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Mechanisms of cytoplasmic-nuclear male sterility in pigeongea wide cross *Cajanus cajan* × *C. acutifolius*

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Abstract

Cytoplasmic-nuclear male sterile plants (CGMS) were obtained as a result of crossing cultivated *Cajanus cajan* with wild species *C. acutifolius*. There were two types of CMS plants which were distinguished by anther morphology. Both the types of CMS plants had complete sterility of the anthers. Type I CMS had partially or totally brown and shriveled anthers and the process of microsporogenesis was inhibited at the pre-meiotic stage. Type II CMS plants had pale while shriveled anthers and the break down in microsporogenesis was at the post-meiotic stage after the formation of tetrads caused sterility of the plants.

Key words: Pigeonpea, cytoplasmic-nuclear male sterility, microsporogenesis, meiosis

Introduction

Cytoplasmic-nuclear male sterility (CGMS) is a maternally inherited trait, in conjuction with nuclear genome, suppresses the production of viable pollen grains, while not affecting the female fertility. It provides a means of pollination control for commercial production of F_1 hybrid seeds. CMS is reported in a wide range of plant species, in more than 150 plant species [1, 2]. Most CMS types have occurred naturally or in interspecific crosses. There are many instances where CMS has been induced by interspecific crosses, which is more common in the dicots than the monocots [2].

Reddy *et al.*, [3] reported genetic male sterility (CMS) in pigeonpea which was characterized by translucent anthers. Non-separation of tetrads associated with persistent tapetum, was attributed as underlying mechanism for male sterility. Wallis *et al.* [4] reported male sterility in an advanced breeding line, where the anthers were brown and shriveled. A study of microsporogenesis in this line was carried out by Dundas *et al.* [5]. The study revealed that degeneration of microspores occurred at the young tetrad stage with the rupture of nuclear membrane and collapse of outer cell wall resulting in male sterility. In both the systems put forward by Reddy *et al.* [3] and Dundas *et al.* [5], meiosis proceeded normally till the stage of formation of tetrads and then abortion of tetrads occurred. Dundas *et al.* [6] reported yet another male-sterile system where male sterility occurred before the commencement of meiosis in pigeonpea.

Cultivated *Cajanus cajan* cultivar ICPL 2 is cross compatible with *C. acutifolius* ICPW 15613 and gives rise to mature seeds, however 30% of the hybrids were male sterile. On the other hand when F_1 sterile plants were crossed with 5 pigeonpea cultivars their progeny were all fertile. F_1 sterile plants when crossed with wild species *C. acutifolius* ICPW 15613 all the resultant progeny were 100% sterile. Hence, the type of male sterility observed in the cross *C. cajan* × *C. acutifolius* was identified as cytoplasmic-nuclear male sterility on cultivated pigeonpca cytoplasm, unlike the CMS systems reported earlier which needed wild species cytoplasm [7, 8, 9 & 10]. The factors causing CMS are present in the cultivated cytoplasm which upon interaction with nuclear genes from wild species *C. acutifolius* gave rise to completely male sterile plants in lower frequency. While many pigeonpea cultivars restored male-fertility and wild species *C. acutifolius* maintained male sterility (Mallikarjuna and Saxena, unpublished). In this paper we report two types of microsporogenesis observed in male sterile plants resulting from the cross *C. cajan* × *C. acutifolius*, one at pre-mciotic stage and the second one at post meiotic stage after the formation of tetrads.

Materials and methods

Cajanus acutifolius accession 15613 and pigeonpea cultivar ICPL 2 were grown in the glasshouse in plastic pots filled with sterilized alfisol mixture with farm yard manure and sand. Emasculations in cv ICPL 2 followed by pollinations with pollen from *C. acutifolius* were carried out before 10 am in the morning. To prevent abscission of floral buds from cross pollinations, 50 mg/l of GA was applied to the base of the pistils. In the sterile F_1 plants, some of the plants had pale white translucent and shrunken anthers (type I) and some others had totally or partially dark brown and shrunken

anthers (type II). Each anther type was selected for the study. For cytological study of meiocytes, floral buds at different stages of maturity were fixed in Carnoy's II solution (acetic acid 1: chloroform 3: and ethanol 6) for 24 hr at 4°C and transferred to Carnoy's I solution (acetic acid 1: ethanol 3). Meiocytes were squashed and stained with 2% aceto-carmine solution and well spread preparations were studied. Floral buds of different sizes ranging from 1-5 mm were fixed in FAA (70% alcohol 90 cc+ formaldehyde 5cc+ acetic acid 5 cc) for 48 hrs or more. Buds were dehydrated through a tertiary butyl alcohol series, and embedded in paraffin at 58-60°C. Transverse and longitudinal sections 10-12 µm thick were hydrated and stained in safranin and fast green and mounted in Canada balsam.

