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Implications of selection in common bean lines in contrasting environments concerning nitrogen levels

Isabela Volpi Furtini^{1*}, Magno Antonio Patto Ramalho² and Ângela de Fátima Barbosa Abreu³

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Abstract – Grain productivities of 100 bean lines were evaluated in the presence and absence of nitrogen fertilizer in order to identify those with high nitrogen use efficiency (NUE) and to determine the correlated response observed in a stressed environment following selection in a non-stressed environment. The genetic and phenotypic characteristics of the lines, as well as the response index to applied nitrogen, were determined. The average grain productivities at both locations were 39.5% higher in the presence of nitrogen fertilizer, with 8.3 kg of grain being produced per kg of nitrogen applied. NUE varied greatly between lines. Lines BP-16, CVII-85-11, BP-24, Ouro Negro and MA-IV-15-203 were the most efficient and responsive. The results showed that it is possible to select bean lines in stressed and non-stressed environments. It was inferred that common bean lines for environments with low nitrogen availability should preferably be selected under nitrogen stress.

Key words: *Phaseolus vulgaris* L., nitrogen use efficiency, correlated response, selection gain.

INTRODUCTION

A range of different types of cropping systems are available for the cultivation of the common bean (*Phaseolus vulgaris* L.). Although many small farmers do not apply modern technologies in crop production, a number of large rural businesses make use of novel irrigation methods, advanced agricultural techniques and modern implements.

Although *P. vulgaris* is a member of the family Leguminosae (Fabaceae), the quantities of nitrogen fixed is insufficient for the daily requirements of the plant (Cassini and Franco 2006, Brito et al. 2011). Thus, nitrogen supplementation to common bean cultures is essential in achieving increased yield. However, while many farmers employ fertilizers in crop production, others choose to cultivate their crops in the absence of such supplements. Considering that fertilizers represent a significant proportion of production costs (Skalsky et al. 2008), that the continuous use of N can cause environmental impacts (Hirel et al. 2007) and since farmers plant similar cultivars regardless of the cropping system used, it is important to develop bean lines that offer high nitrogen use efficiency.

The nitrogen use efficiency (NUE) of a crop may be

determined on the basis of either the grain yield produced per unit of nitrogen supplied, or the grain yield produced under conditions of low nitrogen availability (Lopes and Guilherme 2000, Hirel et al. 2007). Generally speaking, most breeding programs evaluate cultivars or progenies in the presence of nitrogen fertilizer since, under such conditions, productivity is high and the control of environmental variabilities is more efficient. Moreover, because the assessment of genotypic differences between cultivars and progenies is more straightforward, it is considered that heritability estimates can be determined more accurately (Ceccarelli et al. 1998, Emede and Alike 2012). However, there are contradictory reports concerning the efficiency of direct and indirect selection of cultivars and progenies. For example, some results obtained demonstrated that the selection under favourable conditions did not reveal gains expressed under unfavourable conditions (Banziger et al. 1997, Brancourt-Hulmel et al. 2005, Mandal et al. 2010, Weber et al. 2012). In contrast, some reports (Gallais et al. 2008, Anbessa et al. 2010) have shown that such indirect selection can be more efficient than direct selection. Atlin and Frey (1989), on the other hand, report that direct and indirect selection are equally efficient since there was a high

¹ Embrapa Arroz e Feijão, Rodovia MT 222, km 2,5, CP 343, 78.550-970, Sinop, MT, Brazil. *E-mail: isabela.furtini@embrapa.br

² Universidade Federal de Lavras (UFLA), Departamento de Biologia, Setor de Genética e Melhoramento de Plantas, CP 3037, 37.200-000, Lavras, MG, Brazil

³ Embrapa Arroz e Feijão, Current address UFLA

genetic correlation between oat lines cultivated under low and high nitrogen availability, and the responses in both environments were similar. The use of alternate direct and indirect selection procedures has been proposed by some researchers (Van Ginkel et al. 2001).

According to Falconer and Mackay (1996), indirect selection is advantageous when the square root of the heritability value (h_x) obtained under non-stressed conditions is larger than that obtained under stressed conditions (h_y), or when the genetic correlation between the two conditions (r_{xy}) is strong ($h_y < r_{xy} h_x$).

