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ALTERNATIVES FOR OBTAINING DOUBLE CROSS MAIZE HYBRIDS[1]

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ABSTRACT- In the search for an alternative to reduce the price of hybrid maize seeds the present experiment was carried out to compare the performance and variability among double cross hybrids (DC) derived from the F_1 and F_2 generations, and from intercrossed plants of the F_2 generation of some single cross hybrids (SC). Experiments were carried out in two agricultural seasons. In the first, 1997-98, 42 double cross hybrids were obtained from seven commercial single cross hybrids, that is**,** 21 from F_1 and 21 from F_2 generations of the respective single cross hybrid. These hybrids were evaluated in three locations, in two experiments/location, one of them with DC hybrids from F_1 generation, and the other with DC from F_2. Four SC hybrids were used as controls in each experiment. In the 2000/2001 season 36 DC hybrids were evaluated, 15 derived from the F_1 generation, 15 from the F_2 generation of the same SC hybrids and six from the F_{2i} generation. The 36 DC hybrids and six controls were evaluated in two locations. The grain yield of some DC hybrids was as high as the yield of the best SC hybrid recommended for the region. The double cross hybrids, from different origins, that is, the F_1, F_2 or F_{2i} generations were similar in their yield performance and variability what shows that it is possible to reduce the cost of double cross hybrid seed production using the intercrossed population derived from the SC hybrid, so line multiplication is not needed nor does the SC F_1 hybrid have to be obtained every year.

Key words: Quantitative genetics, maize, double cross hybrids

ALTERNATIVAS PARA A OBTENÇÃO DE HÍBRIDOS DUPLOS DE MILHO[1]

RESUMO- Na busca de alternativas para a redução do preço das sementes híbridas de milho, foi conduzido o presente trabalho, com o objetivo de se comparar o desempenho e a variabilidade existente dentro dos híbridos duplos (HD) oriundos das gerações F_1, F_2 e de um intercruzamento da geração F_2 (F_{2i}) de alguns híbridos simples (HS). Foram realizados experimentos distintos em duas safras agrícolas. Na primeira, conduzida em 1997/98, a partir de sete HS comerciais, foram obtidos HD, sendo 21 com a geração F_1 e 21 com a F_2 dos respectivos HS. Esses híbridos foram avaliados em três locais, com dois experimentos em cada um deles, sendo um referente aos HD oriundos da geração F_1 e o outro da geração F_2. Em cada experimento foram utilizados quatro HS como testemunhas. Na safra 2000/01, foram avaliados 36 HD, sendo 15 oriundos da geração F_1, 15 da geração F_2 dos mesmos HS e mais seis da geração F_{2i}. Os 36 HD, juntamente com seis testemunhas, foram avaliados em dois locais. Observou-se que alguns híbridos duplos foram tão produtivos quanto os melhores híbridos singles recomendados para a região. Constatou-se, também, que os HD das diferentes origens, ou seja, das gerações F_1, F_2 ou F_{2i}, são semelhantes tanto em relação ao seu

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desempenho produtivo quanto à variabilidade existente dentro deles. Isso evidencia ser possível a redução no custo de produção das sementes dos HD utilizando as populações intercruzadas derivadas dos HS, não necessitando, portanto, da multiplicação das linhagens e a obtenção da geração F_1 dos HS todos os anos.

Palavras-chave: Genética quantitativa, milho, híbrido duplo.

Double cross maize hybrids have been used predominantly in Brazil for several years due to the lower seed cost . This is because four lines are involved in obtaining them, which are crossed in pairs to create two SC hybrids. Later these SC hybrids are crossed to obtain the F_1 generation of the commercial DC hybrid. As the SC hybrids are more productive than the inbred lines, the commercial seed production of the DC hybrid is much greater than that of a SC hybrid reducing the costs for farmers (Paterniani and Miranda Filho, 1987).

However, the cost of hybrid seeds could be reduced further if the stages of maintaining the four lines and obtaining the SC hybrid were eliminated, permitting the dissemination of their use even among subsistence farmers. One of the options would be the use of the F_2 generation of the SC hybrid to obtain the DC hybrid. This is perfectly feasible, as theoretically the proportions of gametes produced in the F_2 generation are the same as those from the F_1 generation. This reasoning was described by Kiesselback at the beginning of the 1930s (Sanchez, 1988). However, information on this is very limited. The only two reports found refer to studies carried out in Mexico in the 1960s (Vasques, 1969 and Manrique and Nevado, 1970, cited by Sanchez, 1988). In both cases the comparison of the double cross hybrid showed that those from F_1 generation were slightly superior (less than 5%) to those from F_2. They did not mention if the difference was significant, but pointed out that it could be due to sampling problems, because the number of F_2 generation individuals involved in obtaining the double cross hybrid was small.

