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Self-incompatibility in ryegrass. XI. Number and frequency of alleles in a cultivar of *Lolium perenne* L.

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An assay of the number of incompatibility alleles in an experimental cultivar of *Lolium perenne* found 17 *S*- and 13 *Z*- alleles in samples of 40 and 43 plants examined, respectively, which yielded estimates of 26 *S*- and 18 *Z*-alleles in the population. This cultivar was founded, however, on only five plants that between them could have contained no more than 10 alleles at each locus. It is suggested that the additional alleles originated by migration of pollen from neighbouring populations of ryegrass during seed-multiplication rather than from mutation.

Keywords: *Lolium perenne*, number of alleles, two-locus self-incompatibility polymorphism.

Introduction

Cultivars of forage grasses are usually founded on a small number of plants, referred to by breeders as basic plants, that have been selected for their superior agronomic performance. These basic plants are not infrequently descended from a very small number of families, so that they may be related by descent. For these reasons, the number of *S*- and *Z*- alleles present in a cultivar could be very small and their frequencies unequal. While only two alleles are required at each locus to maintain the polymorphism, difficulties with seed-set could be experienced during seed multiplication if the number of alleles at each locus is very small, especially if their frequencies are unequal, because at least five equally frequent alleles are required at each locus if the cross-incompatibility between plants is to be reduced to less than 1 per cent (Lundqvist, 1963; Fearon *et al.*, 1994).

The purpose of the investigation reported in this paper was to determine the number and frequency of *S*- and *Z*-alleles in an experimental variety of perennial ryegrass, Ba8674, which had been founded from five diploid plants that had been selected from the long-established and successful W.P.B.S. variety, S23. In principle, this variety could have contained as many as 10 alleles at each locus, provided that each of the basic plants were double heterozygotes and none possessed alleles that also occurred in the others; if this is the case, the population founded from these plants would be expected to have a very high level of cross-compatibility of 99.9 per cent (Fearon *et al.*, 1994). In practice, however, it is unlikely that each allele would occur only once, even if the sample of five plants had been drawn at random from a population containing a large number of equally frequent alleles at each locus. The chief question with which we are concerned in this paper is the extent to which the theoretical maximum of 10 alleles at each locus is realized in this variety.

Materials and methods

In order to provide enough seed of a potentially new variety of a species of forage grass for comparative trials with other varieties and, assuming these are successful, to provide enough seed to market the variety, it is necessary to advance the material through a series of stages of a seed multiplication programme. In the first stage, five clonal replicates of each of the five basic plants are brought into flower in an isolation chamber in a glasshouse in which the plants are allowed to open pollinate to provide what is referred to as breeder's seed. This seed is then used to sow a plot in open ground, where the plants are again allowed to open pollinate, in isolation from other populations of ryegrass, to provide what is termed prebasic seed. This process is essentially repeated in the following seasons to provide, firstly, basic seed and, finally, certified seed,

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except that these stages of the seed multiplication programme are usually carried out, under contract, on a number of commercial farms. The certified seed used by the farmer is, thus, the product of at least three successive generations of seed multiplication.

The seed used to raise the plants of the variety Ba8674 used in the present investigation was certified seed obtained from a semi-commercial source. Fifty or so of these plants were, when they came into flower, crossed with the $S_{1.1}Z_{1.1}$ tester to initiate the crossing programme described in the previous paper (Fearon *et al.*, 1994). At the end of this programme, 40 plants were analysed in the *S*-diallel and 43 in the *Z*-diallel, 40 of the latter being descended from the same ancestors as the former. All other details were the same as described previously (Fearon *et al.*, 1994).

Results

Tables 1 and 2 show that the plants of this sample contain, between them, more than the expected maximum number of alleles at each locus of 10. Thus, 17 different *S*-alleles were identified among the 40 plants analysed in the *S*-diallel and 13 *Z*-alleles among the 43 plants of the *Z*-diallel. The frequencies of these alleles are, furthermore, very unequal. Thus, while nine of the *S*-alleles occurred only once, one occurred nine times in the sample of 40 *S*-alleles examined. Again, while five of the *Z*-alleles occurred only once, one occurred no less than 21 times in the total of 43 alleles examined.

As in the previous paper (Fearon *et al.*, 1994), these data can be used to obtain estimates of the number of alleles in the variety. As the allele frequencies are significantly unequal at both loci, the E_2 estimator of O'Donnell & Lawrence (1984) appears, again, to be the most appropriate expression for this purpose; this yields estimates of 26 alleles at the *S*- and 18 alleles at the *Z*-locus in this cultivar. For reasons given earlier (Fearon *et al.*, 1994), however, these estimates are almost certainly biased downwards, particularly that concerning the number of *Z*-alleles. The Ba8674 population is likely to contain, therefore, perhaps as many as three times the number of *S*-alleles and twice the number of *Z*-alleles than the maximum that could have been present in the five basic plants on which the variety was founded.

