QTL Conditioning Physiological Resistance and Avoidance to White Mold in Dry Bean

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ABSTRACT

Physiological resistance is an important component of integrated strategies used to control white mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary], a major disease of common bean (*Phaseolus vulgaris* L.) in North America. Information pertaining to inheritance of physiological resistance, as detected by the greenhouse straw test, and its relationship with field resistance is lacking. The objectives of this study were to compare physiological resistance as detected by two separate straw tests with field resistance, evaluate heritability of physiological resistance, and to characterize the disease reaction of G 122 by quantitative trait locus (QTL) analysis. This was done in a recombinant inbred population (A 55/G 122) consisting of 67 F_8-derived lines. The greenhouse tests with five and six replications, respectively, and the field test with three replications were conducted in randomized complete block designs. Moderate heritability for disease reaction (scored from 1 = no symptoms to 9 = severe disease) was observed across the straw tests (0.65) and in the field (0.78). Inheritance of disease reaction was further investigated with a framework linkage map composed of 74 markers. Interval mapping detected a QTL on linkage group B7 near the phaseolin seed protein (*Phs*) locus that explained 38% of the phenotypic variation for disease score across the straw tests. The same B7 QTL (26%), and an additional QTL (18%) on B1 near the *fin* gene for determinate growth habit, conditioned field resistance. A QTL (34%) for canopy porosity, a measure of potential disease avoidance, also mapped to the *fin* locus. Results confirmed that physiological resistance as detected by the straw test was an integral component of field resistance, and that both physiological and avoidance mechanisms contributed to field resistance in the A 55/G 122 population. The landrace G 122 clearly provides breeders with a heritable source of physiological resistance to combat white mold disease.

White mold is one of the most important fungal diseases of common bean worldwide. Combining physiological resistance with avoidance mechanisms (upright plant architecture, open canopy) is the current breeding strategy for minimizing yield losses due to white mold in common bean. Upright architecture promotes air and sunlight penetration into the plant canopy creating a drier microclimate less conducive to white mold epidemics (Blad et al., 1978; Coyne et al., 1974, 1977; Fuller et al., 1984a; Park, 1993a; Schwartz et al., 1978, 1987). Avoidance, in the absence of physiological resistance, can be overcome in the presence of severe disease.

Germplasm lines and cultivars with upright architecture have become commonplace (Hosfield et al., 1995; Kelly et al., 1992a, 1992b), whereas development of cultivars which combine physiological resistance to white mold with upright architecture has progressed relatively slowly. Cumbersome screening methods, low heritability, and few available resistance sources have contributed to the lack of progress for enhancing physiological resistance. This situation may soon be alleviated, however, as the recently developed Bean White Mold Nursery (Steadman, 1995, 1997; Steadman et al., 1999) and novel, less cumbersome screening methods (Petzoldt and Dickson, 1996; Steadman, 1997; Kolkman and Kelly, 2000) are helping to identify, characterize, and select for physiological resistance to white mold in common bean (Miklas et al., 1998, 1999). The straw test (Petzoldt and Dickson, 1996) is a simple procedure for evaluating physiological resistance primarily within stem tissue. However, little is known about the inheritance of this trait.

The landrace cultivar G 122, has exhibited field resistance to white mold in the Bean White Mold Nursery (Steadman, 1997; Steadman et al., 1999) and elsewhere (Kmiecik and Nienhuis, 1998; Park et al., 1999a). The field resistance of G 122 likely results from physiological resistance. The breeding line A 55 expresses avoidance due to its upright architecture and narrow growth habit. Our objectives were to gain a better understanding of resistance to white mold in G 122 using QTL analysis, evaluate heritability of physiological resistance as detected by the straw test, and compare physiological resistance as detected by the straw test with field reaction.

