Toward an Integrated Linkage Map of Common Bean. III. Mapping Genetic Factors Controlling Host-Bacteria Interactions

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ABSTRACT

Restriction fragment length polymorphism (RFLP)-based genetic linkage maps allow us to dissect the genetic control of quantitative traits (QT) by locating individual quantitative trait loci (QTLs) on the linkage map and determining their type of gene action and the magnitude of their contribution to the phenotype of the QT. We have performed such an analysis for two traits in common bean, involving interactions between the plant host and bacteria, namely Rhizobium nodule number (NN) and resistance to common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli*. Analyses were conducted in the progeny of a cross between BAT93 (fewer nodules; moderately resistant to CBB) and Jalo EEP558 (more nodules; susceptible to CBB). An RFLP-based linkage map for common bean based on 152 markers had previously been derived in the F_2 of this cross. Seventy F_2-derived F_3 families were inoculated in separate greenhouse experiments with *Rhizobium tropici* strain UMR1899 or *X. c.* pv. *phaseoli* isolate isolate W18. Regression and interval mapping analyses were used to identify genomic regions involved in the genetic control of these traits. These two methods identified the same genomic regions for each trait, with a few exceptions. For each trait, at least four putative QTLs were identified, which accounted for approximately 50% and 75% of the phenotypic variation in NN and CBB resistance, respectively. A chromosome region on linkage group D7 carried factor(s) influencing both traits. In all other cases, the putative QTLs affecting NN and CBB were located in different linkage groups or in the same linkage group, but far apart (more than 50 cM). Both BAT93 and Jalo EEP558 contributed alleles associated with higher NN, whereas CBB resistance was always associated with BAT93 alleles. Further investigations are needed to determine whether the QTLs for NN and CBB on linkage group D7 represent linked genes or the same gene with pleiotropic effects. Identification of the QTLs raises the possibility of initiating map-based cloning and marker-assisted selection for these traits.

STUDIES of host-pathogen interactions have focused mainly on qualitative gene-for-gene relationships (CRUTE 1985; GABRIEL and ROLFE 1990; KEEN 1990). Yet, for many host-pathogen interactions, the genetic control appears to be quantitative rather than qualitative. Until recently, genetic analysis and breeding of quantitative traits (QT) has been generally based on biometrical approaches (MATHER and JINKS 1977; FALCONER 1981; MAYO 1987). These approaches deal mainly with the collective characterization of the multiple factors affecting a QT and allows us to partition the overall phenotypic variance into its genotypic and environment components. Biometrical methods are generally, however, not capable of characterizing or manipulating specific loci.

Individual loci involved in quantitative traits can be characterized with linked marker genes as shown first by SAX (1923) in common bean (*Phaseolus vulgaris* L.) and later by THODAY (1961) in Drosophila. The limiting factor in these studies was the number of (morphological) markers available. Genetic linkage maps based on molecular markers–principally, restriction fragment length polymorphisms (RFLPs) and rapid amplified polymorphic DNA (RAPDs), and to a lesser extent, isozymes, and seed proteins–allow us to map and estimate the effects of individual factors controlling a quantitative trait (QT) because only molecular markers are sufficiently numerous in any one cross to provide adequate genome coverage (PATERSON, TANKSLEY and SORRELLS 1991).

Two statistical approaches have been used to map and measure the effects of putative quantitative trait loci (QTL). Regression analysis using a general linear fixed effects model, estimates the statistical significance of the association between the phenotypic expression of the QT and a marker locus region (either a putative QTL or a marker locus linked to it) as illustrated by the studies of EDWARDS, STUBER and WENDEL (1987) and REITER *et al.* (1991) in maize, NIENHUIS *et al.* (1987) and MARTIN *et al.* (1989) in

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tomato, and KEIM *et al.* (1990) and KEIM, DIERS and SHOEMAKER (1990) in soybean. Using the LOD (logarithm of the odds ratio) score method developed for genetic linkage analysis, LANDER and BOTSTEIN (1989) developed an interval mapping method to locate QTLs. They suggest that interval mapping approach overcomes three weaknesses of regression analysis: (a) underestimation of the phenotypic effects of QTLs; (b) confounding effects between linkage distance and magnitude of the QTL effect, which results in imprecise QTL map location; and (c) the large number of progeny required to detect QTLs. Interval mapping analysis has been used to map several Mendelian factors associated with QTs in tomato (PATERSON *et al.* 1988, 1991).

In the work reported here, we used regression and interval mapping analyses to examine the genetic control of two host-bacterium interactions that display a quantitative inheritance in common bean ($2n = 2x = 22$): nodulation by *Rhizobium tropici* (nodule number, NN) and resistance to *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight (CBB). We were interested in determining whether any genetic correlation existed between the host plant interactions with two distinct bacteria, which could reflect a common signal transduction mechanism at the molecular or biochemical level. To our knowledge, no information is available so far on the relationship between these two interactions in common bean or other crops. The specific objectives of this study were to determine: (1) the number of statistically significant associations between marker loci and Rhizobium nodule number or CBB resistance; (2) the individual contribution of each QTL to the expression of their respective QT; and (3) the location of QTLs on an RFLP-based linkage map of the common bean genome. This map consists, in its current stage, of 152 molecular markers assigned to 15 linkage groups and covers 827 cM (NODARI *et al.* 1993; GEPTS 1993).

