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Efficiency of methods for conducting segregating populations in the breeding of common beans for protein quality

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ABSTRACT - *Common bean (*Phaseolus vulgaris *L.) is part of the daily diet of over 300 million people in the world and is a staple food of low-income populations, due to its high protein content. Many authors have proposed a further improvement by breeding methods that exploit the existing genetic variability. The objective of this research was to evaluate the genetic potential of segregating bean populations with variability for several traits and conducted by different breeding methods. The F_2 segregating population was developed from a cross of the parental genotypes CNFC 7812 and CNFC 8056. One hundred and fifty randomly taken F_2 plants originated the segregating populations, which were conducted until the fifth generation by the following breeding procedures: Single Seed Descent (SSD), Bulk and Bulk within F_2. The total grain protein content of the F_5 families was then analyzed. It was concluded that there was enough genetic variability in the cross under study. The Bulk method proved to be the most efficient since it generated families with superior averages for the trait protein content in comparison with the other breeding methods.*

Key words: protein content, SSD, bulk, bulk within F_2 families.

INTRODUCTION

Common bean ((*Phaseolus vulgaris* L.) is part of the daily diet of over 300 million people throughout the world and the species is known as a key nutritional crop for low-income populations since it is the cheapest protein source available. The per-capita consumption is high in regions of low-income families, e.g., in Northeastern Brazil (18.5 Kg inhabitant^{-1} year^{-1}) (Yokoyama and Stone 2000).

An increase in the nutritional quality of common bean in terms of protein content may make its consumption more appealing, which would in turn increase the profit of small-scale bean farmers and enable them to invest more in their plantations. Furthermore, a better quality of the common beans served in meals at public schools or distributed in food distribution programs would specifically benefit the poorer segment of the Brazilian population.

Brazil is the main common bean producer and also its greatest consumer. With the increase of the nutritional standard high-quality beans could be made available on the national market without additional costs, and moreover benefit the part of the population that relies on common bean as a cheap protein source.

Worldwide the demand is high for cultivars of annual crops such as common bean that should be high-

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yielding and have grains of high nutritional quality. It is therefore of paramount importance to explore the genetic variability of the plant populations efficiently in order to maximize the possibilities of success in programs of cultivar development. Based on the knowledge of germplasm bearing the genes of interest, these may be used in appropriate breeding methods, along with information on the genetic potential of the populations, to reduce time and costs for the development of the final product of the breeding process.

Buratto et al. (2005) evaluated four cultivars and three strains of common bean at four locations and observed genetic variability for protein content in the genotypes, from 22.68 to 25.52%. Lemos et al. (2004) found variability for protein content between 17 and 23.9% in 31 genotypes evaluated in two growing seasons. Furthermore, Filho et al. (2005), observed that the group with the greatest gradient of variation is "carioca", followed by the black group with a protein content between 13.7 to 30.3%. The major part of the kernel protein consists of phaseolin, a storage protein, which is deficient in sulfur amino acids such as methionine (Broughton et al. 2003).

The success of breeding programs depends on the efficient exploration of the existing genetic variability in the segregating populations. This variability is a function of the genetic diversity of the parents used in the crosses, as well as of the methods applied in the segregating populations (Castanheira 2004). Comparisons among the methods of conducting variable populations in common bean are still scanty and focus mainly on the trait grain yield (Raposo et al. 2000). This fact may be due to the dependence of the studies on environmental and infrastructural factors and on the relative long time needed to obtain information. It is important to evaluate the relative efficiency of the methods available, since the success of programs depends on the differences between the newly released cultivars and those used by growers. It is getting the longer the more difficult to detect this difference.

Estimates of genetic and phenotypic parameters are useful information for breeders and help them in decision-making processes. Underlying the choice of parents, they are an orientation for the conduction and comparison of segregating generations, as well as in the evaluation of the effects of selection in other not directly selected traits. Some of the most used parameters are: heritability, correlations between traits and genetic progress expected by selection.

