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QTL mapping for nodule number and common bacterial blight in *Phaseolus vulgaris* L.

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Abstract

A recently developed bean RFLP linkage map was used to identify genetic elements affecting quantitative trait loci (QTLs) in two contrasting common bean genotypes, BAT-93 and Jalo EEP558, under two levels of mineral nitrogen: low – 0.25 m*M* NH_4NO_3 and a high – 6 m*M* NH_4NO_3. QTLs affecting nodule number (NN) and response to *Xanthomonas campestris* bv. *phaseoli*, which causes common bacterial blight (CBB) were identified and mapped. Analyses of 70 F_2-derived F_3 families, using the F_1, the two parents, and a nodulation-defective mutant (Nod^-) inoculated with *R. tropici* UM1899 under both levels of N showed significant differences ($P < 0.0001$) among the F_3 families for NN.

Under low N, three genomic regions influenced both traits, with seven linked markers. In three of the six regions influencing NN, higher NN was associated with the Jalo EEP-558 allele, whereas in only two regions was the BAT-93 allele associated with higher NN. One-way analysis of variance, with each marker as the independent variable and NN as the dependent variable, and interval mapping analysis identified four QTLs, which accounted for 45% of the total variation, and two additional QTLs near to yet unassigned loci. In linkage group D7, one QTL mapped to the same region as a QTL for CBB.

Under high N, three additional regions were linked to NN, one where the BAT-93 allele was closely associated with CH18 (chitinase), and the others where the Jalo EEP-558 allele was associated with CHS (chalcone synthetase) and PAL-1 (phenylalanine ammonia lyase). Four regions for CBB were mapped adjacent to or in the same region as a QTL for NN. Thus, N showed dual and opposite effects on the expression of NN and CBB. Analysis of these RFLP markers revealed these 'hidden' favorable alleles and can serve as an indirect selection tool to increase NN and resistance to CBB.

Introduction

Breeding for increased N_2-fixation can improve legume crops that are normally dependent on N fertiliser for significant yields, and will promote development of low-input cropping systems. The idea that biological nitrogen fixation (BNF) in common bean (*Phaseolus vulgaris* L.) can be improved through breeding has received attention in the recent years, due to the availability of superior populations with good N_2-fixation characteristics (Bliss, 1993). BNF has been characterised as a quantitatively-inherited (McFerson, 1983), complex trait (Mytton, 1984), and no simply-inherited characters have been described (Rosas and Bliss, 1986). Moreover, an increased susceptibility to one or more important diseases was observed among the selected N_2-fixing plants. Due to the low disease-resistance patterns of most of the selected material, BNF traits were not effectively transferred to other breeding programmes. It is not clear whether breeding for one BNF trait affects the expression of

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other traits, such as disease resistance, in common bean.

During the past decade, several attempts have been made to improve N_2-fixation traits in common bean (Bliss, 1993; CIAT, 1983; Graham, 1981). Because those efforts proved to be laborious and expensive, only a few genotypes were successfully released (Bliss et al., 1989). One of the main constraints for BNF improvement is that quantitatively inherited traits such as nodulation are greatly influenced by the environment (Bliss, 1985; McFerson, 1983). Two main options for improving N_2 fixation are: management of the legume to maximise growth and minimise stresses such as mineral nitrogen shortage (Tsai et al., 1993) and other soil factors (Peoples et al., 1995), and breeding legumes with enhanced capacity for N_2 fixation, as discussed by Bliss (1993) and Herridge and Danso (1995). The selection process could be greatly enhanced by the use of genetic markers, such as restriction fragment length polymorphisms (RFLP), linked to factors controlling BNF and which are not influenced by the environment. RFLPs could be useful as markers to determine genetic relationships, to identify and map loci affecting quantitative traits, and to monitor these loci during introgression or crosses between two divergent parents (Nodari et al., 1992, 1993a, b; Paterson et al., 1991a, b).

