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Self-compatibility in *Lolium temulentum* L: its genetic control and transfer into *L. perenne* L. and *L. multiflorum* Lam

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The segregation of self-compatibility from *L. temulentum* was examined in backcross generations derived from hybrids between this species and the self-incompatible species, *L. perenne* and *L. multiflorum*, the latter being used as recurrent parents. Segregation patterns for self-compatibility were determined by percentage self seed set and by *in-vitro* self-pollination scores in the resulting backcross generations. Self-compatibility in *L. temulentum* is controlled in a gametophytic manner by a single gene mutation of either the *Z* locus or a locus tightly linked to it. Self-compatibility can be fixed in the homozygous form (i.e. 100 per cent pollen-tube growth on selfed stigmas) by selfing half compatible backcross plants. The *S* locus is still present in *L. temulentum* and functions when combined with a functional *Z* locus derived from either *L. perenne* or *L. multiflorum*. This has evolutionary significance for the relationship of the self-compatible and -incompatible *Lolium* species.

Keywords: incompatibility, *Lolium multiflorum*, *Lolium perenne*, *Lolium temulentum*, self-compatibility.

Introduction

The two locus (*S*, *Z*) multi-allelic, gametophytic incompatibility system has been found to operate in most outbreeding grasses including the agriculturally important *Lolium perenne* (Cornish *et al.*, 1979) and *L. multiflorum* (Fearon *et al.*, 1983). This system, although normally very efficient, occasionally breaks down such that self seed may be produced. Enforced selfing of plants can result in seletion of genotypes capable of self-fertilization (Jones & Jenabzadeh, 1981). One inbred line of *L. perenne* has been shown to posses a single gene mutation, independent of the *S* and *Z* loci, which acted gametophytically such that, on selfing, only pollen grains that possess the mutated allele (Sc^1) were able to penetrate the style and effect fertilization (Thorogood & Hayward, 1991). In addition, within the *Lolium* genus, naturally self-fertile species occur, such as *L. temulentum*, which hybridize relatively easily with both *L. perenne* and *L. multiflorum* (Jenkin, 1935,1954). Unilateral incompatibility (UI), a genetic mechanism which isolates self-incompatible and self-compatible species, is rarely encountered in the *Lolium* genus and so should not present a barrier to the transfer of self-compatibility genes from one species to another. Indeed, Nitzsche (1983) has shown that such transfer can be effected between *L. temulentum* and *L. perenne*. However, to increase the efficiency of transfer for the production of self-fertile lines of the two agricultural species, it is necessary to have information on the genetic control of self-compatibility. Such knowledge may also indicate the mode of evolution of self-compatibility in the *Lolium* genus.

The experiments described here follow the segregation of self-compatibility through the early generations obtained by backcrossing the hybrids, *L. temulentum* × *L. perenne* or *L. multiflorum*, to the outbreeders as recurrent parents (RPs) and examines the relationship of self-compatibility to the *SZ* incompatibility system.

Hypotheses for the control of self-compatibility in *L. temulentum*

Self-fertility is generally regarded as a derived condition (Jeffery, 1916; Ames, 1939) and has arisen as an essential step in the colonization of new habitats where the initial absence or scarcity of other pollen parents

would otherwise prevent a further spread and success of the immigrant species (Stebbins, 1957). Jenkin (1935) suggested that *L. temulentum* is derived from *L. perenne*. Until now, the genetic control of self-compatibility in *L. temulentum* has been unknown, although a number of models, from knowledge of the functioning of the two locus incompatibility system in *Lolium* spp. and of the evolutionary relationships between self-fertile and self-incompatible species in general, may be proposed.

