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Comparison between the SPD and the SPDS Methods for Segregating Generation Advancement in Soybean

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ABSTRACT

The purpose of this work was to compare the SPD (Single Pod Descent) and SPDS (Single Pod Descent with Selection) methods for segregating generation advancement in soybean breeding. Sixteen populations derived from crosses among Japanese and adapted Brazilian soybean were used. The traits, number of days to flowering (NDF) and to maturity (NDM), plant height at flowering (PHF) and at maturity (PHM), and individual plant yield (IPY) were assessed. The populations obtained by SPDS showed values close to the variation in the populations obtained by SPD. Regression through the origin showed that both methods were equivalent for NDM in the F_3 generation and for NDF in the F_4 generation. For all other trait/generation combinations, SPDS yielded superior means. Therefore, it was preferable to use SPDS because it allowed the advance of generations with smaller population size.

Key words: *Glycine max*, breeding methods, food type soybean

INTRODUCTION

Soybean [Glycine max (L.) Merrill] is an important crop in Brazil due to its large cultivation area in different regions of the country. The importance of soybean worldwide is due mainly to its high oil and protein contents (Marega Filho et al., 2001). Cross-breeding between pure lines is performed in order to obtain new cultivars, usually more productive and/or with other desirable characteristics. The segregating generations may be conducted by any of the methods frequently used for self-fertilization crops: pedigree, bulk, single seed descent (SSD) or single pod descent (SPD). The SSD method is implemented by collecting a single seed from each plant to form the next generation (Brim, 1966). When the number of F₂ plants is limited, a method derived from SSD can be used, in which each plant is represented by multiple seeds in all the segregating generations (Fehr, 1987). To obtain a sample of seeds in which all plants are equally represented in later generations, a pod with 3 seeds is cultivated, characterizing the SPD method. This method is an easy and economic alternative for the advance of segregating populations, aiming at a quick homozygosis.

A modification of the SPD method is the SPDS method (Single Pod Descent with Selection). It is similar to SPD, but no pods are collected from agronomically inferior plants in the F_2 to F_4 generations. Such selection in segregating generations must be performed only for qualitative or quantitative traits with high heritability, and which have high phenotypic and genotypic correlation with grain yield.

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In studies comparing the main advantages and disadvantages of several generation advancement methods, Toledo et al. (1994) concluded that the methods had similar probabilities to generate descendants with high grain yield when the sample of genetic variability was comparable. This explains the preference for simpler methods, such as the SPD. In order to obtain plants with a long juvenile period and based on the information that the inheritance of this character is simple (few genes involved) and that there is the possibility of selecting genotypes with this characteristic under short day conditions, the following procedure has been used in the breeding program at the National Research Center of EMBRAPA - Brazilian Company for Agronomic Research - in Londrina, PR (Kiihl and Garcia, 1989): a) genotypes used in crosses are planted in November and December, to coincide with the flowering period; the crosses are carried out in January and February and the seeds are harvested in March or April; b) the F₁ are cultivated during the winter in greenhouses with supplementary light, for 20-30 days, to avoid early flowering; c) the F_2 populations are grown in the field, with early October planting. One pod is collected per plant, but only from plants with a long juvenile period (SPDS method); d) the F₃ populations are grown during winter, from which one pod per plant is harvested (SPD method); e) the F₄ populations are planted early in October, and the best plants with long juvenile period are harvested individually; f) F₅ progenies are grown also in early October planting, and the best lines are selected.

The Brazilian cultivars are not adequate for direct human consumption. To obtain such cultivars, industrial sovbean is usually crossed with food type genotypes. Pure lines of food type soybean were developed in Asia (approximately 40° N latitude) and showed a short juvenile period. When they are cultivated in Brazil, where the days are shorter due to low latitude, there is a early flowering. Such fact, associated with the determinate growing habit reduces the number of nodes, plant height and grain yield (Manfredini et al., 1998). Destro et al. (1994) observed that the soybean introduced from Japan had disadvantage of having low physiological seed quality which reduced the germination and emergence capacity of the seeding in the field.

The purpose of this work was to compare the SPD and SPDS methods for segregating generation advancement in soybean cross-breeding for human consumption with industrial soybean.