The most studies on nitrogen use efficiency (NUE) was performed with grasses, especially with crops of corn (Banziger et al. 1997, Presterl et al. 2003, Emede and Alike 2012, Weber et al. 2012) and wheat (Le Gouis et al. 2000, Brancourt-Hulmel et al. 2005). As the vast majority of common bean breeding programs in Brazil evaluates the progeny and/or lines in the presence of N, it is important to check whether the selection made under favorable conditions to culture, is also effective for stress conditions. Since information relating to these aspects of the culture of bean is limited, the objective of the present study was to identify bean lines presenting high NUE and to determine the correlated response of grain productivity observed in a stressed environment following selection in a non-stressed environment.

MATERIAL AND METHODS

The experiments were conducted in Lavras (lat 21° 14' S, long 44° 59' W and alt 919 m asl), on a Dystroferic Red Oxisol and in Ijaci (lat 21° 10' S, long 44° 55' W and alt 805 m asl), on a Red-Yellow Ultisol, both in the State of Minas Gerais, Brazil. The chemical characteristics of the soils at these two locations are presented in Table 1.

A total of 100 bean lines derived from the germplasm bank of the Universidade Federal de Lavras (UFLA) were evaluated. Most of these lines were of the carioca type and some were commercial cultivars originating from breeding programmes conducted at UFLA over the last 30 years. Each bean line was submitted to two separate, but adjacent, experiments. In the first experiment no nitrogen fertilizer was applied, whilst in the second experiment 100 kg ha⁻¹ of nitrogen [(NH₄)₂SO₄] was applied (1/3 before sowing, 1/3 20 days after sowing and 1/3 27 days after sowing). In both experiments, 80 kg ha⁻¹ of phosphorus (P₂O₅) and potassium (K₂O) were applied to the soil before sowing.

A 10 x 10 triple lattice design was employed. The plots consisted of two lines (2 m each) spaced at 50 cm from each

other and seeds were sown at a density of 15 seeds/m. The grain yields (kg ha⁻¹) of the bean lines were determined for each nitrogen level and location, and the results were subjected to individual and combined analysis of variance involving the two N levels per location as well as all levels and locations, according to the model:

$$y_{ljtu} = \mu + p_l + n_t + a_u + q_{j(tu)} + (pn)_{lt} + (pa)_{lu} + (na)_{tu} + (pna)_{ltu} + e_{ljtu}$$

in which y_{ljtu} is the observation relating to line l , repetition j , nitrogen level t , location u ; μ is the average value; p_l is the effect of line l ($l = 1, 2, 3, \dots, 100$); n_t is the effect of nitrogen level t ($t = 1, 2$); a_u is the effect of location u ($u = 1, 2$); $q_{j(tu)}$ is the effect of repetition j , nitrogen level t , location u ; $(pn)_{lt}$ is the effect of the interaction between bean lines x nitrogen level; $(pa)_{lu}$ is the effect of the interaction between bean lines x locations; $(na)_{tu}$ is the effect of the interaction between nitrogen levels x locations; $(pna)_{ltu}$ is the effect of the interaction between bean lines x nitrogen levels x locations; and e_{ljtu} is the experimental error $e_{ljtu} \sim N(0, \sigma^2)$.

Nitrogen level (presence or absence), location and the average value were considered as fixed effects, whilst the others were considered to be random effects. The genetic and phenotypic parameters were estimated from the expected mean square values according to the literature (Falconer and Mackay 1996, Bernardo 2002). The errors associated with the expected gains from selection by the expression proposed by Bridges et al. (1991) were also calculated.

The response index to applied nitrogen (α) was calculated from average grain yield values (kg.ha⁻¹) using the equation (Thung 1990):

$$\alpha_i = (N_{1i} - N_{2i})/Q$$

in which α_i is the response index of line i ; N_{1i} is the average grain yield of line i in the presence of nitrogen fertilizer; N_{2i} is the average grain yield of line i in the absence of nitrogen fertilizer; and Q is the amount of nitrogen applied (100 kg ha⁻¹).

