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ORIGINAL PAPER

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Genetics of nodulation and nitrogen fixation in Brazilian soybean cultivars

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Abstract The objective of this work was to study the genetics of the nodulation and biological nitrogen fixation (BNF) characteristics of Brazilian soybean cultivars. Four cultivars were identified with different capacities for BNF: J-200 and Bossier (high), Embrapa 20 (medium) and Embrapa 133 (low), and all possible crosses, including reciprocals, were carried out to obtain the F1, F2 and F3 generations. Three experiments were performed simultaneously, under greenhouse conditions, with the restricted set of generations P1, P2, F2 and F3, and plants were evaluated for nodulation (nodule number, NN, and nodule dry weight, NDW) and plant growth (shoot dry weight, SDW). No significant differences between reciprocal and direct effects were observed, therefore all data from F2, as well as from F3 plants were pooled. The frequency distributions for the tested variables in the F3 families were normal, with no evidences of discontinuities, consistent with polygenic inheritance. In the J-200 × Embrapa 133 and Bossier × Embrapa 133 crosses, the significance of the models for means and variances was less frequent, but was eventually observed for NN and NDW. In the other two experiments, there was a predominance of genetic additive (*d*) and/or genetic additive variance (*D*) effects for most of the tested variables, except for NDW in the cross of J-200 × Bossier. Genetic dominant effects (*h*) and/or genetic dominance variance (*H*) were detected for all variables in the cross Embrapa 20 × Embrapa 133 and for NDW/NN for Bossier × Embrapa 20. Additive × additive epistatic (*i*) and interaction genotype × microenvironment effects were less important in all experiments. The narrow-sense heritabilities (h_n^2) estimates ranged from 39% to 77%, with higher values for NN and NDW in Bossier × Embrapa 20 and for SDW in Embrapa 20 × Embrapa 133; these were high values when compared to other legumes. The prediction of the genetic potential to generate superior inbred lines for nodulation and BNF capacity indicated that selection could be more effective for crosses Bossier × Embrapa 20, and Embrapa 20 × Embrapa 133.

Keywords *Bradyrhizobium japonicum* · *Bradyrhizobium elkanii* · Dinitrogen fixation · *Glycine max* · Nodulation

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Introduction

Soybean (*Glycine max* L. Merrill) is an exotic species introduced to Brazil about 120 years ago, and today the country produces 31 million tons of grain on 13 million hectares, being the second largest producer of this legume worldwide. High technology is used in its cultivation, resulting in a national mean yield of 2,375 kg ha^{-1}. The capacity of soybean to satisfy its nutritional N requirement via biological nitrogen fixation (BNF) with *Bradyrhizobium japonicum*/*B. elkanii* strains selected by Brazilian microbiologists is well documented, and today no N fertilizer at all is recommended for the crop, resulting in an economy estimated at U$ 1.5 billion per crop season (Hungria and Vargas 2000; Hungria et al. 2000). However, higher yielding soybean cultivars and high technology demand a continuous selection of more efficient and competitive bradyrhizobial strains and of plant genotypes with higher BNF, so that there will still be no need to apply N-fertilizers.

Although there is no doubt about the economical importance of BNF to soybean production in Brazil, breeders often do not evaluate the symbiotic performance of newly released soybean lines, with greater attention paid to resistance to diseases and yield. However, recently, differences among cultivars were observed when 152 genotypes recommended for various regions of Brazil were evaluated for BNF capacity: some of them accumulated up to four times more nodule dry weight and two and a half times more N in tissues (Bohrer and Hungria 1998; Hungria and Bohrer 2000). These results indicate that,

during the last 20 years, losses in BNF capacity may have resulted from the introduction of new and less efficient cultivars. For example, cultivar Davis, a North American genotype introduced to Brazil in the 1960s showed up to one-third greater nodule number and total N in tissues than some recently released cultivars that have Davis as a parental genotype (Bohrer and Hungria 1998). Therefore, in Brazilian soybean breeding programs, more attention should be directed at understanding and evaluating plant mechanisms that control and regulate root-nodule formation and activity, otherwise BNF capacity may be compromised. These observations agree with van Kessel and Hartley (2000), who after analyzing 362 experiments, the majority conducted in North America and Australia, verified a trend of declining N_2-fixation by soybean.

Recent advances have been achieved in rhizobial genetics, including the sequencing of the whole symbiotic plasmid of *Rhizobium* strain NGR234 (Freiberg et al. 1997), followed by the transcriptional analysis of the genes (Perret et al. 1999) and the determination of the complete genome structure of *Mesorhizobium loti* (Kaneko et al. 2000). However, plant genetic traits related to nodulation and BNF are still poorly understood, mainly due to the larger size of the plant genome. For soybean, a few studies have identified alleles responsible for restriction of nodulation to certain serogroups of *Bradyrhizobium* (Caldwell 1966; Vest et al. 1972; Devine 1976; Qian et al. 1996) and for the ability to nodulate with the fast-growing species *Sinorhizobium fredii* (Devine 1984). Plant mutants with altered symbiotic performance are also useful for gaining a better understanding of plant-microbe interactions: supernodulating soybean mutants, which form very large numbers of nodules, as well as "nitrate-tolerant-symbiotic" (*nts*) mutants have been obtained and studied (Harper and Gibson 1984; Carroll et al. 1985; Gremaud and Harper 1989; Akao and Kouchi 1992; Lohrke et al. 1996). Recently, an integrated genetic linkage map of the soybean genome was published, indicating some loci related to nodulation (Cregan et al. 1999).

