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ABSTRACT

Six male-sterile plants, found in two apparently unrelated sources in pigeon pea (*Cajanus cajan* (L.) Huth) did not produce pollen. Crossing success was normal, indicating that the plants were female-fertile. White translucent anther color distinguished male-sterile plants from fertile plants, which had yellowish anthers. A single recessive allele was responsible for the male sterility described here. Study of microsporogenesis revealed that tetrads in male sterile plants did not separate and they completely disintegrated. The tapetum and the intercellular wall of the two adjacent microsporangia persisted in the mature anthers. The potential value of male sterility in breeding programs is indicated.

Additional index words: Selfing, Microsporogenesis, Male gametophyte, Tapetum.

PIGEON pea (*Cajanus cajan* L. (Huth) is a food legume grown in almost all tropical countries. Most of the acreage is planted to local landraces. While its floral biology appears to favor self-pollination, insect pollen vectors are sufficiently effective to put pigeon pea in the category of "often cross-pollinated crops." Sufficient heterozygosity exists to permit differentiation of the local landraces in different environments, and to make each landrace a potentially rich gene pool.

Recognizing the utility of male sterility in population breeding and possibly in commercial hybrid seed production, we made a deliberate search for male-sterile plants in 3,659 germplasm collections and 3,557 sublines grown at ICRISAT in 1974. A total of 72 plants were found with reduced pod set, and these could be classified into five aberrant floral types (Reddy et al., 1977). While all five types are being maintained, we have concentrated our efforts on the one in which the only floral abnormality was absence of pollen. To our knowledge, male sterility had not been found previously in pigeon pea. Sterile plants reported by Deshmukh (1959) were apparently female-sterile.

MATERIALS AND METHODS

Six male-sterile plants that appeared identical in their flower morphology were found in two apparently unrelated sources. Those identified as MS-3.A to E were found in ICP-1555 and two of its sublines that had been separated on the basis of seed color. ICP-1555 had been collected in Andhra Pradesh, India. MS-4.A was in ICP-1596, a collection from Maharashtra.

Because of the indeterminate nature of flowering in the two sources, it was possible to utilize flowers on the original plants for various studies. From each plant three to five buds were fixed in 75% alcohol and the anthers squeezed in 2.2% aceto orcein stain for microscopic examination for the presence of pollen. All plants were further tested for pollen presence by covering an entire branch with a cloth bag (after removing pods and previously opened flowers) and by covering buds with glassine bags.

Each male-sterile plant was crossed with eight normal parents that were diverse in origin and maturity. Each male sterile was also sibbed with fertile plants within the row, but unfortunately, male parents were not identified individually, so meaningful data could not be obtained from these sib progenies. Open-pollinated seed was collected from the individual fertile plants within each row.

