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Cytoplasmic Male Sterility in Common Bean[1]

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Abstract. **The inheritance of male sterility was studied in germplasm of common bean (*Phaseolus vulgaris* L.) obtained from the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. The source was selected for plants with high pollen abortion rates (mean = 91%) and for failure to set any seed or pods by self-pollination when grown in screened greenhouses. These male-sterile plants were crossed with the snap bean 'Sprite', and the resulting F_1 progenies were all male-sterile under greenhouse conditions. The F_1 plants were backcrossed to 'Sprite' and the BC_1 progenies did not segregate for male-fertile plants under field conditions. Four more backcrosses to 'Sprite' have produced progenies that were uniformly male-sterile. It was concluded that the CIAT source of male sterility is inherited through the maternal parent and is cytoplasmic. Nineteen commercial cultivars of snap beans and dry beans were crossed onto BC_3 plants, and none of these genotypes restored the pollen fertility in F_1 progeny.**

The common bean is normally a highly self-pollinated crop. Recently, genic male sterility was reported to be controlled by 2 genes producing 100% aborted pollen but with completely normal female fertility (1). Another source of male sterility was discovered at the Centro Internacional de Agricultura Tropical (CIAT) (3). This report describes the results of an inheritance study of the male sterility source discovered at CIAT.

Materials and Methods

A composite of seed from field-grown, male-sterile plants was sent to the authors by Dr. Shree Singh, CIAT. About 150 seeds from the CIAT composite were planted in a screened greenhouse in September, 1979, and the resulting plants were used as seed parents in crosses with 'Sprite'. Pollen abortion rates were determined for each seed parent plant by crushing mature anthers in acetocarmine stain. A single mature bud was used as the pollen source for each plant. Mature F_1 seed was harvested from only the 18 plants that failed to set any pods by self-pollination after 5 weeks of flowering.

A single F_1 seed from each of the 18 male-sterile selections was planted in a screened greenhouse in December, 1979. Pollen abortion rates were determined in the F_1 by using a single mature flower bud from each plant. The F_1 plants were examined at maturity (13 weeks after planting) for any pods produced by self-pollination. 'Sprite' was backcrossed onto each of the 18 F_1 plants.

In March, 1980, 15 backcross progenies (14 seeds each) were planted in the field at 10-cm spacing in a single-row plot, which was covered with a netting made of opaque fibers (strand count of about 8 × 10 per 2.5 cm^2) to exclude bumble bees and honey bees. Mature buds were harvested from only 4 plants in each backcross progeny. A population of about 60 plants (4 × 15) was considered sufficient to detect segregation for any recessive restorer genes that may be present. The buds were fixed in Carnoy's solution [6:3:1 v/v/v of ethanol (95%):chloroform:glacial acetic acid] and transferred after 24 hr to 70% ethanol for storage. Pollen abortion rates were determined by using 2 or 3 buds from each plant. Counts were made of the pods set by self-pollination and the seeds/pod for 144 BC_1 plants after 7 weeks of flowering. The second backcross to 'Sprite' was made in the BC_1 field plots.

The 3rd and 4th backcrosses with 'Sprite' were made in the greenhouse in the Winter of 1980–81. A 5th backcross was made in the field in May, 1981. Pollen abortion counts were made for 33 plants in the BC_2 population grown in the greenhouse in October, 1980; 53 BC_2 plants were examined for pods set by self-pollination. All advanced backcross populations were observed for pod set and for any segregation for fertile plants.

In a BC_2 plant we found a raceme with 3 well-filled, mature pods that apparently resulted from self-pollination. From a composite of this seed, 10 plants were grown and pollen abortion counts were made. The fertile plants in this progeny were crossed to BC_3 plants to test for fertility restoration. The 6 F_1 progenies were grown in the field and examined for pollen abortion.

Nineteen cultivars of snap beans and dry beans were used as pollen parents in crosses onto BC_3 plants to screen these genotypes for fertility restoring genes. The F_1 progenies were planted in the field in March, 1981, and were covered with saran screen (30% shade) to keep out pollinating insects. Pollen abortion counts were made using 2 or 3 buds from each plant in the F_1

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progenies, and counts were made of selfed pods and seeds/pod after 7 weeks of flowering.

An experimental plot was planted on June 5, 1981, at Twin Falls, Idaho, to test the leafcutter bee and other naturally occurring insect pollinators for effectiveness in bean cross-pollination. Two rows of the BC_4 were planted, each having 250 seeds. Two rows of fertile 'Sprite' were planted on either side of the 2 male-sterile rows. The rows were 56 cm apart and 26 m in length. A colony of leaf-cutter bees in a "bee board" (5.1 cm × 10.2 cm × 1.5 m) was placed near the plot when flowering began on July 19. The flowering period in the fertile 'Sprite' rows was artificially prolonged by removing all the young pods once or twice a week. The BC_4 plants were harvested on October 7.

