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NOTE

Effect of allopollen in artificial crosses of white and yellow endosperm maize hybrids

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ABSTRACT - *The search for novel technologies that would boost the yields of hybrid cultivars to yet new heights challenges breeders. One to date little exploited approach is the vigor of fecundation caused by allopollen. In this setting the heterosis in allopollen-induced weight gain was estimated as well as the grain preference for allopollen fecundation in relation to plant-own pollen (selfpollen). The experiment was carried out at the Universidade Federal de Lavras (UFLA) in the crop year 2004/2005. Two single-cross hybrids with white and three with yellow endosperm were crossed using the same amount of pollen. A preference of 80.06% for allopollen fecundation was observed, against 19.94% for autopollen. Heterosis obtained by allopollen in maize plants was 11.90 %, demonstrating that allopollen combined with the xenia effect contributed to a higher grain weight and consequently an increased mean plant yield.*

Key words: *Zea mays*, allopollen, maize hybrid.

INTRODUCTION

The headway made in genetic maize improvement is beyond question. This advance occurred mainly from the moment on when maize hybrids came into use (Duvick 1977). The performance of a hybrid depends on the individual performance of every line and the heterosis derived from crosses (Falconer and Mackay 1997). Genetic gains for grain yield have been systematically obtained for approximately 60 years, since the first maize hybrids were adopted by farmers (Russel 1994, Duvick 1984, 1992). Nevertheless, breeders are still challenged to search for new strategies that would force these gains even higher. One strategy little explored so far is the employment of hybrid vigor, derived from pollen of foreign origin (allopollen). It is known that the embryo and mainly the maize endosperm correspond to over 95% of the grain weight and present the phenomenon of xenia, that is, its expression is a direct result of fecundation. The manifestation of the xenia effect in the traits related to endosperm and embryo has been described in several studies (Davarynijad et al. 1994, Seka et al. 1995a, Bulant et al. 2000).

It has been observed that allopollen is not only preferred in the pollination but contributes to a higher grain weight as well (Seka and Cross 1995b, Lopes and Larkins, 1993, Mercer 2002) and that it is variable according to the lines and/or hybrids used in the cross

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(Weiland 1992). The gain in weight through allopollen is related with the early activity of enzyme ADPGppase (E.C. 2.7.7.27) that acts in the starch synthesis. This activity is 19% higher in allopollen-fecundated grains 14 DAP (days after pollination) and drops to 8% 74 DAP (Bulant et al. 2000). The kernel weight of parents in maize has no correlation with quantitative traits such as yield. Plants derived from lighter seeds, normally from the region of the tip of the ear, have the same performance as plant descents of heavier seeds from the ear base (Bignotto et al. 2003). The kernel weight does therefore not influence the plant gene expression and as the maize endosperm presents the xenia effect, the pollen of a single-cross hybrid would have the same expression in the endosperm, independently of the seed weight and size of this hybrid, since the kernel weight depends on the position on the ear.

Based on this observation some studies were conducted with the aim of increasing yields on commercial plantations through hybrid mixtures (Bulant and Gallais 1998, Mercer 2002). There is also evidence that certain hybrids perform better in cultivar competition trials than in separate plantations. This is most likely a consequence of pollination by different pollen in the experiments, which increases hybrid vigor. The only way to corroborate this fact is by means of a genetic marker. The most commonly used is gene *Y*, which is responsible for the endosperm color. The present study targeted an estimation of heterosis in the development of allopollen-induced grains and the identification of allo and autopollen-derived grains by the use of a morphological marker for the trait endosperm color.

MATERIAL AND METHODS

The experiment was conducted on the experimental area of the Department of Biology of the Universidade Federal de Lavras (UFLA), Lavras, MG, Brasil. The city lies in the southern region of the state of Minas Gerais (lat 21º 45'S, long 45º 00" W, alt 910 m asl).

Five single-cross maize hybrids were used in this study. Three had yellow endosperm (DKB333B, A2555 and 30F90), genotype *YYY,* and two white endosperm (2223 and 2324), genotype *yyy*. The white endosperm hybrids were obtained from the maize improvement program of the Sector of Genetics and Plant Improvement, of the Department of Biology of UFLA while the yellow ones were purchased from local produce.

