Theor Appl Genet (1995) 91:1107–1110

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A method for isolating and characterizing homozygous S-allele lines in Brassicae

Received: 4 March 1995 / Accepted: 17 March 1995

Abstract A method for isolating and characterizing homozygous *S*-allele lines of brassicas is described. The tester plants used are produced by crossing the parent plants with a recessive *S*-allele homozygote. The full method uses reciprocal crosses to identify and characterize the lines immediately. When flowering is prolonged and enables further tests to be carried out, a more efficient method that only uses single crosses initially can be used.

Key words Self-incompatibility · Brassicae

Introduction

Members of the Brassicae possess a self-incompatibility system with a multi-allelic series of *S* genes having sporophytic control. The system has been successfully used for producing F_1 hybrid cultivars, especially in the vegetable brassicas. Because of the sporophytic control, however, one of the main problems in breeding hybrid brassicas is isolating and characterizing homozygous *S* allele lines.

Thompson and Howard (1959) suggested that one method of identifying *S*-allele lines would be to use a parent plant as a tester and cross it reciprocally with its selfed progeny. This parent-tester method assumes that the parent plant is heterozygous for its *S* alleles, although the sporopytic system does made it possible to have homozygous plants. Any first-generation inbred (I_1) plant giving compatible crosses would have to be homozygous for a recessive *S* allele. There are four types of *S* allele interactions which can be recognized by their behaviour with the parent tester (as heterozygous tester in Table 1). Type-I interactions give compatible reactions with both reciprocal crosses between the recessive homozygote and the heterozygote. Type-II and Type-III interactions give differing results with the reciprocal crosses, and Type-IV interactions do not give any compatible crosses. Thompson and Howard (1959) suggested that an improvement could be made by using two sib plants as testers, one as male and the other as female. By chance, one or both of the testers could be homozygous and would produce compatible crosses with a Type-IV interaction. If both testers were heterozygous, then the situation was considered to be no worse than using reciprocal crosses with the parent. The second stage of the method is to cross the plants giving a compatible reaction with the parent to find which class is homozygous, and the third stage is to use the identified homozygote to isolate the other homozygote.

Thompson and Howard (1959) were working with kale (*Brassica oleracea* var 'acephala') in which the flowering seasons can be greatly extended and which can also be maintained by propagating cuttings. The situation is different in turnips (*B. campestris*), which are far less amenable to inbreeding and maintenance in the glasshouse. Because of this, Mackay (1977) considered it necessary to use a system that ensured the highest probability of success with only one round of crossing, and he carried out complete diallels with as many plants as possible during the flowering period.

The methodology of isolating homozygotes proposed by Wallace (1979) combines the parent-tester method with a sequence of sib-testing when necessary. The sib-testing involves making reciprocal crosses with each I_1 plant as it comes into flower, once with an I_1 plant which has previously been tested, and once with an I_1 which has not previously been tested. This process produces a chain of crosses with the possibility of picking up compatible crosses with a Type-IV interaction. However, it is only when the parent-tester method has not picked up any compatible pollinations after five or six crosses that Wallace suggested using sib-testing, unless the parent is not available or it is not flowering.

Communicated by H. F. Linskens

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Table 1 Results of all possible reactions with the four main types of dominance interaction when sibs are used as testers (*I* incompatible, *C* compatible)

Tester genotype:	S_1S_1			S_1S_2			S_2S_2		
Plant genotype:	S_1S_1	S_1S_2	S_2S_2	S_1S_1	S_1S_2	S_2S_2	S_1S_1	S_1S_2	S_2S_2
Type I									
♂ S_1 dominant	I	I	C	I	I	C	C	C	I
♀ S_1 dominant	I	I	C	I	I	C	C	C	I
Type II									
♂ S_1 dominant	I	I	C	I	I	I	C	C	I
♀ Co-dominant	I	I	C	I	I	C	C	I	I
Type III									
♂ Co-dominant	I	I	C	I	I	C	C	I	I
♀ S_1 dominant	I	I	C	I	I	I	C	C	I
Type IV									
♂ Co-dominant	I	I	C	I	I	I	C	I	I
♀ Co-dominant	I	I	C	I	I	I	C	I	I

