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USE OF NATURAL OUTCROSSING TO IMPROVE THE ANTHRACNOSE RESISTANCE OF STYLOSANTHES GUIANENSIS

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ABSTRACT

Experiments were planted at two sites in southeast Queensland to determine whether dominant genes for resistance to the anthracnose disease pathogen, *Colletotrichum gloeosporioides*, could be transferred by natural outcrossing from resistant genotypes of *Stylosanthes guianensis* to a set of susceptible selections. The smooth stem/bristly stem phenotypic contrast, shown to be controlled by a single gene (Bri/bri bri), and disease resistance itself were used to detect outcrosses. Disease resistance was transferred to 244 of the 385 susceptible progenies tested. Estimates of outcrossing rate based on stem bristles varied from 1.6 to 21.9% between selections ($P < 0.001$) but differences between sites were not significant. The advantages of increasing outcrossing rate through selection and an outline for a recurrent selection scheme based on outcrossing are briefly discussed.

KEYWORDS

Inheritance, stem bristles, outcrossing rate, disease resistance.

INTRODUCTION

A breeding programme has been developed to combine the best agronomic attributes of sub-tropical and tropical forms of *Stylosanthes guianensis*, a predominantly self-pollinating, perennial pasture legume (Cameron, 1977). Three cycles of crossing have been used to help overcome severe infertility which reduced chances of selecting superior recombinants. During the current phase of agronomic selection from the third crossing cycle we wish to improve resistance to the Type B anthracnose disease caused by the fungus *Colletotrichum gloeosporioides* (Irwin and Cameron, 1978). Type A disease causes only slight damage to *S. guianensis* but Schofield and Endeavour cultivars have been devastated by Type B disease. Two dominant genes for resistance to Type B are present in the breeding population (authors, unpub. data), but races which match these genes appear in Australian populations of the fungus. Four races of the Type B disease have already been identified in Australia (Irwin *et al.*, 1986) and a wider range of races is known to occur in South America (Lenne *et al.*, 1982). Development of cultivars with durable resistance to this variable pathogen will be a difficult task but incorporation of several more resistance genes into the breeding populations may allow selection of cultivars with acceptable levels of field resistance.

Dominant genes with resistance to three or four of the Australian Type B races (authors, unpub. data) have been found in four tropical accessions which have not been used in the breeding programme. Since selections from the breeding programme are still highly variable for fertility and agronomic characters, very large numbers of hand crosses would be required to introduce resistance genes from these four tropical accessions into an adequate sample of genotypes from the breeding population. Gene transfer by natural outcrossing, as advocated by Miles (1985), could be much more efficient than the slow and difficult procedure of hand crossing, particularly if outcrossing rates similar to the 13.8% reported from Colombia (Miles, 1985) can be achieved in Australian conditions. In this paper we report the results of an experiment designed to transfer anthracnose resistance genes to the breeding population using natural outcrossing. A stem bristle character was used to estimate outcrossing rate.

MATERIALS AND METHODS

Inheritance of stem bristles

The inheritance of a stem bristle character used in the outcrossing study was examined in two pot experiments. A total of 1089 F_2 progeny from two crosses between a smooth-stemmed cultivar (Graham), and two bristly lines (L1 and L2), three crosses between Graham and three other smooth lines, and four crosses among five bristly lines were grown in the first experiment. Additional F_2 plants from Graham x L1 and Graham x L2, and 50-100 F_3 progeny

from each of 29 smooth and 14 bristly F_2 plants were grown in the second experiment. Plants in both experiments were scored for the presence/absence of stem bristles eight weeks after planting (Table 1).

Table 1. Segregation for stem bristles in F_2 populations of *Stylosanthes guianensis*.

Cross	Type combination	Smooth plants	Bristly plants
Graham x L_1	smooth x bristly	115	49
Graham x L_2	smooth x bristly	96	29
Graham x 81278	smooth x smooth	69	0
Graham x L_3	smooth x smooth	58	0
Graham x 18750	smooth x smooth	148	0
79639 x L_2	bristly x bristly	0	120
L_4 x 79639	bristly x bristly	0	139
L_1 x L_2	bristly x bristly	0	113
L_1 x L_4	bristly x bristly	0	153

Outcrossing study

The four accessions carrying dominant resistance genes flower later than the breeding population so it was necessary to cross the accessions with the earlier flowering cultivar, Graham, to improve synchronisation of flowering. One of the accessions, CPI 79637 (CPI = Commonwealth Plant Introduction number), carries two dominant genes for Type B resistance (authors, unpub. data) but the cross with Graham is only partly fertile. A single backcross to Graham and selection for disease resistance, early flowering, and fertility in BC_1S_1 provided more suitable lines for the outcrossing experiment. Each of the other three accessions carries a single dominant resistance gene (allelic relationships for these genes have not been determined) and produces fertile hybrids with cv. Graham. F_2 populations of these three crosses were used.