MATERIALS AND METHODS

Plant Materials

G 122 is an upright determinate bush (Type I, Singh 1982) bean of Andean origin with elongated, cranberry bean-type seeds. In addition to white mold resistance, G 122, collected from India as PI 163120, and also known as 'Jatu Rong', exhibits heat tolerance (Shonnard and Gepts, 1994). A 55 is an indeterminate, upright short vine (Type IIa), advanced black bean breeding line of Middle American origin developed at CIAT (Center for Tropical Agriculture, Cali, Columbia) that exhibits field avoidance to white mold under moderate disease pressure; however, under heavy disease pressure A 55 is known to be susceptible to white mold in the field (Park, 1993b; Steadman, 1995). A population of 67 F_8-derived recombinant-inbred lines (RILs) from a cross between A 55 and G 122 were generated using the single seed descent method. The white mold resistant black bean line I9365-31 (Miklas et al., 1998) and susceptible 'Othello' pinto bean were included as checks in the greenhouse test.

Genetic Linkage Map

A linkage map for the A 55/G 122 RIL population was previously developed by Johnson (1997, http://agronomy.ucdavis.edu/gepts/mapdata2.htm#framework; verified October

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Abbreviations: cM, centimorgan; MAS, marker-assisted selection; QTL, quantitative trait locus or loci; RIL, recombinant inbred line.

28, 2000). In a first step, a molecular linkage map consisting of 245 markers [222 AFLPs (amplified fragment length polymorphism), 10 RFLPs (restriction fragment length polymorphism), 3 RAPDs (random amplified polymorphic DNA), 2 SCARs (sequence characterized amplified region), 4 phenotypic markers, 3 isozymes, and 1 seed protein] was established in the A 55/G 122 population using Mapmaker/EXP 3.0 (Lander et al., 1987). A pairwise linkage analysis of the marker data, imposing a minimum LOD score of 3.0 and maximum distance of 30 centimorgans (cM), was used to establish the linkage groups. Three-point and multi-point log-likelihood thresholds (LOD) of 2.5 and 2.0, respectively, were used to order the markers within linkage groups with the Order and Ripple commands. Centimorgan distances between linked loci were based upon recombination fractions using the Kosambi (1944) mapping function.

Twelve linkage groups were identified, ten of which were anchored to the core map for common bean using previously linked markers (Freyre et al., 1998; http://agronomy.ucdavis.edu/gepts/mapdata2.htm#Coordination; verified October 28, 2000). The total map length was 853 cM. This is less than the map length of 1200 cM reported for common bean (Freyre et al., 1998), suggesting that more markers other than AFLPs, which tend to cluster around the centromere, are needed to obtain a more complete map. As a result of clustering among the AFLP markers, only 74 framework markers from the original 245 markers were chosen for QTL analysis. The markers were chosen so as to have as few missing data as possible and also have a regular 10-15 cM spacing. The markers for the framework map were composed of 60 AFLPs; 5 RFLPs; 3 phenotypic markers *Asp*, *fin*, and *I*; 2 RAPDs; 2 SCARs; 1 isozyme *RBcS*; and 1 seed protein *Phs*. The primers for the AFLP markers found to be linked to a white mold resistance QTL were A05 (5′-G ACT GCG TAC CAA TTC ACA-3′), A12 (5′-G ACT GCG TAC CAA TTC AGT-3′), P9 (5′-G ACG ATG AGT CCT GAG TAA AGA-3′), P10 (5′-G ACG ATG AGT CCT GAG TAA AGC-3′), and P11 (5′-G ACG ATG AGT CCT GAG TAA AGG-3′). A combination of restriction enzymes, *Eco*RI and *Mse*I, was used to digest the genomic DNA prior to PCR amplification.

