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Selection of popcorn inbred lines based on performance and genealogy of S_5 progenies and plants

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ABSTRACT - *The development of hybrids is the most important objective in a corn breeding program. The objectives of this study were to select and assess inbred lines, and to discuss the efficiency of four cycles of among and within inbred family selection. Two experiments were evaluated, one with 144 S_5 progenies of the popcorn population Beija-Flor and another with seven inbred lines. Based on expected gains in expansion volume, the best selective procedure was mass selection. The 14 selected inbred lines should be divergent, because only two pairs were derived from the same S_3 or S_4 family. The analysis of selection efficiency demonstrated that it is important to select superior plants in families with poorer performance to minimize the loss of superior genotypes. The quality of the evaluated inbred lines was comparable to commercial populations and the yield satisfactory. The information about genealogy did not increase the inbred line selection efficiency.*

Key words: pedigree method, inbred family, genetic gain.

INTRODUCTION

Maize breeding programs of research institutions, including the improvement of specialty corn, must mostly if not exclusively, as in the private seed producing companies, focus on the development of hybrid varieties, exploiting heterosis for grain yield. The development of open-pollinated varieties is restricted to public research and educational institutions. They are indeed indispensable, since they provide farmers who do not purchase seeds annually with genetic superior plant material. It is predominantly the pedigree method that is used in the development of inbred lines. Since the number of possible inbred lines is high, the disadvantages are limited to factors after the establishment, mainly concerning the evaluation of a vast number of inbred lines in hybrid combinations (Bernardo 1996). According to Rademacher et al. (1999), the reciprocal recurrent selection has not found widespread use among maize breeders in view of the efficiency of the pedigree method in the development of superior inbred lines.

Sawazaki et al. (2000) crossed S_5 and S_6 families of variety Guarani and S_3 progenies of variety IAC 64 with a single-cross hybrid of inbred lines of the population SAM to evaluate the potential of inbred lines of Brazilian popcorn varieties to generate superior hybrids. Yields of the topcross hybrids were high in all trials (means of 5,002 t ha^{-1} and 4,241 t ha^{-1} for the hybrids of Guarani and IAC 64 progenies), outmatching the controls Zélia and Composto USA (average of 2,864 t ha^{-1}). Besides, the quality of the hybrids was higher. The means of expansion volume (EV) of the two groups were 32.9 mL g^{-1} and 33.8 mL g^{-1}, comparable to the mean of the controls, 35.8 mL g^{-1}. Vilarinho et al. (2003) used the

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index of Mulamba and Mock, with weight 2 for EV and 1 for yield, to obtain superior S_2 and S_3 progenies. The realized gains with S_1 family selection were 2.52 mL.g^{-1} and 72.4 kg ha^{-1} higher than the estimated (1.02 mL g^{-1} and 27.2 kg ha^{-1}). With S_2 progeny selection the gains were predicted at 2.04 mL g^{-1} and 68 kg ha^{-1}, while gains of 1.56 mL g^{-1} and 133 kg ha^{-1} were obtained. Based on a realized gain of 1.5 mL g^{-1}, Santos et al. (2004) concluded that the best selection strategy to establish superior S_3 popcorn families is among and within selection, using the Mulamba and Mock index, with weight 3 for EV and 1 for yield. The same criterion was used by Viana et al. (2007), with S_3 families. The among and within selection targeting superior S_4 progenies resulted in total estimated gains in EV and yield of 8.72 mL g^{-1} and 5.56 mL g^{-1}, and of 390 kg ha^{-1} and 176 kg ha^{-1}, in two programs. The realized gains, justified as underestimated for EV, evidenced a yield loss of 45 kg ha^{-1} and quality increase of 0.57 mL g^{-1}. Arnhold and Viana (2007) evaluated and confirmed the efficiency of selection within S_4 popcorn families by the predicted and realized gains. The selection of one plant in each superior progeny maximized the gain in EV, but did not allow or minimize indirect gains in yield. The authors obtained best results for EV by direct selection, with a predicted gain of 2.6 mL g^{-1}. The indirect predicted yield gain was 68.3 kg ha^{-1}. The real gains were 1.35 mL g^{-1} and 143.37 kg ha^{-1} with direct selection in EV and 1.19 mL g^{-1} and 256.61 kg ha^{-1}, when using the Mulamba and Mock index, with weight 3 for EV and 1 for yield.

