Effect of the number of intermatings on genetic properties of a segregant common bean population

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ABSTRACT - *Objective of this study was to verify whether the intermating of F_2 plants improves the efficiency of the selective process in common bean. A multiple cross involving eight parents was obtained for this purpose. Three populations were derived from this cross (S_0): without intermating, with one, and with two intermatings. One hundred and thirty families were taken from each population to assess the $S_{0:2}$ and $S_{0:3}$ generations. The magnitude of the trait grain yield was not affected by intermating, while the genetic variance presented a small increase that could be due to the linkage in repulsion of some genes involved in the trait control. The conclusion was drawn that the enhancement in the efficiency of the selective process with intermating does not justify the required time and cost investment.*

INTRODUCTION

Grain yield and other economically important traits in the cultivated species are controlled by innumerous genes and it is difficult to accumulate all favorable alleles into a single individual. Breeders therefore try to associate the favorable alleles to different individuals by means of crossing. There are several alternatives for the improvement of autogamous plants to combine parents, that is, to obtain a segregant population. One possibility is through biparental crosses, which gives rise to single-cross hybrids, or else, involving three, four or several parents (poly-cross hybrids) (Fehr 1987, Fouilloux and Bannerot 1988). There is no consensus on which alternative is the best. However, if a thorough choice of the parents is not possible, multiple hybrids should be chosen, as shown by Carneiro (2002) for grain yield in the common bean crop.

One of the questions in relation to obtaining a multiple hybrid is whether it is necessary to intermate S_0 plants to increase the chance of recombination. Some studies with simulation show that intermating is advantageous (Hanson 1959, Pederson 1974, Fujimaki 1979). On the other hand, Bos (1977) mentioned contrary results. The effect of intermating under field conditions was also studied in some crops, as in cotton (Meredith and Brigde 1971), wheat (Altman and Busch 1984), soybean (Guimarães and Fehr 1989), and rice (Marin-Garavito 1994, Cordeiro 2001). In general, intermating did not appear to be beneficial.

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However, most of these studies were realized by intermating in recurrent selection programs by means of male sterility, where the crosses are completely randomized. By manual intermating the hybridizations can be manipulated and attain a greater efficiency. No information in relation to intermating for the bean crop was found, so the aim of the present study was to verify whether the genetic properties and the efficiency of the selective process in segregant populations of the common bean, provenient from a multiple cross, increase when S_0 plants are manually intercrossed.

MATERIAL AND METHODS

The experiments were conducted at the Departamento de Biologia, da Universidade Federal de Lavras (UFLA), Lavras, MG, Brasil. The segregant population was obtained from a cross of eight lines, all of the carioca grain type, i.e., cream with brown stripes (main characteristics see Table 1). The eight parents were initially crossed by pairs, which brought forth four single-cross hybrids: (PÉROLA x FEB 200), (MAR 2 x H 4-10), (AN 9022180 x IAPAR 31), and (PF 9029975 x A 805). Double-cross hybrids were obtained thereafter, by crossing the four F_1's: [(PÉROLA x FEB 200) x (MAR 2 x H 4-10)] and [(AN 9022180 x IAPAR 31) x (PF 9029975 x A 805)]. Finally, using the two double-cross hybrids, a multiple cross hybrid was obtained involving the eight parents. The crosses were carried out so that an amount of around 300 seeds could be obtained from the multiple cross hybrid.

The F_1 seeds of this hybrid were sown on the field to obtain generation F_2 (S_0). A part of the S_0 seeds were stored to originate the population without intermating (I_0). The remanescent seeds were used for the intermating of the plants. The process was carried out manually in a greenhouse, randomly, with 250 plants, bringing forth 100 cross pairs. The obtained seeds were blended, one part to make up the population with a

cycle of intermating (I_1) and the rest for the second intermating (I_2), analogically to the first. The bulk seeds of the segregant populations from I_0, I_1, and I_2, were used for the evaluation and extraction of families.

The three S_0 populations from the I_0, I_1, and I_2 intermatings, besides two controls 'H 4-10' and 'Pérola', were evaluated in February 2000 in a randomized complete block design, in six replications. Each plot consisted of four rows of five meters, with 15 seeds per meter, to obtain grain yield data. The experiment was installed in an area under no tillage and water supply via irrigation. Four hundred kg ha^{-1} of the fertilizer formula 8-28-16 were applied at planting and 25 days after plant emergence 150 kg ha^{-1} of ammonium sulphate were applied in cover. The other crop treatments were realized according to regional recommendations for common bean.

To obtain the $S_{0:1}$ families, samples from 130 plants were randomly collected at each cycle (I_0, I_1, and I_2) at the harvest of the bulk of the segregant S_0 populations. The 390 families were multiplied (sowing in July 2000). Each family was represented in a 2 m row. At harvest, the seeds within every row were mixed, which gave rise to the origin of the $S_{0:2}$ families.

