FURTHER EVIDENCE ON GRAFT INDUCED TRANSMISSION TO PROGENY OF CYTOPLASMIC MALE STERILITY IN PETUNIA[1]

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GRAFT induced transmission to progeny of cytoplasmic male sterility in Petunia has been previously demonstrated (FRANKEL 1956), when it was found that normal male fertile plants grafted on male sterile stocks, though remaining phenotypically unaltered, yielded some male sterile progeny. This phenomenon in Petunia was debated—and doubted—by various workers (SAND 1960; GABELMAN, personal communication); recently it has been checked by EDWARDSON and CORBETT (1961), and they state in their report that their results verify in general lines asexual transmission of cytoplasmic male sterility thru grafts.

The aim of the present note is a corroboration of the initial results published (FRANKEL 1956). It provides a section of the information assembled by the present author, through comprehensive investigations since 1956, illustrating results mainly by the pedigree of one graft combination.

Male sterile lines used as graft components were P-431-53 (ms)[2] backcrossed for 3–6 generations to a male fertile maintainer—the variety "Rosy Morn". Independence of the cytoplasmic male sterility in these lines from nuclear control was verified as follows: (1) ten backcrosses to "Rosy Morn" variety yielded male sterile progeny only (a total of 844 plants); (2) crosses with ecotypes of the wild "species" *Petunia axillaris* (Friess) and *P. violacea* (Lindl.) yielded male sterile plants only (84 plants); (3) crosses with six commercial petunia varieties yielded a total of three male fertile and 476 male sterile plants (the three male fertile plants were obtained from the fifth backcross of P-431-53 (ms) × "Black Prince" giving a progeny of three fertiles:93 male steriles). The fertile petunia varieties used by the author (including the "Rosy Morn" variety) have upon selfing or sibbing yielded only an occasional male sterile plant. Ten successful reciprocal cleft grafts were made in November 1957 between male sterile and fertile "Rosy Morn" (maintainer) components, planted in August 1957. Cuttings of both types of components served as control plants (Figure 1). In this study sterility was visually determined and verified by microscopical pollen analysis.

Genotypic control on the transmission phenomenon: DUVICK (1959), citing a

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[2] The original male sterile line was kindly furnished by W. Atlee Burpee Co.

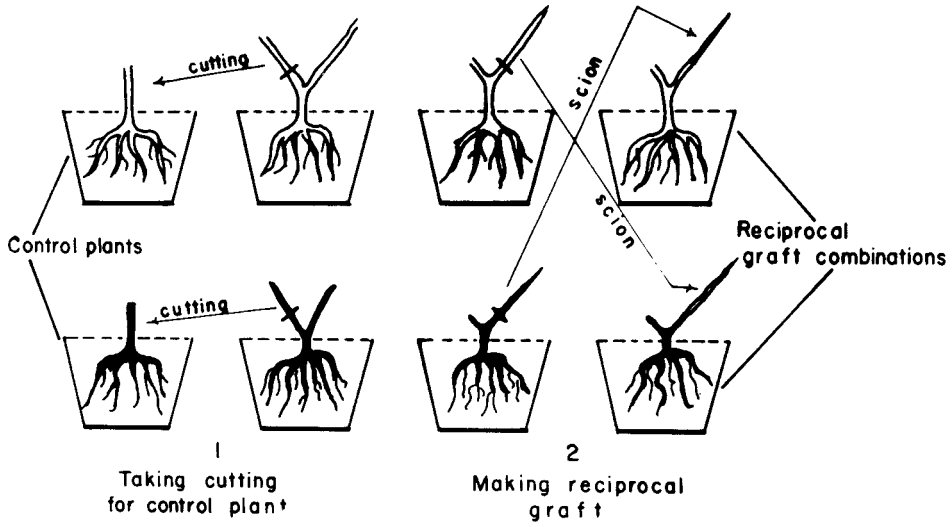

FIGURE 1.—Procedure of reciprocal grafting and securing control plants.

personal communication with G. A. GOLDSMITH, states that the cytoplasmic male sterility in petunia is influenced by the genotype. On the other hand J. LOWE (personal communication 1961) states that "so far as I know, no one has as yet developed lines of petunia carrying cytoplasmic male sterile factor and pollen restoring genes". It has been shown that within the material used in the present study cytoplasmic male sterility is not subject to nuclear control. But two pieces of evidence indicate the presence of genotypic control on the graft induction of male sterility in the progeny of fertile graft components. First, only part of the fertile graft components (25%) yielded some male sterile F_1 progeny from selfing. Second, even in fertile graft components yielding some male sterile progeny, different pollen sources resulted in different proportions of male sterile progeny (Figure 2). This, if restorer genes are excluded, indicates the presence

