RESEARCH

QTL Mapping of Ineffective Nodulation and Nitrogen Utilization-Related Traits in the IC-1 Mutant of Cowpea

Erik W. Ohlson, Sirando L. Seido, Suheb Mohammed, Carlos A. F. Santos, and Michael P. Timko★

ABSTRACT

Biological nitrogen fixation is a valuable component of sustainable agriculture. Improved understanding of nodulation pathways can facilitate enhancement of nitrogen utilization-related traits. Previously, a cowpea [*Vigna unguiculata* (L.) Walp.] mutant, 'IC-1', with ineffective nodules was identified. Heritability studies indicated that ineffective nodulation in IC-1 was controlled by a single gene designated *cpi*. To map *cpi*, F_2 mapping populations were developed from crosses between IC-1 and two commercial cowpea cultivars. The F_2 populations were evaluated for leaf and nodule color, nitrogen content, dry plant weight, nodule number, and nodule fresh weight. Nitrogen content, aboveground plant dry weight, and nodule fresh weight were each significantly lower in individuals with ineffective nodulation, whereas nodule number was significantly higher. Ninety individuals from one population were selected and genotyped with the Cowpea iSelect Consortium single-nucleotide polymorphism (SNP) array. In total, 6083 polymorphic SNPs were used for linkage map construction and quantitative trait loci (QTL) analysis. A major QTL accounting for nearly 50% of the phenotypic variance was identified on linkage group (LG) 4 associated with all traits except nodule fresh weight, whereas a minor QTL for nodule fresh weight was mapped to LG 6. The major QTL was validated in a second F_2 population with several polymerase chain reaction markers. Identification of these QTL provides a foundation for future identification of the *cpi* gene and potential loci for targeted improvement of nitrogen-related traits in cowpea.

E.W. Ohlson, S. Mohammed, and M.P. Timko, Dep. of Biology, Univ. of Virginia, Charlottesville, VA 22904; S.L. Seido, Melhoramento Vegetal de Plantas, Univ. Federal Rural de Pernambuco, Recife, Brazil; S. Mohammed, Daylight Foods, 660 Vista Way, Milpitas, CA 95035; C.A.F. Santos, Embrapa Semiárido, CP 23, Petrolina, PE, 56302970, Brazil. E.W. Ohlson and S.L. Seido contributed equally to this work. Received 21 July 2017. Accepted 13 Sept. 2017. ★Corresponding author (mpt9g@virginia.edu). Assigned to Associate Editor Randy Dinkins.

Abbreviations: ARMS, amplification-refractory mutation system; F_2^{PJ}, F_2 population derived from the cross 'BRS Pujante' × 'IC-1'; F_2^{MA}, F_2 population derived the cross 'BRS Marataoã' × 'IC-1'; fix+, effective nodules and nodulation; fix−, ineffective nodules and nodulation; ICIM, inclusive composite interval mapping; LG, linkage group; LOD, logarithm of odds; PCR, polymerase chain reaction; QTL, quantitative trait locus/loci; SNP, single-nucleotide polymorphism.

The symbiotic relationship formed between legumes and rhizobia is a major contributor to the global nitrogen cycle. In exchange for biologically available nitrogen, legumes provide rhizobia with a microaerobic environment and carbon supply. Symbiotic nitrogen fixation is estimated to inject ~40 Tg of nitrogen into agricultural systems annually (Herridge et al., 2008). When used in crop rotations, legumes can alleviate the burden of artificial fertigation on farmers and the environment. Furthermore, legumes are a primary source of protein in many diets. Increased understanding of pathways involved in rhizobial colonization, nodule development, and nitrogen fixation is vital for improvement of symbiotic nitrogen fixation in legume crops (El Msehli et al., 2011; Udvardi and Poole, 2013).

Rhizobial establishment and development of functional nodules involves a complex series of steps beginning with host recognition, followed by nodule development, maintenance, and regulation. Plants attract rhizobia through secretion of flavonoids,

activating the release of nodulation factors by rhizobia. Host recognition of nodulation factors induces infection thread formation. The bacteria travel through the infection thread, where they are ultimately deposited within the cytoplasm of cortical cells, forming the symbiosome. Finally, rhizobia in the symbiosome differentiate into their nitrogen-fixing form, where they are maintained and regulated by the host.

