



NOTE

Genetics of phosphorus content in common bean seeds

Nerinéia Dalfollo Ribeiro^{1*}, Simone Saydelles da Rosa¹, Evandro Jost¹, Daniele Piano Rosa¹, Nerison Luís Poersch¹ and Sandra Maria Maziero¹

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ABSTRACT – The common bean is an important source of minerals and protein in human nutrition. The objective of this study was to investigate possible maternal effects on phosphorus contents in seeds of the common bean, to estimate the heritability in early hybrid generations for the phosphorus content character and to evaluate the potential for genetic improvement. The controlled crossings were performed among Pérola x Guapo Brillhante and TPS Nobre x Guapo Brillhante cultivars. Reciprocal F_1 , and F_2 generations as well as backcross populations were produced by each hybrid combination. The range of variation in phosphorus content among tested progenies was from 3.38 to 5.78 g kg⁻¹ dry matter, and no significant maternal effect was discovered. Narrow sense heritability was of an intermediate 65.54% to low 21.37% value. An increase of 19.17% was obtained for phosphorus content in the seeds using just the three parents tested in this study.

Key words: *Phaseolus vulgaris* L., maternal effect, heritability, early generation.

INTRODUCTION

Phosphorous is a mineral present in practically all foods and their deficiency is rare (Fennema 2000). However, when phosphorous deficiency is diagnosed in the human organism, it has been observed to cause neuromuscular, skeletal, hematological and renal abnormalities (Kathleen 1998). Phosphorous also plays a part in energy metabolism, since it is a constituent of adenosine triphosphate (ATP) and is therefore important in absorbing and transporting nutrients, in regulating protein activity and in the acid-base balance (Cozzolino 2007). For this reason, 0.8 to 1.2 g phosphorous is recommended, according to age, to supply the daily requirements for an adult (Kathleen 1998).

The common bean (*Phaseolus vulgaris* L.) has a high seed phosphorous content (Beebe et al. 2000, Mesquita et al. 2007) and genetic variability has been observed for phosphorous content character. Accessions with the range

of variation from 3.60 to 6.55 g kg⁻¹ dry matter (DM) were tested in Burundi (Barampama and Simard 1993) and from 5.86 to 8.84 g kg⁻¹ DM in Colombia (House et al. 2002). In Brazil, values ranging from 4.50 to 7.30 g kg⁻¹ DM were observed among 21 common bean genotypes in the Middle American gene pool (Mesquita et al. 2007). Common bean seeds from the Middle American gene pool normally present a higher phosphorous content in comparison with seeds of the Andean gene pool (Islam et al. 2002). The existence of genetic variability showed that selection is possible for the common bean germplasm with a high phosphorous content for diet enrichment. Another advantage in that its genotypes with high seed phosphorous contents also have an increase in grain yield (Silva et al. 2003).

The phosphorus content in seeds/grain can be increased by applying fertilizer to the soil (Silva and Vahl 2002) or by treating the seeds with P₂O₅ (Silva et al. 2003). However, other alternatives should be considered, since

¹ Universidade Federal de Santa Maria, Departamento de Fitotecnia, 97.105-900, Santa Maria, RS, Brasil. *E-mail: nerineia@hotmail.com

phosphorous is a non-renewable natural resource and, in Brazil, there are extensive areas of soils with low phosphorous availability and high capacity for retaining this mineral (Sfredo et al. 1997). Bearing this in mind, using genetic improvement to increase phosphorous content in common bean seeds is a low-cost option with significant social benefits, since 8 to 27 % of the phosphorous necessary to satisfy daily nutritional requirements in Latin America is obtained through the consumption of beans (Pennington and Young 1990).

The common bean seed is made up of the seed-coat, representing around 9 % dry matter, cotyledons at 90 % of total dry matter, and the embryonic axis at only 1 % of the total dry matter. The seed-coat contains more than 80 % of the entire calcium content and only 1.9 to 3.6 % of the phosphorus content (Moraghan et al. 2002). Hence, most phosphorus is in cotyledons. However, as the seed-coat represents maternal tissue (as opposed to the zygotic tissue of the cotyledons and the embryo), significant maternal effects are possible. Such an effect was observed in common bean seeds for the calcium content (Jost et al. 2009a). From the genetic point of view, the seed-coat and the embryo/cotyledons are tissues that represent different generations and the expression of the genotype is delayed by one generation (Ramalho et al. 2008).