If the use of the F_2 generation is feasible, there is the possibility of making the process of obtaining DC hybrids less expensive. This is because once the F_2 generation has been obtained, it could be kept by intercrossing in isolated fields and be used every year to obtain the F_1 generation of the double cross hybrid. Experiments were carried out to evaluate this hypothesis comparing the performance of the DC hybrids derived from the F_1 and F_2 generations of the SC hybrids and from an intercross of the F_2 (F_{2i}) generation of the respective SC hybrid.

Material and Methods

The experiments were carried out in two different agricultural seasons, 1997/98, and 2000/2001. In the 1997/98 season the experiments were carried out in three locations in the south of Minas Gerais state: Lavras, Ijaci and Lambari.

Seven SC hybrids, P3069 from the Pioneer company, C901, C909 and C333 from Cargill, AG9012 from Agroceres and Z8452 and Z8392 from Zeneca were used. From each SC hybrid the F_2=S_0 generation was obtained by selfing the F_1 generation. Controlled crosses were set up and 42 possible DC hybrids were obtained: 21 DC hybrids from crossing the F_1 SC hybrid, and 21 from crossing the F_2. For this, each SC hybrid was sown in five 10m long rows and ,at flowering ,the tassels **w**ere collected from about 100 plants, the pollen was extracted and used to make the controlled pollination. Two contiguous experiments were carried out in each location, one for the 21 DC hybrids from SC of the F_1 generation and the other for the 21 DC hybrids from SC of the F_2 generation. In both experiments

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the SC hybrids C909, C333, AG9012, and Z8392 were used as controls. Each experiment was set up in randomized block design with three replications in plots of two 4-meter-long rows. The trials were fertilized with 600 kg/ha of 4-14-8 plus zinc and 200 kg/ha of ammonium sulfate was applied 35 days after planting date.

The 2000/2001 experiments were carried out in two locations in the South of Minas Gerais state: Lavras and Ijaci. Among the SC hybrids used in the previous season the hybrids Z8452 and P3069 were withdrawn and the C333B was added. The F_2 generation of SC hybrids was obtained from selfing 20 F_1 plants of each SC hybrid. Later, F_2 plants of the C901, C333B, and AG9012 hybrids were intercrossed to obtain the F_{2i} generation. In this case, 400 seeds of each F_2 generation were sown. At flowering time ,approximately 200 tassels were collected, and the pollen obtained was mixed for pollination by a salt cellar/shaker, of approximately 150 plants per hybrid. The ears were collected, shucked and mixed to thus compose the F_{2i} generation.

Commercial hybrids were also used to obtain the double cross hybrids, following the diallel scheme. As there were F_1 seeds from six SC hybrids, 15 double cross hybrids were obtained. The same procedure was performed in the case of the F_2 generation. As there were F_{2i} seeds from only four cultivars, six double cross hybrids were synthesized. To obtain all DC hybrids the parents F_1, F_2 or F_{2i} generations were sown in five 10m lines and ,at flowering time, the pollen from 100 plants was collected, mixed and used for controlled pollination. Later, when the hybrid seeds were harvested, the reciprocals were mixed to attain the necessary amount of seeds to set up the experiments.

The 36 DC hybrids, 15 from the F_1, 15 from the F_2 and 6 from the F_{2i} generation, and as control the parent hybrids, C901, C909, C333, C333B, AG9012 and the three-way cross hybrid C747, were tested in experiments using the alpha lattice design with three replications and plots with three 4-meter-long rows . The trial was fertilized with 400kg/ha of 08-28-16 plus zinc and 200 kg/ha of ammonium sulfate were applied 35 days after sowing.

The data for stand and grain yield were recorded and grain yield was adjusted to 13% moisture. The grain yield data, from both years, was transformed to t/ha, and submitted to analysis of variance (Ramalho, Ferreira and Oliveira, 2000). It is important to point out that as the alpha lattice design used in the season 200/01 was inefficient, the analysis were carried out following a complete randomized block design. Individual analyses of variance was set up for each location, and later it was made the joint analysis involving the double cross hybrids and the controls evaluated in the different generations and locations tested.