Natural and artificial mutants in model legumes *Medicago truncatula* Gaertn. and *Lotus japonicas* L. have been key tools for dissecting components of the nodulation pathway. A large proportion of reported nodulation genes were identified within these two species, from which at least 26 were cloned (Kouchi et al., 2010). These include genes facilitating nod signaling (Kouchi et al., 2010; Madsen et al., 2010; Murray, 2011; Domonkos et al., 2013; Oldroyd, 2013; Venkateshwaran et al., 2013), nodule number regulation (Krusell et al., 2002; Nishimura et al., 2002; Schnabel et al., 2005; Varma Penmetsa et al., 2008), bacterial infection (Arrighi et al., 2008; Yano et al., 2009; Kiss et al., 2009), nodule differentiation (Wang et al., 2010), nodule maintenance (Kumagai et al., 2007; Bourcy et al., 2013; Domonkos et al., 2013), and nutrient transport (Krusell et al., 2005; Bagchi et al., 2012). Additional nodulation genes and quantitative trait loci (QTL) have been reported in several crop species including pea (*Pisum sativum* L.) (Bourion et al., 2010), common bean (*Phaseolus vulgaris* L.) (Nodari et al., 1993; Tsai et al., 1998), and soybean [*Glycine max* (L.) Merr.] (Tanya et al., 2005; Nicolás et al., 2006; Santos et al., 2013; Hwang et al., 2014). More than 40 genes related to rhizobial symbiosis have also been reported in pea (Borisov et al., 2007), and QTL for root, nodule, and shoot variability were mapped (Bourion et al., 2010). In soybean, QTL governing nodule number, size, and weight were identified (Tanya et al., 2005; Nicolás et al., 2006; Santos et al., 2013; Hwang et al., 2014), as well as QTL regulating acetylene reduction and plant dry weight (Tanya et al., 2005). Several QTL for nodule number have also been mapped in common bean (Nodari et al., 1993; Tsai et al., 1998; Heilig et al., 2017).

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most important legumes grown in the semiarid tropics including Asia, Africa, Southern Europe, the Southern United States, and Central and South America (Timko and Singh, 2008). Cowpea is especially valued in sub-Saharan Africa, where it is a primary source of protein. Improving the efficiency of nitrogen fixation in cowpea is an important objective due to the impact of nitrogen on yield-related traits and cowpea's ability to enhance and grow in marginal environments. Previously, a cowpea mutant designated 'IC-1' was identified with ineffective nodules (fix−) (Pemberton et al., 1990). Acetylene reduction assays indicated no nitrogenase activity, consistent with the fix− phenotype. Additionally, IC-1 plants

exhibit lighter leaf and nodule color and reduced root and shoot weights. Inheritance studies suggested the presence of a single, recessive gene, which was designated *cpi* (Pemberton et al., 1990). Despite the dearth of nitrogenase activity, IC-1 nodules contain both infection threads and bacteroids. Northern blot analysis of four nodulin genes indicated that VuENOD2, an early nodulin, was initially reduced, whereas VuB mRNA levels declined more slowly in IC-1. Additionally, late nodulins leghemoglobin and uricase were expressed at reduced levels. Combined, these results suggest that *cpi* may play a role in synthesis or maintenance of the peribacteroid membrane (Purdom and Trese, 1995). However, further research is needed to determine the function of *cpi*.

The objectives of this study were to improve the characterization of the fix− cowpea mutant IC-1 and map QTL associated with the ineffective nodulation phenotype.

MATERIALS AND METHODS

Plant Material and Population Development

An F_2 mapping population (F_2^{MA}, $n = 174$) was derived from a cross between the effectively nodulating (fix+) commercial cowpea cultivar 'BRS Marataoã' and the fix− mutant IC-1. Quantitative trait loci validation was performed in a second F_2 population (F_2^{PJ}, $n = 175$) developed from a cross between commercial cowpea cultivar 'BRS Pujante' (fix+) and IC-1. For each population, IC-1 was used as the male parent. Both populations were grown at Embrapa Semiárido, Petrolina, Brazil.

Phenotypic Analysis

The two populations were grown in a protected environment under 70% shade cloth in 3-L plastic pots. Plants were irrigated daily with no supplemental fertigation. To ensure uniform colonization, all plants were inoculated with a cocktail of nitrogen-fixing bacterial strains BR 3262, BR 3267, and BR 3299. At planting and 5 d after emergence, inoculum was prepared and adjusted to ~10^9 cells mL^{-1} and 2 mL was added to each pot. Bacterial strains were provided by Embrapa Agrobiologia, Seropédica, Rio de Janeiro, Brazil.