No significant maternal effect was discovered for insoluble dietary fiber content in common bean seeds (Londero et al. 2009). For this reason, selection for high insoluble dietary fiber content in the common bean should begin on the F_2 seeds (cotyledons in F_2 generation). This observation alone has direct implications on the selection process and the progression of segregating populations in genetic improvement programs.

It was postulated that the zinc content in the navy bean seeds was controlled by a single dominant allele and additive gene action was responsible for most of the observed (Cichy et al. 2005). In this case, a dominant monogenic inheritance and high heritability for zinc content was verified. To phosphorus content in common bean seeds no genetic study has been described.

No maternal effects have been investigated on the expression of the phosphorus content in the common bean seeds and there is some doubt as to whether the phosphorus content presents qualitative or quantitative inheritance. This study was designed to investigate possible maternal effects on the expression of the phosphorus content, to estimate the heritability of the character and selection gains, and to evaluate the potential for genetic

improvement in high phosphorus common bean cultivars from the Middle American gene pool.

MATERIAL AND METHODS

Parents were selected based on their seed phosphorous content and agronomic value from among the accessions of the common bean maintained in the Bean Germplasm Bank of Santa Maria Federal University (UFMS), Brazil. The pattern of inheritance, including any possible maternal effects, was studied in hybrids obtained from the following crosses between three cultivars in the Middle American gene pool in: cv. Pérola x Guapo Brilhante and TPS Nobre x Guapo Brilhante. Pérola represented the Carioca type seeds (beige with brown streaks) and has an indeterminate growth habit with long guides (type III). Guapo Brilhante and TPS Nobre cultivars belonged to the black commercial group and have an indeterminate growth habit with a small to medium guide development (type II).

The crosses were made in a greenhouse, with emasculation of the flower bud using the interlacing method (Peternelli and Borém 1999). The F_1 ($P_1 \text{♀} \times P_2 \text{♂}$) and F_1 reciprocal ($P_2 \text{♀} \times P_1 \text{♂}$) seeds were produced for each hybrid combination in autumn-winter 2007. In spring-summer 2007, natural self-fertilization of F_1 hybrids produced reciprocal F_2 generations, while backcrossing of F_1 (♀) to P_1 (BCP_1) and F_1 (♀) to P_2 (BCP_2) produced the backcross generations. The process was replicated in autumn-winter 2008, so that the seeds of the F_1 , F_1 reciprocal, F_2 , F_2 reciprocal and backcross (BCP_1 and BCP_2) generations could be evaluated under the same cultivation conditions.

In all stages, the seeds were sown in 5 liter capacity plastic pots in a mix of soil + Plantmax® commercial substrate + carbonized rice husk, mixed in proportions 3: 1: 1. The soil was typical alitic Argisol (Argissolo Bruno-Acinzentado), with the following chemical composition: pH (H_2O): 5.8; organic matter: 1.9 %; zinc: 15.3 mg dm^{-3} ; potassium: 84 mg dm^{-3} ; calcium: 5.8 $\text{cmol}_c \text{dm}^{-3}$; magnesium: 2.4 $\text{cmol}_c \text{dm}^{-3}$; sulfur: 11.6 mg dm^{-3} . Fertility was corrected in accordance with the chemical soil analysis. Plants were watered daily to maintain the soil water contents close to the field capacity. Diseases and insects were controlled as necessary in order to avoid compromising the normal development of the common bean plants and to maintain the integrity of the flower buds.

The pods were harvested at maturity and the seeds were removed manually. The seeds were dried in a greenhouse (65-70 °C) until the average moisture content of 13 %.

Samples of dry seeds were ground in a micro-mill to the particle size of less than 1 mm. The phosphorous content of the raw bean flour was determined by nitric-perchloric digestion (HNO₃ + HClO₄, in proportion 3:1), as described by Miyazawa et al. (1999). The phosphorous content was measured using a UV-VIS spectrophotometer set to the wavelength of 660 nm.