A genetic linkage map is a tool with many applications in basic and applied genetic research. First, it provides information on the genetic control of traits, especially those with complex inheritance, and on their linkage relationships to other traits (Lander and Botstein, 1989). Molecular markers can also be used as indirect selection tools to simplify breeding or to provide information about genome evolution (Gepts et al., 1993). Other markers with limited polymorphism, such as a smaller number of morphological traits or isozymes, may also provide additional information for linkage mapping in common bean. This work used a recently developed RFLP linkage map between BAT-93 (Middle American origin) and Jalo EEP558 (Andean origin) for genotypic differentiation (Nodari et al., 1993a, b). Identification of genetic loci affecting quantitative trait loci (QTLs) such as number of root nodules formed (NN) and their possible associations with other QTLs involved in responses to the common bacterial blight (CBB) pathogen *Xanthomonas campestris* bv. *phaseoli* were studied in F_3-progenies, grown at two levels of mineral N nutrition that are optimum (0.25 m*M* N) or partially inhibitory (6 m*M* N) for root-nodule formation.

Material and methods

A. Analyses of parents

Plant material

For RFLP mapping BAT-93 and Jalo EEP558 were chosen because of their divergent evolutionary origins and contrasting interactions with pathogens and *Rhizobium*. BAT-93 is a breeding line developed by S Temple at the Centro International de Agricultura Tropical (CIAT, Cali, Colombia) and derived from a double cross involving four Middle American genotypes (Veranic 2, PI 207262, Jamapa, and Great Northern Tara). Jalo EEP558 was selected from the Andean landrace Jalo obtained from the Estação Experimental de Pato de Minas (Guazelli, Minas Gerais, Brazil). BAT-93 is resistant to bean common mosaic virus (BCMV), rust (*Uromyces phaseoli*), common bacterial blight (*Xanthomonas campestris* bv. phaseoli), and anthracnose (*Colletotrichum lindemuthianum*), but is susceptible to angular leaf spot (*Phaseoisariopsis angularis*). Jalo EEP558 exhibits opposite reactions to the same pathogens (CIAT, 1983). BAT-93 and Jalo EEP558 were crossed to produce F_1, F_2, and F_3 families used in this study. Nodule number was analyzed among 16 bean parents and compared to a bean mutant with defective nodulation CIAT-125 (Davis et al., 1988). The parental lines were chosen from several programmes for morpho-agronomic traits including BNF. The N experiment was carried out in the greenhouse and consisted of a completely randomised design with four replicates with two plants per pot. Seeds were sown in Leonard jars, containing a mixture of vermiculite, sand and perlite (1:1:1, v:v:v) supplied with N-free nutrient solution (20% strength) in the bottom reservoir.

Rhizobium inoculation and nodule evaluation

After germination, seedlings were thinned to two per jar and inoculated with a stationary-phase broth culture of *Rhizobium tropici* strain UM1899 (Martínez-Romero et al., 1991, originally supplied by P H Graham, University of Minnesota, U.S.A.). Rhizobia were grown in yeast extract mannitol media (Vincent, 1970). Equal 5-mL amounts of inocula (10^9 cells mL^{-1}) were added to the jars. The surface of each jar was covered with 1 cm of perlite to protect against direct exposure to sunlight. NN data were collected from plants grown at low N (0.25 m*M*) or high N (6 m*M*) supplied as NH_4NO_3 in the second week after

germination, and nodules were counted 32 days after planting.

B. Analyses of F_2-derived F_3 families

Xanthomonas campestris bv. phaseoli inoculation

CBB tests used a broth containing *Xanthomonas campestris* bv. phaseoli, isolate W18 (Dr Robert Gilbertson, University of California at Davis, U.S.A.) as inoculum source. Two bean seeds were planted in each 2-kg pot containing UC-mix substrate and supplied with 8 m*M* N, with four replicates per family. Ten-day old trifoliolate leaves were chosen and inoculated after making two incisions on the leaflet with a razor blade. Lesions were evaluated on a scale (1 = resistant to 6 = susceptible) two weeks later.

Nodule number evaluation

At low N, segregation NN and shoot dry matter (SDM) were analyzed in a population of 70 F_2-derived F_3 progenies, the F_1, the parents (BAT-93 and Jalo EEP558), and the Nod^- bean mutant. After 32 days, plants were harvested, the shoots were dried at 65 °C for 72 h, and roots were stored in a cold room until the day of nodule analysis. For the high N test, only NN data were collected because the plants showed uniform development. All data are based on four replicates per treatment with two plants per replicate. One previous experiment was carried out to determine the inhibitory levels of N on nodulation. Increasing levels of N, from 0 to 16 m*M*, supplied as NH_4NO_3 were applied to both parents – Jalo EEP558 and BAT-93. 32 days after germination, nodules were counted to determine the N level which could partially inhibit the trait 6 mM.