Few genetic studies of BNF with soybean have been performed (Ronis et al. 1985; Greder et al. 1986; Burias and Planchon 1990; Herridge and Rose 1994, 2000; Herridge and Danso 1995), and none with Brazilian cultivars. In this context, our objective was to study the inheritance of quantitative traits of BNF with Brazilian cultivars.

Materials and methods

Screening of parental cultivars

Soybean cultivars were screened with the objective of identifying different BNF capacities. Cultivars with high (Bossier, J-200, OCEPAR 4, Coodetec 201, Davis, BR-9 Savana, FT-Cristalina, Numbaíra), medium (Embrapa 20, Doko) or low (EMGOPA-313, FT-Seriema, MS BR-34, FT-Canarana) BNF capacity were chosen. Most of these were selected from 152 cultivars tested for BNF capacity by Bohrer and Hungria (1998) and Hungria and Bohrer (2000), who also described their genealogy. The exceptions were Coodetec 201 [OCEPAR 4-Iguaçú (5) × W 20], BR 9 (Selection in Lo 874–2) and Embrapa 133 (FT-Abyara × BR 83–147), which were included in this study due to their importance in programs of selection for disease resistance and high yield.

Cultivars were evaluated for BNF parameters under greenhouse conditions. *Bradyrhizobium elkanii* strain SEMIA 587 and *B. japonicum* strain SEMIA 566, both of which are established in the majority of Brazilian soils cropped to soybean (Bohrer and Hungria 1998), were grown in yeast mannitol medium (YM; Vincent 1970), for 7 days. The cultures were equalized at 10^9 cells ml^{-1} and mixed. Information about the strains may be obtained elsewhere (Bohrer and Hungria 1998). Five seeds were sown per pot of 5-kg capacity containing 4 kg of non-sterile soil and sand, with an established population of *B. japonicum*/*B. elkanii* estimated at 10^4–10^5 cells g^{-1} soil. Before planting, soil received the required nutrients, except for N, according to a soil chemical analysis. After 5 days, plants were thinned to two per pot. To guarantee good nodulation, the roots were inoculated at the V2 stage (completely unrolled leaf at the first node above the unifoliolate node; Fehr and Caviness 1977) by adding 1 ml of the mixed inoculum (SEMIA 587:SEMIA 566, 1:1, v:v) per plant. The experiment was performed in a complete randomized design, with four replicates.

Five weeks after emergence, plants were harvested and divided into shoots, roots and nodules. Nodule number (NN) was determined and shoot (SDW) and nodule (NDW) dry weight were evaluated after drying at 65°C for 4 days; finally, total N (Kjeldahl) accumulated by shoots (TNS) was determined as previously described (Bohrer and Hungria 1998). Cultivars were selected based on the parameters of nodulation (number and dry weight) and BNF capacity (total N accumulated in shoots). From this experiment, the following cultivars were selected: J-200, due to higher nodulation and BNF capacity; Bossier, with high BNF capacity, but lower nodule mass than J-200; Embrapa 20, with medium values for nodulation and BNF; and Embrapa 133, with medium nodule number but lower BNF capacity.

Obtaining segregant generations

All possible single crosses, including reciprocals, were made between the four selected cultivars. In the summer of 1998 (January) two seeds of each parent were planted per pot and were grown as described in the screening cultivars item; hybridizations were performed late in the afternoon. The F2 and F3 generations were obtained by March of 1999, after self pollination (single-seed-descent method, SSD). At harvest, seeds were taken manually from each pod, and three to five seeds were randomly sampled to produce the next generation. The remaining seeds were kept at the Embrapa Soja soybean germplasm bank. In the summer (January) of 1999, seeds of the parents, F1 and F2 generations, were planted simultaneously with the production of the segregant generation F3, to produce seeds of the same age, thereby minimizing intrinsic and environmental effects.

Morphological markers were used to verify the parenthood of F1, as described by Destro et al. (1990), for all crosses except Bossier × Embrapa 20, Bossier × Embrapa 133, J-200 × Embrapa 133 and Embrapa 20 × Embrapa 133, because the pollen donor was not dominant for any of the markers in these crosses. For these crosses RAPD (random amplified polymorphic DNA) markers had to be used, and DNA was extracted from the first trifoliolate leaves of the F1 generation. The DNA was amplified with 38 primers of Operon Life Technologies (Calif.) and three primers were obtained (OPS-09, OPS-13 and OPS-18) which allowed the identification of parental cultivars.

The experiment on quantitative genetics was performed in May of 1999, with seeds of the parental and F2 and F3 generations all produced in March of 1999. Inoculation and growth conditions were as described in the screening cultivars section. The experiment used a completely randomized design, and was performed simultaneously in three different greenhouses, due to the large space required. In each greenhouse the experiment had 2 combina-

tions of crosses plus the 4 parents and included 20 pots for each parent, 30 pots for each F2 and RF2 (reciprocal), 30 F3 and RF3 families, with 4 plants per family, summing 340 pots for each cross, 680 pots for each experiment and 2,040 pots for all 6 crosses used in this study. The first experiment was performed in 2-kg ceramic pots, and the two others in 2-kg plastic pots, all filled with a mixture of non-sterile soil and sand, as describe in the screening cultivars section. The plants were harvested 40 days after germination, proceeding to the evaluation of nodule number and dry weight and shoot dry weight, as described in the screening cultivars item. Total N in shoots was not evaluated because Bohrer and Hungria (1998) and Hungria and Bohrer (2000) found statistically significant correlations between SDW and TNS, of r =0.90 and r =0.91, respectively, in experiments performed in N-free sterile substrate or with low-N non-sterile soil.

