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The relationship between a nuclear restorer gene and the nuclear gene for male sterility in *Petunia**

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Summary. This paper describes the relationship between the restorer gene and the gene for male sterility in the background of normal cytoplasm. We combined these two traits by crosses in one plant, thus making genetic analysis possible. Two main conclusions can be drawn: 1. The restorer gene and the gene for male sterility are located at different loci which segregate independently one from the other. 2. The *Rf* allele does not affect the expression of the *e* allele.

Key words: Cytoplasmic male sterility (cms) – Nuclear male sterility (nms) – Fertility restorer (*Rf*) – Normal cytoplasm-Cytoplasmic male sterility inducing elements (ste)

Introduction

Male sterility (ms) in *Petunia* is characterized by the lack of pollen grains in otherwise normal anthers. Three types of genetic control systems on male sterility have been described in petunia: a multigenic control (Izhar 1983), a nuclear-cytoplasmic control (Edwardson and Warmke 1967; Izhar 1978, 1983), and a single nuclear recessive allele control (Frankel 1962, 1971).

The first type is apparently a result of the accumulation of several undefined deleterious alleles. This sterility is not stable as far as expression and inheritance are concerned; fertility is restored simply by crossing ms plant with any normal plant (Izhar 1983). The nuclear-cytoplasmic sterility is expressed as an interaction between cytopasmic male sterility (cms) inducing elements (ste) and a nuclear genome which lacks male fertility restoration alleles (mfr) (Duvick 1959; Edwardson and Warmke 1967; Izhar 1977, 1978, 1983). A single *Rf* dominant allele and several other quantitative genes which restore fertility are known in petunia (Izhar 1978). The third type, nuclear male sterility (nms), was discovered by Frankel (1962, 1971). The *e* allele in a homozygous condition was found in a F_2 segregant of a fertile scion grafted onto a cms stock (Frankel 1962). The *e* allele was suggested to be the "integrated state" of the original cytoplasmic sterility elements – the "autonomous state" of cms. The *e* allele is the only single recessive nuclear sterility factor known today in the genus *Petunia* (Izhar 1983). Other investigators have obtained the transmission of cms via grafting similar to Frankel (1956) in *Petunia* and other species: Edwardson and Corbett (1961), Bianchi (1963), Izhar (unpublished data) and Hanson (unpublished data) in *Petunia;* Curtis (1967) in sugarbeet, and Thompson and Axtell (1978) in alfalfa.

In all the above-mentioned graft transmission of cms, the transmissible trait was maternally inherited. Possible integration of the ste into the nucleus was suggested by Frankel (1962), to explain the obtainment of nuclear recessive male sterility by grafting. This observation remains unique. A long-term goal of our laboratory is to verify, using genetic and molecular methodologies, whether the *ste* and *e* are indeed the "autonomous state" and the "integrated state", respectively, of the same element. In only a few cases are the two kinds of sterility known to exist in the same species (Gottschalk and Kaul 1974).

The *Petunia* system offers a unique opportunity to study the interaction between cms, its restoration genes and nms. In the present paper we present genetic analysis which describe the relationship between the fertility restorer allele *Rf* and the allele for male sterility *e* in the background of a normal plasmon.

Materials and methods

All plant material was of *Petunia hybrida* Hort. Vilm. cv. 'Rosy Morn' (RM). The following abbreviations are used to designate the specific lines:

(S) – a male sterility inducing plasmon
(N) – normal fertile plasmon

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Rf – the dominant allele of the *Rf/rf* locus which restores fertility in the background of the (S) plasmon (Edwardson and Warmke 1967). *rf* – is the alternative allele in the *Rf/rf* locus.
e – The recessive allele of the *E/e* locus. *e* induces male sterility in normal cytoplasm (Frankel 1971).