RFLP linkage map

An RFLP linkage map of common bean covering 827 centiMorgan (cM), constructed by Nodari et al. (1993a,b) and by Gepts et al. (1993) was obtained from 194 RLFP markers (from *Pst*I, *Eco*RI-*Bam*HI and *Mbo*I genomic libraries, the latter from Dr Michael Dron, Paris, France), 11 RAPD markers, 7 isozyme loci (*Aco, Diap, Lap-3, Me, Mue, Rbcs* and *Skdh*), 9 clones with known function (CHI, CHS, PAL, PHS, rDNA, V591, V765, n100, V861, provided by C Lamb, J Slightom, W Thompson and D P S Verma – U.S.A.) and three agronomic traits (flower color, corona and resistance to bean common mosaic virus – *I* gene). Distances and order of the markers were established by using MAPMAKER (Lander et al., 1987). The markers were distributed in 14 linkage groups at an average interval of 6.5 cM.

Statistical analysis

NN and SDM from both N-treatments were subjected to one-way analyses of variance using the SAS PROC GLM procedure (SAS Institute Inc., 1988) and the means were grouped according to Duncan's Multiple Range test. Correlation coefficients between NN and SDM were estimated using SAS PROC CORR (SAS Institute Inc., 1988). Data for NN were also subjected to pairwise comparison with each molecular maker and the phenotypic trait to one-way analysis of variance using the SAS PROC GLM procedure. Significant ($P < 0.05$) *F*-values and differences in mean values among marker genotypic classes located potential linkage regions on the RFLP map.

Results

A. Analyses of parents

Nodule number

Analyses for NN (Figure 1) produced three major genotypic groupings: high (168–322 nodules per plant) – Puebla-152 through Jalo EEP558; medium (45–115 nodules per plant) – BAT-93 through Mexico-309; and low (8 and 0 nodules per plant) – G12873 and the Nod^- which did not nodulate any of the four replicates. The wide range observed for nodule number indicates that this parameter, although highly affected by environmental stresses, gives significant differences ($P < 0.05$) between Jalo EEP558 and BAT-93, the parents selected for the nodulation studies. From our observations on other traits used for BNF evaluation, nodule biomass seems to be affected by the nutritional status of the plant, as a compensatory effect of increased nodule size may be due to a surplus of C-sources from shoot, or nutrient from soil. Pereira et al. (1993) were the first to identify nodule number as a highly heritable BNF trait in common bean.

NN × NH_4NO_3 interactions

Concentrations of mineral N of 4 m*M* or less stimulated root nodule formation in Jalo EEP558, whereas higher concentrations decreased NN (Figure 2). Despite the reduced sensitivity of NN in BAT-93 to mineral N, the same general response was observed in Jalo EEP558.