Genetic analysis

The means and variances of the studied traits for each parent and derived generations were calculated. After testing for reciprocal effects, the cross and reciprocal data in each generation were pooled. Genetic and environmental effects were estimated from the means and variances of generations. A simple additive-dominant model was used, according to Mather and Jinks (1982), involving the mean values of the parents, *m*; *d*, representing the algebraic sum of additive effects of all k loci that differed among P1 and P2; *h* being the algebraic sum of all dominant effects in the same k loci. Epistatic effects like *i*, representing additive × additive; and *l*, representing dominant × dominant interaction effects were included in the model whenever necessary. The genetics were estimated by the WLS (Weighted Least Squares) scale of Cavalli (1952), with Genfit software (de Toledo 1991).

Estimates of the additive genetic (*D*), dominance (*H*) and additive environmental (*E*) variances were obtained by the method of WLS, according to Hayman (1960) and Mather and Jinks (1982). The method was used for all fitted models, with the Genfit software. Variances (σ^2) were estimated as a function of *D*, *H* and *E* for each parental as well as between and within families (Mather and Jinks 1982). Heritabilities were estimated according to Mather and Jinks (1982) and Lynch and Walsh (1998). When the genetic model with *D*, *H* and *E* was not adjusted, another model involving genotype × microenvironment, including *E1* and *E2* (Mather and Jinks 1982), was used.

Narrow-sense heritability (h_n^2) was estimated as follows: $h_n^2 = 1/2D/(1/2D + 1/4H + E/n)$, with *n* representing the harmonic mean of the number of individuals composing the families of the F3 generation. The broad-sense heritability (h_b^2) of F3 families was estimated from the variance components of F3 families, as follows: $h_b^2 = \sigma^2b/(\sigma^2b + \sigma^2w/n)$, with σ^2b representing the genetic variance component between families (*b*), σ^2w the genetic variance component within families (*w*) and *n* the harmonic mean of the number of individuals. The estimation of these variance components was obtained from the analysis of the variance of F3 progenies in each cross. The standard error estimate for heritability was calculated after Hallauer and Miranda (1981), as follows: SE $(h_b^2) = 2\ (1-h^2)\ [(1/n_1+2)+(1/n_2+2)]$, where n_1 and n_2 are degrees of freedom between and within F3 families, respectively.

The method of prediction of superior inbred lines of a given cross was estimated according to Jinks and Pooni (1976, 1982) and de Toledo (1987), aiming to evaluate the expected frequency of superior inbred lines with a higher capacity for nodulation and BNF. The estimations of *m* and *D* obtained in the genetic models were used to calculate the predictions. The phenotypic proportion of pure lines with a superior performance in relation to a reference value was obtained using the following equation: Z = ref. – m/vD. The values taken as reference were 3 g for SDW, 120 for NN, 300 mg for NDW and 4 mg nodule^{-1} for NDW/NN. After obtaining the prediction values, the values were compared with the values of a *Z* table that indicates the proportion of expected pure lines for each character.

Results and discussion

Brazil ranks second in soybean production worldwide, and BNF plays an essential role in crop establishment and yield, since no N fertilizer is applied. Effects of soybean genotypes on N_2 fixation have often been described (e.g. Kvien et al. 1981; Senaratne et al. 1987; Mytton and Skøt 1993), and pioneer studies reported differences among genotypes grown in Brazil (Döbereiner and Arruda 1967; Brose et al. 1979; Vargas et al. 1982). However, few studies have been performed in the past two decades.

With respect to the genetics of BNF in soybean, few studies have been reported, and most of these were related to restriction in nodulation of North American genotypes to certain serogroups of *Bradyrhizobium*, or to mutagen-induced genotypes with higher nodulation capacities, including higher nodulation in the presence of nitrate (Caldwell 1966; Vest et al. 1972; Devine 1976; Harper and Gibson 1984; Gremaud and Harper 1989). Some of these and a few other studies have reported breeding of soybean to increase nodulation and/or N_2 fixation, and successful results were achieved using either mutation (e.g. Carroll et al. 1985; Akao and Kouchi 1992; Lohrke et al. 1996; Qian et al. 1996) or classical breeding techniques (Ronis et al. 1985; Greder et al. 1986; Burias and Planchon 1990). In all of these studies, nodulation was the parameter usually evaluated. However, nodule number does not always show a good correlation with total N accumulated in shoots or in plants (Döbereiner 1966; Nutman 1981), while nodule dry weight is regarded as a more reliable parameter (Döbereiner 1966; Bohrer and Hungria 1998; Hungria and Bohrer 2000). The improvement in N_2 fixation results in a better plant growth and high correlations between SDW and TNS have been reported for soybean and other nodulated legumes (Haydock et al. 1980; Bohrer and Hungria 1998; Hungria and Bohrer 2000). Therefore, a simple evaluation of SDW can be used to evaluate BNF capacity of plants growing in a low-N content substratum, speeding breeding programs aiming at that trait, while decreasing research costs.