The development of maize hybrids, used since 1930 in popcorn improvement, was responsible for the great advances in popcorn yield and quality in the United States (Ziegler and Ashman 1994). Likewise, the development of improved populations should contribute to the growth of popcorn industry in Brazil, which is still an importer of popcorn grain and seed. The objectives of this study were to select and evaluate inbred lines, with selection based on the performance and pedigree of S_5 progenies and plants, aiming at the development of hybrids and synthetics, and discuss the efficiency of four cycles of among and within inbred family selection.

MATERIAL AND METHODS

Similarly as in the studies of Arnhold and Viana (2007), Viana et al. (2007) and Santos et al. (2004), 144 S_5 progenies of the popcorn population Beija-Flor were evaluated in an experiment with replication of the controls IAC 112 and Zélia only, on an isolated plot, in Viçosa, MG, in the 2002/03 growing season. Each plot consisted of one 5-m row, with 30 plants and inter-row spacing of 0.9 m. The control plants were detasseled, to ensure the recombination of families only. Ten superior plants of each progeny were selfed. The following traits were measured in each plot: plant and ear height; number of ears, of poorly hulled ears and of ears affected by plagues and diseases; number of root lodged and stalk lodged plants, final stand, grain moisture, weight of 100 grains, yield, and expansion volume. The grain weight and EV of the selfed and the three plants sampled in each row of IAC 112 were measured. The EV was evaluated with samples of 30 g per plot and of 10 g per plant, in a hot air popper (1,200 w).

Prior to analyses, the yield data were corrected to a moisture of 14.5% and to ideal stand. The weight of 100 grains was adjusted to standard moisture as well. The analyses of variance were performed in an incomplete block design. The genetic analyses included estimation of genotypic variance among families, heritability and genotypic correlation, and gain prediction, using selection differential (Hallauer and Miranda Filho 1988). For population improvement, among selection was based on the index of Mulamba and Mock (1978) with weight 3 for EV and 1 for yield. Targeting the development of 14 superior inbred lines to test the specific combining ability (diallel) and of 30 inbred lines for the development of a synthetic, the following selection strategies were evaluated:

1. Mass selection – selection of selfed plants of superior EV; in this case, the genetic variability within each progeny was assumed as negligible;

2. Family selection based on the best plant – progeny selection based on EV of the best selfed plant; to minimize the within genetic variability, the phenotypic values of selected plants maintained the amplitude within that of IAC 112, where the variation is due to the environment only (assuming that in this modified single-cross hybrid the phenotypic variability is due to the environment only);

3. Selection among and within – family selection based on EV of plot and within selection based on EV of selfed plant; this procedure also assumes a negligible within genotypic variability;

4. Selection among families – family selection based on EV of plot; to minimize the genetic variability within, the phenotypic values of selected plants maintained the amplitude within that of IAC 112;

5. Family selection based on EV of plot and EV of selfed plant – the index of Mulamba and Mock was used with weight 1 and 3, respectively;

6. Mass selection with index – equivalent to mass selection, using the index of Mulamba and Mock with weight 3 for EV and 1 for yield; this procedure also considers the within genotypic variability as negligible;

7. Family selection based on the best plant, using index – best selfed plant selection based on the index of Mulamba and Mock with weight 3 for EV and 1 for yield;

8. Family selection based on EV, yield and pedigree of the best plant – the family pedigree, available in the studies of Vilarinho et al (2003), Santos et al. (2004), Viana et al. (2007), and Arnhold and Viana (2007) was used to generate a variable, computed as follows: value 1 was attributed from S_1 or S_2 to S_4, in the cases of selected progeny and selected plant, and value 0 in the cases of non-selected progeny and non-selected plant; the number used in the index of Mulamba and Mock was the sum of these values; weights of the variables were equal; this procedure also admits the within genotypic variability as negligible.

The evaluation of the efficiency of among and within inbred family selection, in relation to the four previous selection cycles, was performed based on the pedigree of the advanced families, selected or not. The among selection efficiency was evaluated by the percentage of selected S_{n+1} families, derived from selected S_n families, calculated as follows:

$$Ef_a = \frac{NSF}{NSF + NNSF} x100$$

where:

NSF is the number of selected S_{n+1} families, derived from selected S_n families; and

NNSF is the number of selected S_{n+1} families, derived from non-selected S_n families.