Model 1

It has been suggested by Nitzsche (1983) that self-compatibility in *L. temulentum* arose due to the absence or inactivation of both self-incompatibility loci. If this is the case, assuming that the genes act gametophytically, backcross families produced by crossing *L. temulentum* × *L. perenne* or *L. multiflorum* F_1 hybrids to self-incompatible *L. perenne* or *L. multiflorum* plants would give rise to 1:2:1 ratios of self-incompatible (−), half (H) self-compatible and three-quarters (T) self-compatible plants (Table 1). If we assume that the self-incompatible parent used was heterozygous at both incompatibility loci, 64 possible backcross genotypes would be formed. Sixteen of these would possess fully functional incompatibility alleles (therefore self-incompatible), 16 would possess a non-functional allele at both *S* and *Z* loci and a further 32 would possess a non-functional allele at either the S or the *Z* locus. On male gamete formation, any pollen grain with a non-functional *S* or *Z* allele would cause the incompatibility reaction to break down as alleles at both loci are required to be matched in the pollen and the stigma for an incompatibility reaction to occur (Hayman, 1956). Thus, those plants with a single non-functional *S* or *Z* allele would be half self-compatible and those with a non-functional *S* and *Z* allele would be three-quarters self-compatible.

Table 1 Model 1. Compatibility genotypes obtained on crossing plants with and without incompatibility alleles and when backcrossing the hybrids to self-incompatible plants

$S_{ij}Z_{ij}$ × $S_{..}Z_{..}$

*$S_{i.}Z_{i.}$ etc. × $S_{kl}Z_{kl}$

		♂				
		S_kZ_k	S_kZ_l	S_lZ_k	S_lZ_l	
♀	S_iZ_j	$S_{ik}Z_{ik}$	$S_{ik}Z_{il}$	$S_{il}Z_{ik}$	$S_{il}Z_{il}$	(−)
	$S_iZ_.$	$S_{ik}Z_{-k}$	$S_{ik}Z_{-l}$	$S_{il}Z_{-k}$	$S_{il}Z_{-l}$	(H)
	$S_.Z_j$	$S_{-k}Z_{ik}$	$S_{-k}Z_{il}$	$S_{-l}Z_{ik}$	$S_{-l}Z_{il}$	(H)
	$S_.Z_.$	$S_{-k}Z_{-k}$	$S_{-k}Z_{-l}$	$S_{-l}Z_{-k}$	$S_{-l}Z_{-l}$	(T)

− = *SI*:H = half *SC*:T = three-quarters *SC*.
1 : 2 : 1

*Four different F_1 genotypes may be obtained, each giving rise to a different range of genotypes on backcrossing but which display the same pattern of segregation of self-incompatible to half and three-quarter self-compatible plants.

Model 2

It is possible that self-compatibility in *L. temulentum*, as a step to colonization of new habitats, may have arisen by a single gene mutation of one of the incompatibility loci.

Model 3

Self-compatibility may have arisen as a single epistatic gene, independent of the two incompatibility loci, which overrides the action of the *S* and *Z* loci.

These two single gene models assume that self-compatibility is controlled either gametophytically or in a combined sporophytic/gametophytic manner, whereby only pollen that possesses the self-compatibility gene is capable of affecting self-pollination, yet on the female side both the compatibility and the incompatibility factors are transmitted. Similar models were earlier proposed for the control of self-compatibility in an inbred line of *L. perenne* in which segregation for self-compatibility was assessed in F_1, F_2 and F_3 generations following the crossing of the self-fertile line with a self-incompatible line (Thorogood & Hayward, 1991). An alternative method proposed to study the genetic control of self-compatibility in *L. temulentum* would be to follow segregations for self-compatibility in backcross generations following the initial production of the F_1 hybrids. On both single gene models, two classes of compatibility can be expected to occur in each backcross generation. The F_1 hybrids, heterozygous for a single self-incompatible gene, when backcrossed to an unrelated self-incompatible recurrent parent, would produce progeny both with and without the self-compatibility allele in equal proportions. The self-fertile backcross plants would be heterozygous and therefore would give a half self-compatible reaction. The remaining backcross plants would not possess a self-compatible allele and therefore would give a self-incompatible (−) reaction. The advantage of this type of segregation is that not only

can the two compatibility types be distinguished by carrying out *in-vitro* self-pollinations but percentage seed-set on selfing plants, under isolating conditions, would confirm the two classes.