MATERIAL AND METHODS

The experiment was developed and conducted at the Londrina State University (UEL) School Farm (Londrina, PR, Brazil), located at 23° 22′ LS, 51° 10′ LW and 585 meters above sea level on a structured purple soil, classified as eutrophic. The 16 populations used were supplied by the UEL Soybean Breeding Program (Table 1). Generations advance were accomplished through SPD and SPDS methods.

Table 1 - Assessed segregating populations.

Population	Cross
1	Paranagoiana X F82-5722
2	Paranagoiana X F82-5782
3	Paranagoiana X F83-7843
4	Paranagoiana X Sel Stwart
5	Paranagoiana X Tadasha
6	BR-27 X Late Giant
7	BR-27 X Kitamusume
8	BR-27 X F82-5722
9	BR-27 X F82-5782
10	BR-27 X F83-7843
11	BR-27 X Natto
12	BR92-15360 X Tambagura
13	BR92-15360 X F82-5722
14	BR92-15360 X F82-5782
15	BR92-15360 X F83-7843
16	BR92-15360 X Wilami

In the F_2 generation, a pod containing three seeds was collected from all the population plants for generation advance and another pod was reserved, using the SPD method. The same procedure was used only on plants with superior agronomic worth (AW), characterizing the SPDS method. The agronomic worth was visually analyzed at maturity. This grade scale varied from one to five, in which one corresponded to a bad plant and five to an excellent one. It represents a visual rate of the plant global merit for a series of adaptative traits such as: number of pods; plant strength and health, resistance lodging, to shattering resistance, and reducted leaf retention after maturity (Guerra et al., 1999). The SPDS method caused a reduction in the population size by the elimination of inferior genotypes, before time and effort were spent on reaching homozygosis. Approximately 15% of the total of plants used initially remained. It is important to emphasize that the selection occurred only in generation F_2 , as in the procedure used by Kiihl and Garcia (1989).

In the F_3 and F_4 generations, the seeds for generation advancement by the SPD and SPDS methods were sown separately to allow their comparative evaluation. In the agricultural year of 1997/98, the F₃ populations were sown in the field on December 22, in six-meter rows for the SPD method and two-meter rows for the SPDS method. The spacing between the rows was 0.9 meter. Three seeds per plant were harvested from the F₃ populations for advance to generation F₄, both for the SPD method and the SPDS method. In the agricultural year of 1998/99, the F₄ populations were sown in the field on December 10. Each population was represented by a five-meter row for the SPD method and a one-meter row for the SPDS method. The spacing between lines was 0.9 meter. The sowing density was 10 plants per linear

In the two agricultural years, the preparation of the soil was conventional, with one plowing and two harrowing. Fertilization was done with 400 kg/ha of NPK, in the 8-28-16 formulation. After the sowing, the fields were hand hoed to control weeds. Insecticides were used to control soybean leaf-shedding caterpillars (*Anticarsia gemmatalis*) and soybean bugs (*Nezara viridula*).

In F₃ and F₄ generations, all plants were harvested and thrashed individually. Five quantitative characters were assessed to compare SPD and generation advance methods. characters were: a) number of days to the beginning of flowering (NDF) corresponding to the period between the sowing and the opening of the first flower (R₁ stage of the scale of Fehr and Caviness, 1977); b) plant height at the beginning of flowering (PHF) which corresponds to the distance in cm measured between the soil level and the most distal inflorescence insertion on the main stem, assessed at the R_1 stage; c) number of days to maturity (NDM) which corresponds to the period between the sowing and the day in which approximately 95% of the pods appeared to be mature (R₈ stage); d) plant height at maturity (PHM) which corresponding the distance (cm) measured from the soil surface and the farthest flower bud on the main stem, evaluated at the R₈ stage; and e) individual plant yield (IPY), evaluated after thrashing and by the grain weight. The data were collected in grams per plant, but only in the F₄ generation. Still were assessed costs of labor, breeder's work and experimental area, but these data are not presented here.

Correlation estimates, means and coefficients of variation of each population were used to compare the methods. Correlation estimates from individual plants among the traits were obtained as described by Mode and Robinson (1959). The significance of the correlations was tested by the *t* test, with n-2 degrees of freedom (Venkovsky and Barriga, 1992). The coefficients of variation (cv) were obtained by:

cv = (standard deviation / mean) * 100.

