QUANTITATIVE TRAIT LOCI LINKED TO WHITE MOLD RESISTANCE IN COMMON BEAN

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Introduction

Introduction:
White mold disease of common bean (*Phaseolus vulgaris* L.), caused by *Sclerotinia sclerotoirum* (Lib.) de Bary, is a serious disease. White mold causes an annual average yield loss of 20 to 30% worldwide, due to reduced seed number, seed weight, and seed quality. Development of cultivars with physiological resistance combined with avoidance mechanisms, such as upright plant architecture, is the current strategy to minimize yield losses due to white mold. Progress in breeding to improve white mold resistance has been hindered by environmental conditions and avoidance mechanisms that confound the expression and reliable detection of physiological resistance in the field. The development of polygenetic physiological resistance to white mold would be enhanced if molecular markers linked to resistance genes were identified. The objectives of this research were to 1) characterize a recombinant inbred line population for reaction to white mold in the straw test and validate the QTL on B7, and 2) Develop a genetic linkage map based on molecular markers to identify additional QTLs associated with white mold resistance.

Materials and Methods

Genetic Materials:
A RIL population from the cross between adapted pinto line CO72548 and G122 was developed to verify the effect of a QTL found in G122 located on B7 (Miklas et al., 2000), and search for additional markers linked to the QTL.

White Mold Screening:

Straw Test:
The RIL population was screened in the greenhouse using the straw test (Figure 1) (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 on a cut stem, and is considered to be a good assessment for physiological resistance.

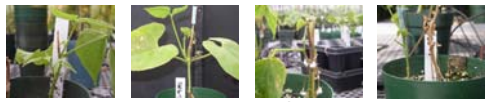
Field Testing:
A subset of the RIL population was screened in an artificially induced white mold nursery at the Carrington Research and Extension Center, Carrington ND (Figure 2). Field reaction was based on disease severity (% of plant tissue with mycelial growth) and is considered to assess both physiological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

Molecular Marker and DNA Analysis:
DNA was extracted from 1 or 2 immature leaflets of the first trifoliolate leaves of each RIL, then purified following the method of Skroch and Nienhuis (1995). Polymerase chain reactions were conducted to amplify and separate DNA fragments as 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).

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



Score=3 | Score=5 | Score=7 | Score=9

Figure 2. Field Evaluation in North Dakota

NDSU Research and Extension Center | Signs of White Mold in the field | Susceptible vs. Resistant

References:

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Kami J, Becerra Velásquez V, Debouck DG, Gepts P. 1995. Identification of presumed ancestral DNA sequences of phaseolin in Phaseolus vulgaris. Proc Nat Acad Sci 92: 1101-1104.

Miklas, P.N., W.C. Johnson, R. Delorme, and P. Gepts. 2001. QTL conditioning physiological resistance and avoidance to white mold in dry bean. Crop Sci.

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Results: Objective 1

Objective 1: Characterize a recombinant inbred line population for reaction to white mold in the straw test.

Resistance among RILs:
Among all RILs, ASI varied from 3.22 = resistant to 8.22 = susceptible . Two lines had significantly higher resistance levels than the resistant parent, G-122 (Table 1). The straw test results from two greenhouse evaluations were highly correlated (r = 0.596, P<0. 001) (Table 2) indicating that the test results are repeatable. The correlation between the mean of the straw tests and field evaluation was also highly correlated (r = 0.418, P = 0.0006). Although these correlations are not as high as desired, it indicates that the test results are associated. Because the straw test is considered to only evaluate physiological resistance, and the field test evaluates both physiological resistance and avoidance, previous studies have also found them to be only moderately associated.

Effect of B7 QTL:
The QTL on B7 reported by Miklas et al. (2001) was tightly linked to the phaseolin locus, therefore we used the SCAR marker that is liked to T *Phs* loci in the resistant Andean parent G122 to tag the QTL. Segregation among our RILs for S and T phaseolin alleles conformed to a 1:1 expected ratio (P = 0.054). Isozyme results validated that the T *Phs* SCAR marker correctly identified the correct *Phs* allele among RILs tested.

A significant relationship (P < 0.01) was found between the QTL on B7 and the white mold reaction in the greenhouse straw test and field severity. Composite interval mapping (CIM) results indicated strong evidence (LOD >2.9) for the B7 QTLs affecting physiological resistance to white mold based on the greenhouse straw test. The B7 QTLs accounted for 16.3% of the phenotypic variation for the white mold reaction. Our results show a much lower effect of the B7 QTL than Miklas et al. who reported that the QTL accounted for 38% of the phenotypic variation for white mold reaction.

Table 1. Phaseolin, mean ASI based on the Straw Test, and Field Infection among the most resistant recombinant inbred lines and the checks.

Entry	Phaseolin Type	Mean ASI Straw Test	Field Infection %
RIL 31	T	3.22 a‡	20
RIL 67	T	3.40 a	25
RIL 32	T	3.46 ab	55
RIL 7	T	3.48 ab	43
RIL 21	T	3.64 ab	53
G 122	T	4.50 bc	27
CO72548	S	5.79 c	60
PC-50	T	5.92 c	27
Montrose	S	8.03 d	87

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

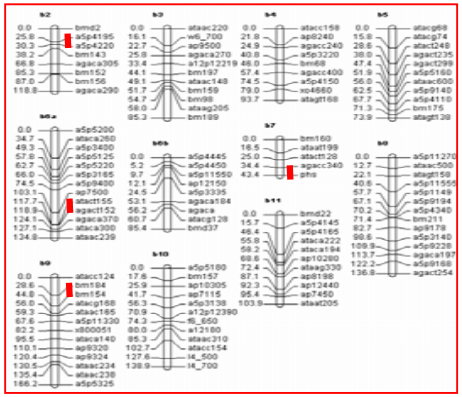
	Correlation coefficients	P-value
Straw test 1 *vs* straw test 2	0.596	< 0.0001
Straw test ave. *vs* field severity	0.418	0.0006
Field severity *vs* Phaseolin type	0.307	0.016

Results: Objective 2

Objective 2: Develop a genetic linkage map based on molecular markers to identify additional QTLs associated with white mold resistance.

RAPD, AFLP and SSR markers generated 124 polymorphic markers in the G122 X CO72548 RIL population. Composite interval mapping results indicated strong evidence (LOD >2.9) for three QTLs affecting physiological resistance to white mold in the greenhouse straw test. The QTLs were linked most closely with marker loci a5p4195, ataca300, and *Phs* on linkage groups B2, B6a, and B7, respectively (Fig. 3). The ataca300 region of B6a had the largest effect and accounted for 19.3% of the phenotypic variation for white mold reaction in the straw test. The a5p4195 region of the B2 linkage group accounted for 17.6%, and the *Phs* region of the B7 linkage group accounted for 16.3% of the phenotypic variation for the white mold reaction in the straw test. All three of these loci were contributed from G122. One other marker was significant at a genome-wide empirical threshold of LOD = 2.8 at the BM184 region of the B9 linkage group.

Figure 3. Linkage map with 124 markers, constructed with Mapmaker/Exp. Solid bars indicate QTLs identified by composite interval mapping and single factor analysis of variance.



Summary

- Average severity index (ASI) among RILs varied from 3.22 to 8.22.
- Field and greenhouse evaluations for reaction to white mold were highly correlated.
- The QTL on B7 reported by Miklas et al. accounted for 16.3% of the phenotypic variation for the white mold reaction based on the straw test.
- Composite interval mapping (CIM) results indicated strong evidence (LOD >2.9) for three additional QTLs affecting physiological resistance to white mold in the greenhouse straw test.