In this study, all possible single crosses, including reciprocals, were performed among four soybean cultivars with different capacities for nodulation and BNF, to study genetic traits related to nodulation and BNF capacity under low N conditions. Figure 1a and c shows the frequency distributions of the SDW and NDW traits, respectively, for F3 plants of Embrapa 20 × Embrapa 133. It is noteworthy that there are several phenotypic classes resulting from the segregation in F3, tending to a continuous distribution, commonly shown by polygenic systems. The same was observed for the NN trait (Fig. 1b). The frequency distributions of SDW and NDW in Fig. 1a and c also show the values for the parents, Embrapa 20 and Embrapa 133, indicating that the F3 families showed transgressive segregation. For this cross, values in F3 ranged from 40 to 438 mg pl^{-1} for NDW (Fig. 1c), from 1.0 to 5.6 g pl^{-1} for SDW (Fig. 1a),

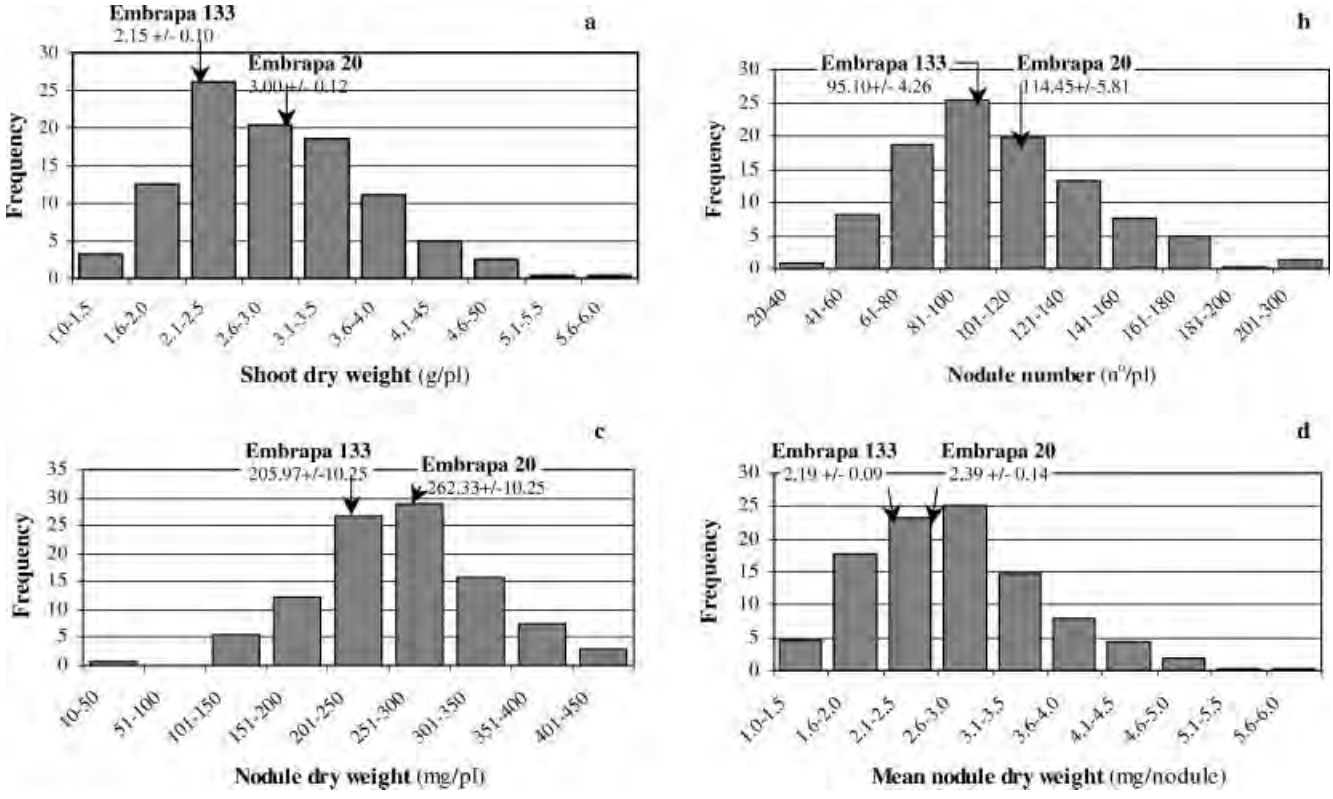

Fig. 1 Frequency distributions for shoot dry weight (**a**) and nodulation (**b**–**d**) parameters in F2-derived F3 families of the cross of soybean cultivars Embrapa 20 × Embrapa 133 inoculated with *Bradyrhizobium elkanii* strain SEMIA 587 and *B. japonicum* SEMIA 566. *Arrows* indicate the values for the parents with standard error

and from 22 to 269 nodules pl^{-1} for NN (Fig. 1b). The ratio NDW/NN was calculated to investigate the effect of mean NDW per plant; mean values for the parental genotypes were 2.39 mg nodule^{-1} for Embrapa 20 and 2.10 mg nodule^{-1} for Embrapa 133, while for the F3 families they ranged from 1.2 to 5.7 mg nodule^{-1} (Fig. 1d). For all the other crosses, the frequency distributions of SDW, NDW, NN and NDW/NN were similar to those observed for Embrapa 20 × Embrapa 133 (data not shown). Therefore, normal distributions and transgressive segregations were obtained for the nodulation parameters and plant growth under low mineral-N availability, indicating quantitative inheritance for those traits.

