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Attempted Pollen-transmission of Cytoplasmic Male Sterility and the Spontaneous Occurrence of Male Sterility in O-type Lines of Sugar Beet (*Beta vulgaris* L.)

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Summary. Several instances of the occurrence of male-sterile individuals in progenies of O-type sugar beet lines are described, and the theoretical explanations considered. An attempt was made to transmit cytoplasmic male sterility through massive pollination by plants carrying *S*-plasm and fertility-restoring genes. No male sterility was demonstrated in the F_1 generation, nor in the backcross to the O-type line used as the original recipient. It was concluded that pollen-transmission did not provide an acceptable explanation of the observed degree of contamination of the O-types, and that *N*-plasm may have changed into *S*-plasm in these cases. Segregation of the monogerm character in the same material could not be satisfactorily explained in terms of simple genetic control.

Introduction

Heritable male sterility is widely employed by sugar beet breeders in the commercial production of hybrid varieties. The most useful form of male sterility for this purpose is that conferred by the plasmic factor *S* described by Owen (1942) since this theoretically allows the production of wholly male-sterile progenies. There is, however, additional genetic control of the expression of this sterility by dominant genes which Owen designated *X* and *Z*; full male sterility being manifested only by the double recessive genotype *xxzz*. It is necessary to establish for each male-sterile breeding line a pollinator line possessing normal cytoplasm (*N*-plasm) and the genotype *xxzz*, and such a pollinator is known as an O-type or zero-restorer since it will not restore male fertility to the progeny of the male-sterile line.

For many years this male sterility has been employed successfully in the manner suggested by Owen (1948), but it is generally acknowledged by breeders that the genetic control of sterility expression is more complex than that postulated by Owen, and much influenced by the environment. More recent investigations by Bliss and Gabelman (1965) and Rohrbach (1965) have supported modifications of this theory to take account of different types of genes and *S*-plasm respectively, while the Genetics and Breeding study group of the Institut International de Recherches Betteravières has recently completed an investigation into climatic influence on sterility expression (*in litt*).

The reported transference of cytoplasmic male sterility through graft unions in *Petunia* Juss. by Frankel (1956 and 1962) and Edwardson and Corbett (1961) and the suggestion by Atanasoff (1964) that plasmic factors may be seed-transmissible viruses, resulted in attempts by many workers to transfer male sterility to fully fertile beets by various means. Grafting experiments by Cleij (1967) failed to demonstrate transmission, as had those reported earlier by Owen (1949), and although two male-sterile plants were obtained by Theurer, Hecker and Ottley (1968), these were considered by the authors to be the result of misclassification or mutation. Curtis (1967) described the successful transmission of cytoplasmic male sterility from SLC O2 MS to its equivalent O-type[1] on a scale inconsistent with the explanation of mutation; but graft-chimaera formation could have produced this result since scion and stock were only distinguishable in respect of the sterility itself. Cytoplasmic change was cited by Cleij (1967) as a possible explanation of his thermal induction of male fertility in plasmatic male-sterile material, and also the segregation of male-sterile plants by certain O-type lines.

The first reports of male sterility in O-types came in 1962 independently from Hawkins and Laby, during a meeting of the Genetics and Breeding study group of the I.I.R.B. (unpublished). Hawkins encountered this phenomenon among monogerm lines developed from United States Department of Agriculture material, and although the possibility of seed-contamination by a male-sterile line could not be ruled out on this occasion it was considered to be remote. Laby obtained male-sterile progeny from isolated O-type plants under experimental circumstances that appeared to preclude the possibility of seed-contamination. Subsequently Hawkins (1971) observed sporadic occurrence of male-sterile plants among O-types at the Anglo-Maribo Station, until in 1971 one or two small progenies proved almost totally male-sterile.

