Euphytica 3 (1954): 117–124

MALE STERILITY AND ITS IMPORTANCE IN BREEDING HETEROSIS VARIETIES

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Received 14 April 1954

1. Introduction

Up to the present, commercial exploitation of hybrid vigour has mainly been limited to crops that produce a large number of seeds per flower or per inflorescence as far as they cannot be propagated vegetatively. A rational application of heterosis to nearly all other crops not so abundant in seed production is handicapped by the technical and economical difficulties involved in annual emasculations.

A method which renders emasculation superfluous would therefore be important to the breeder of heterosis varieties. A similar method is rendered possible by the aid of male sterility.

The author gives a survey of the occurrence, mode of inheritance, and application of male sterility to cultivated plants.

2. Male sterility (1,5)

In a large number of crops (see Table 1), plants have been found that completely or almost completely fail to produce viable pollen as a result of their genetical constitution. These plants produce less than 0.1–0.5 % viable pollen under conditions in which normal fertile specimens produce it abundantly. Their development is at first completely normal in every respect; during the formation of pollen, however, a hypertrophy of the tapetum is induced by genetical factors, leading to a degeneration of the pollen grains in process of formation during or after meiosis. Most of the pollen sacs contain only shrivelled pollen grains and tapetal remains.

Other types of pollen sterility are known, for example that form which is believed to occur in the potato. This article, however, deals only with the type of male sterility outlined in the above paragraph. The term male sterility (MS) is used to denote plants displaying the symptoms we have described.

3. Identification of male-sterile plants

Male-sterile plants can be identified before pollen dispersal in fertile plants by the fact that their anthers are empty or only partly filled and feel flabby to the touch. In the case of beets, however, it is necessary to make a cross section of the buds at anther level. By this means the empty translucent anthers of MS plants can easily be distinguished from the well-filled anthers of fertile plants. In the case of the tomato it is sufficient to examine with a pocket-lens anthers previously opened with a needle just prior to flowering. Similar methods of identification may be developed for other crops, as they are required.

[1]) The author wishes to thank Prof. Dr R. Prakken for his kind permission to publish this study which has been carried out under his direction, and is grateful to Prof. Dr J. C. Dorst for his helpful criticism.

4. Inheritance of male sterility

Nearly all known types of MS are caused by one or more recessive nuclear alleles (see Table 1). Only a few forms of cytoplasmically inherited MS are known in the case of cross fertilized plants and hybrid species, which are, however, all modified by nuclear alleles. This is mainly a consequence of the reproductive rate of the different MS genotypes, although the mutability of the plasmatic genes also plays a role.

Table 1. Survey of the known forms of male sterility

	Author	Inheritance			Number of non allelomorphic factors, known up to now
		Plasm factor	Nuclear alleles		
			dom.	rec.	
Allium cepa	Monosmith	+	0	1	
Antirrhinum	Lewis				
Beta vulgaris	Owen	+	0	2	
Beta vulgaris	Owen	+	0	1	various (1945)
Capsella spp.	Lewis				
Capsicum frutescens	Martin				
Canna spp.	Offerijns				
Castanea sativa	Mac Kay				
Centhranthus ruber	Sobrinko	–	1	1	
Cirsium oleraceum	Correns				
Coleus spp.	Ford	–	1	0	
Cucumis melo	Bohn	–	0	1	
Cucurbita maxima	Scott	–	0	1	
Cucurbita pepo	Shifriss	–	0	1	
Dactylis glomerata	Myers	–	0	1	
Daucus carota	Welch	+	1	0	
Hebe townsoni	Frankel				
Hordeum vulgare	Suneson	–	0	1	
Lathyrus odorata	Fabergé				
Linum (hybrid spec.)	Bateson	+	0	1	
Lolium perenne	Jenkin				
Musa spp.	Dodds				
Nicotiana (hybrid spec.)	East	+	0	1	
Origanum vulgare	Lewis	+	1	1	
Oryza sativa	Ramanyam				
Plantago lanceolata	Correns				
Prunus communis	Crane				
Prunus domestica	Crane				
Prunus persica	Crane				
Rubus idaeus	Lewis				
Satureja hortensis	Correns				
Sorghum spp.	Stephens	–	0	1	two (1937)
Sorghum (hybrid spec.)	Stephens	+			
Solanum esculentum	Crane	–	0	1	thirteen (1945)
Solanum esculentum	Lesley	–	0	2	
Viola orphanides	Clausen				
Zea mays	Eyster	–	0	1	twenty (1935)
Zea mays	Rhoades	+			
Zea mays	Josephson	+			
Zea mays	Schwarz	+	1	1	

Explanation: *a.* By "author", is meant the writer who first published information (as far as known) on the subject. *b.* "0" indicates the absence of alleles; when there is not enough information as to the mode of inheritance, one or more of the relevant columns have been omitted. *c.* "Number of recessive alleles" signifies the number of recessive factors necessary to cause the relevant species to be MS. So with the onion, (S)mm is male sterile.

