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Genetic parameters of agronomic and nutritional traits of common bean (*Phaseolus vulgaris* L.) populations with biofortified grains

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Abstract

The objective of this study was to estimate genetic parameters for agronomic and nutritional traits in four common bean populations with biofortified grains for minerals, and to identify the superior population for these traits. Thus, four common bean populations were obtained by controlled crossings between parents contrasting for potassium (P_1), phosphorus (P_2), zinc (P_3) and copper (P_4). A total of 40 lines in $F_{6:8}$ generation of each population and nine parents were evaluated in two field experiments (rainy season of 2012 and dry season of 2013) in the state of Rio Grande do Sul, Brazil. The studied populations presented genetic variability for cycle, insertion of the first pod, grain yield, and potassium, phosphorus and zinc concentrations in grains. Heritability estimates and selection gain of intermediate and high magnitude were obtained for cycle, insertion of the first pod, grain yield, and potassium concentration in grains in P_2. In P_4, insertion of the first pod, grain yield and phosphorus and zinc concentrations in grains presented high heritability and selection gain estimates. From the tested crossings, it is possible to select common bean inbred lines biofortified for potassium, phosphorus and zinc and with high agronomic performance.

Keywords: agronomic performance; heritability; line x environment interaction; minerals; *Phaseolus vulgaris*; selection.
Abbreviations: P_{1}_segregating population for potassium, P_{2}_ segregating population for phosphorus, P_{3}_ segregating population for zinc, P_{4}_ segregating population for copper

Introduction

Brazil is a major producer of beans, wherein in the rainy season of 2012, 2.8 thousand tons of grains were produced in an area of 3.1 million hectares (CONAB, 2014). In this scenario, the development of bean cultivars with erect plant architecture, early cycle and high grain yield represents technological advantages for bean farmers. In addition, the possibility of adding nutritional value to the high agronomic performance of bean cultivars is a recent trend in breeding programs. Genetic biofortification of food crops is an important strategy to combat deficiencies of iron, zinc, vitamin A and E in humans (Prasad, 2012). Bean presents high mineral concentration in grains (Ribeiro et al., 2008; Tryphone and Nchimbi-Msolla, 2010; Silva et al., 2012; Ribeiro et al., 2013). Bean grains do not require milling, polishing, pearling or decortication in the processing, as cereal grains. Therefore, bean grains are high potential for mineral biofortification. Iron and zinc deficiencies affect about 40% and 33%, respectively, of the people in the world (The Word Bank, 2006). The potential of beans for biofortification for iron and zinc has been evaluated, and the obtained results are promising (Blair, 2013; Jost et al., 2013; Petry et al., 2015; Teixeira et al., 2015). Biofortification for potassium, phosphorus and copper has not been reported in the literature for beans. Biofortification for potassium is important since higher potassium intake causes decrease in blood pressure, reduces cardiovascular diseases, and enables prevent the development of diabetes (Intersalt, 1986; He and MacGregor, 2008).

Phosphorus and copper deficiencies are unusual in humans. However, symptoms as painful bones, irregular breathing, fatigue, anxiety, numbness, skin sensitivity and changes in body weight have been described in cases of phosphorus deficiency (Martínez-Ballesta et al., 2010). Copper deficiency can cause several hematological symptoms, such as normocytic, hypochromic anemia, leucopenia and neutropenia, and skeletal disturbances (Guerrero-Romero and Rodríguez-Morán, 2005; Huskisson et al., 2007). Currently, over two billion people worldwide suffer from “hidden hunger”, a term used to describe malnutrition of nutrients (Kennedy et al., 2003). In this scenario, the biofortification of beans for iron, zinc, potassium, phosphorus and copper is a promising practice to improve human’s health. Common bean inbred lines with high iron and zinc concentrations in grains were obtained by plant breeding (Blair, 2013; Jost et al., 2013; Pereira et al., 2014; Teixeira et al., 2015). Preliminary studies showed that it was possible to increase phosphorus and copper concentrations in common bean grains in 19.17 and 46.78%, respectively, in F_2 generation (Ribeiro et al., 2011; Poersch et al., 2013). Since common bean is self-fertilizing, is it expected that these values are reduced in each self-fertilizing generation. For this reason, the evaluation in advanced generations will be necessary to assess segregation for this trait. Since selection of superior common bean inbred lines for agronomic performance and mineral concentration in grains is efficient, it is necessary to know the magnitude of genetic parameters estimates. In common bean inbred lines, it was obtained high heritability for cycle (Faleiro et al., 2002), insertion of the first pod (Ribeiro et al., 2009; Jost et al., 2013), grain yield (Ribeiro et al., 2009; Torga et al., 2010; Jost et al., 2013), and zinc concentration (Teixeira et al., 2015). For potassium, phosphorus and copper concentrations in common bean, the genetic parameters estimates are restricted to early generations. Narrow-sense heritability was of high magnitude for phosphorus and copper concentrations in common bean grains in F_2 generations (Ribeiro et al., 2011; Poersch et al., 2013), and of low magnitude for potassium concentration (Poersch et al., 2011).