Outcrossing blocks were grown in 1984/85 at the Samford (27°22'S., 152°53'E) and Narayen (25°41'S, 150°52'E) Research Stations using a three-row arrangement. Single rows of selections (females) from the breeding programme were grown on either side of a single row (males) of smooth stemmed plants carrying resistance to 22269 (Queensland Department of Primary Industries, Plant Pathology Branch accession number), an isolate of Race 3 of Type B *C. gloeosporioides*. The male selections were from the crosses between cv. Graham and the four disease resistant accessions. The 59 female selections were smooth, bristly, or mixed and most were susceptible to the 22269 isolate.

Each of the female selections was represented by a row of five plants in each of four replicates and the male entries were randomly allocated to individual positions in the male rows. The plants in the male rows were expected to include some plants flowering later than the plants in the female rows. Two plants were grown at each planting position in the male rows to increase the probability that flowering of adjacent male and female plants would be synchronised. In both male and female rows, planting positions were spaced at 1.5 m in the row with rows 1.5 m apart. Presence/absence of stem bristles was recorded for all the female plants. Seed samples were collected from a small portion (*c.* 0.06 m^2) of each selected female plant, immediately adjacent to the nearest male plant to maximise the chances of recovering crosses due to bee transfer of pollen. Seed from the most fertile female plants (197 from Samford, 225 from Narayen) was scarified and sown in 12.5 cm pots, one progeny of *c.* 100 seeds per pot, in a controlled environment room.

Six weeks after planting all pots were inoculated with a spore suspension (*c.* 1 x 10^6 spores/ml) of a Race 3 Type B isolate, UQ62 (University of Queensland accession number; substituted for 22269 which had lost virulence). The spores were harvested from oatmeal agar plates which had been streaked with conidia and incubated at 25-27 °C under near-ultraviolet light for five days. Disease severity and stem bristles were scored 9-12 days after inoculation. Disease was scored on a 1-10 scale (1 = <1% necrosis, 2 = 1-10% necrosis, ...10 = >80% necrosis) and plants with a score of 1 were classed as resistant. Outcrosses in the progenies were identified from one of two phenotypic contrasts, either disease resistant/disease susceptible (resistance controlled by dominant genes) or smooth stem/bristly stem (smooth dominant to bristles).

Outcrossing rates were calculated using the smooth stem/bristly stem phenotypic contrast. The frequency, p, of the dominant smooth allele was inferred on the assumption that smooth homozygotes comprised one third of the number of smooth plants in each male or female selection that was segregating smooth and bristly plants. Assuming that all plants contributed equally to the pollen pool, outcrossing rate, t, was estimated for the individual progenies of bristly plants as:

$$t = \frac{H}{p}$$

where H is the proportion of smooth stem heterozygotes in the progeny of a bristly plant. Arcsin transformations for 125 bristly progenies from 17 selections (5-14 progenies/selection) were used for an analysis of variance of selections, sites, and the selection x site interaction. The other 38 bristly progenies were omitted from the analysis because they came from selections sampled at only one site or with less than five progenies sampled over the two sites.

RESULTS

Inheritance of stem bristles

In the first experiment the crosses among smooth lines and among bristly lines did not segregate and the two smooth x bristly crosses segregated 211 smooth to 78 bristly (P for 3:1 of 0.25-0.50). Single gene control with smooth dominant to bristly was confirmed by the results from the second experiment with F_2 and F_3 populations (Table 2). In the F_3 generation all plants of the 14 families from bristly F_2 plants were bristly. Segregation of families from smooth F_2 plants is shown in Table 2. The 19 segregating families from smooth F_2 plants contained 976 smooth and 308 bristly

Table 2. Segregation for smooth and bristly stems in two *Stylosanthes guianensis* crosses.

Cross	F_2 generation No. of plants			F_3 generation No. of progenies of smooth plants		
	Smooth stems	Bristly stems	P(3:1)	All smooth	3 smooth: 1 bristle	P(1:2)
Graham x L1	60	27	0.10-0.25	5	13	0.50-0.75
Graham x L2	61	21	>0.90	5	6	0.25-0.50

plants (P for 3:1 of 0.25-0.50). We propose that the alleles for the stem bristle gene be designated Bri (smooth allele) and bri (bristly allele).