Each F_2 population was evaluated 40 d after emergence for six traits: leaf color (green/yellow), nodule color (dark/white), nitrogen concentration, aboveground plant dry weight, nodule number, and fresh nodule weight. Nitrogen concentrations were determined using the Kjeldahl method (Mendonça and Matos, 2005). Green leaf and dark nodule color or yellow leaf and white nodule color were used to classify F_2 individuals as fix+ or fix−, respectively.

Genotypic Analysis

DNA was extracted from parental replicates and F_2 individuals using a modified cetrionium bromide (CTAB) protocol (Doyle and Doyle, 1987). Ninety of the F_2^{MA} individuals and three replicates of each parent were genotyped using the Cowpea iSelect Consortium Array, which consists of 51,128 single-nucleotide polymorphisms (SNPs, WG-401-1002, Illumina). Genotyping was performed on an iSCAN System bead chip reader at

the Center for Public Health Genomics (University of Virginia School of Medicine, Charlottesville, VA). The SNPs were called and filtered in GenomeStudio 2.0 (Illumina, 2016) based on guidelines outlined in the Illumina genotyping technical notes (Illumina, 2014) and a previously developed cowpea cluster file (Muñoz-Amatriaín et al., 2017). Genotype data were exported from GenomeStudio and additional filtering was applied based on parental calls and expected F_2 segregation. An IC-1 replicate was removed from consideration due to high heterozygosity (>20%), and markers were excluded if parental genotypes were heterozygous or polymorphic among replicates. Informative SNPs were identified between IC-1 and BRS Marataoã and used for linkage map construction and QTL analysis. A single F_2^{MA} sample was excluded due to suspected sample contamination or outcrossing during population development.

Chi-square tests ($\alpha = 0.01$) were performed to identify and remove markers for which F_2 segregation deviated significantly from the expected ratio.

Linkage Map Development

A genetic linkage map was developed using MSTmap with Kosambi mapping function and grouping logarithm of odds (LOD) 7 (Wu et al., 2007, 2008). The map order for each F_2 individual was inspected, and questionable SNP calls were excluded to reduce the effects of genotyping error and residual heterozygosity in parental lines on linkage and QTL analysis. Linkage group (LG) number and orientation were assigned according to the cowpea reference genome (Vigna unguiculata v1.0, NSF, UCR, USAID, DOE-JGI, http://phytozome.jgi.doe.gov/).

QTL Analysis

The QTL mapping was performed in Windows QTL Cartographer 2.5 (Wang et al., 2012) and QTL IciMapping 4.1 (Meng et al., 2015). Leaf and nodule colors were mapped using categorical trait interval mapping implemented in Windows QTL Cartographer using a walk speed of 1 cM and LOD threshold of 5.5, as determined by permutation tests ($n = 1000$, $P = 0.05$). Inclusive composite interval mapping (ICIM) was employed for analysis of nitrogen content, dry plant weight, nodule number, and nodule fresh weight using QTL IciMapping and the parameters: missing phenotype = deletion, step = 1 cM, and probability in stepwise regression = 0.001. A LOD threshold of 3.58 was assigned on the basis of permutation tests ($n = 1000$, $P = 0.05$). In addition to mapping each trait separately, multiple-trait composite interval mapping was performed in Windows QTL Cartographer for joint analysis of nitrogen content, dry weight, nodule number, and nodule weight. Model 6 was used with background markers identified using forward stepwise regression and probability thresholds of 0.05. A window size of 10 cM and walk speed of 1 cM were assigned. A LOD threshold of 7.8 was determined by permutation tests ($n = 1000$, $P = 0.05$).

QTL Validation

After the detection of a major QTL on LG 4 for five of the six measured traits, validation of the QTL was performed using F_2^{PJ}. Five tetra-primer amplification-refractory mutation system (ARMS)-polymerase chain reaction (PCR) markers (Ye et al., 2001) were developed from SNPs tightly linked to the QTL and used for genotyping. The PCR was performed in 20-μL reactions containing 1× Taq buffer, 200 μM deoxynucleotides, 50 ng of genomic DNA, appropriate primer, and $MgCl_2$

Table 1. Tetra-primer amplification-refractory mutation system (ARMS) markers on linkage group 4 used for validation of ineffective nodulation quantitative trait loci (QTL) in the 'BRS Pujante' × 'IC-1' F_2 population. The primer concentrations, annealing temperature (T_A), and magnesium (Mg^{2+}) concentrations were optimized for each marker.