Greenhouse Straw Tests

The straw test described by Petzoldt and Dickson (1996) was used to screen the RIL population, parents, and checks in two separate greenhouse environments. Straw Test 1 (planted 14 Oct. 1997) and Straw Test 2 (planted 24 Feb. 1998) consisted of five and six replications, respectively. An individual plant of each line represented a replicate and the replications were randomized in complete blocks. The parents were inadvertently excluded from the first test. For both tests the greenhouse environment was maintained at 20°C/night to 28°C/day with a 14-h daylength provided by sunlight and supplemental artificial lighting. Plants were watered and fertilized for normal growth. The *S. sclerotiorum* culture T001.1, hyphal-tip isolated from a sclerotia collected from 'Newport' navy bean in Quincy, WA, in 1996, was the source of inoculum. Inoculation took place approximately 28 d after planting following the procedure of Petzoldt and Dickson (1996). Briefly, the growing tip of the main stem of a single plant, sown in a 10- to 15-cm-diameter pot containing a soilless potting mix (Sunshine No. 1; Fison Hort., Vancouver, BC), was discarded and a plastic straw containing an agar plug of mycelium of the pathogen was fitted over the intact cut stem of the whole plant. The entire petri plate of growing mycelium was used as inocula soon after mycelium had grown from the center to the periphery of the plate. Eight days after inoculation, the white mold reaction was scored from 1 to 9, where 1 = no symptoms, 2 = invasion of the stem past the site of inoculation but not to the first node, 3 = invasion of the stem to the first node, 4 = invasion of the internode slightly past the first node, 5 = invasion to the middle of the internode, 6 = invasion to the second node, 7 = invasion slightly past the 2nd node, 8 = invasion to the middle of the second internode and beyond, and 9 = total plant collapse (Petzoldt and Dickson, 1996; Miklas et al., 1999).

Field Test

The field plot at the USDA-ARS Cropping Systems Research Farm at Patterson, WA, has a history of *S. sclerotiorum* disease in potato (*Solanum tuberosum* L.) and pea (*Pisum sativum* L.). Nevertheless, 120 kg ha^{-1} of sclerotia mixed with seed tailings obtained from a local bean elevator (Central Bean, Quincy, WA) were incorporated across the plot in October 1998. The soil is a Quincy sandy loam (mixed, mesic Typic Torripsamments). The RILs and parents were planted 24 May 1999 in a randomized complete-block design with three replications. A plot consisted of four rows, 3 m long, and spaced 0.56 m apart. Planting density was 234 848 seeds ha^{-1}. To promote white mold disease, approximately 6.3 mm of water was applied on a daily basis from the onset of flowering to late pod-fill by overhead center-pivot irrigation. To maintain vigorous plant growth, nitrogen was foliar applied at a rate of 22 kg ha^{-1} on 14 June, 2 July, 22 July, and 11 August by chemigation.

Disease reaction within the middle two rows was scored from 1 to 9 on the basis of combined incidence and severity of infection at physiological maturity (8 September), where 1 = no diseased plants; 2 = 1 to 20% diseased plants and/or 1 to 5% infected tissue; 3 = 20 to 30% diseased plants and/or 5 to 10% infected tissue; 4 = 30 to 40% diseased plants and/or 10 to 20% infected tissue; 5 = 40 to 50% diseased plants and/or 20 to 30% infected tissue; 6 = 50 to 60% diseased plants and/or 30 to 40% infected tissue; 7 = 60 to 70% diseased plants and/or 40 to 50% infected tissue; 8 = 70 to 80% infected plants and/or 50 to 60% infected tissue; and 9 = 80 to 100% diseased plants and/or 60 to 100% infected tissue. Traits measured at mid-pod fill (9 August) included canopy height (cm) and canopy porosity (Deshpande, 1992) scored from 1 to 5, where 1 = an open canopy with the soil surface between rows completely visible and 5 = completely closed canopy over the furrow with no soil visible.

Inheritance and QTL Analysis

Analysis of variance for each trait was performed by PROC GLM (SAS, 1987). A narrow-sense heritability estimate for disease score for each test was computed with variance components on an F_8-derived line-mean basis (Hallauer and Miranda, 1981). This h^2 approximates a narrow-sense estimate because coefficients for nonadditive genetic components in the expectation of genetic variance are near zero for F_8-derived lines. Exact 90% confidence intervals were calculated for h^2 according to the procedures of Knapp et al. (1985). Error mean squares for the separate analyses of variance for the two straw tests were homogeneous based on Bartlett's test (Steel and Torrie, 1980); therefore, a combined analysis of variance was conducted to obtain h^2 for disease score across straw tests. Frequency distributions of the RIL means for disease score were tested for normality by the Shapiro and Wilk test statistic W (PROC Univariate, SAS, 1987). A probability of $P < 0.001$ was used to indicate lack of fit.

Simple interval mapping performed by MQTL (Tinker and

Table 1. Heritability estimates for physiological (straw tests) and field resistance to white mold, and agronomic traits associated with disease avoidance in a dry bean population of 67 F_8-derived RILs from the cross A 55/G 122.