MATERIALS AND METHODS

Plant material: Segregation analyses were conducted in the progeny of the cross BAT93 × Jalo EEP558. High levels of polymorphism at the molecular level distinguished these two parents (NODARI *et al.* 1993). BAT93 had low levels of nodulation after inoculation with *R. tropici* strain UMR1899 and moderate levels of resistance to *X. c.* pv. *phaseoli*, whereas Jalo EEP558 had high levels of nodulation and was very susceptible to *X. c.* pv. *phaseoli* (see RESULTS).

Segregation for Rhizobium NN was analyzed in a population of 70 F_2-derived F_3 families of the BAT93 × Jalo EEP558 cross. Control genotypes included the parents, the F_1, CIAT 125 (a nodulation defective or Nod$^-$ mutant: J. KIPE-NOLT, personal communication) as negative control, and cultivar "Puebla 152," a strong nodulator (PEREIRA, BURRIS and BLISS 1989). The 70 F_3 families were derived by selfing from the same F_2 mapping population that was used to generate an RFLP-based linkage map (NODARI *et al.* 1993). Therefore, the parental genotype of each F_3 family had been previously determined at each of 152 loci used to develop the linkage map. Segregation analysis for CBB resistance was conducted on the same set of F_2-derived F_3 families, but with different plants than those inoculated with Rhizobium.

Rhizobium NN experiment: The experiment was conducted in a greenhouse. The two parents, the F_1 generation, and the F_3 families were planted in a completely randomized block design with four replicates, and two plants per replicate. Three seeds were sown per Leonard jar wrapped with aluminum foil. The top half of the jar contained a mixture of vermiculite, sand and perlite (1:1:1, v:v:v basis), whereas the bottom half contained a nitrogen-free solution (JOHNSON *et al.* 1957; 1:5 strength). After germination, the jars were thinned from 3 to 2 seedlings. Seven days after sowing, seedlings were inoculated with *R. tropici*. A highly infective strain (UMR-1899, originally supplied by P. GRAHAM, University of Minnesota) of *R. tropici* (MARTÍNEZ ROMERO *et al.* 1991) was used to provide good discrimination among the segregating genotypes. Rhizobia were grown in shake culture to stationary phase in a medium containing mannitol (0.1%), yeast extract and salts (VINCENT 1970). Ten milliliters of inoculum (approximately 10^9 cells/ml) were added into each jar. After inoculation, the surface of the jars was covered with 1 cm of perlite to protect the substrate and inoculum from direct exposure to sunlight. Nutrient solution was added weekly. Thirty-two days after initiation of the experiment, plants were harvested and analyzed for nodule number. The roots were stored with the substrate in a cold room until the number of nodules was counted. The average of the nodule number for two plants in each jar constituted the experimental unit.

CBB experiment: The experiment was conducted in a greenhouse and consisted of a completely randomized block design with three replicates, and three plants per replicate. The planting design included the parental genotypes, the F_1 generation, and the F_3 families. Three weeks after sowing, the first expanded trifoliolate leaves were inoculated with *X. c.* pv. *phaseoli* isolate W18 (GILBERTSON, RAND and HAGEDORN 1989) by the razor blade method (PASTOR-CORRALES, BEEBE and CORREA 1981; SILVA, SINGH and PASTOR-CORRALES 1989). To prepare the inoculum, an aqueous bacterial suspension was prepared in sterile distilled water from *X. c.* pv. *phaseoli* grown on sucrose peptone agar for 48 hr and diluted to obtain an optical density of 0.5 at 600 nm in a spectrophotometer which results in about 1×10^7 colony-forming units/ml. The reaction to *X. c.* pv. *phaseoli* was evaluated 15 days after inoculation according to a 1–9 scale where 1 and 9 identified no visible symptoms and very severe disease symptoms, respectively; these evaluations were then converted into a disease index representing the percentage of leaf tissue affected (CIAT 1987). Scores of each experimental unit represented averages for three plants.

Statistical analyses: Data for Rhizobium nodule number and common bacterial blight of each F_3 family were subjected to regression analysis using the SAS PROC GLM procedure and the means were grouped according to Duncan's multiple range test (SAS 1988). Each pairwise combination between a quantitative trait and a molecular marker was also subjected to regression analysis. Significant F values ($P < 0.05$) and significant differences in mean values (Duncan's multiple range test) among genotypic classes for a marker locus were interpreted to indicate segregation of genotypes at a QTL linked to that marker locus (EDWARDS, STUBER and WENDEL 1987). In addition to the main effects, the interactions between two marker loci were examined using the PROC GLM procedure, in which the 8 d.f. were

partitioned into (a) the additive effects, (b) the dominance deviation (for both marker loci) and (c) interactions between the two marker loci namely: additive × additive, additive × dominant, dominant × additive, and dominant by dominant. Those interactions, in which at least one marker locus was scored in a 3:1 ratio or missing data restricted the number of degrees of freedom, were not analyzed.