Results and Discussion

Male sterility inheritance. The 18 male-sterile plants used as seed parents had a mean pollen abortion rate of 91%. (Table 1). These plants did not set any pods under greenhouse conditions. The remaining 9% of the pollen is stainable, but usually fails to achieve pollination. The F_1 hybrids between the male-sterile selections and 'Sprite' also had a mean pollen abortion rate of 91%, and no pods were set on any of the F_1 plants (Table 1). Clearly, the male sterility in the seed parent plants is not controlled by a single recessive gene.

None of the 15 BC_1 progenies segregated for plants with pollen abortion rates in the normal range (Table 1). The lowest mean pollen abortion rate for a single plant in the BC_1 progeny was 86%. Pollen abortion counts in the BC_2 also failed to detect any fertile plants. Normal pollen abortion rates under Spring field conditions rarely exceed 10% at Gainesville. If the male sterility in the CIAT source was controlled by a single dominant gene, by 2 to 4 dominant complementary genes, or by 2 to 4 duplicate recessive genes; the BC_1 population would have segregated for fertile plants. The lack of segregation for fertility in the F_1 and backcross progenies is consistent with the hypothesis that the male sterility source discovered at CIAT is controlled in part by cytoplasmic factors and that 'Sprite' has a maintainer genotype.

The male-sterile plants in the various generations studied (Table 1) shed virtually no pollen on the stylar hairs. Examination of flowers from male-sterile plants, using a 30× hand-held loupe, revealed that the anthers did not shed on the stylar hairs, and no attempts were made to use male-sterile plants as pollen parents in test crosses.

Selfing and somatic reversion rates. In the cytoplasmic male-sterile 'Sprite' population in the BC_1 (cyto-*ms* 'Sprite' BC_1), 144 plants were examined for pods produced by self-pollination. The mean pod set per plant after 7 weeks of flowering was 0.42 pods, and most of the pods observed were immature and had only 1 or 2 seeds with a maximum number of 3 pods per plant.

Table 1. Pollen abortion rates in 18 male-sterile seed parent plants (CIAT-*ms*) and in the F_1, BC_1, and BC_2 progenies derived from crosses with 'Sprite'.

Generations	Pollen abortion (%)		
	Mean	SE	N
CIAT-*ms* seed parents	91	4.79[z]	18
CIAT-*ms* × Sprite (F_1)	91	5.11	18
(CIAT-*ms* × Spr) × Spr (BC_1)	94	4.44	56
(CIAT-*ms* × Spr) × Spr (BC_2)	96	2.97	33

[z]Range of observations was from 82 to 99% pollen abortion.

Apparently under field conditions the cyto-*ms* 'Sprite' BC_1 either had a very low selfing rate after prolonged flowering or there was a very low level of outcrossing that the netting failed to control.

The cyto-*ms* 'Sprite' BC_2 was grown in a screened greenhouse, and 53 plants had a mean selfed pod set per plant of 0.51 after 6 weeks of flowering and a mean seeds per pod of 3.5. It appears that the cyto-*ms* 'Sprite' BC_2 had a very low rate of self-pollination late in the flowering period, but this selfing rate would produce an insignificant percentage of selfed seeds (probably less than 1%) in an F_1 hybrid seed production field. The subsequent BC_3, BC_4, and BC_5 populations did not segregate for any fertile plants, but detailed data were not taken on pod set and seeds per pod.

The cyto-*ms* 'Sprite' BC_4 plots planted at Twin Falls, Idaho, were harvested after more than 8 weeks of flowering. A total of 452 cyto-*ms* 'Sprite' plants produced only 1094 pods, or 2.4 pods/plant. These pods had an average of 2.4 seeds/pod. These values are only slightly higher than the expected rates from selfing (Table 2). There was very little cross-pollination between the fertile 'Sprite' rows and the male-sterile rows. If these results are representative of the Snake River Valley, it is unlikely that F_1 hybrid seed can be produced in this region. Areas of California and Florida with high bumble bee populations would have the necessary insect vectors, but bean diseases transmitted through seed would be a problem in these areas. At Gainesville, Fla., where the bumble bee population is high, cyto-*ms* plants grown in the field have full pod set, unless protected by netting from the bumble bees.

There is evidence that the CIAT source of sterile cytoplasm has a very low reversion rate from sterile to fertile cytoplasm in small sectors of the plant, i.e. a low somatic reversion rate. A plant in the cyto-*ms* 'Sprite' BC_2 population had a single raceme bearing 3 well-filled pods. The 10 plants grown from a composite of this seed segregated for 6 fertile and 4 sterile plants.

Table 2. Pollen abortion rates, pod set, and seeds per pod in F_1 hybrid progenies derived from 19 bean cultivars crossed with the CIAT male-sterile source in BC_3 to 'Sprite'.