The two single gene models can be distinguished by carrying out pollinations between self-fertile BC1 plants and the original recurrent parents (RP) used to produce them. With a self-compatibility mutation of either the *S* or *Z* locus, backcross pollinations can only give rise to three-quarters compatibility (Table 2a). However, when a separate self-compatibility locus is involved, which segregates independently of the two incompatibility loci, the backcross pollinations would show a reciprocal difference in compatibility proportions. When the self-fertile BC1 plant is the pollinator, seven-eighths (7/8) compatibility would be expected whereas the reciprocal would exhibit a three-quarters compatible reaction. The self-incompatible BC1 plants would give reciprocal three-quarters compatible reactions with their RP (Table 2b).

Model 4

Self-compatibility may also have arisen by the accumulation of polygenic modifiers, in which case self-compatibility in the first backcross generation would be expressed as a continuous distribution of plants ranging from low or zero self-compatibility up to 100 per cent self pollen-tube growth. Alternatively, first backcross generation plants may not possess enough of the *L. temulentum* genome to allow expression of self-compatibility at all. This would be especially so if genes essential for the expression of self-compatibility were spread throughout the *L. temulentum* genome. Clearly, from a practical point of view, transfer of polygenically controlled self-compatibility may be less feasible and more unpredictable than if it is controlled by only a few genes.

Table 2(a) Backcross pollinations expected when F_1 plants possess a heterozygous self-compatibility mutation at the *Z* locus

		♂		
$F_1 = S_{ij}Z_{-i}$		RP $S_{kl}Z_{kl}$	*SC* BC1 $S_{ik}Z_{-k}$ etc.	*SI* BC1 $S_{ik}Z_{ik}$ etc.
♀				
RP	$S_{kl}Z_{kl}$	—	T	T
SC BC1	$S_{ik}Z_{-k}$ etc.	T		
SI BC1	$S_{ik}Z_{ik}$ etc.	T		

Table 2(b) Backcross pollinations expected when F_1 plants possess a heterozygous self-compatibility mutation at a locus separate to *S* or *Z*

		♂		
$F_1 = S_{ij}Z_{ij}$Ff		RP $S_{kl}Z_{kl}$ff	*SC* BC1 $S_{ik}Z_{ik}$Ff etc.	*SI* BC1 $S_{ik}Z_{ik}$ff etc.
♀				
RP	$S_{kl}Z_{kl}$ff	—	**7/8**	T
SC BC1	$S_{ik}Z_{ik}$Ff etc.	T		
SI BC1	$S_{ik}Z_{ik}$ff etc.	T		

Materials and methods

F_1 hybrids between *L. temulentum* on the one hand and *L. perenne* and *L. multiflorum* on the other (produced by Dr G. M. Evans, Department of Agricultural Science, University of Wales, Aberystwyth) were used as the female recipient parent on to which recurrent *L. perenne* and *L. multiflorum* parents were backcrossed. The hybrid plants (Table 3) were produced by crossing hand-emasculated Ba3081 *L. temulentum* plants with *L. perenne* and *L. multiflorum* plants and culturing resultant embryos on a modified Gamborg and Miller B5 medium.

In order to aviod the possibility of cross-incompatibility on backcrossing, the RP plants were not of the same incompatibility genotype as the self-incompatible parents used to produce the original hybrids but had similar flowering dates so that no problems with synchronizing for crossing occurred. They were also practically self-incompatible, although some plants produced a small number of self seed when bagged as isolated units. The *L. multiflorum* variety, Bb2067, was used as the pollen parent to backcross on to the 'A' and 'C' hybrids, *L. perenne* cv Cropper on to the 'D' and 'F' hybrids and *L. perenne* cv Aurora on to the 'E' hybrids.

