Theor Appl Genet (1987) 74:642–645

Genetics of fertility restoration in cytoplasmic male sterile *Phaseolus vulgaris* L.

1. Cytoplasmic alteration by a nuclear restorer gene *

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Received March 23, 1987; Accepted April 2, 1987
Communicated by R. Hagemann

Summary. Restoration of fertility in cytoplasmic male sterile *Phaseolus vulgaris* by line R-351 was controlled by a single gene. The restorer gene (*Fr*) displayed incomplete dominance leading to partial restoration of fertility in F_1 generations; full restoration was not achieved until the F_2 generation. Once full restoration of fertility was produced in the F_2 generation, no segregation for sterility was observed in subsequent generations derived from heterozygotes *Frfr*, either by testcrossing (restored × maintainer) or in F_3 progenies. Implications of the irreversible nature of this restoration are discussed.

Key words: Common bean – Cytoplasmic-nuclear interaction

Introduction

Cytoplasmic male sterility (CMS) in *Phaseolus vulgaris* was first reported by Bassett and Shuh (1982). The discovery of a source of CMS in *Phaseolus* is of interest for two reasons. CMS has been useful in development of hybrid seed cultivars in a number of important crops including maize, sugar beet, onion, and carrot. A source of CMS also provides a useful system for the study of cytoplasmic-nuclear interactions in higher plants.

The source of CMS in *P. vulgaris* was originally derived from accession line G08063 (Singh et al. 1980). The male sterile phenotype is stably maintained using the 'Sprite' snap bean nuclear genotype, and the original source of CMS has now been backcrossed for 10 generations to 'Sprite' (CMS-Sprite).

In order to evaluate the potential for hybrid seed development using this source of CMS, efficient sources of fertility restoration are required. In searching for restorers, breeding line R-351 was included and observed to effect restoration of fertility. This study was undertaken to determine the genetics of restoration using line R-351 as restorer and CMS-Sprite as a source of sterility.

Materials and methods

In Florida, three bean plant generations per year can be grown by planting two generations in screened greenhouses, the first in September and the second in January, and one generation in the field in April. Greenhouse plants were grown in 4-l pots. In-row spacing of field plantings was 10 cm. The single row plots were covered with netting prior to flowering to prevent insect pollinations.

In October, 1983, pollen from breeding line R-351 was used to hand-pollinate open flowers of CMS-Sprite. The following January F_1 seed was grown in the greenhouse and plants were observed for seed set. Seed was harvested from F_1 plants, and individual F_2 populations were grown in the field in April.

Within F_2 populations, fertile segregants were selected on the basis of 3 criteria described below: pollen stainability (IKI stain), pollen shed, and seed set. Pollen from fertile F_2 segregants was used to hand-pollinate CMS-Sprite. Therefore, the initial backcross population (BC_1) was derived from a cross using selected fertile F_2 plants as pollen donors, some of which were probably homozygous for the restorer gene. Individual BC_1 populations were grown in the greenhouse and evaluated for fertility using the same 3 criteria. Those plants within the BC_1 populations that had the highest fertility were backcrossed to CMS-Sprite (BC_2) and were also allowed to self and set seed (BC_1F_2). Similarly, BC_2 and BC_1F_2 populations were evaluated for fertility, and BC_2 plants with the highest fertility were backcrossed to CMS-Sprite (BC_3). The backcrossing process was repeated to BC_4 and F_2 populations for each backcross generation were also evaluated for fertility segregation.

* Florida Agr. Exp. Sta. Journal Series No. 7733

Unusually high temperatures that persisted over the period of bud initiation in the field occurred during evaluation of the BC_3 and BC_2F_2 generations. A second BC_3 population from remnant seed was later grown in the greenhouse.

Five selected fertile BC_3F_2 plants were reciprocally crossed to a maintainer genotype. This was done by using 'Sprite' snap bean pollen to pollinate fertile BC_3F_2 plants. Pollen from open flowers of the same 5 BC_3F_2 plants was used to pollinate CMS-Sprite. These BC_3F_2 plants were also allowed to self-pollinate for BC_3F_3 seed. The F_1 populations produced from $BC_3F_2 \times$ 'Sprite' were allowed to set F_2 seed.