In his publication Cleij (1967) noted that the O-type of Owen's SL 9460 sometimes segregated up to ten per cent male-sterile plants, and that subsequent-

[1] Lines developed by the United States Department of Agriculture in Utah.

ly the sterility was inherited plasmically. Cleij attempted mechanical transmission of male sterility by sap- and aphid- infection to test other explanations of the phenomenon, but without success.

At Cambridge occasional male-sterile individuals were encountered in two monogerm 0-type progenies flowering in the field in 1969, and numerous examples were furnished by two other monogerm lines flowering in the field in 1971. One of these latter also flowered under glass, and it was possible to examine anthesis and pollen production closely. In this population, consisting of 111 plants, 20 were male-steriles of the 'white anther' type while the remainder were classified as fully fertile.

After the first accounts by Hawkins and Laby it was decided to test the theory that the heritable particles in the cytoplasm could be transmitted in small amounts to the zygote through the pollen. Such a phenomenon might assume practical importance in circumstances where massive pollination occurred (e.g. in pollination bags) if many pollen grains were able to contribute cytoplasm simultaneously to one egg cell.

Material and Method

In order to test pollen transmissibility of the plasmic factor *S* it was necessary to develop a strain of beet carrying the plasmic factor and the dominant genes which restore male fertility. A further requirement was the presence of a distinctive dominant marker gene so that any selfed progeny could be identified. It was decided to employ a table beet as the source of fertility-restoring genes, as this would also provide dominant genes for red anthocyanin pigmentation in the progeny. The 0-type was a monogerm line homozygous for green hypocotyl colour, and its male-sterile equivalent provided the *S*-plasm (Table 1). This latter arrangement might be expected to enhance the expression of any male sterility in subsequent backcross generations, owing to the special compatibility of the male-sterile with its own 0-type.

During 1963 seedlings of SLC 91 MS and Blood Red Globe were raised in the glasshouse and vernalized in a refrigerator to induce flowering. In 1964 individual plants of SLC 91 MS were bagged with plants of Blood Red Globe as first flowers opened, and the resulting seed harvested from the male-sterile plants was bulked and designated (MS × BRG) (Fig. 1). This seed was sown under glass, and all seedlings proved to be deeply coloured with anthocyanin. The plants were vernalized and

Table 1. *Description and origin of sugar beet material used in the pollen-transmissibility experiment*

Population	Description
cv. Blood Red Globe	Commercial multigerm red table beet, possessing fertility-restoring genes
Line SLC 91	United States Department of Agriculture, monogerm 0-type line; Cambridge reselection homozygous for green hypocotyl
Line SLC 91 MS	Cytoplasmic male-sterile equivalent of SLC 91; Cambridge reselection homozygous for green hypocotyl

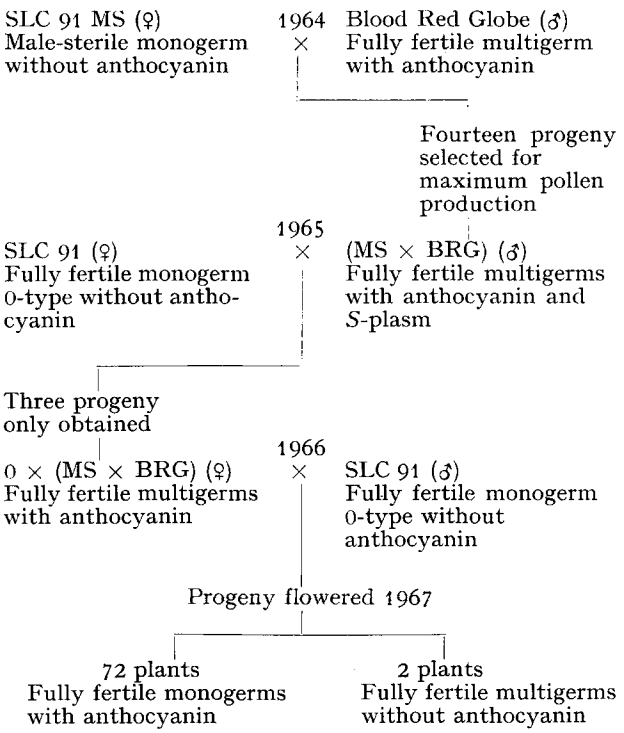

Fig. 1. Chart showing derivation of plant material, with notes on pollen fertility, anthocyanin pigmentation and monogermity