Genetic parameter estimates are important to identify the nature of the action of genes involved in the control of quantitative traits, and enables the evaluation of the efficiency of different breeding strategies for obtaining genetic gains and maintaining the genetic variability (Cruz and Carneiro, 2006). Genetic parameter estimates for potassium, phosphorus and copper concentrations in common bean inbred lines were not found in the literature. Therefore, the objective of this study was to estimate genetic parameters for agronomic and nutritional traits in four common bean populations with biofortified grains for minerals, and to identify the superior population for these traits.

Results

Analysis of variance for agronomic and nutritional traits

The efficiency of the simple lattice design compared to randomized block design varied from 99.88 (cycle) to 120.95% (phosphorus concentration) (Table 2), and can be considered low. Thus, to correct the effect of blocks within each replicate, variance analysis was carried out in randomized block design. For this, adjusted mean squares of treatments and effective error of the lattice were used.

Furthermore, variance of the experimental error for the two growing seasons was homogeneous. Thus, the joint variance analysis for all traits was carried out. Cycle, insertion of the first pod, grain yield, and zinc concentration in grains presented significant treatment × environment and line × environment interactions (Table 2). For these traits, parent × environment interaction was also significant, except for zinc concentration. For potassium and phosphorus concentrations, significant interaction was observed only for treatment × environment. On the other hand, for copper concentration in grains, the main effects and interactions tested were not significant in most cases; therefore, this mineral was not used to obtain estimates of genetic parameters. Considering the decomposition of line effect within population (L/P), significant differences were observed for all traits, except for copper concentration (Table 2). In addition, within each population, it was found that there is genetic variability among common bean lines for most populations and traits, except for copper concentration.

Genetic parameters of agronomic and nutritional traits in beans

Heritability estimates for cycle ranged from 49.7 (P_4) to 74.7% (P_2) (Table 3), and were considered of intermediate to high magnitude. These estimates correspond to narrow-sense heritability, since they were determined in $F_{6:8}$ generation, which presents high homozygosis.

Selection of common bean lines with differentiated cycle is promising in population 2 (P_2), considering the genetic parameters estimates obtained for cycle in magnitude and sign. This population had the highest heritability (h^2= 74.7%) and CVg (3.9%) estimates for cycle among the evaluated populations. CVg/CVe ratio was the closest to the unit in P_2, and the highest estimate of selection gain for cycle was obtained in P_2 (SG= -4.1%). In P_2, it is possible to select common bean lines with intermediate cycle, similar to parents cultivars with shorter cycle: Guapo Brilhante, TPS Nobre and TPS Bonito (Table 4). Also, it is possible to selected lines with longer cycle, compared to parents cultivars (Tables 3 and 4).

Heritability of the insertion of the first pod presented from intermediate (56.4% in P_3) to high magnitude (71.1% in P_4) (Table 3). The highest genetic variance values for the insertion of the first pod were found in P_2 and P_4. These populations also presented the highest heritability (h^2= 69.4 and 71.1%) and selection gain (SG= 13.3 and 12.8%) estimates for the insertion of the first pod, indicating favorable conditions for selection. For grain yield, it was obtained high heritability estimates, of 62.6 to 81.5% (Table 3). In P_1, P_2 and P_4 populations, it was obtained selection gain estimates superior to 23.2% for grain yield. In these three populations, the mean of the five lines selected for grain yield was higher than that of Pérola cultivar, which was the parent with the highest grain yield (Table 4). Potassium concentration in common bean grains in $F_{6:8}$ generation had low to intermediate heritability (h^2= 28.8 to 59.1%) (Table 3). The highest heritability and selection gain values obtained for potassium concentration were observed in P_2 (h^2= 59.1% and SG= 6.1%), indicating greater chances of success if selection is carried out in this population. However, when considering the range of minimum and maximum values for potassium concentration in grains, it is possible to select common bean lines with high potassium concentration in any of the populations evaluated (Table 3). The heritability of phosphorus and zinc concentrations (h^2= 23.9 to 61.9%, and h^2= 21.8 to 64.3%, respectively) varied from low to high magnitude (Table 3). P_4 is the most suitable population for selection of common bean lines with biofortified grains for phosphorus and zinc, due to higher heritability and selection gain obtained for these traits.