Degrees of freedom, means with standard errors and variances for the SDW, NN, NDW traits, and NDW/NN ratio, for the parents and derived generations in each of the three experiments involving the crosses of complete dialleles between the cultivars J-200, Bossier, Embrapa 20 and Embrapa 133 are shown in Table 1. The experiments were performed simultaneously, but in three different greenhouses, due to their large size. There were also not enough pots of the same kind, therefore the first experiment was performed in ceramic pots, usually used in the plant breeding program of Embrapa Soja, while the two others were performed in plastic pots, used by the microbiology department; all pots had the same capacity. Although pots received the same amount of water daily, losses of water through the pores of the ceramic pots were much higher, therefore plant growth in the first experiment was lower than in the other two. No reciprocal effects were observed, therefore data of F2 and F3 generations represent a pool among the straight and reciprocal crosses.

In the first experiment, no significant differences were observed among the parents, for SDW and NDW/NN ratio. However, NDW and NN in Bossier were significantly higher than in the other three parents, although not statistically different from Embrapa 133 for NDW (Table 1). In the second and third experiments (Table 1), differences among the parents were detected in all parameters analyzed. In both experiments, Bossier confirmed a superiority for the NDW trait that resulted in higher SDW in the second, but not in the third experiment. The lowest values of NDW in both experiments 2 and 3 were achieved with cultivar Embrapa 133. NN was a parameter with high variability among the cultivars, with some (e.g. Embrapa 20 in the third experiment) showing almost twice the number of nodules than others (J-200). The NDW/NN ratio also varied among parents, and as an average of the three experiments, the lowest values were recorded in Embrapa 20, and the highest in J-200. Although showing a higher NDW/NN ratio, J-200 had poor plant growth, therefore, heavier nodules did not result in higher BNF rates. The highest SDW was observed in Bossier in the first two experiments.

The genetic parameters adjusted for the SDW, NN and NDW means and variances of each cross in the

Table 1 Degrees of freedom (*df*), means with standard error (*SE*) and variances (σ^2) of the parents, F2 and F3 generations for shoot dry weight per plant (*SDW*, g plant^{-1}), nodule number (*NN*, no. plant^{-1}) and nodule dry weight (*NDW*, mg plant^{-1}) and the *NDW/NN* ratio obtained for soybean plants inoculated with *Bradyrhizobium elkanii* strain SEMIA 587 and *B. japonicum* SEMIA 566

Generation	*df*	SDW		NN		NDW		NDW/NN	
		Mean ±SE[a]	σ^2	Mean ±SE[a]	σ^2	Mean ±SE[a]	σ^2	Mean ±SE[a]	σ^2
Experiment 1									
J-200	17	1.28±0.09a	0.14	66.72±6.41b	740.33	85.26±9.17b	1,513.85	1.31±0.12a	0.27
Bossier	19	1.53±0.11a	0.23	101.40±9.6a	1,841.94	125.59±13.75a	3,779.40	1.34±0.16a	0.52
Embrapa 20	19	1.52±0.05a	0.06	64.60±5.35 b	572.99	89.57±10.88b	2,368.76	1.32±0.11a	0.24
Embrapa 133	19	1.46±0.09a	0.18	63.50±7.16b	1,025.21	103.86±12.43ab	3,089.04	1.67±0.13a	0.36
J-200 × Embrapa 133									
F2	58	1.51±0.05	0.18	76.27±3.77	839.37	117.37±5.71	1,924.68	1.63±0.07	0.31
F3 total	238	1.45±0.02	0.15	66.19±1.95	910.91	100.63±3.39	2,742.68	1.60±0.05	0.54
Between (families)	59		0.14		1,149.95		2,411.85		0.56
Within (families)	179		0.16		830.69		2,853.70		0.53
Bossier × Embrapa 133									
F2	59	1.59±0.06	0.21	81.30±4.26	1,087.91	120.57±5.96	2,131.05	1.66±0.11	0.71
F3 total	237	1.49±0.02	0.15	69.32±2.09	1,036.57	104.07±3.21	2,453.53	1.65±0.06	0.87
Between (families)	59		0.18		1,365.72		3,015.82		0.87
Within (families)	178		0.14		925.17		2,263.21		0.87
Experiment 2									
J-200	19	2.13±0.11b	0.26	83.70±8.36b	1,397.48	241.93±15.24b	4,645.13	3.21±0.24a	1.22
Bossier	19	2.74±0.17 a	0.57	98.40±6.0ab	727.73	286.61±13.40a	3,586.71	3.11±0.23a	1.07
Embrapa 20	19	2.55±0.12a	0.29	115.10±5.87a	689.15	244.11±12.30b	3,027.72	2.17±0.11b	0.24
Embrapa 133	19	2.35±0.15ab	0.46	90.45±7.81b	1,221.00	232.70±16.04b	5,148.40	2.90±0.31a	1.93
J-200 × Embrapa 20									
F2	59	2.49±0.09	0.44	96.68±3.64	793.94	257.79±7.20	3,116.36	2.80±0.09	0.54
F3 total	239	2.60±0.04	0.46	96.77±1.75	734.83	264.31±3.94	3,719.12	2.88±0.05	0.67
Between (families)	59		0.76		945.68		6,248.16		1.02
Within (families)	180		0.36		664.55		2,876.11		0.55
Bossier × Embrapa 20									
F2	59	2.33±0.10	0.64	88.98±4.23	1,074.82	250.98±9.69	5,634.23	3.07±0.13	1.07
F3 total	238	2.39±0.05	0.61	102.00±2.36	1,333.87	257.87±4.61	5,090.82	2.72±0.06	0.81
Between (families)	59		1.25		2,373.18		10,655.00		1.02
Within (families)	179		0.40		985.10		3,223.48		0.74
Experiment 3									
J-200	18	1.93±0.14b	0.37	67.16±5.94c	670.47	243.39±20.78ab	8,201.20	3.74±0.72a	1.41
Bossier	19	1.89±0.12b	0.29	91.30±7.26b	1,054.33	266.03±22.53a	10,149.29	2.99±0.15b	0.47
Embrapa 20	19	3.00±0.12a	0.27	114.45±5.81a	675.21	262.33±10.25a	2,102.88	2.39±0.14c	0.37
Embrapa 133	19	2.15±0.10b	0.20	95.10±4.26b	363.88	205.97±10.25b	2,102.43	2.19±0.09c	0.15
J-200 × Bossier									
F2	58	2.07±0.09	0.50	78.83±4.08	981.79	254.30±8.71	4,475.55	3.52±0.15	1.25
F3 Total	235	2.31±0.05	0.72	87.28±2.52	1,500.19	262.50±5.54	7,241.59	3.32±0.08	1.35
Between (families)	59		1.43		2,179.00		10,571.84		1.64
Within (families)	176		0.47		1,266.92		6,097.17		1.25
Embrapa 20 × Embrapa 133									
F2	59	2.84±0.11	0.71	104.60±4.68	1,315.81	269.76±10.60	6,748.42	2.67±0.08	0.35
F3 Total	239	2.84±0.05	0.71	103.06±2.34	1,313.80	262.71±4.63	5,148.39	2.71±0.05	0.65
Between (families)	59		1.32		1,451.62		6,391.24		0.76
Within (families)	180		0.51		1,267.87		4,734.11		0.65