To compare the variability within the hybrid, according to the seed origin, the phenotypic variance was estimated for plant height in the season 1997/98, and for plant height, ear height and ear weight in the season 2000/01. A sample of ten plants was taken from each plot. Then the mean of the variances was estimated per plot and for each type of hybrid and generation. The mean was compared to the maximum F test (Ramalho, Ferreira and Oliveira, 2000). In this case, the variances of each hybrid were ranked in the different generations, dividing the greatest value by the least. For those hybrids evaluated in two generations, that is, derived from F_1 and F_2 seeds of the SC hybrids, the result obtained was compared to the F_{max} theoretical value, with two generations and 27 degrees of freedom ($F_{max} = 2.18$). For the hybrids assessed in three different generations, the theoretical F_{max} was obtained for 3 generations and 27 degrees of freedom ($F_{max} = 2.56$).

Results and Discussion

In any breeding program it is fundamental that the experiments are planned and executed with

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care to obtain the greatest accuracy and consequently greater confidence in the decisions to be made. The coefficient of variation is the most used measurement of experimental accuracy. In this study, the coefficient of variation obtained for ear and grain yield were similar or inferior (Tables 1 and 2) to those commonly reported in the literature for maize hybrid assessment (Scapim, Carvalho and Cruz, 1995; Gonçalves et al. 1999), permitting us to infer, comparatively, that the experiments presented good accuracy.

TABLE 1. General means of the treatments, in the different generations assessed for the ear yield trait (t/ha) in Lavras, Ijaci and Lambari, in the 1997/98 season.

Treatment	Set		
	F1	F2	Mean
AG9012xP3069	7,7	8,5	8,1 b
AG9012xC901	8,9	8,3	8,6 a
AG9012xC909	8,6	9,1	8,9 a
AG9012xC333	8,8	9,8	9,4 a
AG9012xZ8392	8	8,4	8,2 b
AG9012xZ8452	9	9,4	9,2 a
P3069xC901	8,1	7,4	7,7 b
P3069xC909	9	8,2	8,6 a
P3069xC333	8	9,1	8,6 a
P3069xZ8392	7,9	8	8,0 b
P3069xZ8452	8,2	9,4	8,8 a
C901xC909	7,2	7,4	7,3 b
C901xC333	9,2	9,2	9,2 a
C901xZ8392	8,2	8,4	8,3 b
C901xZ8452	8,7	9,7	9,2 a
C909xC333	9	8,6	8,8 a
C909xZ8392	8	8,6	8,3 b
C909xZ8452	9,1	9,1	9,1 a
C333xZ8392	8,9	9,2	9,0 a
C333xZ8452	8,5	9	8,8 a
Z8392xZ8452	8,7	8,2	8,5 b
Mean	8,5 B	8,7 A	8,6
C909	9,1	9,1	9,1 a
C333	9	8,6	8,8 a
AG9012	8,2	9	8,6 a
Z8392	9	9	9,0 a
Mean	8,8 A	9,0 A	8,9
CV(%)	12,9		

Means followed by different letters indicate significant differences among the hybrid by the Scott Knott test (5% probability).

TABLE 2. Means of the double cross hybrid in the F_1, F_2 and F_{2i} generations and the controls, for grain weight (t/ha). Mean data of the experiments at Lavras and Ijaci, in the 2000/01 season.

Treatment	Mean grain yield (t/ha)			Mean
	F1	F2	F2i	
AG9012xC333	8,1 A	7,9 A	7,8 A	8,0 a
AG9012xC901	7,4 A	7,5 A	7,3 A	7,4 b
AG9012xC909	8,0 A	6,7 B	7,2 B	7,3 b
C333xC901	7,4 A	6,5 A	7,2 A	7,0 c
C333xC909	7,8 A	7,3 A	7,1 A	7,4 b
C901xC909	6,8 A	6,6 A	6,5 A	6,7 c
Mean	7,6 A	7,1 B	7,2 B	7,3
AG9012xC333B	7,9 z	7,5 z		7,7 z
AG9012xZ8392	7,4 z	7,4 z		7,5 z
C333xC333B	7,6 z	7,8 z		7,7 z
C333xZ8392	8,1 z	7,5 z		7,8 z
C333BxC901	8,0 z	7,5 z		7,8 z
C333BxC909	8,3 z	8,3 z		8,3 w
C333BxZ8392	8,7 z	8,2 z		8,4 w
C901xZ8392	7,7 z	8,1 z		7,9 z
C909xZ8392	7,5 z	7,0 z		7,3 z
Mean	7,9 z	7,7 z		7,8
AG9012	7,8			7,8
C333	8,4			8,4
C333B	8,7			8,7
C901	7,1			7,1
C909	8			8
C747	7,6			7,6
Mean				8
CV(%)	9,9			