RESULTS AND DISCUSSION

In experiments testing fertilizer levels it is advisable to use borders surrounding the plots. However, when 100 bean lines are evaluated, the plots would be large, and it would hardly be possible to evaluate homogeneous areas, particularly in terms of organic matter content. For this reason, two contiguous experiments were performed without borders surrounding the plots. With this strategy it was expected that the two experiments close to each other would differ predominantly due to the N application levels. The strategy was apparently appropriate, since the experimental precision

was good, when considering the experiments separately.

The differences between the N levels were significant ($P < 0.01$). In the mean of the two locations, the experiments with N fertilization produced 39% more than where no N was applied, ie, there was a mean grain yield increase of 826 kg ha⁻¹, which corresponds to 8.3 kg grain per kg N applied (Table 2). Nitrogen is the most required nutrient by most crops, including common bean. For this reason, N response to nitrogen in common bean can frequently be observed (Furtini et al. 2006, Binotti et al. 2007). In a total of 80 field experiments with common bean, the response to N application was positive in 64% of the trials (Vieira 2006). However, a comparison of the response between experiments is difficult because it depends on a number of factors, related to the environment as well as the genotype.

The interaction locations x N levels was also significant ($P < 0.01$). The response to N application was most significant in Ijaci, where grain yield increased by 11.1 kg grain per kg N (Table 2). The conditions of soil fertility at the experimental locations, although close to each other, were somewhat different (Table 1). It was therefore not surprising, that the response of the lines differed between locations.

For this kind of experiments the existence of genetic variability in the lines for the trait is essential. The differences between lines were significant in all experiments ($P < 0.01$). The lines tested had been bred in the last 30 years in the UFLA improvement program as well as in other breeding programs in Brazil. Differences in yield, as in fact observed, had therefore been expected.

Table 1. Chemical analysis of soil sampled from the layer 0 - 20 cm in the experimental areas in Lavras and Ijaci¹

Characteristics	Lavras		Ijaci	
	Values ²	Evaluation ²	Values ²	Evaluation ²
pH (water)	5.1	Regular acidity	5.2	Regular acidity
P (mg dm ⁻³)	13.6	Good	24.9	Very good
K (mg dm ⁻³)	70	Regular	108	Good
Ca ²⁺ (cmol _c dm ⁻³)	0.8	Low	1.3	Regular
Mg ²⁺ (cmol _c dm ⁻³)	0.3	Low	0.4	Low
Al ³⁺ (cmol _c dm ⁻³)	0.6	Regular	0.3	Low
H + Al (cmol _c dm ⁻³)	5.6	Good	4.5	Regular
Total bases (cmol _c dm ⁻³)	1.3	Low	2.0	Regular
Effective CEC (cmol _c dm ⁻³) ^{3/}	1.9	Low	2.3	Low
CEC at pH 7.0 (cmol _c dm ⁻³) ^{3/}	6.9	Regular	6.5	Regular
Base saturation (%)	18.6	Low	30.6	Low
Aluminium saturation (%)	32	Regular	13	Very low
Organic matter (dag kg ⁻¹)	2.2	Regular	1.9	Low
Residual P (mg L ⁻¹)	12.2	Good	20.5	Very good
Zn (mg dm ⁻³)	3.8	Very good	5.0	Very good
B (mg dm ⁻³)	0.4	Regular	0.3	Low
S (mg dm ⁻³)	59.4	Very good	0.3	Very good

¹ Chemical analyses were performed at the Departamento de Ciência do Solo, UFLA, Lavras, MG, Brazil, according to the methodology described by Empresa Brasileira de Pesquisa Agropecuária (1997).

² The values were interpreted following Alvarez et al. (1999).

³ Cation exchange capacity.

Table 2. Mean grain yield (kg ha⁻¹), relation between the yield with and without N application and N response index (α) at the evaluation locations

Locations	Mean grain yield		With N/ Without N	α
	With N	Without N		
Lavras	2529	1991	1.27	5.4
Ijaci	3301	2187	1.51	11.1
Mean	2915	2089	1.39	8.3

The genetic variability between lines was also shown by the estimates of genetic variance (σ_G^2) in the lines (Table 3). In all environments (locations and N levels), σ_G^2 differed from zero ($P < 0.05$).

