GENETICS AND PLANT BREEDING

Strategies to select soybean segregating populations with the goal of improving agronomic traits

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ABSTRACT. The success of breeding programs depends on selection procedures and on the breeding methods adopted for selecting segregating populations. The objective of this study was to evaluate the efficiency of the Bulk method with selection in the F_3 generation (BulkF3) compared to that of Bulk method as well as determine the most effective selection strategy in terms of genetic gain. Twenty segregating populations were selected by two methods. The 60 best families of each method were selected according to their average agronomic performance. An augmented block design was used. The following agronomic traits were evaluated: insertion height of first pod, plant height at maturity, number of branches and of pods per plant, 100-seed weight, and grain yield. For comparison of the methods, genetic component estimates, genetic gain and predicted breeding values were calculated using mixed models (REML and BLUP). The results showed the families obtained with the BulkF3 method were more productive, showed suitable plant height, a larger number of branches and pods, and higher 100-seed weight. The BulkF3 method was found to be an effective selection strategy for soybean improvement.

Keywords: genetic gain; *Glycine max*; breeding methods; mixed model.

Estratégias de seleção em populações segregantes de soja com características agronômicas desejáveis

RESUMO. O sucesso dos programas de melhoramento genético depende dos procedimentos de seleção e dos métodos de melhoramento adotados para a condução das populações segregantes. O objetivo deste trabalho foi avaliar a eficiência do método Bulk com seleção em F_3, (BulkF3) em relação ao método Bulk, bem como comparar qual estratégia de seleção é mais eficiente em termos de ganho genético. Para a realização do trabalho foram utilizadas 20 populações segregantes, conduzidas pelos dois métodos. Foram selecionadas 60 melhores famílias de cada método, de acordo com a performance média agronômica. O delineamento utilizado foi de blocos aumentados. Foram avaliadas as seguintes características agronômicas: altura da inserção da primeira vagem e da planta na maturidade, número de ramos e de vagens por planta, peso de cem sementes e produção de grãos. Para efeito de comparação dos métodos, foram utilizadas as estimativas dos componentes genéticos, ganho genético e predição dos valores genéticos, calculados via modelos mistos (REML e BLUP). Os resultados demonstraram que as famílias obtidas pelo método BulkF3 foram as mais produtivas, com menor porte, maior número de ramos, vagens e peso de cem sementes. O método BulkF3 mostrou-se uma estratégia de condução eficiente para a seleção no melhoramento genético de soja.

Palavras-chave: ganho genético; *Glycine max*; métodos de melhoramento; modelo misto.

Introduction

Soybean (*Glycine max* (L.) Merrill) is an economically important crop worldwide. Brazil is the second largest producer of this legume with a production of 112 million tons (Conab, 2017). In this respect, most of this production is due to the cultivation of genetically modified soybean, with 111 million tons produced in an area of 33.87 million hectares (Cêleres, 2017). Soybean production is increasing in Brazil, mainly as a result of the efforts of plant breeding programs (Borém & Miranda, 2009; Ramalho, Abreu, Santos, & Nunes, 2012), which have led to the improvement of different traits of agronomic interest, including increases in productivity, lodging resistance, adaptability to different soil and climatic regions, tolerance to herbicides, and resistance to the main pests and diseases.

The success of breeding programs depends on selection procedures that allow increased genetic

gain and on breeding methods adopted for selecting segregating populations, as well as on the effective exploration of genetic variability released over generations and how much of this phenotypic variability is due to genetic and environmental differences (Raposo, Ramalho, & Abreu, 2000; Ramalho et al., 2012). Different selection strategies of populations have been employed in breeding programs in attempts to obtain lines with superior agronomic traits (Martin & Geraldi, 2002; Borém & Miranda, 2013; Ramalho et al., 2012). In this respect, an efficient estimation of genetic parameter estimates such as variance components, heritability and genetic gain are of the utmost importance for the guidance of breeding programs to obtain promising genotypes from segregating populations (Farias Neto, Clememt, & Resende, 2013; Silva, Melo, Pereira, & Melo, 2014).

