Genetics of the concentration of copper in common bean seeds

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ABSTRACT. The common bean (*Phaseolus vulgaris* L.) is an important source of copper in human nutrition. The objective of this research was to investigate the possible maternal effects on copper concentrations in the seeds of the common bean, to estimate the heritability and the selection gains in early hybrid generations for the characteristic of copper concentration and to evaluate the potential for genetic improvement. The controlled crossings were performed among IAPAR 44 x IAPAR 31 and Diamante Negro x TPS Bonito cultivars. The reciprocal F_1, and F_2 generations, as well as the backcross populations, were produced by each hybrid combination. The range of variation in the copper concentration among the tested progenies was from 1.07 to 7.75 mg kg^{-1} dry matter, and no significant maternal effect was discovered. A narrow-sense heritability was of an intermediate (51.98%) to high (61.99%) value. A 46.78% increase in the copper concentration was obtained in the seeds using only the four parents tested in this study.

Keywords: *Phaseolus vulgaris*, embryo, maternal effect, heritability, early generation.

Genética da concentração de cobre em sementes de feijão comum

RESUMO. O feijão comum (*Phaseolus vulgaris* L.) é uma importante fonte de cobre na nutrição humana. O objetivo deste trabalho foi investigar a existência de efeito materno para a concentração de cobre em sementes de feijão comum, estimar a herdabilidade e os ganhos na seleção em gerações precoces para o caráter concentração de cobre e avaliar o potencial para o melhoramento genético. Os cruzamentos controlados foram realizados entre as cultivares IAPAR 44 x IAPAR 31 e Diamante Negro x TPS Bonito. As gerações F_1, F_1 recíproco, F_2 e F_2 recíproco, bem como as populações de retrocruzamentos foram obtidas para cada combinação híbrida. A faixa de variação da concentração de cobre entre as progênies testadas foi de 1,07 a 7,75 mg kg^{-1} de matéria seca, e nenhum efeito materno significativo foi detectado. A herdabilidade em sentido restrito foi de intermediária (51,98%) a alta (61,99%). Um incremento de 46,78% foi obtido para a concentração de cobre nas sementes utilizando os quatro parentais testados nesse trabalho.

Palavras-chave: *Phaseolus vulgaris*, embrião, efeito materno, herdabilidade, geração precoce.

Introduction

The common bean (*Phaseolus vulgaris* L.) represents 50% of the grain legumes consumed worldwide (TALUKDER et al., 2010). The common bean grains contain a high concentration of copper in its seeds, and a genetic variability has been observed in the copper concentration character. In the germplasm of the common bean cultivated in Brazil, copper values ranged from 11.37 to 17.73 mg kg^{-1} of dry matter (DM) (MESQUITA et al., 2007).

Genetic breeding is a strategy often used to increase the mineral concentration in common bean seeds. Controlled crossing and selection among segregated progenies resulted in concentration increases of 19.1% in phosphorus (RIBEIRO et al., 2011), 33.6% in calcium (JOST et al., 2009a), 94.0%

in iron (JOST et al., 2009b) and 37.3% in zinc (ROSA et al., 2010) of the common bean seeds of early hybrid generations. There is no documentation concerning the use of genetic breeding to increase the copper concentration in common bean seeds. Increasing the common bean seeds' copper concentration is important for human health because this micronutrient is essential for the satisfactory operation of the immune system, carrying iron, metabolizing glucose and cholesterol, myocardial contractility and brain development and is also a component of various enzymes (CUNHA; CUNHA, 1998).

In common bean seeds, the embryo (cotyledons and embryonic axis) represents approximately 90.1% of the entire DM, while the seed coat consists of 9.9% of the entire DM (MORAGHAN et al., 2006).

These tissues accumulate minerals in different ways. The embryo contains 93% of the zinc, 76% of the iron and 16% of the entire calcium content of the whole seed (MORAGHAN; GRAFTON, 2002). The embryo originates from fertilization, and the seed coat represents the maternal tissue (RAMALHO et al., 2008). That these tissues are present in different generations of the same seed this will have implications for the selection of segregating populations in breeding programs.