brought to flower the following year, and fourteen individuals selected for maximum pollen production were placed around a single flowering plant of SLC 91 to ensure that the latter received massive pollination. Fruit from the SLC 91 was mostly inviable, and only three seedlings were obtained; all with red hypocotyls confirming (MS × BRG) as the pollen parent. These plants were vernalized and brought to flower in 1966. All flowering plants in each generation were grown in the vicinity of fully fertile plants, both monogerm and multigerm, which acted as controls in the assessment of pollen production as a measure of male fertility.

Results

Male fertility in the table beet *cv.* Blood Red Globe was similar to that of multigerm sugar beets; copious pollen being liberated from well-developed anthers. Pollen production in the 0-type SLC 91 was considerably less, but similar to that of monogerm 0-types used as controls. The SLC 91 MS material was fully male-sterile, with shrunken pale green or white anthers which rapidly darkened to brown or black after the flowers opened.

Progeny from the cross of SLC 91 MS × Blood Red Globe were multigerm, and demonstrated a wide range of male fertility; some being totally male-sterile with shrunken bright crimson anthers. Some semi-sterile forms with reddish anthers shed small quantities of deep orange pollen, but many appeared fully fertile with normal yellow pollen. Only these latter were employed in the backcross, since the intention was to achieve pollen saturation of SLC 91.

The three backcross individuals were all multigerm, not demonstrably different in pollen production from neighbouring multigerm sugar beet plants; and clearly greatly superior to their monogerm parent in this respect. There was no sign of any deformity or abnormal pigmentation on the anthers of these plants throughout the flowering period, so the expression of any *S*-plasm present must have been nullified by the influence of restoring genes. For this reason it was necessary to use SLC 91 O-type as a donor of *x* and *z* genes and seek male sterility among the second backcross progeny.

The second backcross provided 74 plants; 72 of which were red-pigmented and monogerm while the other two were green-hypocotyl multigerms. These plants were raised in smaller pots than had been used for previous generations, and for this reason they produced fewer flowers and less pollen; as did also the ten control plants of Blood Red Globe. None, however, produced any male-sterile flowers or deformed anthers.

Conclusions and Discussion

The experiment showed that in this material there was no pollen-inheritance of *S*-plasm detectable, either in the immediate progeny, nor in the backcross to an O-type pollinator. It must be concluded that if *S*-plasm is pollen-transmissible in sugar beet, it is not normally thus transmitted in sufficient concentration to be demonstrated in an experiment of this nature. It would be still less likely to account for the observed instances of male-sterile contamination of O-types in the field, in view of the relative scarcity of *S*-plasmic pollen in breeders' multiplication plots. The possibility remains that pollen-transmission occurs only occasionally; perhaps as the result of abnormal behaviour of the pollen tube and egg-apparatus at time of fertilization; and consequently would be difficult to demonstrate with small-scale experiments. It will, nevertheless, be of value to consider the other alternative explanations for the anomalous behaviour of these O-types.

The risk of seed contamination by threshing and processing equipment is usually considerable with large-scale equipment, but when seed samples are also large the proportional carry-over of seed is negligible. In the case of small experimental seed lots as used by Laby and in the work at Cambridge, special small machines are employed so that the contamination fraction again is slight. The proportion of male-sterile progeny both at Anglo-Maribo and Cambridge would appear to be well outside the expected limits of contamination. The chance of accidental contamination in the pair-bagging experiments conducted by Laby would be minimal.

