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HISTOCHEMICAL STUDIES ON MICRO AND MEGASPOROGENESIS IN THE INTERSPECIFIC F_1 HYBRID OF *ABELMOSCHUS ESCULENTUS* x *A. TETRAPHYLLUS*

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Abstract: In the interspecific hybrid between *Abelmoschus esculentus* and *A. tetraphyllus* the developmental stages of male gamete were abnormal. Pollen mother cells underwent normal meiosis and restitution division. The restitution division gave raise to fertile pollen grains. The developmental stages of female gamete were not complete. The study revealed the degeneration of megaspores of the linear tetrad and thus formation of empty ovules. The megaspore tetrads were low in nucleic acids and protein. The sterility of the interspecific hybrid is attributed to break down of entire megaspores.

INTRODUCTION

Okra *(Abelmoschus esculentus)* occupies a pride position among vegetable crops. A great limitation to the cultivation of this crop is the susceptibility to yellow vein mosaic virus (YVMV) disease. It was necessary to look for resistance in the related wild species and transfer the resistant genes to the cultivated species. *A. tetraphyllus*, a related wild species of okra was found promising source of resistance to YVMV (Ugale *et al.*, 1976). In an attempt to transfer the resistance from *A. tetraphyllus* to *A. esculentus* hybridisation work was undertaken. The crosses were successful but F_1 plants were sterile eventhough it had 76.42 per cent of its pollen, fertile. This necessitated to conduct the cytological and developmental studies in the interspecific hybrid. The present studies on the micro and megasporogenesis in the F_1 hybrid elucidate the primary causes of its sterility.

MATERIALS AND METHODS

The interspecific F_1 plants were raised in the field during October 1984 to March 1987 at the Indian Institute of Horticultural Research, Bangalore. The developmental phases of flower buds were sampled in such a way that all the representative stages of development of anther and ovule are included. Carnoy's fixative containing six parts of alcohol, three parts of chloroform and one part of acetic acid was used for fixing the tissue to localise RNA, DNA, proteins and polysaccharides. Flower buds were fixed in the above fixative for three hours. The standard procedures of plant microtechnique (Jenson, 1962) were followed for making slides. The sections were subjected to periodic acid Schiff's (PAS) method (Hotchkiss, 1948) to estimate total insoluble polysaccharides, mercuric bromophenol blue method (Mazia *et al.*, 1953) to estimate the total proteins and toludine blue method for localising DNA and RNA. The slides were observed under a binocular microscope attached to a camera for taking the microphotographs. The observations made in the slides and microphotographs revealed a vivid pattern of carbohydrate, protein and nucleic acids mobilisation in the developmental stages of flower buds.

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RESULTS AND DISCUSSION

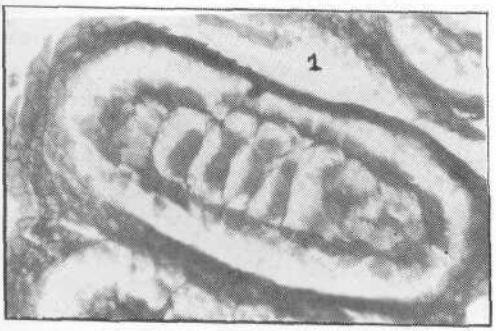
In anthers, during early stages of its development, the early sporogenous cells, middle layers, endothecium, epidermis, connective tissue and vascular strand showed rich cytoplasmic polysccharide, nucleic acids and protein content (Fig. 1). At later stages of sporogenous tissue development, sporocytes and tapetum had rich cytoplasmic polysaccharide, nucleic acids and proteins. With the differentiation of pollen mother cells (PMCs) from the sporogenous cells in anthers, callose deposited around the PMCs and then sister meiocytes were separated from each other. Intense polysaccharides (Fig. 2), nucleic acids and proteins in PMCs and tapetal cells emphasised the high metabolic activity in them. Pollen mother cells underwent two types of division, normal meiosis presumably with disjunctional abnormalities forming tetrads and first division restitution (FDR) forming dyads (Fig. 3). This is in agreement with the observations by Wagenaar (1960) and Galeiva (1971) in many interspecific hybrids.

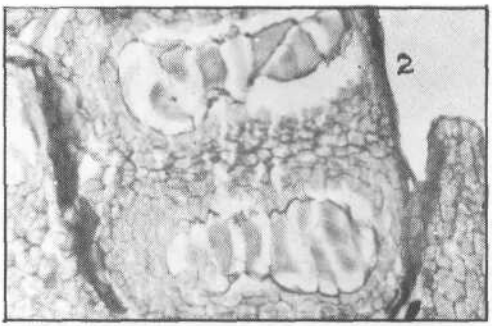
According to Ramanna (1983) and Hermson (1984) two distinct types of restitution division occur, the first division restituion (FDR) and the second division restitution (SDR). FDR gametes originate from an equational division of the entire chromosome complement after completion of prophase I, whereas SDR gametes result from chromosome doubling in the haploid nuclei after completion of the first meiotic division. In both cases dyads instead of tetrads of spores will result. In interspecific and intergeneric hybrids generally FDR gametes can only survive (Ramanna, 1983). The further observations revealed that the thickness of tapetum had slightly reduced and was peripheral, indicating that the PMCs undergoing division might have drawn nutrients from tapetal cells. The tapetal cells had rich polysaccharides, intense nucleic acids and proteins.