Traditional breeding methods of self-pollinating plants are used to develop new soybean cultivars through the formation, conducting and selection of genotypes. Among the methods for selecting segregating populations, the Bulk method is commonly used in breeding programs because of its simplicity, easy handling of populations, rapidity, and low cost. However, this method has the disadvantage in that promising progenies used in the advancement of subsequent generations may be lost from one generation to the next due to sampling deficiencies (Allard, 1971; Ramalho et al., 2012). In attempts to overcome this obstacle, Frey (1954) proposed a Bulk method with selection in F_2 or F_3 families in which different traits of interest are selected in each generation. This method involves harvest of individual plants in the F_3 generation, with each plant giving origin to a family that will be used to form the next generation. The Bulk method with F_3 selection has the advantage of exploring 1.5 of the additive variance, thus increasing its utilization by 50%. Selection in the F_3 generation is therefore more advantageous and, consequently, more appropriate than selection in the F_2 generation (Pimentel et al., 2014; Ramalho et al., 2012; Resende, 2002).

The Bulk method with selection in the F_3 generation is commonly used in most breeding programs of bean (Raposo et al., 2000; Costa, Rava, & Zimmermann, 2002; Silva et al., 2008; Ramalho et al., 2012) and wheat (Wang et al., 2003). However, studies demonstrating the efficiency of this method in soybean crops are rare. Therefore, the objective of this study was to evaluate the efficiency of the bulk method with selection in the F_3 generation (BulkF3) compared to that of the traditional Bulk method with respect to traits of economic interest in soybean improvement, as well as to determine the most effective selection strategy in terms of genetic gain.

Material and method

The experiments were conducted in three agricultural years (2011/2012, 2012/2013, and 2013/2014) on the Farm for Teaching, Research and Extension of the Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP-FCAV), Jaboticabal Campus, northern region of the State of São Paulo, Brazil.

The segregating populations were obtained by biparental crossings using conventional lines as parents, which were commercial Monsoy cultivars (Monsanto) carrying the RR gene. The lines belonged to the breeding program of the UNESP-FCAV, had good agronomic attributes, and were adapted to the cultivation conditions of São Paulo State. For this study, 20 segregating populations that generated the respective families in the F_3, F_4, and F_5 generations, as well as two control cultivars (CD 216 and V-Max), were used (Table 1).

Table 1. Genealogy and identification of 60 families obtained from 20 soybean segregating populations.

POP	IF	GENEALOGY	POP	IF	GENEALOGY
1	3,4,5	JAB.00-04-1/5A4D x M 8230 RR	11	33,34,35	JAB.00-05-1/5C3B x M 8336 RR
2	6,7,8	JAB.00-03-3/1H1D x M 7908 RR	12	36,37,38	JAB.00-05-1/5C3B x M 8360 RR
3	9,10,11	JAB.00-03-3/1H2D x M 8360 RR	13	39,40,41	JAB.00-05-8/2D3C x M 7211 RR
4	12,13,14	JAB.00-01-21/4M1D x M 8336 RR	14	42,43,44	JAB.00-05-8/2D3C x M 7636 RR
5	15,16,17	JAB.00-05-5/4A2D x M 7578 RR	15	45,46,47	JAB.00-05-8/2D3C x M 7908 RR
6	18,19,20	JAB.00-05-5/4A2D x M 7908 RR	16	48,49,50	JAB.00-05-8/2D3C x M 8336 RR
7	21,22,23	JAB.00-05-5/4A2D x M 8221 RR	17	51,52,53	JAB.00-06-2/3I3D x M 8230 RR
8	24,25,26	JAB.00-05-1/5C3B x M 7211 RR	18	54,55,56	JAB.00-06-2/3I3D x M 8236 RR
9	27,28,29	JAB.00-05-1/5C3B x M 7639 RR	19	57,58,59	JAB.00-02-30/1G4A x M 8360 RR
10	30,31,32	JAB.00-05-1/5C3B x M 8211 RR	20	60,61,62	JAB.00-02-3/6A4D x M 8360 RR

POP: Populations; IF: Family identification; Cultivars 1: CD216 and 2: V-Max.

Families were selected from the F_3 to the F_5 generation using two breeding methods: the Bulk method with selection in the F_3 generation (BulkF3) and the traditional Bulk method, in which 60 families were selected per method according to their average agronomic performance. A total of 122 treatments were evaluated, including 120 segregating families and two controls (Coodetec 216 and V-Max). Generation advancement using the different selection methods and evaluation of the families were performed in three agricultural years (2011/12, 2012/13, and 2013/14).