The two QTs were also analyzed by the interval mapping approach with MAPMAKER/QTL (LANDER and BOTSTEIN 1989; E. S. LANDER and S. E. LINCOLN, personal communication). The threshold LOD score was approximately 1.6 corresponding to a nominal significance level of 0.01 (LANDER and BOTSTEIN 1989). After the first scan for putative QTLs, the locus with the highest LOD score was fixed and additional scans were performed to detect further regions with a significant contribution to the expression of the QT. In this process, an additional region was considered to contribute significantly to the trait variance when the LOD score of the scan exceeded by two units the score of the previous scan. This procedure was repeated until no additional significant scores could be detected. Gene action was tested by evaluating the relative likelihood of additive ($d = 0$), dominant ($d = +a$), or recessive ($d = -a$) models. Any model showing a one-LOD-score unit (10-fold) reduction was deemed unlikely (PATERSON *et al.* 1991).

RESULTS

In order to determine the genetic control of Rhizobium nodulation and resistance to CBB, segregation for these two traits was analyzed in F_3 families which were derived by selfing from individual plants of the F_2 generation used to establish an RFLP-based linkage map (NODARI *et al.* 1993). Segregation analysis of quantitative traits in the F_3 instead of the F_2 allowed us to conduct replicated trials and, hence, obtain a presumably more accurate phenotypic value for each of the F_2 plants. In addition, remnant F_3 seed, which were not used in the destructive nodulation and bacterial blight resistance assays, were used to develop a recombinant inbred or single seed descent population from this cross for future studies.

Rhizobium NN: The parents BAT93 and Jalo EEP558 had 103 and 336 nodules/plant, respectively (Figure 1a), and standard deviations of 18 and 58 nodules/plant, respectively, whereas the Nod$^-$ genotype had no nodulation (not shown). The F_1 plants showed heterosis for NN, with an average of 443 nodules/plant (standard deviation of 53 nodules/plant). F_3 families showed transgressive segregation, with NN ranging from 58 to 451 nodules/plant (average of 247 nodules/plant) (Figure 1a). The values for NN in the F_3 did not deviate significantly from a normal distribution as determined by the absence of significant kurtosis and skewness (not shown). A regression analysis with the F_3 family as independent variable and NN as dependent variable indicated that there were significant differences ($P < 0.0001$) among F_3 families for nodule number (Table 1). There were no significant differences among replicates (not shown). The coefficient of variation of 21.0% was considered to be low given that both environmental variation and genetic heterogeneity within F_3 families could cause differences between replications.

Regression analyses with individual marker loci as the independent variable and nodule number as the dependent variable identified 12 significant associations (Table 2). Nine marker loci were distributed in four genomic regions: one region on linkage group D1 (markers *D1228*, *D1290* and *D1593*), two regions on linkage group D3 (*D1128* and *D1132*, *Skdh*), and one region on linkage group D7 (*D1390*, *D1861* and *Phs*). The other three loci showing a significant association with NN (*D1683a*, *D1683b* and *D1737*) are unassigned on the genetic linkage map. Under the assumption that each of the four mapped genomic regions contain a single QTL, the contribution of these putative QTLs to NN were 13%, 11%, 9% and 18%, respectively (Table 2).

At five loci, alleles of Jalo EEP558, the high NN parent, were associated with higher NN in comparison with BAT93 alleles (region on linkage group D7 and unassigned markers *D1683a* and *D1683b*; Table 2). BAT93 contributed the allele associated with higher NN at the remaining loci, except the *Skdh* locus (linkage group D3) where the heterozygote marker class showed the highest NN. Two-way analyses of variance were conducted among markers showing a significant association with NN by regression analysis in order to identify significant interactions. The frequency of significant interactions (4.9%) was similar to the frequency of α errors when a 5% level test is used, suggesting that interlocus interactions may not have been important in the genetic control of NN.

Interval mapping analysis revealed a pattern similar to that found using regression analysis. Four genomic regions located on linkage groups D1 (with *D1290* as the closest marker), D3 (*Skdh* and *D1038*), and D7 (*D1861*) were associated with significant effects on NN phenotypic expression (Table 3 and Figure 2). The LOD scores for regions marked by *D1861*, *D1290* and *D1038*) were above the threshold score of 1.6, whereas the LOD score of the region marked by *Skdh* was nearly so (Table 3). The contribution to NN of the four putative QTLs was 12%, 12%, 11% and 17%, respectively (Table 3), which amounted to a total of 52% of the variation. These values were very similar to those obtained by regression analysis.