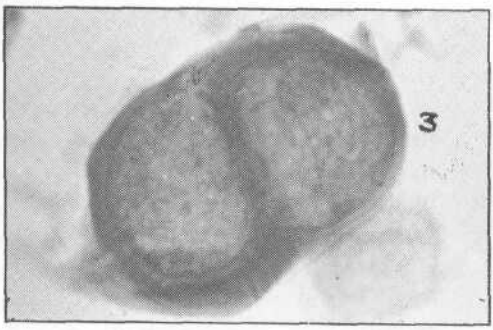
Callose disappeared after the formation of tetrads and dyads, then microspores were released into the anther locules. The bigger sized microspores in the dyads had rich polysaccharides, intense nucleic acids and proteins compared to that in the tetrads. This suggests the possibility of nonviability of pollen grains formed from tetrads. At this stage tapetal cells had intense cytoplasmic polysaccharides, nucleic acids and protein content, which are needed for the pollen wall formation. The cytoplasm of tapetal cells diffused and invested around the developing microspores. This was followed by the development of pollen wall into exine and intane. The tapetal cells completely degenerated at the time of pollen grain formation.

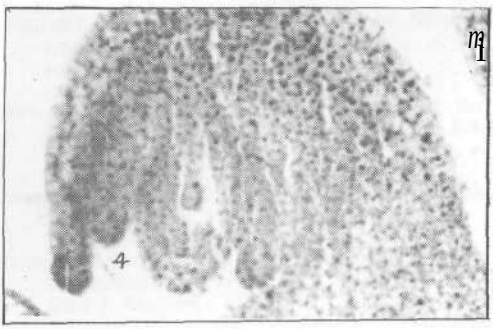
The small sized pollen grains formed from the tetrads did not take any stain whereas bigger and well shaped pollen grains formed from dyads had intense polysaccharides, nucleic acids and proteins, indicating that they are fertile thus contain nutrients which are required for their further development. So the sterility in the interspecific hybrid cannot be attributed to male factors.

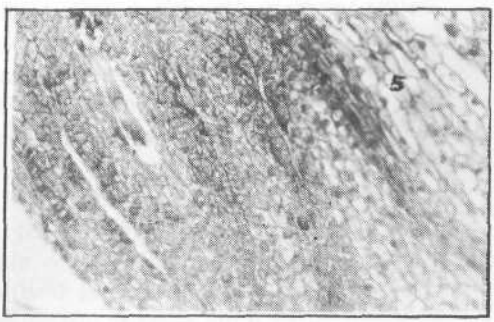
In ovules, during early stages of ovule development, the sporogenous cells had intense nucleic acid content. In the later stages of development, archesporium and megaspore mother cells had low polysaccharide content but











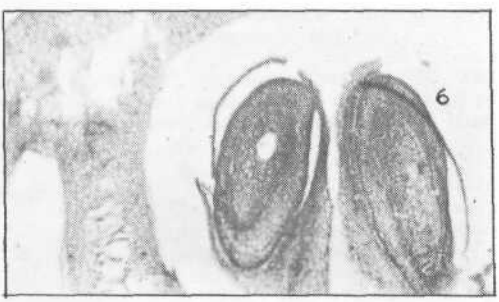


Fig. 1. Sporogenous tissue showing rich protein content (x 120)
Fig. 2. Sporogenous tissue with intense polysaccharides (x 120)
Fig. 3. Dyads due to first division restitution (x 400)
Fig. 4. Megaspore mother cell of the interspecific F_1 showing intense nucleic acid content (x 120)
Fig. 5. Megaspore tetrad of interspecific F_1 showing low protein (x 120)
Fig. 6. Empty ovule after the degeneration of megaspores (x 200)

had intense nucleic acids (Fig. 4) and proteins. At the megaspore tetrad stage, it had low polysaccharides, nucleic acids and proteins (Fig. 5).

The ovule degeneration at terad stage was observed in all the ovules studied in the interspecific hybrid. At this stage all the four megaspores degenerated, followed by disintegration of the ovules (Fig. 6).

Considerably high amount of pollen fertility, pollen germination in the style upon selfing but non-seed setting in the interspecific hybrid raised doubts which were solved by the present investigation.

The degeneration of tetrad occurred in ovulues by degenerating all the megaspores. Miki-Hirosige (1964) in *Lilium longiflorum*, Pritchard (1964) in *Stellaria media* and Panchaksharappa and Syamasundar (1975) in *Dipcadi nostanum* observed that megaspore tetrad contained rich nucleic acids and intense protein which indicated high rate of metabolic activity in them. In contrary to the above, during the present investigation, it was observed that the megaspore tetrad was low in nucleic acids and proteins which may be the reason for its degeneration.

In interspecific hybrids, the male gametes are more easily upset than the female ones (Stebbins 1958) but female sterility had been observed in several interspecific crosses such as in *Nicotiana sylvestris x N. tomentosiformis* and related hybrids by Greenleaf (1942) and Ar-Rushid (1956) and in *Allium cepa x A. fistulosam* hybrid by Davis (1955). So the sterility observed in the interspecific hybrid of *A. esculentus x A. tetraphyllus* is thus solely due to the failure of the development of female gamete.

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