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DIVERSE TYPES OF MALE STERILITY IN CASSAVA

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ABSTRACT

While screening the germplasm of cassava for pollen fertility, diverse types of pollen sterility were noticed. The male sterile clones were more frequent among the indigenous than exotic genotypes. Though the male sterile clones of indigenous origin showed empty anthers, those of exotic origin, except Ce 539, had 100% sterile pollen of small size. Male sterility among the indigenous strains resulted from nonseparation of microspores from the tetrads, which may be due to the suppression of callase activity. In the exotics, delayed separation of microspores leads to the production of small sterile pollen. The partial pollen sterility resulted from the cryptic structural hybridity manifested through aberrant pachytene pairing.

Key words: Male sterility, tapetum, late disjunction, microspores, callase, cassava.

Cassava (*Manihot esculenta* Crantz) is an important tropical tuber crop raised through stem cuttings. Because of vegetative propagation in this crop, types with different pollen sterility ranges are perpetuated. Since the tuber is the economic produce, the presence of full or partial male sterility does not discourage the selection and propagation of such clones in cassava. Cassava exhibits complete male sterility due to nondisjunction of microspores [1], abnormal tapetum [2], asynapsis [3], and partial pollen sterility due to cryptic structural differences [4], in addition to functional male sterility [5]. The present communication reports the occurrence of small sterile pollen leading to total or partial male sterility and compares it with other types of male sterility observed in cassava.

MATERIALS AND METHODS

Of the 1214 genetic stocks screened for flowering during the last two seasons, only 692 flowered. Different types of pollen sterility were noted and categorised. The sequence of microsporogenesis in the male sterile (MS) and male fertile (MF) clones was compared. Besides, since such male sterility is occasionally found along with partial sterility due to cryptic structural hybridity, the occurrence of the latter type of partial sterility among the exotic clones was also studied. The following clones were included for a detailed study of microsporogenesis.

Male fertile: CE–86 (exotic)
Male sterile: Type A: Production of empty anthers: CI–51 (indigenous) and CE–539 (exotic)
Type B: New type: CE–406 and 560 (exotic)
Type C: Sterile pollen large: CE–209 and 240 (exotic)
Type D: Combination of B and C: CE–578

The buds were fixed in 1 : 3 acetic-alcohol, processed following the usual procedures, and 8 μm thick sections were stained in Heidenhain's hematoxylin. For cytological studies, propiono-carmine was used. Pollen fertility was determined from the percentage of stainability. A combined stain of malachite green and acid fuchsin [6] was also used for differentiating the sterile and fertile pollen.

RESULTS

Pollen fertility was determined in 692 genetic stocks. The fertile clones were classified into high, medium and low pollen fertility groups as per the norms standardised earlier [4]. However, this classification was made with a permissible variation of 5% in the same clone. Among the exotics 70.2% clones were highly fertile and 3.2% were male sterile (Table 1) as compared to the corresponding values of 64.1% and 7.1%, respectively, in the indigenous stocks.

Among the sterile clones, different causes of male sterility were noted (Table 2). The indigenous clones showed empty anthers without any pollen, while exotics showed 100% sterile small pollen grains, except in clone CE-539, where the anthers were empty as in the indigenous clones. Among the exotics, three cases of tapetal abnormalities and asynapsis and nine clones with anthers having small sterile pollen were obtained. Interestingly, such male sterility was absent in the indigenous genetic stocks.

Table 1. Range of pollen fertility in cassava clones

Pollen fertility (range)	Indigenous		Exotic	
	No.	%	No.	%
High (71–100%)	182	64.1	287	70.3
Medium (31–70%)	68	23.9	81	19.9
Low (up to 30%)	14	4.9	27	6.6
Male sterile	20	7.1	13	3.2
Total	284	—	408	—

Table 2. Different causes of sterility among MS clones of cassava

Cause of male sterility	Indigenous	Exotic
Nondisjunction of microspores from tetrads	20	1
Late disjunction of microspores	0	9
Other causes:		0
tapetum asynapsis	0	1

However, in the partially sterile clones the sterile pollen grains were large and occasionally both large and small sterile pollen grains were found, apparently due to different causes, along with the normal fertile pollen.

MALE FERTILE

CE-86. The microsporogenesis was normal, producing tetrads (Fig. 1: 1–3) and the microspores separated normally and developed into fertile pollen grains. The tapetum was utilized by the developing microspores (Fig. 1: 4). The mature anther developed endothecial thickenings and the pollen grains were liberated on dehiscence. In the highly fertile clones up to 99% pollen grains were viable (Fig. 1: 5). The fertile pollen grains were globular with a diameter of 141–148 μm.

