

## Performance of testers with different genetic structure for evaluation of maize inbred lines

### Desempenho de testadores com diferentes estruturas genéticas para avaliação de linhagens endogâmicas de milho

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#### ABSTRACT

The objective of this study was to evaluate four maize testers for identification of superior inbreed lines in testcross. The four testers evaluated are cultivars with narrow or wide genetic base (single-cross hybrids and open pollinated varieties) and two kernel types (flint or dent). SynD and SynF testers are open pollinated varieties with dent and flint kernels, respectively, and FSH and DSH testers are single-cross hybrids with flint and dent kernels, respectively. SynD tester showed the biggest genetic variance among the maize inbreed lines in crosses. The effects of general combining ability (GCA) for lines and specific combining ability (SCA) for lines x testers were significant, whereas GCA effects for testers were not significant. SynD and SynF testers identified the largest number of lines with higher GCA. The DSH and FSH testers showed suitable to identify lines with high SCA. It was concluded that SynD and SynF testers are adequate to identify inbreed lines with high GCA effects, and it's possible to identify new lines with high heterotic potential in each one of the four testers.

**Key words:** *Zea mays*, diallel, testcross, combining ability.

#### RESUMO

O objetivo deste estudo foi avaliar quatro testadores de milho quanto à capacidade de identificar linhagens superiores em testcross. Os quatro testadores avaliados são cultivares com base genética ampla (variedades de polinização aberta) e estreita (híbridos simples) e dois tipos de grãos (duro e dentado). Os testadores SynD e SynF são populações de milho de polinização aberta com grãos dentados e duros, respectivamente, e FSH e DSH são híbridos simples com grãos duros e dentados, respectivamente. O testador SynD possibilitou a maior expressão de variância genética entre os

cruzamentos com as linhagens. A capacidade geral de combinação (CGC) das linhagens e a capacidade específica de combinação (CEC) entre linhagens e testadores foram significativas, enquanto que os efeitos de CGC dos testadores foram não significativos. Concluiu-se que os testadores SynD e SynF mostram-se adequados para identificar linhagens com maiores efeitos de GCA e é possível identificar novas linhagens com alto potencial heterótico com cada um dos quatro testadores utilizados.

**Palavras-chave:** *Zea mays*, diallelo, testcross, capacidade de combinação.

#### INTRODUCTION

High maize yield can be obtained by crossing of two parents that show high heterosis in hybrid combinations (CARENA & HALLAUER, 2001; GUIMARÃES et al., 2007; MIRANDA et al., 2008) and with the choice of adequate cultivars aiming to capitalize genotype x environment interaction (FRITSCHÉ-NETO et al., 2010a; FALUBA et al., 2010).

The diallel matting can be used in maize breeding programs to identify parents with high favorable alleles frequencies and greater heterosis patterns in crosses (SILVA et al., 2003; PATERNIANI et al., 2008; CHAVES et al., 2008). Various methods of diallel analysis are used to determine genetic parameters of the parents, helping breeders to choice appropriated

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testers (MIRANDA FILHO & VENCOSKY, 1999; PFANN et al., 2009; OLIVEIRA et al., 2011).

In maize breeding programs is essential to select appropriate testers that correctly classify specific and general combining abilities (SCA and GCA) of inbred lines to produce hybrids with high yield potential for seed market exploitation, even for wide recommendation (DEITOS et al., 2006) or for specific environments such as soils with low availability of nitrogen (SOUZA et al., 2010), low phosphorus (FRITSCHÉ-NETO et al., 2010b) and aluminum toxicity (CANÇADO et al., 2002).

Testers have been used in maize breeding programs to form heterotic groups, assess the combining ability and to identify superior hybrid combinations. In addition, information about performance and combining ability of lines can be useful to further selection in early generations, with a good prediction of performance in advanced generations. GUTIÉRREZ-GAITAN et al. (1986), VASAL et al. (1992) and LI et al. (2007) emphasized the importance of testers; therefore, the success of a maize breeding program depends on the choice of the most appropriated testers to select superior lines with a significant reduction of costs and labor.

Ideal testers should allow great expression of genetic variability in their progeny (RUSSELL, 1961). Moreover, recessive homozygous lines and populations with low frequency of favorable alleles should be successful testers in plant breeding programs, because new lines with high frequency of favorable genes could be better identified by using that kind of testers (SMITH, 1986; HALLAUER et al., 2010).