Marker		Primer sequence (5′ → 3′)	Primer concentration	T_A	Mg^{2+}	Amplicon size
			μM	°C	mM	bp
2_00349	Forward inner	AACTAGATGATGTATAATCCTCTTTCTGCG	0.25	55	2.0	202
	Reverse inner	AGCAGTGCCTAAATTATGTTGACAACGT	0.25			236
	Forward outer	GTGTTCTTTCAGGTTTTTCTTTCACCAT	1.50			380
	Reverse outer	GGTTATGGTAGAGAGGGATGAACTTTTG	1.00			
2_00352	Forward inner	TGGCAAAAGTTCTTGGAACCATTTCC	0.25	55	3.0	206
	Reverse inner	AGTAATTACTGTGGCACCCCCCACAATA	0.10			235
	Forward outer	TTTTGCTTCTGCATTGCAAAACTCAGTT	1.00			387
	Reverse outer	AAAACTATCCAACCAGCCCATGACAGAC	2.00			
2_15618	Forward inner	TTTAAGATGTGGTGTGATGACTGGTA	0.25	60	2.5	234
	Reverse inner	GTCCAATCTATATGTAAAAATTCAATCCC	0.25			192
	Forward outer	GTATTGTTTGGAAACGGAGATGTATTTA	1.00			371
	Reverse outer	TTTTTTCTACTTTGAACCCGTTCTAAACT	1.50			
2_16203	Forward inner	TTTGATGTTATTATGATGAGATCTGCAA	0.25	55	3.0	236
	Reverse inner	ATGCCCAAAAAATAGAAATAGACCAAC	0.25			261
	Forward outer	TTATAATGATTTATACGAGCTTGTTCTTGG	1.50			442
	Reverse outer	AATTACGATGTCGGAATAGTAGAGTTCC	2.00			
2_24723	Forward inner	ATGATCCTCCAGCTGAGAATTCTTGCAT	0.10	55	2.0	246
	Reverse inner	TTGTAGCATGATTGAAATCAATGGAGCAG	0.10			223
	Forward outer	TTCATCACAGGCTACGGAGCTACAATTG	1.00			412
	Reverse outer	AGGTTGTCAGATGCATGGGAATGTAGAA	1.00			

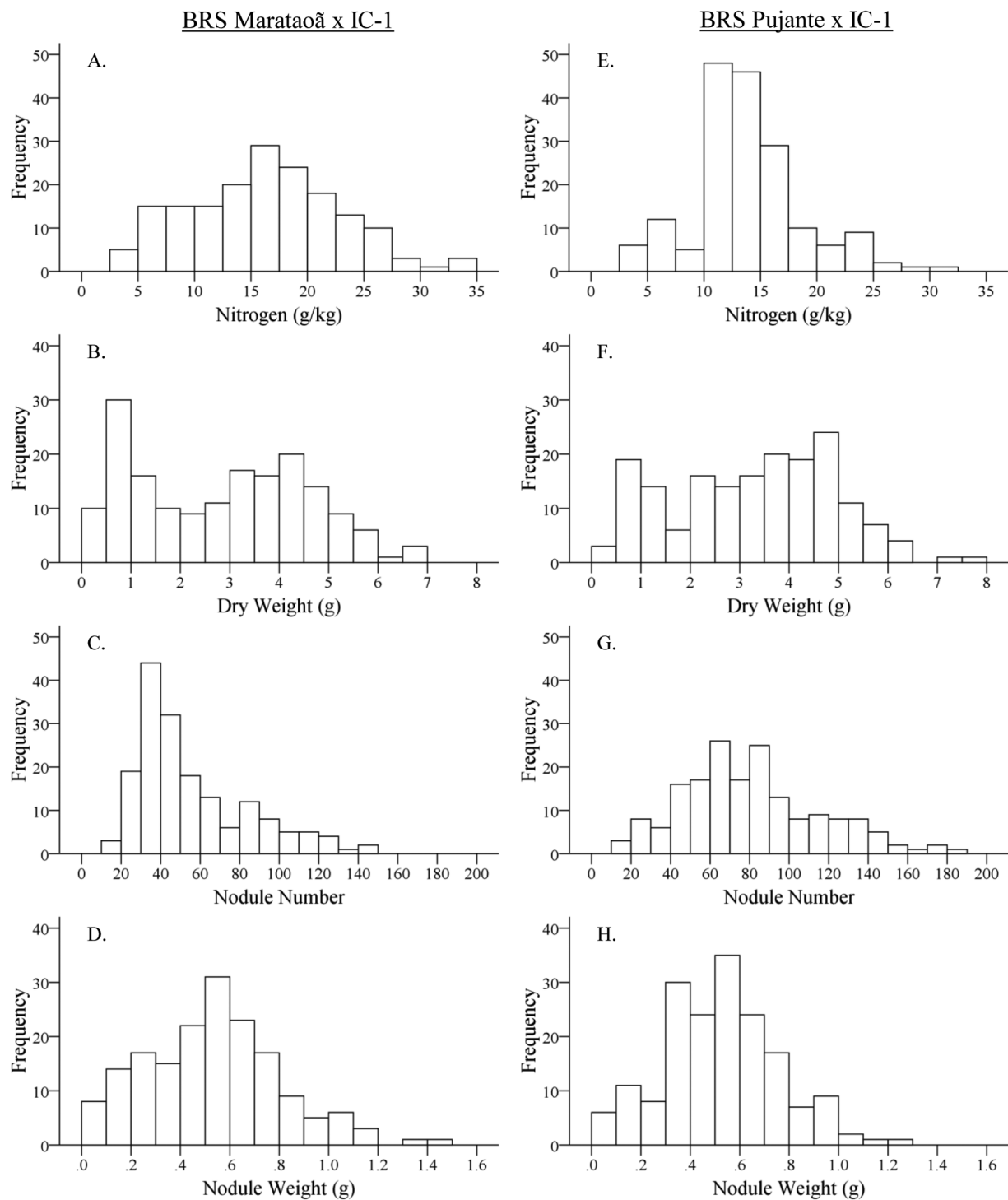Fig. 1. Frequency distributions of nitrogen-related traits in two cowpea F_2 populations derived from the crosses 'BRS Marataoã' × 'IC-1' (n = 174) and 'BRS Pujante' × 'IC-1' (n = 175). Phenotypic data were collected from F_2 cowpea grown in pot trials at Embrapa Semiárido, Petrolina, Brazil.

