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Evaluation of *Phaseolus vulgaris* germplasm; screening for resistance to common blight

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Summary

Initial screening of *Phaseolus vulgaris* L. germplasm for resistance to common blight (*Xanthomonas campestris* pv. *phaseoli*) has indicated nine accessions with promising levels of field resistance. Effectiveness of mass selection in the germplasm was estimated at 42.8%. A supplementary study with an F_2 population suggested a broad sense heritability of 62%, and that expression of resistance was partially dominant.

INTRODUCTION

A collection of *Phaseolus vulgaris* L. germplasm was assembled at Hermitage Research Station in 1982 to assist development of a new navy bean breeding programme. The main sources of material were; the Queensland Department of Primary Industries (DPI) collection at Kingaroy; the Victorian Department of Agriculture collection; the CSIRO collection; and new introductions from the International Centre for Tropical Agriculture (CIAT) in Colombia and the USA. The materials included navy beans, french and processing beans, Borlotti and other culinary bean types, breeding lines from the USA and CIAT, and accessions from CIAT which originated from farmers fields in Central and South America. One hundred seed weight ranged from below 10 to over 60 g, with primary colours grading from white to tan, yellow, pink, red, brown and black, plus assorted secondary colour patterns.

The initial objectives of the navy bean breeding programme defined after a survey of the previous DPI programme (Redden *et al.* 1984) were to obtain increases in grain yield and pod height, and resistances to rust (*Uromyces appendiculatus*), common blight (*Xanthomonas campestris* pv. *phaseoli*), peanut mottle virus, and sclerotinia rot (*Sclerotinia sclerotiorum*). Further longer term objectives included improved tolerance to low levels of soil zinc, increased ability to nodulate and fix nitrogen, and drought tolerance. Acceptable canning quality and white seed colour were other requirements for new navy bean cultivars.

The genetic base of the current navy bean varieties is narrow (Redden *et al.* 1984), but to utilise variation from a germplasm collection it must first be evaluated for the relevant characters. This article reports on the first two stages of screening the germplasm for resistance to common blight.

Genetic resistance to common blight is quantitatively inherited (Webster *et al.* 1980; Valladares *et al.* 1983) over a range from moderate resistance to high susceptibility. Pods and foliage may have different reactions to the pathogen (Coyne and Schuster 1974*a*). The foliage reaction is the more important for grain production in Queensland. Screening of cultivars for foliage resistance has been carried out in the USA by Rands and Brotherton (1925), Burkholder and Bullard (1946) and Coyne and Schuster (1973). CIAT is currently screening over 20 000 accessions for foliar reaction to common blight.

MATERIALS AND METHODS

Stage 1 screening

A collection of 1462 accessions plus 14 blight tolerant cultivars from CIAT were sown unreplicated in order of accession at Queensland Agricultural College (QAC), Lawes, on 29 December 1982. A susceptible check (Gallaroy) and a tolerant check (Borlotti) were sown side by side after every 10th accession. Plots were single rows 3 m long and 50 cm apart, with 1 m alley ways which were pre-sown on 6 December with Gallaroy as a spreader for both rust and common blight. Three additional rows of Gallaroy were sown as guard rows on both sides of the trial. Standard agronomic practices for herbicide and fertiliser applications were used, and supplementary irrigations were given at 4 and 6 weeks after sowing. The accessions were inoculated with a suspension of *X. campestris* pv. *phaseoli* cells using one petri dish culture per litre of water (grown in vitro) and *U. appendiculatus* urediniospores in a suspension of 0.2 g/L applied by knapsack 30 days after sowing.