No significant maternal effect was discovered for the zinc concentration in the common bean seeds (ROSA et al., 2010). Accordingly, most zinc is in the embryo and the F_1 generation seeds (embryo in the F_1 generation) exhibited the products of crossing between the parents. However, because most iron is in the seed coat, a significant maternal effect was observed for the iron concentration in the common bean seeds (JOST et al., 2009b). The iron concentration in the seed coat varies from 47.8% to 83.2% of the total DM of seeds of the common bean cultivars grown in Brazil (RIBEIRO et al., 2012). In this case, the appearance of the genotype is delayed for one generation. As the iron concentration was dependent on the seed coat, the selection for high iron in the common bean seeds begins with the F_3 seeds (embryo in F_2 generation) (JOST et al., 2009b).

A maternal effect on the copper concentration in the common bean seeds has not yet been investigated, and there is doubt as to whether the copper concentration displays a qualitative or quantitative inheritance. The objective of this work was to investigate the possible maternal effects on the copper concentration of the common bean seeds, as well as to estimate the heritability and selection gains in the early hybrid generations and to evaluate the potential for genetic improvements in high copper common bean germplasm.

Material and methods

The copper concentration inheritance pattern was studied in hybrids obtained from crosses between four cultivars from the Mesoamerican gene pool: IAPAR 44 x IAPAR 31 and Diamante Negro x TPS Bonito. These crosses were produced in a greenhouse at the Plant Science Department of the Federal University of Santa Maria (UFSM) in Santa Maria, Rio Grande do Sul State, Brazil. The seed mass of the cultivars varied from 22 to 23 g per 100 seeds. The cultivars were selected from common bean accessions of the UFSM Bean Germplasm Bank based on their different seed copper concentration and their agronomic value. IAPAR 44

and Diamante Negro cultivars represented the black commercial group and have an indeterminate growth habit with a small to medium guide development (type II). Also, IAPAR 31 and TPS Bonito cultivars belonged to the Carioca type seeds (beige with brown streaks) and have an indeterminate growth habit with long guides (type III).

An interlacing method with emasculation of the flower bud was used to obtain 50 F_1 ($\female$ P_1 x $\male$ P_2) and 50 F_1 reciprocal ($\female$ P_2 x $\male$ P_1) seeds for each hybrid combination in the fall/winter of 2008. In the spring/summer of 2008, natural self-fertilization of the F_1 and F_1 reciprocal plants produced the 48 F_2 and 48 F_2 reciprocal generations, while backcrossing of the F_1 ($\female$) to the P_1 (BC_1) and F_1 ($\female$) to P_2 (BC_2) produced the 36 backcrossed generations. This process was repeated in the fall/winter of 2009, meaning that the seeds of the F_1, F_1 reciprocal, F_2, F_2 reciprocal and backcrossed (BC_1 and BC_2) generations could be evaluated under the same cultivation conditions.

The seeds were sown in plastic pots with a 5 L capacity with two plants per pot. Pots were filled with soil, carbonized rice husks and Plantmax® commercial substrate that was mixed at a 2:1:1 ratio according to volume. The soil was typical alitic Argisol, Hapludalf with the following chemical composition: pH (H_2O): 6.5; organic matter: 2%; phosphorus: 14.4 mg dm^{-3}; potassium: 40 mg dm^{-3}; sulfur: 17.6 mg dm^{-3}; calcium: 5.2 $cmol_c$ dm^{-3}; magnesium: 2.1 $cmol_c$ dm^{-3}; copper: 0.2 mg dm^{-3}; zinc: 0.6 mg dm^{-3}.

Soil fertility was corrected, and nitrogen was applied to the surface based on the chemical soil analysis. Plants were watered daily to maintain the soil water content close to that of field capacity. Plant disease and insect infestation were controlled as necessary to avoid compromising the normal development of common bean plants and to maintain the integrity of the flower buds.