Two cycles of backcrossing were carried out and, in addition, each backcross was selfed, by bagging inflorescences from plants as single selfing units, to produce the BC1S1 generation to assess the segregation of

Table 3 Hybrid plant material

Cross type	Number of plants	Pedgree
A	20	Ba3081 × (Bb1232/4 × Titania)
C	20	Ba3081 × (Bb1232/4 × Trident)
D	20	Ba3081 × (Lp19 × S24)
E	20	Ba3081 × (Lp19 × Aurora)
F	6	Ba3081 × (Lp19 × Premo)

self-compatibility genes as determined by the seed set data. BC1 progeny were obtained by embryo-culture or from mature seed and subsequent generations were obtained from mature seed only.

In-vitro self pollinations of BC1 and BC1S1 plants, and cross-pollinations between BC1 plants and the RP genotypes used to produce them, were carried out using the Petri-dish method of Lundqvist (1961) and analysed using the aniline blue fluorescence technique of Lalouette (1967).

Isozyme phenotypes of BC1 plants at five loci, namely *PG1/2, GOT/2, GOT/3, AcP/2* and *SOD* were determined using electrophoretic separation systems developed for the forage grasses by Hayward & McAdam (1977) in order to investigate the possibility of linkage between these loci and any *L. temulentum*-derived self-compatibility genes.

Results

The F_1 hybrid plants were, as expected, male-sterile (Jenkin, 1935, 1954) but backcross progeny could be obtained by using these hybrids as female parents.

The first generation back-crosses to *L. multiflorum* (Bb2067) yielded 18 plants all of which were male-fertile with fully dehiscent anthers producing plentiful quantities of pollen. The *L. perenne* cv Aurora back-crosses were less successful, with only 22 of the 52 plants producing enough viable pollen to effect fertilization. Not one of the 14 backcross plants resulting from 'D'-type hybrids produced dehiscent anthers. Of the 40 BC1 plants from which viable pollen could be collected, 21 were fully self-incompatible and 19 showed some self-compatible grains on *in-vitro* self-pollination. These results fit a 1:1 ratio ($P > 0.80$) as proposed in models 2 and 3 and give significant deviation from the 1:3 ratio ($P < 0.001$) expected on model 1 (Table 4).

Counts of compatible and incompatible pollen grains of two of these self-fertile BC1 plants were carried out after *in-vitro* self-pollination. A large proportion of inviable pollen grains showing no fluorescence reaction made counts difficult in other cases. All pollinations could be classified as half self-compatible with most pollinations giving segregation ratios that differed significantly from a 1:3, SI:SC ratio but with all pollinations agreeing with a 1:1 ratio (Table 5).

Self-compatibility is ultimately expressed in terms of the number of florets setting seed in the absence of pollen from other plants. None of the self-incompatible BC1 plants produced more than 0.67 per cent (5) seeds, whereas those plants classified as self-fertile gave a range of seed set figures from 1.41 to 53.57 per cent. Thus percentage seed set agreed fully with the compatibility status of the plants as classified by *in-vitro* self-pollination (Fig. 1).

Table 4 Number of self-incompatible, self-fertile and male-sterile plants in BC1 and BC2 generations

Cross type	Self in-compatible	Self-compatible	Model 1 χ^2 (1:3)	Models 2 and 3 χ^2 (1:1)	Number of male-sterile plants
BC1†					
A	4	8	0.11^{ns}	0.75^{ns}	—
C	4	2	3.56^{ns}	0.17^{ns}	—
D	—	—			14
E	12	9	9.92***	0.19^{ns}	23
F	1	—			—
Total	21	19	14.70***	0.02^{ns}	37
BS2††					
A	16	19	6.94**	0.11^{ns}	—
C	7	3	8.53**	0.90^{ns}	—
E	13	12	8.33**	0.00^{ns}	—
Total	36	34	24.69***	0.01^{ns}	—

** = Significant difference at 1 per cent level
*** = Significant difference at 0.1 per cent level.
ns = No significant difference from expected ratio.
†Based on *in-vitro* self-pollination scores.
††Based on seed set data only. Plants derived from self-fertile BC1 plants.

Some of the BC2 seed was sown and plants, when not produced from emasculated crosses, were identified as resulting from BC1 × RP crosses and reciprocals by the use of isozyme markers. The overall fertility of the plants was vastly increased with no male-sterile plants being identified.