The experiment using the BulkF3 method was started in the F_3 generation by sowing 1,800 plants from 20 segregating populations. Each population consisted of a variable number of lines depending on the number of seeds. During harvest, six plants from each line were selected, with each plant generating one family. The seeds derived from each family were used to obtain the next generations (F_4 and F_5). This method is referred to as BulkF3 because selection occurs in the F_3 generation.

In the experiment using the traditional Bulk method, the plants were harvested in bulk, i.e., all seeds from the F_3 to F_5 generation are mixed together. For sowing, one seed sample was removed to obtain the next generation.

For comparison of the methods, 360 plants were chosen; of these, 60 families were selected. This procedure was repeated for each generation until the F_5. Approximately 16.67% of the best families were selected in each generation based on their average agronomic performance.

The augmented block design, in which the population were arranged in 5 m long rows spaced 0.5 m apart was used for the two experiments. The controls were intercalated between the populations evaluated. The seeds were sown mechanically in furrows in November, and 300 kg ha^{-1} of 2-20-20 fertilizer was applied. The sowing density was 20 seeds per meter. All agricultural procedures followed the technical recommendations for soybean crop (Embrapa, 2013).

The following agronomic traits were evaluated for the selection of the six plants: insertion height of first pod (IHP): distance, in cm, from the soil surface to insertion of the first pod in the main stem; plant height at maturity (PHM): distance, in cm, from the soil surface to the last node of the main stem at maturity; number of branches (NB): total number of branches on the main stem of the plants; number of pods per plant (NP): total number of pods with formed seeds; 100-seed weight (100SW): weight, in grams, of 100 seeds of an individual plant determined after threshing the pod , and grain yield (GY): total seed weight, in grams, after threshing.

Individual and combined analyses of variance were used for the traits studied. For the combined analysis, the means adjusted for the families in each year were calculated, in addition, the effects of block and generation were included as fixed effects and the effects of family were included as random effects.

The genetic gain provided by the methods and t-tests were used for comparison of the selection methods. Mixed models were applied to estimate the genetic parameters (Henderson, Kempthorne, Searle, & Krosigk, 1959). Variance components were estimated by the restricted maximum likelihood method (REML) developed by Patterson and Thompson (1971). The best linear unbiased prediction (BLUP) was used for the prediction of breeding values. The genetic parameters were estimated using the Mixed procedure of Statistical Analysis System software SAS 9.3 (2011) and analysis of variance was performed using the PROC GLM procedure of the same software.

The broad-sense heritability was calculated using the following equation: $h^2 = \frac{\sigma_g^2}{\sigma_f^2} x100$, where: h^2 is the broad-sense heritability, σ_g^2 is the genotypic variance, and σ_f^2 is the phenotypic variance. The genetic gains for each trait and generation were estimated taking into considering a selection pressure of 16.67% based on the average agronomic performance calculated with the following equation: $GG = SD \times h^2$, where: GG is the genetic gain; SD is the selection differential, which corresponds to the mean of the 10 best families according to the t-test minus the mean of the families, and h^2 is the heritability.

For PHM and IHP, decreases in genetic gain were desired considering that breeding is generally aimed at genotypes with medium heights (80 to 100 cm) and an insertion height of 12 cm or higher, both of which are suitable for mechanical harvesting. Thus, for these traits, a decrease in values during selection was desired since higher values are present in the data.

For better interpretation of the results in terms of genetic gain, for methods comparisons, a confidence interval was constructed using the following equation (Resende, Araújo, Sampaio, & Wiecheteck, 1995): $GG \pm t\sqrt{\frac{1}{N}\left(\sigma_g^2(1-r_{A\hat{A}})\right)}$, where: GG is the genetic gain by selection, t is the table value of Student's t-distribution, N is the number of families selected, σ_g^2 is the genetic variance, and $r_{A\hat{A}}$ is the accuracy of selection. In this study, the accuracy of selection was determined by the square root of heritability. R software (R Development Core Team, 2013) was used to construct the confidence intervals.

Table 2. Combined analysis of variance for the insertion height of first pod (IHF), plant height at maturity (PHM), number of branches (NB), number of pods per plant (NP), 100-seed weight (100SW) and grain yield (GY) of the families obtained by the BulkF3 and Bulk methods.