5. Principles in the application of male sterility (5, 6, 7, 10, 15)

Male-sterility is most easily applied in cross-fertilized plants, cytoplasmically inherited forms of MS being used successfully. The system described below is the one being applied.

If F_1 seed is to be used commercially, as is the case with beet and onion, all mother plants must possess the plasmatic factor causing MS. When sufficient father plants are at hand, the MS plants obtained in this way produce practically 100 % hybrid seed without emasculation. The fact that this seed produces plants which are also male sterile is not a disadvantage in crops grown for their vegetative parts.

In the case of crops actually grown for seed, the breeder usually provides for commercial purposes the so-called "double cross" seed. In this case only half of the lines to be used as mother plants need possess the plasm factor causing MS. From the pollination of an MS inbred line A with a normal fertile inbred line B, we obtain the male sterile single cross AB. The latter can be pollinated with a single cross CD which originated from a cross between two normal fertile inbred lines, so that the commercial seed ABCD can develop on the AB-hybrid. From this commercial seed, however, new MS plants will grow, so that (if seed setting is desired) one will have to provide a sufficient amount of pollen in the field. This is most easily done by mixing the MS commercial seed with seed of a fertile hybrid flowering at the same time e.g. the normal ABCD. It is, however, safer to counteract the MS genetically although this requires some extra work at first.

In *self fertilized plants*, only horticultural crops offer possibilities for the utilization of MS on a commercial scale.

6. Advantages of male sterility (5, 6, 10, 14)

Male sterility permits the production of hybrids on a commercial scale in crops which formerly could not be treated in this way owing to the high cost of emasculation, while existing hybrids can in future be produced more economically.

If the method described in § 8 is used correctly, the hybrids obtained will have a greater degree of purity than the classical ones. Even with the most careful emasculation more than 5 % selfing usually occurs, whereas in MS lines this percentage amounts to about 0.1–0.5 %. Moreover, the plants are not damaged by emasculation, so that they suffer less from various diseases, can be fertilized more easily and produce higher yields.

7. Problems involved in the use of MS (7, 9, 10, 13)

In order to exploit the above advantages to the full, the breeder of cross-fertilized varieties must change the genetical constitution of at least 25 % of his strains by the transference of the plasm factor for MS. In addition, he has to ensure that the MS lines finally to be utilized do not produce viable pollen under any of the conditions prevailing in practice. Factors capable of partially or completely restoring fertility in these lines should therefore be absent. Apart from this, however, such fertility restoring factors can render very valuable service in restoring fertility in the fields genetically (see § 8).

The breeder has to be very careful in this matter, however, as impure hybrids and/or unsufficient seed set in the fields may lead to large reductions in yield. Constant inspection of all the lines and hybrids used is therefore necessary, even when genetically pure material is employed.

These and other problems, for example the maintenance of MS lines in a homozygous state – have been solved satisfactorily in the case of *cross-fertilized plants.* In the case of *self-fertilized plants* a good seed set cannot be obtained without artificial pollination, with the result that only in some horticultural crops (for example the tomato) can MS be used on a commercial scale.

8. Application of MS in cross-fertilized plants (3, 5, 6, 7, 8, 9, 12, 14, 15)

The transference of MS to fertile lines is effected by converting the available MS line into one that is useful to the breeder by repeated backcrossing with a good and fertile inbred line. For example:

Onion = [(Smm × Nmm) × Nmm] × Nmm...[1]
Beet = [(Sxxzz × Nxxzz) × Nxxzz] × Nxxzz...

As the required number of backcrossing generations depends on the degree of resemblance between the original MS line and the old inbred line, it is more effective to look for MS plants in the variety itself than to derive this factor from other varieties. In cases where MS plants have been sought on a large scale (In the Netherlands the onion; in the U.S.A. the tomato) they have almost always been found.

If the inbred line which was used for backcrossing contains segregating factors which counteract the effect of the MS plasm factor, one has to ensure that a sufficient number of plants is always used for backcrossing, in that after the first backcrossing generation some flowers of each plant are selfed in each generation.

This method, of which a sketch is given below, can be practised with different maize varieties.