concentrations (Table 1), 50 ng DNA, and 1 U *Taq* polymerase. The PCR conditions consisted of 2 min denaturation (95°C); 35 cycles of 1 min denaturation (95°C), 1 min annealing (55–60°C), and 1 min extension (72°C); and 2 min final extension (72°C). Amplicons were visualized on 2% agarose gels stained with ethidium bromide.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 24 (IBM Corporation, 2016). Multiple comparisons of mean phenotypes were tested via Tukey's honest significant difference test with α = 0.05. Visual representations of LGs and LOD scores were developed in MapChart 2.3 (Voorrips, 2002).

RESULTS

Phenotypic Analysis

Nodule and leaf color cosegregated in all F_2 plants and were used to designate individuals from each population into fix+ and fix− phenotypic classes. The F_2^{MA} consisted of 128 fix+ and 46 fix− phenotypes and approximated a 3:1 distribution ($\chi^2 = 0.10$). However, a higher–than–expected number of plants were fix+ ($n = 149$) in F_2^{PJ}, compared with just 26 fix− individuals. Similarly, genotypic segregation on LG 4 was skewed towards the BRS Pujante genotype in F_2^{PJ}. Genotyping of F_2^{PJ} using markers located near the top of LG 4 indicated that 23 plants were homozygous IC-1, 78 were heterozygous, and 74 were homozygous BRS Pujante.

Mean nitrogen content differed significantly between the three parental genotypes, averaging 6.2 g kg^{-1} in IC-1 compared with 19.6 and 16.3 g kg^{-1} in BRS Marataoã and BRS Pujante, respectively. No statistical differences were found between F_1 generations, fix+ F_2 individuals, or their respective fix+ parents. Contrastingly, the mean nitrogen concentrations of fix− individuals in the two mapping populations were significantly diminished and most similar to IC-1 (Fig. 1, Table 2).

Plant dry weight for each parent ranged from 1.1 g in IC-1 to 3.4 g in BRS Pujante. No statistical differences were observed in mean dry weight among the fix+ parents, F_1 generations, or fix+ F_2 individuals. Additionally, no statistical differences were identified between the F_2 fix− individuals and IC-1 (Fig. 1, Table 2).

Mean nodule number varied significantly among all three parents, ranging from 51 nodules in BRS Marataoã to 125 in IC-1. The F_1 and fix+ F_2 individuals in both populations presented similar nodule numbers as their respective fix+ parents. Interestingly, in F_2^{MA}, significantly fewer nodules were found on fix− individuals than in IC-1. However, nodule number remained significantly higher in fix− individuals compared with the overall F_2 population mean. Although most traits were consistent between F_2^{MA} and F_2^{PJ}, significantly more nodules ($P < 0.01$) developed in F_2^{PJ}, averaging 80 nodules per plant compared with just 55 nodules per plant in F_2^{MA}. Nodule number and nitrogen concentration appeared inversely proportional in both populations (Fig. 1, Table 2).

