alfert 1953)*	deep blue
6.	Lignin	Azure B test Acid phloroglucinol test (Siegel 1953) Basic fuchsin—Schiff's reagent test	greenish pale pink magenta

* Referred from original.

in polysaccharides (Figs. 10, 13), RNA (Figs. 11, 14) and proteins (Fig. 12). The numerous starch grains appearing in the vegetative cell of the young pollen grain (Fig. 15) disintegrate to form a homogeneous PAS positive cytoplasm (Fig. 16) in the shedding pollen. The nucleus in the generative cell is more Feulgen positive than the one in the vegetative cell (Fig. 17). The cytoplasm of the shedding pollen is rich in RNA (Fig. 18) and proteins (Fig. 19).

With the differentiation of PMCs a thick protein (Fig. 6) and polysaccharide (Fig. 4) positive wall material starts depositing internal to meiocyte wall. This deposition increases in dyads, specially near the poles (Figs. 7, 9) and in tetrads (Figs. 10, 12) and finally disappears prior to the separation of microspores. This deposition does not stain with azure B (Figs. 5, 8, 11).

At PMC stage the cells of the tapetum show rich cytoplasmic RNA (Fig. 5) but medium concentration of polysaccharides (Fig. 6). Polysaccharides and proteins together increase gradually during meiosis and reach a rich level at microspore stage prior to the disintegration of the tapetum. The RNA concentration remains at rich level only, throughout meiosis. The nucleus in each tapetal cell increases in size considerably during meiosis retaining the same intensity for azure A staining (Figs. 4, 7, 10, 13).

The wall layers and connective commence storing starch (Fig. 1) very early in

Figs. 1-9. 1, periodic acid Schiff's (PAS) test for total insoluble polysaccharides. The cytoplasm of sporogenous tissue has low concentration of polysaccharides. The cells of wall layers and connective store starch grains. 2, azure B test for RNA. The sporogenous tissue has rich cytoplasmic and nucleolar RNA. The wall layers and epidermis have less RNA. 3, mercuric bromophenol blue test for proteins. Note the low concentration of proteins in sporogenous tissue and wall layers. 4, azure A-PAS test for DNA and polysaccharides. The cytoplasm of PMCs and tapetal cells shows an increased staining with PAS test. The walls of PMCs are thick and densely PAS positive. Anther wall layers store starch. 5, azure B test. Tapetal cells and PMCs are with rich RNA. RNA has decreased to a very low level in wall layers and epidermis. 6, mercuric bromophenol blue test. The PMCs and tapetal cells show an increase in cytoplasmic proteins. The additional wall layer around PMC is protein positive. 7, azure A-PAS test. Concentration of cytoplasmic polysaccharides in dyads is the same as that of PMCs. Note that the nucleus in tapetal cell has increased in size. 8, azure B test. The dyads and tapetal cells have rich cytoplasmic RNA. 9, mercuric bromophenol blue test. The dyads and tapetal cells show normal cytoplasmic proteins. Note the increase in the nuclear size in tapetal cell. Sn—nucleus in a wall layer cell; Tn—nucleus in a tapetal cell.

the ontogeny of the anther. Such storage starch is observable in these tissues till microspore stage (Fig. 13). Later, concomitant with the differentiation of fibrous thickenings in the endothecium, the wall layers lose the starch grains whereas the

Figs. 10-19. 10, azure A-PAS test. Spore tetrad has rich PAS positive cytoplasm. 11, azure B test. Rich RNA both in tapetum and spore tetrads. 12, mercuric bromophenol blue test. Spore tetrad has rich proteins while tapetum has slightly less. 13, azure A-PAS test. Tapetal cells and microspores have rich PAS positive cytoplasm. 14, azure B test. Spores and tapetal cells with rich RNA. 15, azure A-PAS test. Young 2-celled pollen grains with numerous starch grains. 16, azure A-PAS test. 2-celled pollen at the time of shedding. Cytoplasm is uniformly PAS positive but devoid of starch grains. The generative nucleus is more densely stained with azure A than the vegetative nucleus. 17, Feulgen test for DNA. The generative nucleus is more densely Feulgen positive than the vegetative nucleus. 18, azure B test. Pollen grain is rich in RNA. 19, mercuric bromophenol blue test. Pollen grain has rich proteins.

connective cells still retain them. The rich RNA content in the epidermis and wall layers of young anther primordium decrease rapidly at PMC stage and very low concentration is retained at later stages of anther development. The proteins are at very low level in these tissues during microsporogenesis. The fibrous endothelial thickenings stain green with azure B, pink with acid phloroglucinol and magenta with basic fuchsin-Schiff's reagent even without the pretreatment with periodic acid.