VF	Mean squares					
	IHP (cm)	PHM (cm)	NB	NP	100SW (g)	GY (g)
Block	3.56 [ns]	48.57 [ns]	0.31 [ns]	149.55 [ns]	3.49 [ns]	10.08 [ns]
Control vs Fam.	79.57**	4560.52**	162.83**	121095.39**	59.68**	7275.17**
Generation	81.79**	1618.65**	84.52**	29807.80**	65.47**	4764.84**
Family	17.80**	467.66**	3.55**	1620.04**	8.23**	89.37**
Methods	4.45 [ns]	238.63*	15.98*	5367.92*	62.75**	2118.46**
Fam. x Methods	12.85**	123.45**	1.69 [ns]	774.95*	3.04 [ns]	29.88 [ns]
Fam. x Gen.	14.15**	160.63**	2.22*	1201.42**	4.06*	88.18**
Gen. x Method	36.98*	1167.94**	19.85*	27289.75**	3.79**	2213.82**
Control	0.01 [ns]	371.37 [ns]	189.47**	116.26 [ns]	10.83*	35.20 [ns]
Error	6.89	57.96	1.29	420.33	2.91	27.73
R^2	0.76	0.87	0.84	0.88	0.73	0.89
CV%	19.54	7.67	31.34	21.95	11.74	19.78

** Significant at 1%; * significant at 5%; [ns] not significant at 5%; CV%: coefficient of variation and R^2: coefficient of determination.

Result and discussion

The combined analysis of variance revealed a significant effect ($p < 0.01$) of generation on all traits studied, characterizing the influence of generations. A significant difference ($p < 0.01$) was also detected for the effect of family on all traits, indicating the existence of genetic variability between families irrespective of the selection method used. Differences were observed between families derived from the different populations, with at least one family differing from the others (Table 2).

The selection method as a source of variation was significant at the 5% level for PHM, NB and NP and at the 1% level for 100SW and GY, indicating differences between the selection methods used to obtain families of the segregating populations (Table 2).

With respect to interaction effects (Table 2), the family x method interaction was significant ($p < 0.01$) for IHP and PHM, while a significant interaction at the 5% level was observed for NP. However, this effect was not significant for NB, 100SW or GY. The effect of this interaction indicates differences in the responses of soybean families obtained with either method.

The family x generation interaction was significant at the 1% level for IHP, PHM, NB, and GY and at the 5% level for NB and 100SW, demonstrating differences in the behavior of the families among the three generations (Table 2). It should be noted that this interaction altered the ranking of families between methods, as the best families obtained with the BulkF3 method were not necessarily the same as those obtained with the Bulk method. For example, among the 10 best families for GY, family 21 was the most productive according to the BulkF3 method (Table 3), while the Bulk method the most productive was family 57 (Table 4). Interactions between generation and

family have been reported in the literature (Raposo et al., 2000; Menezes Júnior et al., 2011).

With respect to the generation x method interaction, significant effects were observed for IHP, PHM, NP, NB, and GY (Table 2). These results demonstrate the existence of variability between families within each method and demonstrate that the methods adopted generated different families, i.e., there was no agreement in the behavior of families between generations or methods. Similar results have been reported by Jost, Ribeiro, Rosa, Possobom, and Mazieiro (2014), Mendes, Ramalho, and Abreu (2012), Rosal, Ramalho, Gonçalves, and Abreu (2000), Silva, Melo, Melo, Bassinelo, and Pereira (2013), who compared the bulk method and bulk selection with families in the F_2 and F_3 generation of common bean crops.

The coefficients of variation ranged from 7.67% for PHM to 31.24% for NP (Table 2) and were compatible with the values reported by other authors (Bárbaro et al., 2007; Lima, Crusciol, Cavariani, & Nakagawa, 2009; Pinheiro et al., 2013).

Tables 3 and 4 show the phenotypic values based on the adjusted means and the genotypic values predicted by the BLUP, respectively, of the 10 best families obtained with the BulkF3 and Bulk methods in the F_5 generation. In both methods, there was little agreement among families within each generation, i.e., the phenotypic values and predicted breeding values differed between the Bulk and BulkF3 methods. These differences in families over generations are probably due to generation x family interaction effects. Lima, Ramalho, and Abreu (2012) and Mendes et al. (2012), also described the occurrence of this type of interaction.