On the other hand, the use of testers with a high frequency of favorable alleles allows identifying the best crossings, the ones with the highest specific combining ability with those testers. In this situation, the testers should be the best elite-lines of the breeding program, one of dent and other of flint heterotic groups, for example: the two parental lines of a good commercial hybrid (HALLAUER & CARENA, 2009). So, new lines identified in superior crossings could become parents directly of commercial hybrids.

The objective of this study was to evaluate four testers with different genetic structures (single cross hybrids, with narrow genetic basis, and open pollinated varieties, with broad genetic basis) and different types of grains (flint and dent) for identification of superior inbred lines in testcross.

## MATERIAL AND METHODS

It was obtained 426 testcross hybrids, synthesized from crosses of four testers with a set of

162 inbred lines belonging to University Federal of Viçosa's maize breeding program, but only 45 lines were crossed with all the four testers. The testers used were: SynD and SynF, that are open-pollinated cultivars with dent and flint kernels, respectively, and FSH and DSH, that are single hybrids with flint and dent kernels, respectively.

A total of 441 treatments, comprising the 426 testcross hybrids and 15 checks, were evaluated in nine trials in 7x7 lattices with two replications. The experimental plot consisted of a row of four meters long with 0.90m spacing between rows, which six seeds per meter for a population 65,000 plants per hectare. The following traits were evaluated: male flowering (MF), female flowering (FF), plant height (PH, cm), final stand (ST) and grain yield (GY, kg ha<sup>-1</sup>).

Preliminary analyses of variance were carried out for each experiment. The joint analysis of experiments was performed after to check the homogeneity of variance of all traits. It was also used a hierarchical statistic model to test the effects of different groups of genotypes. For this analysis, the sums squares of treatments were partitioned into the sum of squares of testcross, testers, lines within each tester, checks and contrast between testcross hybrids and the checks. A Scott-Knott test was performed for all 426 testcross hybrids to grouping genotypes according to means of grain yield, using a significance level of 1% of probability.

It was evaluated the testcross hybrids obtained from crosses between two groups of parents (testers - group 1 and lines - group 2). Therefore, 11 partial diallel analyses were carried out for all combinations of testers with lines in common. The genetic effects were determined by partial diallels according to methodology of GRIFFING (1956), adapted by GERALDI & MIRANDA FILHO (1988). It was determined only for the trait grain yield. The genetic-statistic model used was:  $y_{ij} = \mu + g_i + g_j + s_{ij} + \epsilon_{ij}$ , where:  $y_{ij}$  = mean of the hybrid combination between the  $i^{\text{th}}$  parent of group 1 (testers) and  $j^{\text{th}}$  parent of group 2 (lines);  $\mu$  = geral mean;  $g_i$  = effect of general combining ability of the  $i^{\text{th}}$  parent of group 1 (testers);  $g_j$  = effect of general combining ability of the  $j^{\text{th}}$  parent of group 2 (lines);  $s_{ij}$  = effect of specific combining ability between the parents  $i$  and  $j$ , of groups 1 and 2, respectively;  $\epsilon_{ij}$  = random error associated with the mean  $ij$ .

## RESULTS AND DISCUSSION

In the combined analysis of trials, it was found that the effects of treatments (testcross hybrids

and checks) were significant for all traits that suggest the existence of genetic variability and the possibility of success in obtaining superior hybrids (Table 1). The general average for grain yield was 9 524kg ha<sup>-1</sup>, and the best testcross hybrid yielded 13 863kg ha<sup>-1</sup>. The group of 15 checks showed grain yield mean equal to 11 367kg ha<sup>-1</sup> and the 15best testcross hybrids showed grain yield mean of 12 730kg ha<sup>-1</sup>.

The effects of testers were not significant for GY and ST (Table 1). The effect of GY, MF, PH and ST were significant for inbred lines within each tester, indicating that the effects of lines were very important in the expression of genetic variability. These results also show that all testers allowed the expression of genetic variability since the lines were randomly sampled in their crosses which each tester.

The Scott-Knott test allowed the discrimination of three groups, among the 426 testcross hybrids, according to its grain yield mean. The first group was formed by the 326 best hybrids, which showed average grain yield ranging from 8 560 to 13 862kg ha<sup>-1</sup>, where the highest yield was achieved for the hybrid synthesized by crossing line 74 and DHS tester. The second group included 70 testcrosses hybrids with averages between 6 369 and 8 536kg ha<sup>-1</sup>, while the third group was formed by 30 testcross hybrids showing the worst performance in terms of grain yield, ranging from 1 535 to 6 193kg ha<sup>-1</sup>. Obviously, there is much more interest in new lines identified in the first group due to higher grain yield potential. So, these inbred lines are the best candidates