At the end of the growth cycle, seeds from each generation were collected and dried in a greenhouse (65 to 70°C) until the average moisture content was 13%. The samples (13% moisture) were ground in a micro-mill to obtain particles less than 1 mm in diameter. Samples (0.5 g) of the bean flour from each generation were digested in 5 mL of a nitric-perchloric solution (HNO_3 + $HClO_4$ at a 5:1 ratio by volume). After 12 hours of cold digestion (no heat applied), the temperature was gradually elevated by 30°C every 30 minutes until it reached 180°C. At the end of the digestion, when 1 mL of the transparent solution remained, the sample was diluted by adding distilled water to a total volume of

50 mL. Approximately 20 mL of this dilution was removed, and the copper concentration was measured by atomic absorption spectrophotometer (Perken Elmer®, model Analyst 200®) at wavelength settings of 324.8 nm.

The copper genetic study was performed in a randomized complete block design with five replications for parents and F_1 reciprocal generations and 24 replications for the F_2 reciprocal generations. To test the maternal effect hypothesis, a comparison was made between the P_1 vs. P_2, the F_1 vs. F_1 reciprocal and the F_2 vs. F_2 reciprocal mean values by Student's t-test at 5% probability.

The genetic parameters were estimated from the variances of the parents' (P_1 and P_2) and the F_1, F_2, BC_1 and BC_2 generations based on embryo generation for each hybrid combination. The broad-sense heritability and the narrow-sense heritability were estimated with the backcross method (WARNER, 1952), by the following expressions:

$$h_a^2 = \sigma_G^2/\sigma_P^2 \text{ and } h_r^2 = \sigma_A^2/\sigma_P^2 \text{ respectively,}$$

where:

σ_G^2 refers to the genotypic variance, estimated by: $\sigma_G^2 = \sigma_P^2 - \sigma_E^2$, where the σ_E^2 represents the environmental variance in F_2 ($\sigma_E^2 = (\sigma_{F_1}^2 + \sigma_{P_1}^2 + \sigma_{P2}^2)/3$); σ_P^2 is the phenotypic variance ($\sigma_P^2 = \sigma_{F_2}^2$); and σ_A^2 is the additive variance, estimated by: $\sigma_A^2 = 2\sigma_{F_2}^2 - (\sigma_{BC_1}^2 + \sigma_{BC_2}^2)$.

Heterosis in the F_1 generation was quantified as a percentage of both the heterosis related to the mean of the parents:

$$H\% = [(F_1 - P)/P] \times 100$$

and of the heterobeltiosis:

$$HT\% = [(F_1 - MP)/MP] \times 100$$

where:

P is the average of parents ($P = (P_1 + P_2)/2$), and MP is the best male parent.

To predict selection gains, we took a selection of 25% of plants with F_2 seeds (embryos in the F_2 generation) with the highest copper concentration. The gain expected when accounting for the selection and recombination of superior plants with F_2 seeds (embryos in the F_2 generation) was estimated by the following equation:

$$\Delta G(\%) = (\Delta G \times 100)/\overline{F_2}$$

where:

ΔG refers to the selection gain, obtained by $\Delta G = DS \times h_r^2$, in which DS is the selection differential, expressed by $\overline{X}_s - \overline{X}_0$, where $\overline{X}_s$ is the average of selected plants with seeds in F_2 and $\overline{X}_0$ is the average of plants with seeds in F_2. Analyses were performed with the aid of Microsoft Office Excel spreadsheets and Genes software (GENES, 1998).

Results and discussion

In the reciprocal hybrids IAPAR 44 (5.28 mg copper kg^{-1} DM) x IAPAR 31 (3.49 mg copper kg^{-1} DM) and Diamante Negro (4.36 mg copper kg^{-1} DM) x TPS Bonito (1.60 mg copper kg^{-1} DM), the contrast of the copper concentration between P_1 vs. P_2 was significant (Figure 1), demonstrating a genetic difference between the parents in their copper concentration.

No significant difference was observed between the mean copper concentration values of the reciprocal F_1 hybrids (Figure 1), suggesting that there was no significant maternal effect on the copper concentration phenotype in the common bean seeds. This result was observed because the embryo from the common bean seeds contains 40.4% to 72.0% of the entire copper content (RIBEIRO et al., 2012). Thus, the copper concentration of common bean seeds is dependent on the embryo and the F_1 generation seeds represent the fertilized product of the parents. Similarly, the common bean seeds' zinc concentration exhibited no significant maternal effect (ROSA et al., 2010). Zinc, similar to copper, was accumulated predominantly in the seeds of the common bean's embryos (MORAGHAN; GRAFTON, 2002; MORAGHAN et al., 2002).