Results and discussion

Meiosis in cultivated pigeonpea cv ICPL 2, used as female parent had 22 chromosomes with normal disjuction of 11 chromosomes at each pole by the end of meiotic division. The highly fertile F_1s/siblings from the cross *Cajanus cajan* × *C. acutifolius* followed the normal meiotic procedure with the formation of viable pollen grains at the end of meiotic division.

In fertile siblings, enlargement of the pollen mother cells (PMCs) continued till the bud was 2.5 mm long (Figs. 1-1 & 1-3). In 3 mm PMCs, meiosis had advanced to the stage of diakinesis. Prominent metaphase plates were seen in bud size of 3.5 mm, leading to anaphase I. In 4 mm long buds tetrads were observed (Figs. 1-5) and by the time the buds were 4.5 mm long, pollen grains were seen in fertile anthers. All the tetrads developed into pollen grains and the anther locule showed the depression in the anther wall which was the line of dehiscence (Figs. 1-7). The line of dehiscence was where the wall of the anther ruptured to release the pollen grains at anthesis. Mature anthers were bright yellow and plump.

In the type I sterile plants, anthers were partially or totally brown and shriveled. PMCs remained in prophase and did not undergo the different stages of meiosis (Figs. 1-2, 1-4 & 1-6). The PMCs enlarged as they would in a normal PMC undergoing meiosis. Once the nucleus enlarged, further division in the PMCs was not observed. A prominent tape-turn was seen in 4.5 mm buds (Figs. 1, 8a), in contrast to fertile plants, where the tapetum had completely degenerated in a 4.5 mm bud. In type II sterile plants anthers were pale white and shriveled. The PMCs proceeded with meiosis as in the fertile plants. In the fertile as well as in the sterile plants, bud size of 2.5 mm had PMCs at prophase (Figs. 2-E, 2-F) and 3.5mm buds had the PMCs at metaphases (Figs. 2-G, 2-H). Tetrads were seen in 4.0 mm buds of sterile plants which did not separate to form pollen grains (Figs. 2-I and 2-L). Anthers began to degenerate in buds of 5 mm, and a shriveled pale while anther was seen. Anther squash preparations revealed that the anthers did not release the pollen grams but the cell contents oozed out. In anthers from fertile plants, by the time tetrads had formed tapetal layer had degenerated, while in sterile plants with type II sterility, by the time the buds were of 6mm in length, the anthers were completely dark or with patches of dark brown and were shriveled (Fig. 2-K). The male sterile system presented in the cross *C. cajan* × *C. acutifolius* is different from the systems reported previously. The CMS being reported in this paper results from the interaction of the cytoplasm of cultivated species with the nuclear genes from *C. acutifolius*, unlike the earlier reports [7 & 8]. In the present system wild species *C. acutifolius* was used as the pollen parent, whereas in the previous systems either *C. serecius, C. scarabaeoides* or *C. volubilis* were used as the female parent whose cytoplasm interacting with nuclear genome of *C. cajan* gave rise to CMS. The mechanism of CMS, which obstructs microsporogenesis, is different from the previous reports

In one type of GMS system reported earlier sterility resulted at tetrad stage [3, 5]. In another type of male sterile system, disruption of microsporogenesis occurred at pre-meiotic stage [6]. None of the male sterile systems reported by the earlier authors had two mechanisms of male sterility together. In the cross *C. cajan* × *C. acutifolius*, two mechanisms of male sterility operated, type I at pre-meiotic stage and type II after the formation of tetrads at late meiotic stage. The advantage of two systems of male sterility in a cross can be envisaged as a double check system, wherein even before the commencement of meiosis there is breakdown of PMCs. If any of the PMCs escaped breakdown of microsporogenesis at pre-meiotic stage, the post meiotic or the type II system of CMS would render PMCs sterile. If both type I and type II systems of CGMS can be brought together, by crossing the two types of microsporogenesis systems, there would be a fool proof system of CMS in pigeonpea. It needs to be seen if the two systems are stable across different environments. Further research will be directed to test the coupling of two systems of CMS and their stability.