The purpose of this study was to evaluate the genetic potential of common bean families derived from the cross CNFC 7812 X CNFC 8056 to raise the protein content, conducted by different methods for generation advance.

MATERIAL AND METHODS

The parents used to obtain the segregating populations were the advanced strains CNFC 7812 and CNFC 8056 both developed by Embrapa common bean breeding program. These strains are contrasting in relation to protein content (23% in CNFC 7812 and 16% in CNFC 8056). Plants of the F_1 generation were selfed to generate the F_2 segregating population. To initiate the process of breeding segregating populations, F_2 was sown in November 2004 in a green-house of Embrapa Arroz e Feijão, in Santo Antonio de Goiás, state of Goiás (lat 16º 28' 00'' S, long 49º17'00", 823m asl).

From 150 plants chosen randomly in the F_2 generation, the segregating populations were obtained that were then conducted by the methods: single seed descent (SSD), Bulk and Bulk within F_2. For the SSD method, three seeds of each one of the 150 F_2 plants were sown to constitute the F_3 generation. This procedure was carried out in a green-house until 136 F_5 families were established in March 2006. At this point, the protein content was analyzed.

For the Bulk method, 750 seeds were harvested from 150 F_2 plants (five seeds per plant) which were then mixed into a sample to plant the F_3 generation. These were sown in June 2005 in ten 5m-long rows spaced 0.5m apart. Plants were harvested and a sample was retained for the F_4 generation. This sample was sown at the same location in March 2006, resulting in the F_5 generation, which was re-sown in July 2006. In October 2006, 150 plants of this generation were harvested for the protein content analysis.

For the within- F_2 -Bulk method, remnant seeds of each F_2 plant were sown to represent the 150 $F_{2:3}$ families that were sown in March 2005 on the experimental farm of the Federal University of Lavras, in the South of Minas Gerais state (lat 21°14'00" S, long 45º00'00" W, 910 m asl). The seed increase plots consisted of 2m-long rows, 0.5m apart with 15 seeds per meter. The $F_{2:4}$ families harvested in the multiplication plots were

planted in two 2m long rows at Embrapa Rice and Beans, in July 2005. The $F_{2:5}$ families were planted in a field trial using a randomized complete-block design with two replications and plots consisting of 2m-long rows in Ponta Grossa, Paraná state (lat 25º05'42", long 50º09'43", altitude 969m asl), in February 2006. A total of 134 families were evaluated and their seeds harvested for protein analysis.

In order to evaluate the protein content, a sample was taken of each genotype derived from the families of the F_5 generation conducted by the three methods for segregating populations. The sample with a number of grains between 5 and 100 grains family^{-1} and per method, was ground and used for the chemical analyses of the protein content.

The the protein content was determined by the total nitrogen content and the micro-Kjedahl method proposed by Aoac (1995). The protein contents for each family were estimated by means of three measurements of nitrogen quantities. The procedure is based on the heating of a sample in digestion with sulfuric acid until carbon and hydrogen are completely oxidized. The protein nitrogen is then reduced to ammonium sulfate. Concentrated NaOH is added under heat to liberate ammonia into a known volume of boric acid solution in order to produce ammonium borate. The latter is produced and dosed in a standardized acidic solution of HCl. This HCl titration determines the nitrogen quantity, which, multiplied by the factor 6.25, permits the conversion of N values into protein values.

In order to compare the performance of the families obtained by the different methods, estimates of means, phenotypic and genotypic variances, heritabilities and descriptive measures were utilized. To make the process of comparison more efficient, these estimates were compared by means of a divergent selection procedure (the best and the worst) in the families established by each method.

The Pearson correlation was estimated between F_2 plants and the correspondent families obtained through the different methods of population conduction. The statistical tests of significance were applied according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

The results obtained for protein content in the three different methods of conducting populations as well as the F_2 generation data are shown in Table 1. The number of families evaluated in each method was different due to the loss of families along the process. It was observed that in the F_2 generation, the mean value was lower than the best parental which contained 23% protein, but was superior to the parent mean (19.5%).