Outcrossing study

Some 32 490 seedlings were scored from the 422 progenies. Of the 259 progenies from smooth plants, 37 were resistant or segregating for resistance to isolate UQ62, so outcrosses to the disease resistant males could not be distinguished. Outcrosses were detected in 254 of the remaining 385 progenies (Table 3), and 244 progenies contained at least one resistant outcross per progeny. A total of 749 resistant outcrosses was identified, 362 from Samford and 387 from Narayen, and the proportion of resistant outcrosses (no. of resistant outcrosses per total no. of progeny plants) for the Samford and Narayen sites was similar at 0.024 and 0.027, respectively. However, the proportion of resistant outcrosses recovered from different female selections varied widely from 0.006 to 0.086 (averaged over sites). Forty five percent of the 862 smooth outcrosses detected in progenies of bristly plants were susceptible to disease. Since most of the selections in the male rows were segregating for disease resistance, pollination of bristly plants with pollen from smooth plants in either the male or female rows could give rise to smooth susceptible outcrosses.

The frequency, p, of the dominant smooth allele at each site was estimated as 0.66. In the analysis of outcrossing rate, based on progenies of bristly plants from the female rows, there were highly significant differences between the 17 breeding population selections ($P<0.001$) but no significant effects for sites or for the selection x site interaction. Outcrossing rate estimates for selections ranged from 1.6 to 21.9% with an overall mean of 7.0 ± 0.2%. Outcrossing rates could not be calculated from the proportion of disease-resistant outcrosses in progenies because the frequency of disease-resistance alleles in the female rows was unknown. However, there was a very close association ($r=0.96$, $P<0.001$) between the proportion of resistant outcrosses in each of the 17 bristly selections and the outcrossing rates estimated from the smooth outcrosses.

DISCUSSION

In the experiments at these two sites 2360 plants from the breeding programme were exposed to natural outcrossing from the same number of plants in the male rows. The best 422 plants were selected from the female rows and 749 resistant outcrosses were recovered from 244 of the 422 progenies. Based on an estimated frequency of 0.71 for gametes from the male rows carrying a dominant resistance gene and assuming three seeds per cross and a success rate of 25% for hand crossing (Cameron *et al.*, 1984), the number of hand crosses, C, required to sample the same number of male and female genotypes is given by:

$$C = \frac{244}{422} \times 2360 \times \frac{100}{25} \times \frac{3}{1} \times \frac{100}{71} = 23\,063$$

Since about 700 man hours would be needed to perform 23 000 emasculations and pollinations, natural outcrossing is an effective alternative for transferring resistance genes to the breeding population. The wide variation among selections of 1.6 to 21.9% outcrossing meant, however, that transfer of resistance genes to the different selections was very uneven. It should be possible to select for higher rates of outcrossing to improve the transfer of genes to selections. If outcrossing rates are related to floral characters, such as flower size and duration of blooming (Stace, 1984), the ease and efficiency of selection for outcrossing rate could be improved. Cultivars with high rates of outcrossing may also be better equipped to counter disease pressures from the variable anthracnose pathogen through rapid reassortment of resistance genes present in the cultivar population. In a study of a natural *S. guianensis/C. gloeosporioides* pathosystem in Columbia, Miles and Lenne (1984) found evidence of genetic diversity and outcrossing in the host population. They suggest that this diversity in the host could contribute to its persistence

Table 3. Numbers of outcrosses identified in progenies of smooth and bristly plants from Samford and Narayen.

Site	Smooth plants		Bristly plants		
	No. of progenies	No. of resistant outcrosses	No. of progenies	Total no. of outcrosses	No. of resistant outcrosses
Samford	118	154	63	369	208
Narayen	104	123	100	493	264

and stability in the pathosystem.

Miles (1985) advocates the use of a systematic, recurrent selection scheme based on natural outcrossing to improve anthracnose resistance in *S. guianensis*. The field and controlled environment experiments described in this paper represent the first cycle of a similar recurrent selection scheme. Selfed seed from resistant plants identified in the controlled environment will be used to establish a second set of outcrossing blocks in the field. Resistant selections from the second cycle will then undergo agronomic testing to identify elite plants for cultivar development. In this scheme the use of a controlled environment for the winter generation is an attempt to complete the two generations for each cycle in one year. Early identification of natural outcrosses in the seedling stage, using stem bristle and disease resistance markers, is essential because of space restrictions in the controlled environment.

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