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HISTOCHEMICAL LOCALIZATION OF ENZYME ACID PHOSPHATASE IN THE ANTHERS OF MALE-FERTILE, CYTOPLASMIC, GENIC AND INDUCED MALE-STERILE PLANTS

HISTOCHEMICAL localization of the enzyme acid phosphatase during different developmental stages in the anthers of male-fertile (MF), Cytoplasmic (CMS), Genic (GMS) and induced male-sterile (IMS) plants of *Allium cepa, Capsicum annuum, Cucumis melo, Cucurbita maxima, Daura alba, Solanum melongena* and *Triticum aestivum* was undertaken.

Flower buds of the above mentioned plants were fixed in 80% alcohol at 25° C for 12 hours. These were quickly dehydrated, cleared and embedded by usual procedure. Serial microtome sections of 6–15 μ thick were cut and Haupt's adhesive was used for affixing the sections to the slides. Fresh sections of the anthers of some of the above mentioned plants were also cut with a sledge microtome using dry ice. For the localization of enzyme acid phosphatase procedure of Jensen[1] was used as summarised in Table I.

These sections were rinsed in water, placed in 95% ethanol and then in 100% ethanol and finally transferred to pure xylene and mounted in balsam.

Table II shows the distribution pattern of enzyme acid phosphatase in the anthers of MF, CMS, GMS and IMS plants at different developmental stages. Anther development had been divided into six stages. The sporangial cells were initially evident, and the last stage ended near anthesis, after the tapetal protoplasts in MF anthers have degenerated and the anthers were ready to dehisce. The intensity of enzyme reaction has been arbitrarily divided into five parts : low (+), moderate (++), high (+++), higher (++++) and highest (+++++).

A. Sporogenous tissue stage : At this stage (Fig. 1), in the anthers of MF plants, the enzyme reaction was high in the cells of epidermis, tapetum, pro-cambial strand and pollen mother cells (PMCs); moderate in other wall layers and connective parenchyma of the

TABLE I

Method used for the localization of enzyme acid phosphatase

Incubation of sections (fresh or fixed)	pH	Temperature	Time	Dilute yellow ammonium sulphide	Control
Substrate + sodium glycerophosphate (0·6 gm lead nitrate in 500 ml of 0·5 M acetate buffer at pH 4·5, added 50 ml of 0·1 M sod. glycero-phosphate)	5	37° C	4–6 hours	15 min. (1–2 ml of yellow amm. sulphide in a coplin jar of water)	1. Sections placed in the substrate minus sod. glycerophosphate. 2. Section placed directly in yellow ammo sulphide. 3. Heated sections were carried throughout.

TABLE II

Evaluation of enzyme acid phosphatase in the anthers of MF and MS (CMS, GMS and IMS) plants at different stages of development. Enzyme activity has been arbitrarily evaluated into five parts : Low (+), Moderate (++), High (+++), Higher (++++) and Highest (+++++)

Stage of Development	Strain	Epidermis	Endothecium	Middle layers	Tapetum	Sporogenous tissue	PMCs	Microspore tetrad	Microspore	Pollen grains	Connective parenchyma	Vascular tissue
A.	MF	+++	++	++	+++	+++	..	..	..	..	+++	+++
	MS	+	+	+	+	+	..	..	..	..	+	++
B.	MF	++++	++	++	++++	..	++++	..	..	..	+++	++++
	MS	++	+	+	+	..	+	..	..	..	+	++
	MF	++++	+++	+++	+++++	..	..	+++++	..	..	++++	+++++
C.	MS	++	+	+	++	..	..	++	..	..	+	++
D.	MF	++++	++++	+	++++	..	..	..	++++	..	+++	++++
	MS	++	+	+	++	..	..	..	++	..	+	++
E.	MF	+++	++	..	..	..	..	..	..	+++++	++	++++
	MS	+	+	+	+	..	..	..	..	++	+	++
F.	MF	+++	+	..	..	..	..	..	..	+++++	++	++++
	MS	+	+	+	+	..	..	..	..	++	+	++

anthers. On the other hand, in the anthers of CMS, GMS and IMS plants, the concentration of enzyme was low in all the parts of an anther (Fig. 2), except pro-cambial strand of the connective, where it was moderate.

B. Meiosis I and II stage : The concentration of enzyme in various parts of an anther in MF plants increased from the preceding stage (Fig. 3). It was higher in epidermal cells, tapetum, vascular tissue and PMCs, while in other parts it was either moderate or high (Fig. 3). On the contrary, enzyme activity in various parts of the anther in CMS, GMS and IMS plants failed to accelerate from the earlier stage.

