proline is synthesised from glutamate and, thus, serves as energy reserve. After 5 months of inoculation when considerable rotting occurred in the internode, proline content reduced in the rotted portion and adjoining nodal buds. This trend was suggestive of proline oxidation to fulfil the energy demand at the place of high metabolic activity.

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GENETICS OF MALE STERILITY IN A GENOTYPE OF CASSAVA

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THE germplasm of cassava (*Manihot esculenta* Crantz) maintained at this Institute comprises 1,320 genetic stocks assembled from different cassava growing countries of the world, and among them 60 clones are found to be male sterile. Cassava is heterozygous, cross-pollinated and generally propagated vegetatively. Male sterilities resulting from pachytene abnormalities[1], desynapsis[2], aberrant behaviour of tapetum[3], non-separation of microspores from tetrad[4] and functional male sterility[5] have already been reported. Among these about 40 clones exhibit male sterility caused by the non-separation of microspores and, due to its apparent importance an attempt has been made to understand the inheritance of male sterility in cassava.

The male sterile (MS) clone Ce-539 showing the characteristic non-separation of microspores, but having high female fertility, was selected and crossed with the male fertile (MF) clone Ce-613. Crosses were made between two other MF clones Ce-584 and Ci-326 and open-pollinated (OP) seeds were also collected from the MS 539, MF 584 and OP-4 clones. The resulting seedling progenies were screened for male sterility. Microsporogenesis and the sequence of pollen development in MS and MF clones were also compared.

Male fertile: The microsporogenesis was normal and tetrads lasted only for a short time (figure 1). The microspores separated and developed their own intine and exine. The tapetum showed signs of shrinking as the microspores separated and completely disappeared later (figure 2). The anthers were filled with pollen and the pollen fertility was above 90% (figure 3).

Male sterile: In the MS clone 539, the anthers were somewhat shrivelled and empty at the time of anthesis. The meiosis and early microsporogenesis was normal as in the case of MF clones. However, the microspores failed to separate from the tetrads and gradually degenerated (figure 4). The empty shells of the tetrads were discernible for a longer period (figure 5) but they also disappeared later. The tapetum was healthy and comparable with that of MF even at the time of early degeneration in the tetrads, but, later the tapetum also degenerated and disappeared leaving empty anthers (figure 6). In cassava, when male sterility was caused by the non-separation and degeneration of microspores,