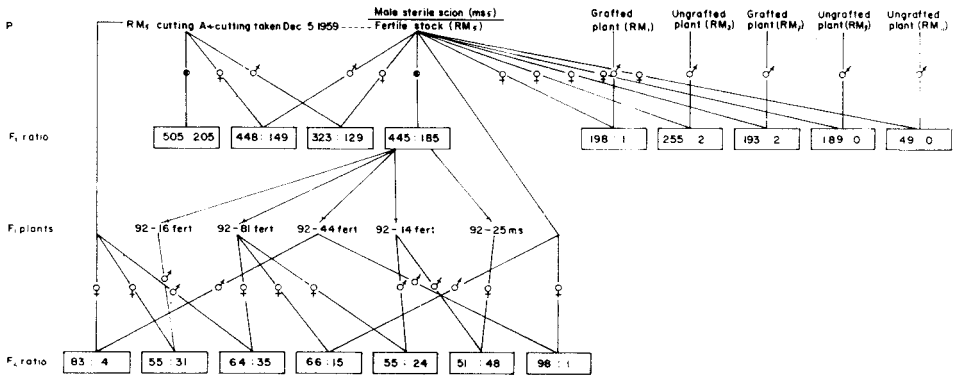

FIGURE 2.—Diagram of one of the pedigrees.
Graft combination shown in upper middle. Ratios (shown in boxes) are fertiles:male steriles. F_1 ratios of cutting and selfed stock are bulk results of progenies shown in Table 1.

of genotypic control on the graft induction of male sterility in the progeny of fertile graft components. In the case illustrated in the pedigree, segregation into male steriles and fertiles occurred also in the F_2, suggesting monogenic control of the transmission. In other cases (as in the case reported by FRANKEL 1956) male sterile F_1 progeny yielded only male sterile progeny for nine generations independent of the pollen parent used. Fertile progeny yielded only fertile progeny for ten generations. This behavior is identical with the stability of maternal transmission of male sterility exhibited by the male sterile material used as graft components.

Environmental influences on the transmission phenomenon: Multidirectional transmission between graft symbionts has been indicated (Table 1). All genotypes gave equal proportions of male sterile F_1 progeny, whether serving as stocks or scions in grafts with male sterile plants. Proportions of fertile and male sterile plants obtained by selfing fertile graft components were shown to be independent of the time lapse between grafting date and seed harvest. Proportions were uniform during a period of over 3½ years for a plant, in which the fertile component served as a stock for a sterile scion (Table 1). However, a general tendency for a somewhat higher proportion of male sterile plants in the progenies of fertile graft components was found in seeds harvested during the winter months, than during the summer months. By selfing a graft component serving as stock to a male sterile scion F_1 bulk results were significantly different (at the five percent level): summer progeny (seed harvested May to October) 1267 fertiles to 469 (26.4%) steriles; winter progeny (seed harvested November to April) 433 fertiles to 201 (31.7%) steriles. Similar results have been obtained with other graft combinations. When a cutting is separated from a fertile graft component, the transmission phenomenon usually ceases in the cutting a short time after the separation from the male sterile component. But in the case illustrated in Table 1, where a graft component served as stock for a male sterile scion, the transmission effect stayed latent in the cutting after its separation from the stock, and continued to express itself unimpaired one year after separation. An additional cutting (RM_5 cutting II, in Table 1), taken from the first cutting (RM_5 cutting A, in Table 1) continued to give the same proportions of male sterile F_1 progeny as the original graft component and the first cutting. All cuttings stayed autonomous in their own male fertility.

Phenotypic autonomy of graft components: In all graft combinations phenotypic autonomy of fertility on the fertile components and of male sterility on the male sterile components of the graft combinations have been observed unaltered during the lifetime of the grafts. Thus, the fertile components always remained normal and produced normal anthers, as did the control plant, and the male sterile component remained unaltered as its control plant. It should be emphasized, that all graft combinations lived for at least one year, and two combinations were maintained for 2 and 3½ years, respectively, still showing their autonomy in male fertility or sterility by producing abundant viable pollen or no pollen at all.