The within selection efficiency was evaluated by the percentage of selected S_{n+1} families, derived from selected S_n plants, by:

$$Ef_w = \frac{NSP}{NSP + NNSP} x100$$

where:

NSP is the number of selected S_{n+1} families, derived from selected S_n plants; and

NNSP is the number of non-selected S_{n+1} families, derived from selected S_n plants.

Seven groups of S_6 families, considered as inbred lines, were evaluated in Viçosa, including 3 of the 14 selected by at least two strategies, 03-548-(1, 3, 4 and 5), 03-566-(2, 3 and 4) and 03-583-(1, 3 and 4), two selected in one of the strategies, 03-597-(1, 2, 3 and 4) and 03-701-(1, 2, 3 and 4), and two non-selected, but derived from a family and plants with superior EV and good genealogy, 03-594-(2, 3 and 5) and 03-693-(2, 3 and 4), all randomly sampled in their respective classes. The experiment corresponded to a diallel, used to obtain 21 single-cross hybrids. The distinct pairs of inbred lines were planted on three different days, September 1, 10 and 17, 2003, in rows of 5 m, with 25 plants and rows spaced 0.9 m apart. Thus, on each sowing date six rows of each inbred line were planted, for a total of 42 plots. The resistance level to *Exserohilum turcicum* (helminthosporiosis), *Puccinia sorghi* (rust), *Helminthosporium maydis* (helminthosporiosis) and *Phaeosphaeria maydis* (maize leaf spot), was evaluated on December 18, for the first and second sowing date. The third planting was evaluated on December 30. All evaluations were performed between 15 and 20 days after the end of flowering, using a grade scale of the Centro Nacional de Pesquisa de Milho e Sorgo and Instituto Agronômico de Campinas. The value of the expansion volume of each inbred line in all replications was established based on one or more selfed plants, using 10 g samples. In the plots without selfed plants, the 10 g sample was obtained from one or two manually pollinated plants or from the seed bulk of non-pollinated plants.

RESULTS AND DISCUSSION

In the test of S_5 families, there were no root lodged or stalk lodged control plants, so it was not possible to perform the analyses of variance for these traits. Significant genetic variability was verified for plant height (at 2%), proportion of disease-affected ears (at 1%), proportion of plague-affected ears (at 1%), weight of 100 grains (at 7%), and expansion volume (at 3%). The residual variation for yield, calculated based on the controls, was unproportional to that of the S_5 progenies, although the experimental procedure was the same as in studies of Santos et al. (2004), Viana et al. (2007), and Arnhold and Viana (2007) with S_2, S_3

and S_4 progenies of the population Beija-Flor. Despite the considerable amplitude of variation of the families, 3,192.11 kg ha^{-1}, the estimated residual variation based on IAC 112 and Zélia was very high, 651,183.1837 (kg ha^{-1})^2, associated to an equally high mean of the controls, of 4,499.67 kg ha^{-1}. The progeny mean was 1,791.38 kg ha^{-1}. Comparing these values to those obtained in the cited studies, it may be concluded that the residual variation of yield, measured in one or more hybrids, can be unproportional to that associated with inbred families when hybrid yields are very high. The consequence is the non-significance of the test of genotypic variability in the population, combined with a wide amplitude of the phenotypic values of progenies. The solution is to repeat at least part of the inbred families and/or use equivalent plant material as controls, that is, inbred lines. The analysis of the grain weight of the selfed plants also showed absence of genotypic variability in S_5, among as well as within families.