In the IAPAR 44 x IAPAR 31 hybrid, no significant difference was observed for the F_2 vs. F_2 reciprocal contrast (Figure 1), reinforcing that the phenotypes of these seeds were similar in terms of copper concentration and represented the expression of the genotype of the F_1 generation. Therefore, F_2 seeds showed embryos in the F_2 generation. For this reason, selecting for a high copper concentration in the common bean seeds should begin with the F_2 seeds, a generation where ample genetic variability is observed. A similar result was observed for the zinc concentration in common bean seeds (ROSA et al., 2010). As no significant maternal effect for the zinc concentration was observed, the selection of the F_2 seeds harvested from the one plant was efficient.
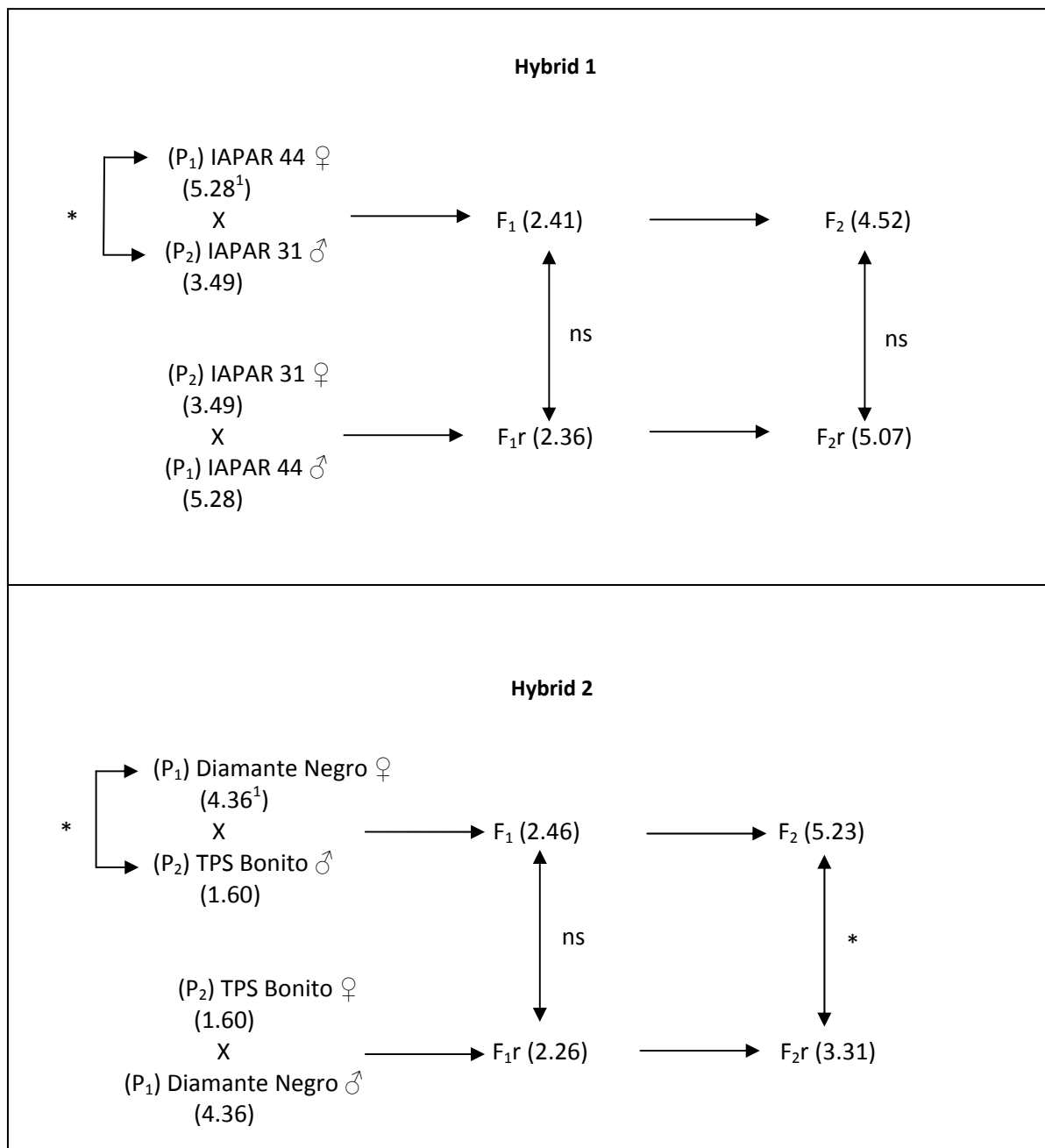
Figure 1. Breeding and selection process schematics for copper concentration of common bean seeds of the IAPAR 44 x IAPAR 31 and Diamante Negro x TPS Bonito hybrids. The mean copper concentration (mg kg^{-1} of DM) is in parenthesis. P_1 and P_2 are the parental seeds, F_1r is the F_1 and F_1 reciprocal seeds, and F_2r is the F_2 and F_2 reciprocal seeds; the significance level for differences in the mean values was evaluated for P_1 vs. P_2, F_1 vs. F_1 reciprocal and F_2 vs. F_2 reciprocal from the IAPAR 44 x IAPAR 31 and Diamante Negro x TPS Bonito hybrids. [1]Copper concentration (mg kg^{-1} of DM); [ns]non-significant; *Significant by Student's test at 5% probability.

