Validation and Introgression of White Mold Resistance from Andean Into Middle American Germplasm

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Introduction

Mechanisms to reduce common bean (*Phaseolus vulgaris* L.), crop losses to white mold caused by *Sclerotinia sclerotiorum* Lib de Bary include genetic resistance and architectural avoidance. There is a need to identify sources of genetic resistance to white mold in common bean. Miklas et al. (2001) reported a QTL that accounted for 38% of the phenotypic variation and 58% of the genetic variation in common bean line G122. The QTL was tightly linked to the T phaseolin seed protein in parent G 122. There is a need to validate the effect of this QTL in other breeding populations, and find new markers linked to resistance genes (QTLs). The objectives of this research were to: 1. Determine the level of resistance among lines derived from G122 and validate the effect of the QTL found in G122 among RILs derived from a cross between G122 and an improved pinto line, 2. Identify molecular markers that can be used to map the RIL population and linked to QTLs for resistance to white mold.

Our immediate goal is to identify RIL lines that possess resistance genes from G 122 in a population derived from crosses between G 122 and pinto germplasm, and to find molecular markers more closely linked to resistance genes.

Materials and Methods

Genetic Materials:
The Colorado State University Dry Bean Breeding project has developed a RIL population from a cross between adapted pinto line CO72548 and G122. The RIL population consists of 115 $F_{5:7}$ lines and were used to verify the effect of the QTL reported by Miklas in G122, and search for additional QTLs for resistance.

White Mold Screening:

Straw Test:
The RIL population was screened twice in the greenhouse using the straw test (Figure 1), a common greenhouse procedure well documented in the literature developed by Petzoldt and Dickson (1996). The straw test rates lines from 1 (resistant) to 9 (susceptible) based on the progression of mycelial growth from the point of infection and is considered to be a good assessment for physiological resistance.

Field Testing:
A subset of the RIL population was screened in a artificially induced white mold nursery at the Carrington Research and Extension Center, Carrington ND (Figure 2). Field reactions were based on disease severity (% of plant tissue with mycelial growth) to assess a combination of physiological resistance and disease avoidance.

Molecular Marker and DNA Analysis:
DNA was extracted from 1 or 2 immature leaflets of the first trifoliolate leaves of RILs, then extracted and purified DNA following the method of Skroch and Nienhuis (1995) with minor modifications. Polymerase chain reactions were conducted to amplify and separate DNA fragments specified by Kami et al. (1995). Amplified DNA was separated by electrophoresis on either 4% agarose gels or 6% denaturing polyacrilamide gels. Only AFLP primer combinations that provided three or more polymorphic bands were considered for use in this study. All RAPD primers considered for this study were present on the core map developed by Freyre et al. (1998). SSR reactions were performed as described by Blair et al. (2003). AFLP reactions were performed as described by Inventrogen Life Technologies (AFLP analysis system II). RAPD reactions were performed as described by Freyre et al. (1998).

Figure 1. Straw Test Inoculation Procedure


Plants were cut just below third node


Inoculum loaded into a straw


Inoculated plants


Plants in mist chamber

Disease Rating Scores for the Straw Test


3


5


7


9

Figure 2. Field Evaluation in North Dakota

NDSU Carrington Research and Extension Center


Signs of White Mold in the field


Susceptible vs. Resistant

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Results

Objective 1: Determine the level of resistance and validate the effect of the QTL found in G122 using a RIL population from the cross G122 x CO72548.

Resistance among RILs:
Among all RILs, average severity index (ASI) varied from 3.3 to 9. Lines with resistance levels as good as G-122 (ASI ~5.0) were found with both S and T phaseolin (Table 1). Lines that expressed physiological resistance in the straw test also expressed resistance in the field (Table 1). Straw tests from two evaluations were highly correlated (Table 2), indicating that the test results were repeatable. The correlation between the mean of the straw tests and field evaluation was also significant (r = 0.418, P = 0.0006). Although these correlations are not as high as desired, it indicates that the test results were associated. Because the straw test only evaluates physiological resistance, where the field test evaluates both physiological resistance and avoidance, previous studies have also found them to be only moderately associated.