to become progenitors of commercial hybrids. Moreover, beyond those good crosses between testers and lines, these best lines should be crossed among them to test new hybrids with high potential to seed market exploitation. In table 2 are presented the parental lines that produced the 35 testcross hybrids with highest grain yield, selected among the 426 hybrids. The average of GY of these 35 hybrids was 12 133kg ha<sup>-1</sup>, and the top ten yielded 12 868 kg ha<sup>-1</sup>. The lines 74, 117, 53, 24, 122, 56, 49, 34, 113 and 23 stood out as parents of the top ten testcrosses. So, these lines should be considered as good candidates to form single cross hybrids with high grain yield potential. Furthermore, among this group of 35 best testcross hybrids it can be seen the lines 24, 122, 56, 113, 23, 26, 40, 4 and 32, that were identified by the average of four testers as the most promising inbred lines (Tables 2 and 3), with high estimates of GCA and SCA.

ELIAS et al. (2000) worked with open pollinated maize cultivars and found differences in absolute values for GCA, ranging from -56.05 to 90.30, which differ in the frequency of favorable alleles for GY. DUARTE et al. (2003) found significant difference in the GCA for testers with high values, suggesting that the higher frequency of favorable alleles for improving the GY was in testers than lines. However, if a tester is used only to evaluate the combining abilities of lines, negative GCA estimate for this tester can be more interesting because the better expression of the favorable alleles from different lines depends on the frequency of unfavorable alleles from the testers (BARATA & CARENA, 2006).

Table 1 - Analysis of variance for grain yield (GY, kg ha<sup>-1</sup>), male flowering (MF, days), female flowering (FF, days), plant height (PH, cm), and final stand (ST, plantas ha<sup>-1</sup>).

SV	DF	-----Mean Square-----				
		GY	MF	FF	PH	ST
Treatments	440	7 379 516 <sup>**</sup>	3.026 <sup>**</sup>	2.623 <sup>**</sup>	409.8 <sup>**</sup>	295 838 227 <sup>**</sup>
Testcrosses	425	7 266 333 <sup>**</sup>	3.020 <sup>**</sup>	2.621 <sup>**</sup>	403.5 <sup>**</sup>	292 349 562 <sup>**</sup>
Testers	3	3 337 526 <sup>ns</sup>	45.953 <sup>**</sup>	51.487 <sup>**</sup>	2 763.0 <sup>**</sup>	678 809 057 <sup>ns</sup>
Lines/SynF	121	6 204 683 <sup>**</sup>	3.052 <sup>**</sup>	2.541 <sup>**</sup>	297.1 <sup>**</sup>	278 508 681 <sup>**</sup>
Lines/SynD	93	8 457 303 <sup>**</sup>	2.648 <sup>**</sup>	2.144 <sup>**</sup>	322.6 <sup>**</sup>	339 083 954 <sup>**</sup>
Lines/ FSH	99	6 498 218 <sup>**</sup>	2.843 <sup>**</sup>	2.894 <sup>**</sup>	504.2 <sup>**</sup>	261 402 714 <sup>**</sup>
Lines/ DSH	109	8 234 490 <sup>**</sup>	2.280 <sup>**</sup>	1.526 <sup>ns</sup>	434.5 <sup>**</sup>	285 311 101 <sup>**</sup>
Checks	14	3 813 239 <sup>ns</sup>	3.390 <sup>**</sup>	2.857 <sup>*</sup>	627.0 <sup>**</sup>	215 232 739 <sup>ns</sup>
Top vs check	1	105 410 202 <sup>**</sup>	0.618 <sup>ns</sup>	0.064 <sup>ns</sup>	4.7 <sup>ns</sup>	2 906 997 000 <sup>**</sup>
Error	441	2 905 876	1.517	1.489	123.58	132 445 356
General mean		9 524	60.73	60.95	232.28	59 461
CV(%)		17.9	2.03	2	4.79	19.35

<sup>ns</sup>: not significant at P>0.05 by F test; \*, \*\* significant at P<0.05 and P<0.01, respectively, by F test.

Table 2 - Ranking of grain yield (GY) for the 35 best hybrid among 426 testcross evaluated, and identification of their testers and parental lines, entry number and trial that they were selected, and this set of top 10 hybrid crosses.