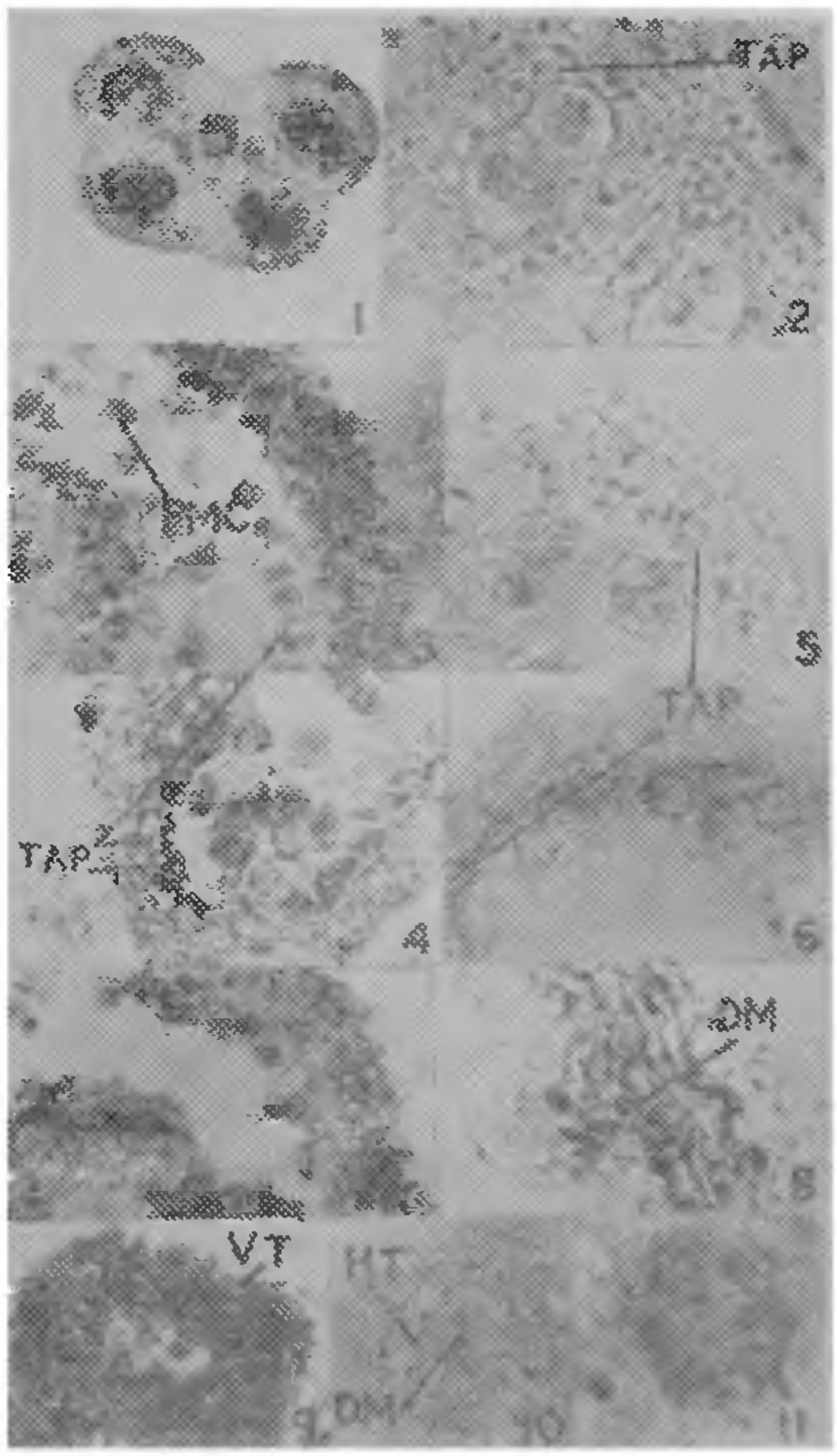
C. Tetrad stage : During the formation of microspore tetrads, the enzymatic activity in MF anthers reached its peak (Fig. 4). It was highest in tapetal cells, vascular tissue; higher in microspore tetrads, epidermal cells and high enzyme activity had been observed in other wall layers and parenchyma cells of anthers connective showed moderate enzyme reaction. In the anthers of CMS, GMS and IMS plants the activity of enzyme even at this stage failed to increase and remained low in almost all the parts (Fig. 5), except tapetum and microspore tetrads. These parts, in a limited number of anthers of IMS plants, showed high enzyme activity.