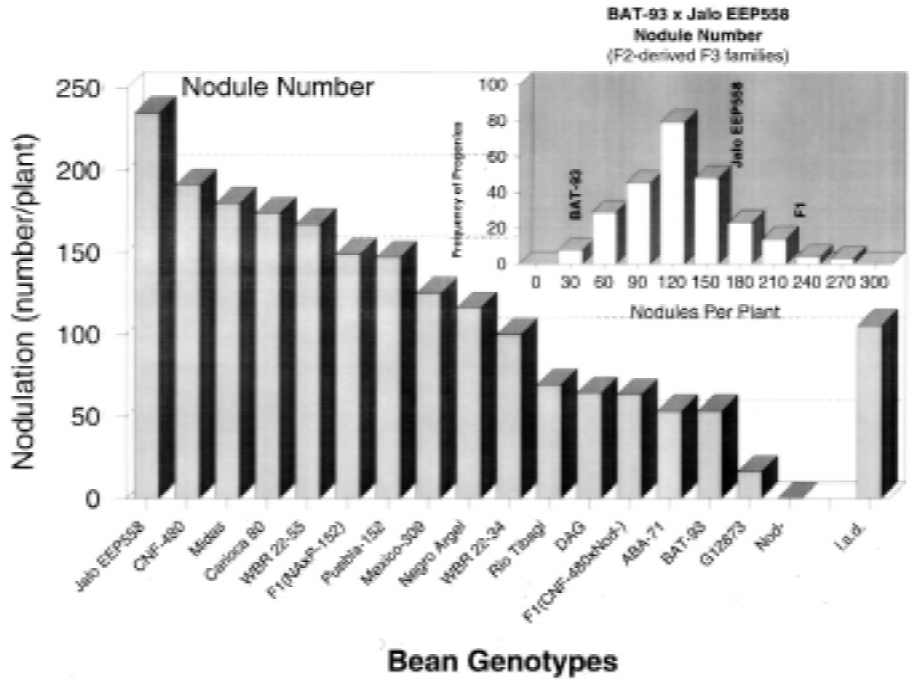

Figure 1. **Left:** Effects of host plant genotype on root nodulation in common bean cultivars inoculated with *R. tropici* UM 1899. **Right:** The distribution of the nodule number frequencies within 70 F_2-derived F_3 families from BAT-93 (Middleamerican) and Jalo EEP558 (Andean) cross.

Table 1. Summary of ANOVA data for NN and SDM, under low N

Source	df	Mean square	*F*	*P* > *F*	R^2	CV (%)
ANOVA						
NN						
Genotypoes	72	94860	8.50	0.0001	0.76	21.2
Error	180	11161				
SDM						
Genotypes	72	5.03	8.26	0.0001	0.77	20.4
Error	180	0.61				
Covariance model[a]						
Genotypes	73	98815	10.88	0.0001		
Error	179	9080				
ANOVA adjusted covariance						
Genotypes	71	50302	5.54	0.0001		
SDM	1	383595	42.24	0.0001		

[a] Dependent variable: Nodule number; Covariant: Shoot dry matter.

Table 2. Segregation of NN and CBB phenotypes in a cross between two common bean cultivars differing in these traits

Trait	Parents B*	J	F_1 B×J	F_3-Families Average	Range
NN[a]					
0.25 m*M* N	103	336	443	247	58–451
6 m*M* N	3.4	44	56	17.1	1–97
CCB[b]					
8 m*M*N	22	75	75	44	19–75

*B = BAT-93; J = Jalo EEP558.
[a] NN = Nodule Number, expressed as number of nodules per plant.
[b] CCB = Common Bacterial Blight, expressed in index units (see Material and methods).
[c] Mean square, $P < 0.0001$.

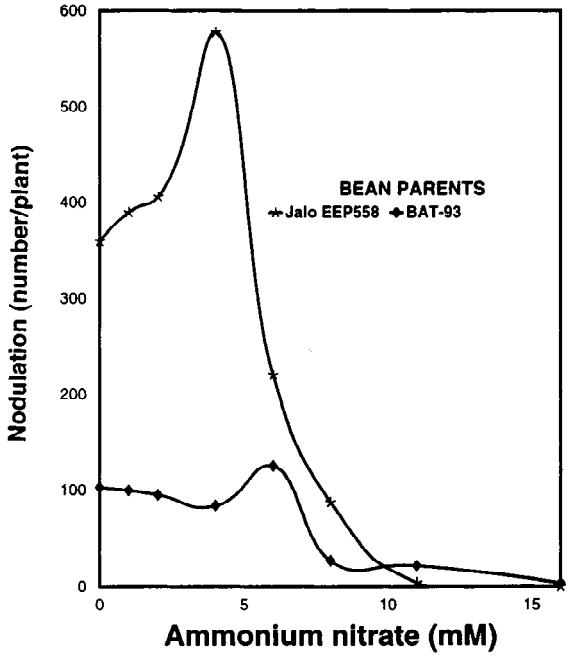

Figure 2. Effects of mineral N on root nodulation of Jalo EEP558 and BAT-93 common beans.