Mendelian male sterility resulting from the action of the recessive major gene *a*, described by Owen (1952), differs from the cytoplasmic type in that it is regularly transmitted by pollination. Since stray pollen may be carried by air currents for great distances there is a risk of contamination of any field seed crop with this form of male sterility, and subsequent selections could concentrate the gene in a breeding line. Although some reported instances of spontaneous appearance of male sterility may have arisen in this way, this is unlikely to segregate much more than 50 per cent male-steriles, since the gene is recessive. For this reason the populations obtained by Hawkins in 1971 are clearly most unlikely to be Mendelian male-steriles. The occasional male-sterile plants found in O-type SL 9460 by Cleij (1967) were shown to be cytoplasmically controlled.

Other explanations considered by Cleij (1967) were the transmission of *S*-plasm with sap applied mechanically or carried by aphids, but experiments failed to demonstrate this convincingly. Some success was achieved in the thermal induction of male fertility in plants with *S*-plasm, but whether this was due to changes in the cytoplasm, the nucleus, or the relative abundance of cells with differing plasmic factors was not clear. This latter experiment was designed to determine whether the *S* factor could be eliminated by methods found effective in treatment of virus diseases (Kassanis, 1957), and it was not anticipated that thermotherapy might induce male sterility in O-types.

Generally Cleij appears to favour the explanation of cytoplasmic change to account for the appearance of male-steriles among O-types and *vice versa*. On the whole this does provide an attractively simple explanation of the reported instances of apparent transition from one form to the other and does not conflict with any of the experimental results so far described. If this proves to be the case then the practical implications to the breeder are considerable. Clearly the time-consuming procedures of progeny testing to identify new O-types and subsequent backcrossing to establish equivalent male-sterile lines are redundant if it is possible to isolate equivalent O-types from male-steriles and equivalent male-steriles from O-types by inspection, with or without thermal induction. Furthermore, in experiments on graft-transmission of *S*-plasm the statistical treatment of results becomes more important in respect of scions and stocks used as controls if these can be expected to change also.

There remains the nature of the environmental influence on plasm changes to be elucidated. Cleij commented on the "fertile" years in which more male-fertile plants appear among male-sterile progenies than usual, and it seems from the observations at Cambridge in conjunction with those of Hawkins and Laby that there are also "sterile" years when the O-types segregate more male-steriles. Whether the common environmental factor operates on the developing flowers, or upon some earlier stage in the life cycle, or even upon the previous generation, awaits further investigation. It is possible that the plasmic

factor *S* is normally present in many fully fertile beet in a concentration below the threshold at which it affects anther development, but that local accumulation can occur giving rise to male-sterile flowers and progeny. The reverse process in a male-sterile plant would explain the occasional full fertility of lateral branches, first noted by Owen (1942). This condition would not require frequent mutation to regenerate the alternative forms, and would be likely to respond to climatic and seasonal changes by increase or decrease of male fertility in both male-sterile and O-type lines. From a practical breeding viewpoint this hypothesis differs little in effect from the cytoplasmic mutation theory, as considerable care must be exercised in single-plant selection in either case.

Mention must be made of the segregation of monogerm and multigerm floral types in the last two generations of the experiment. The monogerm character of SLC 91 originates from the monogerms isolated from United States material by Savitsky (1952), and is conferred by the recessive gene *m*. The cross SLC 91 MS × Blood Red Globe should have yielded heterozygotes (*Mm*), and the progeny were indeed all multigerm. The backcross to SLC 91 gave only three fertile seeds — probably due to the influence of the *S*-plasm on pollen development and viability — but the fact that these were all multigerm does not constitute a significant departure from the expected 1:1 segregation. A second backcross of these apparently heterozygous individuals to the monogerm parents, however, did not provide a satisfactory segregation of monogerms and multigerms, and these results cannot be explained on the basis of simple recessive gene action. It is possible that the three seed-parents were homozygous for the *m* gene, but that the monogerm character was masked by the action of modifiers or an expression of heterosis in respect of flower production.

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