Means followed by different letters indicate significant differences among the hybrids by the Scott Knott test (5% probability).

When the mean yield of the double cross hybrids was compared with the controls SC hybrids, it was found that the superiority of the single cross hybrid was only 2.8% in the 1997/98 season and 4.7% in the 2000/01 season (Tables 1 and 2). These results are according to theoretical probability studies that indicate that the best single cross hybrid obtained will be at most 5% superior to the best double cross hybrid (Wricke and Weber, 1986). Furthermore, it was observed that some double cross hybrids were as or more productive than the best single cross hybrid used as a control (Tables 1 and 2). As these single cross hybrids are among the best commercial cultivars recommended for the region (Ribeiro, 1998) the performance similarity of these two kinds of

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hybrids enables inference that the double cross hybrids evaluated show high yield potential.

It is known that the use of SC hybrids in the southeastern region is not fully justified because, by the results obtained in the literature (Ribeiro, 1998), it is possible to recommend DC hybrids with mean yield equal to the best SC hybrid. The advantage of greater uniformity in the SC hybrid is also questioned because most producers who adopt this kind of maize do not use technologies that allow them to benefit from this greater uniformity.

In both seasons, regardless of origin, significant differences were detected among the DC hybrids. In 1997/98 season the mean ear yield varied from 9.1 t/ha, for the C901xC909 to 11.6 t/ha for the AG9012xC333 (Table 1). For the 2000/01 season the variation in the grain yield was 6.7 (C901xC909) to 8.4 t/ha (C333BxZ8392) (Table 2). It is pointed out that the SC hybrids C901 and C909 had a small genetic divergence (Souza Sobrinho, Ramalho and Souza, 2001), due, probably, to the presence of a common line that explains the low grain yield of the DC hybrid derived from this cross.

In the 1997/98 season the source of variation origin of the seeds was significant, and a slight superiority (2.8%) was found in the hybrid derived from the F_2 generation compared with those derived from F_1 generation (Table 1). For the 2000/01 season, considering the hybrids evaluated in two generations (F_1 and F_2), no significant difference was detected for the origin of the DC hybrid. However, considering the F_1, F_2 and F_{2i} generations the source of origin variation was significant. In this case, a mean superiority of 6% was observed in the F_1 generation hybrids compared to those of the F_2 and F_{2i} generations (Table 2). It should be pointed out, however, that although the DC hybrid x origin interaction was not significant, the partitioning of the origin effect was performed for each hybrid and there was difference among the origins only for the AG9012xC909. In this specific case the F_1 generation was 15% superior to the mean of the F_2 and F_{2i} generations (Table 2).

The results above confirms the hypothesis of equality among the DC hybrids derived from the F_1 and F_2 generations of the SC hybrids. In this case, the mean of the two years, the grain yield of DC hybrids derived from the F_1 generation was 8.20 t/ha versus 8.18 t/ha to F_2 (Tables 1 and 2) that is, practically the same mean. This result **i**s more expressive when it is considered that in the first year 21 hybrids, three locations and three replications were evaluated, while in the second year 15 hybrids, two locations with three replications were evaluated, that is, there were 279 observations. There are few studies about this in the literature. The two reports found were by Vasques (1969) and Marquie and Nevado (1970) cited by Sanchez (1988). In those cases the authors found that the DC hybrids derived from the F_1 generation were about 5% more productive than those derived from the F_2 generation. The authors reported that this probably occurred because of deficient sampling when obtaining the F_2 SC hybrid generation.

