Original paper Open Access

Topcrosses in the selection of testers and inbred lines S3 for the yield and bromatological quality of silage maize.

Alex S. T. Figueiredo[1*], Ronald J. B. Pinto[2], Carlos A. Scapim[2], Diego A. Rizzardi[2 3], Rodrigo I. Contreras-Soto[4], Robson A. Matsuzaki[2], Clóves C. Jobim[2], Marcos V. Faria[5]

[1*] Sakata Seed Sudamerica, Bragança Paulista, 12906-840, São Paulo, Brazil
[2] Departamento de Agronomia, Universidade Estadual de Maringá – UEM. Maringá, Paraná, 87020-900, Brazil
[3] Geneze Sementes S/A, Cambé, Paraná, 86181-010, Brazil
[4] Instituto de Ciencias Agronómicas y Veterinarias, Universidad de O'Higgins – UOH. Rancagua, 2820000, Chile
[5] Departamento de Agronomia, Universidade Estadual do Centro Oeste – UNICENTRO. Guarapuava, Paraná, 85040-080, Brazil
*Corresponding author : E-mail: alexstfigueiredo@gmail.com

KeyWords *Zea mays L, silage maize, hybrid topcrosses, combining ability.*

Abstract

The study aiming to evaluate the combining ability of maize partially inbred lines (S3) in crosses with testers of narrow genetic base aiming at the selection of the inbred lines, testers and topcross hybrids. Three simple lattice trials with 81 treatments were carried out in the 2014/2015 and 2015/2016 growing seasons. For each growing season, trials were used to evaluate the topcross hybrids obtained in combination with the testers AG8088, DKB330 (single-cross hybrids) and 9.H33.3 (line). The hybrid AG8088 and line 9.H3.33 were the best testers for grain yield. The line 9.H3.33 was the best tester for the traits related to yield and quality of silage maize. The inbred lines that stood out for their combining abilities and capacity of generating great topcross hybrids with the testers (i.e., 201-23.2, 201-59.1, 201-80.2, 201-81.5, 203-195.3, 201-100.4, 201-145.4, 201-169.3, 202-155, 202-159, 203-23, 203- 31, 203-32, 203-38, 203-75, 203-98, 203-111, 203-135, 203-139, 203-150, 203-188, 203-235, and 203-237) should be maintained in the UEM silage maize breeding program. Progenies 201-59.01, 201-100.4, 203-135, 203-150, 203-235 and 203-254 were selected as tester lines of progenies derived from selfing of the testers AG8088 and DKB330 to improve the silage maize quality. The topcross hybrids 201-95.3 x 9.H3.33, 203-71 x 9.H3.33, 203-72 x 9.H3.33, 203-88 x 9.H3.33, 203-139 x 9.H3.33, and 203-150 x 9.H3.33 were selected for good performance for agronomic and bromatological silage maize traits. These hybrids are indicated for evaluations in more environments, with a view to recommending commercial cultivars for grain and silage production in the future.

KeyWords *Zea mays L, silage maize, hybrid topcrosses, combining ability*

Introduction

Maize is the reference specie for silage production in the world and also in Brazil. Maize breeding for silage production is complex, for requiring the simultaneous improvement of several traits related to silage yield and bromatological quality (Coors and Lauer, 2001). It is generally agreed that a good maize hybrid recommended for silage production must have a high biomass yield, high proportion of total grain dry matter, improved nutritional value of the fibrous portion, aside from features such as yield stability, broad adaptation and resistance to the main pests and diseases (Argillier et al, 2000).

The structure of maize breeding programs is divided into four basic stages: formation and selection of the base populations, line development, selection of the lines in crosses with testers and, finally, assessment of the hybrid combinations (Hallauer et al, 2010). However, the stage of evaluation and selection of the inbred lines based on their genetic merit is the most onerous (Souza Júnior, 2001), since the value of a line is determined by its ability to generate valuable hybrids for a breeding program (Castellanos et al, 1998).

When applying inbreeding over successive generations in a given base population by the traditional breeding methods, the result can be a very large number of lines. The *per se* performance of an inbred line, on the other hand, is of little use for breeders, as the correlation between the performance of an inbred line and its hybrids is very low (Souza Filho, 2001). In this context, Davis (1927) suggested topcrosses methodology, which consists of combining a large number of inbred lines in still partially inbred generations, with one or more testers in common, selecting and advancing only the promising lines over more generations.

The selection of the best tester to discriminate the genetic potential of lines still ignites discussions in maize breeding programs (Guimarães et al, 2012). Many authors conclude that the choice of the best tester should take the genetic merit (high or low frequency of favorable alleles) into account, as well as the genetic background (broad *versus* narrow), development stage of the breeding program, availability of testers, and which type of hybrids is being exploited by breeding program. However, Hallauer et al (2010) emphasizes that the best tester is one that unites simplicity in use and the ability to correctly classify the genetic merit of lines.

Studies with a partial diallel mating design between maize lines for traits related to breeding for silage maize can be consulted in the literature (Wolf et al, 1993; Ertiro et al, 2013). However, there is a lack of studies that discuss the choice of lines and testers for silage maize breeding programs. The objective of this study was to evaluate the combining ability of maize inbred lines (S_3) in crosses with narrow-genetic base testers in order to select maize lines, testers and topcross maize hybrids with a view to improving of yield and quality of silage maize.

Materials and Methods

Genetic materials

A total of 72 partially inbred lines S_3 (supplementary material) derived from the commercial hybrids P30F53, AS1560 and SG6015, previously selected by Gralak et al (2017), were crossed by manual pollination with the testers AG8088, DKB330 and 9.H3.33 to establish topcross hybrids. The testers AG8088 and DKB330 are single-cross hybrids and were selected in view of their phenotypic adaptability and stability and also for being recommended for grain and silage production. The elite line 9.H3.33 was developed by UEM maize breeding program, derived from silage hybrid AG8080.

Agronomic trials

Six trials (three per season) were carried out in the growing seasons of 2014/2015 and 2015/2016 on the Experimental Farm Iguatemi (EFI - UEM) belonging the Maringá State University (UEM). Each trial was used to assess the topcross hybrids obtained with the testers AG8088, DKB330 and 9.H3.33. The experimental area is located at an altitude of 550 m asl and the climate and soil are classified, respectively, as Cfa, according to Köppen, and dystroferric red Latosol (Oxisol).

Pre-planting and cover fertilization were identical in the 2014/2015 and 2015/2016 growing seasons, based on official recommendation criteria for silage maize fertilization, for a high fresh matter yield (> 60 t ha^{-1}). As pre-planting fertilization, 450 kg ha^{-1} of the N-P-K 08-20-20 + Zn fertilizer was applied. The first cover fertilization was applied between stagesV_4 and V_5 (90 kg N ha^{-1}+ 45 kg K_2Oha^{-1}), and the second between stages V_7 and V_8 (90 kg N ha^{-1}). At 25 days after sowing, the plants were thinned to a final plant density of 55,555 plants ha^{-1}.

The experimental unit in both growing seasons consisted of two contiguous 5m rows spaced 0.9 m apart. The first row was used for assess the fresh matter yield, dry matter yield and the bromatological quality of maize fodder at ensiling (measurable area 4.5 m2). The second row was used to evaluate grain yield (area 4.5 m2).