	Check means		Parental means		Recombinant inbred population		
	Othello	I9365-31	A 55	G 122	Mean	Range	h^2/(90% CI)
Straw test 1 (1–9)†	6.6	4.4	–	–	7.2	3.2–9.0	0.75/(0.82–0.64)
Straw test 2 (1–9)	6.8	2.0	8.8b‡	5.3a	6.0	2.3–9.0	0.76/(0.83–0.65)
Combined (1–9)	6.7	3.1	–	–	6.6	2.3–9.0	0.65/(0.82–0.57)
Field (1–9)	–	–	2.7a	3.8b	3.8	1.3–7.5	0.78/(0.84–0.67)
Canopy porosity (1–5)	–	–	1.8a	2.5b	2.4	1.0–4.5	0.91/(0.94–0.86)
Plant height (cm)	–	–	59.0a	55.0b	52.4	41.0–68.5	0.81/(0.86–0.71)

† Straw test reaction scored from 1 to 9, where 1 = no symptoms and 9 = total plant collapse; field reaction scored from 1 to 9, where 1 = no diseased plants and 9 = 80 to 100% diseased plants and/or 60 to 100% infected tissue; and canopy porosity scored from 1 to 5 where 1 = an open canopy with the soil surface between rows completely visible and 5 = completely closed canopy with no soil visible.
‡ Parental means within a row followed by the same letter are not significantly different at the 5% level of probability according to LSDs.

Mather, 1995) was used to detect QTL conditioning physiological resistance in the straw tests, field resistance, and agronomic traits associated with disease avoidance, along the framework map of 74 markers (Johnson, 1997). We performed permutation analyses (1000 permutations) of the disease score data sets (Doerge and Rebai, 1996) from the straw tests and field in order to identify a significance threshold of the test statistic for a QTL based upon a 10% experiment-wise error rate. The threshold value thus determined for the test statistic was 12.4 for the straw tests and 12.8 for the field which when multiplied by 0.22 equates to LOD scores of 2.7 and 2.8 respectively. Significant QTL were then declared if the test statistic calculated by MQTL was greater than 12.4 (Straw tests) or 12.8 (field). The R^2 values for describing the phenotypic variation explained by a significant QTL were calculated as (variance explained) / (total variance). The effects of QTL and agronomic traits on disease score in the field was modeled by multiple regression (PROC STEPWISE, SAS, 1987). A significance level of 0.15 was required for a trait to be included in the model.

RESULTS AND DISCUSSION

Greenhouse Straw Tests

A difference ($P < 0.05$) in disease score between the parents was observed in Straw Test 2 (Table 1), as a moderate level of physiological resistance to white mold was detected for G 122 which had a score of 5.2 in comparison to A 55 which was highly susceptible with a score of 8.8. The straw tests also effectively differentiated ($P < 0.05$) the white mold reaction of the resistant (I9365-31) and susceptible (Othello) checks, which averaged scores of 3.2 and 6.7, respectively.

The disease score means of the RILs were positively correlated ($r = 0.45$, Table 2) between the straw tests indicating uniform genetic expression of physiological resistance across greenhouse environments, though a significant line × test interaction ($P < 0.01$) suggested otherwise. The high correlation ($r = 0.87$, 16 df, $P < 0.01$) for the nine RILs with the highest and nine with the lowest scores for Straw Test 1 with the corresponding RIL means for Straw Test 2 revealed that the interaction primarily resulted from differential reactions between tests for lines with intermediate scores. A difference in the magnitude of response also contributed to the interaction. Differential reactions may occur from a slight change in prevailing temperature between tests. For instance slightly cooler temperature which would favor infection and subsequent spread of the pathogen could explain the higher mean disease score observed for the RIL population in Straw Test 1. On the basis of the correlations between tests, straw test results are primarily reported for the combined analysis.

The frequency distribution of the RILs for mean disease score across the straw tests was normally distributed (Fig. 1), such that there was a lack of discrete resistant and susceptible segregation classes. The h^2 (0.65) for disease score across straw tests was moderately high (Table 1).