Table 1 Partial incompatibility genotype of the 43 plants classified in the *S*- and *Z*-diallels

	Genotype			Genotype	
Plant no.	S_a	Z_c	Plant no.	S_a	Z_c
1	9	9	24	4	1
3	3	1	26	17	1
4	14	1	27	1	1
5	12	1	28	8	4
6	1	1	29	7	1
7	—	1	30	1	1
8	2	5	31	1	7
9	13	3	32	1	8
10	2	2	33	1	2
11	2	1	34	5	7
12	3	1	35	1	10
13	2	2	36	1	3
14	4	1	37	10	6
15	2	1	38	2	1
16	7	11	40	5	2
17	—	1	41	8	13
18	3	1	43	6	1
19	3	1	44	1	4
20	15	1	45	11	3
21	4	5	46	16	12
22	6	6	47	—	1
23	3	8			

Discussion

The obvious question that these results raise is where did the extra alleles come from? Did they appear in the population by mutation or by immigration?

Now if the additional alleles arose by mutation, the rate of mutation must have been very high to account for the very considerable excess of those observed at

Table 2 Summary of the data of Table 1

(a) *S*-alleles
No. of plants sampled = 40
No. of alleles found = 17
Repeatability = 0.61

Frequency distribution of these alleles:

No. of occurrences	1	2	3	4	5	6	7	8	9
No. of alleles	9	4	1	0	1	1	0	0	1

$P(\chi^2_{16} \geq 34.800) = 0.0028$

(b) *Z*-alleles
No. of plants sampled = 43
No. of alleles found = 13
Repeatability = 0.73

Frequency distribution of these alleles:

No. of occurrences	1	2	3	4	21
No. of alleles	5	5	1	1	1

$P(\chi^2_{12} \geq 105.442) < 0.000003$

See Table 4 of Fearon *et al.* (1994) for further details.

each locus compared with the maximum number expected. Furthermore, most of this mutation would have had to have occurred in the early generations of seed multiplication for the frequencies of the mutant alleles to have been high enough to have been captured in a sample of 40 or so plants. The problem with this explanation is that the spontaneous mutation rate at incompatibility loci of species with gametophytic systems appears to be very low and that no mutations to new functional alleles have ever been obtained (Lewis, 1948, 1951; Lewis & Crowe, 1954; Hayman & Richter, 1992). The most likely explanation of these results is, therefore, that the additional alleles have appeared in the population *via* immigration of pollen from neighbouring populations during seed multiplication. This conclusion has, of course, quite serious practical implications because while a mutational origin of these alleles would involve the contamination of the population by these alleles alone, their arrival in the population *via* pollen would entail contamination by whole genomes.

The final point worth making about these results concerns the unequal allele frequencies observed at each locus. Thus, while with a sample taken from a natural population there is an *a priori* expectation that the alleles at each locus should be equally frequent, no such expectation holds for a population founded from a very small number of plants. Furthermore, simulation of this situation on the computer shows that when a population is increased in size as quickly as the Ba8674 population was during seed multiplication, which was typical of the rate of multiplication used in commerce, the allele frequencies in the final generation are virtually identical to those in the founding generation, which implies that, in these rather special circumstances, frequency-dependent selection has had insufficient time to equalize allele frequencies and that the number of gametes sampled in each generation is too great for drift to have had much effect. It is probable, therefore, that the alleles that occurred at a relatively high frequency in the *S*- and *Z*-diallels are those that were present in the original five basic plants. Inspection of Table 1 suggests that S_1, which occurred nine times and S_2, S_3 and S_4, which occurred six, five and three times, respectively, in the sample of 40 plants examined in the *S*-diallel probably occurred also among the five foundation plants, the remaining 13 alleles being immigrants; and that of the *Z*-alleles, Z_1, which occurred 21 times and Z_2 and Z_3, which occurred four and three times, respectively, among the sample of 43 plants analysed in the *Z*-diallel were among the foundation alleles, the remaining ten being immigrants. Indeed, as Z_1 occurred in nearly half of the plants of the *Z*-diallel sample, it is probable that two or more of the basic plants were homozygous for that allele. The obvious way of testing these surmises would be to determine the incompatibility genotype of each of the five basic plants. Herbage seed multiplication procedures are based on strict guidelines that are designed to minimize the likelihood of contamination of seed crops (Griffiths, 1950). Nevertheless, subsequent tests, using isozyme markers, have indicated that some contamination of this variety had occurred. There is, of course, no reason for supposing that the contamination of this material by immigrant pollen is peculiar to this particular cultivar. While contamination of this magnitude is clearly undesirable for agronomic reasons, it at least suggests that in practice the current isolation of seed crops of wind-pollinated grasses may sometimes be less effective than was previously assumed.

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