Alipieva (1984) used an extended sib-testing method which was a modification of that used by Haruta (1962). Three random sibs were used as testers: one was crossed reciprocally with all of the plants tested, and the other two were used just as pollen parents. This method has a much greater chance of identifying a homozygote with a Type-IV interaction than the method of Thompson and Howard (1959), and it may also identify the dominance interaction; however, it requires twice the number of pollinations.

If homozygous recessive *S* alleles are available, testers can be produced and used in what appears to be the most effective method for any situation. The full method is a substitute for diallel testing, but when supplementary crosses can be made a much shorter and more efficient method can be used.

Methodology

When the parent plant of an inbred line is initially self-pollinated, it is also crossed with a recessive homozygous *S* allele line. This process gives progeny that are heterozygous for the recessive *S* allele (S_r) and a parental *S* allele (S_1, S_2), and these progeny are used as testers. The testers are sown out along with the I_1 progeny and therefore come into flower at the same time.

Full method

One plant of the tester line is crossed with other plants of the tester line to identify the two heterozygotes, S_1S_r and S_2S_r. Five crosses of 1 plant with other plants should give a 97% probability of identifying the other heterozygote, which will give a compatible reaction. If no compatible crosses are produced, then the original plant must have been homozygous. The two heterozygotes are used as testers by crossing reciprocally with the I_1 progeny. Thirteen I_1 plants are needed to give a 95% probability of obtaining both homozygotes. The possible results are shown in Table 2. A unique pattern of results is obtained with each of the four dominance interactions, and both homozygotes are identified except in the case of a Type-I reaction.

With a Type-I reaction, progeny testing is necessary to identify the homozygous dominant *S* allele plants, and this is the case with all methods. Selfed progeny can be tested by crossing with the recessive homozygote, but it is more efficient to cross the I_1 plants with the recessive homozygote and test their progeny by backcrossing with the recessive homozygote. Only 7 plants are needed with the backcross to give a 99% probability of detecting a recessive homozygote in the progeny, as opposed to 16 plants being required with selfed progeny.

Table 2 Results expected from families with the four main types of dominance interaction when crossed reciprocally with heterozygous testers (*I* incompatible, *C* compatible)

	Ratio: plant genotype					
	1:S_1S_1		2:S_1S_2		1:S_2S_2	
Reaction when used as:	♂	♀	♂	♀	♂	♀
Type I phenotype	S_1	S_1	S_1	S_1	S_2	S_2
Crossed with: S_1S_r	I	I	I	I	C	C
S_2S_r	C	C	C	C	I	I
Type II phenotype	S_1	S_1	S_1	S_1S_2	S_2	S_2
Crossed with: S_1S_r	I	I	I	I	C	C
S_2S_r	C	C	C	I	I	I
Type III phenotype	S_1	S_1	S_1S_2	S_1	S_2	S_2
Crossed with: S_1S_r	I	I	I	I	C	C
S_2S_r	C	C	I	C	I	I
Type IV phenotype	S_1	S_1	S_1S_2	S_1S_2	S_2	S_2
Crossed with: S_1S_r	I	I	I	I	C	C
S_2S_r	C	C	I	I	I	I

Short method

With a lengthy flowering period, which allows supplementary crosses to be made, a single tester can be used just as the pollen parent. With this method, 13 plants would again be used to give a 95% probability of obtaining both homozygotes. Only one-quarter of the number of initial crosses are required compared to the full method, but compatible crosses are identified with all four types of interaction (Table 3). If no compatible crosses are obtained, then the original plant is assumed to have been homozygous. When one-quarter of the crosses are incompatible, these identify the recessive homozygote. In the other cases, one-quarter of the crosses are compatible, and these identify one of the homozygotes, but it could be either one depending on the dominance interaction. With only 13 plants being tested, the frequency obtained will often not match a 1:3 ratio. In practice, the portion with the smallest number of plants (6 or less out of 13) should be taken as that expected to have one-quarter of the plants, and this has a 98% probability of being correct.