Sxxzz × NXXZZ → SXxZz
→SXxZz × NXXZZ → { SXXZZ, SXxZZ, SXXZz } *when selfed: producing* no MS progeny; are discarded
{ SXxZz } 6.25 % MS progeny (returns to backcrossing)

By this means a limited number of backcrossing generations followed by one selfed generation results in a genotype Sxxzz which differs only in its MS from the original types used by the breeder in creating his hybrids.

a. Maintenance of the MS genotypes

MS genotypes obtained in this way may best be maintained by vegetative propagation. If this is difficult or impossible the MS plants may be backcrossed each year

[1]) It is customary to designate the plasm factor for male sterility by *S* and the factor normal plasm by *N*.

with their fertile sister-strains Nmm and Nxxzz, respectively. If the breeder does not have these genotypes at his disposal, as in the case of the beet last mentioned, this is first effected by pollinating the NXXZZ genotype with a genotype SXxZz from the last available backcross generation. NXXZZ crossed with SXxZz then gives 25 % NXxZz plants, which after a test cross with the MS genotype Sxxzz as maternal parent may be distinguished from the other genotypes NXXZZ, NXXZz and NXxZZ by the fact that they produce 25 % MS plants. In the year in which this cross takes place, all the genotypes mentioned are also selfed which makes NXxZz produce 6.25 % seeds of the desired genotype. This genotype can also be distinguished by test crossing from the other genotypes produced. With the aid of the Nxxzz plants to be homozygously increased by selfing, one can maintain the genotype Sxxzz according to the formula:

$$\rightarrow \text{Sxxzz} \times \text{Nxxzz} \longrightarrow \text{Sxxzz} \;\lrcorner$$

b. Breeding of the single cross

The breeding of the single cross with the aid of MS differs only in the absence of the need to emasculate the rows of mother plants as in the classical method.

c. Breeding of the double cross and restoring fertility

If seed is not to be mixed in order to restore fertility in the field this holds for the breeding of the double cross as well. If this is really the case, however, a number of rows CD, male sterile AB and normal fertile AB, are alternatively sown in the ratio 2:4:2 to 2:2:4 in order to produce double crosses. The normal AB rows are emasculated and the inbred seed of CD is kept apart; the hybrid seed ABCD of both types AB rows is harvested as a mixture and sold on a commercial scale.

Owing to the danger of demixing and the difficulties attached to the emasculation of AB it is preferable, however, to counteract the MS in the field genetically. With a view to this the hybrid CD is composed in such a way that it is homozygous for one or more dominant alleles which entirely counteract the effect of plasm factor for MS. These fertility restoring factors are then introduced into the lines C and/or D by repeated backcrossing.

In order to prevent the loss of the fertility restoring factors desired to transmit, test crosses are in addition carried out every year with the available MS lines. After a number of backcrossing generations lines are available which cause normal fertility in the double crosses to which they belong. An example of this is summarized below:

Inbred lines		Single crosses		Double crosses	
Genotypes	Fertility	Genotypes	Fertility	Genotypes	Fertility
A = Sxxzz	MS	AB = Sxxzz	MS	ABCD = SXxZz	normal
B = Nxxzz	normal				
C = NXXZZ	normal	CD = NXXZZ	normal		
D = NXXZZ	normal				

d. Control system

The following control system for the production of hybrid seed has been evolved by INGERSOLL: The individual plants occurring in the propagation and single cross fields of the MS (maize) plants, and which produce some fertile pollen, are wholly discarded before the flowers appear. In the double cross field less than 0.5 % pollen is produced when the above-mentioned measures are carefully carried out. This double cross field is then inspected and the pollen producing plants standing in MS rows are emasculated but not removed, because superfluous pollen can only be of advantage in the fields the following year.

9. APPLICATION OF MS IN SELF-FERTILIZED HORTICULTURAL CROPS (3, 14)

One difficulty involved in the method described in § 5 and § 8 for cross fertilized plants is the long time needed for preparatory breeding work. RICK has reduced this to one year in the case of the tomato. His method is based on the discovery that every tomato variety has about 0.1 % MS types in which the mode of inheritance is mainly monofactorial recessive. These MS plants can easily be identified by their small fruit set and consequently greater vegetative development; to make perfectly sure, however, that it is indeed an MS plant one has to dissect the anthers [1]).