Effect of QTL:
The QTL reported by Miklas et al. (2001) was tightly linked to the T phaseolin locus, therefore we used a SCAR marker linked to T *Phs* to tag the locus. Segregation among our RILs for S and T phaseolin alleles conformed to a 1:1 expected ratio. (S=70 and T=49, P = 0.05). To confirm that the T-phaseolin SCAR marker correctly identified RILs, we evaluated the parents and twelve lines for phaseolin proteins (isozymes) according to the method of Gepts et al. (1992). These results validated that the T *Phs* SCAR marker correctly identified the correct *Phs* allele among all RILs tested.

The SCAR marker was also correlated with the straw test (r = 0.26, P <0.009) and field reaction (r=0.307, P< 0.016) (Table 2). The phaseolin locus accounted for 7% of the resistance found among lines in the straw test and 9% of the resistance found in the field test. These results show a lower level of association between resistance and the QTL reported by Miklas et al.(2001).

Table 1 Growth habit and reaction to white mold among resistant RILs.

Entry	Growth Habit †	Field Severity ‡	Straw Test §	*Phs* Type ¶
CO72548	II	NA	5.83	T
G122	I	40	4.62	S
MONTROSE	II	90	7.76	T
RIL 102	II	5	4.00	T
RIL 106	II	30	4.36	S
RIL 160	II	45	5.00	S
RIL 164	III	30	4.33	T
RIL 20	II	NA	4.13	S
RIL 21	I	40	3.46	T
RIL 26	I	25	3.29	T
RIL 27	II	10	4.36	S
RIL 29	II/III	17.5	5.21	S
RIL 32	I	10	3.43	T
RIL 67	II	30	3.60	T
RIL 130	I	NA	3.90	S
RIL 35	II/III	10	3.67	S
			LSD = 2.52	

† I, Determinate; II, Indeterminate; III, Indeterminate true climber; II/III, segregated for Indeterminate and Indeterminate true climber
‡ Entries were grown in North Dakota and visual infection severity were taken on whole plots
§ Averaged over two straw test (Petzoldt and Dickson, 1998)
¶ Determined by SCAR marker linked to the *Phs* locus

Table 2. Correlations between the straw tests, field severity and phaseolin type

	Correlation coefficients	P-value
Straw Test 1 *vs* Straw Test 2	0.596	< 0.0001
Straw Test (mean) *vs* Field Severity	0.418	0.0006
Straw Test (mean) *vs* Phaseolin Type	0.262	0.018
Field Severity *vs* Phaseolin Type	0.307	0.016

Results

Objective 2: Develop molecular markers that can be used to map the RIL population and identify markers linked to QTLs for resistance to white mold..

RAPD, AFLP and SSR markers generated 278 polymorphic bands between parental lines, with 105 primer combinations for a total of 2.4 polymorphic bands per primer combination. Among the polymorphic markers, 78 have been evaluated for linkage to candidate QTLs on the entire set of RILs. Seven AFLP bands have shown a significant (P< 0.05) relationship with disease scores based on the mean Straw Test rating (Table3). A preliminary linkage map using the 78 markers provided 21 linkage groups with several anchors from the core common bean map, including the *Phs* SCAR marker on linkage group B7. The remainder of the RILs will be screened to develop a map of the population and seek additional markers associated with resistance. Many of the markers used in this study have been anchored on the bean core map and should be useful to identify linkage groups on the core map.

Table 3. Relationships between molecular markers and resistance among RILs and location on the bean core map.

Marker †	R^2	P-value	Linkage Group ‡
Pat Mact_128	0.08	0.006	B7
Pat Maac_500	0.08	0.01	Unknown
Pat Maac_239	0.07	0.012	Unknown
Pat Maag_305	0.10	0.003	Unknown
Pag Maca_370	0.10	0.0024	Unknown
Pag Macc_340	0.08	0.005	B7
Phs	0.09	0.016	B7

† First five markers are AFLP, where P = Pst 1 and M = Mse 1. Letters that follow are the selective primers for each restriction enzyme and the band size; *Phs* is a SCAR marker linked to the *Phs* locus.

‡ Linkage groups are based anchor markers from the core common bean map.

Summary

- Average severity index among RILs varied from 3.3 to 9.
- Field and greenhouse evaluations for reaction to white mold were correlated.
- The *Phs* SCAR marker reported by Miklas et al. (2001) was found to be weakly associated with resistance among lines. The *Phs* SCAR marker accounted for 7% of the resistance in the straw test and 9 % of the resistance in the field test.
- RAPD, AFLP and SSR markers provided 278 polymorphic bands from parental lines.
- Seven markers have been found linked to disease reaction, and are candidate markers to identify new QTLs for resistance.