The agronomic traits fresh matter yield (FMY: kg ha-1), dry matter yield (DMY: kg ha^{-1}) and grain yield (GY: kg ha^{-1}) were evaluated in the 2014/2015 and 2015/2016 growing seasons. When the plants reached 30 and 35% of total dry matter content (2/3 of the kernel milk line), corresponding to the phenological stage characterized as pasty to farinaceous grain, all plants of the first row were cut at a standard height of 0.2 m. The cut plants were weighed to calculate the fresh matter yield at ensiling (FMY: kg ha^{-1}). Six plants were separated and chopped in a shredder to produce a mean particle size of 1.5 cm. A 0.3 kg (± 0.1 kg) sample was dried in a forced air circulation oven at 55°C to constant weight. The pre-dried samples were processed in a knife mill, sieved (diameter <1 mm) and a 2.0 g (± 0.025 g) aliquot was taken from the original sample and oven-dried at 105°C to obtain the total dry matter. Based on the fresh matter yield and final dry matter content, the dry matter yield at ensiling (DMY, kg ha^{-1}) was estimated.

Twenty days after the plants had reached the point of physiological grain maturity, the ears from the second planting row were harvested and estimated the total grain yield (GY, kg ha^{-1}). After the harvest, grain moisture was measured and the total grain yield (kg ha^{-1}) calculated, correcting the final moisture content to 13%.

Bromatological quality evaluation

The bromatological quality of silage maize fodder at ensiling was evaluated in the 2014/2015 growing season, based on the following traits: crude protein (CP), neutral detergent insoluble fiber (NDF), acid detergent insoluble fiber (ADF) and lignin (LGN). To estimate the total protein content, the total N content of the plants was determined by the micro Kjeldahl method and the total N values were converted to CP content by multiplication with a conversion factor of

6.25 (Silva and Queiroz, 2012). The neutral and acid insoluble detergent fibers were determined using to the non-woven fabric filter bags (density 100 g dm-3) applying neutral and acid detergent solutions, respectively (Van Soest et al, 1991). To determine neutral insoluble detergent fiber, 0.5 mL thermo stable α-amylase was used for each sample. The lignin content was determined after treatment with acid detergent and sample digestion in 72% concentrated sulfuric acid solution to determine the lignin content as described by Van Soest and Wine (1968).

Statistical analyses

For analysis of variance of the data, the treatment sources of variation [hybrid topcrosses established by combining the i-th inbred line (random effect) with the j-th tester (fixed effect)], blocks and blocks/locations were considered of random nature, while the source of variation of growing season was considered fixed. Initially, the individual analyses of variance were performed for the agronomic and bromatological traits of silage evaluated in the 2014/2015 and 2015/2016 growing seasons, in a simple lattice design (9x9), using the analysis strategy with intrablock variance with recovery of interblock information to obtain the adjusted means with the effective lattice variance.

Before proceeding with the join analysis of variance for the traits GY, FMY and DMY evaluated in the 2014/2015 and 2015/2016 growing seasons, the homogeneity of variances of individual analyses was tested. As criterion for the combined analysis, a ratio between residual variances less than or equal to 7:1 was adopted, as suggested by Pimentel Gomes (1990). The combined analysis was carried out in a complete block design with randomized treatments, using the adjusted means and the mean effective variance of the individual analyses in simple lattice as mean square of the residue.

The components of genetic and phenotypic variance and broad-sense heritability for the characters CP, NDF, ADF, and LGN were estimated for the 2014/2015 growing season. For the characters GY, FMY and DMY evaluated in the 2014/2015 and 2015/2016 growing seasons, the components of genetic and phenotypic variance of the genotype-environment interaction were estimated, with the broad-sense heritability based on join analysis, in a complete block design with randomized treatments.

Individual analyses of the partial diallel were performed with only the F_1 combinations (Griffing, 1956), in which the square sum of the topcross hybrids for agronomic and bromatological traits of silage quality evaluated in the 2014/2015 and 2015/2016 growing seasons were partitioned in general combining ability of inbred lines (Group: I), testers (Group: II) and specific combining ability. For the individual analysis of the partial diallel, the error degree of freedom consisted of the sum of the error degrees of freedom of the individual analyses of variance in the experiments with the topcross hybrids. As effective error in the individual analysis of the partial diallel, the mean square arithmetic mean of the effective error of the analyses of individual variance of the experiments with the topcross hybrids was used. The general (inbred lines and testers) and specific combining ability of the traits GY, FMY and DMY were estimated based on the combined analysis of the partial diallel with only the F_1 hybrid combinations (Griffing, 1956). The total variability in the join analysis of the partial diallel was partitioned into main effects of growing season, general (inbred lines: group I and testers: group II) and specific combining ability as well as the interaction of combining ability (GCA and SCA) with growing seasons. As combined error degree of freedom of the join partial diallel analysis we used the sum of degrees of freedom of the mean effective error of individual analyses of the partial diallel for the 2014/2015 and 2015/2016 growing seasons. As mean square of the mean effective error in the combined analysis of the partial diallel, the arithmetic mean of the mean effective errors of the individual analyses of the partial diallel was used.

The statistical software Genes (Cruz, 2013) was used to process the individual and combined variance analysis of lattice, estimation of the genetic and phenotypic parameters and the individual and combined analysis of the partial diallel with only F_1 combinations.

Results and Discussion

The estimates of the component of genetic variance among topcross hybrids indicates the capacity of a given tester to express the genetic variability contained in the progenies (Hallauer et al, 2010; Guimarães et al, 2012; Bolson et al 2016; Miotto et al, 2016). In this sense, the tester that provides the highest estimates of genetic variance among its topcross hybrids is the most appropriate, for allowing the expression of genetic variability of the progenies, highlighting the lines with highest genetic merit (Castellanos et al, 1998; Ortiz et al, 2010).

The testers that proved most efficient in releasing genetic variability of the progenies in terms of CP content of silage maize were AG8088 and 9.H33.3 (Table 1). The total amplitude between the CP means of the topcross hybrids with the testers AG8088 and 9.H33.3 were 3.22% and 2.55% respectively, and the topcross hybrids with the lowest CP content at ensiling were derived from tester AG8088 combined with the progenies 203-23 (CP: 10.59%) and 203-

Table 1 Estimates of genetic and phenotypic parameters of agronomic and bromatological traits of maize silage quality evaluated in trials with topcross hybrids obtained with the testers AG8088, DKB330 and 9.H3.33 and 72 inbred lines

Tester AG8080

Genetic parameters	CP	NDF	ADF	LGN	GY	FMY	DMY
	(% of dry matter)^2				(kg ha^{-1})^2		
Broad-sense heritability (h^2_{ampla})	0.688	0.5	0.57	0.736	0.52	0.13	0.09
Genetic $\hat{\sigma}^2_g$	0.218	5.93	1.58	0.245	428,104,86	994,127,26	90,02752
Phenotypic $\hat{\sigma}^2_f$	0.317	11.7	2.73	0.333	817,169,02	7,480,382,27	951,212,40
Genotype x environments $\hat{\sigma}^2_{gxa}$	-	-	-	-	455,993,21	7,069,991,87	734,345,92

