The genetic control of self-compatibility in an inbred line of *Lolium perenne* L

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The segregating generations derived from a cross of a self-compatible with an incompatible inbred line of *Lolium perenne* were analysed for the incompatibility reaction as revealed by the pollen/stigma fluorescence test. All F_1 plants showed a half-compatible reaction whilst segregation occurred in the F_2 into half- and fully-self-compatible classes. The latter plants bred true in the F_3 whilst the former once again showed the same pattern of segregation. These results indicate that the control of self-compatibility is by a single gene (Sc) which is gametophytic in action. Further analysis of an intra-F_2 pollination diallel and of reciprocal cross-pollinations between F_2 and F_1 plants reveal that this gene is additional to the SZ incompatibility system.

Keywords: gametophytic, incompatibility, *Lolium perenne*, pollen, self-compatibility.

Introduction

Self-incompatibility in *Lolium perenne* is known to be controlled by a two-locus (S,Z) multiallelic gametophytic system (Cornish *et al.*, 1979) in common with most grasses studied (Hayman, 1956; Lundqvist, 1956, 1961, 1962a, 1965; Murray, 1974; Fearon *et al.*, 1983). This self-incompatibility mechanism, although usually very efficient, occasionally breaks down so that a small number of self seed can be obtained. Early extensive studies by Jenkin (1931) led him to conclude that self-fertility was genotype-dependent and that it should be possible to produce a fully self-fertile plant. Subsequently, many workers have undertaken inbreeding studies on *Lolium perenne* (Wexelsen, 1952; Beddows *et al.*, 1962; Bean & Yok-Hwa, 1972). In most cases inbreeding was associated with a reduction in seed set, although Utz & Oettler (1976) observed an increase in seed production over six generations of selfing. From studies on the early generations of inbreeding, Jones & Jenabzadeh (1981) suggested that some self-fertile lines had achieved fertility either by selection for polygenic modifiers or by mutation in one of the incompatibility loci.

Mutations of the S locus are well known to give rise to self-fertility in a number of species such as *Petunia* (Mather, 1943), *Nicotiana* sp. (East, 1932) and *Trifolium* (Atwood, 1942; Townsend, 1965) which all possess single-locus gametophytic incompatibility systems (see review of de Nettancourt, 1977). Alternatively, self-fertility may arise by mutation at a locus

independent of the S locus as in *Trifolium hybridum* (Townsend, 1966) or be conditioned by polygenic background modifiers such as those found in interspecific hybrids of *Petunia* (Mather, 1943) and *Lycopersicon* (Martin, 1968). Relatively little is known about the genetic control of self-fertility in the Gramineae. In inbred rye, Lundqvist (1960, 1968) showed that self-fertility arose by changes in one of the incompatibility loci, however some of the results he obtained were suggestive of 'the existence of an additional kind of major gene, independently inherited and able to turn into an sf determinant'. The present investigation seeks to elucidate the genetic basis of self-fertility in an inbred line of *Lolium perenne*.

Materials and methods

The basic parental plant material was a pair of inbred lines selected from a number which had previously been screened for their pollen/stigma reaction (Thorogood, 1991). Parent Ba 10354, a ninth-generation inbred derived from the German cultivar Odenwalder, was selected as showing full self-compatibility, whilst parent Ba 10355, a sixth-generation inbred from the WPBS cultivar S23, was completely self-incompatible. Following the crossing of these two parents the 24 F_1 plants obtained were selfed to produce F_2 and F_3 families. Within each generation, plants were tested for self-compatibility using the in-vitro method of Lundqvist (1961) and the aniline blue fluorescence technique of Lalouette (1967). Ten F_1 and 128 F_2 plants

were assessed for their self- and cross-compatibility. Within the F_2, a partial diallel of pollinations was carried out amongst 39 plants. Self-compatibility was examined in 19 F_3 families derived from single F_2 plants chosen at random, each family consisting of from six to thirty individuals.

Results and discussion

The results of the self-pollinations are summarized in Fig. 1. All F_1 plants showed a half-compatible reaction when selfed. Intermating of 10 of these plants revealed complete uniformity for pollen tube growth, all crosses displaying the same half-compatible reaction as the selfs. In the F_2 generation segregation was observed; 73 individuals showing a fully compatible and 65 a half compatible reaction when selfed. There was no evidence of heterogeneity amongst the 16 groups to which the F_2 plants could be assigned according to which F_1 plant they derived from. The fully self-compatible F_2 plants, when selfed, gave rise to F_3 progeny all of which were themselves fully self-compatible. In contrast, there was segregation amongst the F_3 progeny of the half self-compatible F_2 into either half or fully self-compatible classes.