The experiments were run in a randomized complete block design with five replications for the parents and the reciprocal F₁ generations, and in 24 replications for the reciprocal F₂ generations. To test the maternal effect hypothesis, a comparison was made between the means using the *t*-test at the 5 % significance level for contrasts P₁ vs P₂, P₁ vs F₁, P₂ vs F₁ reciprocal, F₁ vs F₁ reciprocal and F₂ vs F₂ reciprocal.

Estimates of the genetic parameters were obtained with the variances of parents P₁ and P₂ and F₁, F₂, BCP₁ and BCP₂ generations, based on the generation of cotyledons, for each hybrid combination. The broad-sense heritability

$$h_a^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

and the narrow-sense heritability

$$h_r^2 = \frac{\sigma_A^2}{\sigma_p^2}$$

were estimated using the backcross method (Warner 1952).

Heterosis in the F₁ generation was quantified as a percentage, for both heterosis relative to the mean for the parents

$$H \% = \frac{F_1 - P}{P} \times 100$$

and for heterobeltiosis

$$HT \% = \frac{F_1 - MP}{MP} \times 100$$

where

$$P = \frac{P_1 + P_2}{2}$$

and MP = best male parent. For predicting selection gains, we took a selection of 25 % of plants with F₂ seeds (cotyledons in F₂ generation) with the highest phosphorus content. The gain expected, taking into account selection and recombination of superior plants with F₂ seeds (cotyledons in the F₂ generation), was estimated using the formula: $\Delta G + DSxh_r^2$ and

$$\Delta G(\%) = \frac{\Delta G \times 100}{\bar{F}_2}$$

where is the selection differential expressed by $\bar{X}_s - \bar{X}_0$, where \bar{X}_s = average of selected plants with seeds in F₂ and \bar{X}_0 = average of plants with seeds in F₂. Genetic-statistical analyses were carried out using the software Genes (Cruz 2006).

RESULTS AND DISCUSSION

In reciprocal hybrids Pérola (P₁: 4.15 g kg⁻¹ dry weight - DW) x Guapo Brilhante (P₂: 4.85 g kg⁻¹ DW) and TPS Nobre (P₃: 4.16 g kg⁻¹ DW) x Guapo Brilhante (P₂: 4.85 g kg⁻¹ DW) the contrast P₁ vs P₂ was significant, demonstrating a genetic difference between the parents in phosphorus content (Table 1).

No significant difference was observed between reciprocal F₁ hybrids suggesting no significant maternal effect for phosphorus content in common bean seeds (Table 1). The common bean seed phosphorus content is dependent on the cotyledons, products of fertilization. This occurs because in common bean seeds more the 96 % phosphorus is concentrated in the embryo (cotyledons and embryonic axis) (Moraghan et al. 2002).

No significant difference was observed for the F₂ vs F₂ reciprocal contrast, reinforcing that the phenotype these seeds were similar in terms of phosphorus content and represented the expression of the genotype F₁ generation. Therefore, F₂ seeds showed embryo in the F₂ generation. For this reason, selection for high phosphorus content in the common bean seeds should begin on the F₂ seeds (cotyledons in F₂ generation), since ample genetic variability was observed in this generation. To dietary fiber content in the common bean seeds no significant maternal effect was discovered, therefore the selection the F₂ seeds, harvested the one plant, was efficient (Londero et al. 2009).

However, significant maternal effect was observed for the calcium (Jost et al. 2009a) and iron content in common bean seeds (Jost et al. 2009b). Since calcium and iron contents were dependent on the seed-coat, selection was begun only on the F₃ seeds (cotyledons in F₂ generation), when segregation was verified. Therefore, investigation of the existence of maternal effect for characters that confer nutritional and functional quality for the common bean was appropriate, since it will have direct implications on the selection process and the progression of segregating populations in improvement programs.