Tester DKB330

Genetic parameters	CP	NDF	ADF	LGN	GY	FMY	DMY
	(% of dry matter)^2				(kg ha^{-1})^2		
Broad-sense heritability (h^2_{ampla})	0.383	0.621	0.65	0.764	0.69	0.05	0.05
Genetic $\hat{\sigma}^2_g$	0.105	4.34	1.72	0.342	326,573,17	349,726,08	40,991,31
Phenotypic $\hat{\sigma}^2_f$	0.276	6.98	2.65	0.447	476,250,40	7,406,455,88	846,355,76
Penotype x environments $\hat{\sigma}^2_{gxa}$	-	-	-	-	92,589,84	6,918,115,45	639,134,83

Tester 9.H3.33

Genetic parameters	CP	NDF	ADF	LGN	GY	FMY	DMY
	(% of dry matter)^2				(kg ha^{-1})^2		
Broad-sense heritability (h^2_{ampla})	0.592	0.7	0.76	0.796	0.38	0.52	0.38
Genetic $\hat{\sigma}^2_g$	0.222	7.92	2.93	0.54	145,131,00	6,293,725,11	569,772,64
Phenotypic $\hat{\sigma}^2_f$	0.375	11.24	3.85	0.678	379,144,31	12,138,534,80	1,515,739,62
Genotype x environments $\hat{\sigma}^2_{gxa}$	-	-	-	-	177,434,92	3,049,392,17	830,934,81

CP: crude protein content (% DM); NDF: content of insoluble fiber in neutral detergent (% DM); ADF: content of insoluble fiber in acid detergent (% DM); LGN: lignin content (% DM); GY: grain yield (kg ha^{-1}); FMY: fresh matter yield (kg ha^{-1}),

108 (CP: 7.37%). For the traits NDF, ADF and LGN, tester line 9.H33.3 provided the highest component of genetic variance among topcross hybrids (Table 1). In the ranking of the 216 topcross hybrids, 7 of the 10 lowest-lignin topcrosses were obtained by combining progenies 203-72, 201-95.3, 202-104, 203-03, 203-31, 202-11, and 202-108 with tester line 9.H33.33, generating hybrids with lower lignin contents, and consequently raising the nutritional value of silage.

Hybrid AG8088 was the tester that provided the highest component of genetic variance among the topcross hybrids for character GY in both growing seasons (Table 1). Analyzing the mean GY data of topcross hybrids in the 2014/2015 growing season, the overall mean was 7,256 kg ha-1 and the mean amplitude 5,043 kg ha-1 among the hybrids 201-100.4 x AG8088 (9,802 kg ha-1) and 203-55 x AG8088 (4,759 kg ha-1), both of which had hybrid AG8088 as tester in common. The topcross hybrid 201-100.4 x AG8088 had a similar GY to hybrid SG6030 (9,494 kg ha-1), the control with highest GY of this growing season. In 2015/2016, the overall mean GY of the topcrosses was 5,603 kg ha-1, with a total amplitude of 4,236 kg ha-1 between hybrids 203-73 x 9.H3.33 and 201-17.01 x DKB330, respectively. The topcrosses obtained in this growing season with tester AG8088 had a amplitude of 5,355 kg ha-1 between GY means and the hybrid with highest GY was 202-159 x AG8088, with an approximately 31% higher yield than the general mean of the all topcross hybrids. In the 2015/2016 growing season, the mean GY of the controls was 5,518 kg ha-1 and again, hybrid SG6030 stood out as control with the highest grain yield (6,306 kg ha-1).

The estimates of genetic variance between topcross hybrids for FMY and DMY were highest for tester line 9.H33.3 (Table 1). The topcross hybrids derived from

Table 2 Analysis of individual and join variance of a partial diallel with only F1 hybrids between inbred lines (group I) and testers (group II) for agronomic and bromatological traits of maize silage

Analysis of individual variance of the partial diallel - bromatological characteristics (growing season 2014/2015)

Source of variation	DF	Mean Square			
		CP	NDF	ADF	LGN
		(%dry matter)^2			
Topcross hybrid	215	0,59*	22,55*	6,18*	0,88*
GCA (group I)	71	0,76*	22,20*	7,93*	0,85*
GCA (group II)	2	0,43ns	491,16*	9,42*	3,12*
SCA	142	0,51*	16,12*	5,26*	0,86*
Mean effective error	192	0,28	7,82	1,99	0,22

Analysis of individual variance of partial diallel – agronomic traits (growing season 2014/2015)

Source of variation	DF	Mean Square		
		GY	FMY	DMY
		(kg ha^{-1})^2		
Topcross hybrid	215	1,850,286,88*	41,040,749,96*	5,151,481,53*
GCA (group I)	71	2,160,827,73*	49,356,306,70*	6,476,640,85*
GCA (group II)	2	13,255,466,56*	300,901,185,67*	42,985,047,00*
SCA	142	1,534,380,12*	33,222,965,45*	3,956,034,75*
Mean effective error	192	691,598,63	17,756,678,02	2,291,439,66

Analysis of individual variance of a partial diallel– agronomic traits (growing season 2015/2016)

Source of variation	DF	Mean square		
		GY	FMY	DMY
		(kg ha^{-1})^2		
Topcross hybrid	215	1,394,611,05*	29,573,126,50*	4,013,215,95*
GCA (group I)	71	1,015,749,26*	22,193,711,35*	2,867,798,02*
GCA (group II)	2	44,007,693,33*	587,105,948,09*	106,582,945,22*
SCA	142	983,857,68*	25,410,259,13*	3,141,280,84*
Mean effective error	192	401,056,61	11,227,441,32	1,736,049,74

Analysis of combined variance of a partial diallel– agronomic traits (growing seasons 2014/2015 and 2015/2016)

Source of variation	DF	Mean square		
		GY	FMY	DMY
		(kg ha^{-1})^2		
Topcross hybrid	215	2,004,975	41,071,816	5,499,200
GCA (group I)	71	1,938,438*	37,637,382ns	5,034,866ns
GCA (group II)	2	52,783,835ns	843,107,798ns	135,062,344ns
SCA	142	1,323,048ns	31,492,751ns	3,906,534ns
Seasons	1	590,173,697*	63,121,802,299*	5,221,930,905*
Topcross hybrid x Seasons	215	1,239,922*	29,542,060*	3,665,496*
GCA (group I) x Seasons	71	1,238,138*	33,912,635*	4,309,572*
GCA (group II) x Seasons	2	4,479,324*	44,899,334*	14,505,648*
SCA x Seasons	142	1,195,189*	27,140,473*	3,190,780*
Mean effective error	384	546,327	14,492,059	2,013,744

Significant by the F test (P <0,05), CP: crude protein content (% DM); NDF: content of neutral detergent insoluble fiber (% DM); ADF: fiber content insoluble in acid detergent (% DM); LGN: lignin content (% DM); GY: grain yield (kg ha^{-1}); FMY: fresh matter yield (kg ha^{-1}),