The following plasmotypes were used as starting material, they were derived from a line of RM. At the beginning of this study an assumption was made that the restorer gene and the sterility gene are located at separate loci *Rf/rf* and *E/e*.
(N) *rfrfee* – plasmotype with normal cytoplasm, recessive non-restoring allele and male sterility allele under homozygous condition. Such a genotype was first obtained and described by Frankel (1962, 1971).
(N) *rfrfEE* – plasmotype with normal cytoplasm, non-restoring allele under homozygous condition and the homozygous fertile allele *E* (Izhar and Frankel 1976).
(S) *rfrfEE* – plasmotype with (S) cytoplasm, a cytoplasmic male sterile plant
(S) *RfRfEE* – a fertile phenotype with the (S) plasmon carrying the dominant restorer *Rf* allele.

Results

1 The relationship between the restorer gene and the gene for male sterility

One of the goals of this research was to define the relation between the restorer gene and the gene for ms in the background of a normal plasmon. To answer this question we made a cross between a ms plant and a plant with a homozygous allele for fertility restoration.

parental lines: (N) *rfrfee* (male sterile plant) × (S) *RfRfEE* (fertile plant)
↓
genotype of F_1: (N) *RfrfEe*

We assumed that the expected F_1 genotype must be (N) *RfrfEe* (this assumption is affirmed later). This is the first time the *Rf* allele and the *e* allele are present in the same genotype. Thereafter, we test-crossed the F_1 to its maternal homozygous recessive male sterile parent. The expected genotypes and phenotypes of such cross are shown in Table 1. Assuming (N) *Rfrfee* to be male sterile, the expected ratio between the progenies of the test cross would be 1 fertile to 1 sterile. Test crosses involving 26 F_1 plants yielded 328 fertile to 300 sterile plants. This ratio was found to fit the 1 : 1 ratio ($X_1^2 = 1.25$, homogenic $X_{25}^2 = 13.7$).

Table 1. The expected genotypes, phenotypes and their frequencies from the test cross (N)*rfrfee* × (N)*RfrfEe*

Expected genotypes	Expected phenotypes	Expected frequency
(N)*RfrfEe*	fertile	1/4
(N)*rfrfEe*	fertile	1/4
(N)*Rfrfee*	?	1/4
(N)*rfrfee*	sterile	1/4

These results indicated that (N) *Rfrfee* is phenotypically sterile. However, further analysis of the plasmotype was required to confirm that the genotype (N) *Rfrfee* indeed exists. Selfing the double heterozygote (N) *RfrfEe* was ecpected to produce the pattern of genotype distribution typical of two independent genes.

If the genotypes (N) *RfRfee* and (N) *Rfrfee* are male sterile, the expected ratio between the progenies of the selfed plant should be 3 fertiles to 1 sterile. If the genotypes (N) *RfRfee* and (N) *Rfrfee* are male fertile the expected ratio between the progenies of the selfed plant should be 15 fertiles to 1 sterile. Selfing F_1 plant yielded 170 fertile and 58 sterile plants. The ratio was found statistically to fit a 3 : 1 fertile to sterile ratio ($X_1^2 = 0.026$).

The F_2 plant population obtained from (N) *RfrfEe* selfed were genetically analyzed as described below.

2 Genetic analysis of the male fertile progenies

In order to determine the allele composition of each of the loci in each F_2 segregant, two test crosses were performed. To determine the genotype for the ms locus (*E/e*) crosses were made as described in Table 2. The test crosses performed to determine the genotype for the *Rf/rf* locus are described in Table 3. The test crosses to determine the genotypes of the fertile progeny are given in Table 4. Note that progenies that carry the *EE* or *Ee* alleles in their genome were fully fertile, independent of the *Rf/rf* background. The *E* allele in one or two dosages yield the same level of pollen fertility.

Table 2. Test crosses to determine the genotypes for the *E/e* locus in fertile plants

Genotype to be identified	Genotypes obtained by test cross to the maternal parent (N)*rfrfee*	Expected progeny phenotypes
(N)*rfrfEE*	(N)*rfrfEe* – fertile	all fertile
(N)*rfrfEe*	(N)*rfrfEe* – fertile (N)*rfrfee* – sterile	1 : 1 fertile to sterile

Table 3. Test crosses to determine the genotypes for the *Rf/rf* locus in fertile plants