The observed pattern of compatibilities allows us to draw some conclusions concerning the mode of action and genetic control of self-compatibility in this pair of inbred lines. The presence of both compatible and incompatible pollen in the selfings of the F_1 indicate gametophytic control in the pollen, although the possibility that the action may be controlled by a combined sporophytic/gametophytic system, as was shown for a case of self-fertility in *Trifolium hybridum* (Townsend, 1966), cannot be ascertained from the present experimental material. The segregation in the F_2 into half and fully self-compatible classes agrees closely with the 1:1 ratio expected if the control is by a single gene ($\chi^2 = 0.46$, $P = 0.50$) and it is assumed that only pollen carrying the self-compatibility gene can effect fertilization whilst on the female side both the compatibility and incompatibility factors are transmitted. The absence of self-incompatible plants in the F_2 would confirm that this assumption is correct. The pattern in the F_3 confirms this hypothesis in that fully self-compatible F_2 only give rise to fully self-compatible progeny and segregation occurs among the progeny of the half self-compatible parents. Amongst these latter families, however, there was a slight but significant excess of half compatible plants (76:52, $\chi^2 = 4.5$, $P = 0.05-0.03$). Such deviation from expectation may be accounted for by selection in favour of the heterozygous state which has been shown to occur during the early generations of inbreeding in related lines of the present material (Albini, 1982).

The identification of a single gene controlling self-compatibility in the parental line Ba 10354 raises the
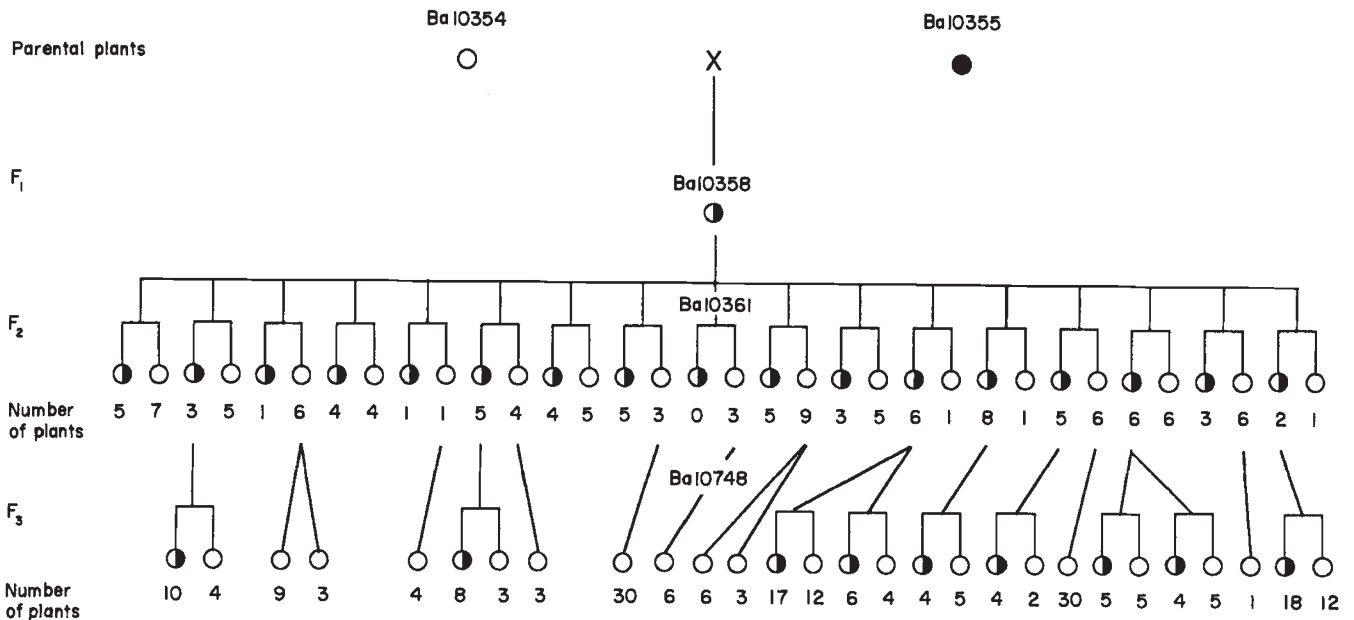

Fig. 1 Segregation of self-compatibility in Cross 1. (○) Fully compatible, (◑) half compatible, (●) incompatible.