CBB resistance: Resistance to CBB showed a normal distribution (Figure 1b) without significant kurtosis or skewness (not shown). Disease severity indices among F_3 families ranged from 19 to 75 (average of 44) similar to the range determined by the parental indices (22 and 75 for BAT93 and Jalo EEP558, respectively, with standard deviations of 8 and 0, respectively). The 7 F_1 plants included in the experiment had a disease index of 75 (SD = 0) and were as

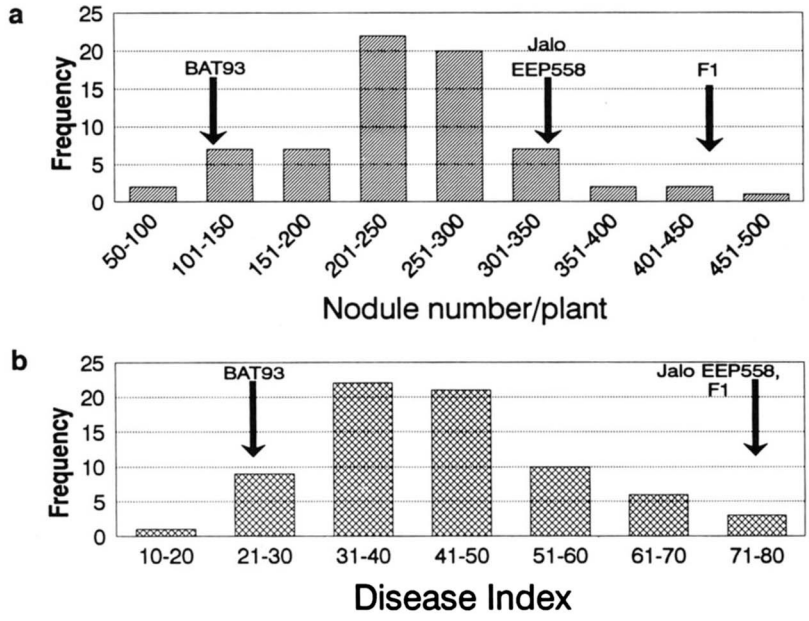

FIGURE 1.—Frequency distributions for nodule number per plant (a) and resistance to common bacterial blight (b) in F_2-derived F_3 families of cross BAT93 × Jalo EEP558.

TABLE 1

Summary of the regression analysis for Rhizobium NN and CBB resistance in the F_3 of cross BAT93 × Jalo EEP558

Trait	Mean square	*F*	*P*	R^2	C.V. (%)
NN	21,936.05	7.81	<0.001	0.76	21.0
CBB resistance	519.22	3.31	<0.001	0.66	28.5

susceptible as the susceptible parent (Jalo EEP558), indicating that CBB resistance was recessive. Highly significant ($P < 0.0001$) genotypic differences in CBB resistance among F_3 families were detected by regression analysis (Table 1). There were no significant differences among replicates (not shown). The coefficient of variation was 28.5%, presumably due to environmental variation and genetic heterogeneity within F_3 families.

Regression analyses using individual marker loci as independent variable and reaction to *X. c.* pv. *phaseoli* as the dependent variable identified 15 significant combinations. Fourteen marker loci were distributed in seven genomic regions: one on linkage group D1 (*D1512*), two on linkage group D2 (*D0108* and *D0166*; *ChS*), one each on linkage groups D3 (*SS*), D5 (*D1461*, *D1080* and *Diap*), D7 (*ChI*, *D1390*, *Phs* and *D1861*), and D9 (*D1101*, *D0157* and *D1831*) (Table 2). In addition, *D1683a*, an unassigned marker, also showed a significant association with this trait. Under the assumption that each of the seven mapped genomic regions contain a single QTL, the contribution of these putative QTLs to CBB resistance would be 13%, 17%, 10%, 11%, 11%, 35% and 15%, respectively (Table 2).

With two exceptions, alleles of BAT93, the resistant parent, were associated with resistance (Table 2). The first exception involved the sucrose synthase (*SS*) locus on linkage group D3 where the Jalo EEP558 allele was associated with resistance. The second exception involved locus *D1512* on linkage group D1 where the heterozygous marker class revealed the highest level of resistance. Because these two loci were not identified by interval mapping (see below), they were not considered any further.

Additivity was the main component of gene action. However, dominant or recessive deviations from the additive model appeared to be involved in three regions (D2, D5 and D7) (Tables 2 and 3). Overall, a small proportion (2.6%) of all possible interlocus interactions were significant, below the threshold level of $\alpha = 0.05$ when a 5% level test is used. This suggests that interlocus interactions were not important in the genetic control of CBB resistance.

Interval mapping analyses detected four regions, which represented a subset of the seven regions revealed by regression analysis: factors on linkage groups D2, D5, D7, and D9 were detected in both analyses (Table 3 and Figure 3). The respective LOD scores were above the threshold score of 1.6 (Table 3). The contribution to CBB resistance of the four putative QTLs was 17%, 15%, 32% and 13%, respectively (Table 3), which were very similar to the values obtained by regression analysis. Jointly, these four putative QTLs explained 75% of the variation.