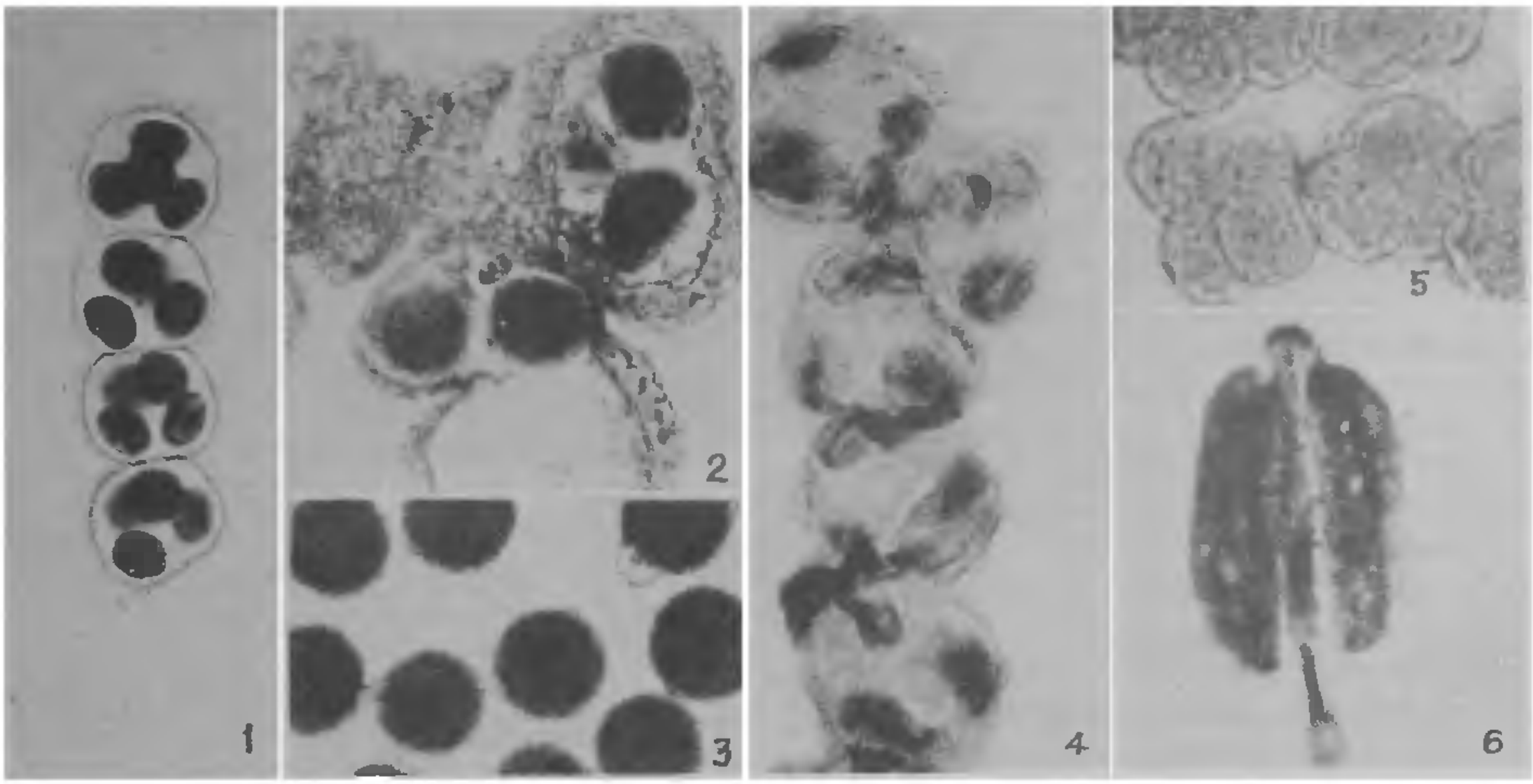

Figure 1–6. 1–3. *Male fertile.* **1.** Normal microspores, × 225. **2.** Anther with developing pollen grains where tapetum has disappeared, × 150. **3.** Fertile pollen, × 180, **4–6.** *Male sterile.* **4.** Non-separated and degenerating tetrads, × 300. **5.** The shells of degenerated tetrads, × 250. **6.** Empty anther, × 40.

invariably it was total which facilitated the screening for MS plants in the seedling progeny.

Because of the heterozygous nature of few parents selected in this study, segregating progeny occurred in the F_1 generation itself. A total of 477 seedlings of different combinations were raised, of which only 205 flowered (table 1).

In the population of 68 plants raised by crossing MS-539 × MF-613, only 47 plants flowered, out of which 23 were found to be completely MS and 24 MF. Among the open-pollinated population of 310 plants established from MS-539, of the 95 plants flowered 17 were MS and 78 MF; the low frequency of MS plants seen in this OP population is probably due to the availability of only few heterozygous MF clones around this clone in the germplasm. When the MF clones 584 and 326 were crossed all the seedlings produced were MF. Similarly in the OP progeny of the MF clone 584 all the seedlings which flowered were found to be MF. However, among the OP progenies of another highly MF clone OP-4, one MS plant appeared among the 14 plants flowered. The MF clone Ce-613 is partially female sterile and hence only after repeated selfings very few seeds could be obtained; when the plants were raised, they did segregate into 3:1 ratio establishing that the clone Ce-613 is heterozygous for male fertility. Consequently, the segregation in the selfed progenies of Ce-613, with the absence of any partial MF plants in the progeny in

Table 1 *Segregation of MS and MF plants in the progeny*

Combination	Characteristic of parents		No. of seedlings established	No. of plants flowered	Male sterile	Male fertile
	Female	Male				
539 × 613	MS	MF	68	47	23	24
539 (OP)	MS	OP	310	95	17	78
584 × 326	MF	MF	26	21	—	21
584 (OP)	MF	OP	44	28	—	28
OP-4 (OP)	MF	OP	29	14	1	13

different combinations and also the absence of any environmental effect on the frequency of pollen fertility, ruled out cytoplasmic-genetic factor while the observations suggested genic male sterility.

The segregation pattern of the different progenies (table 1) showed that the MF clone 584 was homozygous for MF because of the absence of any MS plant in the OP progeny. However, another MF clone OP-4 was apparently heterozygous because one MS plant appeared in the OP progeny. Besides, the occurrence of the MS plants in the OP progeny of MS clone 539 in the F_1 progeny itself clearly established the presence of heterozygous MF genotypes around the clone in the germplasm. When the MS clone 539 was crossed with the MF clone 613, the occurrence of 23 MS: 24 MF plants in the F_1 progeny suggested that it was only a backcross ratio of 1:1, wherein the MS clone 539 was homozygous recessive (ms ms) while the MF clone 613 was heterozygous (MS ms), and the male fertility was dominant over male sterility.

Genetics of male sterility controlled by monofactorial recessive inheritance has been reported in more than 100 cultivated plants[6]. It is concluded that the male sterility in the clone 539 is controlled by single recessive gene pair, which is being reported for the first time in cassava.

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DIFFUSIVITY OF PROLINE AND ITS SIGNIFICANCE

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MANY solutes are known to accumulate in leaf tissue under moisture stress conditions and proline is one such. Recently, the solubility of proline and its ability to co-exist with other solutes and its biological significance have been shown[1]. Proline has certain other unusual properties such as prevention of denaturation of enzymes by heat[2,3], micelle formation in solution[4] and increasing the solubility of proteins[5].

Under moisture stress conditions, proline has been shown to accumulate essentially in the cytoplasm[6]. However, accumulation in the vacuole is not ruled out. The concentration of proline in the leaves of *Triglochin maritima* expressed on the basis of cell water content may exceed 200 mM. Moreover, if it is assumed that this entire accumulated proline is confined to the cytoplasm, which occupies around 10% of the cell volume, the calculated osmotic potential for this compartment would be -5.6 MPa. This value is very much less than the osmolarity of the cell sap, -2.9 MPa[7]. Hence, some of the proline must be located in the vacuole also. If accumulation in the vacuole can take preferentially, then diffusion from cytoplasm to the vacuole across the tonoplast membrane must be occurring at a fast rate. Perhaps, this compartmentation may be the reason for the lack of a feedback inhibition in proline accumulation under stress.

Experiments were conducted to study the rate of diffusivity of proline and other solutes in plant tissue.

Experiment No. 1: Cylindrical pieces of potato, 2 cm in diametre and 1.5 cm in height were cut. The thickness of the sides and the bottom of the potato cup was 5 mm in each case. Such potato cups were placed in distilled water for 16 hr to remove the easily diffusible endogenous solutes present within the tissue. Each of the test solutions (0.4 ml), proline, glycine, glucose and potassium chloride (0.1 M) and distilled water were taken in the cups and were placed in petri plates with 5 ml of distilled water for 2 hr. Three such osmometers were kept in a single petri plate and three replications per treatment were taken. At the end of the two-hour period, the different solutes in the ambient medium were estimated and used as a measure of differential diffusivity.