A single major-effect QTL was detected by the straw tests (Table 3 and Fig. 2). This QTL explained 38% of the phenotypic variation for disease score, and was equally expressed in Straw Test 1 (36%) and 2 (35%).

Table 2. Simple correlation coefficients (r) among white mold disease score means from greenhouse straw tests and the field and agronomic trait means from the field in a population of 67 F_8-derived RILs from the cross A 55/G 122.

	Straw Test 2	Field reaction	Canopy porosity
Straw Test 1 (1–9)	0.45**	0.43**	
Straw Test 2 (1–9)		0.25*	
Straw Tests Combined		0.38**	
Canopy porosity (1–5)		0.16ns	
Plant height (cm)		−0.37**	0.10ns

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† ns = not significant.

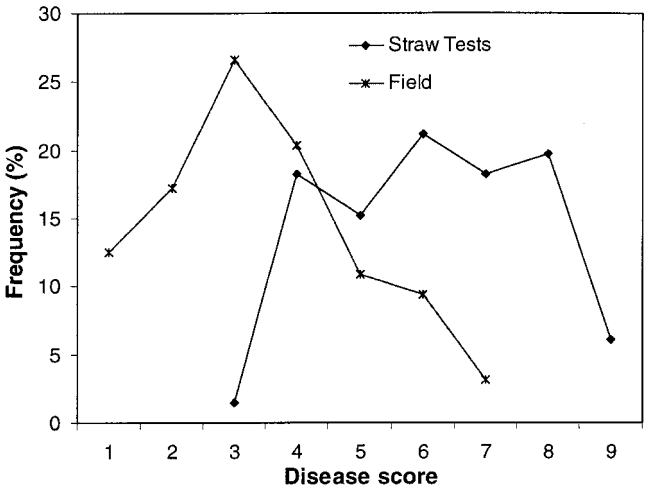

Fig. 1. Frequency distributions of the mean scores for white mold reaction among 67 dry bean RILs (A 55/G 122) across greenhouse straw tests and in the field.

Table 3. Markers linked with major QTL (>LOD 2.7 and 2.8 for straw tests and field, respectively) conditioning physiological resistance to white mold across straw tests and in the field, and linked with agronomic traits conditioning avoidance to white mold in the field in a dry bean population of 67 F_8-derived RILs from the cross A 55/G 122.

Trait	Marker	Linkage group	LOD	R^2(%)	Means†		t-Test
					A 55	G 122	
Straw tests (disease score 1–9)	*Phs*‡	B7	6.2	38	7.46	5.70§	**
Field (disease score 1–9)	A05/P09 T	B7	4.3	26	4.36	2.89	**
	fin	B1	2.8	18	4.39	2.95	**
Agronomic traits							
Canopy porosity (1–5)	*fin*	B1	5.9	34	2.98	1.70	**
Plant height (cm)	None detected						

† Means of RILs possessing the parental alleles for the QTL-linked marker, with ** indicating means different at the 0.01% probability level.
‡ *Phs* is a seed protein, *fin* a gene for growth habit, and A05/P09 T is an AFLP marker.
§ Actual mean values reflect the units of each particular trait (disease scores from 1–9 for the straw tests and field; scores from 1 to 5 for canopy porosity; etc.).

The QTL explained 58% of the genotypic variation. The statistically significant region (LOD > 2.8) for this QTL included the *Phs* locus for seed storage protein and linked AFLP markers located on linkage group B7 (Johnson, 1997; Freyre et al., 1998). The G 122 and A 55 parents have the respective Tendergreen 'T' and Sanilac 'S' phaseolin haplotypes at the *Phs* locus. The linked AFLP markers were A05/P09 T, A05/P11 O, and A12/P10 B. The letter designations T, O, and B represent the size of the fragments in ascending order from A (= shortest base-pair fragment) in relation to the pool of AFLPs generated by the specific primer pairs. Exact size of the AFLPs are available upon request. The allele for resistance at the B7 QTL was derived from the white mold resistant parent G 122.