However, a significant maternal effect was observed for the iron concentration in common bean seeds (JOST et al., 2009b). Because the iron concentration was dependent on the seed coat, selection began only on the F_3 seeds (embryos in the F_2 generation) when segregation was verified. Ribeiro et al. (2012) found that 47.8 to 83.2% of the whole iron content accumulated in the seed coat of the common bean seeds. The migration of the iron was lower for the embryos of the common bean seeds. Thus, a breeding program should investigate of the existence of a maternal effect on mineral concentrations in germplasm subjected to selection. The embryo generation should be considered for the selection process and the progression of segregating populations in genetic breeding programs.

A breakdown of the phenotypic variance showed the predominance of genetic effects in relation to the environmental effects for each hybrid combination (Table 1). As result, a high broad-sense heritability was observed in the IAPAR 44 x IAPAR 31 hybrid (h^2a: 71.90%) and Diamante Negro x TPS Bonito hybrid (h^2a: 79.65%). This finding was observed because the genetic variance was responsible for most of the phenotypic variance.

Table 1. Genetic parameters and predicted selection gains for copper concentration in the common bean seeds of the IAPAR 44 x IAPAR 31 and Diamante Negro x TPS Bonito hybrids.

Estimate	Copper concentration (mg kg⁻¹ of DM)	
	IAPAR 44 x IAPAR 31	Diamante Negro x TPS Bonito
Mean	4.32	3.78
Environmental coefficient of variation (%)	26.39	24.96
Phenotypic variance (σ^2_P)	1.86	1.99
Parent 1 variance ($\sigma^2 P_1$)	0.53	0.20
Parent 2 variance ($\sigma^2 P_2$)	0.35	0.36
F$_1$ variance ($\sigma^2 F_1$)	0.69	0.66
Environmental variance ($\sigma^2_E F_2$)	0.52	0.40
Genetic variance (σ^2_G)	1.34	1.59
Additive variance (σ^2_A)	0.97	1.23
Broad-sense heritability (h^2_a)	71.90	79.65
Narrow-sense heritability (h^2_r)	51.98	61.99
Heterosis (H%)	-44.97	-17.48
Heterobeltiosis P$_1$ (HT%)	-54.28	-43.63
Maximum value in parents	6.27	5.00
Minimum value in parents	2.60	1.07
Maximum value in F$_2$	7.75	7.53
Minimum value in F$_2$	2.47	1.98
Selection gain (ΔG %)	18.55	29.03

A narrow-sense heritability of an intermediate (h^2r: 51.98%) to high magnitude (h^2r: 61.99%) was observed in the IAPAR 44 x IAPAR 31 and Diamante Negro x TPS Bonito hybrids, respectively (Table 1). Previous studies have shown that iron and zinc concentrations in seeds of the common bean have a narrow-sense heritability ranging from an intermediate to high value (JOST et al., 2009b; ROSA et al., 2010). As the additive variance represented the higher fraction of the genetic variance, the selection for high copper, iron and zinc in the seeds of the common bean is possible. The fixation of these characters could be observed in advanced generations.