	♂						F₁
♀	SiiZiF	SiiZFF	SijZiF	SijZFF	SjjZiF	SjjZFF	SijZiF
SiiZiF	H	+	T	+	+	+	T
SiiZFF	+	+	+	+	+	+	+
SijZiF	H	+	H	+	H	+	H
SijZFF	+	+	+	+	+	+	+
SjjZiF	+	+	T	+	H	+	T
SjjZFF	+	+	+	+	+	+	+
F₁ SijZiF	H	+	H	+	H	+	H

Fig. 2 Theoretical pattern of pollinations amongst F_2 plants. Self-compatibility mutation at S or Z locus (Model I) H = half compatible, T = three-quarters compatible, + = fully compatible. Shaded area = fully compatible male arrays (vertical) and female arrays (horizontal).

mating types which would appear in the F_2 and depends on two assumptions; homozygosity at the S and Z loci and proper functioning of these loci. If self-compatibility is due to a mutation at S or Z (Model I) six different genotypes are expected in the F_2 whilst the alternative, namely a mutation outside of the SZ system (Model II), will give rise to 18. A pollination diallel of members of the F_2 generation will lead to differing patterns of inter-compatibility according to which model applies as shown in Figs 2 and 3. As can be seen from Fig. 2, on Model I, plants fully self-compatible are also fully cross-compatible whereas on Model II (Fig. 3), full cross-compatibility is only expressed throughout the male arrays. Reciprocal differences of the form partially/fully compatible are expected with Model II whenever the self-compatibility gene is homozygous in one parent and heterozygous in the second. By use of the F_1 as a pollen parent onto the members of the F_2, fully compatible reactions are expected in half of the cases for Model I, whereas none are expected for Model II.

question of its relationship to the two-locus SZ incompatibility system. Self-compatibility may have arisen by two alternative pathways, namely a mutation at either of the two incompatibility loci or by a mutation at a third locus. Differentiation between these two hypotheses requires consideration of the differing

The results of the intra-F_2 pollination diallel are shown in Tables 1(a) and (b) from which it can be seen that, while all plants identified as fully self-compatible are cross-compatible as male parents, they are sometimes partially compatible as female parents. On the other hand F_2 plants which are half self-compatible

		♂ FF									Ff									F₁* Ff
		SiiZii	SiiZij	SijZii	SiiZjj	SijZij	SjjZii	SijZjj	SjjZij	SjjZjj	SiiZii	SiiZij	SijZii	SiiZjj	SijZij	SjjZii	SijZjj	SjjZij	SjjZjj	SijZij
♀ FF	SiiZii										H	T	T	+	7/8	+	+	+	+	7/8
	SiiZij										H	H	T	H	T	+	T	+	+	T
	SijZii										H	T	H	+	T	H	+	T	+	T
	SiiZjj										+	T	+	H	7/8	+	T	+	+	7/8
	SijZij										H	H	H	H	H	H	H	H	H	H
	SjjZii										+	+	T	+	7/8	H	+	T	+	7/8
	SijZjj										+	T	+	H	T	+	H	T	H	T
	SjjZij										+	+	T	+	T	H	T	H	H	T
	SjjZjj										+	+	+	+	7/8	+	T	T	H	7/8
♀ Ff	SiiZii										H	T	T	+	7/8	+	+	+	+	7/8
	SiiZij										H	H	T	H	T	+	T	+	+	T
	SijZii										H	T	H	+	T	H	+	T	+	T
	SiiZjj										+	T	+	H	7/8	+	T	+	+	7/8
	SijZij										H	H	H	H	H	H	H	H	H	H
	SjjZii										+	+	T	+	7/8	H	+	T	+	7/8
	SijZjj										+	T	+	H	T	+	H	T	H	T
	SjjZij										+	+	T	+	T	H	T	H	H	T
	SjjZjj										+	+	+	+	7/8	+	T	T	H	7/8
F₁* Ff	SijZij										H	H	H	H	H	H	H	H	H H

Fig. 3 Theoretical pattern of pollinations amongst F_2 plants. Self-compatibility mutation at a locus additional to the S and Z loci (Model II) H = half compatible, T = three-quarters compatible, 7/8 = seven-eighths compatible, + = fully compatible, shaded area = fully compatible male arrays, *reaction of the F_1 when used as a pollinator or stigmatic parent.