Discussion

The common bean lines responded differently to changes in farming environments, because a significant treatment × environment, line × environment, and parent × environment interactions were observed for cycle, insertion of the first pod and grain yield. Significant genotype × environment interaction was previously described for cycle (Ribeiro et al., 2004), insertion of the first pod (Moura et al., 2013), grain yield (Ribeiro et al., 2008; Moura et al., 2013; Ribeiro et al., 2013), and zinc concentration (Cichy et al., 2005; Ribeiro et al., 2008; Silva et al., 2012) in experiments that evaluated bean lines performance. This represents difficulties in the selection of superior lines for traits that confer high agronomic performance and nutritional quality to beans. For this, breeding programs need to evaluate inbred lines in different environments before registering them as new common bean cultivars.

The evaluated inbred lines presented genetic variability for all traits, except for copper concentration in grains. However, in the F_2 generation, it was observed wide genetic variability for copper concentration in the recombinant obtained from crossings between the common bean cultivars IAPAR 44 × IAPAR 31, and between Diamante Negro × TPS Bonito (Poersch et al., 2013). In the present study, the variation coefficient obtained for copper concentration was of high magnitude (CV= 33.18%), providing low experimental accuracy, and it did not allow the differentiation between common bean inbred lines for this mineral. Therefore, from the crossings tested in this study, it is possible to select common bean inbred lines with differentiated cycle duration and which are superior regarding insertion of the first pod, grain yield, and potassium, phosphorus and zinc concentrations in grains.

Cycle presented intermediate to high heritability in common bean lines in $F_{6:8}$ generation. In inbred lines, heritability was estimated in narrow-sense, since variation corresponds to the variability resulting from additive gene action (Ribeiro et al., 2009). Similar heritability values were obtained for cycle determined in 154 common bean lines in F_8 generation (Faleiro et al., 2002). However, Jost et al. (2014) observed lower estimates values for cycle in 272 common bean lines in F_7 generation. The observed differences may be justified by the range of variation observed for cycle in the lines evaluated in different populations, by the experimental design (Faleiro et al., 2002), and by the method of advance of segregating generations (Jost et al., 2014).

Selection of common bean inbred lines with differentiated cycle in P_2 may be effective by fixing of this trait in the next selection cycles. This is because this population had the highest heritability estimate for cycle, which indicates that genetic variance was mostly formed by additive variance. Additionally, CVg presents the highest magnitude among populations evaluated for cycle. CVg provides an idea regarding the proportionality of the gain in relation to the mean in the case of selection (Faleiro et al., 2002), and it is an estimate that needs to be considered by the breeder. CVg/CVe ratio was the closest to the unit in P_2, indicating that in this population, environmental effects were less significant among the evaluated populations, which favors selection. The highest selection gain estimate for cycle was obtained at P_2. In this case, the negative value indicates a shorter cycle, which is of great interest for the Bean Breeding Program of UFSM.

Heritability estimates from 56.4% in P_3 to 71.1% in P_4 for insertion of the first pod were obtained. Therefore, insertion of the first pod showed little uncontrollable variation, which favors the selection of common bean inbred lines with higher values for this trait, which is in agreement with previous results obtained by Ribeiro et al. (2009) and Jost et al. (2013). Insertion of the first pod varied from 11.6 cm (P_3) to 22.6 m (P_1). Thus, it is possible to select common bean inbred lines with plant standard that allows mechanized harvesting in all the evaluated populations. This is because plants with insertion of the first pod superior to 9.3 cm favor the direct harvest of beans with combine harvester (Silva et al., 2009). Identification of common bean lines with higher insertion of the first pod is important for the breeding program for representing marketing advantages to beans producers.

Grain yield showed high heritability estimates in $F_{6:8}$ generation. High heritability values for this trait were not expected, since, according to Ramalho et al. (1993), grain yield in common bean presents quantitative inheritance. However, the magnitude of heritability estimates for grain yield obtained in the present study were similar to those values previously described for grain yield in common bean lines in F_7 generation (Ribeiro et al., 2009; Jost et al., 2014), and $F_{3:7}$ (Torga et al., 2010). Thus, despite the fact that it was used parents that had already been selected, and some cultivars have already been grown by farmers in the present study, genetic variability and selection gains ranging from 14.1 (P_3) to 25.4% (P_1) were obtained for grain yield.