DISCUSSION

Comparison of regression analysis and interval mapping analysis: Regression analysis and interval mapping analysis are different yet related algorithms used to detect QTLs. Interval mapping becomes identical to regression analysis when the QTL is located at the marker locus (LANDER and BOTSTEIN 1989). It was, therefore, not surprising that the two methods

TABLE 2

Linkage distribution of markers showing significant associations with phenotypic differences for NN and CBB as determined by regression analysis

		NN					CBB				
Linkage group	Marker	Significance level of *F* test	R^2	BB	BJ	JJ	Significance level of *F* test	R^2	BB	BJ	JJ
D1	*D1228*	*	0.12	278a	249ab	208b					
	D1290	**	0.13	294a	228b	230b					
	D1593	*	0.12	293a	226b	236b					
	D1512						*	0.13	45.6ab	39.1b	49.2a
D2	*D0108*						**	0.17	35.3b	46.8a	46.8a
	D0166						*	0.10	36.6b	45.6a	47.3a
	ChS						*	0.10	36.9b	44.4ab	49.6a
D3	*D1132*	*	0.11	288a	251ab	210b					
	D1128	*	0.11	288a	247ab	209b					
	SS						*	0.11	49.2a	43.4ab	37.8b
	Skdh	*	0.09	232ab	287a	218b					
D5	*D1461*						*	0.11	39.6b	41.3b	49.2a
	D1081						*	0.11	38.8b	42.0ab	49.4a
	Diap						*	0.10	38.5b	42.3ab	48.9a
D7	*Chl*						*	0.05	38.9b	45.6a	
	D1390	**	0.16	215b	231b	304a	***	0.35	30.5c	45.1b	52.7a
	Phs	**	0.18	201b	233b	298a	***	0.27	30.5b	45.4a	49.8a
	D1861	*	0.14	199b	240ab	288a	**	0.20	31.9b	45.0a	46.2a
D9	*D1101*						*	0.11	36.9b	43.3ab	49.7a
	D0157						*	0.12	36.2b	44.4a	49.0a
	D1831						**	0.15	37.6b	45.0ab	51.6a
Not assigned	*D1683a*	*	0.11	194b	263a		*	0.09	36.4b	45.9a	
	D1683b	*	0.11	201b	265a						
	D1737	**	0.15	294a	229b						

For map location of loci, see Figure 2. Genome regions within linkage groups that are distant by more than 30 cM are separated by single lines. Italicized D numbers represent loci identified by random genomic clones (NODARI *et al.* 1993; GEPTS 1993); *Chl*, chalcone isomerase; *ChS*, chalcone synthase; *Phs*, phaseolin seed protein; *Skdh*, shikimate dehydrogenase; *SS*, sucrose synthetase. B, BAT 93 allele; J, Jalo EEP558 allele. NN, nodule number/plant; CBB, resistance expressed as disease index (see MATERIALS AND METHODS). Significance levels: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. For each trait and within each line, values followed by the same letter are not significantly different according to Duncan's multiple range test.

gave highly similar results. With one exception, genomic regions identified with MAPMAKER/QTL as carrying putative NN QTLs coincided with the regions revealed by regression analysis (Table 4). A discrepancy occurred on linkage group D3, where the interval mapping method identified significant effects associated with marker *D1038* whereas the regression analysis method identified significant effects associated with markers *D1128* and *D1132*. However, the latter markers are separated from *D1038* by only 7 cM. Therefore, our results indicate the existence of at least one putative QTL for NN in the genomic region marked by *D1038*, *D1128* and *D1132* on linkage group D3, in addition to the putative QTLs for NN identified on the other linkage groups (see below).

The number of regions (seven) detected by regression analysis that have significant effects on CBB resistance was higher than the number of regions (four) revealed by the interval mapping method. However, each of the four regions identified by interval mapping analysis corresponded to one of the seven detected by regression analysis (Table 4). This discrepancy may be due to the different significance levels adopted for the regression and interval mapping analyses (0.05 and 0.01, respectively). In the three regions identified by regression analysis but not by interval mapping, only one marker showed a significant association with common bacterial blight resistance; in addition, the significance level of each of these markers only reached the 0.05 level. This indicates that, when using regression analysis to locate a locus controlling a QT to a particular genomic region, confidence in the results is increased when several linked markers in that region show a significant association with the QT.