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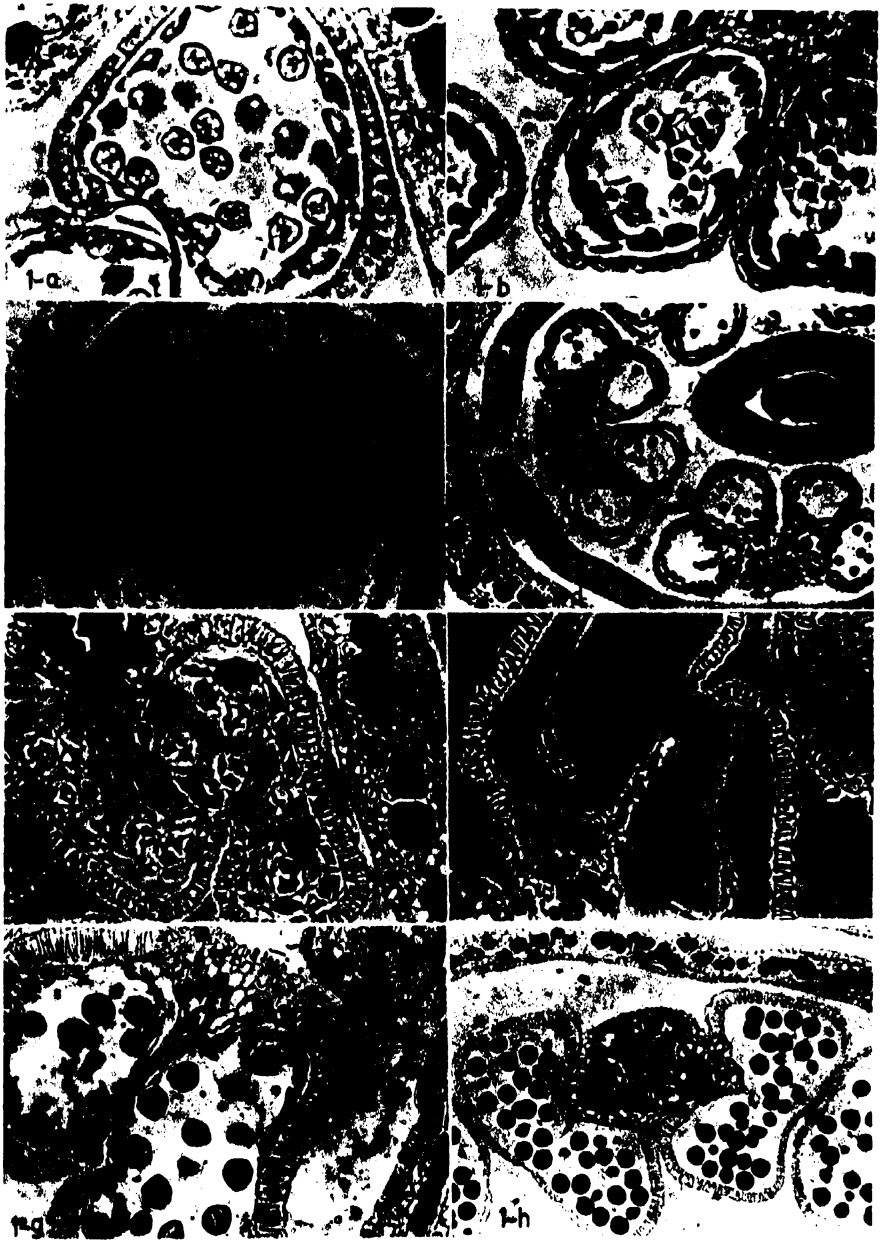

Fig. 1. Development of male gametophyte in fertile and male sterile anthers of pigeon pea. 1a. Tetrads (160×) in fertile. 1b. Tetrads (160×) in male-sterile. 1c. Immature male gametophytes (40×) in fertile. 1d. Immature male gametophytes (40×) in male-sterile. 1e. Degenerated male gametophyte within the spore mother-cell sac (160×) in male sterile. 1f. Completely degenerated male gametophytes and the persistent tapetum (160×) in male sterile. 1g. Release of male gametophytes from spore mother cell sac (160×) in fertile. 1h. Complete developed male gametophytes (160×) and no trace of tapetum in fertile.

In 1975 all the F_1's, sibs, and progenies of fertile plants of the parent lines were grown. Male-sterile plants were sibmated with individual fertile plants. In 1976 all F_1's and sib progenies were grown and plants were classified for male sterility.

In the progeny of a fertile plant harvested in the subline 1555-2, which segregated for fertile and male sterile, buds were collected from male-sterile and fertile plants for comparative studies of microsporogenesis. Buds of various lengths up to 5 mm were fixed in FPA (70% alcohol 90cc, formaldehyde 5cc, propionic acid 5cc) for at least 48 hours. After dehydration through an ethanol series followed by tertiary butyl alcohol, the buds were embedded in paraffin at 60 to 62 C. Transverse sections 10 μ thick were hydrated and stained with toludine blue 0 according to the procedure of O'Brien et al (1964). These were examined under an ordinary light microscope and photomicrographs were made.