The trial was rated for blight on foliage during 16 to 18 March 1983, using a 1 (resistant) to 9 (susceptible) scale based on visual assessment of overall disease level in each plot. A rating of 1 indicated absence of common blight. A score of 2 was for occasional small light lesions in the lower canopy; 3 for small to medium lesions on up to 20% of leaves in the lower canopy; 4 for up to 40% of lower canopy leaves plus scattered upper canopy leaves with lesions; 5 for up to 20% infection of the upper canopy; 6 for up tp 40% infection in the upper canopy; 7 for 50% and more upper canopy infections; 8 for noticeable levels of dead leaves in the upper canopy; and 9 for a high frequency of dead leaves. Approximately 200 accessions were not rated due to poor germination or lack of vigor. The grid layout of checks at the site was used to provide disease contour maps of incidence levels. From the mean value of the two checks predicted values were estimated for accessions which were then ranked on the calculation of (observed–predicted value+10) in ascending order.

Stage 2 screening

A total of 122 accessions were sown in 2 non-randomised replicates at Inglewood Field Station on 20 January 1984. Plots were single rows 5 m long and 75 cm apart, with Gallaroy sown as a spreader on the same day to intersect the ends of all plots. Within each replicate, plots were machine sown in blocks of 24 plots, in serpentine sequence. Four rows of Gallaroy check were sown in each block between the replicates placed side by side. The accessions included 115 selections, for which seed was available, from the most blight tolerant 125 accessions in the QAC trial, plus the accessions; Jules, Refugee AD11, two accessions of PI 207 262, and Great Northern Nebraska No. 1 Sel 27 (for which two extra accessions were in the above 125) which showed tolerance in other studies (Zaumeyer, 1930; Coyne *et al.* 1973).

Inoculation of plants was done on 28 February (day 39 after sowing) with a high pressure spray using an aqueous suspension exudates from water soaked cuttings of common blight diseased bean plants.

Disease rating for common blight at Inglewood were made on a plot basis on a scale of 1 (resistant) to 9 (susceptible) as described earlier. Ratings were made 49, 59, 70 and 75 days after sowing.

Selection effectiveness was estimated by the shift in population mean in response to mass selection.

Genetic study

In a separate study on the inheritance of resistance, parental and F_2 plants of the cross ML49/2×Regal Rouge were inoculated with common blight (as described above) at Inglewood on 28 February (day 36 after sowing). Parental plots of 12 plants 1984 in a single 3 m row and F_2 plots of 36 plants in 3×3 m rows were sown as randomised plots in rows 1.5 m apart with the susceptible cv. Kerman sown as a standard guard row between all rows of experimental material. There were three replicates. Individual plants were rated for common blight infection at 67 and 80 days after sowing on a 1 to 9 scale as outlined above.

RESULTS

At QAC, Gallaroy check consistently had higher blight ratings than Borlotti, with a range of 4 to 8 for Gallaroy and 2 to 6 for Borlotti with a mean difference of 2.6. The means of the checks, which were used as the predicted values over the site, ranged from 4.09 to 6.88 with an overall mean of 5.41. Thus according to the contours of predicted values, the original ratings for accessions were mainly adjusted upwards or downwards by less than 1.0. This adjustment was relatively small for the range of data (1–9) amongst the accessions (Figure 1). The most resistant 10% of accessions at QAC correspond with over half the most resistant 5% of accessions at Inglewood 1983. Here they were grown in the same season for agronomic assessment, and common blight infection was natural. The correspondence in ratings between sites reinforced confidence in the assessment at QAC. The disease scores at Inglewood 1984 ranged from 1 to 6 on day 49, and steadily increased to a range of 2 to 8 by day 70. Over the two replicates only 16 entries had a mean rating of 3.5 or less at day 70, and only 9 of these were below this score at day 75 (Table 1). The distribution of resistance scores was continuous and unimodal, with at day 70, 33 entries at or below a mean of 4.0, and 52 below the overall mean score of 4.54. An analysis of variance on day 70 data showed highly significant differences between entries with an l.s.d. of 1.5 clearly separating the most resistant 9 from susceptible entries and checks. The correlation between replicates on day 70 was 0.67.