Grain yield of the 10 best families was higher for the BulkF3 method than for the Bulk method. The same was observed for 100SW, NB, and NP (Table

3). The BulkF3 method also provided a greater amplitude for the following traits: GY (14.36 g), 100SW (4.33 g), and NP (99.0) (Table 3).

The predicted genotypic values obtained with the BulkF3 method were generally better than those from the bulk method for the identification of superior families. In addition, the best phenotypes had also the best genotypic values. A difference in the ranking of the families according to their phenotypic and genotypic values was observed between methods. Population 4 provided the largest number of productive families in both selection methods. Costa et al. (2002) also reported that selection methods of segregating populations differ in terms of identification of more productive families.

Table 3. Phenotypic and genotypic values of the top 10 families obtained by the BulkF3 method in the F_5 generation.

	IHP (cm)			PHM (cm)			NB	
Fam	AM	BLUP	Fam	AM	BLUP	Fam	AM	BLUP
15	12	-1.60	29	90	-0.85	12	7	1.86
19	12	-1.60	25	91	-0.40	61	7	1.86
22	12	-0.72	47	91	0.03	6	6	1.48
60	12	-0.51	24	92	0.04	51	6	1.04
61	12	-0.51	3	92	-0.84	21	6	0.99
62	12	-0.51	61	92	0.16	22	6	0.99
32	12	-0.35	10	94	0.05	23	6	0.99
39	12	-1.03	44	94	1.36	58	6	0.88
46	12	-1.03	46	95	1.80	59	6	0.88
12	13	-0.81	56	95	0.63	5	5	0.93
Amp.	1	1.25		5	2.65		2	0.93
Me.	12.10	-0.87		92.60	0.20		6.10	1.19
	NP			100SW (g)			GY (g)	
Fam	AM	BLUP	Fam	AM	BLUP	Fam	AM	BLUP
12	217	113.16	45	20.86	2.58	21	41.94	14.06
21	195	92.48	47	19.86	2.08	12	41.04	12.49
51	191	85.49	46	18.86	1.58	61	40.46	12.75
23	146	49.25	37	17.66	1.58	48	38.74	11.13
61	133	38.45	27	17.13	1.17	23	34.70	8.50
50	131	32.55	26	16.93	1.07	13	33.92	7.02
56	126	28.14	42	16.86	0.58	14	32.59	6.00
37	122	33.43	59	16.72	1.15	51	31.03	5.21
39	122	32.22	38	16.66	1.08	55	30.68	4.94
52	118	21.08	7	16.53	0.98	37	27.58	4.00
Amp.	29.19	28.72		19.09	14.44		14.36	10.06
Me.	59.86	18.04		40.81	9.07		35.27	8.61

Fam: Families selected for calculating the genetic gain; AM: adjusted mean; BLUP: best linear unbiased prediction; Amp: amplitude; Me: means; IHF: insertion height of first pod; PHM: plant height at maturity; NB: number of branches; NP: number of pods per plant; 100SW: 100-seed weight, and GY: grain yield.

Table 4. Phenotypic and genotypic values of the top 10 families obtained by the Bulk method in the F_5 generation.

	IHP (cm)			PHM (cm)			NB	
Fam	AM	BLUP	Fam	AM	BLUP	Fam	AM	BLUP
5	12	-0.40	25	91	-3.72	6	6	1.00
26	12	0.18	7	93	-5.17	57	6	0.79
51	12	-0.43	19	93	-2.46	7	5	0.63
54	12	-0.43	20	93	-2.46	14	5	0.67
32	12	0.54	8	94	-4.74	52	5	0.52
44	12	0.18	46	94	-2.04	29	5	0.53
11	13	0.40	50	94	-1.86	58	5	0.42
14	13	0.84	18	94	-2.03	11	4	0.26
23	13	0.98	62	94	-4.41	39	4	0.43
27	13	0.98	28	95	-2.01	13	4	0.29
Amp.	1	1.38		4	-3.16		2	0.74
Me.	12.40	0.28		93.50	-3.09		4.90	0.55
	NP			100SW (g)			GY (g)	
Fam	AM	BLUP	Fam	AM	BLUP	Fam	AM	BLUP
57	135	40.97	10	18.13	3.73	57	32.24	8.46
39	132	36.67	12	16.94	2.70	54	29.02	4.94
42	132	36.67	33	16.91	2.93	53	30.93	6.18
52	125	27.93	28	16.78	2.39	52	27.99	4.28
53	120	24.42	45	16.31	1.92	42	25.52	4.27
54	117	22.31	46	16.29	1.90	39	27.62	5.62
33	112	26.76	13	16.13	2.06	16	25.90	3.50
56	112	18.76	5	16.08	2.11	14	35.42	9.65
26	108	17.85	32	15.61	1.90	13	25.67	3.35
29	105	15.74	43	15.28	1.10	12	32.52	7.78
Amp.	30	25.23		2.5	2.63		6.72	6.30
Me.	119.80	26.81		16.45	2.27		29.28	5.80