TABLE 3

Linkage distribution and gene action of factors associated with phenotypic differences for Rhizobium nodule number and resistance to CBB as determined by interval mapping analysis using MAPMAKER/QTL

Linkage group	Marker	LOD score	Variation explained (%)	Additivity (*a*)	Dominance (*d*)	Mode
NN						
D1	*D1290*	1.93	12	−22.5	−17.6	A, D
D3	*D1038*	1.80	12	−27.3	11.3	A, R
D3	*Skdh*	1.53	11	−11.2	46.1	A, R
D7	*D1861*	2.81	17	50.4	−1.6	A, R
Resistance to CBB						
D2	*D0108*	2.66	17	3.5	1.2	A, D
D5	*D1081*	2.12	15	4.6	−3.5	A, R
D7	*D1390*	5.95	32	9.2	6.7	A, D
D9	*D0157*	2.23	13	7.4	−0.7	A

Italicized D Numbers represent loci identified by random genomic clones (NODARI *et al.* 1993; GEPTS 1993); *Skdh*, shikimate dehydrogenase.

This similarity between regression analysis and interval mapping analysis has also been observed by DOEBLEY and STEC (1991).

Having identified genomic regions with statistically significant associations with the two QTs, we will infer the existence of putative QTLs controlling these QTs and discuss our results in light of previously published information on the inheritance of the two QTs studied. It should be pointed out that the number of putative QTLs identified for these QTs should be considered a minimum estimate for the following reasons. The QTLs identified in this study account for only part of the genetic variation for the QTs. In addition, other QTLs may be identified in different populations or environments. Moreover, the relatively small population size ($n = 70$) would be expected to allow only for the detection of genomic regions with larger contributions to the phenotypic variation (LANDER and BOTSTEIN 1989; KEIM *et al.* 1990). Lastly, we cannot determine at this stage whether each QTL corresponds to a single gene with a larger effect or to several linked genes with smaller effects.

Rhizobium NN: Loci located in four regions on three different linkage groups of the common bean genome showed a major effect on the number of nodules per plant (Table 4 and Figure 2). These four regions were responsible for about 50% of the genetic variation for the trait. On linkage group D1, three markers (*D1228*, *D1290* and *D1593*) representing at least one putative QTL, were clustered in a genome segment of 16 cM. On linkage group D3, two loci (*D1128* and *D1132*) were separated by only 0.8 cM, but the third locus (*Skdh*) was located more than 50 cM from the first two. The large linkage distance and the reduction of the LOD scores by 1.5 for markers located between these two regions suggest that at least two QTLs for NN are located on linkage group D3. The fourth putative QTL for NN was located close to marker *D1861* (linkage group D7), which is situated near *Phs* (2 cM). In addition to these four putative QTLs, three other significant marker-trait combinations remain to be mapped, which may identify additional factors controlling the number of nodules in this population.

Alleles with positive effects in NN were present in both parents. The BAT93 alleles were associated with higher number of nodules in three regions, located on linkage groups D1 and D3. On the other hand, Jalo EEP558 alleles associated with a positive effect were located on linkage group D7. This pattern of gene action is consistent with the greater number of nodules found in the F_1 generation than in those of the parents, and the transgressive segregation observed in the F_3 generation. Polygenic inheritance with dominance of the abundant nodulating habit has been previously reported in red clover (NUTMAN 1984).

CBB resistance: Both regression and interval mapping analyses identified at least four putative QTLs affecting CBB resistance, located on linkage groups D2, D5, D7 and D9 (Table 4 and Fig. 2). These four QTLs explained 75% of the variation for CBB resistance. Regression analysis, but not interval mapping, detected additional significant associations between CBB resistance and marker loci which mapped to linkage groups, D1, D2 and D3. One additional significant association involved an unassigned marker (*D1683a*). Our findings are in agreement with previous studies indicating both quantitative and qualitative inheritance for CBB resistance [reviewed by SINGH (1991)]. Varying magnitudes of gene effect may explain why in certain genetic backgrounds or environmental conditions, major genes can be detected. Significant dominance deviations and additive by additive interactions were also detected by SILVA, SINGH and PASTOR-CORRALES (1989). However, the complete recessiveness of resistance observed in our experiment stands in contrast with the complete dominance observed by COYNE and SCHUSTER (1974). In addition, unlike VALLADARES-SANCHEZ, COYNE and MUMM (1983), no transgressive segregation was observed in our experiment.

Interactions between the two traits: A genomic segment on linkage group D7, containing the *Phs* (phaseolin seed protein) locus and other markers, was associated with the two QTs. However, the statistically most significant marker in this region, as determined by MAPMAKER/QTL was different for each QT: NN and CBB were most significantly associated with *D1861* and *D1390*, respectively. This suggests that this genomic region may contain multiple QTLs that affect different traits; alternatively, one factor with