The mean total protein content of the families obtained through the bulk method was 20.37 and 20.44% for the families by the $F_{2:5}$ Bulk method. Similar values are normally expected for the cultivar group carioca, whose protein contents for such cultivars has been founded between 18.13 and 22.51%, as well as Lemos et al. (2004), who stated variation from 17 to 23.9% in 31 genotypes studied for the two growing seasons. In this research the effect of genotype- environment interaction was also demonstrated. Filho et al. (2005) however, found values with greater variation (11.5 - 30%) for the same group of cultivars. These results reinforce the existence of genetic variability for this trait in common bean germplasm, so it is possible to obtain superior genotypes for the trait by conventional breeding methods.

There is great variability within the F_2 population for protein content, in which it was possible to identify 34 plants (32.07% of the total) with higher means than the parent with the highest protein content (23%) and 74 plants with higher means than then the parent mean. The maximum value obtained was 29.23% (Figure 1A).

For the SSD method, the average protein content was 16.58%, also below the parent mean (19.5%). In this method, one family was found superior to the best parental, with a protein content of 25.55% and 16 families (11.76% of the total) with averages exceeding the parent mean (19.5%) (Figure 1B).

The highest protein content of the families in the Bulk method, was 24.12%, which was greater than the best parent; four out of the evaluated families exceeded the best parent and 105 families (70% of the total) surpassed the parental mean (Figure 1C).

By the Bulk $F_{2:5,}$ the protein content was not higher than in the best parent (23%) in any family. The highest protein content was 22.84% and 77 families (51.68% of the total) surpassed the parent mean (Figure 1D).

In the overall analysis, it was verified that the SSD released the highest variability, although some values were extreme, in particular the low protein contents (Figure 1). The second highest variability and a greater number of

Table 1. Descriptive measures for protein content of F_2 plants and families conducted by different breeding methods; analyses performed by the grain quality laboratory of Embrapa Rice and Beans for the F_2 generation in 2004 and for the families in 2006

Populations	Number of Observations	Mean (%)	Minimum (%)	Maximum (%)	CV (1) (%)	σ^2_F(2)	σ^2_G (3)	h^2 (4) (%)
F_2	106	21.58	13.58	29.23	15.92	11.802	7.472	63.31
SSD F_5	136	16.58	10.82	25.55	13.78	5.219	0.885	16.96
Bulk F_5	150	20.37	17.04	24.12	7.15	2.123	0	-
Bulk $F_{2:5}$	134	20.44	18.17	22.84	5.69	1.044	0.366	35.06

(1): Coefficient of variation, (2): Phenotypic variation, (3): Genotypic variation and (4): Broad-sense heritability