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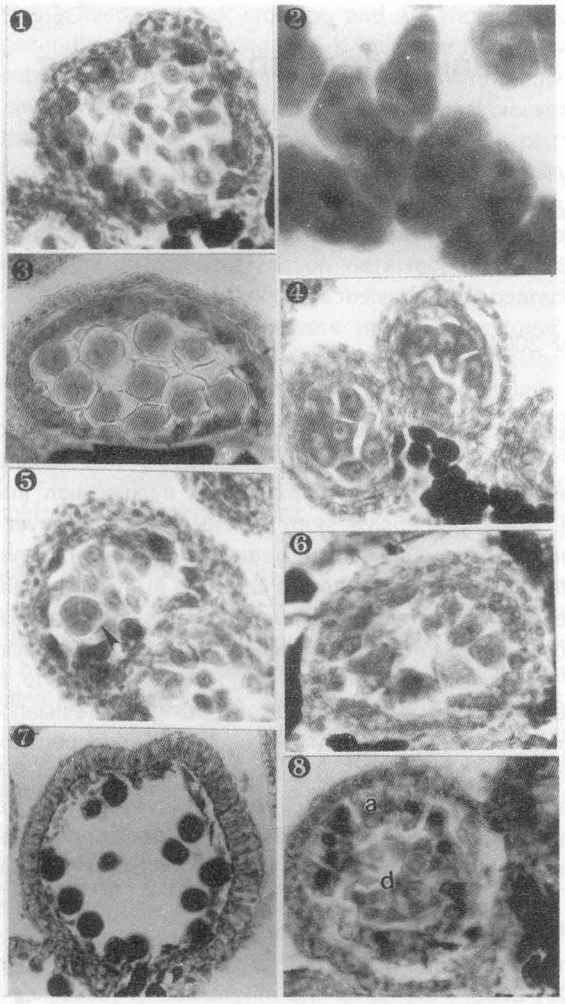

Fig. 1. Type 1 male sterility in the cross *Cajanus cajan* × *C. acutifolius*. 1. Cross section of 2.0 mm fertile anther showing PMC at prophase, 2. Squash of 2.0 mm sterile anther showing the PMCs at prophase, 3. Cross section of 2.5 mm fertile anther showing the enlargement of PMCs preparing for meiosis, 4. Cross section of 2.5 mm sterile anther showing enlargement of the nucleus, 5. Cross section of 4.0 mim fertile anther with PMCs at tetrad stage of meiosis, 6. Cross section of 4.0 mm sterile anther with PMCs still at prophase, 7. Cross section of 4.5 mm fertile anther with the dissolution of the tapetal layer and the formation of pollen grains. Note the line of dehiscence, 8. Cross section of 4.5 mm sterile anther with PMCs still at prophase with a prominent tapetum.

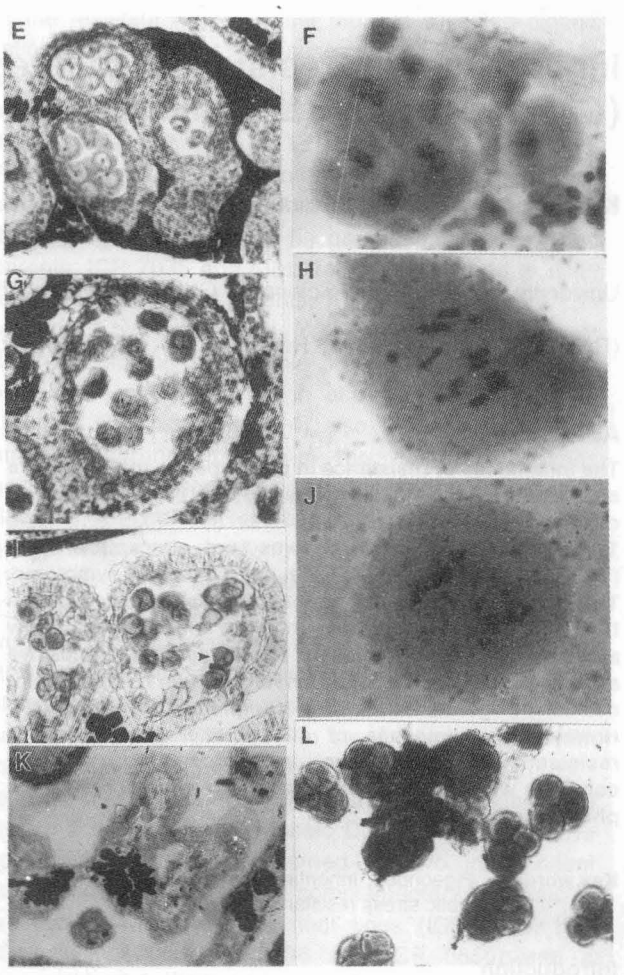

Fig. 2. Type II male sterility in the cross *Cajanus cajan* × *C. acutifolius*. E. Cross section of anther from 2.5 mm bud with PMCs at prophase, F. Whole mount of PMCs from 2.5 mm sterile bud showing prophase, G. Cross section of an anther from 3.5 mm sterile bud at metaphase, H. Whole mount of PMCs from 3.5 mm sterile bud at mctaphase, I. Cross section of 4.5 mm sterile bud showing PMCs at tetrad stage, J. Whole mount of PMCs from 4.0 mm sterile bud showing anaphase plates, K. Cross section of a 5.0 mm sterile anther which has shriveled, L. Squash of 5.0 mm anther allowing PMCs at tetrads. Note that the tetrads have not separated and are held together.

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