The three hundred and ninety $S_{0:2}$ families, the eight parents and controls, LH-11 and CII-102, making up 400 treatments, were evaluated in a 20 x 20 simple lattice design. Each plot was represented by a 2 m row, spaced 0.5 m apart in a density of 15 seeds m^{-1}. The seeds were sown in February 2001, under the aforementioned fertilization and crop treatments and the grain yield data per plot were obtained.

At sowing in July 2001 the 390 $S_{0:3}$ families, together with the parents and controls of the previous evaluation, were evaluated again in the same design, but in three replications. The plot size and crop management were similar to the reported in the previous experiment. The data were subjected to variance analysis by software MSTAT-C (1991). Considering the generation and the mean effect as fixed and the others as random,

Table 1. Characteristics of common bean lines used to obtain the segregant population

Lines	Growth habit	Plant habit	AN[1]	ALS[2]	FU[3]	CBB[4]	Origin
PÉROLA	II/III	Climbing	S[5]	T[6]	T	S	Embrapa
FEB 200	II	Erect	R[7]	S	-	S	CIAT
MAR 2	III	Climbing	-	R	-	S	CIAT
H 4-10	II	Erect	R	S	-	S	UFLA
AN 9022180	II	Erect	S	T	-	S	Embrapa
IAPAR 31	II	Erect	R	T	-	T	IAPAR
PF 9029975	II	Erect	S	T	-	S	UFLA
A 805	II	Erect	R	S	-	S	CIAT

[1]Anthracnose; [2]Angular leaf spot; [3]Fusarium wilt; [4]Common bacterial blight; [5]Susceptibility resistance; [6]Partial resistance; [7]Resistance reaction

the genetic and phenotypic parameters were estimated according to procedures described by Aguiar (2003).

RESULTS AND DISCUSSION

The variance analysis of the grain yield of the segregant S_0 populations showed that the F test did not detect any significant difference among the three populations, indicating that intermating did not alter the mean performance of the populations. As observed in Table 2, the mean population yields were very similar, whether subjected to intermating or not.

The joint analysis of the evaluation of the $S_{0:2}$ and $S_{0:3}$ families (Table 3) shows that the effect of the generations and the interaction generations x harvests was significant ($P \leq 0.01$). The mean yield of the $S_{0:3}$ families was reasonably higher than that of $S_{0:2}$ (Table 4). The generation effect alone did not explain the difference in the mean performance of the families; if the trait grain yield were controlled by additive genes, the mean would not change owing to endogamy. With dominance, because of the endogamy, the mean of the $S_{0:3}$ generation should be lower and not higher, as happened here. The explanation for the difference is the environmental effect.

Table 2. Grain yield means of the S_0 populations with different numbers of intermatings and percentage of the controls.

Number of intermatings		Grain yield	fron controls
		— kg ha⁻¹—	— % —
0		2391 a[1]	100.0
1		2247 a	94.0
2		2302 a	96.3
Mean		2313	
Controls	PÉROLA	2530	
	H 4-10	2272	
Mean		2401	

[1]means followed by the same letter are not significantly different at a level of P<0.05 in the Tukey test

Table 3. Joint variance analysis of the grain yield (kg ha⁻¹) obtained in the evaluation of the families of the generations $S_{0:2}$ and $S_{0:3}$

Sources of variation	df	Mean squares	Prob.[1]
Generations (Harvests)	1	1375852342.21	0.000
Treatments	399	914618.89	0.000
Families	389	916966.00	0.000
Families I_0	129	808271.19	0.000
Families I_1	129	1011184.74	0.000
Families I_2	129	894146.22	0.000
Among family types	2	1902409.40	0.017
Controls	9	1168264.65	0.006
Families vs. controls	1	232937.76	0.479
Treatments x generations	399	742677.89	0.000
Families x generations	389	754660.84	0.000
I_0 families x generations	129	725419.72	0.000
I_1 families x generations	129	799714.21	0.000
I_2 families x generations	129	748122.96	0.000
Among family types x generations	2	156464.88	0.718
Controls x generations	9	285519.04	0.789
Families vs. controls x generations	1	195738.34	0.517
Mean effective error	1482	465660.89	
VC (%)	15.31		
Mean	3619.00		

[1]Significance level in the F test

Table 4. Mean of the grain yield (kg ha^{-1}) of the populations with different numbers of intermatings of the generations $S_{0:2}$ and $S_{0:3}$

Generations	Intermating cycles			Mean
	0	1	2	
$S_{0:2}$	2708	2836	2831	2791
$S_{0:3}$	4434	4512	4497	4481
Joint	3571	3674	3664	3636
%	100	102.9	102.6	.