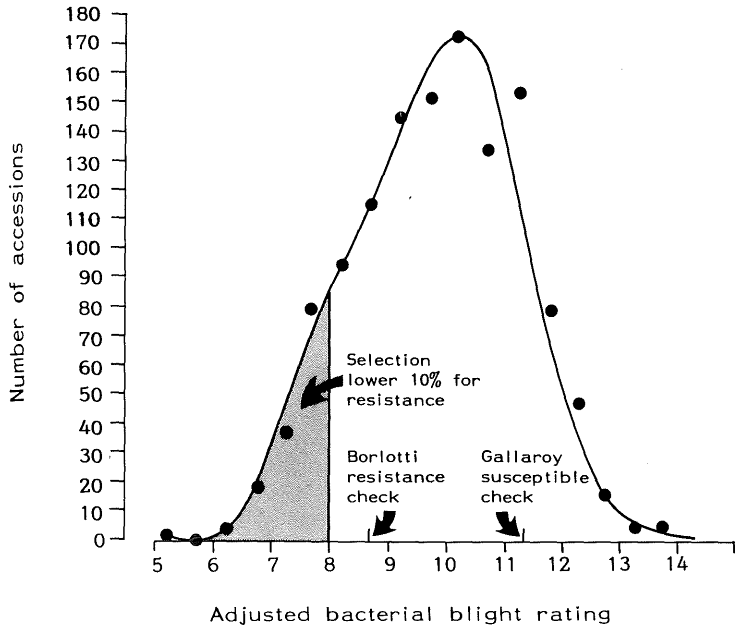

Figure 1. Frequency plot for common blight screening of bean germplasm, QAC 1983. Adjusted rating based on scale of 1=resistant to 9=susceptible.

Less than 10% of the 115 selections showed a promising level of field resistance to common blight infection of foliage. It is to be expected that some of the 115 selected originally from an unreplicated screening would be escapes. In the second stage of evaluation at Inglewood only 6 entries were equally or more susceptible than Gallaroy, and 39 more were not significantly different from Gallaroy. On this basis 39% of the original selections can be regarded as susceptible escapes. The remaining 52% of entries displayed intermediate to low levels of field resistance. The range in disease ratings in the second stage was continuous and similar to that for the first stage (Figure 1), indicating quantitative inheritance.

Table 1. Common blight ratings at Inglewood for field resistant cultiars at different time intervals after sowing (inoculation at day 40)

Entry	Cultivar	49 days	59 days	70 days	75 days
31	Liborino	3.0	3.5	3.5	4.0
32	Bridgeton	2.0	2.5	3.5	3.5
42	Antioquia 23	2.5	3.5	3.0	3.0
62	PI 207 262	2.5	2.5	3.5	3.5
85	CPI 95894	2.0	3.0	3.5	3.5
88	ICA21611M23PMM4	3.0	2.5	3.5	4.0
100	Brown resel. K534	2.0	2.5	2.5	4.0
103	Brown bean	2.5	3.5	3.5	4.0
105	BAC 57	1.0	2.0	2.5	2.5
108	BAT 910	1.5	3.0	3.5	4.5
109	BAT 76	3.0	3.0	3.0	3.0
110	BAC 125	1.0	1.0	2.0	2.0
111	BAC 134	1.5	3.0	3.5	4.5
112	BAC 135	2.0	3.0	3.0	3.0
113	BAC 51	2.0	1.0	1.0	2.0
115	CPI 169868	2.5	3.5	3.5	4.5
	Gallaroy (check)	3.0	5.0	7.0	7.0
9	Cannelini (susceptible)	4.0	6.5	7.5	7.5
Overall mean (115 entries)				4.54	
l.s.d. P=0.05				1.55	

The effectiveness of mass selection can be estimated with reference to Gallaroy, the common check in both stages of evaluation. At QAC the population mean was less than Gallaroy by 1.46 units, while the Selection differential (S) between the lowest 10% selected and the mean was 2.43. At Inglewood the population mean at 70 days was 4.5, or 2.5 units lower than Gallaroy, giving a value of 1.04 units for (R) the response to selection after estimation of the difference from the previous generation mean. Thus effectiveness of selection is given by R/S as 42.8%.