The variability between lines can also be confirmed by the estimates of heritability (h^2). In all experiments, the lower limit of the confidence interval (CI) of the h^2 estimates was positive. It was also inferred that the h^2 estimates were similar under most conditions (Table 3).

It was often reported that h^2 estimates are lower in stressed environments due to the lower accuracy under such experimental conditions (Banziger et al. 1997, Ceccarelli et al. 1998, Brancourt-Humel et al. 2005, Emede and Alika 2012). However, it was observed that the h^2 estimates were similar in conditions with and without N stress (Table 2). Similar results were reported by Presterl et al. (2003). It can therefore be inferred, in principle, that the conditions for success with selection do not depend on the presence or absence of stress.

Estimates of the expected gains with selection (GS) were established for 10% of the best lines, at each N level. It appears that the estimates of GS were significant (Table 4), both with or without N application. It follows therefore that the success can be high, regardless of whether selection is performed with or without N stress.

It is questionable whether indirect selection under high N would result in gains for performance under low N. The correlated response to indirect selection only exceeds

direct trait selection, if the square root of h^2 , under N-stress conditions (h_y) is lower than the product of genetic correlation of line performance in both conditions by the square root of heritability (h_x) between lines in the presence of N ($r_{xy} h_x$) (Falconer and Mackay 1996). In this study, the h_y estimates always exceeded the product $r_{xy} h_x$. Under this condition, the estimates of correlated response by the selection in the presence of N and the response to environmental stress were all lower than in direct selection (Table 3). These results are similar to those reported by Banziger et al. (1997), Brancourt-Humel et al. (2005), Mandal et al. (2010) and Weber et al. (2012). These authors observed that if the goal is the selection of cultivars for N-poor environments, the selection must be performed under N stress to maximize the gain with selection.

The estimates of genetic correlation of line performance in environments with or without N application (r_G) were intermediate at both locations (Table 4). The estimates of correlated response ($RC_{y/x}$) by selection in the environment with N application was relatively high (Table 3). The relationship between $RC_{y/x}$ and the gain with direct selection in the stressed environment (GS_y) showed that the highest and lowest estimates of the gain with indirect selection accounted for 62% and 46% of the gain with direct selection, respectively.

Although the correlated response to indirect selection was lower than to direct selection, it was significant (Table 4). Some lines that performed well in stressed as well as unstressed environments could therefore be identified. Based

Table 3. Heritability estimates (h^2), genetic variance (σ_G^2), variance of the interaction lines x N levels (σ_{GN}^2), ratio between σ_{GN}^2 and σ_G^2 and genetic correlation (r_G) between the performance of lines with and without N fertilization, at the evaluation locations

Locations	h^2 (%)		σ_G^2		σ_{GN}^2	σ_{GN}^2/σ_G^2	r_G
	With N	Without N	With N	Without N			
Lavras	34 (5,53) ¹	58 (39,70)	1679.69 (1294.87, 2266.73)	5014.32 (3865.52, 6766.78)	1422.41 (1096.53, 1919.53)	0.74	0.6
Ijaci	52 (31,66)	49 (27,64)	3406.19 (2625.82, 4596.63)	1482.94 (1143.19, 2001.22)	1498.32 (1155.05, 2021.97)	1.58	0.6
Mean	43	54	2542.94	3248.63	1460.37	1.16	0.6

¹ Values in brackets indicate the estimates of the lower and upper limits of the confidence intervals, at 5% probability.