RESULTS AND DISCUSSION

The male-sterile plants were apparently morphologically identical to the fertile plants in each source culture in maturity, height, branching, leaf size, etc. The male-sterile plants were slower to senesce than the fertile plants, and they could be distinguished by their greener and more abundant leaves at normal harvest time. Brim and Young (1971) reported male-sterile soybean plants, *Glycine max* (L.) Merr., were greener with thick leaves. It seems likely that in both species delayed senescence resulted from reduced pod set.

All flower parts, except anthers, were normal in size and color in the male-sterile plants. Their anthers were smaller than normal, flat, and translucent with whitish scaly surfaces. These anthers did not dehisce and they dried faster than the normal anthers. The anthers of sterile plants could be distinguished from the plump, yellowish normal anthers 3 to 4 days before anthesis. Differences in the appearance of anthers were used to distinguish male-sterile and fertile plants.

Pollen was not observed in the anthers of any of the six original male-sterile plants when tested in aceto-orcein stain. There was no pod setting on protected branches or buds of five of the male-sterile plants; on one plant, two of approximately 80 buds inside the cloth bag set pods. A progeny test indicated the seeds were hybrid. We assumed that either two open flowers or young pods were not removed at the time of bagging or a bee entered through an undetected hole in the bag. The general failure of pod set upon selfing corroborates the absence of pollen. Self-incompatibility is not considered to be a factor, since wide-scale selfing in the germplasm collection has been successful.

Open-pollinated seed from the fertile plants in the two source cultures were planted in progenies. From the MS-3 source (ICP-1555 and sublines), 29 progenies were grown, of which 24 segregated for male sterility. Within each progeny, the segregation fit a 3:1 ratio. For the 24 progenies pooled, there were 240 fertile and 78 male-sterile plants with $p = 0.75$ to 0.90 for goodness of fit to 3:1. In the MS-4 source, one of seven progenies segregated for 11 fertile to three male-sterile, also a good fit to 3:1.

Crossing success on the male-sterile plants was normal, demonstrating female fertility. Of the 48 attempted crosses involving the six male-sterile plants and eight normal parents, 41 were successful. All F_1's were fertile, indicating dominance of the normal. In the F_2's only one of the 41 combinations did not fit a 3:1 ratio. The total for all crosses was 2,243 normal to 737 male-sterile, $P=0.50$ to 0.75 for goodness of fit to 3:1. It seems safe to conclude that this male sterility is controlled by a single recessive gene. The symbol ms_1 is proposed.

Microsporogenesis was similar in the fertile and male-sterile plants in the premeiotic and meiotic stage. Tetrad formation (Fig. 1a, b) was the latest stage observed in which fertile and sterile did not differ. After the formation of tetrads, the course of development differed in the sterile and fertile plants. Tetrads were released from the spore mother cell sac in the fertile plants (Fig. 1c); in sterile plants (Fig. 1d) the tetrads were not released, and they started degenerating by vacuolation (Fig. 1e). Later the tetrads degenerated completely, while the tapetum persisted (Fig. 1f). By contrast, in the normals the tapetum started degenerating at the time of tetrad formation, and during male gametophyte development no tapetum remained (Fig. 1g, h).

The cause for failure of pollen development in the male-steriles cannot be inferred from the data presented here. Persistent tapetal cells in male-sterile plants have been reportd in *Allium* by Singh and Kobabe (1969), in orchardgrass (*Dactylis glomerata* L.) by Filion and Christie (1966), and in barley (*Hordeum vulgare* L.) by Kaul and Singh (1966). Degeneration of tetrads while in the spore mother cell sac as observed in this study was reported in two-row barley by Kaul and Singh (1966). They reported release of microspores before degeneration in six-row barley, and Filion and Christie (1966) reported release of the tetrads in *Dactylis* before degeneration occurred.

Our staining and cytological studies showed that the mutant plants were completely male-sterile. The inheritance studies indicated that the male sterility is governed by a single recessive allele. The visible marker, white translucent anther color, provides an efficient and fast way of recognizing these plants in the field. Further, pod setting on male sterile plants under open pollination appears to be adequate for seed production. Therefore, the male sterility reported here is considered potentially useful in the production of hybrids and in population improvement programs.

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