Owing to casual cross fertilization such MS plants always produce some seeds. The latter are sown out in the greenhouse immediately after harvesting, while the mother plant is propagated by cuttings. In this way cuttings from the mother plant can be pollinated as early as the following winter with pollen from the heterozygous plant Mm grown from the seed. The artificially pollinated mother plant mm gives 50 % seed of the MS genotype mm, which can be distinguished from the fertile heterozygotes Mm just before the flowering stage. These MS plants are pollinated by an adequate pollinator and produce hybrid seed that can be sold commercially. The price of such seed in the U.S.A. is about $ 1 per 1000 seeds.

The MS genotypes are maintained by artificial pollination with the fertile heterozygote Mm as mentioned above. Twice as many plants have thus to be sown as are thought necessary for the production of hybrid seed.

Owing to the amount of labour in identification and pollination of the MS plants the method is not suitable for agricultural crops; it may be used, however, for many horticultural crops.

10. RESULTS OBTAINED WITH MALE STERILITY (2, 8, 11)

In the U.S.A., heterosis varieties of tomato have appeared in the catalogues of Asgrow Inc. since 1942, and heterosis varieties of onion since 1949. The increased yields of the hybrids obtained with the aid of MS amounted in the tomato and the onion to more than 200 and 20 to 51 %, respectively, compared with open pollinated varieties.

Preparatory measures to introduce heterosis varieties of sugar beet and maize are already in an advanced stage, while in the case of beet increased yield of 10 % sugar/ha have been established in cases where MS was used. This percentage might have been considerably higher if the hybrids used had not proved to be extremely susceptible to *Cercospora beticola*.

[1]) We cannot deal in this article with the application of functional (male) sterility which is known, inter alia, in the tomato and there called the "John Baer type".

These encouraging results make it conceivable that more important results can be obtained with other cross fertilized plants and self fertilized horticultural crops, including tobacco.

SUMMARY

On the basis of data from the literature, a survey of the occurrence and mode of inheritance of male sterility in a large number of plant species is presented (Table 1).

The importance of male sterility as a means of breeding heterosis varieties is indicated and the advantages attached to the use of MS, and the problems arising from it, are discussed.

It is contented that the rarely occuring types of plasmatically inherited MS offer good possibilities of utilization in the case of cross fertilized plants. The procedure to be adopted in using this type of MS is described in considerable detail in § 5 and 8.

It is regrettable that, in self-fertilized field crops under conditions of natural pollination, the small seed set obtained presents serious difficulties in breeding hybrids on a scale which is commercially justified. In horticulture, however, where artificial pollination can be made to pay, hybrids have already been created with the aid of MS types possessing a monofactorial recessive system of inheritance and have been put on the market. The procedure adopted is described in § 9.

A survey is given of the results already obtained with MS in tomato, onion, sugar beet and maize, and it is anticipated that important increases in yield may also be obtained in other cross fertilized plants and self-fertilized horticultural crops with the aid of MS.

SAMENVATTING

Manlijke steriliteit en haar betekenis voor het kweken van heterosisrassen

Aan de hand van literatuurgegevens werd een overzicht gegeven van het voorkomen en de wijze van vererving van mannelijke steriliteit in een groot aantal plantensoorten. (tabel 1).

Gewezen werd voorts op de betekenis van mannelijke steriliteit als hulpmiddel bij het kweken van heterosisrassen, terwijl de aan het gebruik van MS verbonden voordelen en de zich daarbij voordoende problemen werden besproken.

Betoogd werd dat de schaars voorkomende typen van plasmatisch verervende MS goede toepassingsmogelijkheden bieden voor gebruik in kruisbevruchters; de bij het benutten van dit type MS te volgen werkwijze werd uitvoerig aangegeven in de paragrafen 5 en 8.

Helaas vormt in zelfbevruchtende akkerbouwgewassen bij natuurlijke bestuiving de geringe zaadzetting van MS planten een ernstig beletsel voor het op commercieel verantwoorde wijze kweken van hybriden; in de tuinbouw, waar men veelal kunstmatige bestuiving rendabel kan maken, werden echter met behulp van monofactorieel recessief verervende vormen van MS reeds hybriden verkregen en in het verkeer gebracht. De hierbij gevolgde werkwijze werd beschreven in paragraaf 9.

Een overzicht werd gegeven van de reeds met behulp van MS bereikte resultaten bij tomaat, ui, suikerbiet en mais, terwijl de verwachting werd uitgesproken dat ook bij andere kruisbevruchters, zelfbevruchtende tuinbouwgewassen en tabak met behulp van MS nog belangrijke meeropbrengsten in de practijk te behalen zullen zijn.

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Restricted as we are by the amount of space available, we can mention here only the more important articles of those we have studied and which amount to about 125; a complete list will gladly be sent upon application.

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