Given that genetic factors exert a large effect on the phenotype and an intermediate to high narrow-sense heritability was observed, the copper concentration appears to exhibit a qualitative inheritance. Thus, we expected to easily select for the measured character. Consequently, it is likely that it will be easy to develop common bean cultivars with higher nutritional quality for the use in foods based on the identification of seeds with a high copper concentration. A more in-depth study of the

genetic effects and the number of genes that control the copper concentration was not carried out in this study, as the efficiency of hybridization was low, and little replications for the F$_2$ generation and backcross (BC$_1$ and BC$_2$) populations were obtained.

For conventional heterosis, negative values in two of the hybrids were observed (Table 1). The mean value for the F$_1$ generation was lower compared to those of the parents (Figure 1). A similar response was observed for heterobeltiosis with negative estimates for the IAPAR 44 x IAPAR 31 (-54.28%) and Diamante Negro x TPS Bonito (-43.63%) hybrids. Negative heterosis estimates were also obtained for plant height (-9.54%), number of pods per plant (-27.87%), and seeds per pod (-18.57%) in the common bean (GONÇALVES-VIDIGAL et al., 2008). Therefore, the epistasis effect on the phenotype of the F$_1$ generation must be considered and included in the model for the partition of the genetic components of variance (BARONA et al., 2009).

Copper concentrations ranged from 1.07 mg kg⁻¹ (Diamante Negro) to 7.75 mg kg⁻¹ (F$_2$ plant from IAPAR 44 x IAPAR 31 hybrid) (Table 1). The observed values were approximately half those verified in the genotypes cultivated in Brazil (MESQUITA et al., 2007). Despite this difference, the copper concentration values verified in the present study are sufficient to supply approximately 20% of the daily requirement for an individual (ANDRADE et al., 2004). Thus, populations of high nutritional value for the human diet were obtained.

Transgressive segregation was observed for the common bean seed's copper concentration. Among the F$_2$ generation seeds examined, individuals with 7.75 mg copper kg⁻¹ DM were identified, which represented an increase of 46.78% in the copper concentration relative to the IAPAR 44 parent (5.28 mg copper kg⁻¹ MS). This was the first report of an increased copper concentration in the common bean seeds by a genetic improvement. There have been reports of increases of 94.0% in iron (JOST et al., 2009b) and of 37.3% in zinc (ROSA et al., 2010) in the common bean seed by use of controlled crossing and selection among segregation progenies in early hybrid generations.

In relation to selection gains, assuming a retention of 25% of plants with F$_2$ seeds (embryo in F$_2$ generation) with the highest copper concentration, gains of 18.55 and 29.03% could be expected for the IAPAR 44 x IAPAR 31 and Diamante Negro x TPS Bonito hybrids, respectively (Table 1). Thus, an early selection of the common bean germplasm with a high copper concentration can be a successful strategy, given

that significant gains in the first cycle after selection were obtained.

Given that it was possible to increase the copper concentration in common bean seeds by genetic breeding, this strategy could result in health benefits because copper is an essential mineral for humans (CUNHA; CUNHA, 1998). Additionally, increasing the seed copper concentration could contribute to agricultural sustainability, thereby reducing the amount of copper-based fertilizer required and the accumulation of heavy metals in the soil.

Conclusion

The early generation narrow-sense heritability of the copper concentration of the common bean seed falls between intermediate (51.98%) and high (61.99%) values, and there is no maternal effect on the expression of this characteristic. It is possible to increase the copper concentration in common bean seeds by 46.8% using genetic breeding.

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