Ranking	Tester	Line	GY (kg ha <sup>-1</sup> )	Entry	Trial
1	DHS	74	13 862	18	7
2	DHS	117	13 640	37	7
3	SynD	53	13 028	45	4
4	DHS	24*	12 919	9	6
5	FHS	122*	12 821	45	5
6	SynD	56*	12 762	24	9
7	DHS	49	12 731	39	8
8	FHS	34	12 701	45	8
9	SynD	113*	12 607	8	4
10	FHS	23*	12 604	8	5
11	SynF	133	12 422	13	4
12	FHS	48	12 299	24	5
13	FHS	11	12 271	37	9
14	DHS	26*	12 217	11	6
15	FHS	25	12 062	10	5
16	FHS	42	12 061	20	5
17	DHS	92	11 989	4	7
18	FHS	83	11 984	12	8
19	DHS	31	11 964	25	8
20	DHS	116	11 889	14	7
21	SynF	52	11 881	23	1
22	FHS	120	11 857	46	8
23	SynF	40*	11 784	17	1
24	DHS	4*	11 781	1	6
25	SynD	99	11 766	14	4
26	SynF	32*	11 640	15	1
27	DHS	6	11 605	3	6
28	SynD	19	11 549	46	3
29	DHS	98	11 514	1	7
30	SynF	119	11 489	28	3
31	SynD	63	11 462	27	2
32	SynD	156	11 418	11	9
33	SynF	22	11 392	5	3
34	DHS	121	11 383	44	6
35	SynD	110	11 280	9	2

\*Elite lines identified as superior in the partial diallel analysis with all four testers.

These results from the partial diallel involving the four testers and the same 45 inbred lines showed no significant effect for testers' GCA ( $P>0.05$ ) and significant ( $P<0.01$ ) for GCA for lines and for tester x lines SCA. It means that additive and non-additive genetic effects (dominance and epistasis) were important in this genetic pool and that the lines have different performances according to the tester used. Thus, different lines can be selected according to the tester and promising hybrids can be synthesized crossing lines with complementary genetic pools, allowing best exploitation of heterosis, as suggested by HALLAUER et al. (2010).

Similarly, to the diallel analysis performed for the 11 groups of crossings of lines by testers, it was only found significance to GCA for lines and to SCA for hybrid combinations. Although there were no significance to GCA for testers, FSH showed positive estimates in all seven partial diallel involved, with estimates ranging from 65.3 to 284.6. On the other hand, each one of the two dent testers showed only one positive, but of low magnitude, estimate for GCA (10.6 for SynD, and 82.8 for DHS), while the other six estimates were negative, ranging from -39.8 to -155.6 for SynD, and from -10.6 to -165.9 for DHS. It indicates that these dent testers have low frequency of favorable alleles

Table 3 - Classification of grain yield (GY, kg ha<sup>-1</sup>) for 45 maize lines for each tester, and mean testers.