Pollen parent	Mean percent pollen abortion	Mean pods/ plant[z]	Mean seeds/pod	No. of plants
Astro	91 ± 3.7[y]	0.9	2.1	7
Blue Crop	90 ± 5.5	0.4	3.3	9
Burly	88 ± 5.4	0.2	1.0	5
Bush Blue Lake 274	88 ± 6.9	2.1	1.9	9
Gallatin 50	92 ± 1.1	0.8	2.0	5
Harvester	89 ± 2.8	0.4	1.0	8
Improved Tendergreen	91 ± 4.2	2.2	2.7	10
Oregon 1604	93 ± 5.4	1.7	1.5	9
Provider	88 ± 3.8	0.7	2.7	8
Roma	89 ± 4.6	2.3	1.9	6
Slenderwhite	93 ± 2.7	1.9	1.7	8
Spartan Arrow	91 ± 7.5	5.2	2.2	9
Sprite	94 ± 3.2	0.4	4.3	7
Tendercrop	96 ± 2.0	1.4	1.9	9
Topcrop	92 ± 5.1	5.2	1.7	9
Triumph	97 ± 1.1	2.9	4.9	9
Great Northern UI 59	92 ± 2.3	7.2	2.5	9
Pinto UI 111	91 ± 4.1	10.8	2.4	9
Great Northern Valley	90 ± 3.9	2.9	3.0	10

[z]No. pods set after 7 weeks of flowering.
[y]Mean ± SD.

The 6 fertile plants were crossed onto cyto-*ms* 'Sprite' BC_3 plants, and the 6 F_1 progenies of 30 plants each were tested in the field. All the F_1 progenies consisted entirely of male-sterile plants. This indicates that the 6 fertile parents were the result of a small chimera in which the sterile cytoplasm reverted to normal (back mutation of a cytoplasmic factor), and not the result of a nuclear gene mutation from maintainer to restorer mode of action.

The rate of somatic reversions to fertility in common bean appears to be quite low, but the data are not adequate to make a quantitative estimate. Cyto-*ms* was discovered in 1957 by D. A. Bond in faba beans (*Vicia faba* L.) and has been studied extensively (4). In faba bean the sterile cytoplasm is unstable and subject to high frequencies of somatic reversion to fertility, leading to permanent fertility restoration in progeny from revertant sectors of cyto-*ms* plants.

Screening for restorer genes. The results of crossing 19 commercial cultivars with cyto-*ms* 'Sprite' BC_3 plants are presented in Table 2. The average pollen abortion rate of the F_1 progenies was 91%, which is very similar to that obtained with 'Sprite'. The average pods per plant after 7 weeks of flowering was much more variable. Fifteen cultivars varied from 0.2 to 2.9 pods/plant, 2 had 5.2 pods/plant,and 2 dry bean cultivars had 7.2 and 10.8 pods/plant. Although these cultivars appear to vary in the degree of male sterility, they all should be classified as having maintainer genotypes.

Cytoplasmic male sterility in plants was reviewed recently by Pearson (2). Cyto-*ms* is a rare phenomenon in legumes, and no previous report of cyto-*ms* in common bean is known to us. Given the excellent maintainer genotype of 'Sprite', the conversion of the CIAT male-sterile cytoplasm to the nuclear genotype of 'Sprite' provides a good seed parent for the production of experimental F_1 hybrid snap bean cultivars in regions with high bumble bee populations. The F_1 progeny will be male-sterile unless the pollen parent has a restorer genotype. Much additional work is needed to identify bean accessions with fertility restoring genotypes and to study the genetics of fertility restoration.

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Influence of Leaching, Fertilizer Source and Rate, and Potting Media on Foliage Plant Growth, Quality, and Water Utilization[1]

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Abstract. **Method of fertilization application and leaching had little effect on response of *Dieffenbachia maculata* (Lodd.) G. Don 'Perfection' (dumbcane) and *Brassaia actinophylla* Endl. (schefflera). The recommended fertilizer rate increased top but reduced root growth. Plants utilized 5 to 10% more water when leached. In a second experiment, plant grade and vegetative growth decreased with a decrease in the fertilizer rate but root grade and growth increased. Plants growing in the peat/sand (3:1) potting medium were rated superior to plants in the peat/bark/shavings (2:1:1) medium. Leaching with 10% excess water or fertilizer solution slightly improved plant response of schefflera, but overall data indicate leaching of pots is unnecessary for short term foliage crops fertilized properly. Increased fertilizer rates increased water utilization efficiency and vegetative growth, but reduced root growth.**

Container-grown plants are usually watered with sufficient volume to result in some leachate containing nutrients such as nitrogen and phosphorus, and sometimes residues of insecticides and fungicides draining from pots. If potted plants could be grown without leaching, water usage and contamination of ground water would be reduced. An earlier experiment (2) demonstrated that foliage plants growing in leached pots were of better quality than plants growing in nonleached pots. However, plants growing in pots that were not leached received only 70% of the water received by plants in leached pots. Thus, plants growing in pots that were not leached may have experienced water deficiency. In another experiment, Poole and Henley (3) found that as amounts of fertilizer applied to foliage plants increased, water uptake and root grade decreased.

The 2 experiments discussed in this paper were conducted to determine effects of various fertilizer sources, rates, potting media, and leaching on plant growth and quality, and water utilization.

Materials and Methods

Experiment 1. A $3 \times 2 \times 2$ factorial experiment in randomized block design with 4 replications was established when 3 schef-

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