Phosphorus content ranged from 3.38 g kg⁻¹ DM (F₂ plant from Pérola x Guapo Brilhante hybrid) to 5.78 g kg⁻¹ DM (F₂ plant from TPS Nobre x Guapo Brilhante hybrid)

Table 1. Mean phosphorus content and respective standard deviations obtained in parents (P₁ and P₂) and in F₁, F₁ reciprocal, F₂ and F₂ reciprocal generations in Pérola x Guapo Brillhante and TPS Nobre x Guapo Brillhante hybrids and probability by the *t*-test for contrasts P₁ vs P₂, P₁ vs F₁, P₂ vs F₁ reciprocal, F₁ vs F₁ reciprocal and F₂ vs F₂ reciprocal

Parents and Generation	Phosphorus content (g kg ⁻¹ of DM)	
	Pérola x Guapo Brillhante	TPS Nobre x Guapo Brillhante
P ₁	4.15 ± 0.15	4.16 ± 0.32
P ₂	4.85 ± 0.20	4.85 ± 0.20
F ₁	4.80 ± 0.24	4.47 ± 0.41
F ₁ reciprocal	4.59 ± 0.25	4.48 ± 0.22
F ₂	4.22 ± 0.48	4.68 ± 0.51
F ₂ reciprocal	4.31 ± 0.53	4.26 ± 0.46
Contrasts		Probability
P ₁ vs P ₂	0.03*	0.36*
P ₁ vs F ₁	0.10*	53.43
P ₂ vs F ₁ reciprocal	11.19	2.39*
F ₁ vs F ₁ reciprocal	21.52	98.09
F ₂ vs F ₂ reciprocal	56.28	0.43*

* Significant at 0.05 probability by the *t*-test.

(Table 2). Similar values were obtained in the common bean genotypes evaluated in Burundi (Barampama and Simard 1993). Higher values were observed in the evaluation of accessions cultivated in Brazil (Mesquita et al. 2007) and Colombia (House et al. 2002). As the common bean seeds from the Middle American gene pool present higher phosphorous content in comparison with seeds of the Andean gene pool (Islam et al. 2002), the concentration ranged in the germplasm.

Transgressive segregation was observed in both hybrid combinations (Table 2). Among the F₂ plants examined, individuals with 5.78 g kg⁻¹ DM were identified, which represented an increase of 19.17 % in phosphorus content relative to the Guapo Brillhante parent (4.85 g kg⁻¹ DM). This was the first register of increased phosphorus content in the common bean seeds by genetic improvement. A similar result was obtained by zinc content in common bean seeds (Gelin et al. 2007, Blair et al. 2009).

A breakdown of phenotypic variance showed the predominance of genetic effects in relation to the environment in the Pérola x Guapo Brillhante hybrid, and a high broad-sense ($h_a^2 = 82.69\%$) and intermediate narrow-sense ($h_r^2 = 65.54\%$) heritability were observed. In this hybrid combination the additive variance constituted the higher fraction of the genetic variance, then the fixation of this character could be observed in advanced generations. In the improvement of autogamous plants, additive variance is of great importance since it does not segregate from generation to generation, making it possible to successfully

select it in segregating populations, since the selective gains will depend only on gametic variance (Carvalho et al. 2001). For this reason Pedigree or Single-Seed Descent (SSD) methods are recommended for progression of segregating populations. Therefore, in the TPS Nobre x Guapo Brillhante hybrid, we verified heritability estimates for broad-sense heritability of an intermediate magnitude ($h_r^2 = 59.46\%$) and narrow-sense heritability of low magnitude (21.37%), indicating difficulties for selection because the effects of environmental variance were also significant.

Phosphorus content in common bean seeds is believed to exhibit quantitative inheritance, because higher effects of environmental variance and the intermediate to low narrow-sense heritability were observed in this study (Table 2). Thus, we expected difficulties in making a selection of the character measured. A more in-depth study of the genetic effects and the number of genes that control phosphorus content could not be carried out in this study as the efficiency of hybridization was low and very low numbers of replications for generations F₂ and backcrosses (BCP₁ and BCP₂) were obtained.

Conventional heterosis of 6.69 % and heterobeltiosis of 15.67 % were obtained in the Pérola x Guapo Brillhante hybrid (Table 2). Thus, hybrid vigor was observed for phosphorus content in the common bean seeds and the dominance and superdominance hypotheses should be considered. As the common bean plant is self-fertilizing, heterosis is expected to be reduced by half in each self-

Table 2. Estimates of means, genetic parameters and prediction of phosphorus content selection gains in the common bean seeds of Pérola x Guapo Brillhante and TPS Nobre x Guapo Brillhante hybrids