Lastly, nodule fresh weight was significantly lower in IC-1 than in BRS Marataoã, BRS Pujante, or the F_1 generations. Within each F_2 population, average nodule weight was significantly higher in fix+ plants compared with those that were fix−, despite representing significantly fewer nodules (Fig. 1, Table 2).

Linkage Mapping and QTL Identification

After filtering and identification of polymorphic markers, 6083 SNPs were used in linkage map development and QTL analyses. The linkage map consisted of 11 LGs and 449 bins, spanning 735.6 cM. The largest gap devoid of markers was 22.6 cM, and in total, eight gaps >10 cM occurred within the linkage map (Supplemental Table S1).

The categorical trait interval mapping of leaf and nodule color detected the presence of a single recessive QTL. The QTL mapped to between 0.8 and 4.0 cM on LG 4 with a LOD score of 17.9 (Table 3, Fig. 2). The location corresponded with QTL for three of the four continuous traits detected via ICIM, as well as the joint-trait analysis. Major QTL for nitrogen content, dry plant weight, and nodule number mapped between SNP markers 2_12850 and 2_54418 near the top of LG 4 and accounted for 42.6 to 49.3% of the phenotypic variance. A single QTL for nodule fresh weight mapped between 33.5 and 34.5 cM on LG 6, flanked by markers 2_11936 and 2_49231, and accounted for 21.4% of the phenotypic variance. The joint-trait analysis mapped a single QTL to a similar interval on LG 4, as

Table 2. Means and standard deviations (mean ± SD) of nitrogen-related traits in parental lines and F_1 and F_2 generations derived from 'BRS Marataoã' × 'IC-1' and 'BRS Pujante' × 'IC-1'. Populations were phenotyped 40 d after emergence. Nodulation effective (fix+) and ineffective (fix–) phenotypic classes were assigned according to leaf and nodule color where yellow leaves and white nodules were considered fix– and green leaves and dark nodules were considered fix+. Multiple comparisons of phenotypic classes were performed via Tukey's honest significant difference and groups are indicated by the letters a through e.

Genotype or population	No. of plants	Nitrogen	Shoot and leaf dry mass	Nodule no.	Nodule mass
		g kg^{-1}	g		g
IC-1	45	6.2 ± 1.2a	1.10 ± 0.41a	125 ± 34a	0.40 ± 0.17a
BRS Marataoã	45	19.6 ± 3.5e	3.38 ± 0.82b	50 ± 16b	0.59 ± 0.24b
BRS Pujante	45	16.3 ± 3.6cd	3.41 ± 1.14b	72 ± 22c	0.54 ± 0.19b
BRS Marataoã × IC-1 F_1	60	18.5 ± 4.9de	3.41 ± 0.98b	51 ± 23b	0.59 ± 0.21b
BRS Pujante v IC-1 F_1	60	15.4 ± 3.6c	3.39 ± 0.83b	75 ± 26c	0.56 ± 0.18b
F_2 BRS Marataoã × IC-1 (fix+)	128	19.1 ± 5.6e	3.57 ± 1.42b	44 ± 17b	0.62 ± 0.25b
F_2 BRS Marataoã × IC-1 (fix–)	46	8.9 ± 3.7b	0.83 ± 0.30a	86 ± 30c	0.28 ± 0.17a
F_2 BRS Marataoã IC-1 (Total)	174	16.4 ± 6.9bc	2.84 ± 1.73b	55 ± 28b	0.53 ± 0.27b
F_2 BRS Pujante × IC-1 (fix+)	149	15.0 ± 4.1c	3.69 ± 1.42b	72 ± 28c	0.54 ± 0.23b
F_2 BRS Pujante × IC-1 (fix–)	26	7.0 ± 3.0ab	1.00 ± 0.28a	126 ± 36a	0.38 ± 0.20a
F_2 BRS Pujante × IC-1 (total)	175	13.8 ± 4.9b	3.29 ± 1.63b	80 ± 35c	0.52 ± 0.23b

Table 3. Nitrogen-related quantitative trait loci (QTL) identified in the 'BRS Marataoã' × 'IC-1' F$_2$ population. Positions are based on 1-logarithm of odds (LOD) confidence intervals, and the maximum LOD score within the interval is provided.