The EV heritability at the level of progeny and of plant within family were 60.1% and 66.4%, associated to estimates of among and within genotypic variation of 13.2591 (mL g^{-1})^2 and of 34.7600 (mL g^{-1})^2. It is therefore expected that the among and within selection based on EV are equally efficient. In the previous cycles, heritability at the family level varied from 32.3% to 80.7%, and heritability at the plant within progeny level varied from 64.8% to approximately 97%, while the realized gains varied from 0.57 mL g^{-1} to 2.52 mL g^{-1} (Vilarinho et al. 2002 and 2003, Santos et al. 2004, Viana et al. 2007, and Arnhold and Viana 2007). The genotypic variance among families was comparable to the estimated in S_1, of 12.7746 (mL g^{-1})^2 (Vilarinho et al. 2002 and 2003), but lower than the estimated in S_2, of 38.6700 (mL g^{-1})^2 (Santos et al. 2004), S_3, of 21.1686 (mean) (mL g^{-1})^2 (Viana et al. 2007), and S_4, of 20.0804 (mL g^{-1})^2 (Arnhold and Viana 2007), with a tendency to decrease from S_2 onwards. The variability within families is higher than the estimated in S_4, of 29.5537 (mL g^{-1})^2 (Arnhold and Viana 2007), comparable to the estimated in S_3, 35.5787 (mL g^{-1})^2 (mean) (Viana et al. 2007), though lower than the estimate in S_2, of 53.9835 (mL g^{-1})^2 (Santos et al. 2004).

The analysis of the genotypic correlations evidenced that EV-based selection can, indirectly, increase resistance to ear plagues (- 0.42) and reduce the ear disease resistance (0.65) and grain weight (- 0.69), in spite of the irrelevant magnitude of the indirect predicted gains.

Aiming at population improvement, 43 families were selected, using Mulamba and Mock index with weight 3 for EV and 1 for yield, with a predicted EV gain of 1.65 mL g^{-1} (95% of the maximum gain). The estimated heritabilities based on the EV values of plots and selfed plants (60.1% and 70% at the progeny level, 66.4% at the plant within family level and 68.4% at the level of S_5 plants) showed that the among, within and mass selection procedures are equally efficient to develop superior S_6 families. In terms of predicted gains (Table 1), based on the EV values of the selfed plants, mass selection proved superior (strategies 1 and 6), owing to a high selection differential. These procedures (as well as strategy 3), should minimize the genotypic variance within S_6 families, but not the genotypic variance among progenies and among groups of S_6 families. The family selection procedures resulted in the selection of, on average, three to five plants in each progeny, with positive estimates of mean within family genotypic variance, for EV as well as for grain weight. They should therefore determine a greater genotypic variability in S_6. The methods that stood out were based on mass selection (strategies 2 and 7). The best S_6 progenies are therefore apparently identified by mass selection. Comparing methods of popcorn inbred line development, Arnhold et al. (2009) concluded that combined selection was the best procedure, compared to among and within selection and to mass selection. Then, assuming genotypic variability for EV and grain weight within several S_5 progenies (Table 1), and that superior genotypes could get lost through selection of a single plant in each selected family, it was decided to select 14 S_5 families, identified as superior in at least two of the evaluated strategies, by eliminating plants with reduced EV values, to maintain the amplitude in agreement with that observed at the IAC 112 (13 mL g^{-1}). For the development of synthetic, 30 S_5 families were selected, 17 identified as superior in at least two of the selection strategies and 13 identified as superior in one of the selection criteria.

Considering each selected group of S_6 families as inbred line, the pedigree analysis showed that two had been derived from the same S_4 progeny, 03-580-(1, 2, 3, 4, 5, 6, 7 and 8) and 03-548-(1, 4 and 5), and two others from the same S_3 family, 03-589-(1, 2, 7 and 9) and 03-546-(3, 4 and 7). The others were originated from distinct S_0 plants. Based on the results of Viana et al. (2007), who evaluated S_3 families of Beija-Flor popcorn population, per se and in topcross, 3 of the 14 inbred lines were derived from progenies with outstanding performance in crosses with tester Viçosa, which are 03-579-(1 and 9), 03-602-(1, 2, 4, 6, 7 and 8) and 03-628-(1 and 2).

The efficiency of S_1 family selection was low, since only 30.7% of the selected S_2 progenies were derived from selected S_1 families. In this stage, within selection was more efficient, since 51.1% of the selected S_2 families were originated from selected S_1 plants. Selection among and within S_2 families was considerably more efficient. Of the selected S_3 progenies, 65.3% were derived from selected S_2 families. For within selection, the efficiency practically attained the maximal value: 98.4% of the selected S_3 progenies were originated from a selected S_2 plant. In the two following cycles, the among selection efficiency increased and within selection efficiency was reduced. In an evaluation of the selected S_4 progenies, 81.6% were derived from a selected S_3 family and 75.1% were originated from a selected S_3 plant. Of the 14 families and 57 selected S_5 plants, 75.8% of the progenies had been derived from a selected S_4 family and 27.1% of the plants from a selected S_4 plant. There is therefore evidence that it is most adequate, in the process of inbred line development, to work systematically with the selection of a larger number of families, select all superior plants within the best progenies and select at least one superior plant in the families of inferior performance, comparatively to the best ones.

