Variability of nodulation traits in Andean and Mesoamerican common bean gene pools

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Abstract – The objective of this work was to evaluate the genotypic diversity for nodulation in common bean (*Phaseolus vulgaris*) genotypes of Andean and Mesoamerican gene pools present in the core collections of Embrapa, in Brazil. Evaluations were carried out in two stages: the first one with 879 genotypes, taking the cultivar 'Ouro Negro' as reference; and the second one with 116 genotypes with greater nodulation, to identify those showing stability in nodulation in the two stages. *Rhizobium* strains were inoculated in pre-germinated common bean seed. Plants received weekly a nutrient solution without N, and nodulation was evaluated 35 days after planting. The percentage of genotypes that exceeded the reference cultivar was 22% for number of nodules, 46% for nodule dry weight, and 33% for one nodule dry weight. The combined analysis of data from the two evaluation phases showed wide differences among genotypes, and significant interactions between genotypes and stages. By the orthogonal contrasts analysis, the Mesoamerican genotypes showed a greater nodulation than the Andean ones. In both stages, twenty-six more stable genotypes were identified, most of them belonging to the Mesoamerican gene pool. There is a large variability in nodulation traits among the common bean genotypes present in the core germplasm collections of Embrapa, which suggests that there are genotypes with improved nodulation.

Index terms: *Phaseolus vulgaris*, biological nitrogen fixation, diversity, germplasm bank.

Variabilidade das características de nodulação em feijoeiro-comum dos conjuntos gênicos andino e mesoamericano

Resumo – O objetivo deste trabalho foi avaliar a diversidade genotípica quanto à nodulação em genótipos de feijoeiro-comum (*Phaseolus vulgaris*) pertencentes aos conjuntos gênicos andino e mesoamericano presentes nas coleções nucleares da Embrapa, no Brasil. As avaliações foram realizadas em dois estágios: o primeiro com 879 genótipos, tendo-se a cultivar 'Ouro Negro' como referência; e o segundo, com 116 genótipos de maior nodulação, para identificar aqueles com estabilidade de nodulação nos dois estágios. Estirpes de *Rhizobium* foram inoculadas em sementes de feijão pré-germinadas. As plantas receberam, semanalmente, uma solução nutritiva isenta de N, e a nodulação foi avaliada 35 dias após o plantio. O percentual de genótipos que superaram a cultivar-referência foi de: 22%, quanto ao número de nódulos; 46%, quanto à massa de matéria seca de nódulos; e 33%, quanto à massa de matéria seca de um nódulo. A análise conjunta dos dados das duas fases de avaliação identificou amplas diferenças entre genótipos e interações significativas entre genótipos e estágios. Na análise de contrastes ortogonais, os genótipos mesoamericanos apresentaram maior nodulação do que os andinos. Foram identificados 26 genótipos mais estáveis nos dois estágios, a maioria deles pertencente ao conjunto gênico mesoamericano. Há uma ampla variabilidade em caracteres relacionados à nodulação entre os genótipos de feijoeiro presentes nas coleções nucleares da Embrapa, o que é indício de que há genótipos com maior nodulação.

Termos para indexação: *Phaseolus vulgaris*, fixação biológica de nitrogênio, diversidade, banco de germoplasma.

Introduction

The genetic diversification of common bean (*Phaseolus vulgaris* L.) had two main centers of domestication, the Andean e Mesoamerican (Bitocchi et al., 2013) ones, which can be distinguished by plant morphology (Singh et al., 1991; Rana et al., 2015), as well as by molecular approaches (Bitocchi et al., 2013; Gaut, 2014). The Mesoamerican gene pool consists of small seed, with 100-seed weight of less than 25 g, and

all type of plant growth habits, while the Andean gene pool have middle seed, whose 100-seed weight ranges from 25 to 40 g, and big seed with 100-seed weight above 40 g, with plants growth habit types I, II, and III (Singh et al., 1991). Mesoamerican materials provided the genetic basis of most Brazilian commercial cultivars (Blair et al., 2013), and most of the landrace genotypes (Burle et al., 2010).