Discussion

The presence of rich cytoplasmic and nucleolar RNA in sporogenous tissue, PMCs, dyads, tetrads and microspores is suggestive of high rate of metabolism which is essential for the further differentiation of the concerned cells.

The deposition of protein and PAS positive layer internal to the original PMC wall at the beginning of meiosis coincides with the differentiation of the tapetum while the disintegration of such deposition at the end of meiosis is also coincidental with the disintegration of tapetum. These facts support the theory that such a deposition acts as a selective molecular filter (Heslop-Harrison 1964) regulating the inflow of nutriment from the highly metabolically active tapetum into the differentiating PMCs.

The appearance of starch grains in the vegetative cell of young 2-celled pollen grain followed by the disintegration of those grains to form dense PAS positive cytoplasm is a special feature. This perhaps is due to the increase in metabolic activity of the pollen as it matures at the time of shedding. Such a dramatic appearance of starch succeeded by its immediate disintegration is also suggestive of the high rate of enzymic activity concerned with these phenomena (it has to be confirmed further). The rich RNA, proteins and polysaccharides present in the shedding pollen grain perhaps act as storage substances to be utilised during further differentiation of male gametophyte and in pollen germination.

The tapetum is unequivocally described as a layer providing the required nutrition to the meiocytes (Maheshwari 1950). The presence of rich polysaccharides, RNA and proteins in tapetum while meiosis is progressing in PMCs substantiates this speculation. The considerable increase in the size of the nucleus with no change in azure A positiveness suggests the increase in DNA quantity which is essential to govern the increased metabolic activities of the glandular tapetal cells. The phenomenal differentiation of tapetum with the beginning of meiosis and its disintegration at the end of meiosis evidently suggest its role as a secretory layer mainly providing the necessary nutriment to the meiocytes.

With the differentiation of fibrous thickenings in endothecium, the starch grains disappear in the wall layers. Perhaps stored starch may have been utilized during such differentiation. The green stainability of fibrous endothelial thickenings with azure B staining suggests the presence of lignin or acid polysaccharides. However, the appearance of pink colour with acid phloroglucinol and magenta with basic fuchsin-Schiff's reagent even without pretreatment with periodic acid clearly indicates the presence of lignin in these thickenings.

The disappearance of RNA and proteins from the wall layers and connective very early in the ontogeny of the anther may be perhaps because of the precocious but complete differentiation of these layers. However, their role as regions of starch storage is evident.

It is an established fact that the synthesis of RNA precedes that of proteins. Thus, in any metabolically active cell or tissue generally the variations in concentrations of RNA and proteins go hand in hand. But no such parallel variations in the concentrations of these macromolecular substances are observed in the tissues of this plant. The concentration of RNA in any specific cell or tissue is always found to be higher than that of proteins except in pollen grains where both of them are equally rich in concentration.

Summary

Microsporogenesis and anther development in *Iphigenia pallida* Bak. were investigated using the cytochemical staining methods for the demonstration of insoluble polysaccharides, RNA, proteins and DNA. The insoluble polysaccharides and proteins are at low level in sporogenous tissue and PMCs. Both of them gradually increase in dyads, tetrads and microspores. Large number of starch grains appearing in the vegetative cell of the young 2-celled pollen grain disintegrate and form densely PAS positive cytoplasm in the shedding pollen grain. A protein and PAS positive layer deposited internally around the PMCs at the beginning of meiosis, disappears prior to the separation of microspores from the tetrads. The cytoplasm of PMCs, dyads, tetrads, microspores and pollen grains is rich in RNA. The nucleus of the generative cell is more Feulgen positive than that of the vegetative cell. The tapetal cells have rich RNA and considerable concentration of proteins as well as polysaccharides. The fibrous endothelial thickenings are lignified.

Acknowledgements

The author (J. S.) is grateful to C. S. I. R. (India) for the financial support.

Literature cited

- Heslop-Harrison, J. 1964. Cell walls, cell membranes and protoplasmic connections during meiosis and pollen development. In "Pollen Physiology and Fertilization". Ed. H. F. Linskens, North Holland Publishing Co. Amsterdam.
- Himes, M. and Moriber, L. 1956. A triple stain for deoxyribonucleic acid, polysaccharides and proteins. Stain Tech. 31: 67-70.
- Jensen, W. A. 1962. Botanical Histochemistry. Principles and Practice. Freeman and Co. London.
- Maheshwari, P. 1950. An Introduction to the Embryology of Angiosperms. McGraw-Hill, New York.
- Mazia, D., Brewer, P. A. and Alfert, M. 1953. A cytochemical staining and measurement of protein with mercuric bromophenol blue. Biol. Bulletin 104: 57-67.
-