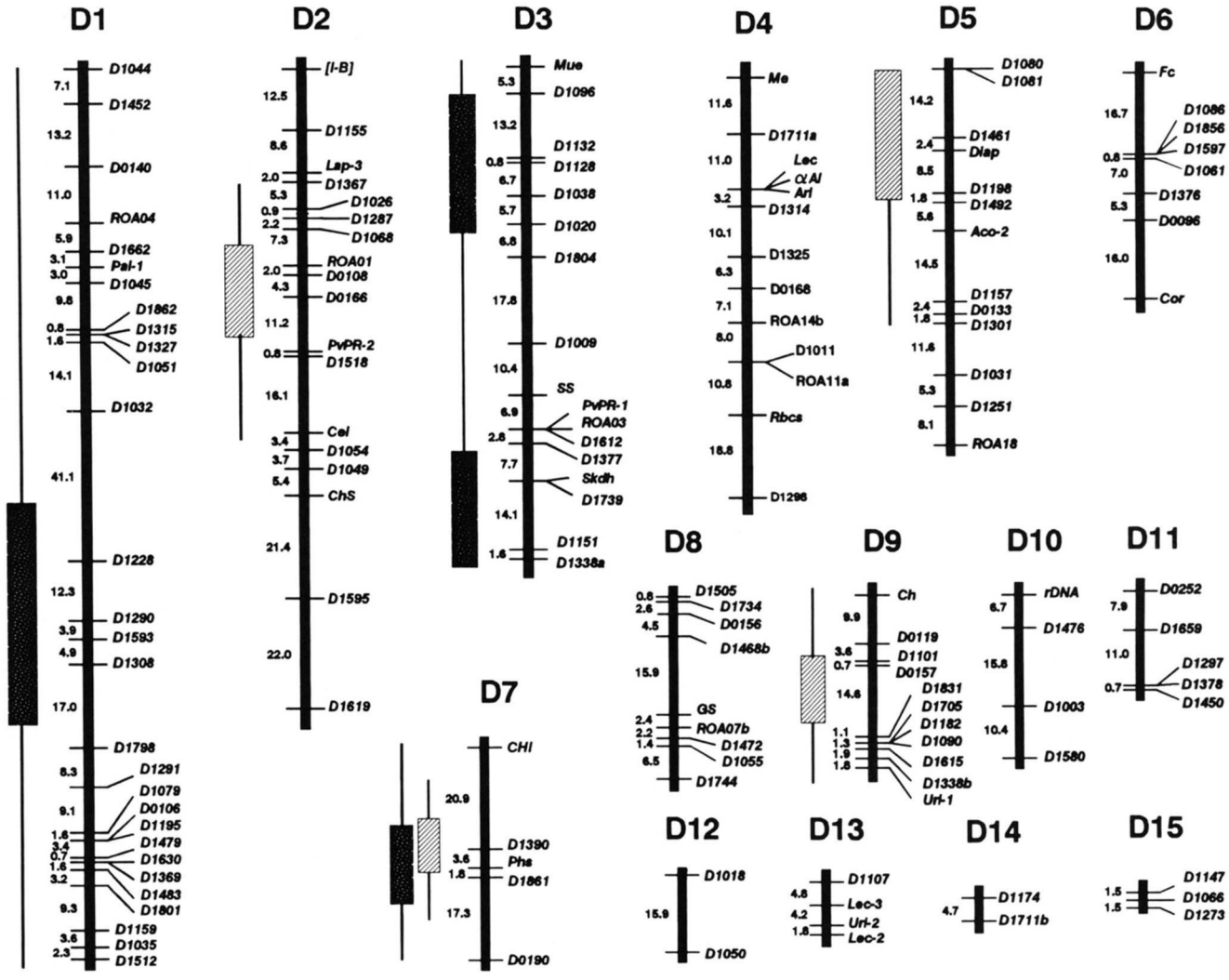

FIGURE 2.—Genetic linkage map of common bean with location of putative QTLs for Rhizobium NN and resistance to CBB. Map distances are KOSAMBI distances. Boxes to the left of linkage groups represent the 1-LOD (10-fold) likelihood intervals, *i.e.*, the interval over which the position of the QTL is at most 10 times less likely than the most likely position as determined by MAPMAKER/QTL. Lines extending beyond the boxes represent the 2-LOD interval. Filled boxes indicate putative QTLs for NN and hatched boxes putative QTLs for CBB.

pleiotropic effects is located in this genome region and our experimental design did not allow us to measure and locate this factor with sufficient precision. In support of the latter hypothesis is the observation that this region has a consistent effect on both NN and CBB resistance, in that alleles of the BAT93 parent in this region are associated with both low NN and resistance to CBB. Therefore, a common molecular and biochemical mechanism may underlie the interaction between the common bean host and these two bacteria.

This potential common mechanism involved in the bean-bacteria interactions could be mediated by flavonoids. These compounds have been shown to be involved in plant-to-bacterium signaling. For example, the expression of the *nodD* gene in Rhizobium is activated by flavonoids exuded by legume roots in general [reviewed by PETERS and VERMA (1990) and FISHER and LONG (1992)] and common bean roots and seeds in particular (HUNGRIA, JOHNSTON and PHILLIPS 1992; HUNGRIA, JOSEPH and PHILLIPS 1991a,b). *Vir* genes of *Agrobacterium tumefaciens* are activated by phenolics produced by wounded tissue [reviewed by WINANS (1992)]. Although no specific information is available to our knowledge about gene activation by flavonoids or related compounds in Xanthomonas, it is possible that common flavonoids could activate both Rhizobium and Xanthomonas gene expression. Conversely, flavonoids could have a deleterious effect on both Rhizobium and Xanthomonas. The flavonoid phytoalexins phaseollinisoflavan and kievitone inhibited strongly *X. c.* pv. *phaseoli* (WYMAN and VAN ETTEN 1982). A related compound, glyceollin, has been shown to be deleterious to *Bradyrhizobium japonicum* and *Sinorhizobium fredii* (PARNISKE, AHLBORN and WERNER 1991).