Due to an incomplete reproductive isolation, most crosses between the Mesoamerican and Andean gene pools generate F1 unfeasible families (Hannah et al., 2007). Epistasis for the pod number, seed number, and seed weight was observed in progenies derived from crosses between the Andean and Mesoamerican genotypes, and it is suggested as one of the determining factors in the failure of crosses between genotypes from different origins (Moreto et al., 2012). Therefore, the genetic variability from one cultivated gene pool has a limited use for improving quantitative traits in the other gene pool. As a consequence, the access to the available diversity within each of these two gene pools is required for identification of the genotypes with desirable alleles to be introduced into breeding programs (Singh, 2001; Rana et al., 2015).

Common bean is a leguminous plant that can obtain N through symbiosis with rhizobia, although such symbiosis is often considered insufficient to fulfill the entire crop demand (Graham et al., 2003; Fageria et al., 2014). Since common bean domestication and selection may have resulted on loss of genetic diversity over time (Singh, 2001), its ability to fix N_2 may also have been reduced, particularly across Brazil breeding efforts which have been conducted using N fertilization. The genetic diversity in collections conserved in the gene banks has helped breeders to identify eligible genotypes for potential sources of desirable genes (Blair et al., 2010; Rana et al., 2015), and the use of wild and weed common bean germplasm can offer a great potential to enhance the variation in the crop (Araújo et al., 1998; Acosta-Gallegos et al., 2007). However, since the selection of lines derived from crosses between the Andean and Mesoamerican gene pools of *P. vulgaris* is reported as problematic, the wild germplasm from within the same gene pool should be prioritized in breeding over the wild lines from the other gene pool (Acosta-Gallegos et al., 2007).

Nitrogen fertilization is one of the main factors inhibiting the development of symbiotic process in legumes, thus plant assessments under conditions of low N availability are recommended to provide an appropriate selection pressure for highly effective nodulation and N_2 fixation (Kraiser et al., 2011). Nodulation is the main plant trait related to the ability for high N_2 fixation in common bean (Graham et al., 2003; Lazali et al., 2016), and increased nodulation is an important and heritable component of symbiosis for selection of bean lines capable of fixing more N_2 (Pereira et al., 1993).

Many efforts have been made for screening new microorganisms with improved symbiotic capacity, but the assessment of genotypic variation in host plant also assumes a paramount importance (Graham et al., 2003; Akter et al., 2014). The exploitation of plant diversity by identifying genotypes presenting a high nodulation potential is advised for choosing parents for breeding programs aimed to increase the biological N_2 fixation of common bean. A large-scale phenotypic characterization provides important information for breeders (Rana et al., 2015), and the present study comprises one of the most extensive investigations focusing on nodulation traits in Brazilian common bean germplasm collections.

The objective of this work was to evaluate the genotypic diversity for nodulation in common bean genotypes of the Andean and Mesoamerican gene pools present in the core collections of Embrapa, in Brazil

Materials and Methods

Genotypes belonging to two core collections of Embrapa Arroz e Feijão were evaluated in response to inoculation of strains that are currently recommended as inoculants for the common bean crop: *Rhizobium tropici* Semia 4077 (Ciat-899) and Semia 4088;, and *R. freirei* Semia 4080. A group of 879 genotypes was evaluated, out of which 261 from the Mexico core collection, and 618 from the International Center of Tropical Agriculture (Ciat, Colombia) core collection. Mexico's core collection consists mostly of materials from pure lines, obtained by Embrapa Arroz e Feijão from the United States Department of Agriculture (USDA). In both collections, there were Mesoamerican and Andean materials. Both collections are currently kept in the Banco Ativo de Germoplasma de Feijão (*Phaseolus vulgaris*) (active common bean germplasm bank) at Embrapa.

The experiments were conducted in two stages in a greenhouse at Embrapa Arroz e Feijão, in

Santo Antônio de Goiás, GO, Brazil. The first stage consisted of screening 879 genotypes in greenhouse, in a randomized block design with three replicates, in eight successive sowings, from September 2011 to March 2012. In each of the eight sowings, 'Ouro Negro' was the cultivar used as reference, due to its ability to fix N_2 (Henson et al., 1993). In the second stage, 116 genotypes with a greater nodulation, selected at the first stage, were evaluated to identify those showing nodulation stability in the two phases. The experiment was carried out in a greenhouse, in a randomized block design with three replicates, from August to October 2012.