All the above populations were classified for fertility using the following 3 criteria: pollen stainability, pollen shed, and seed set. Pollen stainability estimates involved squashing anthers from a mature unopened bud in a drop of IKI (Nagata and Bassett 1985; Jensen 1962). Viability was recorded as the percentage of darkly stained individual grains. At least 100 pollen grains per bud were counted. Pollen shed data were obtained by observing the amount of pollen on the stigma of an open flower. At least 3 flowers per plant were observed. Seed set data were taken by observing both the total number of pods produced and the number of seeds produced per pod.

Plants classified as "fertile" produced more than 70% stainable pollen, large amounts of gray pollen completely covering the stigma, and numerous well-filled pods with no evidence of parthenocarpy. "Semisterile" plants produced both stainable pollen (20–70%) and aborted tetrads, shed small amounts of pollen barely visible on the stigma, and produced on a single plant at least 4 seed-bearing pods plus numerous parthenocarpic pods. Plants classified as "sterile" produced less than 20% stainable pollen with nearly all pollen aborted in tetrads, shed no visible pollen on the stigma, and produced only parthenocarpic pods except for occasional plants with 1–2 poorly-filled seed pods in addition to the usual number of parthenocarpic pods for a sterile plant.

Table 1. Inheritance of fertility restoration on sterile cytoplasm, using R-351 as restorer, in F_2 populations of common bean

Population	No. of plants	Segregation for fertility		X^2 (3 :1)	*P*
		Fertile	Sterile		
F_2	57	45	12	0.47	0.49
BC_1F_2[a]					
2-19	69	53	16	0.12	0.73
2-24	69	56	13	1.39	0.24
BC_2F_2					
2-19-6	8	7	1		
2-19-29	42	32	10		
2-19-36	45	33	12		
2-19-44	20	18	2		
2-19-48	6	4	2		
	121	94	27	0.46	0.50
BC_3F_2					
2-24-13-7	22	18	4		
2-19-44-20	21	19	2		
2-19-44-3	10	8	2		
2-19-6-1	5	3	2		
2-19-36-48	6	3	3		
2-19-36-38	5	2	3		
2-19-29-14	5	2	3		
	74	55	19	0.017	0.90

[a] BC_1F_2 populations were derived from self-pollination of CMS-Sprite × fertile F_2 plants

Results and discussion

Two characteristics of CMS-Sprite have proven valuable in tests for restoration. One is the fact that pollen aborts at the tetrad stage in CMS material. The aborted tetrads are easily distinguished from aborted individual microspores produced by environmental stress. Another characteristic of CMS-Sprite is production of parthenocarpic pods. Parthenocarpy provides a valuable indicator of semisterility since plants not fully restored produce parthenocarpic and seed-bearing pods on a single plant.

In F_2 populations only a small number of plants (10% or less) could be considered semisterile under normal conditions (Conditions of extreme heat existed during bud formation in the BC_2F_2 generation and the semisterile class was larger). Since the semisterile segregants did not form a distinct Mendelian class, the fertile and semisterile classes were pooled so that F_2 populations were classified into two groups, fertile and sterile. All the F_2 populations clearly segregated in a 3 : 1 (fertile : sterile) ratio characteristic of a heterozygous locus with one dominant allele and the other allele recessive (Table 1).

Backcross populations segregated 1 : 1 (semisterile : sterile) with no fertile plants evident (Table 2). The appearance of a semisterile class, rather than the fertile class that one would ordinarily predict from the F_2 data, may be the result of a single gene with incomplete dominant action. However, with 1 : 1 (semisterile:sterile) segregation in BC populations the conventional prediction for F_2 segregation would be 1 : 2 : 1 (fertile: semisterile : sterile). This was not observed.