[a] Parental means followed by the same letter do not differ significantly (P <0.05) according to Duncan's multiple range test

three experiments are shown in Table 2. In experiment 1 (J-200 × Embrapa 133 and Bossier × Embrapa 133), significant genetic effects (*d*, *h*, D and H) were detected for the NN trait for both crosses and NDW and NDW/NN for J-200 × Embrapa 133. Dominance *h* effects were detected for NN and NDW for J-200 × Embrapa 133. For Bossier × Embrapa 133, genetic variability was detected for the NN trait, with additive *d*, dominant *h* and additive × additive epistatic *i* effects. However, in both crosses, the absence of additive *d* effects for NDW does not guarantee the absence of variability between the parents, since the alleles with additive effects can be dispersed

Table 2 Genetic parameters adjusted to the means and variances of shoot dry weight and nodulation parameters (*m* mean values of parents, *d* sum of additive effects of all k loci, *h* sum of dominant effects of all k loci, *D* additive genetic variance, *H* dominance variance, *E* additive environmental variance, *i* additive × additive epistatic effect, *E1 and E2* genotype × microenvironment effects; *ns* and *s* indicate statistically non-significant and significant, respectively)

	J-200 × Embrapa 133	Bossier × Embrapa 133	J-200 × Embrapa 20	Bossier × Embrapa 20	J-200 × Bossier	Embrapa 20 × Embrapa 133
Shoot dry weight (SDW, g plant^{-1})						
m	1.45±0.02	1.50±0.22	2.44±0.03	2.42±0.04	2.17±0.42	2.63±0.07
d	–	–	0.21±0.08	–	–	0.43±0.08
h	–	–	–	–	–	0.64±0.26
χ^2/g.l	4.91^{ns}/3	2.70^{ns}/3	1.99^{ns}/2	5.87^{ns}/3	15.96^{s}/3^{b}	2.38^{ns}/1
D	–	–	0.22±0.07	0.37±0.10	0.47±0.11	0.56±0.13
H	–	–	–	–	–	–
E	0.16±0.01	0.17±0.01	0.30±0.04	0.34±0.05	0.33±0.05	0.34±0.05
χ^2/g.l	1.19^{ns}/4	5.89^{ns}/4	0.51^{ns}/3	5.14^{ns}/3	1.18^{ns}/3	4.20^{ns}/3
Nodule number (NN, no. plant^{-1})						
m	61.25±3.57	57.34±5.92	96.98±1.50	100.42±1.85	83.96±1.95	103.72±1.81
d	–	18.95±5.99	16.52±4.83	8.52±4.20	13.00±4.61	9.36±3.48
h	25.14±11.85	47.92±18.77	–	–	–	–
i	–	25.11±8.42	–	–	–	–
χ^2/g.l	1.73^{ns}/2	[a]	0.24^{ns}/2	10.01^{s}/2^{b}	4.33^{ns}/2	0.20^{ns}/2
D	–	–	–	718.02±205.27	502.24±198.22	–
H	–	–	–	–	–	2,699.79±868.28
E	895.45±69.50	1,089.58±84.31	784.23±60.50	768.21±100.26	1,027.28±120.73	759.29±139.32
χ^2/g.l	3.42^{ns}/4	8.48^{ns}/4	9.36^{ns}/4	0.40^{ns}/3	3.40^{ns}/3	6.09^{ns}/3
Nodule dry weight (NDW, mg plant^{-1})						
m	88.54±5.65	108.37±2.70	260.55±3.25	258.12±3.79	259.59±4.47	238.66±6.44
d	–	–	–	20.64±9.07	–	28.18±7.25
h	53.85±18.11	–	–	–	–	81.49±24.55
χ^2/g.l	1.92^{ns}/2	7.68^{ns}/3	4.34^{ns}/3	1.18^{ns}/2	1.33^{ns}/3	1.84^{ns}/1
D	–	–	1,335.67±526.51	3,364.51±856.36	–	–
H	–	–	–	–	–	15,136.06±3,463.11
E	2,557.74±198.52	2,506.04±193.92	2,794.15±322.90	2,706.90±377.51	6,975.76±543.06	2,496.78±494.86
χ^2/g.l	4.90^{ns}/4	5.68^{ns}/4	5.15^{ns}/3	2.20^{ns}/3	14.96^{s}/4^{b}	2.62^{ns}/3
Nodule dry weight/Nodule number (NDW/NN, mg nodule^{-1})						
m	1.59±0.04	1.63±0.05	2.84±0.04	2.55±0.10	3.36±0.06	2.42±0.068
d	0.19±0.09	–	0.62±0.10	0.41±0.12	0.37±0.14	0.15±0.078
h	–	–	–	0.84±0.37	–	0.73±0.22
χ^2/g.l	1.56^{ns}/2	3.50^{ns}/3	1.98^{ns}/2	1.53^{ns}/1	1.48^{ns}/2	9.13^{s}/1^{b}
D	–	–	0.19±0.09	–	–	–
H	–	–	–	1.29±0.63	–	–
E	0.47±0.04	0.79±0.06	–	–	–	–
E1	–	–	0.84±0.13	0.99±0.23	2.09±0.25	2.10±0.25
E2	–	–	0.21±0.07	0.23±0.07	0.51±0.16	0.51±0.16
χ^2/g.l	7.35^{ns}/4	5.30^{ns}/4	2.66^{ns}/2	1.18^{ns}/2	3.14^{ns}/3	3.14^{ns}/3