Field Test

A disease score varying from 8 to 9 for the dry bean 'Sutter Pink', used as a border for the field experiment, indicated that adequate and uniform white mold pressure occurred across the trial. The frequency distribution of the RILs for mean disease score in the field was normally distributed (Fig. 1). Together, a normal distribution, lack of discrete segregation classes, and moderately high h^2 (0.64) for disease score (Table 1) affirmed the quantitative inheritance of resistance to white mold epidemics in the field as previously reported (Fuller et al., 1984b; Miklas and Grafton, 1992; Kolkman and Kelly, 1999). Note that h^2 based upon a single environment $(\sigma^2_g + \sigma^2_{ge})/\sigma^2p)$ is biased upward because of the presence of σ^2_{ge} in the numerator.

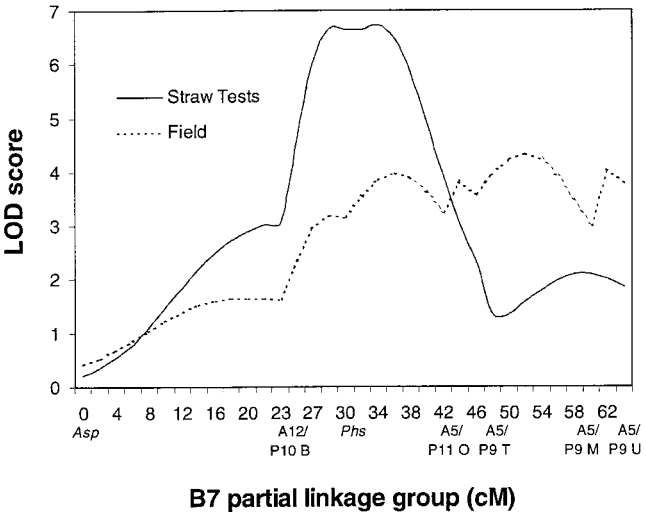

Fig. 2. A quantitative trait locus conditioning physiological resistance to white mold on partial linkage group B7 as depicted by interval mapping (note that the MQTL test statistic multiplied by 0.22 equates to LOD score) in 67 F_8-derived dry bean RILs (A 55/G 122) screened in two separate greenhouse straw tests (combined analysis shown) and in the field.

Both parents expressed field resistance, however, less disease for A 55 (2.7) than G 122 (3.8) indicated avoidance was expressed (Table 1). This result provides further support of the extreme importance that disease avoidance has on reducing white mold disease in the field. Physiological resistance was also expressed. Field reaction to white mold is confounded by physiological resistance and avoidance making it difficult to separate the contribution of each to overall resistance. In this study QTL mapping in the A 55/G 122 population enabled some separation of the different mechanisms.

The same B7 QTL derived from G 122 in the general area of the *Phs* locus conditioning physiological resistance in the straw test was expressed in the field (Table 3 and Fig. 2). Small population size, which renders QTL location less precise, is likely responsible for the slight shift in QTL location away from the *Phs* locus and toward the A5/P9 T AFLP marker in the field test. Positive correlations (Table 2) between disease scores from the field and straw tests support the presence of a common QTL conditioning physiological resistance in the field and greenhouse.

Given the major effect of the QTL detected on B7, it will be worthwhile to determine the potential for *Phs* and associated AFLP markers to indirectly select for physiological resistance derived from G 122 in different segregating populations. The potential use of *Phs* for marker-assisted selection (MAS) of the QTL would be restricted initially to certain snap bean lines and dry beans of Middle American origin that possess S phaseolin because G 122 has the T phaseolin. Caution in the interpretation of the QTL results should be exercised, however, as the small size of the A 55/G 122 RIL population leads to an overestimate of the actual effect of the resistance gene and to a reduction in the precision of the location of the gene (Melchinger et al., 1998). Thus, to achieve efficient MAS, it may be necessary to identify markers more tightly linked or flanking the QTL in larger populations. The identification of a major QTL

conditioning physiological resistance in the straw test located on a different linkage group, B8, and from a different source, landrace Pompadour Checa 50 (Park et al., 1999b), provides an opportunity for examining the usefulness of combining a different source of physiological resistance to white mold with that of G 122.