It has been verified that in the fall-winter crop (sowing in July) the environmental conditions are more favorable. In this season, temperatures are mild and the relative humidity is lower. These conditions contribute to a reduction in pathogens and improvement of the fruit setting and grain filling.

Significant differences ($P \leq 0.01$) were also stated between the families and between the families of populations with 0, 1 and 2 intermatings (Table 3). Despite the difference detected in the mean performance of the families, the variation was very small. That is, the mean superiority of families derived from populations with intermating was only 2.8% in relation to the obtained without intermating (Table 4). The interactions families x generations were all significant, giving evidence that the behavior of the families was not coincident in the two evaluated environments (Table 3). It is worth mentioning the source of variation among family types, which was significant ($P = 0.017$), despite the interaction family types x generations was not significant ($P = 0.72$).

The intermating did practically not alter the population mean for generation $S_{0:2}$ or for $S_{0:3}$ (Tables 3 and 4), as observed earlier in the S_0 generation. Similar results have been reported frequently in literature regarding other species such as cotton (Meredith and Bridge 1971), wheat (Altman and Busch 1984), soybean (Guimarães and Fehr 1989), and rice (Marin-Garavito 1994). However, in a study carried out with rice using genetic male sterility for recombination, an increase in the grain yield mean was stated after 4 intermating cycles (Cordeiro 2001).

Considering a single locus, a population mean (m) is obtained by: $m = (2p-1)\alpha + 2p(1-p)\delta$ (Falconer and Mackay 1996), where p is the frequency of the favorable alleles; α the contribution of the loci in homozygosis, deviation of the homozygotes in relation to the mean; and δ the deviation of the heterozygotes in relation to the mean. The two latter were property components of the locus. Thus, if there are no problems with sampling, the allelic frequency does not alter because of the intermating and, therefore, the mean cannot be altered. However, a large number of genes must be involved in the control of the trait grain yield, and not only one. In this same situation, even if the genes are linked and the population is not in equilibrium, the mean is not affected by intermating. The mean is only influenced by intermating if the two genes are linked and if epistasis occurs. Unfortunately, no reports were found on the occurrence of epistasis in the common bean for grain yield. In the case of rice, where the mean increased through intermating, Cordeiro (2001) explained that, besides the possible effect of the aforementioned disequilibrium of linkage and epistasis, there is also the effect of natural selection. In the present study, as intermating was realized under controlled greenhouse conditions, there was probably no action of natural selection.

Intermating is expected to provide the breakup of linkage blocks and, consequently, a greater release of variability (Hanson 1959, Fujimaki 1979), despite this fact is frequently called into question (Pederson 1974, Bos 1977). The release of the variability can be proved, for instance, by means of the estimate of the genetic variance among families. In case of the evaluation involving generation $S_{0:3}$, the release of the genetic variability was greater with intermating. Nevertheless, in generation $S_{0:2}$ the genetic variance estimates were very similar (Table 5).

It is worth pointing out that when the population is in Hardy-Weinberg equilibrium, the genetic variance is not modified, once the genotypic background is the same. Nonetheless, taking the large number of involved genes into account, the population is certainly not in equilibrium since many loci would have to be linked, as mentioned earlier. Under this condition it is expected that the genetic variance is altered by intermating, as it occurred in $S_{0:3}$, in other words, when the genes are linked in a phase of repulsion the variance decreases, while when the variance are linked in a phase of attraction, the variance increases through intermating.

The heritability estimate (h^2) can also be used to evidence if a greater release of variability occurred, since the families were evaluated in the same experiment and therefore it can be expected that the environmental effect would be similar. The h^2 estimates are in agreement with the observations on the genetic variances (Table 5). That is, h^2 can be considered to have the same magnitude for the

Table 5. Estimates of genetic variances (σ_G^2), phenotypic variances (σ_F^2), variances of families x generations interaction (σ_{GxS}^2), genetic correlations (r_G), heritability (h^2) and upper (LU) and lower (LL) heritability limits

Generations	Parameter	Number of intermatings		
		0	1	2
$S_{0:2}$	σ_G^2	194174 (94481; 376933)*	241146 (145491; 452673)	177165 (112459; 400700)
	σ_F^2	433898	480870	416889
	h^2 (%)	44.75	50.14	42.49
	LL (%)	24.85	31.99	21.82
	LU (%)	58.75	62.67	57.09
$S_{0:3}$	σ_G^2	54543 (43097; 70029)	123117 (97281; 158074)	116805 (92293; 149969)
	σ_F^2	205167	273742	267429
	h^2 (%)	26.59	44.97	43.67
	LL (%)	16.55	22.71	22.47
	LU (%)	54.20	57.58	50.88
Joint	σ_G^2	17261	44056	30422
	σ_F^2	168390	210663	186280
	σ_{GxS}^2	108232	139188	117692
	h^2 (%)	10.25	20.91	16.33

*Values in brackets refer to the confidence interval of the genetic variance considering α = 0.05

different numbers of intermating in generation $S_{0:2}$ and in the case of $S_{0:3}$; estimates were smaller without intermating. We should bear in mind, however, that in the same $S_{0:3}$ generation, the estimates of the lower and higher heritability limits suggest that the estimates can be considered similar with a probability of 95%.