Table 1   (a) Partial diallel of pollinations involving half self-compatible F$_2$ and F$_1$ plants as males. (b) Partial diallel involving pollination of fully self-compatible F$_2$ plants as males

(a) ♂

Comp. group	F₂ plt	2 18/3	4 24/1	7 16/1	8 15/1	9 18/2	11 17/1	15 4/4	15 18/5	17 7/1	18 8/5	19 9/4	? 15/2	? 15/3	? 2/5	F₁ 1	F₁ 17
♀ 1	17/3		P		+							+					
1	12/3		P	+	+	+	P			+	P	+	+			P	
1	17/5		P		+				P		P	+					
2	18/3	H			+						P	+	+				
3	10/3		P	+	P	+	+		+	+	P	P				P	P
3	12/14		P		P	+	+	+	+			P					
4	24/1		H	+	P	+	+		+		P	P				P	P
5	8/2		P	+	P	+	P		+	+	P	P				P	P
6	1/3		P		P		P		P		P	P		+			
6	5/4		P	+	P	+	P		P	+	P	P		+	P	P	P
7	16/1		P	H	+	+	P		+	P	+	+				P	P
8	15/1		P	+	H	P	+			P	P	P				P	P
9	18/2		P	P	+	H	+		P	P	+	+				P	P
10	11/2		P	P	P	P	P		P			P		P	P	P	
10	7/5		P		P	P	P	P		+	P	P				P	P
11	17/1		+		+	P	H	P			+	+					
11	10/1	P	+								+	+	+				
12	2/1		+		+		+		+		+	+					
13	24/4	+	P		+						P	P	+				
13	24/3		P		+	+			+	+	P	P				P	
14	23/5		P		P	+	+		P		P	P	P				P
15	18/5		P		P				H		P	P		+	P	P	
15	4/4		P		P	+	+	H			P						
16	12/1		P		P		+		P		P	P		P	P		
17	7/1	+	P	P	P	P	P			H	P	P	P		P	P	P
18	8/5		P		P						H	P					
19	9/4		P		P						P	H					
?	23/4		P		P	P					P	P					
?	8/1		P		P	+	+		P		P	+			P		
?	5/5		P		P		P		P		P			P			
?	1/4	+	P		P				P			P	P				
?	15/2	P	P		P							P	H				
?	11/5		P		P				P		P	P					
?	2/4													+	+		
?	6/2													+			
?	15/3								P					H	P		
?	2/5								+					P	H		
?	5/3								P					+	P		
?	11/4																
F₁	1	P	P	P	P		P					P			P	H	H
F₁	17		P	P	P		P					P			P	H	H

? = Could be placed in more than one group or may represent a further unique genotype.
Note that male and female compatibility groupings are not interrelated.
+ = Fully compatible.
P = Partially compatible.
H = Half self-compatible.

Table 1 *Continued*

(b)