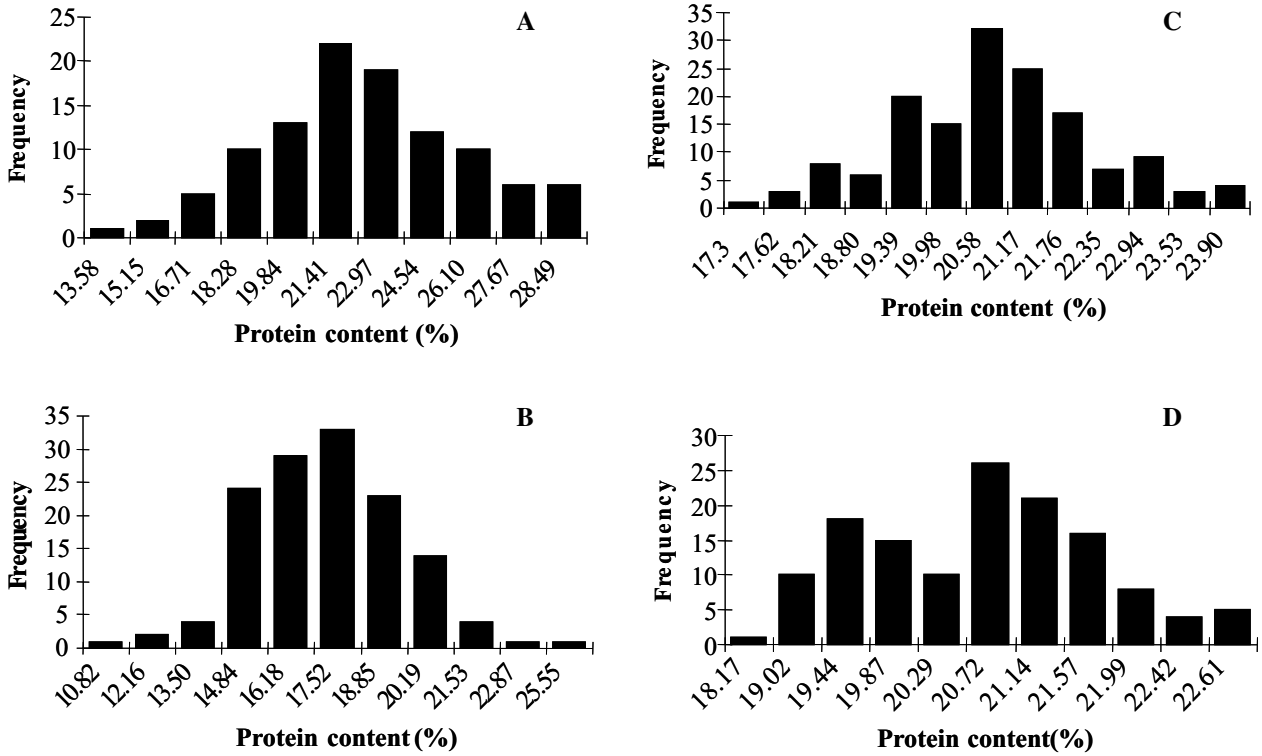

Figure 1. Frequency distribution of total protein content obtained in analyses in 2004 of F_2 generation (A) and in 2006 by the methods SSD (B); Bulk (C) and Bulk $F_{2:5}$ (D)

families with extreme and higher values of protein content were obtained by the Bulk followed by the $F_{2:5}$ Bulk methods. In both of them all families were superior to the parent with the lowest mean. This result shows the advantage of these methods over the previously cited, since they produced a greater number of families with superior performance. These observations are corroborated by the estimates of the phenotypic variances of the families obtained by the three methods (Table1).

Heritability estimates for the trait protein content were: 63.31% for F_2, 16.96% for the families obtained by the SSD method and 35.06% for families obtained by the Bulk $F_{2:5}$. These last two heritability estimates are considered low, demonstrating that this trait is strongly influenced by the environment. The genotypic variance found for the Bulk method was nil, due to the fact that the environmental variance was greater than the phenotypic variance.

Sampling in itself is a problem that may affect the genetic variability in families and affects particularly the Bulk method. This may explain the results found here and indicates the need of evaluating a larger number of families for this trait when this method is chosen. Nevertheless, by this method the highest number of families superior to the parent mean as well as to the mean of the best parent was produced.

The SSD method did not present sampling problems and in this case, was the method with the greatest genetic variance and enabled the identification of a family with the highest total protein content (25.55%). The environmental variation was lowest by the Bulk $F_{2:5}$ method which makes the selection process more efficient. Nevertheless, it was not possible to identify families superior to the best parent, which may certainly be ascribed to the low variability released in families.

The descriptive measures of the 10 and 20 best and the 10 and 20 worst families selected from each method of conducting populations were analyzed (Table 2).