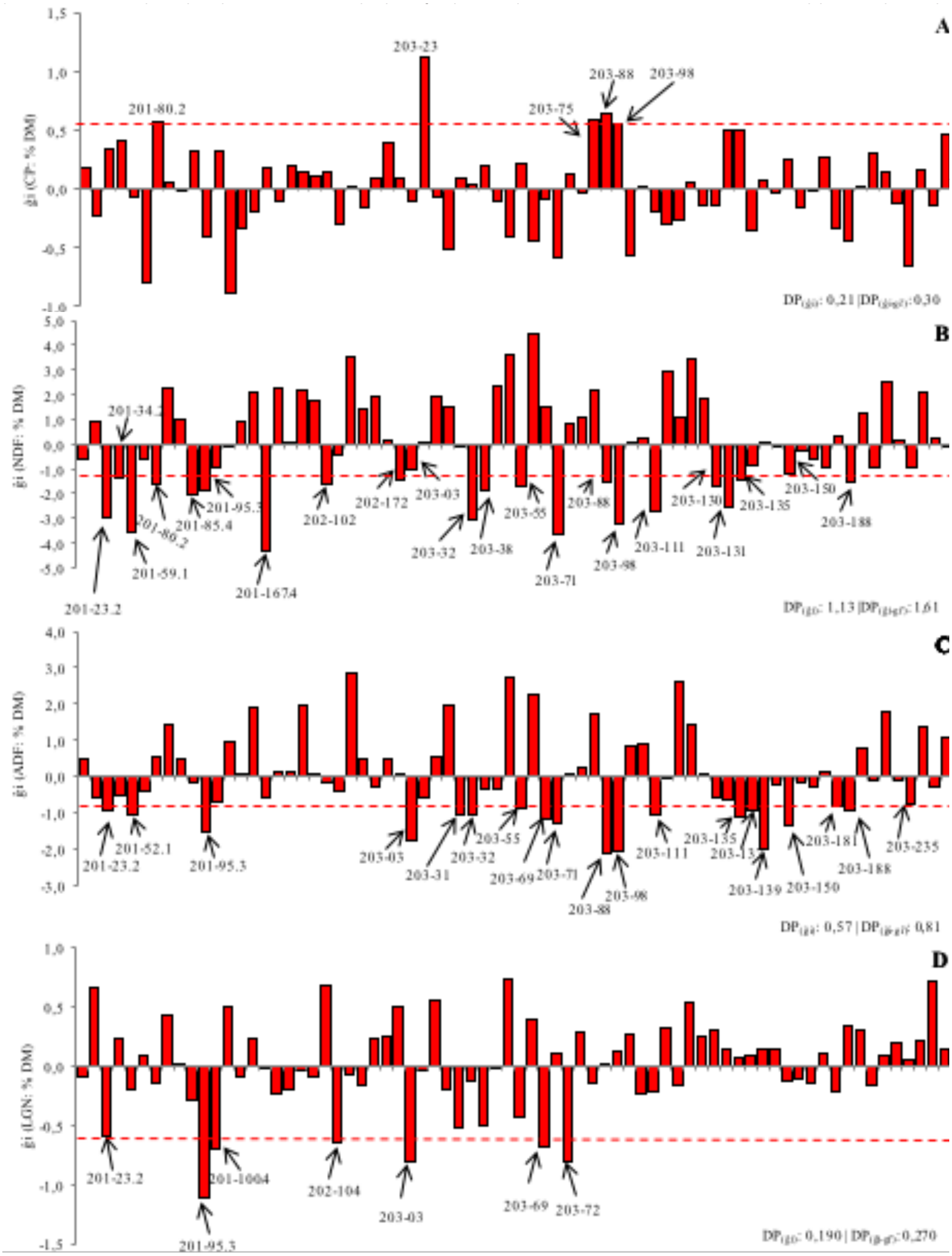


Figure 1 Estimates of general combining ability ($\hat{g}_i$) of inbred lines for bromatological traits of maize silage quality: A: crude protein (CP: % DM); B: neutral detergent fiber (NDF: % DM); C: acid detergent fiber (ADF: % DM); D: lignin (LGN: % DM). Estimates of general combining ability (ĝi) of inbred lines for bromatological traits of maize silage quality: A: crude protein (CP: % DM); B: neutral detergent fiber (NDF: % DM); C: acid detergent fiber (ADF: % DM); D: lignin (LGN: % DM).

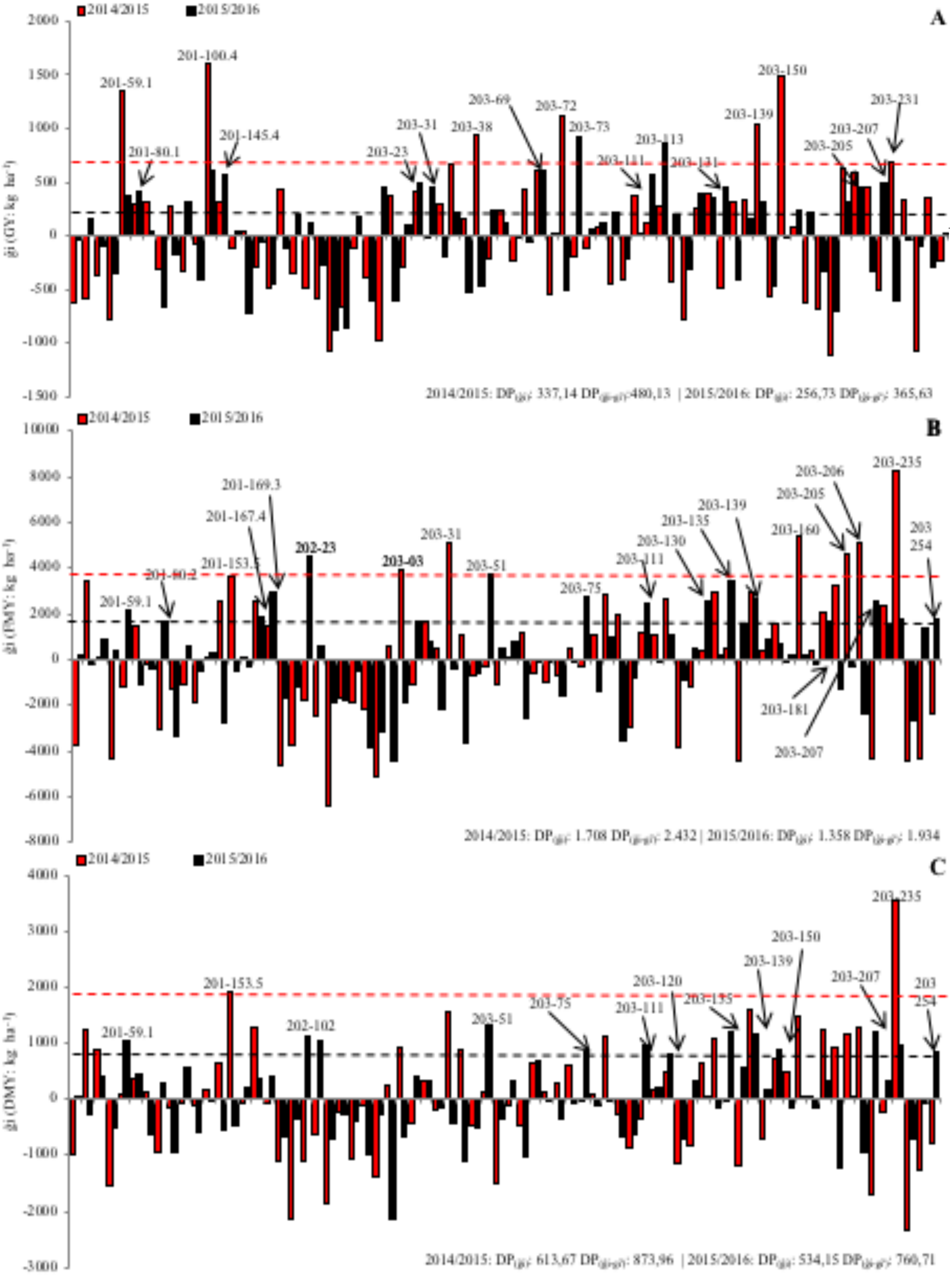
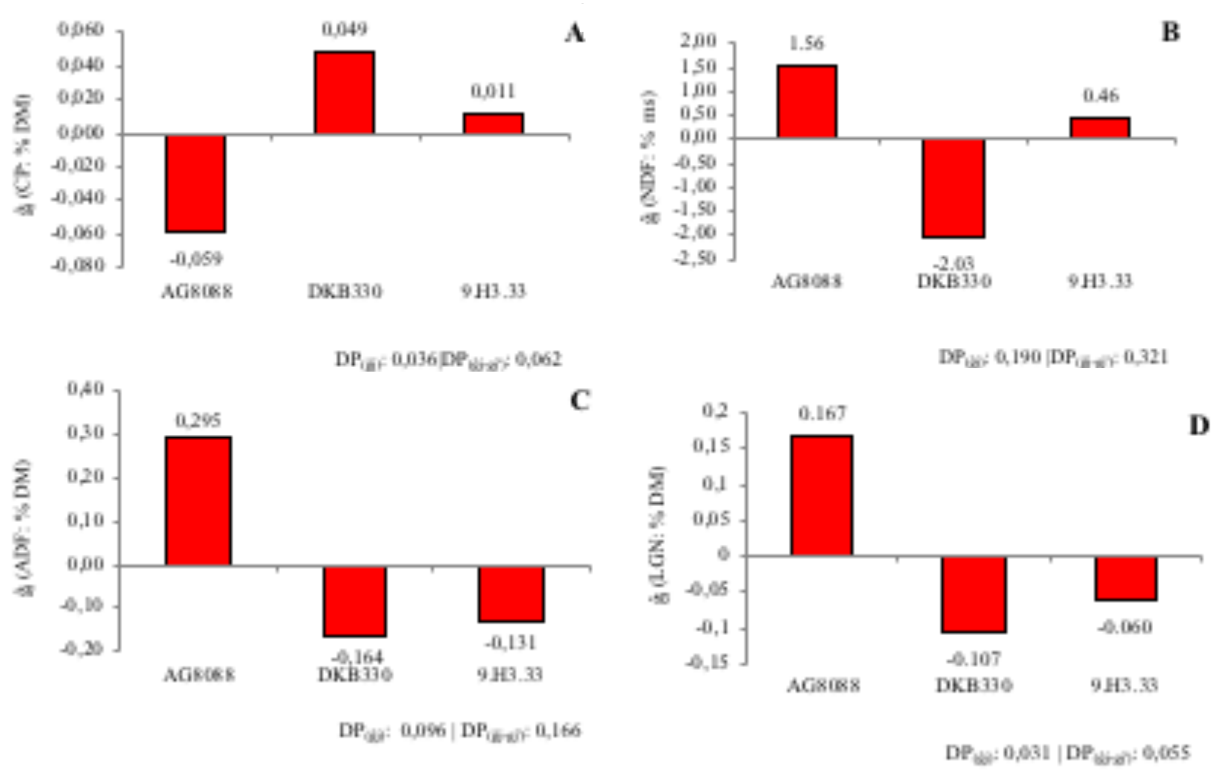