Table 2. Field evaluation of reaction to common blight of parental and F_2 populations of the cross ML49/2×Regal Rouge

	Observations Day 67			Observations Day 80		
	Mean	Variance	Herir-ability	Mean	Variance	Herir-ability
Parent ML49/2	3.33	0.807		3.85	0.454	
Parent Regal Rouge	7.05	1.122		7.31	0.666	
F_2 ML49/2×R.R.	4.17	2.348	59%	4.79	1.575	64%

Results of the genetic study on F_2 material indicated a broad heritability of 62%, based on mean parental and F_2 variance comparisons over two observations (Table 2). The F_2 mean was intermediate between the resistant parent and the mid-parent value on both dates, suggesting that resistance is partially dominant.

DISCUSSION

The estimates of broad sense hereitability in these studies are comparable to estimates of 73 to 78% obtained by Valladares-Sanchez *et al.* (1983) using cultivars with greater resistance than ML49/2. Webster *et al.* (1980) obtained an F_2/F_3 regression estimate of 0.62 when using cv. Jules as the resistant parent, but only 0.27 with PI 207 262 as resistant parent. Inheritance of resistance to common blight is quantitative and narrow sense heritability has usually been found to be low (Coyne and Schuster 1974*b*), however large additive expressions were indicated by Valladares *et al.* (1983). The expression of heterosis for resistance in the F_2 population at Inglewood was similar to genetic studies of Valladares *et al.* (1983), for foliar reaction to common blight. In some cases a delayed flowering response to the interaction of long photoperiod and high temperature has been observed to be genetically linked to tolerance for common blight (Coyne *et al.* 1973). Furthermore these workers observed that susceptibility to common blight in beans tends to increase with plant age. Both of these effects were in agreement with observations at Inglewood.

Accession ML49/2 does not have a very high expression of resistance, being intermediate between the above 9 lines with field resistance and Gallaroy for disease reaction. However, it is much more resistant than Regal Rouge and the estimate of heritability provides supporting evidence for both the genetic basis of resistance in the most promising 9 accessions, and the possibility that these resistances can be used in a breeding programme.

The realised selection effectiveness of 42.8% for one generation of mass selection is much higher than gains per cycle achieved in segregating populations for other self pollinated crops; 1 to 2% per cycle for reduced height in oats (Romero and Frey 1966), and 8% per cycle for hard seededness in clover (Weihing 1962). This high level of apparant heritability for resistance to common blight, possibly reflected the heterogeneous nature of the germplasm evaluated, especially as 15 accessions were introduced on the basis of their blight resistance at CIAT. The results show that mass selection using grid plots is an effective screening procedure, with 60% of those selected being more resistant than the susceptible check.

For individual accessions at Inglewood in 1984, PI 207 262 had greater resistance in both entries (3.5 and 4.0 at day 70) than the three entries of Great Northern No. 1 Selection 27 (5.0, 4.0 and 4.5 at day 70) as in other studies (Valladares *et al.* 1983). However, the BAC lines from CIAT appear to be more resistant than both these and other accessions rated as resistant to common blight at Inglewood in 1984.

Since growth did not overlap laterally between plots in most cases, but all intersected with the spreader, within field variation appeared to be more due to micro-environmental effects than to neighbouring plots. Therefore analyses of variance were considered to be acceptable.

Future breeding strategy will include, (i) stage 3 field trials to better characterise the resistance of the nine selected accessions, (ii) crosses to transfer this resistance to navy beans, and (iii) inter-crosses amongst resistant lines in an attempt to accumulate genes for a transgressive expression of greater resistance. Four of BAC accessions are white seeded, though mainly larger than navy bean size, while other resistant cultiars have coloured seeds. Cultivar Bridgeton is a lima bean (*Phaseolus lunatus*), but it could be used as a source of resistance (Honma and Heeckt 1956). Selection of resistance will be conducted in the field in irrigated and inoculated nurseries, using pre-planted spreader rows.

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