B. Analyses of F_2-derived F_3 families

Genotypic effects on root nodule number

Effects of low mineral N. An analysis of variance showed significant differences among genotypes for NN in the F_2-derived F_3 population from BAT-93 × Jalo EEP-558 (Table 1), with no significant differences among replicates. The CV value of 21% was low considering the type of trait studied. Because of the correlation between NN and shoot dry matter (SDM), an analysis of covariance was performed to remove the confounding effect of SDM on NN. Results indicate that significant genotypic differences in NN exist irrespective of SDM (Table 1). Actual NN data ranged from 58 to 451 nodules per plant in the F_3 families (Table 2).

Effects of high mineral N. Under 6 m*M* N the parental lines BAT-93 and Jalo EEP558 produced 3 and 44 nodules per plant, respectively (Table 2), whereas the Nod^- CIAT-125 did not nodulate in any replicate. The 56 nodules per plant in the F_1plants under high N suggests heterosis. As in the low-N tests, several F_3 families (a total of 14) exhibited transgressive segregation for NN, varying from 1 to 97 nodules. A tendency for assymetry was observed in the F_3-generation distribution curve. Significant differences ($P < 0.0001$) for NN, SDM and CBB among F_3 families were detected by the ANOVA test and no significant differences were observed among replicates. The coefficient of variation of 55.6% (Table 3) was higher than in the low-N test.

Location of factors controlling NN and CBB

NN QTLs. Under low N, loci located in four regions of three different linkage groups (D1, D3 and D7) of the bean genome presented a positive effect on NN (Table 4, Figure 3). These regions were responsible for 45% of the genetic variation of this trait (Table 5). In linkage D1 group, three markers (D1228, D1290, D1593) representing at least one putative QTL were pooled with a genomic segment of 16 cm. In D3, two loci (D1128 and D1132) covered 0.8 cm and a third locus (*Skdh*) located 50 cM away. Due to this great dis-

Table 3. Summary of analyses of variance and covariance for nodule number (NN), shoot dry matter (SDM) and common bacterial blight (CBB)

Source	df	Mean square	*F*	*P*(>*F*)	R^2	CV (%)
ANOVA (NN)						
Genotypes	69	1211.4	13.36	0.0001	0.83	55.6
Error	180	90.69				
ANOVA (SDM)						
Genotypes	69	1.049	8.29	0.0001	0.76	17.4
Error	180	0.126				
ANOVA (CBB)						
Genotypes	72	519	6.64	0.0001	0.66	28.5
Error	180	78.16				

Table 4. Phenotypic differences among segregation classes of molecular markers significantly linked to factors involved in nodule number

Marker-linkage group		Low N			High N				
		BAT allele	F_1	Jalo allele	BAT allele	F_1	Jalo allele	J + J/B	B + B/J
D1[a]	D1228	560a	501a	400b					
	D1290	587a	453b	465b					
	D1593	599a	444b	474b					
	D1308	582a	466b	473b					
D2	D1155	433b	499ab	579a					
D2	D1049				11b	16b	31a		
	CHS				10b	17ab	26a		
	D1595				9b	15ab	26a		
D3	D1739	459ab	552a	431b					
	SKDH	463ab	537a	441b					
D3	D1132	576a	503ab	425b					
	D1128	576a	502ab	418b					
D4	D1298	458b	552a			28a			13b
D5	D1081				12b	10b	28a		
D7	D1390	430b	465b	608a					
	PHS	397b	489ab	576a					
	D1861	389b	471b	596a					
D9	CH18				29a			14b	
NA*	D1737	294a	229b		29a			13b	

[a] D: *Pst*I genomic clones from UCD; [b] SKDH: shikimate dehydrogenase; [c] CHI: chalcone isomerase; [d] PHS: phaseolin; NA* = Not assigned yet.