The next stage in the identification procedure is to carry out reciprocal crosses between the identified homozygote and other previously tested plants. These crosses are to identify the other homozygote and to find the reaction of the heterozygote. It is suggested that 8 plants are tested at this stage to give a 96% probability of obtaining the other homozygote. The possible results from these crosses are given in Table 4. The second homozygote is identified by being reciprocally compatible, except in the case of a Type-I interaction, where all of the crosses are reciprocally compatible. As with all other methods, the dominant homozygote can only be isolated in these cases by progeny testing. If any of the test crosses gives a reciprocal difference, then the dominance interaction is Type II or III, depending on which is the compatible cross.

Heterozygotes with a Type-IV interaction give an incompatible reaction in both reciprocal crosses with the tester homozygote, but so will a Type-II reaction when it is the dominant homozygote that was identified initially. If it is required to distinguish between these two cases then one further cross is required; a plant of the second homozygote is pollinated, as female, with one of the heterozygotes. A compatible cross will occur with a Type-II interaction, but a Type-IV interaction will again give an incompatible cross.

Discussion

In theory, the simplest way of producing *S* allele homozygotes is to produce doubled haploids, which must be homozygous at the *S* locus and all other loci. However, even if a protocol is available for producing them, it is unlikely that doubled haploids will be satisfactory. The crops involved are mainly outbreeders, and high levels of inbreeding depression are likely. The inbreeding will be instantaneous and uncontrollable, unlike normal inbreeding where some selection can be exerted and the inbreeding can be stopped if the depression is getting too severe.

Another simple method, again in theory, is to produce several lines of I_2 plants from selected parents. Half of the I_2 lines will be homozygous. Isolated families of plants would give seed set if they were progeny from heterozygotes, with 20 plants giving a greater than 99% probability that both homozygotes were present. Although this method requires a minimum of hand-pollination, the facilities and labour needed would be prohibitive for anything more than a small number of lines.

The use of 2 sib plants as testers instead of the parent (Thompson and Howard 1959) avoids the problems of matching flowering times with the tester plants, and it provides the possibility of identifying homozygotes with a Type-IV reaction. Apart from this advantage, however, the method is inferior to the parent-tester method because it is not known whether the compatible or incompatible cross is the homozygote, nor what the dominance interaction is. Extending the sib-testing procedure to include more crosses and reciprocals (Alipieva 1984) increases the chances of obtaining a homozygote with Type-IV interactions and of identifying the interaction type, but the numbers of pollinations are greatly increased and it is still a very unpredictable method.

The sib-testing sequence suggested by Wallace (1979) will be successful in identifying compatible crosses in a Type-IV situation if enough plants are tested. However, it would appear that a chain of crosses is slightly less efficient than random pair crosses because when a plant has given an incompatible reaction in its first cross it reduces the probability of it being a homozygote for the next cross. If 13 crosses were used, the probability of picking out the two homozygotes with a chain of crosses would be 78%, whereas it would be 82% with random pair crosses. A 95% probability of obtaining both homozygotes would need reciprocal crosses with 23 random pairs or a chain of 27 plants.