In this study, genetic parameter estimates were obtained using the mean data obtained for each trait in two growing seasons; thus, greater accuracy in selecting superior lines is expected. Since the highest heritability and selection gain estimates for grain yield were recorded in P_1, P_2 and P_4, selection of superior lines is recommended in these populations, since there is a greater expectation of success.

Mineral concentration heritability in common bean grains varied from intermediate to high magnitude for potassium, phosphorus, and zinc in $F_{6:8}$ generation. Heritability estimates of potassium and phosphorus concentrations were not found in literature for common bean lines in advanced generations. However, at early generation, it was observed narrow-sense heritability estimates similar in magnitude for potassium (Poersch et al., 2011) and phosphorus (Ribeiro et al., 2011). High narrow-sense heritability was obtained for zinc concentration in common bean grains in F_2 generation (Cichy et al., 2005; Rosa et al., 2010) and $F_{3:6}$ generation (Teixeira et al., 2015).

The mean of the five lines superior for potassium in the P_1, P_2, P_3 and P_4 was higher than the values recorded by Poersch et al. (2011) for this mineral in the F_2 generation. Therefore, from the tested crossings, it is possible to select common bean inbred lines with high potassium concentration in grains. Common bean inbred lines with biofortified grains for potassium could be incorporated to the diet in order to decrease the risk of occurrence of cardiovascular diseases and kidney stone formation, and also to prevent the development of diabetes (Intersalt, 1986; He and MacGregor, 2008).

When considering the selection of common bean inbred lines with biofortified grains for phosphorus and zinc, P_4 is the most suitable population for selection of superior lines, due to higher heritability and selection gain obtained for these traits. However, mean values obtained for the five superior lines selected in each population were similar to the recombinants selected in the F_2 generation by Ribeiro et al. (2011) for high phosphorus concentration in common bean grains. Since phosphorus is important in bone and teeth mineralization, and participates in energy metabolism (Oliveira, 2007), the use of common bean inbred lines with biofortified grains for phosphorus could be important to prevent symptoms as painful bones, irregular breathing, fatigue, anxiety, numbness and skin sensitivity (Martínez-Ballesta et al., 2010).

In relation to zinc concentration, the mean of the five superior lines for concentration of this mineral was equal to 32 mg kg^{-1} of dry matter in P_2, P_3 and P_4, characterizing high zinc concentration in common bean grains according to the classification of Cichy et al. (2005) and Tryphone and Nchimbi-Msolla (2010). Common bean inbred lines with biofortified grains for zinc could be included in diets to improve the immune system efficacy and the sensibility of taste and smell senses (Guerrero-Romero and Rodríguez-Morán, 2005).

In the present study, selection of superior lines in population 2 favors to obtain new common bean cultivars with shorter cycle, higher insertion of the first pod, higher grain yield and higher potassium concentration in grains. Selection of superior lines in population 4 is promising for the development of new common bean cultivars with higher insertion of the first pod, higher grain yield and higher phosphorus and zinc concentrations in grains. Selection of common bean inbred lines with biofortified grains for potassium, phosphorus and zinc represents human health benefits due to the high nutritional value of this food. However, it is necessary to evaluate polyphenolic and phytate contents in the obtained populations, since these compounds, according to Ariza-Nieto et al. (2007) and Zhao and Shewry

Table 1. Genealogy, seed coat colour and institution of origin of the parents used in the controlled crossings.

Parent	Genealogy*	Seed coat colour	Origin Institution
IAPAR 44	BAC 2/RAI 12//Rio Tibagi/Cornell 49242	black	IAPAR
Guapo Brilhante	XAN 125 {BAT 336 (A83/ICA Pijao)}	black	EMBRAPA
BRS Expedito	CNF 5491/FT Tarumã	black	EMBRAPA
BRS Valente	[(Emgopa 201-Ouro/Ônix)//AN 512586]	black	EMBRAPA
Pérola	Mass selection in Aporé cultivar	Beige with brown streaks	EMBRAPA
TPS Nobre	FT 120/FT 84-1806//FT 84-424	black	FT Sementes
IAPAR 31	BAC 4/RAI 46//BAC 2/IGUAÇÚ/3/BAT/BAC 4	beige with brown spots	IAPAR
Diamante Negro	XAN 87/A 367	black	EMBRAPA
TPS Bonito	IAPAR 44/IAC Carioca	beige with brown streaks	FT Sementes

* /: single crossing; //: double crossing.