In both stages, seed were taken from a cold chamber at -10°C, and then they were scarified, packed in germitest paper humidified with sterile water, and kept in a growth chamber at 24°C. After radicle protrusion, five seed per pot were planted in 3 L pots filled with sterile sand and vermiculite (2:1 v:v). Seven days after planting (DAP), seedlings were thinned to three plants per pot. Each plant was treated with 1 mL of a mixture of three commercial *Rhizobium* strains grown in YM culture (Vincent, 1970), with 10^9 cells mL^{-1}. Once a week, 200 mL per pot of Norris nutrient solution without N was added, and irrigation was performed with sterile water every two days. During the development period, plants were kept in a greenhouse, where the temperature was monitored and regulated with a shading system. Plants were harvested at 35 DAP, and roots were separated from e shoots and carefully washed in running water. Nodules were detached and counted, oven-dried (65°C for 48 hours), and weighed. The one-nodule dry weight was obtained by the ratio between the dry weight and the number of nodules.

Data obtained from 'Ouro Negro', in each of the eight sowings, were subjected to the analysis of variance, in order to evaluate the experimental uniformity across sowing dates, which was confirmed by the lack of sowing effects on nodule traits. Further, the analysis of variance was performed for data of the eight sowings of nodulated genotypes and the cultivar 'Ouro Negro'. Tukey´s test, at 5% probability was used for grouping genotypes in nodulation classes. A scatter diagram displaying nodule dry weight and the number of nodules was drawn, and 116 genotypes were selected for the second evaluation. Based on information such as the genotype origin and the seed size, obtained from the databases of Ciat and Grin-Global (Global Plant Genbank Information Management System – USDA), the 116 genotypes were further related to the Andean or Mesoamerican gene pools.

In the second stage, for sowing and plant development, the same procedures previously described for the assessment of 879 genotypes were adopted. Data obtained from 116 genotypes were joined to the data of the same genotypes obtained in the first stage, and the analysis of variance was performed, considering a double factorial between stage and genotype; means of each stage of evaluation were compared by Student t test (LSD), at 5% probability. This combined analysis was used as an indicator of stability of materials with high nodulation in both evaluations. Further, the analysis of variance was performed for each stage separately, and the sums of squares were rearranged to perform an orthogonal contrast between the genotypes of the two gene pools by the F test. With the average values of nodule dry weight and the number of nodules obtained for 116 genotypes, in the two stages of evaluation, a scatter diagram was carried out for selection of the most promising genotypes.

Results and Discussion

In the first stage of evaluation, from a total of 879 evaluated genotypes, 686 ones presented some nodulation. These 686 nodulated genotypes and the reference cultivar 'Ouro Negro' showed large difference among genotypes (Table 1). The number of nodules per plant ranged from 2 to 646, nodule dry weight ranged from 1 to 601 mg per plant, and one nodule dry weight ranged from 0.10 to 13.24 mg (Figure 1). Differences between common bean genotypes for nodulation traits are often observed in greenhouse experiments, such as those reported by Akter et al. (2014) and Farid & Navabi (2015), who reported a similar magnitude of variation to that observed in the present work.

Considering that a strong nodulation is associated with improved symbiotic N_2 ability in common bean (Graham et al., 2003; Drevon et al., 2011; Lazali et al., 2016), the extensive evaluation of a wide number of accesses allows of the use of nodulation data as a faster way to genotype selection. Additionally, differences in shoot dry weight might not have a strict adherence to N_2 symbiotic rates, particularly in weedy genotypes that can show a poor adaptation to specific growth conditions, and sensitivity to photoperiod (Araújo et al., 1998).

The genotypes were allocated in nodulation classes, as follows: 148 genotypes (22%) were placed in higher classes than the reference cultivar 'Ouro Negro', for the number of nodules; 316 genotypes (46%) were higher than the reference cultivar for nodule dry weight (Figure 1); and 225 genotypes (33%) fall above 'Ouro Negro' for one nodule dry weight. From the scatter diagram of nodule dry weight and the number of nodules of the 686 nodulated genotypes, 116 genotypes with improved nodulation could be identified (Figure 2). Among these 116 genotypes, 53 Mesoamerican and 61 Andean genotypes were identified; however, for two genotypes, information was unavailable.