Figure 2 Estimation of general combining ability ($\hat{g}_i$) of 72 maize inbred lines for the agronomic traits: A: grain yield (GY: kg ha^{-1}); B: fresh matter yield (FMY: kg ha^{-1}); C: dry matter yield (DMY: kg ha^{-1}); evaluated in the 2014/2015 and 2015/2016 growing seasons.



progenies 203-150, 203-71 and 203-181.

The lignin content of silage of the topcross hybrids ranged from 1.45% to 6.08% in the combinations 203-72 x 9.H3.33 and 202-102 x AG8088, respectively. The topcross hybrid 203-72 x 9.H3.33 had the lowest SCA of all topcrosses, while the parents of both combinations were selected for GCA. Topcross 201-95.3 x 9.H3.33 is worth mentioning, for having a negative SCA for LGN (-1.32%) and concomitantly, both parents were selected for the additive effects.

In the 2014/2015, among the inbred lines with a relevant GCA for GY, only 201-100.4 (Ŝij: 1,070 kg ha-1), 201-59.1 (Ŝij: 533.7 kg ha-1), 203-139 (Ŝij: kg ha-1), and 203-31 (Ŝij: 894.1 kg ha-1) had appropriate SCA effects in combination with tester AG8088. The topcrosses 201-100.4 x AG8088 and 203-139 x AG8088 were the two hybrids with highest GY of the 2014/2015 growing season (9,802 kg ha-1 and 9,644 kg ha-1, respectively). Only the progenies 203-72 (Ŝij: 1,136 kg ha-1) and 203-231 (Ŝij: 638.06 kg ha-1) stood out for general and specific combining ability with the testers DKB330 and 9.H3.33, respectively.

For the 2015/2016, the inbred lines 203-98 (Ŝij: 977.9 kg ha-1), 203-51 (Ŝij: 837.4 kg ha-1), 203-131 (Ŝij: 828.5

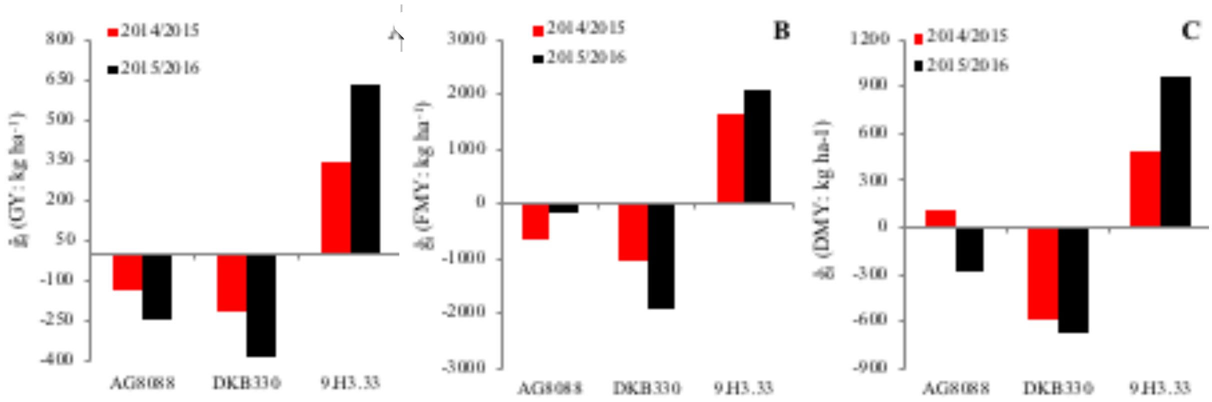


Figure 4 Estimates of the general combining ability ($\hat{g}_j$) of the Testers AG8088, DKB330 and 9.H3.33 for agronomic traits: A: grain yield (GY: kg ha^{-1}); B: fresh matter yield (FMY: kg ha^{-1}); C: dry matter yield (DMY: kg ha^{-1}) evaluated in the 2014/2015 and 2015/2016 growing seasons.

Table 3 Ranking of the maize inbred lines based on general combining ability ($\hat{g}_i$) and the topcross hybrids derived from the testers AG8088, DKB330 and 9.H33.3 ranked for the highest grain yield (GY: kg ha^{-1}) in the 2014/2015 and 2015/2016 growing seasons.