The parent-tester method cannot identify homozygotes with a Type-IV reaction, and it is domi-

Table 3 Results expected from the four main types of dominance interaction when crossed with a tester as pollen parent (*I* incompatible, *C* compatible)

		Female plant genotype			Ratio of compatible: incompatible
		S_1S_1	S_1S_2	S_2S_2	
Type I	Phenotype	S_1	S_1	S_2	
Crossed with:	S_1S_r	I	I	C	1:3
	S_2S_r	C	C	I	3:1
Type II	Phenotype	S_1	S_1S_2	S_2	
Crossed with	S_1S_r	I	I	C	1:3
	S_2S_r	C	I	I	1:3
Type III	Phenotype	S_1	S_1	S_2	
Crossed with:	S_1S_r	I	I	C	1:3
	S_2S_r	C	C	I	3:1
Type IV	Phenotype	S_1	S_1S_2	S_2	
Crossed with:	S_1S_r	I	I	C	1:3
	S_2S_r	C	I	I	1:3

Table 4 The possible results of reciprocal crosses of an identified homozygote with previously tested plants which were not that homozygote (*C* compatible, *I* incompatible)

Initial test result	Homozygote indentified	Dominance interaction	Reciprocal crosses with			
			Homozygous		Heterozygous	
			♂	♀	♂	♀
25% I	S_2S_2	Type I	C	C	C	C
		Type III	C	C	I	C
25% C	S_1S_1	Type II	C	C	I	I
		Type IV	C	C	I	I
	S_2S_2	Type I	C	C	C	C
		Type II	C	C	C	I
		Type III	C	C	I	C
		Type IV	C	C	I	I

nant *S* alleles that are of most interest in a breeding programme. There may also be problems with propagation or coincidence of flowering with the parent-tester method. However, it is a relatively efficient method of isolating *S* allele homozygotes that are recessive. The recessive homozygote is immediately identified with Types I, II and III, and the dominance interaction is given.

The use of reciprocal crosses on 13 plants with the parent-tester method requires 26 pollinations initially to isolate the recessive homozygote, if there is one. The short method presented here requires only half of the initial number of crosses, using 13 single crosses, and will identify one of the homozygotes even with a Type-IV reaction. There should be no problems with coincidence of flowering, as all of the plants are sown at the same time.

When the situation is such that supplementary sets of crosses are not practicable, then the choice is between the diallel test or the full method. Mackay (1977) used between 5 and 10 plants in diallel tests, with an average of 7. With 7 plants there is a 99% chance of obtaining one of the homozygotes, and a 74% chance of obtaining both. The probability of identifying both is somewhat less, because only the recessive allele can be identified with a Type-I interaction. In two-thirds of the diallels carried out by Mackay (1977) both of the homozygotes were identified, which is therefore in agreement with the expected. If 7 plants are used then 42 crosses are needed to complete a diallel set. The full method presented here would require 33 crosses, including the 5 needed to identify both testers. If 13 plants were used, as is usually suggested to give a 95% chance of obtaining both homozygotes, then a diallel would need 156 crosses. The full method here would need 57 crosses, again including the 5 needed to identify both testers.

The proposed methods of using recessive *S* allele heterozygotes as testers for isolating and characterizing *S* allele homozygotes appear to be superior to any other method. The methods do, however, require a previously identified *S* allele with the lowest level of dominance to realize their full potential. In the case of *Brassica oleracea*, the dominance of the *S* alleles is well-established (Thompson 1968), and lines S_2 or S_{15} would be most useful for producing testers. With *B. campestris*, the situation is not as well-defined, but line S_8 of Lee and Yoon (1981) would be suitable.

If no weak *S* allele were available initially, a start could be made using the full method by crossing with any other plant to produce the tester heterozygotes, especially if the main interest is in isolating strong *S* alleles. In such cases, Type-IV interactions would still give isolation of both *S* alleles. With the other interactions, only the recessive *S* allele would be identified initially because the recessive allele in the tester would be masked by a dominant allele and would therefore only give compatible reactions. However, the recessive homozygote could then be used to identify the dominant homozygote. It could also be used as the low-dominance *S* allele to produce the next set of tester heterozygotes and continue for this purpose until it appeared dominant to another *S* allele, which would then take its place as the low-dominance tester.

Acknowledgements The author wishes to thank Andrew Wallace for checking the probabilities quoted in this paper.

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