Parameters	Phosphorus content (g kg ⁻¹ of DM)	
	Pérola x Guapo Brillhante	TPS Nobre x Guapo Brillhante
Mean	4.29	4.53
CVE (%)	0.31	11.06
Phenotypic variance (σ_p^2)	0.23	0.26
Environmental variance ($\sigma_{E F_2}^2$)	0.04	0.10
Genetic variance (σ_G^2)	0.19	0.15
Additive variance (σ_A^2)	0.15	0.05
Broad-sense heritability (h_a^2)	82.69	59.46
Narrow-sense heritability (h_r^2)	65.54	21.37
Heterosis (H %)	6.69	-13.03
Heterobeltiosis P ₁ (HT %)	15.67	-5.86
Maximum value in parents	5.08	5.08
Minimum value in parents	3.94	3.81
Maximum value in F ₂	5.31	5.78
Minimum value in F ₂	3.38	3.90
Selected plant in F ₂	8, 5, 7, 23, 17 and 24	13, 14, 11, 22, 21 and 7
Original mean in F ₂	4.22	4.68
Mean of selected plants	4.83	5.33
Selection differential (SD)	0.61	0.65
Selection gain (ΔG)	0.40	0.14
Selection gain (ΔG %)	9.47	2.97
Predicted mean after the first selection cycle	4.62	4.82

fertilizing generation, and for this reason, the F₃ generation may present lower means than that of F₂. In view of this, evaluation in advanced generations will be necessary to assess segregation for this character.

In relation to selection gains, assuming retention of 25 % plants with F₂ seeds (cotyledons in F₂ generation) with the highest phosphorus content, gains of 9.47 % and 2.97 % could be expected, with predicted means of 4.62 and 4.82 g kg⁻¹ DM, respectively, after the first selection cycle in the Pérola x Guapo Brillhante and TPS Nobre x Guapo Brillhante hybrids (Table 2). Therefore, early germplasm selection of common bean seeds with high phosphorus content could be effective in the development of common bean cultivars with a high nutritional value that could be used in foods to reduce the risk of phosphorus deficiency and lower the number of cases of malnutrition. This also could contribute to increase grain yield (Silva et al. 2003).

Since it was possible to increase phosphorous content in common bean seeds using genetic improvement, this strategy could have positive impacts on the economics of non-renewable natural resources, on the conservation of the environment and on keeping the population healthy. However, the bioavailability of phosphorous will need to

be assessed, since in legumes, this mineral is found mainly in the form of phytic acid, which is very difficult for the human digestive tract to absorb. Phytic acid is considered to be an anti-nutritional factor due to its capacity to form complexes with some minerals, rendering them unavailable and affecting their bioavailability (Cheryan et al. 1980). One of the main physiological functions of phytic acid in the plant is its role as a phosphorous reserve, representing around 60 to 97 % of total phosphorous (Ravindran et al. 1994). This can limit the nutritional quality of diets, especially in Latin America where 8 to 27 % of the phosphorous content necessary to satisfy daily nutritional requirements is obtained by consumption of the common bean (Pennington and Young 1990).

CONCLUSIONS

There is no maternal effect in the expression of the common bean seeds phosphorous content and early generation narrow-sense heritability of the character is between intermediate and low. It is possible to increase phosphorous content in common bean seeds by 19.17 % using genetic improvement.

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Genética do teor de fósforo em sementes de feijão

RESUMO - O feijão é uma importante fonte de minerais e de proteína para a nutrição humana. O objetivo desse trabalho foi investigar a existência de efeito materno na expressão do teor de fósforo em sementes de feijão, obter estimativas de herdabilidade em gerações precoces e avaliar o potencial do melhoramento para esta característica. Os cruzamentos dirigidos foram realizados entre as cultivares Pérola x Guapo Brillhante e TPS Nobre x Guapo Brillhante. As gerações F_1 , F_1 recíproco, F_2 , F_2 recíproco e retrocruzamentos foram obtidas para cada combinação híbrida. O teor de fósforo variou de 3,38 a 5,78 g kg⁻¹ de matéria seca e nenhum efeito materno significativo foi observado. Herdabilidade em sentido restrito de intermediária (65,54 %) a baixa (21,37 %) foi observada. Um incremento de 19,17 % foi obtido para o teor de fósforo em sementes de feijão, usando os três genitores testados neste estudo.

Palavras-chave: *Phaseolus vulgaris* L.; efeito materno; herdabilidade; seleção precoce.

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