In the BC1 generation, the percentage self seed set agreed perfectly with the *in-vitro* pollination scores and thus in the BC2 generation it was considered necessary to obtain seed set data only. Five BC2 plants from each of 14 self-fertile BC1 plants were selfed, five plants being a large enough sample to obtain at least one self-incompatible and one self-fertile segregant in 95 per cent of cases if a 1:1 ratio (models 2 or 3) is expected. If plants segregate according to model 1, there would be an approximately 25 per cent chance of obtaining families with no self-incompatible segregants. Thus such an absence would be likely to occur in about three of the 14 families selected and tested. With an arbitrary level of 1 per cent self seed set to distinguish self-incompatible from -fertile plants, every self-fertile plant produced at least one self-incompatible and one self-fertile plant. The overall segregation of the BC2 agreed closely with a 1:1 ratio (Table 4). The percentage seed set figures of the self-fertile plants followed a normal distribution and data for all BC2 plants indicated a clear bimodal distribution into self-incompatible and self-fertile plants (Fig. 2). Mean seed set was increased in the BC2 self-fertile plants (BC1 = 16.45 per cent, BC2 = 37.02 per cent).

Three BC2 plants from each of 21 self-incompatible BC1 plants were also selfed, of which nearly 80 per cent (50 plants) were also self-incompatible. The remaining 13 plants, each derived from one of eight of the BC1 plants, produced between 7.11 and 50.62 per cent self seed, enough to consider them as self-fertile. Furthermore, there was no obvious relationship between the level of pseudo-self-compatibility (characterized by low self seed set) shown by some of the self-incompatible BC1 plants and the high level of self-fertility in their BC2 derivatives.

Forty-one progeny resulting from the selfing of self-fertile BC1 plants were tested for a self pollen/stigma reaction (Table 6). Pollen quality was poor in some of the pollinations. Some pollen grains exhibited a diffuse

Table 5 Number of self-incompatible and self-compatible grains in self-compatible BC1 plants

Cross type	SI	SC	χ^2 1:3 (model 1)	χ^2 1:1 (model 2)
A	154	129	129.05***	2.04^{ns}
C	51	58	46.29***	0.33^{ns}
E	74	66	56.47***	0.35^{ns}
Total	279	253	212.23***	1.17^{ns}

ns = No significant difference from expected ratio.
*** = Significant difference at 0.1 per cent level.

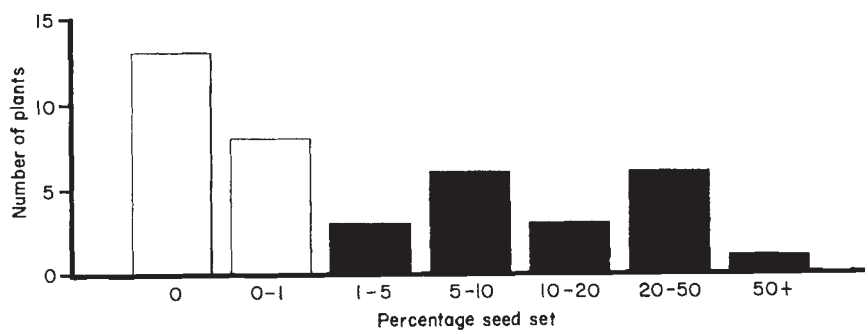

Fig. 1 Percentage seed set by BC1 plants. (□) S1 BC1 selfed, (■) SC BC1 selfed.