Trait	Linkage group	Position	Flanking markers (left/right)	r^2†	LOD	Additive effect
		cM		%		
Leaf or nodule color	4	0.8–4.0	2_12850/2_54418	–	17.9	–
Nitrogen (g kg^{-1})	4	0.0–0.5	2_12850/2_54418	49.3	9.7	−6.6
Dry mass (g)	4	2.5–5.5	2_12850/2_54418	42.6	6.7	−1.2
Nodule number	4	0.0–0.5	2_12850/2_54418	48.4	11.7	28
Nodule fresh weight (g)	6	33.5–34.5	2_11936/2_49231	21.4	3.7	−0.1
Joint-trait	4	0.0–4.0	2_12850/2_54418	–	34.7	–

† The percentage of phenotypic variance explained by each QTL.
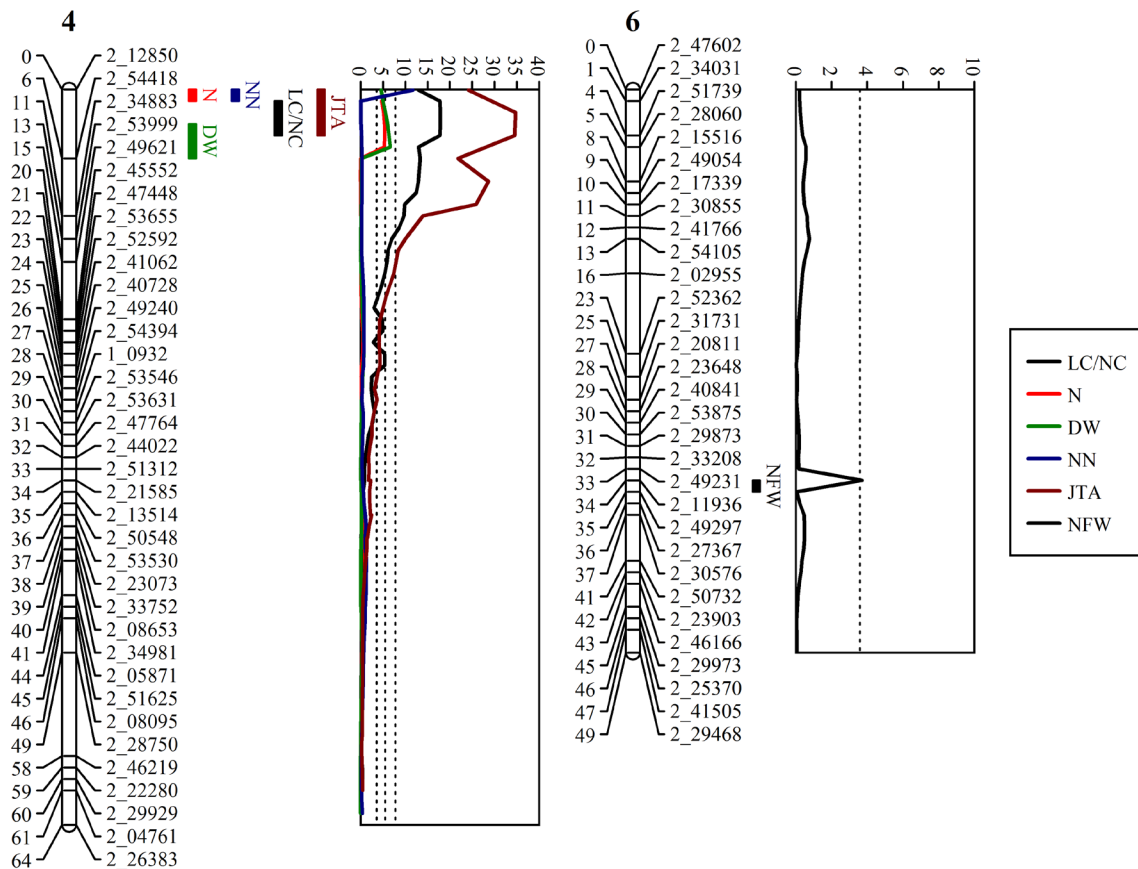
Fig. 2. Cowpea linkage groups 4 and 6 and logarithm of odds (LOD) graphs corresponding to quantitative trait loci (QTL) detected for leaf color (LC), nodule color (NC), nitrogen content (N), aboveground plant dry weight (DW), nodule number (NN), nodule fresh weight (NFW), and joint trait analysis (JTA). LOD thresholds of 3.58 (N, DW, NN, NFW), 5.5 (LC, NC), and 7.8 (JTA) are displayed as constants. QTL mapping was performed in an F$_2$ population derived from a cross between 'BRS Marataoã' and 'IC-1' and grown in pot trials at Embrapa Semiárido, Petrolina, Brazil.

previously identified by ICIM (Table 3, Fig. 2). However, no additional QTL were detected via this method.