It is known that when only one locus is considered, the population reached equilibrium after one generation of random crossing. With many loci it also reaches equilibrium, but over more generations (Falconer and Mackay, 1996). However, when there is an SC hybrid F_1 generation, as the gametes in attraction have the same frequency as those in repulsion, even with multiple independent loci the population reaches equilibrium in a random crossing generation (Mettler and Gregg, 1973). Therefore, the F_2 generation of a SC hybrid not considering the linkage, is in Hardy-Weinberg equilibrium. Not considering the occurrence of epistatic effects, even where there is a linkage disequilibrium the means of

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the F_2 populations do not change with successive intercrosses. Lima Neto (1998) revising studies about the need to intercross the F_2 generation in recurrent selection programs concluded that the current practice of sampling in the F_2 generation without additional genetic recombination by random crossing has been used successfully. Thus, populations derived from intercrossing of the F_2 generation plants should have the same behavior also in crossing, which was confirmed in this study.

It could be argued that when the DC hybrids were obtained by the F_2 or F_{2i} generation the variability within these hybrids would be greater due to the recombination. Although variability among the DC hybrids was different, as was expected, it was also of similar magnitude among the different origins (F_1, F_2 or F_{2i}) ,that is, in this case the F maximum case was not significant (Figures 1 and 2). It is thus understood that there is no restriction in obtaining DC hybrids using the intercrossed population of the respective SC hybrid.

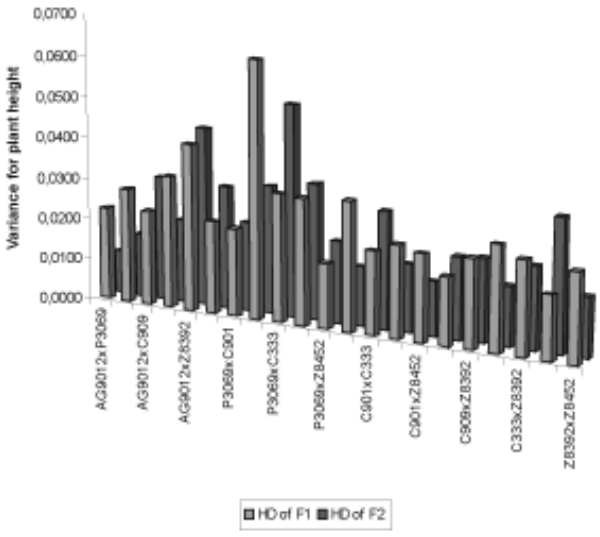

FIGURE 1. Mean variance for double cross hybrid plant height in the F_1 and F_2 generations, Lavras, 1997/98 season.

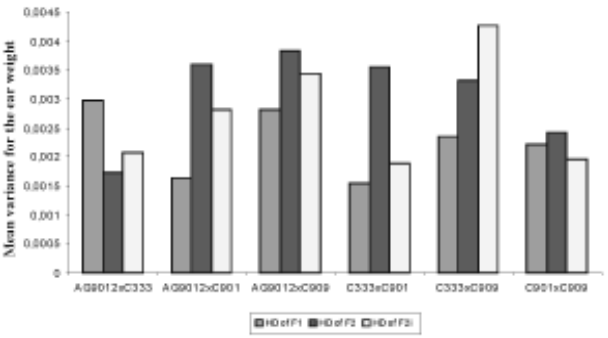

FIGURE 2. Mean of the variances for the ear weight for the double cross hybrid of the F_1, F_2 and F_{2i} generation in Lavras, 2000/01 harvests.

The great advantage of the use of DC hybrids is seed cost as in the seed production field the female is an SC hybrid evidently with greater yield than the lines (Paterniani and Miranda Filho, 1987). It should be mentioned however that two stages are involved when obtaining DC hybrids: maintenance of the four parental lines and obtaining the two SC hybrids and later, the cross of the two SC hybrids to obtain the F_1 seeds of the DC hybrids.

The present study showed it was possible to obtain F_1 seeds of DC hybrid in only one step, without having to maintain the lines or obtain the seeds of the F_1 generation of the single parental hybrid every year. This implies that the same DC hybrid can be obtained every year from the intercrossed population of the respective SC hybrid with substantial savings in time and money. Thus, it will be possible to produce good DC hybrids at more reasonable cost than those presently found on the market.

Conclusions

Some DC hybrids evaluated are as productive as the best SC hybrids recommended for the southern region of Minas Gerais state.

From the genetic point of view DC hybrid seed production cost can be reduced using intercrossed populations of the SC hybrid involved.

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