The orthogonal contrast analysis between the two gene pools showed that, in the second stage of evaluation, the Mesoamerican gene pool showed both higher nodule dry weight and one nodule dry weight than the Andean gene pool, whereas, in the first stage, the number of nodules and nodule weights were similar for both gene pools (Table 2). Akter et al. (2014) also found a higher nodulation ability in Mesoamerican than in Andean genotypes, whereas Farid & Navabi (2015) found a greater number of nodules and nodule weights in Andean genotypes; however, nodules were more active in Mesoamerican genotypes. By the magnitude of the mean squares of variation sources estimated in the analysis of variance, it was not possible to state one of these two gene pools as showing larger genotypic variability for nodulation traits.

Evaluating 158 common bean genotypes, Rodiño et al. (2011) have distinguished groups with large nodules, although they were few in number, which showed plants with higher shoot mass, and genotypes with small and numerous nodules, but with plants with lower shoot growth. Larger and heavier nodules are considered more important than a high number of

them, since many small nodules might not reflect a good N_2 fixation ability (Pereira et al., 1993). Moreover, the occupation of nodules by active bacteroids is also
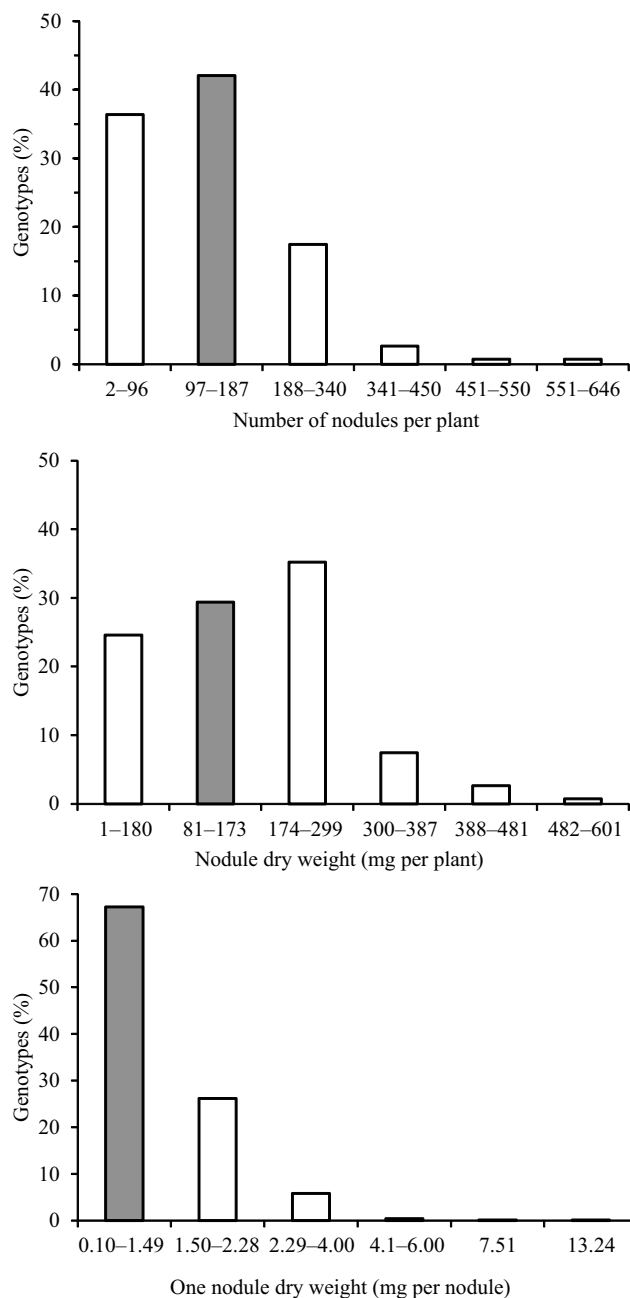

Figure 1. Percentage distribution of 686 nodulated common bean genotypes, according to the number of nodules per plant, nodule dry weight per plant, and one nodule dry weight, in greenhouse experiments; the gray column signals the group including the reference cultivar 'Ouro Negro'. The distribution classes were defined by Tukey's test, at 5% probability.

Table 1. Analysis of variance (values of mean square) of the number of nodules, nodule dry weight, and one nodule dry weight of 686 common bean genotypes, and the reference cultivar 'Ouro Negro', in eight sowings of bean genotypes in greenhouse.

