Inheritance and QTL Analysis of Field Resistance to Ashy Stem Blight in Common Bean

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ASHY STEM BLIGHT or charcoal rot, incited by *Macrophomina phaseolina* (Tassi) Goid. can be a serious disease of common bean (*Phaseolus vulgaris* L.) under drought and high temperature conditions in some regions. The mode of inheritance of valuable sources of resistance is lacking. We studied inheritance of field resistance to ashy stem blight in a recombinant inbred population (‘Dorado’ × XAN 176) consisting of 119 F$_5$$_0$ recombinant inbred lines (RILs) tested in replicated experimental plots across 2 yr. A score from 1 to 9 (no disease to severe disease) was used to measure disease reaction. Moderate H$_{50}$ (0.53 and 0.57) and near-normal frequency distribution of RILs for mean disease score each year indicated a lack of discrete segregation classes. The phenotypic variation across a subgroup composed of 79 RILs was further investigated with 165 randomly amplified polymorphic DNA (RAPD) markers by one-way analyses of variance and interval mapping. Five quantitative trait loci (QTL), explaining 15, 15, 13, and 13% of the phenotypic variation for disease score, were detected in 1993. Three of these QTL, explaining 15, 12, and 12% of the variation in disease reaction, were detected in 1994. Multiple QTL regression models (P < 0.01) explained up to 47% (four loci) of the phenotypic variation for disease score in 1993 and 28% (three loci) in 1994. The five QTL, all derived from XAN 176, generally showed additive effects. These QTL-linked RAPD markers may prove useful for indirect selection of field resistance to ashy stem blight derived from XAN 176.

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Disease score combined incidence and severity where 0 = no visible disease symptoms and 9 = severely infected; and 9 = >80% of the plants infected and/or about 20% of plant area affected; 7 = 60 to 80% of plants infected and/or about 50% of plant area affected; 5 = 40 to 50% of plants infected and/or about 20% of plant area affected; 3 = 20 to 30% of plants infected and/or about 10% of plant area affected; 1 = no visible symptoms.

Results and Discussion

The error mean squares for disease score from analyses of variance across years were homogeneous based on Bartlett's test and the Levene's test. A narrow-sense heritability estimate for disease score was computed with pooled variances on a plot (1 m of row) basis, where 1 = no visible symptoms; 3 = 20 to 30% of plants infected and/or about 10% of plant area affected; 5 = 40 to 50% of plants infected and/or about 20% of plant area affected; 7 = 60 to 80% of plants infected and/or about 50% of plant area affected; 9 = >80% of the plants infected and/or about 20% of plant area affected.

An analysis of variance across years was also conducted. A narrow-sense heritability estimate for disease score among the RILs was recorded from 1 = early to 3 = >60% of the plant area affected. Differences in relative maturity between a RAPD and QTL. Multiple stepwise regression of variance with the disease score means using PROC GLM was conducted, interval mapping (OGene; Nelson, 1996) was performed. A pairwise linkage analysis of the RAPD data, imposing a LOD of 2.0 was used as a significance threshold. An F-test significant at P < 0.002 indicated linkage. Two-way analysis (PROC GLM) for epistatic interactions (additive × additive) were only computed with the exception that no bulked segregant analysis or selection (reaction) procedures followed those of Miklas et al. (1996). The DNA extraction and PCR (polymerase chain reaction) were performed. Frequency distributions of the RIL means for disease score were tested for normality with the Shapiro and Wilk test.

An F5:7 line-mean map of the RIL population was constructed, interval mapping (OGene; Nelson, 1996) was used to establish the linkage groups. Centimorgan distances between linked loci were based upon recombination fractions (Kosambi, 1944). Once the map was constructed, interval mapping (OGene; Nelson, 1996) was used to further analyze the QTL detected by regression. An F-test significant at P < 0.001 was used to indicate lack of fit. A separate and similar analysis of the parents was conducted. A narrow-sense heritability estimate for disease score was computed with pooled variances on a plot basis with relative maturity measured in days earlier than an intermediate (2) and late maturing (5) line. Frequency distributions of the RIL means for relative maturity were analyzed by one-way analysis of variance (ANOVA). An early maturing line generally reached harvest maturity (80% of pods harvestable) four to eight days earlier than an intermediate (2) and late maturing (5) line. An early maturing line generally reached harvest maturity (80% of pods harvestable) four to eight days earlier than an intermediate (2) and late maturing (5) line. An analysis of variance across years was also conducted. Differences in relative maturity between the parents was analyzed by one-way analysis of variance (ANOVA). An early maturing line generally reached harvest maturity (80% of pods harvestable) four to eight days earlier than an intermediate (2) and late maturing (5) line.
Table 2. Linkage group, estimated map distances between marker loci, and phenotypic variation in disease score associated with QTL identified by single-factor regression analysis (P < 0.002) that condition field resistance to ashy stem blight in 79 Fs: RILs (Dorado/XAN 176) tested two separate years.

<table>
<thead>
<tr>
<th>Linkage Group</th>
<th>Marker</th>
<th>Allele</th>
<th>Distance R</th>
<th>R² P</th>
<th>Distance R² P</th>
</tr>
</thead>
<tbody>
<tr>
<td>US-4</td>
<td>Q11</td>
<td>X</td>
<td>15.5</td>
<td>0.000</td>
<td>12.5</td>
</tr>
<tr>
<td>US-6</td>
<td>E</td>
<td>1999</td>
<td>0.000</td>
<td>1.3</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>US-7</td>
<td>AH5</td>
<td>37</td>
<td>0.000</td>
<td>10.0</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>US-8</td>
<td>W12</td>
<td>0</td>
<td>0.001</td>
<td>5.7</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unlinked</td>
<td>W18</td>
<td>X</td>
<td>0.000</td>
<td>12.8</td>
<td>0.001</td>
</tr>
</tbody>
</table>

RAPD marker named for a specific Operon (Alameda, CA) decamer primer (number) from within a kit (letters). Number in subscript represents the base pair size of the marker. D or X = RAPD marker allele derived from Dorado parent or XAN 176 parent, respectively.