♂

♀	17/3	10/3	8/2	1/3	11/2	8/1	7/5	12/4	5/5	1/4	12/3	24/4	23/5	11/5	5/4	12/1	17/5	24/3	2/1	2/4	6/2	5/3	11/4
17/3	+	+	+	+												+							
10/3	+	+	+	+	+	+	+		+	+				+				+	+				
8/2	+	+	+	+	+	+	+											+	+				
1/3	+	+	+	+		+		+		+						+	+		+		+	+	+
16/1	+	+	+	+	+	+	+		+	+		+					+	+		+			
15/1	+	+	+	+	+		+		+				+		+		+	+					
24/1	+	+	+	+		+	+		+	+		+		+			+	+					+
8/5	+	+	+	+																			
9/4	+	+	+	+	+	+	+	+	+														
18/2	+	+	+	+	+	+	+	+	+		+		+				+	+					
23/4	+	+	+	+										+									
4/4	+	+	+	+	+	+	+	+	+				+										
11/2	+	+	+	+	+					+			+					+				+	
8/1	+	+	+	+	+	+	+	+	+				+				+			+			+
7/5	+	+	+	+	+	+	+	+	+		+	+	+	+			+	+		+			
17/1	+	+	+	+	+	+	+	+	+		+	+	+										
12/4	+	+	+	+	+	+	+	+	+	+		+	+										
5/5	+	+	+	+	+	+	+		+	+			+			+	+						+
7/1	+	+	+	+	+		+			+	+	+	+				+		+				+
10/1	+	+	+	+						+	+												
1/4	+	+	+	+		+			+	+		+	+				+						
12/3	+	+	+	+	+					+			+					+					
15/2	+	+	+	+						+			+										
24/4	+	+	+	+						+			+										
10/3	+	+	+	+						+													
23/5	+	+	+	+	+	+				+	+		+	+			+	+					
11/5	+	+	+	+										+					+				
18/5	+	+	+	+		+			+					+	+	+	+		+	+	+	+	+
5/4	+	+	+	+	+	+	+		+		+		+	+	+	+	+		+		+	+	+
12/1	+	+	+											+	+		+		+	+	+	+	+
17/5	+	+	+											+		+	+	+	+			+	
24/3	+	+	+	+	+	+			+	+			+				+	+	+				
2/1	+	+	+	+					+					+					+				
2/4	+													+	+					+	+	+	
6/2														+	+						+	+	+
15/3	+													+	+					+	+	+	+
2/5	+													+	+					+	+	+	+
5/3	+													+	+					+	+	+	+
11/4	+													+						+			+
F$_1$ 1	+						+				+					+	+			+	+		
F$_1$ 17	+						+				+	+					+			+			

+ = Fully compatible.

give rise to both partial and fully compatible reactions both as male and female parents. The number of mating groups to which the F$_2$ plants may be allocated is 19, with a further 12 plants being unclassified. These latter plants may be allotted to more than one group or could represent unique genotypes. The occurrence of more than the 18 classes expected if the second hypothesis, Model II, applies may be accounted for by two possible explanations. First, errors in scoring the large numbers of pollinations cannot be ruled out although this is unlikely in view of the fact that one is differentiating between only partially and fully com-

patible reactions (see Cornish, 1979). Associated with possible misclassification, the excess of partially compatible individuals in the F_3 may reflect a viability disturbance in the pollen which, if manifest in the F_2 although not detected in the overall classification could lead to what in fact were fully compatible pollinations being wrongly scored as partially compatible. Secondly, failure of one of the assumptions, namely heterozygosity at either of the incompatibility loci in one of the original parents leading to a heterogeneous F_1 would also give rise to a greater number of classes than expected in the F_2. As indicated previously, however, no heterogeneity was detected in the F_1 diallel.

The results of using two of the F_1 plants as pollinators are also shown in Table 1. For both pollinators the reaction observed was of partial compatibility throughout. If the origin of self-compatibility is by mutation at either the S or Z locus (Model I) half of the pollinations are expected to be fully compatible. Whilst the observed result could have arisen by chance sampling, the probability of it occurring in the two sets of 10 and 13 individuals is less than 1 in 1000 when considered separately and less than 1 in 10^6 when they are combined. We may safely conclude therefore that Model I does not apply.

The general pattern of results is indicative of a self-compatibility gene (Sc) being operative in this material which is non-allelic to the S and Z loci. In rye ($Secale$ $cereale$) Lundqvist (1968) proposed the existence of a similar additional locus. In a closely related species, $Lolium$ $temulentum$, it has been shown that self-fertility is probably due to a mutation at the Z locus (Thorogood & Hayward, 1988). This gene has been successfully transferred to $L.$ $perenne$ and $L.$ $multiflorum$ and is fully functional in the changed genetic background. It would thus appear that in the $Lolium$ genus self-fertility may arise by at least two alternative pathways. The occurrence of a locus additional to the S and Z loci must raise questions as to whether the two-locus system has arisen by gene duplication, as proposed by Lundqvist (1962b), or whether it represents a remnant of a multilocus mechanism. The possible occurrence of multilocus systems has been suggested for the grasses by Lundqvist et $al.$ (1973) on the basis of their findings of three loci in $Ranunculus$ $acris$ and at least four in $Beta$ $vulgaris$. In the grass species, $Briza$ $spicata$, self-incompatibility is thought to be controlled by more than two loci (Murray, 1979). The expression of any relic locus could well be activated by the change in genetic balance brought about by the enforced inbreeding that has taken place. That such changes in the expression of incompatibility loci can occur when the genetic balance is disrupted is well established in a number of species and their hybrids (Darlington & Mather, 1949; de Nettancourt, 1977).

Acknowledgements

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