Source of variation	DF	Number of nodules	Nodule dry weight	One nodule dry weight
Block	2	13091.89	20890.40*	0.16
Genotype	686	7050.63***	35641.41***	2.03***
Residue	1372	5854.00	6487.37	0.57
CV (%)		55.77	48.52	57.90

*, ***Significant at 5 and 0.1% probability, respectively, by the F test.

very important for the symbiosis (Arthikala et al., 2014). The number of nodules and the nodule dry weight are phenotypic characteristics considered as representatives for identifying quantitative trait loci (QTL) for biological N fixation (Santos et al., 2013). In some limiting conditions for plant growth, such as

P deficiency, large individual nodules can compensate for the decreased number of nodules (Lazali et al., 2016), underlying the relevance of nodule weight as a parameter of evaluation. Notwithstanding, the relationship between nodule size and N_2 fixation ability stands to be clearly established on common bean.

The combined analysis of variance showed significant differences for nodule traits among the 116 genotypes evaluated in two stages, as well as a significant interaction between genotypes and the evaluation stage (Table 3). The nodulation of genotypes was broadly lower in the second stage (Figure 2); however, the Andean genotypes showed a stronger reduction in the number and weight of nodules in this stage than the Mesoamerican genotypes (Table 2), suggesting a higher stability of nodulation in Mesoamerican genotypes.

In common bean breeding programs, the evaluation season is a major factor influencing the interaction between genotype and environment (Pereira et al., 2011). The time of sowing is considered an unpredictable environmental factor, and its effect has been most commonly assessed under field conditions (Rodiño et al., 2011; Torga et al., 2013). Considering that the present work took place in greenhouse conditions, the significant interaction between genotype and evaluation stage (Table 3) may have been affected by subtle environmental variations between the two stages. Terán & Singh (2009) also detected the interaction between year of sowing and different screening methods in the identification of bean genotypes resistant to white mold in greenhouse experiments.

The magnitude of the interaction between genotype and evaluation stage for nodule traits (Table 3) reinforces the adequacy of choosing the most stable genotypes across environments. By the combined analysis of data obtained from 116 genotypes and the scatter diagram of nodule dry mass and number of nodules (Figure 2), 26 promising genotypes could be selected for their high nodulation and repeatability in the two stages(Table 4). Out of these 26 genotypes, 10 belong to the Andean gene pool, 15 belong to the Mesoamerican gene pool, and one could be related to any gene pool (Table 5). Most of these genotypes had black (8) or red tegument (5). Indeterminate growth habit prevailed, appearing in 19 genotypes.

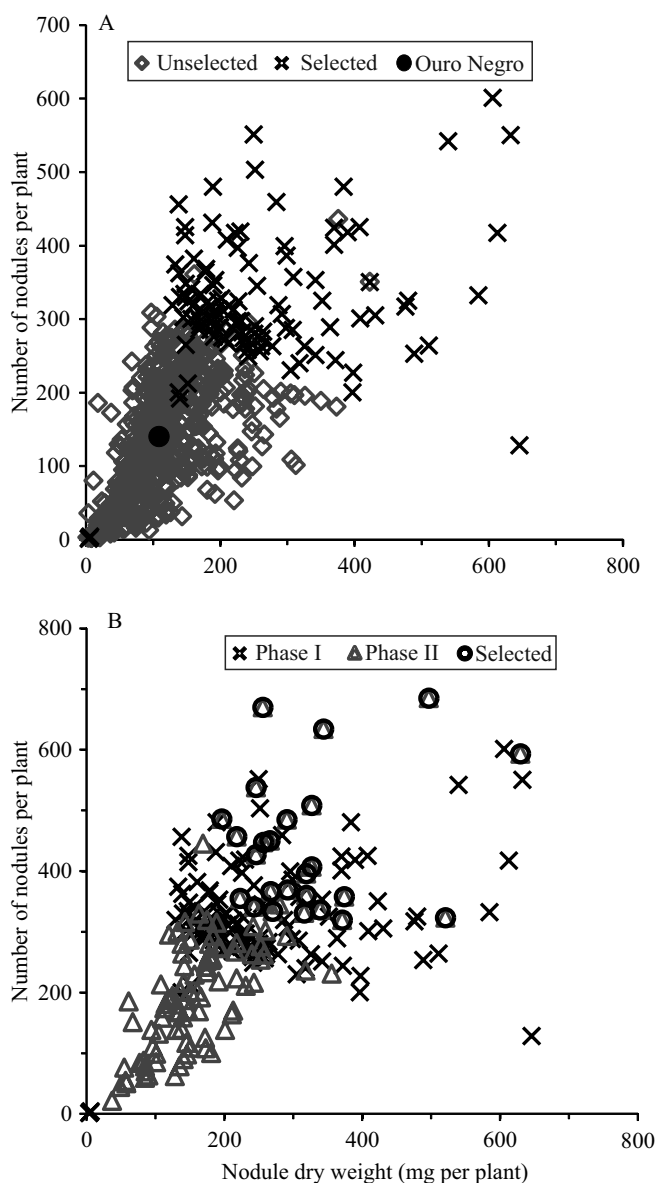The diversity of response obtained in the present study through *Rhizobium* inoculation shows the relevant