Leaf and nodule color QTL were validated by genotyping F$_2^{PJ}$ with several PCR-based SNP markers. Genotyping of 175 F$_2$ individuals with five tetra-primer ARMS-PCR markers (Table 1) confirmed a strong association of the locus with the fix− phenotype. Nearly all F$_2^{PJ}$ phenotypes matched the expected parental genotype (i.e., fix− genotyped homozygous IC-1). However, correspondence of phenotypes and genotypes for seven

individuals (4%) deviated from expectations, suggesting incomplete linkage of the markers with the fix− gene.

DISCUSSION

Pemberton et al. (1990) previously reported a single recessive gene controlled the fix− phenotype in IC-1. This conclusion is supported by 3:1 segregation in F$_2^{MA}$ for leaf and nodule color and consistent with the majority of nodulation ineffective mutants previously identified (Bhatia et al., 2001). However, F$_2^{PJ}$ did not segregate in a 3:1 ratio. Despite abnormal segregation, there was near-complete

correspondence of the fix+ phenotype with the fix+ genotype and the fix− phenotype with the fix− genotype.

The fix− mutation in IC-1 influenced several nitrogen-related traits. Significant differences between fix+ and fix− individuals were found for all reported characters including lower nitrogen content, reduced plant and nodule weight, and increased nodule number. Reduced plant weight and nodule size in conjunction with increased nodule number were consistent with previous characterizations of IC-1 (Pemberton et al., 1990). Furthermore, several studies in soybean have reported strong correlation between greater nodule number and smaller nodule size (Purcell et al., 1997; King and Purcell, 2001; Santos et al., 2013; Hwang et al., 2014).

The F_2^{MA} linkage map (Supplemental Table S1) corresponds well with the cowpea consensus map and previously reported cowpea maps (Lucas et al., 2011; Muñoz-Amatriaín et al., 2017). Although the genome size in this study is shorter than the consensus map (735.6 vs. 837.1 cM), the result is consistent with potential effects of a smaller population size and fewer recombination events occurring in an F_2 population compared with the recombinant inbred line populations used in consensus map development (Ferreira et al., 2006; Muñoz-Amatriaín et al., 2017).

We performed QTL mapping in F_2^{MA} for six traits associated with ineffective nodulation. Mapping of leaf and nodule color identified a major QTL near the top of LG 4, suggesting that the fix− phenotype in IC-1 is controlled by a single gene, which is consistent with the 3:1 phenotypic segregation of leaf and nodule color (Table 3). Validation of the QTL in a second population indicated nearly 100% correspondence between fix− phenotype and genotype, providing a high level of confidence in the fidelity of the QTL. Furthermore, ICIM and multiple-trait composite interval mapping identified QTL for nitrogen content, dry weight, and nodule number at the same locus. However, the nodule fresh weight QTL did not colocalize with the other five traits. It is likely that the reduced individual nodule weights of fix− individuals were masked by the increased abundance of nodules produced by fix− individuals. However, colocalization for five of the six traits measured in this study suggests that a single, pleiotropic gene is most likely responsible for influencing several nodulation and nitrogen utilization traits in IC-1.

Examination of cowpea genome annotations within the LG 4 QTL indicates the presence of ~160 genes (Vigna unguiculata v1.0, NSF, UCR, USAID, DOE-JGI, http://phytozome.jgi.doe.gov/). Intriguing candidate genes include an aluminum-activated malate transporter gene, since malate is the primary source of carbon exported by legumes for rhizobial consumption (Udvardi and Poole, 2013), and a sulfate transporter gene (Krusell et al., 2005). Although large numbers of SNP markers were used in this study, no polymorphic SNPs were found between flanking markers corresponding to the fix− QTL. Consequently, additional markers may be needed to perform fine mapping and cloning of cpi.

The fix− cowpea variety IC-1 is a valuable model for studying ineffective nodules and their effects on nitrogen utilization. In addition to confirming reduced plant and nodule mass and increased nodule number in fix− individuals (Pemberton et al., 1990), this study identified significantly lower nitrogen content associated with the ineffective nodulation phenotype (Table 2). Furthermore, we report the identification of a major QTL associated with several nitrogen-related characteristics (Fig. 2, Table 3). The results of this study provide a strong foundation for future identification of the cpi gene.

Supplemental Material Available

Supplemental material for this article is available online.

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