Finally, to compare the effect of intermating, the percentage of families with higher or lower performance can be used. The twenty families with highest and lowest mean among the evaluated 390 were identified for this purpose, checking whether they were derived from populations with or without intermating (Table 6). Considering the mean performance of the two generations, the number of families with a superior performance was slightly higher with intermating, i.e, of the 20 best families eight came from the population with one intermating, seven with two, and two without intermating. Among the 20 worst families, five were of two intermatings, eight of one intermating and seven without intermating. This variation is probably random, which was expected in view of the independent distribution of the segregant genes in the trait control; as mentioned earlier, the genotypic frequency would not change with intermating under these conditions. The genotypic frequency would only change if there were linkage disequilibrium.

Bos (1977) discusses this aspect considering the two genes A and B, with different recombination frequencies among them, and calculating the expected number of individuals with the genotype AABB or A_B_. As mentioned earlier, if the distribution is independent, the intermating does not alter the expected genotypic frequency. If the genes are linked under repulsion, the frequency of plants wit the desired genotype increases through intermating. This increase is the greater the smaller the recombination frequency. The maximum increase in the genotype frequency AABB is 25%, compared to generation F_∞ (without intermating) and F'_∞ (with intermating).

On this background, it becomes clear that intermating is only advantageous when the genes are linked in repulsion and under a very low recombination frequency. Considering the time required for intermating, that is, one harvest per intermating, it is questionable whether this additionally invested time is of any advantage compared to the more extensive evaluation of the families in one or more additional harvests. Despite this information is not available for common bean, in the case of recurrent selection in soybean, Guimarães and Fehr (1989) showed that it was more advantageous to evaluate the families in two harvests with only one recombination instead of two intermatings, and evaluate in only one harvest. The same was stated for other crops, such as rice (Marin-Garavito 1994), maize (Lima Neto 1998), wheat (Altman and Busch 1984), and cotton (Meredith and Brigde 1971).

Table 6. Grain yield means of 20 families with best and 20 families with worst performance considering the mean performance of the two generations

Best families			Worst families		
Rank	Families	kg ha^{-1}	Rank	Families	kg ha^{-1}
1	B58	5116	1	B19	2270
2	B104	4892	2	C12	2425
3	B77	4843	3	C68	2448
4	C31	4777	4	B67	2623
5	B53	4768	5	A129	2623
6	C24	4713	6	B6	2645
7	B33	4607	7	B14	2662
8	C29	4545	8	A7	2684
9	A15	4522	9	C22	2698
10	C99	4516	10	A36	2754
11	B17	4515	11	C44	2765
12	C103	4510	12	A104	2792
13	A84	4500	13	B41	2794
14	A124	4492	14	A6	2799
15	C30	4482	15	B127	2843
16	B57	4473	16	B125	2867
17	B91	4391	17	B24	2874
18	A37	4386	18	C76	2897
19	C57	4360	19	A65	2908
20	A25	4356	20	A123	2947

A - without intermating; B - one intermating; C - two intermatings

CONCLUSION

The mean of the genetic properties of the segregant population of common bean was not altered by intermating. On the other hand, the genetic variance presented a small increase that may be due to the linkage disequilibrium of involved genes in repulsion. This increase, however, does probably not justify the time and costs required to intermate F$_2$ plants.

Implicações do número de intercruzamentos nas propriedades genéticas de uma população segregante de feijoeiro

RESUMO - *O objetivo do trabalho foi verificar se o intercruzámento de plantas F$_2$ melhora a eficiência do processo seletivo no feijoeiro. Para isso foi obtido um cruzamento múltiplo envolvendo oito pais. Desse cruzamento foram geradas três populações (S$_0$): sem intercruzamento, com um e com dois intercruzamentos. De cada população foram retiradas 130 famílias, as quais foram avaliadas nas gerações S$_{0:2}$ e S$_{0:3}$. Considerando o caráter produtividade de grãos, constatou-se que a média não foi afetada pelo intercruzamento; já a variância genética apresentou um pequeno incremento, que pode ser devido a ligação em repulsão de alguns genes envolvidos no controle do caráter. Conclui-se que a melhoria na eficiência do processo seletivo com o intercruzamento não compensa o tempo e os recursos gastos.*

Palavras-chave: intercruzamento, plantas F$_2$, variância genética, repulsão

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