MALE STERILE

Type A. Production of empty anthers: CI-51 and CE-539. The anthers were somewhat shrivelled and empty at the time of anthesis. Meiosis and early microsporogenesis were normal. However, the microspores failed to separate from the common mucilaginous sheath of tetrads and showed signs of degeneration (Fig. 1: 6, 7) and completely disappeared later, resulting in the development of empty anthers (Fig. 1: 8).

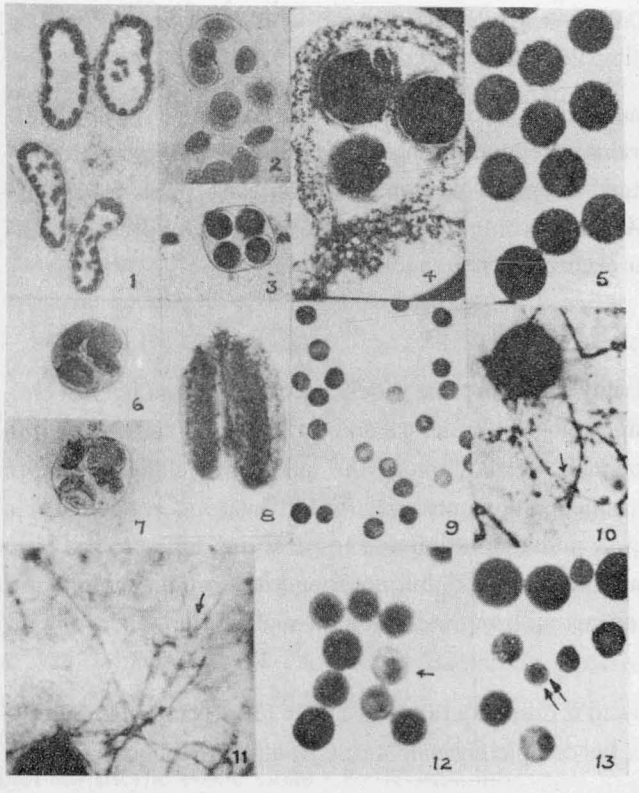

Fig. 1. Microsporogenesis in cassava. 1–5) Male fertile clone CE–86: 1) T. S. of young anther showing microspore tetrad and normal tapetum, x100; 2–3) normal microspore tetrad, x225; 4) T. S. of anther, x250; and 5) fertile pollen, x200. 6–8) Male sterile clone CI–59: 6–7) degenerating, nondisjuncted tetrads, x200; 8) empty anther, x60. 9) Male sterile clone CE–560: completely sterile pollen, x175. 10–12) Chromosomal partial pollen sterility in clone CE–240: 10) pachytene showing interstitial nonpairing (arrow), x950; 11) terminal nonpairing (arrow), x950; and 12) fertile and sterile large pollen (arrow) x200. 13) Partial pollen sterility in clone CE–578: fertile, sterile large (single arrow) and small pollen (double arrow), x200.

Type B. New type: CE-406 and CE-560. Early microsporogenesis was comparable with MF. The pachytene pairing, meiosis and tapetal development were normal and comparable with MF. Occasionally signs of early degeneration were noticed in a few microspores inside the tetrads. Later, the microspores disjuncted and developed normal exine, and the pollen grains were only about 95–105 μm in diameter; all the pollen grains had uniform shape and size (Fig. 1: 9). Though most pollen grains were poorly stained and empty, about 12% were stained in propiono-carmine. To determine whether stainability of these small pollen grains was any indication of fertility, 150 flowers of a highly female fertile clone were pollinated using line CE-560 as male. Complete failure of capsule set in these pollinations confirmed the total sterile nature of pollen. Besides, these pollen grains stained to green in malachite green–fuchsin stain, supporting the conclusion that they were nonviable.

PARTIALLY STERILE POLLEN

Type C. Sterile pollen large: CE-209 and CI-240. Though microsporogenesis was normal, pachytene pairing was rather irregular showing abnormalities like interstitial (Fig 1: 10) and terminal nonpairing (Fig. 1: 11); occasionally loop formation and deletions were also noticed involving different chromosome pairs. The degree of pollen sterility in such clones depends on the extent of pachytene abnormalities [4]. The sterile pollen grains, though smaller than in fertile pollen, were comparatively larger than in Type B with the diameter of about 126 μm (Fig. 1: 12).

Type D. Combination of B and C: CE-578. In this group of partially male sterile clones, both large and small sterile pollen grains were found along with fertile pollen (Fig. 1: 13). They had pairing abnormalities at pachytene as well as late separation of microspores from a few tetrads, but the tapetum was normal. Though both types of sterile pollen were present in the same clone, the pollen sterility never exceeded 85%.