The frequency distributions of the 119 F5:7 lines for mean disease score each year indicated a lack of discrete segregation classes (Fig. 1). Moderate heritabilities, 0.53 in 1993 and 0.57 in 1994, for disease score among F5:7 lines (Table 1), also indicated resistance within this population was quantitatively inherited. The significant deviation (P < 0.05) of the population mean from its midparent value, toward Dorado in 1993, may reflect a difficulty with recovering breeding lines with the same level of resistance of XAN 176.

Polygenic inheritance was further corroborated by RAPD marker analyses which revealed five independent QTL affecting disease reaction in 1993 (Table and Fig. 2-5). These QTL on linkage groups US 4, US 6, US 7, and US 8 and the unlinked marker W18 explained 15, 15, 1.9, 13, and 13% of the phenotypic variation for disease score in 1993. Only the three QTL on US 4, US 6, and US 7, respectively explaining 12, 12, and 15% of the variation in disease reaction, were detected in 1994.

The regression and interval mapping analyses corroborated the relative effect of the identified QTL as RAPD markers associated with higher R² values also had higher LOD scores (Table 2 and Fig. 2-5). The three QTL on US 4, US 6, and US 7, had parallel response intervals between years, differing only by the magnitude of effect being greater in 1993 than 1994. The two significant peaks at E1999 and AS81 explained a single QTL, because when one locus was fixed the second locus had an LOD below 2.0. Perhaps including additional RILs in the mapping population would provide better resolution of this region.
The relatively flat interval response for US 8 in 1994 suggests that the QTL (W12700) detected in 1993 will only be expressed in specific environments. A few lines in 1994 had greater resistance (P < 0.05) than XAN 176. Recombinant inbred lines with QTL-linked RAPD alleles from XAN 176 had a reduced mean disease score and from Dorado an increased mean score both years, indicating none of the five QTL originated from Dorado. Thus, the transgressive segregation observed in 1994 could not be explained by the QTL we detected.

The entire map (900 cM), consisting of 147 RAPD markers assigned to ten linkage groups (US 1 to US 10), two linked triads (LT 11, LT 12), and seven linked pairs (LP 13 to LP 19) is described in detail by Miklas et al. (1998a). All the RAPD markers associated with QTL in this study fit segregation ratios (1:1) expected for undistorted markers.

Multiple QTL models (P < 0.01) explained up to 47% [Q11980 (US 4) + AS81300 (US 6) + AA19600 (US 7) + W12700 (US 8)] of the phenotypic variation for disease score in 1993 and 28% [Q11980 (US 4) + E19990 (US 6) + AH51370 (US 7)] in 1994. The effects were mainly additive. Epistasis was detected (P < 0.05) between the US 4 (Q11980) and AA19600 QTL in 1993. The effect was positive, explaining an additional 4% of the phenotypic variation. Although a significant line × year interaction was detected (P < 0.05), with the exception of W12700 on US and the unlinked W181300, the same QTL were expressed each year; however, they explained more of the variation in disease score in 1993 than 1994. Perhaps, less disease pressure contributed to the lower predictability of the multi-locus model in 1994. Interestingly the phenotypic variation explained by the 1993 model (47%) approached the heritability estimate (0.53), whereas they differed widely in 1994 (28% vs. 0.57). It appears that selection in a single environment may be adequate if ideal disease pressure occurs to facilitate better separation of resistant and susceptible lines.

The quantitative inheritance of disease score in this population suggests high selection intensities, large populations, and strong genetic effects. The genetic basis of resistance to ashy stem blight is complex, with multiple QTL contributing to the phenotype. The results indicate that breeding programs for resistance should focus on a combination of QTL rather than single gene resistance. Further research is needed to understand the genetic architecture of resistance and to develop effective breeding strategies.
Fig. 4. Quantitative trait loci conditioning field resistance to ashy stem blight on linkage group US 7 as depicted by interval mapping (LOD > 2.0) in 79 F₃;7 RILs (Dorado/XAN 176) tested two separate years. Population sizes, and perhaps multiple environments will be required to phenotypically select for the XAN 176-derived resistance to ashy stem blight segregating in other populations. Whether the RAPDs linked to these QTL will have utility for indirect selection of the XAN 176-derived resistance is unknown. Hybridization between a highly resistant inbred progeny line from this study and another susceptible cultivar would provide a useful population for assessing and confirming these QTL-linked RAPDs for marker-assisted selection.

For lasting control, combining quantitative resistance traits with qualitative resistance genes is preferred, but quantitative resistance is difficult to retain and is often lost in a traditional backcross breeding program to introgress major genes. The Mp-1 and Mp-2 resistance genes and linked RAPD markers from the breeding line BAT 477 (Olaya et al., 1996) have not yet been placed on a linkage map. Although it seems unlikely these QTL with relatively minor effects would be associated with major gene resistance, the relationship, if any, between Mp-1 and Mp-2 and the QTL identified in this study cannot be discounted. If independent, the markers for Mp-1 and Mp-2 and the QTL-linked RAPDs described herein would provide initial tools for testing marker-assisted selection approaches to retaining and combining quantitative resistance traits with qualitative resistance genes for control of ashy stem blight in common bean.
Fig. 5. Quantitative trait loci conditioning field resistance to ashy stem blight on linkage group US 8 as depicted by interval mapping (LOD 2.0) in 79 F$_{2}$:7 RILs (Dorado/XAN 176) tested two separate years.