Table 1. Expected gain (G) in expansion volume (EV, mL g^{-1}), considering eight selection strategies[1] of S_5 popcorn families and plants, minimum, mean and maximum values of EV and grain weight (weight, g $(0.15\ m^2)^{-1}$), and of the within progeny genotypic variance (σ^2_G) for EV and grain weight (W), and amplitude of variation (A) of EV, for the selected selfed plants (N)

Strategy	N	G		EV	Weight	$\sigma^2_{G(EV)}$	$\sigma^2_{G(W)}$	A
1	14	12.9	Maximum	57.0	72.0	-	-	-
			Mean	51.0	45.6	-	-	-
			Minimum	48.0	22.0	-	-	-
2	42	9.6	Maximum	52.7	59.0	42.90	45.76	13.0
			Mean	46.2	41.1	13.78	5.02	9.6
			Minimum	40.0	25.0	0.00	3.33	0.0
3	14	6.5	Maximum	53.0	80.0	-	-	-
			Mean	41.9	42.8	-	-	-
			Minimum	33.0	19.0	-	-	-
4	75	2.5	Maximum	46.6	54.5	12.20	63.13	13.0
			Mean	36.2	33.8	3.74	11.05	11.0
			Minimum	28.8	22.0	0.00	0.00	4.0
5	67	5.7	Maximum	46.6	54.4	42.9	67.79	13.0
			Mean	40.5	32.9	10.42	8.11	11.3
			Minimum	32.9	21.0	0.00	0.00	8.0
6	14	10.9	Maximum	57.0	144.0	-	-	-
			Mean	48.1	67.1	-	-	-
			Minimum	42.2	48.0	-	-	-
7	61	7.9	Maximum	52.7	59.9	42.90	1192.46	13.0
			Mean	43.6	45.1	10.79	150.73	11.1
			Minimum	38.2	27.8	0.00	0.00	3.0
8	71	5.5	Maximum	46.7	62.0	24.73	1192.46	13.0
			Mean	40.2	46.8	7.76	85.18	11.0
			Minimum	31.5	32.0	0.00	0.00	3.0

[1] 1 - mass selection; 2 - family selection based on the best plant; 3 - selection among and within; 4 - selection among families; 5 - family selection based on EV of plot and EV of selfed plants; 6 - mass selection with index; 7 - family selection based on the best plant, using index; 8 - family selection based on EV, yield and genealogy of the best plant

In the experiment of evaluation of seven groups of S_6 families, the analyses of variance evidenced significant interaction (at 1%) with planting dates, regarding expansion volume, yield, grade of resistance to *P. sorghi* and plant and ear height. Therefore, regarding these traits, the differences between inbred lines differed in the three periods.

In the first period, the mean expansion volume of the inbred lines was 36.5 mL g^{-1}, the lowest value was 27.2 mL g^{-1} of inbred line 03-566, and the highest 42.0 mL g^{-1}, of 03-701. The mean in the second period was a little lower, 34.2 mL g^{-1}, with a minimum of 27.9 mL g^{-1} for 03-594, and maximum of 41.3 mL g^{-1} for 03-583. The mean of inbred lines was even lower in the third period, 31.5 mL g^{-1}. The lowest value, 23.7 mL g^{-1}, was of inbred line 03-548, and the highest value, 34.0 mL g^{-1}, was of inbred line 03-693. Analyzing the mean values of EV of the inbred lines, considering the three sowing dates, the minimum of 30.0 mL g^{-1} was found for 03-566, and the maximum of 38.5 mL g^{-1} for 03-583. In a study of evaluation of measurement equipments of expansion volume and using a hot air popper, Matta and Viana (2001) observed mean values of 39.5 mL g^{-1} and 34.2 mL g^{-1} for two commercial types, for sample grain weight between 10 and 90 g. The maximum values, obtained with 90 g samples, were 41.7 mL g^{-1} and 36.0 mL g^{-1}. The inbred lines 03-583 and 03-701 therefore have a quality comparable to the commercial grain types, 38.5 mL g^{-1} and 38.4 mL g^{-1}, respectively. The superior inbred lines were 03-597, with 34.9 mL g^{-1}, 03-693, with 34.3 mL g^{-1}, and 03-594, with 33.3 mL g^{-1}. In the study of Pacheco et al. (2001), the EV of commercial varieties, also measured in a hot air popper, was 34.1 mL g^{-1} for the triple hybrid Zélia, 30.3 mL g^{-1} for the modified single-cross hybrid IAC 112, 29.8 mL g^{-1} for population RS 20, and 22.5 mL g^{-1} for population Ângela. The inbred lines therefore have a comparable quality to commercial populations as well. Taking the results obtained by Lyerly (1942) into consideration, it is expected that the resulting hybrids maintain this high quality.