The mean of each population still was used in the analysis of regression. The regression procedure, which fits a straight line passing through the origin (Bussab, 1990), complemented with the test for hypotheses H_0 : $\beta=1$ and H_1 : $\beta<1$ was used. This procedure evaluated the possibility of substitution of the SPD by the SPDS method in the generation advancement of soybean segregating populations. Whenever hypothesis H_0 was accepted by the test, it meant that the methods were equivalent and whenever it was rejected in favor of H_1 , it meant that the methods were not equivalent.

RESULTS AND DISCUSSION

Grain yield has a low heritability in early generations (Bays, 1975). This can be overcome by the use of traits with strong heritability, which have a high association with grain yield. Among these characters, number of days to flowering and maturation and plant height at flowering and maturation, can be mentioned. In our work, the higher number of correlations among these traits and grain yield were significant (Table 2). Positive and significant correlations among these characters also were found different authors (Weatherspoon and Went, 1934; Weiss et al., 1947; Johnson et al., 1955; Anand and Torrie, 1963; Kwon and Torrie, 1964; Harrison et al., 1981).

Soybean is one of the cultivated species which is most sensitive to photoperiod. Usually the cultivars are adapted to an area with a narrow latitude range and they are sensitive to changes in the sowing date (Kiihl and Garcia, 1989). The F_3 generation was sown later than the F_4 generation. This resulted in the F_3 populations in a smaller number of days to flowering and maturation and smaller plant height at flowering and maturation (Tables 3 and 4).

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Table 2 - Correlation estimates among traits assessed in 16 soybean populations in the F_3 (diagonal above) and F_4 generations (diagonal bellow) obtained by advancing from the F_2 by the SPD and the SPDS methods. Data from 1997/98 and 1998/99, respectively ^a.

		PHF	NDF	PHM	NDM
PHF	SPD	-	0.69**	0.71**	0.36*
	SPDS	-	0.74**	0.65**	0.31*
NDF	SPD	0.52**	-	0.55**	0.22*
	SPDS	0.64**	-	0.68**	0.18^{ns}
PHM	SPD	0.62**	0.39*	-	0.29*
	SPDS	0.69**	0.54**	-	0.41**
NDM	SPD	0.57**	0.47**	0.50**	-
	SPDS	0.48**	0.39*	0.33*	-
IPY b	SPD	0.22*	0.10^{ns}	0.23*	0.21*
	SPDS	0.36*	0.19^{ns}	0.35*	0.37*

^a PHF: plant height at flowering; NDF: number of days to flowering; PHM: plant height at maturity; NDM: number of days to maturity; IPY: individual plant yield.

When both methods are compared, the results of the coefficients of variation (Tables 3, 4 and 5) showed that the selection done in the F_2 generation did not affect the variability. The populations conducted by the SPDS method, which suffered a

strong selection in the F_2 generation, continued showing values close to the variation in the populations obtained through the SPD method, which did not suffer any selection. According to Allard (1960), in most hybrids a great number of genes segregate, and thus, each F_2 plant was different from all the others. This fact explained the maintenance of the variation in the populations obtained by the SPDS method.

Averages of populations obtained by the SPDS method for all characteristics evaluated were always superior to those obtained by the SPD method (Tables 3, 4 and 5). The only exception was for the NDM character in the F_3 generation (Table 3), where the SPD method had a slightly superior average compared to the SPDS method (145.3 days for SPD and 144.1 days for SPDS).

For IPY trait, evaluated only in the F₄ generation, the comparison was only possible in populations where the cross-breeding involved early parents. Populations where the cycle was too long suffered a strong bug attack (*Nezara viridula*), which made it impossible to evaluate the IPY in these populations. Hence IPY was evaluated in seven populations (Table 5).

Table 3 - Means and coefficients of variation (cv) for four traits in 16 populations in the F_3 generation obtained by advancing from the F_2 by the SPD and the SPDS methods. Data from 1997/98^a.