TABLE 4

Summary of statistically significant associations and inferred QTLs for Rhizobium NN and resistance to CBB

Linkage group	Marker	NN			CBB		
		ANOVA	Interval mapping	QTL inferred	ANOVA	Interval mapping	QTL inferred
D1	*D1228*	+					
	D1290	+	+	✓			
	D1593	+					
	D1512				+		
D2	*D0108*				+	+	✓
	D0166				+		
	ChS				+		
D3	*D1132*	+					
	D1128	+		✓			
	D1038		+				
	SS				+		
	Skdh	+	+	✓			
D5	*D1081*				+	+	✓
	D1461				+		
	Diap				+		
D7	*ChI*				+		
	D1390	+			+	+	✓
	Phs	+			+		
	D1861	+	+	✓	+		
D9	*D1101*				+		
	D0157				+	+	✓
	D1831				+		
Not assigned	*D1683a*	+			+		
	D1683b	+					
	D1737	+					

Genome regions within linkage groups that are distant by more than 30 cM are separated by single lines. Numbers represent D genomic clones (NODARI *et al.* 1993; GEPTS 1993); *ChI*, chalcone isomerase; *ChS*, chalcone synthase; *Diap*, diaphorase; *Phs*, phaseolin seed protein; *Skdh*, shikimate dehydrogenase; *SS*, sucrose synthetase. B, BAT 93 allele; J, Jalo EEP558 allele. NN, expressed as nodule number/plant; CBB, resistance expressed as disease severity index (see MATERIALS AND METHODS).

Is there evidence that genes involved in the synthesis of flavonoids are located on linkage group D7? In the F_2 population of cross BAT93 × Jalo EEP558 used here, the *ChI* gene, coding for chalcone isomerase, was mapped at one end of linkage group D7, within or near the 2-LOD-score-unit region of both traits (Figure 2). More recently, we have mapped on linkage group D7 the *P* gene conditioning the presence or absence of flavonoid and anthocyanin pigments (*i.e.*, pigmented *vs.* non-pigmented plant parts; FEENSTRA 1960). This gene was mapped in the recombinant inbred population Midas × G12873. F_8 recombination fractions were converted to F_2 fractions with the formula of HALDANE and WADDINGTON (1931) and further transformed into mapping distances using the formula of KOSAMBI (1943). The order of the genes (with KOSAMBI distances in parentheses) was as follows: *Phs*–(1.3)–*D1861*–(4.8)–*P* (E. KOINANGE and P. GEPTS, unpublished results). Marker locus *D0190* was not polymorphic in this population and could therefore not be tested. The *P* gene falls within the 1-LOD-score-unit interval for NN and within the 2-LOD-score-unit interval for CBB resistance. Allelic variation at the *P* locus between parents BAT93 and Jalo EEP558 could account for the quantitative effects observed on NN and Xanthomonas resistance. However, more detailed analyses of this linkage group are needed to determine whether the two QTLs identified here correspond to two distinct, yet linked genes, or if they represent the same gene with pleiotropic effects. In addition, the relationship, if any, between these two QTLs and any gene involved in flavonoid synthesis needs to be further investigated.

Our results set the stage for additional studies to determine to what extent the QTLs identified here (1) can also be observed in other populations of common bean and related legumes (*e.g.*, cowpea, mung bean and soybean) or after inoculation with other Rhizobium or *X. c.* pv. *phaseoli* isolates, (2) control these two traits in other environments, and (3) also control resistance to CBB at other developmental stages, for example the pod stage, which has been shown to be associated (SILVA, SINGH and PASTOR-CORRALES 1989) or not associated (VALLADARES-SANCHEZ, COYNE and MUMM 1983) with resistance at the vegetative stage. In addition, experiments involving both direct and indirect (based on linked markers) selection can be conducted in order to determine the relative efficiency of these two selection methods. In this regard, indirect selection for common bacterial blight resistance using the linked markers on linkage group D7 should be particularly attractive because of the major effect of the putative QTL(s) located on this linkage group. Polyacrylamide gel electrophoresis of the phaseolin (*Phs*) locus or polymerase chain reaction-based analyses of the linked markers (*Phs*: J. KAMI and P. GEPTS, unpublished results), *D1390* and *D1861*) would provide relatively rapid selection tools to obtain increases in CBB resistance.

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