Table 4. Estimates of the expected selection gains with (GS_x) and without (GS_y) N application, the errors related to GS, the correlated response ($RC_{y/x}$) in the environment without N (y) by selection in the environment with N (x) and relation between $RC_{y/x}$ and GS_y , at the evaluation locations

Location	With N (x)			Without N (y)			$RC_{y/x}/GS_y$	
	GS_x	Error	GS_y	Error	$RC_{y/x}$			
	kg ha ⁻¹	--%--	kg ha ⁻¹	kg ha ⁻¹	--%--			
Lavras	209.50	8.29	85.15	472.45	27.73	90.60	10.94	0.46
Ijaci	368.45	11.16	84.05	236.60	10.82	58.20	6.68	0.62

on estimates of the response index to N application associated with line yields in the nitrogen-stressed environment, the lines were grouped into four categories, according to Blair (1993), namely:

Inefficient and non-responsive (INR): lines that produced less than the overall mean of the N- stressed experiments and had a α below the overall mean of the indexes; Inefficient and responsive (IR): lines that produced less than the overall mean of the N- stressed experiments, but α exceeded the overall mean of the indexes; Efficient and non-responsive (ENR): lines that produced more than the overall mean of the N- stressed experiments and had a α below the index mean; Efficient and responsive (ER): lines that produced more than the overall mean of N-stressed experiments and had a α above the general index mean.

The lines were distributed in 29% INR, 23% IR, 21% ENR and 27% ER (Figure 1). The mean grain yield of the lines PF2-53 and P5-9, classified as INR, was low and did not respond to N fertilization. The lines CVII-45-5, P1-103 and RC-II-2-19 however, classified as IR, responded to N application, but had low mean grain yields. The lines BP-16, CVII-85-11, BP-24, Ouro Negro, and MA-IV-15-203 performed best in the category ER. The performance of the lines Pérola, CVIII-6, CVII-215-10 and MA-I-2.5 was also interesting, since they produced high grain yields in the environment under N stress, although they responded poorly to N fertilization (Figure 1).

Implicações da seleção no feijoeiro efetuada em ambientes contrastantes em níveis de nitrogênio

Resumo – A produtividade de grãos de 100 linhagens de feijoeiro foi avaliada na presença e ausência de fertilizante nitrogenado visando identificar aquelas com maior eficiência no uso de nitrogênio (EUN) e estimar a resposta correlacionada em ambientes sob baixa disponibilidade de nitrogênio, pela seleção efetuada sem o estresse do nutriente. Estimaram-se os índices de resposta à aplicação de nitrogênio e os parâmetros genéticos e fenotípicos. Na média dos locais, a produtividade de grãos obtida com N foi 39,5% acima da obtida sem N, correspondendo a 8,3 kg de grãos por kg de N aplicado. A EUN variou entre as linhagens. As linhagens BP-16, CVII-85-11, BP-24, Ouro Negro e MA-IV-15-203 foram as mais eficientes e responsivas. É possível obter sucesso com a seleção em ambientes com e sem estresse de nitrogênio, contudo a seleção de linhagens para ambientes sob baixa disponibilidade de nitrogênio deve ser realizada preferencialmente nesta condição.

Palavras-chave: *Phaseolus vulgaris* L., eficiência na utilização de nitrogênio, resposta correlacionada, ganho com a seleção.

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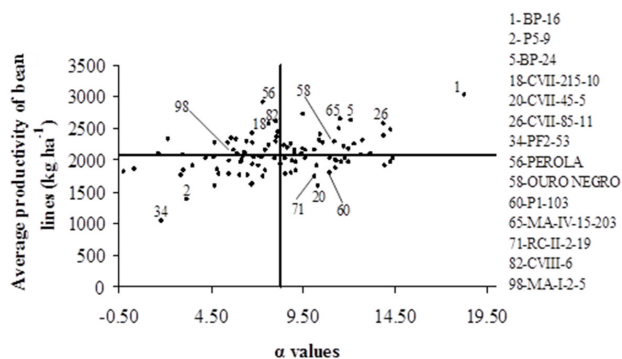


Figure 1. Response index to nitrogen (α) and mean grain yield of lines without N fertilization.

The performance of Ouro Negro and MA-I-2.5, in response to N, has been described elsewhere (Furtini et al. 2006). Firstly, it can be inferred that these lines make better use of available soil N than the others. Possibly these lines also have greater efficiency in biological nitrogen fixation with native soil strains, since the seeds were not inoculated. For the line Ouro Negro line this last aspect has already been reported in the literature (Franco 1995).

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