Table 2. Joint variance analysis containing mean squares, degrees of freedom (DF) and mean values for cycle (days), insertion of the first pod (IFP, cm), grain yield (YIELD, kg ha^{-1}), potassium (K, g kg^{-1} of dry matter - DM), phosphorus (P, g kg^{-1} DM), zinc (Zn, mg kg^{-1} DM) and copper (Cu, mg kg^{-1} DM) concentrations in grains of 160 common bean inbred lines and nine parents evaluated in rainy and dry seasons.

		Mean square						
Sources of variation	DF	Cycle	IFP	YIELD	K	P	Zn	Cu
Treatment (T)	168	51.55 **	18.68 **	468026.49 **	3.18 **	0.68 **	36.09 **	11.52 ^{ns}
Line (L)	159	52.49 **	18.09 **	480897.54 **	3.33 **	0.61 **	38.63 **	13.00 ^{ns}
Population (P)	3	218.45 **	171.00 **	1136523.58 **	6.31 **	0.28 ^{ns}	16.36 ^{ns}	25.72 ^{ns}
L/P	156	49.30 **	15.15 **	468289.35 **	3.27 **	0.62 **	39.06 **	12.75 ^{ns}
L/P_1 - Potassium	39	47.28 **	14.95 **	514792.52 **	2.70 *	0.74 **	21.18 ^{ns}	9.41 ^{ns}
L/P_2 - Phosphorus	39	74.21 **	17.58 **	496448.06 **	4.39 **	0.41 ^{ns}	36.01 **	9.25 ^{ns}
L/P_3 - Zinc	39	52.79 **	8.90 ^{ns}	242569.66 ^{ns}	3.55 **	0.57 **	51.42 **	17.65 *
L/P_4 – Copper	39	22.92 ^{ns}	19.17 **	619347.14 **	2.45 ^{ns}	0.76 **	47.63 **	14.69 ^{ns}
Parents (Pa)	8	35.69 **	21.78 **	164786.19 ^{ns}	5.12 **	0.46 ^{ns}	13.52 ^{ns}	14.11 ^{ns}
Group (Gr)	1	53.39 ^{ns}	52.58 **	636960.53 *	1.12 ^{ns}	0.27 ^{ns}	2.03 ^{ns}	33.65 ^{ns}
Environment (E)	1	112201.78 **	18449.89 **	11615546.69 **	88.20 **	16.88 **	9541.31 **	804.16 **
T × E	168	36.49 **	14.02 **	380798.59 **	1.92 **	0.36 **	23.73 **	16.03 ^{ns}
L × E	159	36.43 **	14.20 **	391097.75 **	2.14 ^{ns}	0.35 ^{ns}	23.50 *	14.38 ^{ns}
Pa × E	8	52.50 **	13.15 **	276812.44 *	1.21 ^{ns}	0.29 ^{ns}	15.74 ^{ns}	25.27 ^{ns}
Gr × E	1	40.61 ^{ns}	5.41 ^{ns}	1071961.33 **	0.03 ^{ns}	1.56 *	26.85 ^{ns}	95.58 *
Residue	288	14.62	4.93	116475.00	1.34	0.26	17.42	10.38
CV (%)(1)		4.20	13.38	18.99	7.90	11.69	15.72	33.18
EF (%)(2)		99.88	101.62	107.15	116.94	120.95	104.26	114.57

(1) Coefficient of variation. (2) Efficiency of the lattice design (mean value of rainy and dry season). * and ** Significant by the F test at 0.05 and 0.01 probability, respectively. ^{ns} Non-significant.

Table 3. Genetic parameters for cycle (days), insertion of the first pod (IFP cm), grain yield (YIELD, kg ha^{-1}), potassium (K, g kg^{-1} of dry matter - DM), phosphorus (P, g kg^{-1} DM) and zinc (Zn, mg kg^{-1} DM) concentrations in grains of four common bean populations (segregating populations for: potassium – P_1, phosphorus – P_2, zinc – P_3, and copper – P_4) evaluated in rainy and dry seasons.