	PHF				NDF				PHM				NDM			
	SPD		SPD	S	SPI	D	SPD	S	SP	D	SPD	S	SPL)	SPI	OS
population	mean	cv	mean	cv	mean	cv										
1	23.1	30.7	25.8	31.8	49.7	12.7	54.0	25.2	28.5	30.5	26.7	30.7	135.8	3.8	136.2	8.6
2	23.5	32.3	37.0	24.1	49.6	12.5	62.0	7.7	28.8	34.7	40.8	18.6	146.0	4.2	145.8	3.9
3	31.2	19.9	33.9	20.4	54.4	13.1	59.0	8.6	37.7	26.5	39.8	22.4	143.5	5.2	146.0	3.3
4	34.7	28.5	36.2	23.2	57.9	10.5	57.0	13.0	43.1	36.4	40.6	16.5	134.0	4.4	136.5	5.3
5	25.2	35.7	35.8	32.4	49.2	14.8	61.0	17.5	30.7	29.0	53.2	16.4	140.8	5.1	148.7	6.5
6	31.4	43.3	33.8	29.0	51.8	14.5	58.0	15.0	40.0	32.7	43.4	35.7	148.2	5.1	147.2	5.5
7	23.9	34.3	21.7	38.7	48.8	18.2	53.0	21.3	27.7	36.1	28.0	28.9	147.7	5.9	141.9	7.6
8	28.5	32.6	42.2	20.9	53.6	12.5	62.0	8.4	33.8	30.8	53.7	21.4	135.5	4.9	136.3	3.7
9	28.5	35.4	41.8	20.6	53.7	13.6	55.0	17.6	39.3	23.4	54.7	23.0	153.0	5.2	156.0	4.6
10	25.8	18.6	34.3	29.7	53.2	6.0	53.0	11.1	36.1	25.2	41.4	33.1	155.0	2.6	148.3	6.9
11	31.5	30.8	34.5	29.0	50.0	16.0	56.0	20.7	42.7	32.8	38.0	25.8	147.5	4.8	140.6	3.4
12	22.0	19.1	21.7	14.8	51.4	6.6	50.0	6.0	21.6	29.2	22.1	15.8	145.8	2.0	135.8	9.1
13	24.0	30.4	28.6	23.4	56.0	10.9	53.3	5.3	28.4	27.1	32.6	23.6	149.1	3.8	148.2	2.0
14	31.8	34.0	29.5	40.7	54.8	16.2	57.4	13.1	34.3	25.1	37.2	33.6	154.0	5.9	154.7	1.2
15	36.9	20.1	36.2	24.6	59.6	11.2	61.7	10.5	38.5	26.0	41.2	21.8	155.1	4.5	148.7	5.6
16	15.5	26.4	20	19.5	47.5	16.4	46.7	15.2	18.9	17.5	19.8	22.7	134.2	5.6	136.0	5.1
Means	27.3	29.6	32.0	26.4	52.5	12.9	56.1	13.5	33.1	28.9	39.1	24.4	145.3	4.6	144.1	5.1

^a PHF: plant height (cm) at flowering; NDF: number of days to flowering; PHM: plant height (cm) at maturity; NDM: number of days to maturity. Number of individuals assessed in each population (N) = 60 for SPD method and 20 for SPDS method.

^b assessed in seven populations in the F₄ generation.

^{*} and ** indicate significance at P<0.05 and P<0.01 by *t* test, respectively.

Table 4 - Means and coefficients of variation (cv) for four traits in 16 populations in the F_4 generation obtained by advancing from the F_2 by the SPD and the SPDS methods. Data from 1998/99^a.