A simple monogenic model with incomplete dominance is not adequate to explain the observations in backcross and F_2 populations. Using *Fr* to designate the restorer allele, a backcross population should be made up of 50% sterile (*frfr*) and 50% semisterile (*Frfr*) plants. The F_2 population should then segregate 1 *FrFr* : 2 *FrFr* : 1 *frfr* and produce 50% semisterile plants. It appeared that in the F_2 population the restorer heterozygotes expressed full fertility.

A possible explanation for the difference in observed phenotypes of BC and F_2 plants heterozygous for the restorer may involve interaction in the maternal parent between the cytoplasm and the nuclear restorer gene. In the F_2 generation the cytoplasm was derived from a plant carrying a dose of *Fr*. In F_1 and backcross

Table 2. Inheritance of partial fertility restoration on sterile cytoplasm, using R-351 as restorer, in backcross populations

Population	No. of plants	Segregation for fertility		X^2 (1 : 1)	*P*
		Semi-sterile	Sterile		
BC_2[a]					
2-19	50	30	20		
2-24	44	16	28		
	94	46	48	0.04	0.84
BC_3 field					
2-19-6	16	11	5		
2-19-29	24	5	19		
2-19-36	46	16	30		
2-19-44	20	12	8		
2-24-13	8	5	3		
	114	49	65	2.24	0.13
BC_3 greenhouse					
2-19-41	40	21	19	0.10	0.75
BC_4					
2-19-41-2	24	11	13		
2-19-41-16	25	14	11		
	49	25	24	0.02	0.89

[a] BC_1 populations were derived from CMS-Sprite × fertile F_2 plants and were therefore not true backcross populations

Table 3. Segregation for partial fertility in F_1 populations produced from testcrosses of CMS-Sprite with 5 selected fertile R-351 derived BC_3F_2 plants

Population	No. of plants	Segregation for fertility		X^2 (1 : 1)	*P*
		Semi-sterile	Sterile		
CMS-Sprite ×					
2-24-13-7-1	14	8	6	0.28	0.60
2-24-13-7-12	15	7	8	0.06	0.81
2-24-13-7-14	15	11	4	3.26	0.07
2-19-44-20-12	14	6	8	0.28	0.60
2-19-44-3-3	14	7	7	0.00	0.99

Table 4. Inheritance of fertility restoration on sterile cytoplasm in testcrosses and derived generations of 5 selected fertile BC_3F_2 plants heterozygous for the restorer gene

Population	No. of plants	Classification
2-24-13-7-1 × Sprite	10	100% fertile
2-24-13-7-12 × Sprite	10	100% fertile
2-24-13-7-14 × Sprite	10	100% fertile
2-19-44-20-12 × Sprite	10	100% fertile
2-19-44-3-3 × Sprite	10	100% fertile
5 fertile BC_3F_2 × Sprite (F_2)	300	100% fertile
5 fertile BC_3F_2 selfed (BC_3F_3)	120	100% fertile

generations, however, the cytoplasm was derived from a plant which lacked the *Fr* allele. This model predicts that a generation of "preconditioning" (interaction between dysfunctional cytoplasm and nuclear restorer) is required to obtain full fertility. Furthermore, a plant heterozygous for a restorer gene (*Frfr*) with dysfunctional cytoplasm would appear less fertile (as in semisterile F_1 or BC populations) than would a restorer heterozygote on "preconditioned" (restored) cytoplasm, as in F_2 populations.

To test this hypothesis of cytoplasmic "preconditioning" by the restorer gene for full fertility, reciprocal crosses were made. Pollen from 5 fertile BC_3F_2 plants was used to pollinate CMS-Sprite. Pollen from 'Sprite' snap bean, a maintainer genotype, was then used to pollinate buds of the same 5 BC_3F_2 plants. Selfed seed from these same 5 plants was also collected.