Genotype to be identified	Genotype obtained by test cross to the maternal parent (S)*rfrfEE*	Expected progeny phenotypes
(N)*RfRfEE*	(S)*RfrfEE* – fertile	all fertile
(N)*RfrfEE*	(S)*RfrfEE* – fertile (S)*rfrfEE* – sterile	1 : 1 fertile to sterile
(N)*rfrfEE*	(S)*rfrfEE* – sterile	all sterile

Table 4. Crosses to determine the genotype of fertile progeny of (N)*RfrfEe* selfed

Plant no.	Progenies of a cross with (S)*rfrfEE*		Progenies of a cross with (N)*rfrfee*		Genotype
	Fertile	Sterile	Fertile	Sterile	
4587-1	20	–	8	–	(N)*RfRfEE*
4587-2	9	–	40	–	(N)*RfRfEE*
4587-3	29	–	17	14	(N)*RfRfEe*
4587-5	29	–	5	3	(N)*RfRfEe*
4587-7	5	10	45	–	(N)*RfrfEE*
4587-9	13	17	28	–	(N)*RfrfEE*
4587-10	8	10	34	–	(N)*RfrfEE*
4587-14	11	6	13	12	(N)*RfrfEe*
4587-15	19	7	16	10	(N)*RfrfEe*
4587-20	16	15	20	13	(N)*RfrfEe*
4587-22	–	17	58	–	(N)*rfrfEE*
4587-24	–	30	41	15	(N)*rfrfEe*
4587-26	–	24	8	11	(N)*rfrfEe*
4587-28	–	19	18	16	(N)*rfrfEe*

3 *Genetic analysis of the male sterile progenies*

In this part we describe the attempts to determine the genotypes of the male sterile segregants obtained by selfing of the double heterozygote (N) *RfrfEe*. The scheme to determine the situation in the *Rf/rf* locus is given in Table 5.

The first step in identifying the *Rf/rf* locus was to cross the sterile plants with (N) *rfrfEE* as male parent to obtain fertile progenies. In the second step we crossed the fertile progeny to (S) *rfrfEE* – any sterile segregant should be homozygous recessive for *rfrf*. The identification of *rfrf*, *Rfrf* and *RfRf* was possible by using the scheme described in Table 5. The test crosses to determine the male sterile plants genotype are presented in Table 6.

The results reaffirmed that all the male sterile plants with normal cytoplasm were homozygous recessive for the *e* allele independent of the *Rf/rf* background. Thus, a direct proof was obtained that the genotypes (N) *RfRfee* and (N) *Rfrfee* were male sterile. Table 7 summarizes the genotypes, phenotypes of the plants obtained by selfing the double heterozygote (N) *RfrfEe*.

Table 6. The test cross to determine the genotypes of three male-sterile plants

1 Tested sterile plant no.	2 Progenies of crosses with paternal parent (N)*rfrfEE*	3 Segregation of the progenies from the cross between plants of column 2 to the maternal parent (S)*rfrfEE*		4 Genotype of sterile plants tested
		Fertile	Sterile	
4587-7S	4587-7S-1	4	1	(N)*Rfrfee*
	4587-7S-2	7	4	
	4587-7S-3	9	9	
	4587-7S-4	11	7	
	4587-7S-5	–	10	
	4587-7S-6	–	13	
	4587-7S-7	–	15	
4587-16S	4587-16S-1	4	5	(N)*RfRfee*
	4587-16S-2	5	7	
	4587-16S-3	8	5	
	4587-16S-4	5	5	
	4587-16S-5	6	4	
	4587-16S-6	5	5	
	4587-16S-7	4	4	
	4587-16S-8	4	5	
4587-4S	4587-4S-1	–	10	(N)*rfrfee*
	4587-4S-2	–	11	
	4587-4S-3	–	3	
	4587-4S-4	–	8	
	4587-4S-5	–	14	
	4587-4S-6	–	10	
	4587-4S-7	–	12	

Table 5. Two-step scheme to determine the allelic composition in the *Rf/rf* locus of the sterile plants