<u></u>	PHF			NDF				PHM				NDM				
	SPI)	SPD	S	SPI)	SPD	S	SP	D	SPI	DS	SP	PD	SPI	S
Population	mean	cv	mean	cv	mean	cv										
1	30.1	22.6	40.8	12.5	53.0	9.4	57.8	5.9	43.3	29.3	47.0	16.0	141.0	8.2	142.6	5.2
2	35.1	22.2	44.0	13.2	56.7	13.8	64.9	8.5	42.4	33.0	55.0	21.8	139.0	2.3	149.3	9.4
3	41.7	16.5	49.1	22.0	61.4	7.7	63.8	11.8	48.9	31.5	53.8	24.3	145.7	4.5	161.1	4.8
4	41.8	17.2	45.5	25.1	64.0	7.7	59.5	11.1	48.3	21.7	48.4	18.6	148.3	3.6	146.0	5.4
5	41.4	15.0	42.0	15.2	64.0	8.6	61.9	4.4	43.6	28.7	44.2	10.6	155.0	3.9	153.0	1.3
6	38.8	21.9	50.8	12.2	59.8	14.7	63.4	5.8	43.0	25.3	60.2	10.1	149.0	5.7	168.0	1.3
7	57.0	14.7	59.3	8.6	63.7	7.1	67.1	3.9	47.3	27.9	47.6	38.4	153.0	6.9	163.9	3.8
8	45.1	19.7	64.1	6.7	62.7	12.4	67.2	5.6	47.8	17.8	61.5	18.0	154.4	7.1	168.0	3.2
9	46.9	16.6	57.5	13.2	58.0	2.4	66.9	3.3	50.5	18.2	54.6	13.6	162.3	5.2	168.0	1.8
10	50.6	18.0	60.2	13.6	64.3	8.6	63.6	10.5	53.5	11.2	59.6	13.9	163.6	4.0	164.0	4.5
11	55.4	12.8	57.1	10.5	62.0	8.4	63.4	3.6	63.5	14.6	70.2	16.1	164.9	4.1	152.2	1.2
12	32.4	17.9	29.8	18.1	55.9	9.8	57.8	7.3	35.0	10.0	38.0	19.5	155.0	1.1	165.5	3.1
13	35.2	4.3	34.4	18.3	63.1	1.7	56.7	2.5	38.3	17.5	39.3	20.9	160.6	4.1	164.0	3.8
14	24.4	9.4	25.7	14.8	62.0	5.6	59.8	9.4	26.3	24.7	27.2	7.7	150.3	3.8	155.8	1.2
15	26.2	29.4	23.6	22.5	60.7	5.8	57.3	8.7	24.6	35.8	30.2	66.5	150.8	7.6	151.0	5.4
16	19.8	22.7	23.2	12.1	54.6	13.6	49.5	11.9	22.9	26.6	26.2	16.8	135.8	4.0	140.8	6.6
Means	38.9	17.6	44.2	14.9	60.4	8.6	61.3	7.1	42.4	23.4	47.7	20.8	151.8	4.7	157.0	4.0

^a PHF: plant height (cm) at flowering; NDF: number of days to flowering; PHM: plant height (cm) at maturity; NDM: number of days to maturity. Number of individuals assessed in each population (N) = 50 for SPD method and 10 for SPDS method.

Table 5 - Means and coefficients of variation (cv) for individual plant yield (in grams) in seven populations in the F_4 generation, obtained by advancing from the F_2 by the SPD and the SPDS methods. Data from $1998/99^a$.

	Weight								
Population	SP	PD	SPDS						
	mean	cv	mean	cv					
1	12.4	68.15	19.57	47.37					
2	11.87	26.62	14.01	94.22					
3	5.95	17.98	6.49	39.45					
4	6.00	57.33	6.30	80.63					
11	1.34	42.54	1.63	29.45					
14	3.50	42.29	3.57	54.90					
16	3.94	75.13	4.18	77.51					
Means	6.42	47.15	7.96	60.50					

^a Number of individuals assessed in each population (N) = 50 for SPD method and 10 for SPDS method.

Taking into consideration all average values, 6.42 grams per plant was obtained by the SPD method against 7.96 grams obtained by the SPDS. As appeared from the coefficients of variation of 47.15 for SPD and 60.50 for SPDS, variation was not reduced by SPDS.

The statistical analysis showed that all regressions were significant and R² values were very high, which meant that the linear equations explained the results obtained (Table 6). For the NDM trait in generation F₃ (average of 145.3 days in SPD and 144.1 in SPDS) and NDF in generation F₄ (60.4 days in SPD and 61.3 in SPDS), the means were too close. In these cases, the *t*-test considered that the ß equation values corresponded to one. In other words, it did not show significant differences and the two methods yielded equivalent mean values. For the NDF, PHF and PHM traits from F₃ generation (Table 3) and PHF, NDM, PHM (Table 4) and IPY (Table 5) from F₄ generation, where the means had bigger differences, the test rejected the hypothesis H_0 : $\beta = 1$. This meant that the methods were not equivalent. As in these cases, the populations obtained by the SPDS method yielded superior means compared to those obtained by the SPD method, the substitution of the SPD by the SPDS method was recommended. These results supported the procedure used in the breeding program by the National Center for Soybean Research of EMBRAPA.