It is noteworthy, that four additional disease resistance genes have mapped to the same region of B7 as the QTL for physiological resistance to white mold. The four loci are major QTL for resistance to common bacterial blight, bean golden mosaic, and ashy stem blight caused by *Macrophomina phaseolina* (Tassi) Goid. (Nodari et al., 1993; Miklas et al., 2000), and a gene for resistance to *Colletotrichum lindemuthianum* (Sacc. et Magn.) Scrib., causal agent of anthracnose (Geffroy, 1997). As has been observed before in other species (Ashfield et al., 1998; Witsenboer et al., 1995) and beans (Geffroy et al., 1998 and 1999; Miklas et al., 2000; Stavely, 1984), disease resistance genes often occur in clusters.

A second QTL (18%) conditioning resistance to white mold in the field was identified on linkage group B1 near the *fin* locus for determinate bush growth habit (Table 3). A 55 has the genotype *Fin*/*Fin* conditioning indeterminancy and G 122 has *fin*/*fin* conditioning determinancy. This QTL could be associated with avoidance to white mold because a QTL (34%) for porous canopy was located in the same region. Note that open canopy was associated with less disease when estimated on an individual plot basis ($r = 0.27$, 189 df, $P < 0.01$), but not when analyzed on a mean basis (Table 2). There was no interaction between the QTL for field resistance on B1 and B7, nor did they appear to have a completely additive effect, as together they explained only 38% of the variation for disease score. The lack of an interaction or completely additive effect provides some additional support that the two identified QTL likely have independent effects, ie., avoidance for the B1 QTL and physiological resistance for the B7 QTL.

The multiple regression model of field reaction to white mold [$y = 6.93 - 0.08$(height) + 0.99(B7 QTL) + 1.08(*fin*)] provides additional support for the importance of both physiological and avoidance factors on the expression of field resistance in the A 55/G 122 population. The B7 QTL (A05/P09 T) had the largest effect (21%) as measured by the partial R^2 contribution to the model, followed by plant height (8.7%), and the B1 QTL or *fin* gene (8.4%). Thus, the quantitative inheritance of field resistance to white mold in the A 55/G 122 population is due in part to both physiological resistance and avoidance mechanisms. The size of the mapping population only enabled detection of QTL which accounted for more than 10% of the phenotypic variation for disease score. Thus, other QTL with a minor influence on resistance could exist in the population.

Oddly, lines with determinate bush growth habit (*fin*) had less disease than the lines with indeterminate vine growth habit (*Fin*), even though A 55 had less disease than G 122 in the field. This suggests that the narrow upright Type II plant profile of A 55 did not result in disease avoidance in the RIL population. This is substantiated by the population mean for plant height (52.4) being less than the parental means and mean canopy porosity (2.4) similar to the less porous parent G 122. Regardless of the growth habit present the importance of increased plant height was associated with less disease ($r = -0.37$) in the field.

The loss of parental ideotype attests to the reported difficulty of obtaining useful germplasm from wide crosses between the Middle American and Andean gene pools (Singh, 1999). Many of the determinate bush RILs seemed to condition avoidance due to a lack of canopy closure and less biomass. Thus, the positive effect of the *fin* gene (bush growth habit) on avoidance in the A 55/G 122 population was primarily an artefact of poor vigor generated by the wide cross. The fact that less disease was partly associated with avoidance resulting from poor vigor attests to the need for simultaneous selection for high yield and resistance to white mold.

SUMMARY AND CONCLUSIONS

Many bean breeding programs are interested in developing cultivars with improved field resistance to white mold disease by combining both physiological and avoidance mechanisms of resistance. Screening methods that enable efficient identification of physiological resistance mechanisms are needed to accomplish this goal. The observations of moderate h^2, a single major-effect QTL on linkage group B7, significant phenotypic correlations between straw test scores and field resistance, and occurrence of a QTL for field resistance in the same region of B7 all indicate that the straw test provides a useful and reliable method for selecting advanced lines in the A 55/G 122 RIL population with a promising level of physiological resistance to white mold. These results support the germplasm surveys conducted by Hall and Phillips (1997 and 1998) and Myers et al. (1999) which showed that the straw test may be used to predict field reaction to white mold.