The experiment was conducted under field conditions with the five above-mentioned simple hybrids. The five hybrids were sown in the crop year of 2004/2005, in 15m rows spaced 0.90m and plants spaced 0.25m apart. To warrant flowering coincidence, the white and yellow hybrids were sown in two portions, split one week apart.

The ears of the white endosperm hybrids were covered before flowering and during flowering the tassels of the plants of white as well as yellow endosperm hybrids were wrapped up with paper bags.

The pollen from both hybrids was collected at about 9 o'clock am, sieved and mixed in equal volume for the crosses 2223XDKB333B, 2223XA2555, 2324XDKB333B and 2324X30F90. These mixtures were taken to the field and 1ml was used to pollinate the white endosperm hybrids with the crosses mentioned above. To confirm the viability of the pollen of both hybrids, the white and yellow endosperm hybrids were selfed as well.

The ears were separated at harvest and the nine fullest ears of each cross selected, that is, the ears with the highest number of fecundated grains, where the identification of the pollen origin on the ear of the white hybrid became viable by means of the xenia effect. The ears were threshed and the grains separated and counted according to the color, providing the percentage of allopollination per ear within each cross. After counting, the grains were weighed to obtain the mean weight of the white and yellow grains per ear. This weight was corrected to 13% moisture and heterosis in the allopollen-derived grains estimated by the expression:

$$h = \frac{(a-b)}{a} \times 100$$

where:

a = mean weight of yellow grains

b = mean weight of white grains

The data obtained for percentage of allopollination were subjected to analysis of variance using a completely randomized design (CRD), where each cross was considered a treatment and each ear a replication. For the trait grain weight we used the randomized block design of (RBD), considering the mean weight of the yellow and white grains as treatment

and each ear with effects of randomized blocks within each cross, which were analyzed separately.

RESULTS AND DISCUSSION

In this experiment the allopollen-derived grains were more frequent; 80.06% of the grains present in the ear were induced by allopollination and 19.94% by autopollination. Maize is a predominantly allogamous plant, i.e., it is cross-pollinated due to the protandry phenomenon, which consists predominantly in wind pollination and the particular arrangement of the leaves above the ear that makes the contact of the pollen grains with the silks of the same plant difficult (Viana et al. 1999). According to our findings, there is some other genetic-physiological mechanism that contributes to a greater viability of the foreign pollen (Table 1). The viability was corroborated by the selfed ears that presented high kernel filling, thus indicating the pollen viability of all hybrids used in the crosses.

The percentage of allopollen-derived grains varied according to the cross, that is, over 90% of allopollination was observed in cross 2223xDKB333B and 71.12% in cross 2223XA2555. In the crosses 2324XDKB333B and 2324X30F90, respectively, 79.22% and 83.14% of allopollination was observed, which allows the conclusion that specific combining ability can also occur in relation to pollen competition in the style-stigma (Table 1). Similar results were obtained by Mercer (2002), who observed a mean allopollination percentage of 73.27% and a mean grain weight gain of 7.1%.

Allopollen did not only contribute to a higher grain weight, but the amplitude of this gain estimated by heterosis also tended to uniformity. Allopollen outmatched autopollen in grain weight in all crosses (Figure 1). This shows that allopollen together with the xenia effect contributed to a higher grain weight and, consequently, increased mean plant yield. Similar results have already been reported in a couple of studies (Kiesselbach 1926, Tsai and Tsai 1990, Bulant and Gallais 1998, Mercer 2002).

A low variation coefficient was observed for mean weight of yellow in relation to white grains (Table 2). Figure 1 shows the statistical difference of these gains.

Heterosis, estimated based on the mean weight of the yellow and white grains, was evident in all crosses. In cross 2223XDKB333B the heterosis was 13.56%, which exceeded the values of the other treatments. Despite small, this difference of heterosis between the cultivars owing to the effect of allopollen had already been expected, since the hybrids as well as the lines differ in the general and specific combining ability, in other words, there is a difference in heterosis, which is function of the existence of dominance and divergence between lines and consequently of the hybrids (Falconer and Mackay 1997). When the overall cross mean was considered heterosis was 11.90%, showing that grain fecundated by foreign