DISCUSSION

The phenomenon of male sterility is known to be widely prevalent in a number of crop plants and is reported to result from cytological, genetical and physiological causes [7, 8]. In cassava, the most common form of male sterility among the indigenous clones is due to degeneration of microspores and production of empty anthers [1], which was noticed only in one exotic genetic stock [9]. However, pollen abortion due to persisting tapetum has been reported in two exotic clones from Tananareive [2], but not found in any indigenous clone. The occurrence of male sterility showing uniform production of small sterile pollen is being reported for the first time.

Screening of germplasm showed a preponderance of highly fertile clones among the exotic and indigenous genetic stocks, but completely male sterile plants were found to be 7.1%

in the indigenous, but only 3.2% in exotics. The type of male sterility prevalent among all the indigenous stocks is the same, including clone CI-51, where the microspores often did not disjunct from the tetrads while the tapetum remained normal and comparable to MF. Release of microspores from the common envelope of tetrad is regarded as the most critical stage for the breakdown of microsporogenesis in male sterile mutants where lack of enzymatic digestion of the callose envelope around the microspores in the tetrad seemingly starves the microspores to degeneration or inhibits proper pollen production in two cytoplasmic and one genic MS lines of *Petunia hybrida* [10, 11]. The nonseparation of microspores from the tetrads resulting in male sterility is reported in broccoli [12], pigeon pea [13], *Lupinus mutabilis* [14] and in two indigenous [1] and exotic clones of cassava [9].

The most common type of male sterility among the exotics is due to late disjunction of microspores from the tetrads, resulting in the production of small, empty and sterile pollen. For the different types of male sterility reported in *Capsicum* [15,16] Daskaloff [17] concluded that pollen abortion takes place after later disjunction from the tetrads in the mutant. The sequence of microsporogenesis in the present study clearly shows that type B might have been the forerunner of Type A. It is apparent that the late separation of microspores resulting in the development of small sterile pollen among exotics progressed to total nondisjunction causing complete degeneration of tetrads among the indigenous and CE-539, the only clone among exotics. The presence of the type A male sterility in CE-539 indicates that both types of sterility may have common origin in the indigenous and exotic stocks.

The delayed callose activity, instead of its suppression, is reported in a few plant species. Accumulation of asparagine and glutamine leading to a drop in pH, altering the timing of phasespecific callose activity and causing breakdown of microsporogenesis is reported in the male sterile anthers of *Petunia* [18]. The dependence of callose activity on pH, lack of glucanase activity in the microsporocytes at all stages of meiosis and its presence in the tapetum and anther locules at the time of normal degradation of the callose matrix of microspore quartet [19, 20] led to the belief that faulty activation of the enzyme in the anther locule may be the primary factor in microspore abortion [10, 11]. Similarly, Chauhan and Singh [21] identified reduced acid phosphatase activity in the tapetum of MS plants of *Cucumis melo.*

The occurrence of two types of sterility among the partially sterile clones of cassava is of special interest. The presence of large sterile pollen grains, about 126 μm in diameter, is invariably linked with pachytene abnormalities, establishing that such partial pollen sterility is due to cryptic structural hybridity noticed in this heterozygous, cross-pollinated and vegetatively propagated crop. The extent and intensity of pachytene abnormalities resulting in variable degree of pollen sterility has been already established in five indigenous clones of cassava [4]. However, in the present study, the occurrence of large and small sterile pollen in the partially sterile exotic clone CE-578 establishes that the same clone may have cryptic

structural hybridity as well as late liberation of microspores due to delayed callose activity where the expression of the latter is only partial.

The type A male sterility has been already established to be double recessive [9], while the sequence of microsporogenesis in type B sterility suggests that it may be due to another wholly independent or modifier gene action which influences MS in homozygous recessive condition. In *Brassica oleracea* with type A sterility, nondisjunction of microspores is known to be influenced by another temperature-sensitive nonallelic gene [22, 23].

While studying the breeding behaviour of cassava, Kawano [24] found that the average yield of open pollinated MF genotypes was lower than under controlled pollination but no such yield differences were noticed in the progeny of MS stocks. In such instances, the extent of sterility due to cryptic structural hybridity is bound to reflect not only in the pollen but also in the female phase, thereby reducing seed set while other types of male sterility mentioned here do not have any impact on female fertility. Hence, partitioning the sterility in cassava based on the respective causes, instead of considering male sterility as a single phenomenon, undoubtedly helps while selecting genetic stocks for breeding and other genetic studies.

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