The landrace cultivar G 122 provides breeders with a heritable source of physiological resistance to white mold disease in common bean. The development of such resistance should follow a strategy whereby selection for physiological resistance is performed first among F_3 or later generation lines using the straw test. Secondly, to combine this resistance with avoidance, the most resistant lines in the straw test would then be tested in a white mold field nursery. Only the highest yielding and least diseased lines would be advanced for additional cycles of testing and crossing. Thirdly, the transferred resistance should eventually be combined with other resistance sources, especially those known to possess different mechanisms and QTL for resistance, using recurrent selection or MAS, to obtain bean cultivars with enhanced resistance to white mold disease.

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Selection of Sugarcane Clones in Small Plots: Effects of Plot Size and Selection Criteria

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ABSTRACT

Sugarcane (*Saccharum* spp.) clones are frequently evaluated in one- or two-row plots in the early stages of selection in sugarcane breeding programs. This study assessed the value of performance in small plots for predicting performance under near pure stands and compared different selection methods and criteria based on measurements made in small plots. Two populations of unselected seedling clones were evaluated in different plot sizes in experiments at two sites over two and three crop-years, respectively. Commercially recoverable sugar content in cane (%), cane yield (kg/ha), sugar yield, and an estimate of relative economic value (REV, $) were determined in each plot. Cane yield was biased by competition effects in the small plots, but this was not the case for sugar content. Genetic correlations between cane yield in one-row plots and the middle two rows of the six-row plots in the same experiment and year averaged 0.49, while the equivalent correlation involving sugar content was 0.91. Measurements of sugar yield and REV were also biased in small plots because of the influence of cane yield. Measurements in small plots were considered in terms of indirect selection criteria for improving REV in large plots (the latter representing REV in pure stands). Selection based on sugar content alone in small plots gave equal or larger gains compared with other selection criteria, including REV itself in small plots. It is suggested that selection in small plots in early stages of selection in sugarcane breeding programs should be based largely on sugar content. Measuring cane yield in such trials may be inefficient and where destructive measurement via mechanical harvesting is involved, may unnecessarily delay progression of selected clones through to the next stages of selection.

Problems associated with the use of small plots are well known in field experimentation. This is particularly so in variety selection trials where measurements in small plots are subject to possible bias due to competition effects when there are significant differences in height between genotypes being compared (see Duncan, 1969; Tovey et al., 1973). Despite these potential problems, a large proportion of resources in sugarcane breeding programs is usually devoted to evaluation in small plots in early selection stages, and selection intensities are often high. In sugarcane breeding programs, small, one-row or two-row plots are usually used extensively for the first two or three stages of selection of seedling clones (Skinner et al., 1987). The reasons for this include the desire to screen large populations of clones within available resource constraints to identify rare, elite recombinants, and the necessity to increase planting material from original seedlings through propagation before planting to larger plots. Given the level of resources usually devoted to early stage selection trials, it is important that optimal procedures are used so that selection is effective and efficient.

There are few published reports of competition in sugarcane. Skinner (1961) and Skinner and Hogarth (1978) examined competition in Australia. Results from both studies highlighted that variance due to competition was potentially large in sugarcane variety trials, and could seriously bias selection trial results. However, interpretation of results in these reports was limited by the methods used. First, these studies involved evaluating clones derived from previous stages of selection. If competition effects were large among seedling clones, either as individual seedlings in the first selection stage, or in subsequent small plots, prior selection pressure would be expected to discard uncompetitive clones. Such studies may underestimate the importance of competition in original populations, and are of unknown relevance to the earliest stages of selection. Second, these studies only examined trials with plots of a single plot size (e.g., three-row plots) and estimated competition effects using certain assumptions about the relative level of competition expressed in different rows in multirow plots. It was assumed that the outside rows of a three- or four-row plot would express half the competition effect expressed in a single-row plot, and that the middle row(s) in a three or four row plot would be free of any effects due to competition. However, this may not be the case: if growth in an outside row(s) of a three- or four-row plot was strongly affected by interplot competition (adversely or favorably), this would result in further inter-row competition effects (in the opposite direction) passed on to the adjacent rows in the same plot.

If the primary objective of variety trials is to select

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Abbreviations: CCS, commercial cane sugar; REV, relative economic value.