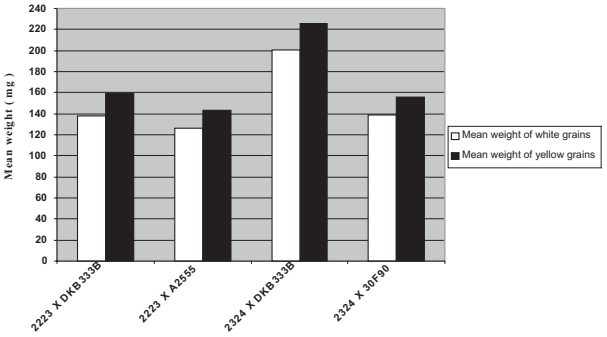

Figure 1. Mean weight of grains derived from allopollen and autopollen within each cross (mg)

Table 1. Percentage of allopollination and autopollination obtained in the different crosses and estimates of the heterosis within each cross and mean heterosis

Crosses	Percentage of allopollination	Percentage of autopollination	*Heterosis %*
2223XDKB333B	90.75 a[1]	9.25	13.56
2324X30F90	83.14a	16.86	11.77
2324XDKB333B	79.22 b	20.78	11.37
2223XA2555	71.12 b	28.88	12.72
Mean	80.06	19.94	11.90
CV (%)	12.06		

[1] Means followed by the same letter did not differ statistically from each other by the Scott- Knott test at 5% probability

Table 2. Mean squares obtained by a comparison of the mean weight of the grains derived from allopollen in relation to those derived from autopollen

		Mean square			
Sources of variation	df	2223XDKB333B	2324X30F90	2324XDKB333B	2223XA2555
Treatment[2]	1	2104.058450*	1359.116006*	2970.406272*	1267.225606*
Ear[3]	8	3261.582256*	1826.373626*	827.758088*	4108.874401*
Error	8	20.262775	76.483068	70.221160	36.106968
CV%		3.03	5.93	3.93	4.46

* Significant at 5% probability by the F test
[2] Mean weight of the white and mean weight of the yellow grains
[3] The effect of Randomized Blocks was considered for each ear

pollen (allopollen) is heavier than grain fecundated by plant-own pollen. These results are in line with those obtained by Bulant and Gallais (1998), who observed gains of 13%, 11% and 11.5% in experiments in 1993, 1994 and in 1995, respectively. As mentioned above, the kernel weight of a maize plant does not influence its productivity. A follow-up experiment with reciprocal crosses or an experiment using seeds of the same size could show whether gain with allopollen is associated with this trait. Earlier studies however indicated no such relation, and in the present study the grains of a same size derived from different pollen (allo and autopollen), i.e., grains of equal size and from the same ear differed only in weight. This allows the conclusion that the only factor responsible for heterosis is related to the pollen origin, confirming results obtained by Bulant et al. (2000).

The conclusion was drawn that allopollen was favored over autopollen in grain fecundation of the maize ear. The fecundation percentage of allopollen-derived grains varied in function of the cross, that is, according to the hybrid combinations, but in all cases the preference of allopollen compared to autopollen was evident. Besides the preference in fecundation, a weight gain was observed in allopollen-derived grains. This is evidence that this gain estimated in heterosis can vary in function of the hybrid; in other words, it is closely linked with the combining ability of the hybrids. Field studies with commercial hybrid cultivars are therefore feasible, if possible using seeds of equal weight, to test their combining ability, which would identify the best combinations and consequently higher gains.

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Efeito do alopólen em cruzamentos artificiais envolvendo híbridos de endosperma branco e endosperma amarelo

RESUMO - *A busca de novas tecnologias que aumentem ainda mais os ganhos das cultivares híbridas é ainda um desafio ao melhorista. Uma técnica ainda pouco utilizada seria o vigor da fertilização causada pelo alopólen. Com esse objetivo procurou-se estimar a heterose em ganho de peso causada pelo alopólen e preferência na fertilização dos grãos em relação ao pólen da própria planta (autopólen). O experimento foi conduzido na Universidade Federal de Lavras (UFLA) no ano agrícola de 2004/2005. Foram utilizados dois híbridos simples de endosperma branco e três híbridos simples de endosperma amarelo de modo a cruzar esses híbridos utilizando o mesmo volume de pólen. Constatou-se uma preferência de fertilização do alopólen da ordem de 80,06%, em relação ao autopólen, 19,94%. A heterose proporcionada pelo alopólen na planta de milho foi de 11,90%, mostrando que o alopólen associado ao efeito de xênia contribuiu com o incremento no peso dos grãos, e conseqüentemente na produtividade média da planta.*

Palavras-chaves: *Zea mays*, alopólen, milho híbrido.

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