Possible genotype of the sterile plants	(N)*RfRfee*	(N)*Rfrfee*		(N)*rfrfee*
First test cross	× (N)*rfrfEE*	× (N)*rfrfEE*		× (N)*rfrfEE*
Expected genotypes (all fertiles)	(N)*RfrfEe*	(N)*RfrfEe*	(N)*rfrfEe*	(N)*rfrfEe*
(S)*rfrfEE* used as tester	(S)*rfrfEE* × (N)*RfrfEe*	(S)*rfrfEE* × (N)*RfrfEe*	(S)*rfrfEE* × (N)*rfrfEe*	(S)*rfrfEE* × (N)*rfrfEe*
Expected genotypes and their phenotype	(S)*RfrfEE*-fertile (S)*RfrfEe*-fertile (S)*rfrfEE*-sterile (S)*rfrfEe*-sterile 1 : 1 fertile to sterile progenies	(S)*RfrfEE*-fertile (S)*RfrfEe*-fertile (S)*rfrfEE*-sterile (S)*rfrfEe*-sterile 1 : 1 fertile to sterile progenies	(S)*rfrfEE*-sterile (S)*rfrfEe*-sterile all sterile progeny	(S)*rfrfEE*-sterile (S)*rfrfEe*-sterile all sterile progeny

Table 7. Genotypes and phenotypes obtained by selfing double heterozygous plants. $\chi^2 = 15.03$; $\chi^2 = 0.05$, 8 = 15.51

Genotype	Phenotype	No. of plants	
		Expected	Observed
(N)*RfRfEE*	fertile	7.75	7
(N)*RfRfEe*	fertile	15.5	6
(N)*RfRfee*	sterile	7.75	8
(N)*RfrfEE*	fertile	15.5	13
(N)*RfrfEe*	fertile	31.0	32
(N)*Rfrfee*	sterile	15.5	26
(N)*rfrfEE*	fertile	7.75	5
(N)*rfrfEe*	fertile	15.5	18
(N)*rfrfee*	sterile	7.75	9
Total		124	124

The results show that all the expected genotypes were available and were in a good agreement with their expected frequency ($X^2 = 15.030$, $X^2 = 0.05$, 8 = 15.51).

Discussion

In the present study we tested whether the restorer gene is different from the male sterility gene and whether the *Rf* allele interacts epistatically with the integrated recessive allele *e* in the background of a normal cytoplasm of *Petunia*. The first indication that the dominant restorer *Rf* allele did not affect the expression of *e* in homozygous condition came from backcrossing the double heterozygote (N) *RfrfEe* to its maternal parent (N) *rfrfee* (Table 1), and from selfing the (N) *RfrfEe* which yielded fertile to sterile progenies at a ratio of 3 : 1.

To generate all the possible combinations between *Rf*/*rf* and *E*/*e* loci in order to provide direct proof about the relationship between them, we analyzed the selfing population of the double heterozygote (N) *RfrfEe*. The data showed that the genotypes *EE* and *Ee* were fully fertile while *ee* were sterile independent of the condition in the *Rf*/*rf* locus. This means that *Rf* did not interact epistatically with *e*, viz., did not affect the expression of *e* in the homozygous condition. *E* expressed complete dominance over *e*, plants that were *Ee* produced normal pollen carrying both types of gametes (half of the polen grains carrying *E* and about half carrying *e*). The same intralocus relationships were found for the *Rf*/*rf* alleles. These results showed that gametic selection did not take place. Selfing the (N) *RfrfEe* produced all the expected genotypes in good agreement to the expected frequency. These results showed that *E*/*e* and *Rf*/*rf* were different and unlinked genes. Frankel (1971) proposed that *e* is actually the "integrated state" of the original cytoplasmic sterility element and showed that *E* (the dominant allele of *e*) did not restore cms, and hence the action of *E*/*e* was not another type of cytoplasmic sterility.

If *Rf* would have restored epistatically the sterility *ee*, we could perhaps conclude that ste and *e* are the same elements. Since this was not the case in the present study, we cannot prove or disprove Frankel's hypothesis.

This work is the first step in finding whether ste and *e* are indeed the 'autonomous state" and the "integrated state", respectively, of the same element. Our next step will be to find whether mutual exclusion between the *e* and the ste, as suggested by Frankel (1971), exists, or if these elements can co-exist in the same cell.

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