Fam: Families selected for calculating the genetic gain; AM: adjusted mean; BLUP: best linear unbiased prediction; Amp: amplitude; Me: means; IHF: insertion height of first pod; PHM: plant height at maturity; NB: number of branches; NP: number of pods per plant; 100SW: 100-seed weight, and GY: grain yield.

For most traits, a higher genetic variance was obtained with the BulkF3 method (Table 5). This fact is important because genetic variability is directly related to genetic gain. In the BulkF3 method, the genetic variance ranged from 1.06 for NB to 1,179.15 for NP, while in the Bulk method, the genetic variance ranged from 0.34 for NB to 404.03 for NP, considering all traits.

In general, the heritability estimates were of medium to high magnitude for most of the traits (Table 5), indicating successful selection. The heritability estimates obtained with the BulkF3 method ranged from 44% for PHM to 88% for NP. In the traditional Bulk method, heritability ranged from 38% for 100SW to 80% for IHP (Table 5). Similar estimates have been reported by Rocha et al. (2015) for PHM and by Chung et al. (2003) and Carvalho, Fristche Neto, and Geraldi (2008) for GY using the BLUP.

Table 5. Genetic parameter estimates for the BulkF3 and Bulk methods in the F_5 generation.

Traits	Method	Genetic parameter			
		σ_g^2	σ_e^2	h^2	Accuracy
Insertion height of first pod	BulkF3	6.37	1.71	0.78	8.83
	Bulk	5.78	1.47	0.80	8.94
Plant height at maturity	BulkF3	56.51	70.88	0.44	6.63
	Bulk	55.48	74.43	0.43	6.56
Number of branches	BulkF3	1.06	0.72	0.60	7.75
	Bulk	0.34	0.57	0.38	6.16
Number of pods per plant	BulkF3	1179.15	157.31	0.88	9.38
	Bulk	404.03	170.27	0.70	8.37
100-seed weight	BulkF3	2.03	2.02	0.50	7.07
	Bulk	6.76	1.78	0.79	8.89
Grain yield	BulkF3	28.48	8.85	0.77	8.77
	Bulk	17.87	9.78	0.65	8.06

σ_g^2: Genetic variance, σ_e^2: residual variance, and h^2: broad-sense heritability.

Higher heritability estimates are associated with greater genetic variability, higher accuracy of selection (Cargnelutti Filho, Storck, & Ribeiro, 2009) and, consequently, a greater chance of successful selection of superior families (Hamawaki et al., 2012; Silva et al., 2014). According to Ramalho et al. (2012), the heritability coefficient is a very useful parameter for breeders because it permits the success of selection and reflects the proportion of phenotypic variation that can be inherited.

A selection method of segregating populations will be more efficient when the magnitude of genetic gain is increased (Raposo et al., 2000). According to Miladinović et al. (2011), genetic gain is considered an indicator of the efficiency of the method. Thus, in addition to the average performance of families, the probability of higher gain with selection should be considered when choosing a selection method. However, the decision of the best method should be based not only on genetic gain but also on the confidence interval of genetic gain (Resende et al.,

1995). According to these authors, the lower limits rather than the upper limits of the confidence interval should be analyzed. Based on this premise, the BulkF3 method resulted in higher genetic gains and extended the lower limit for GY, IHP, PHM, NB, and NP (Table 6). For GY, NB, and NP, the BulkF3 method exhibited a lower limit that was higher than the upper limit obtained with the Bulk method (Table 6).