D. Vacuolate Microspore stage : At this stage, epidermal cells of MF anthers continued to show higher concentration of the enzyme. Endothecial cells exhibited a slight increase from the preceding stage. However, in other parts including tapetal cells, enzyme activity decreased. The microspores in such anthers possessed higher concentration of enzymes. In CMS, GMS and IMS plants, the enzyme activity in various parts of an anther including vascular tissue, tapetum and microspores failed to exhibit any significant change (Fig. 6).

E. Vacuolate Pollen stage : Enzyme activity was maintained in epidermis of the anthers in MF plants, while in other parts enzyme activity decreased. On the other hand, all the parts of an anther including malformed tapetum[2] (Fig. 10) in CMS, GMS and IMS plants continued to show moderate or low enzyme concentration.

F. Engorged Pollen Stage : The activity of the enzyme remained highest in pollen grains (Fig. 7) and vascular tissue (Fig. 9), while rest of the anther showed moderate or low activity in MF plants. The anthers of CMS, GMS and IMS plants showed the same reaction as exhibited in the earlier stage. The degenerated mass of tapetum and pollen grains also exhibited low enzyme activity (Figs. 8, 10). The vascular tissue in such anthers also showed low or moderate enzyme activity (Fig. 11).

The above mentioned account on the localization of enzyme acid phosphatase in the anthers of MF plants indicated that there was a gradual increase in the activity of enzyme until microspore tetrad stage. This was well marked in the cells of epidermal, tapetal, sporogenous and vascular tissues. After this stage, the activity failed to increase in various parts except pollen grains. On the other hand, in the anthers of CMS, GMS and IMS plants, acid phosphatase activity failed to accelerate significantly and remained low.



FIGS. 1–11. Localization of acid phasphata e enzyme in the anthers of MF, CMS, GMS and IMS Plants. Fig. 1 : 45×; Figs. 2–11 : 110×. Fig. 1. *Solanum melongena* (MF) Stage A. Fig. 2. *Cucumis melo* (GMS) Stage A. (TAP : Tapetum). Fig. 3. *Datura alba* (MF) Stage B. (PMC : Pollen mother cell). Fig. 4. *Allium cepa* (MF) Stage C. (MT : Microspore tetrad). Fig. 5. *Capsicum annuum* (CMS) Stage C. Fig. 6. *Datura alba* (IMS) Stage D, showing low activity. Fig. 7. *Capsicum annuum* (MF) Stage E. Fig. 8. *Cucurbita maxima* (GMS) Stage E. (DM : Degenerated mass). Fig. 9. *Triticum aestivum* (MF) Stage E. (VT : Vascular tissue). Fig. 10. *Cucumis melo* (GMS) Stage E. (HT : Hypertrophied tapetum). Fig. 11. *Capsicum annuum* (IMS) Stage E. Vascular tissue.

Higher enzyme acid phosphate activity has been reported in the areas of greater metabolic activity, rapid cellular differentiation, protoplasmic synthesis and vascularization[1-8]. In the opinion of the present author the poor enzyme activity in various parts of an anther including malformed tapetum in CMS, GMS and IMS plants, indicated low metabolic activity in these anthers. This supports a suggestion made earlier regarding the problem of abnormal tapetal behaviour including hypertrophy of these cells which caused pollen degeneration[2,9].