Grain Yields									
Growing season 2014/2015					Growing season 2015/2016				
Progeny	**AG8088**	**DKB330**	**9.H3.33**	**GCA**	**Progeny**	**AG8088**	**DKB330**	**9.H3.33**	**GCA**
201-19.3			11		201-17.1	9			
201-59.1*	3	9	5	3	**201-59.1**			8	15
201-80.1	7				**201-80.1**		2		13
201-80.2	10				201-81.5		8		
201-81.5	15				**201-85.4**	5		11	
201-100.4	1	3		1	**201-100.4**		1		3
201-145.4	5				**201-145.4**	2			5
201-167.2			3		201-167.2	14			
202-07			13	14	201-167.4			12	
202-23			10		202-154		6		
202-155		12			**202-159**	1			10
202-172		7			203-03	13			
203-23				15	**203-23**	10			7
203-30	4				**203-25**			15	12
203-31	8			8	**203-31**			14	
203-38	11		4	6	**203-51**			7	
203-54			15		203-55		5		
203-55		4			203-59	3			
203-59	12		7	13	**203-69**			2	4
203-69			9	10	203-71	7			
203-72		1	8	4	**203-73**		3	1	1
203-110		15			203-88	8			
203-122	9				**203-98**			4	
203-130		11			203-108			9	
203-135		8			**203-111**		7		6
203-139	2			5	**203-113**	6	4		2
203-144		14			**203-120**	11			
203-150	6	6	1	2	**203-121**	12			
203-154		5	12		**203-122**			5	14
203-188				9	**203-130**		15		
203-205			13	11	**203-131**			3	9
203-206		2	6	12	203-135		11		
203-207		13			203-137		9		
203-231		10	2	7	203-144		10		
203-250	13				**203-154**	4			
203-254	14				**203-160**			10	
					203-188	15			
					203-205		13	6	11
					203-206		12		
					203-207		14	13	8

* The bold and underlined progenies were selected for effects of general combining ability based on diallel analysis.

Table 4 Ranking of partially inbred maize progenies based on general combining ability (ĝi) and topcross hybrids derived from the testers AG8088, DKB330 and 9,H33,3 were ranked with lowest acid detergent fiber (ADF: % DM) and lignin (LGN: % DM) in the 2014/2015 growing season.

Progeny	ADF				Progeny	LGN			
	AG8088	DKB330	9.H3.33	GCA		AG8088	DKB330	9.H3.33	GCA
201-23.2*			9	14	**201-23.2**		1		7
201-59.1	13		8	10	**201-59.1**		3		
201-81.5		7	3		201-80.2	4			
201-95.3	9	10		5	201-81.5	10			
201-100.4	3				201-85.4	3			11
201-169.3		4			**201-95.3**		6	2	1
202-23	8				**201-100.4**	2		9	4
203-03	4		6	4	201-153.5		2		
203-25		14			201-167.4		5		
203-31				13	201-169.3			13	12
203-32	5			12	202-07	15	14		
203-51		12			202-11			6	
203-55		8	15		**202-104**			3	6
203-69	6	5		8	202-108			7	
203-71		3	1	7	**203-03**	1		4	2
203-88		13	4	1	203-23	9	11	8	
203-98				2	203-31		10	5	8
203-110	12				203-32	12			
203-111		15		11	203-38		7	11	9
203-113			14		203-55			13	10
203-122		11			203-59	13			
203-130			13		**203-69**	14	4	14	5
203-131			11		**203-72**			1	3
203-135		6	10	9	203-88	8			
203-137	1	9		15	203-110	6			13
203-139	7		7	3	203-111	7			14
203-150	15		5	6	203-139			12	
203-160	10				203-150	5			
203-181			12		203-160		9		
203-188	14	1			203-174		12		
203-231	11	2			203-181		13	10	15
203-235	2				203-235		15		
203-245			2		203-254	11	8		

* The bold and underlined progenies were selected for effects of general combining ability based on diallel analysis.

kg ha- 1), 203-73 (Ŝij: 802.76 kg ha-1), 203-69 (Ŝij: 766.1 kg ha-1), 203-122 (Ŝij: 713.0 kg ha- (Ŝij: 670.5 kg ha-1), 201-59.01 (Ŝij: 651.7 kg ha-1), and 203-205 (Ŝij: 643.7 kg ha-1) were selected for their effects of GCA and SCA with tester 9.H3.33 for GY. With tester AG8088, the progenies selected for the effects of combining ability were 201-145.4 (Ŝij: 810.94 kg ha-1), 203-154 (Ŝij: 771.2 kg ha-1), 201-85.4 (Ŝij: 608.31 kg ha-1), and 203-120 (Ŝij: 390.11 kg ha-1).

The testers AG8088 and DKB330 are single-cross commercial hybrids, consequently, the topcrosses between these testers and progenies have no commercial value. However, the main justification for the use of commercial hybrids as testers is the possibility of selecting lines that have a high combining

Table 5 Description the main agronomic traits from inbred lines, testers and commercial checks used in the trials carried out 2014/2015 and 2015/2016 growing seasons at Maringá-PR