[a] Perfect fit; [b] Better model found

between the parents, as has been observed for soybean seed oil content (Miranda and Arias 1998). Still in the first experiment, none of the crosses showed either effects of additive genetic variance (*D*), or of dominance (*H*), only environmental variance (*E*) was detected. However, the absence of effects could be related to the limitation in plant growth due to a water stress caused by the ceramic pots used in this experiment, as explained before. Therefore it was not possible to choose any of these two crosses for the selection of cultivars with high capacity for nodulation and BNF, or for the search of molecular markers linked to the QTLs (quantitative trait loci) controlling BNF traits.

In the second experiment (J-200 × Embrapa 20 and Bossier × Embrapa 20), genetic effects were detected for the J-200 × Embrapa 20 and Bossier × Embrapa 20 crosses, for both NN trait and NDW/NN ratio; however, for SDW the effects were observed only in the cross J-200 × Embrapa 20 and, for NDW, exclusively in the Bossier × Embrapa 20 cross (Table 2). Additive *d* effects were detected in SDW for J-200 × Embrapa 20. Additive effects for NDW have also been reported in genetic studies for BNF, e.g. for chickpea (*Cicer arietinum*; Miller et al. 1986). Dominance *h* effects were only observed for the NDW/NN ratio in Bossier × Embrapa 20. For the variance components, *D* was detected for SDW

Table 3 Narrow-sense (h_n^2) and broad-sense (h_b^2) heritabilities and expected frequency of superior inbred lines (E) for biparental crosses using the restricted set of generations for the shoot dry weight and nodulation traits. Values taken as the reference for estimating E were 3.0 g for shoot dry weight, 120 for nodule number, 300 mg for nodule dry weight and 4.0 mg/nodule for the ratio of nodule dry weight and nodule number

	J-200 × Embrapa 133	Bossier × Embrapa 133	J-200 × Embrapa 20	Bossier × Embrapa 20	J-200 × Bossier	Embrapa 20 × Embrapa 133
Shoot dry weight (SDW, g plant^{-1})						
h_n^2	–[a]	–	0.59	0.68	0.74	0.77
h_b^2	–	–	0.53±0.01	0.68±0.004	0.67±0.005	0.61±0.007
E	–	–	0.11	0.18	0.11	0.30
Nodule number (NN, no. plant^{-1})						
h_n^2	–	–	–	0.65	0.49	–
h_b^2	0.28±0.023	0.32±0.02	0.30±0.02	0.58±0.008	0.42±0.015	0.13±0.033
E	–	–	0.03	0.24	0.05	0.04
Nodule dry weight (NDW, mg plant^{-1})						
h_n^2	–	–	0.49	0.71	–	–
h_b^2	–	0.25±0.025	0.54±0.009	0.70±0.004	0.42±0.015	0.26±0.024
E	–	–	0.14	0.24	0.11	0.05
Nodule dry weight/Nodule number (NDW/NN, mg nodule^{-1})						
h_n^2	–	–	0.39	–	–	–
h_b^2	–	–	0.47±0.012	0.27±0.023	0.24±0.025	0.20±0.028
E	–	–	<0.01	–	0.08	–

[a] No satisfactory model was found

and NDW in both crosses, for NN just in Bossier × Embrapa 20 and for the NDW/NN ratio in J-200 × Embrapa 20. Therefore those cultivars could be selected based on the broad-sense heritability (h_b^2). Dominance (*H*) was detected exclusively in Bossier × Embrapa 20 for the NDW/NN. Finally, effects of the interaction genotype × microenvironment (*E1*, *E2*) were detected for the ratio NDW/NN for both crosses (Table 2).