Figure 2. Number of nodules and nodule dry weight of 686 nodulated common bean genotypes in greenhouse experiments. A, The reference cultivar 'Ouro Negro', and the 116 genotypes selected for the second stage of evaluation were detached. B, Distribution of the number of nodules and the nodule dry weight of 116 common bean genotypes, in two stages of evaluation; the most promising genotypes were detached as selected ones.

Table 2. Contrasts between Andean and Mesoamerican groups of common bean genotypes for the number of nodules, nodule dry weight, and one nodule dry weight, in two evaluation stages in greenhouse[1].

Group	Stage I			Stage II		
	Number of nodules per plant	Nodule dry weight (mg per plant)	One nodule dry weight (mg)	Number of nodules per plant	Nodule dry weight (mg per plant)	One nodule dry weight (mg)
Mesoamerican	245a	319a	1.47a	188a	264a	1.46a
Andean	267a	320a	1.43a	193a	231b	1.25b

[1]Means followed by equal letters, in the columns, do not differ, by the F test, at 5% probability. Mesoamerican and Andean groups represent the averages of 53 and 61 genotypes, respectively.

Table 3. Analysis of variance (values of mean square) of nodulation traits of 116 common bean genotypes, in two evaluation stages in greenhouse.

Source of variation	DF	Number of nodules	Nodule dry weight	One nodule dry weight
Block	2	4.55*	5.26***	0.04
Genotype	115	4.43***	4.70***	0.73***
Phase	1	72.80***	89.30**	51.46***
Genotype x phase	115	3.16***	3.52***	0.72***
Residue	462	1.14	1.06	0.11
Coeficient of variation (%)		47.46	36.25	28.32

*, **, ***Significant at 5, 1, and 0.1% probability, respectively, by the F test.

Table 4. Number of nodules, nodule dry weight, and one nodule dry weight of the selected, promising common bean genotypes, after two evaluation stages in greenhouse[1].

Genotype	Core collection	Number of nodules per plant		Nodule dry weight (mg per plant)		One nodule dry weight (mg)	
		Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
CNF 0011234	Ciat	633a	497a	550a	685a	0.89a	0.73a
CNF 0011559	Ciat	585a	630a	332b	593a	0.57a	1.05a
CNF 0011239	Ciat	192a	344a	306b	634a	1.53a	0.54b
PI 209491	Mexico	409a	256a	301b	670a	0.74a	0.38a
PI 387865	Mexico	390a	327a	418a	508a	1.02a	0.64b
CNF 0011228	Ciat	384a	246a	480a	538a	1.25a	0.44b
CNF 0011026	Ciat	188a	291a	431a	485a	2.33a	0.60b
CNF 0011137	Ciat	170b	521a	291a	323a	1.72a	1.68a
CNF 0011075	Ciat	262a	266a	273b	450a	1.05a	0.60a
CNF 0011095	Ciat	222a	196a	417a	486a	1.88a	0.40b
CNF 0011240	Ciat	226a	257a	397a	447a	1.74a	0.59b
CNF 0011028	Ciat	364a	327a	289a	407a	0.96a	0.81a
PI 325750	Mexico	218a	218a	288b	457a	1.28a	0.48b
PI 313633	Mexico	187a	319a	299a	396a	2.09a	0.75b
CNF 0011086	Ciat	203a	374a	275a	358a	1.36a	1.05a
CNF 0011102	Ciat	308a	371a	285a	319a	0.93a	1.16a
CNF 0011015	Ciat	178a	339a	364a	336a	1.98a	1.08b
PI 312031	Mexico	145b	319a	302a	360a	2.22a	0.87b
CNF 0011252	Ciat	237a	292a	267a	369a	1.11a	0.79a
PI 309701	Mexico	191a	267a	290a	366a	1.43a	0.74b
CNF 0011027	Ciat	296a	280a	399a	345a	1.67a	0.88b
CNF 0010996	Ciat	190a	270a	346a	334a	1.81a	0.90b
CNF 0011053	Ciat	231a	243a	297a	341a	1.33a	0.75b
CNF 0011097	Ciat	278a	246a	263a	427a	1.07a	0.57a
PI 313495	Mexico	250a	223a	551a	355b	2.12a	0.67b
CNF 0011451	Mexico	251a	316a	280a	331a	1.13a	0.95a