Inbred Line	Pedigree	Grain	Color	Inbred Line	Pedigree	Grain	Color
*201-171	P30F53	Flint	Yellow	203-55	SG6015	Flint	Yellow
201-193	P30F53	Flint	Yellow	203-59	SG6015	Flint	Yellow
201-232	P30F53	Semi-Flint	Yellow	203-69	SG6015	Flint	Yellow
201-342	P30F53	Flint	Yellow	203-71	SG6015	Flint	Yellow
201-591	P30F53	Dent	Yellow	203-72	SG6015	Flint	Yellow
201-801	P30F53	Flint	Orange	203-73	SG6015	Flint	Yellow
201-802	P30F53	Flint	Orange	203-75	SG6015	Semi-Flint	Yellow
201-812	P30F53	Flint	Orange	203-88	SG6015	Flint	Orange
201-815	P30F53	Flint	Orange	203-98	SG6015	Flint	Yellow
201-854	P30F53	Flint	Orange	203-108	SG6015	Flint	Orange
201-953	P30F53	Semi-Flint	Yellow	203-110	SG6015	Flint	Yellow
201-1004	P30F53	Dent	Yellow	203-111	SG6015	Flint	Orange
201-1454	P30F53	Flint	Orange	203-113	SG6015	Flint	Yellow
201-1535	P30F53	Semi-Flint	Yellow	203-120	SG6015	Flint	Yellow
201-1672	P30F53	Semi-Flint	Yellow	203-121	SG6015	Flint	Orange
201-1674	P30F53	Flint	Yellow	203-122	SG6015	Semi-Flint	Yellow
201-1693	P30F53	Flint	Yellow	203-130	SG6015	Flint	Yellow
202-07	AS1560	Flint	Yellow	203-131	SG6015	Dent	Yellow
202-11	AS1560	Flint	Yellow	203-135	SG6015	Flint	Orange
202-23	AS1560	Flint	Orange	203-137	SG6015	Flint	Yellow
202-102	AS1560	Dent	Yellow	203-139	SG6015	Flint	Orange
202-104	AS1560	Flint	Red	203-144	SG6015	Dent	Yellow
202-108	AS1560	Flint	Red	203-150	SG6015	Flint	Yellow
202-154	AS1560	Flint	Red	203-154	SG6015	Semi-Flint	Yellow
202-155	AS1560	Flint	Red	203-160	SG6015	Semi-Flint	Orange
202-159	AS1560	Flint	Red	203-174	SG6015	Flint	Yellow
202-172	AS1560	Dent	Red	203-181	SG6015	Dent	Yellow
203-03	SG6015	Flint	Yellow	203-188	SG6015	Flint	Yellow
203-23	SG6015	Flint	Orange	203-205	SG6015	Flint	Yellow
203-25	SG6015	Flint	Orange	203-206	SG6015	Flint	Yellow
203-30	SG6015	Flint	Orange	203-207	SG6015	Flint	Yellow
203-31	SG6015	Flint	Yellow	203-231	SG6015	Flint	Orange
203-32	SG6015	Flint	Yellow	203-235	SG6015	Flint	Orange
203-38	SG6015	Dent	Orange	203-245	SG6015	Flint	Yellow
203-51	SG6015	Dent	Yellow	203-250	SG6015	Flint	Yellow
203-54	SG6015	Flint	Orange	203-254	SG6015	Flint	Yellow
Tester	**Genetic Base**	**Grain type**	**Grain Color**	**Life Cycle**	**Male Flowering**	**Female Flowering**	**Aptitude**
*AG8088	HS	Flint	Yellow	Early	60	61	WPS/G
DKB330	HS	Dent	Orange	Super Early	56	59	WPS/HGS/G
9.H3.33	Line	Dent	Yellow	Early	63	65	WPS/G
Commercial	**Genetic Base**	**Grain type**	**Grain Color**	**Life Cycle**	**Male Flowering**	**Female Flowering**	**Aptitude**
**P30F53	HS	Semi-Flint	Orange	Early	60	63	WPS/G
30B39	HS	Semi-Flint	Yellow	Early	62	65	WPS/G
LG6030	HS	Semi-Flint	Yellow	Early	59	63	WPS/HGS/G
LG6032	HS	Semi-Flint	Yellow	Early	61	63	WPS/G
PL6880	HT	Dent	Yellow	Normal	63	65	WPS/G
Formula	HS	Flint	Orange	Super Early	57	60	G
AG8041	HS	Semi-Flint	Orange	Early	60	62	G
2B688	HT	Semi-Flint	Orange	Early	61	63	WPS/G
DKB390	HS	Semi-Flint	Orange	Early	61	63	G

WPS: Whole Plan Silage. G: Grain. HGS: Humid Silage Grain. * Dates were obtained by specialty corn from UEM breeding program.
** Dates supplied by holder seed companies

ability with these testers, and can in the future be used as testers of new progenies derived from these hybrids.

The inbred line 201-59.01 is a potential tester for progenies derived from hybrid AG8088, for the selection of lines with good combining ability for GY, FMY and DMY, since it was selected for its significant additive effects for these characters and also had a significant specific combining ability with tester AG8088. The progeny 201-100.4 was selected for its significant additive effects for both GY and LGN. However, when this inbred line was combined with the testers AG8088 and DKB330, it generated topcross hybrids with significant SCA estimates and therefore, progeny 201-100.4 is considered a potential tester of the inbred lines derived from inbreeding of AG8088 and DKB330.

Progeny 203-135 is a potential tester of inbred lines derived from AG8088 for the selection of inbred lines with good combining ability for DMY and ADF. On the other hand, progeny 203-235 can be used as a tester of progenies derived from DKB330, aiming at the selection of inbred lines with good combining ability for FMY and DMY. Progeny 203-150 can be used as tester of progenies derived from hybrids AG8088 and DKB330 for the traits DMY and FDN. Other outstanding lines in this study that may be used as potential testers of new inbred lines derived from hybrids AG8088 and DKB330 are 203-254 and 203-235, since they combined well with these testers for the traits FMY and DMY, and may be use in the future in the selection of lines that provide increases in total silage yield and their topcross hybrids.

To improve the understanding of the choice of the best tester for the traits GY, ADF and LGN, 15 progenies with highest GCA and 15 topcross hybrids with highest performance were ranked with each tester (Tables 3 and 4).

In the 2014/2015, inbred lines 201-59.1 and 203-150 were selected for their GCA effects and concomitantly formed good topcross hybrids with high GY with all testers (Table 3). The progenies 201-100.4, 203-38, 203-59, 203-72, 203-206, and 203-231, simultaneously, a positive GCA for GY and generated good topcrosses hybrids with two testers (Table 3). The testers AG8088 and 9.H3.33 were similar in their ability to identify inbred lines with highest genetic merit for GY in 2014/2105. Fourteen of the 15 topcrosses with highest GY derived from the testers AG8088 and 9.H3.33 were crosses with progenies with positive GCA, of which seven were selected for the high frequency of favorable alleles by diallel analysis (Table 3).

The ranking of 216 hybrid topcrosses for GY highlighted topcrosses 201-100.4 x AG8088 and 203-139 x AG8088 for highest GY in the 2014/2015 growing season (Table 3). At the same time, progenies 201-100.4 and 203-139 presented the two highest estimates of general combining ability for GY, indicating that tester AG8088 was efficient in identifying the progenies with the highest genetic merit for GY (Table 3).

Based on the results of the 2015/2016, AG8088 and 9.H3.33 were similar again in their ability to identify the genetic merit of partially inbred lines for GY. Thirteen of the 15 topcrosses with highest GY derived from tester 9.H3.33 resulted from progenies selected for their GCA effects. The tester 9.H3.33, when combined with progeny 203-73 (with highest GCA for GY), originated the topcross hybrid with highest GY of the 2015/2016. Tester AG8088 also identified progenies with highest genetic merit, e.g, topcross hybrid 201-145.4 x AG8088, which produced 6,740 kg grain ha-1 and was also derived from progeny 201-145.4, with the fifth-highest estimate of GCA for GY.

Information regarding the genetic background of the testers AG8088 and 9.H3.33 may be used in an attempt to explain the similarity of these testers to distinguish progenies. Line 9.H3.33 was derived from the single-cross hybrid AG8080 by successive generations of selfing and selection. Hybrids AG8088 and AG8080 belong to the same breeding company, and consequently, most likely have parents in common, indicating that tester AG8088 and line 9.H3.33 have a certain degree of relatedness and consequently, were able to identify the same progenies with highest genetic merit.

Of the 15 topcrosses of tester AG8088 with lower ADF, 14 were derived from progenies with negative GCA, of which seven were selected by the additive effects to reduce the ADF content of their topcross hybrids (Table 4). All topcross hybrids with lower ADF content derived from tester 9.H33.3 resulted from combinations with progenies with negative GCA estimates (Table 4). This fact indicates that tester 9.H3.33 was efficient in identifying progenies with higher genetic merit in reducing the silage content of ADF, with a high concordance between the hybrid performance and GCA of the partially inbred progenies (Table 4).

Line 9.H3.33 also stood out as the most efficient tester in identifying the genetic merit of the progenies in generating good topcross hybrids with regard to the LGN content (Table 4). Of the 15 low-lignin hybrids obtained with this tester, 11 were derived from inbred lines ranked for lowest LGN content. The two topcross hybrids with lowest LGN content were 203-72 x 9.H3.33 (1.45%) and 201-95.3 x 9.H3.33 (1.78%), both of which were derived from tester 9.H3.33 and at the same time, with the inbred line with 3rd and 2nd lowest GCA to reduce the LGN silage content of the topcross hybrids, respectively.