A 1 : 1 segregation (semisterile : sterile) was observed in all CMS-Sprite × fertile BC_3F_2 populations, suggesting that all 5 selected BC_3F_2 plants were heterozygous for the restorer gene (Table 3). In the reciprocal crosses using the same 5 selected BC_3F_2 plants as females [BC_2F_3 × 'Sprite' pollen (*frfr*)], 100% of the F_1 progeny were fully fertile (10 progeny per cross for 50 plants total observed) (Table 4). To summarize: (1) CMS-Sprite (*frfr*) × selected BC_3F_2 (*Frfr*) segregated 50% semisterile (*Frfr*) and 50% sterile (*frfr*); and (2) selected BC_3F_2 (*Frfr*) × 'Sprite' (*frfr*) produced 100% fertile F_1 progeny.

The fertile F_1 progeny from the cross BC_3F_2 × 'Sprite' were allowed to set F_2 seed. Assuming 1/2 the F_1 progeny to be heterozygous for the restorer gene, one would expect segregation for *frfr* (sterile) progeny in the F_2 generation. One hundred F_2 plants from each of 3 crosses, a total of 300 plants, were grown and all were fully fertile (Table 4). The 5 selected BC_3F_2 plants used in reciprocal crossing were also allowed to set BC_3F_3 seed. Three BC_3F_3 populations of 40 plants each, a total of 120 plants, were grown and again no male sterile plants were produced; all BC_3F_3 plants were fertile (Table 4).

These results are consistent with a hypothesis that restoration of CMS is an irreversible phenomenon. Once full restoration is achieved in the F_2 generation, segregation for sterility in subsequent generations is no longer observed (i.e., plants of the genotype *frfr* are fertile). This would suggest that cytoplasmic-nuclear interaction in semisterile heterozygotes produces a "permanent" (stable) cytoplasmic alteration leading to full fertility in the next generation.

The change in the cytoplasm apparently does not occur immediately upon addition of *Fr*. Since the F_1

generation (CMS-Sprite × *Fr*/–) only results in semisterility, the cytoplasm is not yet fully restored. The condition of semisterility appears to be an intermediate stage, with only partial cytoplasmic function (with respect to microsporogenesis) or perhaps with a heterogeneous population of functional and dysfunctional organelles. Segregation of the nuclear restorer in the F_2 generation may be accompanied by cytoplasmic sorting of functional and dysfunctional organelles. The few F_2 plants (10% or less) appearing semisterile may be those heterozygous for nuclear restorer (*Frfr*) with cytoplasm not yet fully sorted or fully restored.

It should be noted that environment had a marked effect on levels of fertility in backcross populations. BC_3 populations grown in the field during extreme heat produced a larger proportion of sterile plants than did BC_3 plants grown in the greenhouse. Although the extreme temperatures resulted in a dramatic increase in the proportion of semisterile plants in BC_2F_2 populations grown under similar conditions, the proportion of sterile plants remained 25%.

Irreversible restoration has been observed in CMS *Vicia faba,* but full restoration is achieved in the F_1 generation with no subsequent segregation in the F_2 progeny (Bond et al. 1966). The CMS phenotype in *V. faba* is associated with the appearance of virus-like double-stranded RNA molecules in the cytoplasm. These RNAs disappear upon reversion or nuclear restoration (Grill and Garger 1981). Whether virus interaction is involved in expression of CMS in *Phaseolus* has yet to be determined.

Cytoplasmic alteration by a nuclear gene, although not reported before in *Phaseolus,* has been observed in maize. The nuclear gene *iojap,* which results in green and white sectoring of leaves in homozygous (*ij*/*ij*) plants, produces a cytoplasmic alteration such that the striped phenotype is maternally inherited in subsequent generations (Rhoades 1946). The *iojap* gene appears to affect plastids but with no detectable alteration of the chloroplast genome (Walbot and Coe 1979).

In many species, CMS appears to be associated with alterations of the mitochondrial genome (for review, Hanson and Conde 1985). Biochemical characterization of organellar DNA is, therefore, underway in an effort to detect cytoplasmic effects of the restorer gene *Fr* in common bean.

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