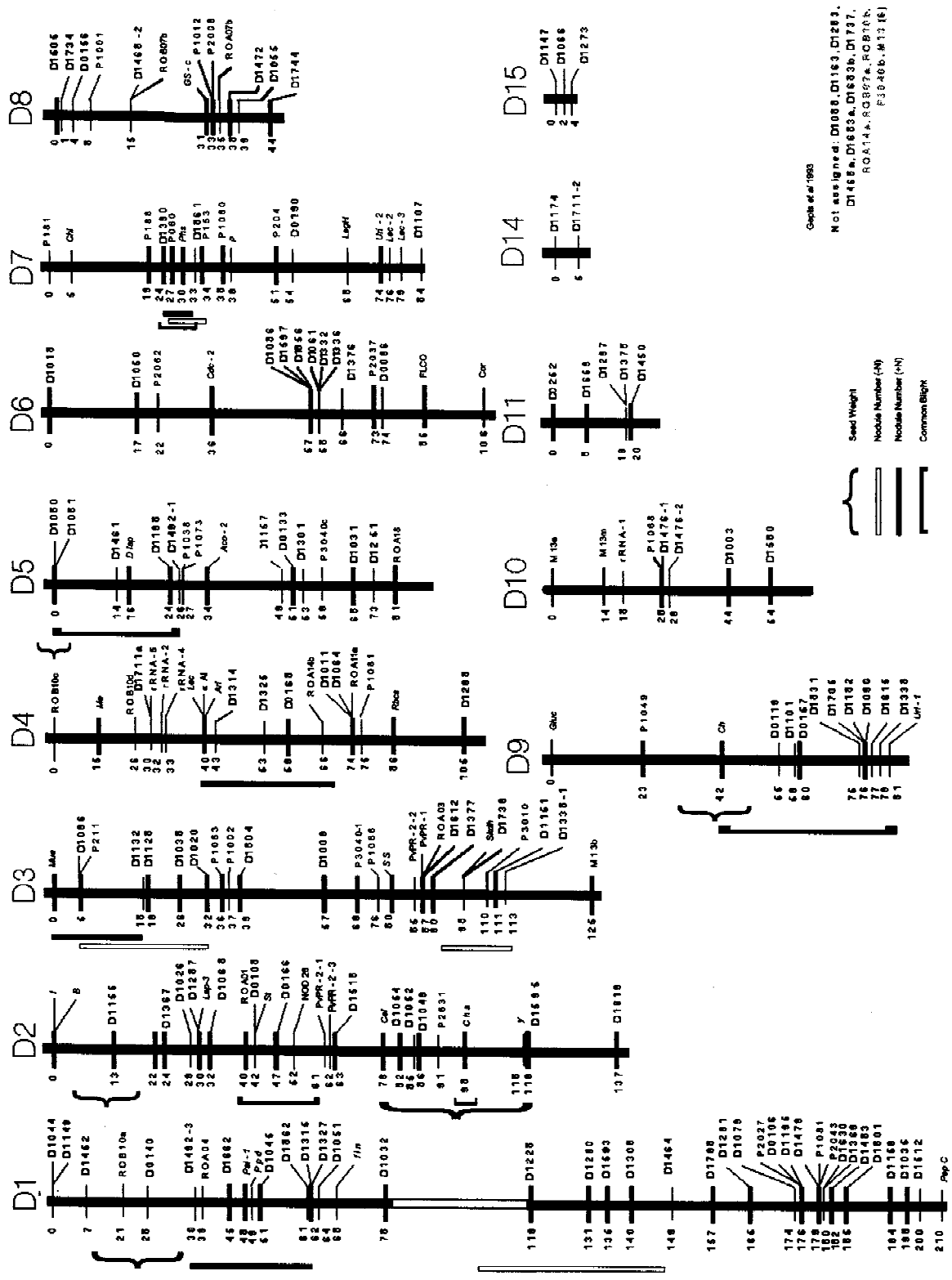

Figure 3. Genetic linkage groups in common bean with codes indicating locations of QTLs in this study.

Table 5. Additivity (A), dominance (D), accumulated variations (%) and type of gene action which affect NN in the F_3 families from the cross BAT-93 × Jalo EEP558, under low and high N levels

Marker linkage group	A	D	Accumulated variation	Type[a]
At 0.25 m*M* N				
D1861 – D_7	52.0	−5.8	19.9	F, A, R
D1038 – D_3	−28.1	18.8	28.0	F, A, R
SKDH – D_3	−8.8	37.0	40.7	F, R
D1290 – D_1	−25.8	−21.8	45.1	F, A, D
At 6 m*M* N				
D1081 – D_5	7.5	−10.2	19.8	F, R
CHS – D_2	13.5	−4.5	29.5	F, R
D0157 – D_9	−2.7	−13.4	45.6	F
D0140 – D_1	8.7	−5.4	59.5	F, R

[a] F = free, A = additivity; D = dominance (d = +a), R = recessive (d = −a).

tance and low LOD (logarithm of the odds ratio) score, this suggests that at least two QTLs for NN are located close to marker D1861 (D7 linkage group), close to the gene *Phs* (2 cm apart). Three other significant combinations not yet mapped indicate the complexity of the trait, and that additional factors may be involved in nodule expression in this population.