In the third experiment (J-200 × Bossier and Embrapa 20 × Embrapa 133), additive (*d*) effects were observed for both crosses when the NN trait and NDW/NN ratio were considered, while for SDW and NDW the effects were observed just in the cross of Embrapa 20 × Embrapa 133 (Table 2). Dominance (*h*) effects were also observed for SDW, NDW and the NDW/NN ratio exclusively in Embrapa 20 × Embrapa 133. Dominant × dominant interaction effects (*l*)were not verified for neither one of the crosses. In relation to the variance genetic components, additive (*D*) effects were observed in both crosses for SDW and only in J-200 × Bossier for NN, while for dominance (*H*) effects were observed in Embrapa 20 × Embrapa 133 for NN and NDW. Effects of genotype × microenvironment (*E1*, *E2*) were also significant for the NDW/NN ratio for both crosses.

The estimates of narrow and broad-sense heritabilities and of the predictions of superior inbred lines are shown in Table 3. Differences in the values of both types of heritabilities were detected, since for the narrow-sense the estimations were based on the parameters adjusted to the variances of all generations of the restricted pool of generations, while in the broad-sense the estimations were based exclusively on the values of F3 families.

In the first experiment (J-200 × Embrapa 133 and Bossier × Embrapa 133), it was not possible to estimate the prediction of superior inbred lines for any of the analyzed parameters, and broad-sense heritabilities (h_b^2) were observed only for NN in both crosses and for NDW in Bossier × Embrapa 133, indicating that there was a predominance of the environmental variance.

In the other two experiments, the values of NN narrow-sense heritabilities were generally larger than those of h_b^2 for the SDW and NN traits (Table 3). For SDW, h_n^2 values ranged from 0.59 (J-200 × Embrapa 20) to 0.77 (Embrapa 20 × Embrapa 133), while for NN narrow-sense heritabilities ranged from 0.49 (J-200 × Bossier) to 0.65 (Bossier × Embrapa 20). Although these values are considered of intermediate magnitude, they are higher than other h_n^2 for NN reported in the literature for other N_2-fixing legumes, such as chickpea (0.55; Miller et al. 1986), common bean (*Phaseolus vulgaris*; 0.30; Miranda et al. 1987) and mung bean (*Vigna radiata*; 0.22–0.46; Miller and Fernandez 1988). The broad-sense heritabilities for SDW ranged from 0.53 (J-200 × Embrapa 20) to 0.68 (Bossier × Embrapa 20), similar to the values of 0.57–0.62 reported by Ronis et al. (1985) for the soybean. Values of h_b^2 for NN ranged from 0.13 (Embrapa 20 × Embrapa 133) to 0.58 (Bossier × Embrapa 20) (Table 3).

For NDW, h_n^2 values varied from 0.49 (J-200 × Embrapa 20) to 0.71 (Bossier × Embrapa 20), while h_b^2 values ranged from 0.26 (Embrapa 20 × Embrapa 133) to 0.70 (Bossier × Embrapa 20; Table 3). In the literature, there are reports of h_b^2 values for NDW in soybean ranging from 0.54 to 0.67 (Greder et al. 1986). The higher values of heritabilities obtained for SDW in the cross of

Embrapa 20 × Embrapa 133 and for NN and NDW in the cross of Bossier × Embrapa 20 were expected, since the additive genetic effects predominated in the models of means and variances for both variables.

For the NDW/NN ratio, the estimations of heritability had low magnitude when compared to the other tested variables, and a h_n^2 value of 0.39 was obtained exclusively for J-200 × Embrapa 20, while h_b^2 values ranged from 0.20 (Embrapa 20 × Embrapa 133) to 0.47 (J-200 × Embrapa 20; Table 3).

Table 3 also shows that the highest expected frequency of superior inbred lines for SDW was for the cross Embrapa 20 × Embrapa 133, while for NN and NDW parameters the highest potential was detected in the cross Bossier × Embrapa 20.

Some conclusions may be drawn from this study. The means of the first experiment with the crosses of J-200 × Embrapa 133 and Bossier × Embrapa 133 were lower than in the two other experiments, resulting in a lower level of expression of the genes controlling the evaluated traits. Therefore, the significance of mean and variance genetic effects was less frequent and occasionally observed only for NN and NDW. In this experiment, there was a predominance of environmental variance.

In experiments 2 (J-200 × Embrapa 20 and Bossier × Embrapa 20) and 3 (J-200 × Bossier and Embrapa 20 × Embrapa 133), there was a predominance of additive effects *d* and/or *D* for the majority of the tested variables, except for NDW in the cross J-200 × Bossier. Dominance effects *h* and/or *H* were present for all variables of the cross Embrapa 20 × Embrapa 133 and for the NDW/NN ratio in the cross Bossier × Embrapa 20 (Table 2). Narrow- and broad-sense heritabilities for NN and NDW traits were superior in the cross Bossier × Embrapa 20, that also showed a high value for SDW (experiment 2). The highest value of h_b2 for SDW was verified in Embrapa 20 × Embrapa 133 (experiment 3; Table 3). Therefore, the results indicate that these crosses would be adequate for use in breeding programs aimed at increasing nodulation and BNF. Furthermore, these crosses show a good potential for the mapping of QTLs related to nodulation and BNF. Effects such as epistasis of the additive × additive *i* type, and interaction genotype × microenvironment were less frequent. Indeed, *i* was observed only once, in experiment 1, for the trait NN of the cross Bossier × Embrapa 133 and *E1* and *E2* only occurred for the ratio NDW/NN in the second and third experiments (Table 2).

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