It should be noted that in the interpretation of confidence intervals, more accurate methods have narrower intervals. In this respect, although the lower limits of the BulkF3 methods were greater than the upper limits of the Bulk method, the amplitude of the confidence intervals was similar. The results of the present study therefore show that, compared with the Bulk method, the BulkF3 method provided higher genetic gain for most traits and that the amplitude of the confidence intervals was similar between both methods (Table 6). It is worth noting that a negative genetic gain is expected for PHM and IHP since height plants of about 80 to 100 cm and with an insertion of the first pod above 12 cm, respectively, are preferred in breeding programs (Sediyama, Teixeira, & Barros, 2015; Sediyama, Teixeira, & Reis, 2013).

Table 6. Rates of genetic gains (GG) and confidence intervals (CI) obtained by the BulkF3 and Bulk methods in the F_5 generation.

Traits	Method	GG	CI
Insertion height of first pod	BulkF3	-0.88	-1.38 a -0.38
	Bulk	-0.28	-0.73 a 0.17
Plant height at maturity	BulkF3	-0.18	-2.71 a 2.34
	Bulk	-3.51	-6.04 a -0.98
Number of branches	BulkF3	1.29	1.00 a 1.57
	Bulk	0.60	0.39 a 0.80
Number of pods per plant	BulkF3	55.28	50.33 a 60.23
	Bulk	29.76	25.05 a 34.46
100-seed weight	BulkF3	1.61	1.16 a 2.06
	Bulk	2.29	1.78 a 2.79
Grain yield	BulkF3	9.23	8.15 a 10.31
	Bulk	6.77	5.69 a 7.85

Results similar to those of the present study, which confirm higher genetic gains with the BulkF3 method compared to bulk and pedigree/bulk selections, were reported by Wang et al. (2003) for wheat using simulated data. Similarly, Silva et al. (2013), who compared Bulk, BulkF3 and SSD selection methods of segregating populations, concluded that the BulkF3 method was the best and most effective method at obtaining bean families based on the results of genetic gain.

One parameter that can be used to compare the superiority of selection methods is the average performance of families selected by the same methods in the last generation (F_5), corresponding

to the end of the selection process (Table 7). In our studies, comparison by the t-tests showed that the BulkF3 method provided the highest means, indicating the superiority of this method compared to the Bulk method (Table 7).

Table 7. Comparison of families obtained from the BulkF3 and Bulk methods throughout the generations with respect to insertion height of first pod (IHF), plant height at maturity (PHM), number of branches (NB), number of pods per plant (NP), 100-seed weight (100SW), and grain yield (GY).

Generation	Method	IHP	PHM	NB	NP	100SW	GY
F_3	BulkF3	14.25 a	105.87 b	4.00 a	132.32 a	14.69 a	41.40 a
F_3	Bulk	14.97 a	104.24 a	2.77 b	95.37 b	14.25 a	27.57 b
F_4	BulkF3	13.57 a	98.66 a	4.72 b	95.03 b	16.13 a	31.61 a
F_4	Bulk	13.69 a	100.99 b	5.16 a	118.71 a	15.09 a	28.13 b
F_5	BulkF3	13.40 a	92.83 a	3.62 a	87.18 a	14.60 a	23.28 a
F_5	Bulk	11.94 b	101.66 b	3.09 b	77.11 b	13.54 b	18.93 b

Values within a column followed by the same letters are not significantly different from each other by according to the t-test.

The percentages of gain were calculated for the F_5 generation for better visualization of the results. The traits studied and their associated percent gains were follows: IHP (12.22%), PHM (0.91%), NB (17.15%), NP (13.06%), 100SW (0.78%), and GY (22.98%). The highest percent gain was obtained for GY, indicating that the BulkF3 method generated more productive families. These results highlight the superiority of the BulkF3 method over the Bulk method.

Thus, compared with those obtained with the bulk method, the families obtained with the BulkF3 method were more productive at the end of the selection process (F_5 generation) and had a suitable height, a larger number of branches and pods, and a higher 100SW. Similarly, Menezes Júnior et al. (2011) reported that the Bulk selection of families derived from F_3 plants is a promising breeding strategy for bean crops because of the larger number of productive families.

Conclusion

The BulkF3 method constituted an effective strategy for soybean improvement when compared to the traditional Bulk method.

The BulkF3 method is more indicated when the objective is to increase the rate of genetic gain in grain yield.

Acknowledgements

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