The phenotypic autonomy of the graft components is hard to interpret after

TABLE 1

Fertile and male sterile progeny (F_1) of the fertile plant RM_5 in its five forms

Seed harvest date		RM_5 ⊗		RM_5 scion ⊗			RM_5 stock ⊗			RM_5 cutting A ⊗			RM_5 cutting II ⊗			RM_5 stock × RM_5 cutting A			RM_5 cutting A × RM_5 stock		
	Lines No.	Fer.	Ste.	Lines No.	Fer.	Ste.	Lines No.	Fer.	Ste.	Lines No.	Fer.	Ste.	Lines No.	Fer.	Ste.	Lines No.	Fer.	Ste.	Lines No.	Fer	Ste.
March 1958	N_{17}	70	0	11+21	46	23	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
February 1959	N_{16}	102	0	86	40	28	92	43	41	..	..	..	..	..	..	..	..	..	..	..	..
August 1959	N_{29}	87	0	..	..	..	67	75	30	..	..	..	..	..	..	..	..	..	..	..	..
May 1960	..	..	..	..	..	..	..	..	..	99	19	16	..	..	..	104	21	10	101	37	13
June 1960	..	..	..	..	..	..	103+107	102	31	100	23	9	..	..	..	..	..	..	102	31	10
July 1960	..	..	..	..	..	..	..	..	..	105	46	19	..	..	..	..	..	..	..	..	..
August 1960	..	..	..	..	..	..	108	20	6	106	70	32	..	..	..	111+127	159	68	109 110 124	200	70
September 1960	..	..	..	..	..	..	122+123	138	45	118 119 120	215	73	..	..	..	128	69	27	125+126	109	31
November 1960	..	..	..	..	..	..	137	67	32	132+133	132	56	140+144	139	54	138	74	24	134	71	25
Total plants	..	259	0	..	86	51	..	445	185	..	505	205	..	139	54	..	323	129	..	448	149

RM_5 = Ungrafted plant obtained as cutting prior to grafting, October 1957.
RM_5 scion = Scion grafted on male sterile stock MS_5 in November 1957.
RM_5 stock = Plant serving as stock to the male sterile scion MS_5 grafted November 1957.
RM_5 cutting A = Cutting taken from RM_5 stock in December 1959.
RM_5 cutting II = Cutting taken from RM_5 cutting A in June 1960.
Fer. = Fertiles.
Ste. = Steriles.

3½ years as being the result of a required adaptation period for the sterility determining entities. Since genotypical control on the transmission phenomenon has been indicated, either suitable genotypes might not behave autonomously, or the transmission occurs on the zygotic (or developing embryo) level only. Since latency of the phenomenon for a long time has been shown in a cutting separated from the male sterile component, and hence no active movement of sterility determining entities or symbiosis is apparently required in all genotypes, the former interpretation of autonomy seems more likely.

On the basis of the partial sterility obtained in their material, and parallels drawn by the transmission methods, EDWARDSON and CORBETT (1961) suggest, that the nature of cytoplasmic male sterility in petunia is indeed analogous to a disease resulting from a virus infection. But it is not easy to explain the autonomy of graft components for 3½ years on the basis of an adaptation period for the virus, required in the maintainer cytoplasm. In the light of the facts reported above, i.e. absence of genotype-cytotype interaction on the trait in the material used but genotypical control on its induction, we would have to assume that genotypes influence this adaptation, but once the "virus" has become adapted, the genotypes do not affect susceptibility. Since in most cases reported occurrence of cytoplasmic male sterility in plants could be traced to wide crosses (usually interspecific), it is obvious, that by this virus infection hypothesis, genotype-cytotype interaction would have to be responsible for the activation or production of the "virus" by development from cellular constituents.

In the light of the evidence available a number of alternative explanations for the transmission phenomenon can still not be ruled out: (a) Movement of sterility determining entities of the cytoplasm; (b) Sterility determining entities must not necessarily move by themselves. Cytoplasmic information could be transmitted across graft unions via a carrier transducing some entity; (c) Symbiotic action might cause an increase or decrease of sterility or fertility determining entities in the cytoplasm; (d) The alteration of a normal constituent of the cytoplasm to a sterility determining entity could be affected thru symbiotic interaction between the graft components. A transformation or mutation of mitochondria to virus-like particles, so not entirely convincing, has been advocated (WOODS and DU BUY 1943; DU BUY and WOODS 1959). Gene induced mutation of a cytoplasmic factor for male sterility has been observed (RHOADES 1950). Genetic material of a virus might be tied to a specific locus of the host and thus behave as a cellular constituent, which in turn may develop into a pathogenic form (JACOB and WOLLMAN 1957).

The altered function, i.e. breakdown of the microspore in cytoplasmic male sterility in petunia, could not yet been correlated with some altered morphology of a cytoplasmic constituent. Evidence which will enable us to discriminate between the alternative explanations for the transmission phenomenon, will shed light on the nature of the cytoplasmic hereditary determinants themselves. Such evidence is sought in studies in progress. Full details of these studies will be published elsewhere in due course.

SUMMARY

Comprehensive investigations confirmed graft induced transmission to progeny of cytoplasmic male sterility in petunia. Evidence shows genotypical control of the transmission, independence of results on the position of the graft components, and the time lapse between grafting date and seed harvest. Latency of the phenomenon in separated graft components has been demonstrated in one case, and not been verified in others. Graft components remain phenotypically autonomous.

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