Rank	-----SynF-----		-----SynD-----		-----FSH-----		-----DSH-----		-----Mean Testers-----	
	Line	GY	Line	GY	Line	GY	Line	GY	Line	GY
1	52	11 859	56*	12 878	122*	12 822	24*	12 919	4	11 061
2	40*	11 780	113*	12 608	23*	12 604	26*	12 218	24	10 830
3	32*	11 643	122*	11 984	48	12 299	4*	11 782	45	10 827
4	159	11 382	63	11 970	42	12 061	121	11 383	26	10 810
5	56*	11 366	26*	11 714	120	11 857	32*	11 201	23	10 797
6	24*	11 228	23*	11 673	55	11 830	131	11 197	122	10 693
7	45*	10 940	156	11 346	4*	11 699	29	11 110	32	10 691
8	4*	10 473	40*	11 095	63	11 560	52	11 031	56	10 656
9	13	10 451	104	11 027	129	11 043	120	10 937	40	10 353
10	26*	10 309	148	10 799	84	11 013	104	10 904	113	10 352
11	121	10 204	105	10 798	29	10 783	45*	10 904	52	10 329
12	29	10 156	45*	10 766	13	10 713	112	10 780	63	10 320
13	161	9 976	52	10 390	45*	10 697	125	10 780	121	10 227
14	120	9 952	4*	10 290	57	10 541	113*	10 751	29	10 024
15	63	9 910	41	10 222	14	10 535	57	10 420	57	9 975
16	122*	9 882	142	10 100	17	10 521	33	10 159	104	9 944
17	14	9 671	24*	9 918	106	10 491	42	9 990	131	9 841
18	104	9 634	139	9 762	41	10 305	44	9 957	105	9 744
19	23*	9 612	32*	9 750	131	10 266	148	9 687	55	9 620
20	48	9 562	121	9 714	32*	10 170	14	9 682	14	9 608
21	150	9 535	125	9 566	107	9 841	55	9 584	125	9 490
22	57	9 525	57	9 413	105	9 645	41	9 520	148	9 416
23	125	9 370	130	9 402	121	9 607	160	9 440	48	9 342
24	105	9 327	42	9 234	148	9 512	23*	9 298	33	9 334
25	138	9 275	138	9 175	138	9 509	105	9 206	129	9 280
26	142	9 239	129	9 122	56*	9 438	129	9 197	42	9 278
27	131	9 220	154	9 087	40*	9 408	40*	9 129	13	9 258
28	17	9 074	33	9 056	113*	9 392	154	9 099	120	9 217
29	55	8 962	17	8 771	161	9 383	161	9 028	17	9 198
30	33	8 777	131	8 682	33	9 342	56*	8 944	41	8 911
31	44	8 720	14	8 543	24*	9 256	84	8 596	161	8 905
32	113*	8 657	48	8 388	26*	9 002	107	8 573	156	8 773
33	107	8 618	13	8 321	160	8 508	17	8 425	112	8 727
34	112	8 536	55	8 103	139	8 330	156	8 172	107	8 624
35	84	8 494	29	8 047	125	8 244	106	8 091	139	8 363
36	139	8 306	160	8 010	104	8 212	122*	8 083	106	8 342
37	106	8 171	112	7 601	142	8 161	63	7 841	138	8 270
38	156	7 841	107	7 463	150	8 077	13	7 549	84	8 229
39	129	7 756	161	7 233	52	8 038	48	7 117	160	8 201
40	148	7 666	44	7 115	112	7 991	159	7 108	142	7 889
41	154	7 212	106	6 616	130	7 783	139	7 053	154	7 457
42	160	6 848	150	5 648	156	7 733	150	6 351	150	7 403
43	42	5 828	84	4 815	159	6 892	130	5 926	159	7 388
44	41	5 599	159	4 171	154	4 432	138	5 120	44	7 380
45	130	4 097	120	4 120	44	3 727	142	4 055	130	6 802
Mean		9 214		9 211		9 628		9 295		9 337
Top 15 Mean		11 143		11 709		11 878		11 468		10 707
Variance		2 631 538		4 269 822		3 538 635		3 509 491		1 236 909

\*Lines classified like the 10 highest producers for four testers.

for GY in comparison with FSH. SynF tester showed three positive estimates for CGA (from 7.1 to 117.5) and four negative estimates (from -65.3 to -103.9). Therefore, hybrids with different heterosis patterns can be formed when lines are selected by testers with divergent estimates of GCA and less agreement in ranking the lines. SynD tester showed the greatest genetic variance for testcrosses among the four testers (Table 3), and the same trend occurred for lines/tester mean square estimates (Table 1). For these reasons and also for its negative GCA estimates, SynD is probably the best tester to discriminate these groups of inbred lines. Furthermore, this tester showed greater concordance in ranking the top lines with average of the four testers, because among its 15 best testcross, eight of those lines were also higher in the general classification for all testers (Table 3). SynF was able to select seven of the best lines in the upper 15 testcross ranked by all testers, while DSH and FSH, selected six and four lines, respectively. Only two lines were selected in common by SynD and SynF, and this could be a consequence of the different types of grain between SynD and SynF, that are traditionally referred as divergent and complementary heterotic groups. So, it can be inferred that this pair of testers can be used to select lines, assessing genotypes with high general combining ability and, simultaneously, arranging them in complementary heterotic groups.

The correlation of ranking between the lines classification by different testers were of low magnitude, ranging from 0.29 (FHS and DHS) to -0.13 (SynD and FHS), but some lines showed superior performance with all the testers (Table 3). Lines 4 and 26, for example, were among the top ten testcross hybrids to three testers and lines 23, 24, 122, 32 and 40 for two testers. This shows that these lines have good general combining ability and, probably, all non-related testers should be able to identify them. Furthermore, it was possible to identify lines that had high SCA estimates with each tester used. The high genetic variability observed in the testcross hybrids, the low frequency of favorable alleles and the high concordance in lines classification with the average of other testers are desirable characteristics for a good maize tester. Therefore, SynD was better suited to inbred line selection with high frequency of favorable alleles, and SynF can be useful as a complement tester with SynD, to arrange selected lines in two heterotic group.

## CONCLUSION

It was concluded that SynD and SynF testers are adequate to identify inbred lines with high

GCA effects and arrange selected lines in two heterotic groups in the maize breeding program, and it's possible to identify new lines with high heterotic potential in each one of the four testers.

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