Pairwise analyses of variance between individual marker loci and NN gave segregation classes for 13 and 14 markers which showed significant differences for NN (Table 4). These markers were grouped in six of the bean genome linkage groups (Figure 3). Most of these regions were unlinked although some were located within the same linkage group. By regression analysis, the contribution of these regions for nodule formation was around 45%, with different types of effect on nodulation (Table 5).

Under high N, NN (data not shown) was inhibited to a large extent, but followed the same pattern as obtained under low N. Loci were located in four genomic regions and distributed in different linkage groups (D2, D4, D5 and D9) of the bean genome (Table 4, Figure 3). In D2, a positive correlation between marker and QTL was associated with D1049 ($P < 0.035$). The other two associations were correlated to the CHS marker ($P < 0.086$) and D1595 ($P < 0.089$), both located in the same linkage group, but 5.4 and 21.4 cm distant from D1049, respectively. It is inferred that one QTL could be assigned for this region. The other three regions were located close to the markers in D4 (D1298), D5 (D1081) and D9 (CH18). By regression analysis, the contribution of these regions for nodule formation was around 59%, with free and recessive types of effects (Table 5). A fifth significant association, linked to D1737 (not assigned), was also observed.

CBB QTLs. ANOVA analysis (Table 3) detected significant correlations ($P < 0.0001$) between the lesion areas and the progenies in 11 marker loci distributed within 7 linkage groups (Table 6). One region gave a correlation with the CHS (chalcone synthetase) marker and NN, with the Jalo EEP558 allele being dominant for both traits. Both regression and interval-mapping analyses identified at least four putative QTLs affecting CBB resistance, located in linkage groups D2, D5, D7 and D9 (Nodari et al., 1993b). These four QTLs explained 75% of the variation for CBB resistance/susceptibility. These data are more extensively discussed by Nodari et al. (1993b).

Discussion

Alleles with positive effects on NN were detected in both parental lines (Table 2). Alleles from Jalo EEP558 (336 nodules per plant) associated with high NN were located in the D7 group and with the unmapped RFLP marker D1737 (Table 4). On the other

Table 6. Phenotypic differences among segregation classes of molecular markers significantly linked to factors involved in CBB, under a high level of N

Marker linkage group		CBB		
		BAT allele	F_1	Jalo allele
D1	[a]D1512	46a	39b	49a
D2	D0108	35a	47a	47a
	D0166	37b	45a	47a
D2	D1049			
	[b]CHS	37b	44ab	50a
	D1595			
D3	[c]SS	49a	43ab	38b
D5	D1461	40b	41ab	49a
	[d]*Diap*	39b	42ab	49a
D7	[e]CHI	39b	46a	
	D1390	31b	45b	53a
	[f]PHS	31b	45b	50a
	D1861	32b	45a	46a
D9	D1101	37b	43ab	50a
	D0157	36b	44a	49a
	D1831	38b	45ab	52a
*NA	D1683a	36b	46a	

[a]D: *Pst*I genomic clones from UCD; [b]CHS: chalcone synthase; [c]SS: sucrose synthase; [d]*Diap*: diaphorase; [e]CHI: chalcone isomerase; [f]:PHS: phaseolin; [g]CH18: chitinase.
*NA: Not assigned yet.

hand, alleles from BAT-93 associated with high NN were located in D1 and D3. This pattern of gene action is consistent with the higher NN found in F_1 (Table 2) than in the progenitors and the transgressive segregation observed in several F_3 families. Polygenic heritability with dominance for NN was previously observed in red clover (Nutman, 1984).