Table 2. Mean values for protein content, variance, coefficient of variation and genetic gain of the 10 and 20 best and 10 and 20 worst families produced by the three breeding methods; analyses performed by the grain quality laboratory of Embrapa Rice and Beans in 2006

Evaluation	Method	Mean (%)	σ^2_F	CV (%)
10 best	SSD F_5	21.15	2.7466	7.83
	Bulk F_5	23.29	2.3524	6.58
	Bulk $F_{2:5}$	23.22	1.3246	4.96
20 best	SSD F_5	20.34	2.0375	7.02
	Bulk F_5	22.85	2.6586	7.13
	Bulk $F_{2:5}$	22.92	1.7382	5.75
10 worst	SSD F_5	12.55	1.1361	8.49
	Bulk F_5	17.61	1.9467	7.92
	Bulk $F_{2:5}$	17.98	1.2480	6.21
20 worst	SSD F_5	13.37	1.2631	8.40
	Bulk F_5	18.05	2.9793	9.56
	Bulk $F_{2:5}$	18.20	1.5289	6.79

σ^2_F: Phenotypic variance and CV: coefficient of variation

The phenotypic variance was highest in the SSD method when the ten best families were selected, whereas the mean protein content was low when compared with the other two methods. For the Bulk F_5 method, the highest values of phenotypic variance were observed when the 20 best families were selected. This method is somewhat influenced by environmental effects, i.e., the increase in the number of sampled families may, in turn, decrease sampling losses and make the identification of families with better performances possible.

In an analysis of the coefficients of variation, with exception of the 20 best families, the variation was greatest by SSD. The method with the lowest coefficient of variation was Bulk $F_{2:5}$, due to the underlying experimental design. However, the lowest estimated values for the phenotypic variances indicated a disadvantage of this method.

Based on what was described and considering the costs involved, the sowing acreage, available labor, number of superior families generated and the mean values of protein content, the Bulk method is most adequate for breeding populations. The SSD, regardless of having produced the family with the highest protein content, generated a low number of families superior to the overall parent mean whereas the overall mean was lowest, which makes it a less efficient method. The Bulk method is more practical and of easy conduction in tropical countries such as Brazil, in accordance with Raposo et al. (2000), who presented similar results for the trait grain yield in common bean.

The correlations between F_2 plants and the best families of either the Bulk $F_{2:5}$ or the SSD methods were not significant (-0.10 and 0.15 respectively), demonstrating that plants of the F_2 generation with higher protein content did not result in the best families in the generation under evaluation (F_5 generation).

This absence of correlation in the SSD method was most likely due to the fact that it was not possible to exploit the within-family genetic variance, since the descent of only one seed was evaluated, generating a family with a mean value that may differ from the overall mean of the family in question. For the Bulk within-family method, the low genetic variability released might have been the cause of the absence of correlation.

CONCLUSIONS

1. For the trait total protein content, the Bulk method was considered preferable over the SSD and the Bulk $F_{2:5}$ methods, due to its practicability and for achieving families superior to the best parent and the parental mean.

2. The cross CNFC 7812 x CNFC 8056 yielded families with sufficient genetic variability for the trait protein content to be exploited by selection.

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Eficiência de métodos de condução de populações segregantes no melhoramento do feijoeiro comum para qualidade protéica

RESUMO - *O feijão (*Phaseolus vulgaris *L.) participa da dieta de mais de 300 milhões de pessoas no mundo todo, sendo alimento essencial para populações carentes, pois possui um teor de proteína elevado, podendo ser melhorado através da variabilidade genética já existente como proposto por vários autores. O objetivo desse trabalho foi avaliar o potencial genético de populações segregantes de feijoeiro para diversos caracteres e conduzidos por diferentes métodos de melhoramento. Dos genitores CNFC 7812 e CNFC 8056, se obteve a $F_{1,}$ e posteriormente a população segregante F_2. A partir de 150 plantas, escolhidas aleatoriamente na geração $F_{2,}$ obtiveram-se as populações segregantes que foram conduzidas pelo método de descendência de uma única planta (SSD), Bulk e Bulk dentro de F_2 até a geração F_5. Foram realizadas avaliações para quantificar o teor total de proteína no grão. Pode-se concluir que existe variabilidade genética suficiente neste cruzamento, com o método Bulk mostrando-se mais eficiente, gerando mais famílias com médias superiores dentre os métodos avaliados.*

Palavras-chave: Proteína, SSD, Bulk, Bulk dentro de F_2.

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