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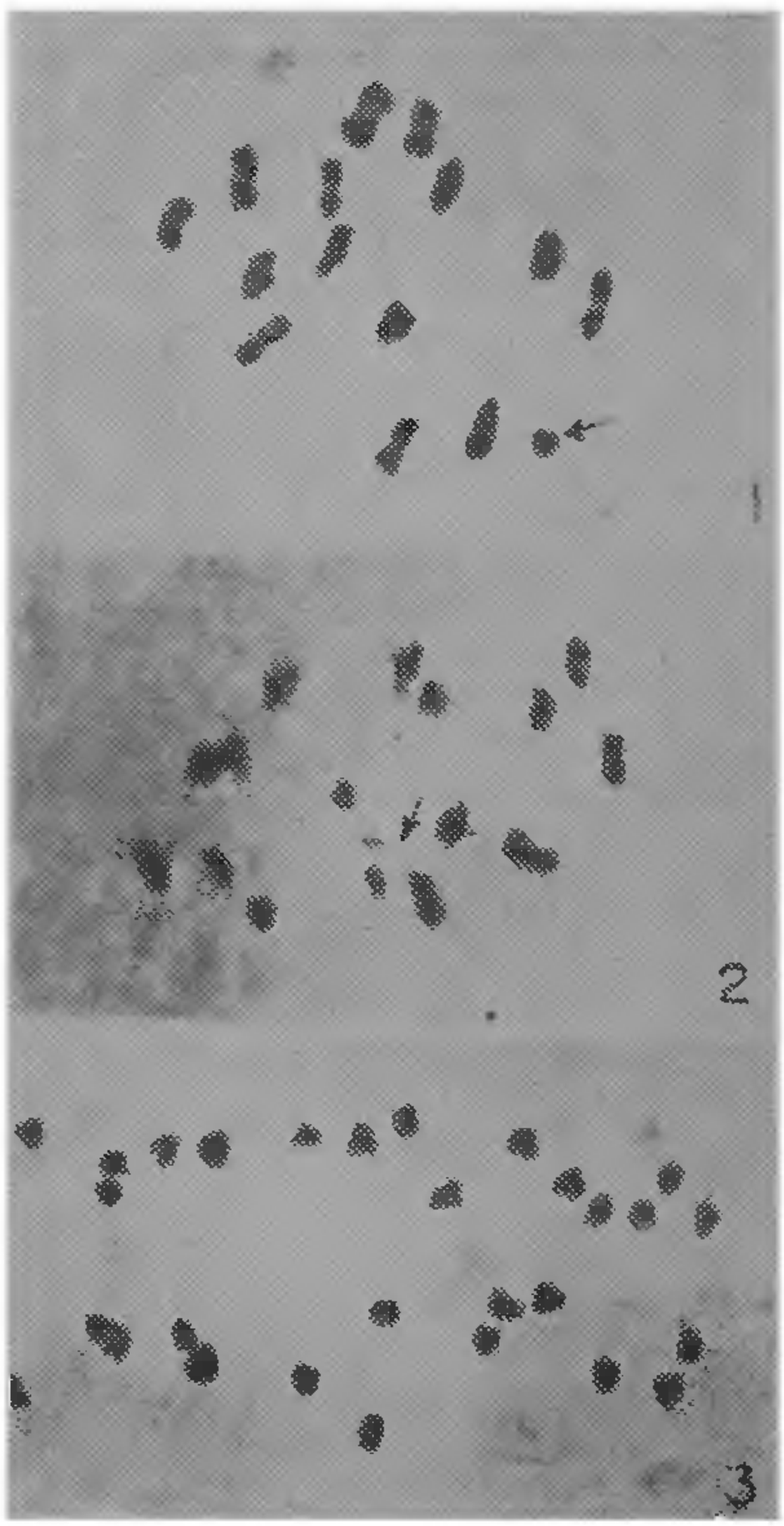
AITRISOMIC *NYMPHAEA* HYBRID

THE genus *Nymphaea* has been subjected to extensive hybridization since about 1850, primarily for the development of new ornamental cultivars. Cultivar 'Director G. T. Moore' is one such hybrid raised at Missouri Botanical Garden by Pring (1941) and was named in honour of the then Director of the Missouri Botanical Garden. One of the parents involved in the origin of this cultivar is *N. colorata* while the other is not known (Innes[5]). The hybrid has been analysed cytologically for the first time, based on the material obtained from Three Springs Fisheries, Lily Pons, Maryland, U.SA., in 1968.

'Director G. T. Moore' is a daily blooming free-flowering tropical water lily, with small fragrant violet flowers 6 to 10 cm across which are carried well above the water level. Leaves are small, numerous and with purplish tinge.

For meiotic studies the anthers were fixed in 1 : 3 acetic-alcohol and were squashed in 1% acetocarmine following the usual technique (Darlington and Lacour[3]).

Out of the 40 pollen mother cells at metaphase I, 85% had 14 II + 1 I (Fig. 1), while the remaining 15% had 13 II + 1 III (Fig. 2). The trivalent configuration was organised in the form of V (60%), chain (20%) and frying-pan (20%). The chiasma frequency per cell was 15·6 at anaphase I while the bivalents disjoined normally and reached their respective poles, the extra chromosome was usually observed to pass to one of the poles undivided, thus resulting in unequal ditsribution of 15 : 14 (Fig. 3). However, in some of the cells the univalent behaved as laggard and thus was eliminated. The pollen stainability was 65%. The cultivar is, however, completely seed sterile.



FIGS. 1–3. Fig. 1. Metaphase I 14 II + 1 I, Fig. 2. Metaphase I 13 II + 1 III, Fig. 3. Anaphase I 15 : 14, All figs. × 1,700.