Genetic parameters	Cycle				IFP				YIELD			
	P_1	P_2	P_3	P_4	P_1	P_2	P_3	P_4	P_1	P_2	P_3	P_4
σ^2_G(1)	8.4	13.9	9.5	2.8	2.6	3.1	1.3	3.4	94591.4	101155.2	37983.2	113015.5
σ^2_E(2)	13.7	18.8	15.0	11.5	4.6	5.4	3.9	5.5	136426.9	91827.2	90636.7	167285.1
h^2 (%)(3)	70.9	74.7	71.6	49.7	69.1	69.4	56.4	71.1	73.5	81.5	62.6	73.0
CVg (%)(4)	3.1	3.9	3.3	1.8	9.2	10.6	7.4	10.8	16.8	16.6	11.2	17.4
CVe (%)(5)	4.0	4.6	4.2	3.7	12.3	14.1	13.0	13.7	20.1	15.8	17.2	21.2
CVg/CVe ratio(6)	0.8	0.9	0.8	0.5	0.7	0.8	0.6	0.8	0.8	1.0	0.6	0.8
Minimum value	88.5	88.0	87.0	87.8	14.5	11.7	11.6	13.2	1267.4	1217.9	1286.5	1207.4
Maximum value	103.3	105.3	105.8	97.3	22.6	21.2	18.0	21.8	3040.8	2582.7	2298.3	2713.4
Mean 5 +(7)	89.5	89.5	88.5	88.5	20.3	19.6	17.6	20.2	2468.5	2460.3	2140.7	2549.9
SG (%)(8)	-2.9	-4.1	-3.0	-1.9	10.9	13.3	9.1	12.8	25.4	23.2	14.1	23.5

Genetic parameters	K				P				Zn			
	P_1	P_2	P_3	P_4	P_1	P_2	P_3	P_4	P_1	P_2	P_3	P_4
σ^2_G(1)	0.2	0.6	0.5	0.2	0.1	0.0	0.1	0.1	1.2	4.5	7.6	7.7
σ^2_E(2)	1.8	1.8	1.5	1.7	0.5	0.3	0.3	0.3	16.6	18.2	21.0	17.0
h^2 (%)(3)	34.2	59.1	56.4	28.8	38.5	23.9	52.0	61.9	21.8	49.6	59.1	64.3
CVg (%)(4)	2.9	4.9	4.2	2.5	5.4	3.2	5.6	7.0	4.0	7.7	10.1	10.1
CVe (%)(5)	8.0	8.1	7.3	7.9	13.6	11.3	10.8	11.0	15.2	15.5	16.8	15.0
CVg/CVe ratio(6)	0.4	0.6	0.6	0.3	0.4	0.3	0.5	0.6	0.3	0.5	0.6	0.7
Minimum value	15.1	15.1	15.3	15.5	4.1	4.2	4.1	4.2	22.6	22.4	20.3	20.5
Maximum value	18.2	19.6	18.8	18.6	5.8	5.6	5.6	5.9	32.9	34.6	36.0	35.5
Mean 5 +(7)	17.9	18.2	18.5	18.0	5.6	5.4	5.3	5.7	29.8	32.7	32.0	32.6
SG (%)(8)	2.6	6.1	5.2	2.2	4.8	2.2	4.2	10.4	2.4	9.5	10.0	11.9

(1)Genetic variance. (2)Environmental variance. (3)Heritability. (4)Coefficient of genetic variation. (5)Coefficient of environmental variation. (6)Genetic variation and environmental variation coefficients ratio. (7)Mean of the best lines within the population. (8)Selection gain (%) considering the selection of five lines. ^{ns} Non-significant by the F test.

Table 4. Mean values for populations and parents for cycle, insertion of the first pod (IFP), grain yield (YIELD), potassium (K, g kg $^{-1}$ of dry matter – DM), phosphorus (P), and zinc (Zn) concentrations evaluated in rainy and dry seasons.

	Mean values for population^{5}					
Population	CYCLE (days)	IFP (cm)	YIELD (kg ha^{-1})	K (g kg^{-1} of DM)	P (g kg^{-1} of DM)	Zn (mg kg^{-1} of DM)
P_1(1)	93.3 ab	17.5 a	1834.1 a	16.7 a	5.0	26.8
P_2(2)	94.7 a	16.5 b	1915.7 a	16.5 a	4.9	27.4
P_3(3)	92.4 ab	15.2 c	1747.3 a	17.0 a	4.9	27.4
P_4(4)	92.1 b	17.1 a	1929.8 a	16.8 a	4.9	27.5
Mean	93.1	16.6	1856.7	16.7	4.9	27.3