[1]Means followed by equal letters, in the lines, do not differ, by Student t (LSD) test, at 5% probability.

Table 5. Some characteristics of the 26 most promising common bean genotypes for nodulation traits, selected after two stages of evaluation in greenhouse.

Genotype	Another identification	Place of collection	Gene pool
CNF 0011234	G22599	Congo	Andean
CNF 0011559	G2573	Ecuador	Andean
CNF0011239	G23568B	Peru	Andean
PI 209491	G18800	Costa Rica	Mesoamerican
PI 387865	W-941 d	Bolivia	Andean
CNF 0011228	G2486	Mexico	Mesoamerican
CNF 0011026	G10966	Mexico	Mesoamerican
CNF 0011137	G21178	Mexico	Mesoamerican
CNF 0011075	G9026	USA	Mesoamerican
CNF 0011095	G148	USA	Mesoamerican
CNF 0011240	G10843	Guatemala	Mesoamerican
CNF 0011028	G19497	Peru	Andean
PI 325750	G19187	Mexico	Mesoamerican
PI 313633	Narino 47	Colombia	Andean
CNF 0011086	G2445	Mexico	Mesoamerican
CNF 0011102	G23840	Mexico	Mesoamerican
CNF 0011015	G6861	Honduras	Mesoamerican
PI 312031	G2352 and G6745	Mexico	Mesoamerican
CNF 0011252	G22647	Congo	Andean
PI 309701	G18934 and G18934A	Mexico	Mesoamerican
CNF 0011027	G19036	Mexico	Mesoamerican
CNF 0010996	G10436	Portugal	Andean
CNF 0011053	G5725	Mexico	Mesoamerican
CNF 0011097	G2276B	Mexico	Andean
PI 313495	Negro Brilhante	Mexico	Andean
CNF 0011451			

role of the host genotypes in the process of biological N_2 fixation. Therefore, the diversity in genebanks should be intensively exploited towards achieving an improved contribution of symbiotic N to common bean crop. In common bean breeding programs, the evaluation across different seasons and years is very important for identifying the most stable genotypes (Pereira et al., 2011; Torga et al., 2013). Henceforth, the insertion of the most promising genotypes identified in the present work into specific breeding programs would require field experiments, in order to assess their agronomic performance and stability. Considering the predominant Mesoamerican genetic basis of the Brazilian cultivars (Blair et al., 2013), the difficulty of crossings between the two gene pools (Moreto et al., 2012), and the nodulation stability of Mesoamerican materials observed in this work, crosses between genotypes of Mesoamerican origin seem more advisable for enhancing nodulation of Brazilian common bean cultivars.

Conclusions

1. There is a large variability in nodulation traits among the common bean genotypes of the Andean and Mesoamerican gene pools, present in core germplasm collections of Embrapa Arroz e Feijão, with many genotypes showing a higher nodulation than the reference cultivar 'Ouro Negro'.

2. Genotypes of the Mesoamerican gene pool show a higher nodule dry weight than those of the Andean gene pool, and should be prioritized in breeding efforts to enhance nodulation of common bean cultivars.

Acknowledgments

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