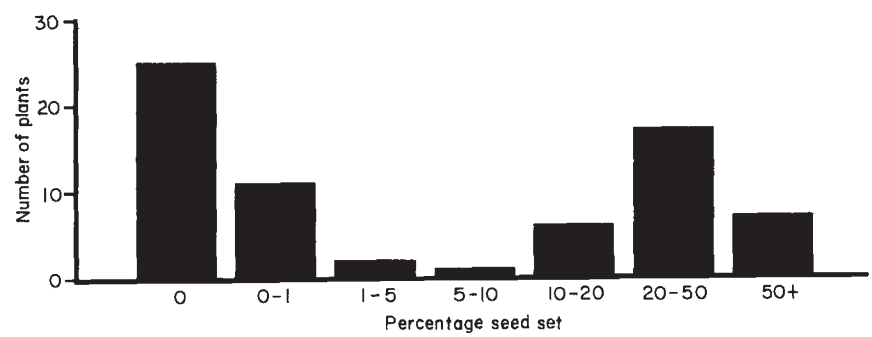

Fig. 2 Percentage seed set by BC2 plants derived from SCBC1 plants.

Table 6 Self pollen reaction of some BC1S1 plants

BC1 plant	Number of partially self-compatible plants	Number of fully self-compatible plants
A7/19/7	4	5
A7/19/9	1	1
E4/13/1	2	1
E4/28/11	3	0
E4/28/19	3	0
E4/28/23	3	0
*E6/5/1	3	4
E6/5/4	8	1
Total	27	12

*Two plants in this family could not be classified as all pollen grains exhibited a diffuse fluorescence which could not be interpreted as either an incompatible or a compatible grain.

fluorescence and could be classified, on casual observation, as incompatible but, as they did not show any sign of pollen-tube tip growth or the cresent-shaped deposition of callose typical of an incompatible reaction, they were deemed to be inviable. A high proportion of dead pollen in other pollinations did not show any fluorescence reaction. The presence of these inviable pollen grains may have caused an over-estimate of incompatible grains but, even so, a number of fully compatible (+) self pollinations were observed (Table 6).

Eight BC1 plants resulted from a cross between the hybrid A7/19 and its RP, Bb2067/7/3. The pollen/stigma reaction of reciprocal crosses between seven of the BC1 plants and the RP were tested to determine the relationship between self-compatibility and the SZ incompatibility system. Pollen counts were made and the ratios of compatible to incompatible pollen grains consistently agreed with a 3:1 ratio (three-quarters compatible pollen). In no pollination, could a 7:1 ratio (seven-eighths compatible pollen) be fitted satisfactorily (Table 7).

Joint segregations of self-compatibility with the *PGI/2, GOT/2, GOT/3, AcP/2* and *SOD* isozyme loci were calcuted for BC1 plants. A significant joint segregation of the *GOT/3* locus with self-compatibility was found where the *L. temulentum* derived 'c' allele was more often than not associated with self-fertile individuals (Table 8).

Discussion

L. temulentum hybridizes relatively easily with *L. perenne* and *L. multiflorum* as either the male or the female parent although embryo-rescue techniques are

Table 7 Segregation of compatible to incompatible (C:I) pollen in backcross pollinations between self-fertile and self-incompatible BC1 plants used as either male or female parent and their RP plants

	C:I grains	χ^2 (3:1)
SC BC1 as ♂	132:46	0.07^{ns}
SI BC1 as ♂	78:36	2.63^{ns}
SC BC1 as ♀	222:77	0.16^{ns}
SI BC1 as ♀	161:61	1.20^{ns}
Total	593:220	1.73^{ns}
Heterogeneity		2.33^{ns}

ns = No significant difference from expected ratio.

Table 8 Joint segregation of the GOT/3 isozyme locus with self-compatibility

	GOT/3 -c	GOT/3 --	
SC(H)	10	5	15
SI	3	12	15
	13	17	

	χ^2 (1:1)	d.f.	*P*
-c:--	0.30	1	ns
SC(H):SI	0.00	1	ns
Joint segregation	5.63	1	<0.05
Total	5.93	3	ns

c = *L. temulentum*-derived allele.
- = *L- perenne/L. multiflorum*-derived allele.
SC(H) = Half self-compatible.
SI = Self-incompatible.

required to obtain adequate numbers of hybrid plants. It therefore follows that no absolute isolation mechanism is commonly present between these members of the *Lolium* genus which would prevent the introduction of self-compatibility genes into normally self-incompatible plants.