	Mean values for parent^{6}					
Parent	CYCLE (days)	IFP (cm)	YIELD (kg ha^{-1})	K (g kg^{-1} of DM)	P (g kg^{-1} of DM)	Zn (mg kg^{-1} of DM)
Guapo Brilhante	91.0 b	15.7 c	2177.9	14.6 b	4.2	29.1
TPS Nobre	91.0 b	17.7 b	1751.6	16.9 a	4.6	29.0
BRS Expedito	95.8 a	18.2 b	2084.6	18.0 a	5.2	29.2
IAPAR 44	94.3 a	16.9 b	2022.0	17.5 a	4.8	26.7
BRS Valente	96.8 a	18.6 b	1814.0	17.6 a	5.2	24.1
TPS Bonito	90.0 b	13.8 c	2108.0	17.4 a	4.7	28.8
IAPAR 31	95.5 a	19.5 a	1945.3	15.9 b	4.7	27.6
Diamante Negro	96.8 a	17.9 b	1719.5	17.9 a	5.3	25.2
Pérola	98.3 a	22.1 a	2317.9	16.3 b	4.7	28.0
Mean	94.4	17.8	1993.4	16.9	4.8	27.5

(1) Segregating population for potassium. (2)Segregating population for phosphorus. (3)Segregating population for zinc. (4)Segregating population for copper. [5]Means followed by the same letter in column do not differ by the Tukey test at 0.05 probability for segregating population. [6]Parents were grouped by the Scott Knott test at 0.05 probability

(2011), could reduce phosphorus, zinc and iron absorption and bioavailability.

Materials and Methods

Plant material and advanced generations

Four common bean populations with biofortified grains for potassium (population 1 - P_1), phosphorus (population 2 - P_2), zinc (population 3 - P_3) and copper (population 4 - P_4) were obtained by Poersch et al. (2011), Ribeiro et al. (2011), Rosa et al. (2010), and Poersch et al. (2013), respectively. For this, controlled crossings were carried out between the cultivars IAPAR 44 × Guapo Brilhante, and BRS Expedito × BRS Valente (P_1), Pérola × Guapo Brilhante, and TPS Nobre × Guapo Brilhante (P_2 and P_3), and IAPAR 44 × IAPAR 31, and Diamante Negro × TPS Bonito (P_4) (Table 1). Segregating generations were advanced using the Single-Seed Descent method (SSD). Seeds obtained in each of these plants were collected individually and stored in paper bags in a cold chamber (2°C and 70% relative humidity) for three months. In the rainy season of 2011, the 288 $F_{6:7}$ lines and nine parents were advanced in an experiment carried out in augmented block design with four replications. This experiment was carried out in the field area of the Bean Breeding Program of UFSM, located in the city of Santa Maria, state of Rio Grande do Sul, Brazil (latitude 29°42'S, longitude 53°49'W, at 95 m above sea level). The experimental plot consisted of one 2 m row, spaced 0.50 m apart, with 30 seeds.

Evaluation of inbred lines

$F_{6:8}$ lines were evaluated in two experiments carried out in the field area of the Bean Breeding Program of UFSM. The first experiment consisted of a 15 × 15 simple lattice design, with sowing in October 27, 2012 (rainy season), and the second experiment was carried out in a 13 × 13 simple lattice design, with sowing in March 02, 2013 (dry season). Each plot consisted of two 1m rows, spaced 0.50 m apart, with 15 seeds per meter. The rainy season of the 2012's experiment was composed of 225 treatments, with 212 $F_{6:8}$ lines, nine parent cultivars and four control cultivars (BRS Campeiro, Carioca, FTS Magnífico and Rio Tibagi). In the dry season of the 2013's experiment, 169 treatments were evaluated, being 160 $F_{6:8}$ lines and nine parent cultivars. In the second experiment, the number of treatments was reduced due to the lack of seeds availability of some lines. Common treatments were evaluated in the experiments, being 40 lines of each of the four populations and the parents. Climate is Cfa humid subtropical with hot summers and no defined dry season, according to the Köppen classification. Soil is classified as typicall alitic Argisol, Hapludalf, and was prepared in conventional manner. The amounts of applied fertilizers were in accordance with the needs identified in the soil chemical analysis, and totaled 275 kg ha^{-1} of the commercial formula 5-20-20 (5% nitrogen, 20% phosphate and 20% potassium) applied at furrow sowing, and 20 kg ha^{-1} of urea (45% nitrogen) applied in the growth stage of the first trifoliate leaf (V3). Thiamethoxam and Lambda-cyhalothrin (100 ml ha^{-1}) insecticides were used to control *Diabrotica speciosa* when it was found 20 insects per beating cloth. Weeds were mechanically removed (with a hoe) when necessary, in order to avoid competition with the crop. Disease control was not carried out. The cycle – number of days from emergence to maturity – was evaluated when half plus one of the plants in the experimental unit reached stage R9 (maturation), i.e., when pods lost their pigmentation and began to dry. Insertion of the first pod was measured in 10 plants randomly collected in the experimental unit. For this, the distance from ground level to the insertion of the first pod was measured, but it did not consider the lodging degree of the plant. The other plants of the experimental unit were manually harvested in order to determine grain yield, which was carried out by calculating the weight of the product obtained in all the experimental unit per hectare, at 13% of average humidity. Covariance – Ideal Stand method, described by Schmildt et al. (2001), was used for correction of grain yield due to the heterogeneity of plant stand. Mineral concentration was determined in random samples of 500 g grains of the $F_{6:8}$ line and of parents from each replication, in each growing season. Grains were ground in a micro-mill (Q298A21, Quimis, Diadema, São Paulo, Brazil), to produce particles smaller than 1 mm in diameter. In the raw bean flour obtained, it was used 0.5 g of the sample for nitric-perchloric digestion ($HNO3 + HClO4$, in a 3:1 ratio by volume), according to the method described by Jost et al. (2013). Minerals were determined by flame photometer (B642, Micronal, São Paulo, São Paulo, Brazil) for potassium concentration, and by UV-VIS spectrophotometer with a capacity of eight cuvettes (UV-Visible Spectrophotometer T60, PG Instruments Ltd., Leicestershire, UK), at wavelengths of 600 nm, for phosphorus concentration. Zinc and copper concentrations were quantified using an atomic absorption spectrophotometer (XplorAA DUAL, GBC Scientific Equipment Pty. Ltd., Braeside, Australia) at wavelengths of 213.9 nm and 324.8 nm, respectively.