Unifactorial ANOVA analyses of the genetic markers as independent variables and NN as the dependent variable identified five statistically significant ($P < 0.05$) associations under high N (Table 4). Four were associated with genetic markers from four distinct genomic regions: one to D2 (D1049); the second region to D4 (D1298); one to D5 (D1081) and the fourth region to D9 (CH18, corresponding to the sequence which codifies the chitinase). The fifth locus associated with D1737 has not yet been assigned. Assuming that each of the four linkage groups contained only one QTL, the estimated contributions from each of these putative QTLs for NN were 11%, 9%, 19% and 9%, respectively. In addition to the significant association with D1049 (D2), regression analysis revealed F values very close to statistical significance ($P = 0.08$), for the CHS marker and D1595, both adjacent to D1049.

Nitrogen plays an important role in BNF and all parameters involved in nodulation. In this study a drastic change in marker loci was found when N was added to the system. When comparing CBB and NN under high N, one can suggest the proximity of both QTLs in D2. We also located CBB loci close to Nodulin-26 and PvPR-2 (protein related pathogenesis) in D2; to CH18 (chitinase) in D9, with the BAT-93 allele dominant for resistance to CBB and to D1080, D1081 in D5. At three loci, high nodulation was associated with the Jalo EEP558 alleles, corresponding to D2, D4 and D5 linkage groups (Figure 3). BAT-93 contributed with al-

leles positively associated to NN at the other two loci, one assigned close to CH18 (chitinase) in D9 linkage group and the other to D1737, not yet assigned.

The interval mapping analysis revealed a similar pattern to that found by regression analysis. Four genomic regions associated with NN were located in linkage groups D1 (at D0140), D2 (CHS, at 5.4 cm of D1049), D5 (at D1081) and D9 (at D0157) (Table 4, Figure 3). The LOD scores of regions associated with the D1081 and CHS markers were above 1.7, whilst in the other remaining two regions the scores were near 1. Overall, the contribution from each of the four located loci for the nodule expression under high N was 14%, 10%, 20% and 14%, respectively, corresponding to a total of about 60% of the phenotypic variation (Table 6). For low N, phenotypic variation for NN reached 45% (Table 6).

In three of the six regions influencing NN, higher NN was associated with the Jalo EEP-558 allele, whereas in two regions, the BAT-93 allele was associated with higher NN (Table 4). The latter observation was not consistent with the difference observed between the parents: BAT-93: low NN; Jalo EEP-558: high NN. This observation is not unexpected as it constitutes the basis for transgressive segregation. RFLP markers allowed us to uncover these 'hidden' favorable alleles and could be especially useful as indirect selection tools to increase NN. One region showed heterosis for NN. This type of gene action may be of less importance because bean cultivars are usually homozygous lines.

As recommended previously by Nodari et al. (1993b), these data suggest that additional studies be done to determine to what extent the QTLs identified here can be observed in other populations of common bean, and with other strains of *Rhizobium* or *Xanthomonas campestris* bv. phaseoli.

Our results indicate that NN in this cross is controlled by at least six chromosomal regions and that the poorly nodulating parent also carries alleles that can contribute to increased nodulation. Some of the factors controlling NN were located in the chromosome regions controlling CBB; this association was stronger than expected by chance alone and raised the possibility of pleiotropy, specially in the case of CHS (chalcone synthetase) in D2, CH18 (chitinase) and PAL-1 (phenylalanine ammnonia lyase) in D9 under high levels of N, and Phs (phaseolin) under a low N. Under both N conditions, flavonoids may be mediating the expression of nodulation (Coronado et al., 1995) or disease resistance/susceptibility. Also, the association of one CBB locus to a protein related to pathogenesis (PvPR-2) and one NN locus expressed under low N to the PvPR-1 probe raises interesting questions about the possibility that nodulation is a modified form of pathogenesis that evolved into symbiosis during bean domestication. Another question that may be raised by this study is that the negative selection for BNF found under N-rich soils has probably been a major contribution to low nodulation capability in commercial cultivars. Most of the materials used in the breeding programmes are highly disease-resistant varieties. Our results suggest that, while BNF has been selected against in past-breeding programmes, future breeding efforts will benefit by considering plant-symbiont relationships as important agronomic traits.

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