Despite the low numbers of BC1 plants produced and the poor fertility status of many of these plants, self-compatibility derived from *L. temulentum* was expressed both in terms of pollen/stigma reaction and ultimately in numbers of self seed set. Self-compatibility was gametophytically controlled as shown by the differential pollen reaction (compatible to incompatible) on the self stigmas. Furthermore, the

bimodal segregation of self-fertile and self-incompatible plants in both BC1 and BC2 generations and the clear half self-compatible pollination in self-fertile BC1 plants indicated that a single gene (*F*) controls self-compatibility. The poor pollen viability especially in the BC1 plants can be accounted for by irregularity in chromosome pairing which had been seen to give rise to univalents and unbalanced complements (M. Scanlon personal communication). Seed set was vastly improved in the BC2 generation as the proportion of RP and, thus, chromosome pairing was increased. The BC2, with its increased fertility, therefore gives a more realistic picture of the percentage seed set possible in this self-fertile material.

The production of a significant number of self-compatible plants from self-incompatible BC1 plants was not further investigated. There was no predictable segregation pattern in these progeny and it is thought that self-compatibility was probably due to polygenic modifiers of the incompatibility system brought into appropriate combinations by recombination of the genetic background (Lundqvist, 1975).

With self-compatibility under single gene control, it is easy to fix it in the homozygous state simply by selfing or intercrossing heterozygous individuals. One hundred per cent pollen-tube growth, indicating homozygosity, was obtained in some BC1S1 progeny by the former method.

Two alternative models have been proposed for the control of self-compatibility by a single gene, which can be distinguished from each other by the differing proportions of compatible to incompatible grains expected in backcrosses when the self-fertile BC1 plants are used as the male parent. Three-quarters compatible pollen reactions are expected in model 2 in all backcross pollinations whereas seven-eighths compatible pollen reactions would have been expected in model 3 in crosses using the self-fertile BC1 plants as males. The lack of heterogeneity detected between the four classes of backcross pollination gives a clear indication that the *F* gene is either a mutation of the *S* or *Z* locus or is at least tightly linked to one of the incompatibility loci (model 2). This of course could be confirmed by carrying out interpollinations of BC1S1 families but, as mentioned previously, the quality of the pollinations was far from adequate for a detailed diallel to be carried out.

The *F* gene showed significant joint segregation with the *GOT/3* isozyme locus, which suggests a loose linkage relationship in the expected phase, where the *GOT/3* 'c' allele from *L. temulentum* was coupled with the *L. temulentum*-derived *F* gene. The estimated recombination frequency was 0.27 ± 0.08. The *PG1/2* locus has been found to be linked to the *S* locus in *L. perenne* (Cornish *et al.*, 1980.) and *GOT/3* and *PG1/2* are known to be on separate chromosomes (2 and 6 respectively) (Lewis *et al.*, 1980). From this it can be deduced that the *F* gene is either allelic to or closely linked to the *Z* locus on chromosome 2. Some corroborative evidence of disturbed allelic ratios at the *GOT/3* locus in certain *L. perenne* paircrosses suggested that *GOT/3* was linked to an incompatibility locus (Hayward & McAdam, 1976).

One of the incompatibility loci (*S*) appears to be still functional in the *L. temulentum* accesssion used in this present study, otherwise segregations would agree with those proposed in model 1. The discovery of a functional incompatibility locus in a self-fertile species is of considerable significance for the possibility of reconstructing a self-incompatible form from genotypes that possess different self-compatibility loci (Larsen *et al.*, 1973). Functional incompatibility loci would also provide additional evidence for the theory that self-fertile species are derived from self-incompatible ancestors. As the two incompatibility loci in the grass system rely on complementation for their expression, the single incompatibility locus in *L. temulentum* is unable to act in the absence of a functional product from the mutated locus. The continued existence of the apparently redundant self-incompatibility locus therefore demands the question as to its role: is it a vestige of the original self-incompatibility system or does it carry out another, as yet, unknown function?

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