Statistical analysis and estimates of genetic parameters

Data normality was verified using the Kolmogorov-Smirnov test at 0.05 probability. Individual variance analysis was carried out considering the simple lattice design. Efficiency of the simple lattice design in relation to the randomized block design was determined by the formula:

$$\mathrm{Ef} = (\mathrm{MS_R}/\mathrm{V_r}) \times 100$$

Where; $\mathrm{MS_R}$ is the mean square of residue analysis of lattice as randomized block design, and $\mathrm{V_r}$ is the mean effective variance of simple lattice analysis with recovery of interblock information. Joint variance analysis was carried out with the treatments common to both experiments, according to the randomized block design, but using adjusted treatment and the effective error of the simple lattice design to correct the effect of blocks. Effects of treatments and experimental error were considered as random, and effects of environment and genotype × environment interaction, as fixed. Homogeneity of residual variances was verified by the Hartley's maximum F test. Effect of treatments was decomposed in lines and parents, and hierarchical method was used to evaluate the effect of lines within each population. The following genetic parameters were estimated in each population: genetic variance, environmental variance, heritability (h^2), coefficient of genetic variation (CVg), coefficient of environmental variation (CVe), CVg/CVe ratio, minimum value, maximum value, mean of the five superior lines, and selection gain (SG). Heritability was calculated based on the components of variance, using the means of treatments, by the formula

$$h^2 = \frac{\hat{\sigma}^2_T}{\hat{\sigma}^2_{\bar{F}}} = \frac{\hat{\sigma}^2_T}{\hat{\sigma}^2_T + \frac{\hat{\sigma}^2_{TA}}{L} + \frac{\hat{\sigma}^2}{JL}}$$

where $\hat{\sigma}^2_T$ is the genetic variance among treatments, which corresponds the additive genetic variance; $\hat{\sigma}^2_{\bar{F}}$ is the phenotypic variance among means of treatments; $\hat{\sigma}^2_{TA}$ is the treatment x growing season interaction variance; $\hat{\sigma}^2$ is the residual variance; L is the growing season number; and J is the number of blocks.

Selection gain for each trait was estimated with the selection of the five lines with the best means for the trait, within each population, according to methodology described by Jost et al. (2013). The Tukey test at 0.05 probability was used for the comparison of means between populations. The Scott-Knott test at 0.05 probability was used to group parents. Analyzis and mean test were carried out using the Genes Software (Cruz, 2013).

Conclusion

Cycle, insertion of the first pod, grain yield and potassium concentration in grains in population 2 presents heritability and selection gain estimates of intermediate to high magnitude. Insertion of the first pod, grain yield and phosphorus and zinc concentrations in grains present high heritability and selection gain in population 4. From the crossings tested, it is possible to select common bean lines biofortified for potassium, phosphorus and zinc, and with high agronomic performance.

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