Conclusions

The testers AG8088 and 9.H3.33 were the most appropriate for GY. Line9.H3.33 was the best tester for the traits grain yield and quality of silage maize.

The inbred lines 201-23.2, 201-59.1, 201-80.2, 201-81.5, 203-195.3, 201-100.4, 201-145.4, 201-169.3, 202-155, 202-159, 203-23, 203 -31, 203-32, 203-38, 203-75, 203-98, 203-111, 203-135, 203-139, 203-150, 203-188, 203-235, and 203-237 had particularly good combining abilities and capacity to generate good topcross hybrids. It is recommended to maintain and advance these progenies in the UEM maize breeding program.

Progenies 201-59.01, 201-100.4, 203-135, 203-150, 203-235, and 203-254 were selected as tester lines derived from progenies obtained by inbreeding of the testers AG8088 and DKB330.

The topcross hybrids 201-95.3 x 9.H3.33, 203-71 x 9.H3.33, 203-72 x 9.H3.33, 203-88 x 9.H3.33, 203-139x9. H3.33, and 203-150 x 9.H3.33 were selected for good performance for the agronomic and bromatological traits of silage maize. It is suggested to evaluate these hybrids in more environments, with a view to establish recommendable commercial cultivar for grain and silage production in the future.

Acknowledgments

The authors are indebted to Capes and CNPq for funding this research project, and wish to thank the Maringá State University (UEM) and the Programa de Pós-Graduação em Genética e Melhoramento at UEM for the financial support.

References

Argillier, O., V. Méchin, Y. Barriére, 2000. Inbred line evaluation and breeding for digestibility-related traits in forage maize. Crop Sci. 40:1596-1600.

Barrière, Y., C. Riboulet, V. Mèchin, S. Matese, M. Pichon, A. Cardinal, C. Lapierre, T. Lubberstedt, J.P. Martinant, 2007. Genetics and genomics of lignifications in grass cell walls based on maize as model species. G3. 1:133-156.

Bolson, E., C.A. Scapim, L.R. Clovis, A.T. Amaral Junior, I.L.J. Freitas, 2016. Capacidade combinatória de linhagens de milho avaliada por meio de testadores adaptados a safrinha. Revista Ceres. 63:492-501.

Castellanos, L.S., A.R. Hallauer, H.S. Cordova, 1998. Relative performance of testers to identify elite lines of corn (Zea mays l.). Maydica. 43: 217-226.

Coors, J.G., J.G. Lauer, 1994. Silage Corn, pp 339-389. In: Specialty Corns. Hallauer, A.R. (Ed.). CRC Press, Boca Raton, USA.

Cruz, D.C, 2013. Genes – a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. Agron. 35:271-276.

Davis, R.L, 1927. Report of the plant breeder. Rep. Puerto Rico Agric. Exp. Stn. 14-15.

Ertiro, B.T., H. Zeleke, D. Friesen, M. Lummel, S. Twumasi-Afriyie, 2013. Relationship between the performance of parental inbred lines and hybrids for food-feed traits in maize (zea mays l.) in Ethiopia. Field Crops Res. 153:86–93.

Guimarães, L.J.M., G.V. Miranda, R.O. De Lima, C. Maia, L.R. Oliveira, L.V. Souza, 2012. Performance of testers with different genetic structure for evaluation of maize inbred lines. Ciência Rural. 42:770-776.

Gralak, E., M.V. Faria, A.S.T. Figueiredo, D. Rizzardi, M.C. Mendes, M. Neumann, S. Galbeiro, C.A. Scapim, 2017. Combining ability and genetic divergence in corn hybrids, and breeding of the agronomic and bromatological quality traits of silage. Genet Mol Res. 10:1-12.

Griffing, B, 1956. Concept of general and specific combining ability in relation to diallel crossing system. Aust J Biol Sci. 9:463-493.

Guerrero, C.G., M.A.G. Robles, J.G.L. Ortega, I.O. Castillo, C.V. Vázquez, M.C. Carrillo, A.M. Resendez, A.G. Torres, 2014. Combining ability and heterosis in corn breeding lines to forage and grain. Am J Plant Sci. 5:845-856.

Hallauer, A.R., J.M. Carena., J.B. Miranda Filho, 2010. Quantitative genetics in maize breeding. New York, Springer. 663p.

Marcondes, M.M., M.V. Faria, M.C. Mendes, A. Gabriel, V. Neiverth, J.C. Zocche, 2016 Breeding potential of s4 maize lines in topcrosses for agronomic and forage traits. Acta Sci. Agron. 3:307-315.

Mendes, M.H.S., C.H. Pereira, J.C. Souza, 2015. Diallel analysis of maize hybrids for agronomic and bromatological forage traits. Acta Sci. Agron. 37:141-146.

Miotto, A.A., R.J.B. Pinto, C.A. Scapim, J.L. Matias Junior, M.M.D. Coan, H.A. Silva, 2016. Comparison of three testers parents in evaluating popcorn families derived from IAC-125. Rev. Cienc. Agron. 47:564-571.

Oliboni, R., M.V. Faria, M. Neumann, J.T.V. Resende, G.M. Battistelli, R.G. Tegoni, D.F. Oliboni, 2013. Análise dialélica na avaliação do potencial de híbridos de milho para a geração de populações-base para obtenção de linhagens. Semina Ciências Agrárias. 34:7-18.

Ortiz, R.L., J.D.M. Gaán, J.J.L. Reynoso, J.A.M. Contreras, D.R. López, 2010. Criteria to choose the best tester of the general combining ability for grain yield of maize inbreed lines. Agrociencia. 44:17-30.

Pimentel-Gomes, F, 2000. Curso de Estatística Experimental. 14 ed. Livraria Nobel, Piracicaba. 477p.

Senhorinho, H.J.C., R.J.B. Pinto, C.A. Scapim, K.F. Milani, T. Ninhei, 2015. Combining abilities and inbreeding depression in commercial maize hybrids. Semina Ciências Agrárias. 36:4133-4150.

Silva, D.J., A.C. Queiroz, 2012. Análise de alimentos: métodos químicos e biológicos. 3.ed. UFV, Viçosa. 235p.

Souza Junior, C.L., 2001. Melhoramento de espécies alógamas, pp 159-200. In: Recursos genéticos e melhoramento de plantas. Nass, L.L.; Valois, A.C.C.; Melo, I.S.; Valadares-Inglis, M.C. (Eds). Fundação MT: Rondonópolis, Brazil.

Souza Neto, I.L., R.J.B. Pinto, C.A. Scapim, C.C. Jobim, A.S.T. Figueiredo, L.S. Bignotto, 2015. Diallel analysis and inbreeding depression of hybrid forage corn for agronomic traits and chemical quality. Bragantia. 74:42-49.

Van Soest, P.J., R.H. Wine, 1968. The determination of lignin and cellulose in acid-detergent fiber with permanganate. J Assoc Off Anal Chem. 51:780-785.

Van Soest, P.J., J.B. Robertson, B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci. 74:3583- 3597.

Wolf, D. P., J.G. Coors, K.A. Albrecht, D.J. Undersander, P.R. Carter, 1993. Agronomic evaluations of maize genotypes selected for extreme fiber concentrations. Crop Sci. 33:1359-1365.