In relation to yield, in the first period the best inbred lines were 03-594, 03-583, 03-566, and 03-548 with 2,374.6, 1,953.5, 1,850.2, and 1,749.9 kg ha^{-1}, respectively The lowest yield was of 03-701. Of the inferior inbred lines of the first period, only 03-693 maintained inferior performance in the second period. The most productive in the second period was 03-566 with 2,244.5 kg ha^{-1}. In the third period, 03-566 and 03-693 produced most and least, respectively, with means of 3,117.2 kg ha^{-1} and 908.9 kg ha^{-1}. According to the mean values of the three periods, the most productive inbred lines were 03-566 with 2,404.0 kg ha^{-1}, and 03-594 with 2,080.5 kg ha^{-1}. The inbred line of lowest yield was 03-693 with 1,186.2 kg ha^{-1}.

The grain shape and color of all inbred lines was according to the commercial standard pearl and yellow grain type and all were uniform in grain weight. The results of the analysis of variance did not indicate differences between inbred lines for weight of 100 grains, whose minimum, mean and maximum values were 12.1 g, 12.4 g and 12.8 g. For plant and ear height, the inbred lines that stood out in the three growing periods were 03-583 and 03-566 in the first, 03-566 and 03-701 in the second, and 03-583, 03-566 and 03-548 in the third. The plant growth of inbred line 03-566 was superior in the three periods (1.9 m and 1.1 m, on average). The inbred line of shortest growth in the three periods was 03-597 (1.1 m and 0.6 m, on average). In spite of the existence of interaction, in the three periods rust-resistance was observed in almost all inbred lines. Exceptions were the inbred lines 03-583 and 03-566 in the third period, which were classified as medium resistant. Moreover, an increase in the level of rust resistance was observed from the first until the third growing season. In relation to the resistance grades to *E. turcicum*, *P. maydis* and *H. maydis*, no difference was verified between the inbred lines, which were medium resistant, independent of the period. *H. maydis* caused most damage in the third period. The damage done by *P. maydis* was greatest in the first period. The incidence level of *E. turcicum* was the same in all three periods. There is thus evidence of good to excellent resistance levels in the group of inbred lines to some leaf spots and to rust.

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Seleção de linhagens de milho-pipoca, considerando desempenho e genealogia de progênies e plantas S_5

RESUMO - *O desenvolvimento de variedades melhoradas, principalmente híbridos, deve contribuir para o crescimento da indústria do milho-pipoca no Brasil. Os objetivos deste trabalho foram selecionar e avaliar linhagens, e discutir a eficiência de quatro ciclos de seleção entre e dentro de famílias endogâmicas. Foram considerados dois experimentos, um com 144 famílias S_5 da população de milho-pipoca Beija-Flor e um de avaliação de sete linhagens. Levando em conta ganhos preditos em capacidade de expansão, o melhor processo seletivo foi seleção massal. Espera-se que as linhagens selecionadas sejam divergentes, pois apenas dois pares derivam de mesma família S_3 ou S_4. O estudo de eficiência de seleção evidenciou que é importante selecionar plantas superiores em famílias inferiores, para minimizar a perda de genótipos superiores. As linhagens avaliadas têm qualidade comparável à de populações comerciais e capacidade produtiva satisfatória. A eficiência de seleção de linhagens não melhorou com a inclusão das informações de genealogia.*

Palavras chave: método genealógico, família endogâmica, ganho genético.

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