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Table 6 - Linear regression equations with respective determination coefficients and t-values for testing H_0 : $\beta=1$ against H_1 : $\beta<1$ for the NDF, PHF, NDM and PHM traits in the F_3 and F_4 generations and IPY in the F_4 generation^a.

1	, ,	3 7	3 7 6				
Generation	Trait	Equation	\mathbb{R}^2	t-values			
	NDF	SPD= 0.932 SPDS **	0.994	t = -3.55 * (b)			
F_3	PHF	SPD= 0.838 SPDS **	0.971	$t = -4.30 * ^{(b)}$			
	NDM	SPD= 1.008 SPDS **	0.999	t = +0.93 ns (b)			
	PHM	SPD= 0.837 SPDS **	0.961	$t = -3.74 * ^{(b)}$			
	NDF	SPD= 0.981 SPDS **	0.995	$t = -1.01^{\text{ns (b)}}$			
F_4	PHF	SPD= 0.866 SPDS **	0.985	t = -4.85 * (b)			
	NDM	SPD= 0.965 SPDS **	0.997	$t = -2.83 * ^{(b)}$			
	PHM	SPD= 0.883 SPDS **	0.990	t = -5.14 * (b)			
	IPY	SPD= 0.744 SPDS **	0.972	t = -4.94 * (c)			

^a PHF: plant height (cm) at flowering; NDF: number of days to flowering; PHM: plant height (cm) at maturity; NDM: number of days to maturity; IPY: individual plant yield (g)

This procedure has been efficient in conducting segregating populations for the development of new cultivars with long juvenile period, based on the SPDS method in the F2 generation (Kiihl and Garcia, 1989).

SPD and SPDS methods are modifications of the SSD method, and they both allow the fast advancement of generations, making it possible to obtain two generations per year (Kiihl and Garcia (1989). Boerma and Cooper (1975) compared the SSD method with the pedigree and the early generation test methods, these two with selection in the F_2 generation. The authors observed that the SSD method was the most efficient since it was fast in the advancement of generations and required less effort in the selection process. In this method, the selection is accomplished only in advanced generations, when it is more efficient. The methodology used in our work showed that although there was a selection in generation F₂ with SPDS method, it did not demand great efforts as in the pedigree and the early generation test methods. The SPDS method allowed the use of smaller population sizes. It reduced labor, breeder's work and experimental area in comparison to the SPD method.

CONCLUSIONS

The populations produced by SPDS tended to have higher means than those produced by SPD. The SPDS method maintained a fenotipic variation comparable to that of the SPD method, even when selection for high heritability traits correlated with grain yield was practiced in the F_2 generation. Thus it was possible to replace the SPD method by the SPDS method in soybean breeding.

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RESUMO

Este trabalho objetivou comparar os métodos de avanço de gerações segregantes SPD (vagem única por planta) e SPDS (vagem única por planta selecionada) no melhoramento da soja. Foram utilizadas 16 populações oriundas de cruzamentos de genótipos japoneses com brasileiros adaptados. Os caracteres número de dias para o florescimento (NDF) e para maturação (NDM), altura da planta no florescimento (PHF) e na maturação (PHM) e produtividade por planta individual (IPY) foram avaliados. As populações obtidas pelo método SPDS continuaram a apresentar valores de variabilidade próximos aos das populações obtidas pelo método SPD. A regressão linear (reta passando pela origem) mostrou que para NDM na geração F₃ e NDF na geração F₄, os métodos são equivalentes. Para as demais combinações caracteres/gerações, **SPDS** produziu maiores médias. O método SPDS permite

^b $t_{(0,05;15)} = 1,75$ (one-tailed test)

 $t_{(0,05; 6)} = 1,94$ (one-tailed test)

^{**} significant at the 1% level of probability.

o avanço de gerações com populações de menor tamanho comparado ao SPD, além de proporcionar ganho genético.

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