Classical and AMMI methods to select progenies, testers and topcrosses hybrids in corn[1]

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ABSTRACT

The objectives were to estimate the potential of S_2 corn progenies for forage-related traits, and use of AMMI analysis to evaluate topcrosses compared to the classic analyzes. Progenies were crosses with four different testers: LG 6030, 2B688, 9.H3.33 and 53F.P37. Topcross hybrids were evaluated in four 9 x 9 simple square lattice design, during the 2017/18 season at Maringa, Parana State. Grain yield, forage fresh matter yield, and forage dry matter yield were measured. Classical approach was composed by variance components, general and specific combining ability, whereas AMMI analysis was performed for progenies x testers interaction, considering additive main effects and multiplicative effects. Considering the classical approach, testers LG 6030 and 2B688 better expressed the genetic variability between progenies for grain yield. AMMI analysis allowed the partitioning of the sum of squares in additive main effects and multiplicative effects, being a complementary result for the classical approach. Progeny 14 was selected due to higher general combining ability for grain yield, forage fresh matter and forage dry matter yield. Topcrosses 14x9.H3.33 and 14x2B688 were selected due to their higher specific combining ability, additive and multiplicative effects. The AMMI analysis was effective and helped in the interpretation of the results.

Keywords: *Zea mays* L.; testcrosses; diallel analysis; tester x progeny interaction.

INTRODUCTION

Corn (*Zea mays* L*.)* is one of the most important crops in the world, being used for human and animal feed, and for industrial purposes. Corn is also considered the reference crop for forage production, mainly because of its favorable agronomic and bromatological traits (Dunière *et al.*, 2013).

Despite the great importance of maize for forage production, most breeding programs do no focus on the selection of genotypes specifically for this purpose; more studies are necessary, especially with regard to the selection of a great number of progenies (Nanavati, 2015).

Topcrosses are one of the most-used mating designs for evaluating a large number of progenies, which have been successfully applied for agronomic and forage traits simultaneously (Guimarães *et al.*, 2012; Rosa *et al.*, 2020). This mating design enables the selection of superior progenies based on genetic parameters, such as general combining ability and specific combining ability (Davis, 1927; Sprague & Tatum, 1942). Although, one of the main limitations of topcrosses designs relies on tester selection, in order to best discriminate the genetic potential of the progenies (Hallauer *at al*., 2010).

One of the most important results of topcrosses designs is the progeny x tester interaction (P x T), obtained from the partitioning of the sum of squares from the analysis of variance. Alternative approaches, such as partitioning the P x T interactions in additive main effects and multiplicative effects, and also partitioning the interaction in a relative contribution of each parent from the significant combination, could be applied for identification of the favorable progenies and testers. (Shah *et al.*, 2015; Heinz *et al.*, 2019).

The additive main effects and multiplicative interaction (AMMI) analysis is an analysis specially designed for two-way factorial data structures, and typically used for evaluating genotype by environment (G x E) interactions (Gauch & Zobel, 1988; Zobel *et al.,* 1988; Silva & Benin, 2012). AMMI analysis could be used for selecting the most promising progenies (P) and testers (T) in topcross design, as well as for partitioning the additive main effects and the multiplicative effects from the P x T interaction (Charcosset *et al.*, 1993; Rosa *et al.,* 2022). To this date, such an approach was described only once in the literature (Rosa *et al.,* 2022).

The objectives of this study were to estimate the potential of S_2 corn progenies for forage-related traits, and use of AMMI analysis to evaluate topcrosses compared to the classic analyzes.

MATERIAL AND METHODS

Seventy-nine S_2 progenies were selfed and selected from a population derived from the commercial hybrid "Impacto". Selection of this base population was obtained on the results from a diallel among commercial hybrids, specially designed for forage traits (Souza Neto *et al.*, 2015).

Each progeny was crossed with four different testers: triple-cross hybrid 2B688; single-cross hybrid LG 6030; inbred line 9.H3.33, obtained from a population-derived of the commercial hybrid AG8080; and inbred line 53F. P37, obtained from a population-derived of the commercial hybrid P30F53.

Progeny and tester crosses were performed in the Fazenda Experimental de Iguatemi (lat 23º 25' S; long 51º 57' W and alt 550 m asl) in Maringa, Parana State, in a soil classified as oxisoil (Nitsche *et al.*, 2019). According to the Köppen classification, the climate of the region is Cfa with an annual average temperature of 19 °C and an annual rainfall of 1500 mm.

Topcross hybrids were evaluated in four field trials, in the second growing season of 2017, in the Fazenda Experimental of Iguatemi (lat 23º 25' S; long 51º 57' W and alt 550 m). Each field trial was performed in a 9 x 9 simple square lattice design, where the treatments corresponds to the the 79 S_2 progenies crossed with one tester plus two commercial checks: single-cross hybrids DKB 330 and P4285, totaling 81 treatments per field trial.

Each plot consisted of two 5-m rows spaced 0.9m apart, with a total of 9 m² of plot area. At forage harvest period, when plants have reached 30 to 35% of total dry matter content (2/3 of the kernel milk line, or R5 scale) corresponding to the phenological characterized as pasty to farinaceous grain, all plants of both rows at a distance of 2.5 m here harvested, totaling half of the plot area (4.5 m²) and destined for forage traits evaluation.

Harvested plants were weighted and used to calculate the fresh matter yield (FMY kg ha⁻¹). Ten plants were randomly separated and chopped in a shredder to produce a mean particle size of 1.5 cm. A 0.5 kg sample was dried in a forced air circulation oven at 55 °C to constant weight, for approximately 72 hours, obtaining the dry matter content (AOAC, 1990). Considering fresh matter yield and final dry matter content, the dry matter yield at ensiling (DMY, kg ha⁻¹) was estimated. At the R8 development stage, the remaining 4.5m² plot area was harvested, and then used for grain yield (GY, kg ha⁻¹) evaluation corrected to a 13% moisture content.

Analyses of variance were performed for the evaluated traits using the analysis with intrablock variance with recovery of interblock information to obtain the adjusted means with the effective lattice variance and error. The following model was fitted:

$$Y_{ijk} = \mu + t_i + b\left(r\right)_{jk} + r_j + \epsilon_{ijk}$$

where: Y_{ijk} is the observed value in the *i*-th treatment at the *k*-th block, and at the *j*-th repetition; μ is the overall mean; t_i is the random effect of the *i*-th $[I = 1, 2, …, I\,(\mathrm{v} = 81)]$ treatment; $b(r)_{jk}$ is the random effect of the *j*-th $[j = 1, 2,…, J\,(\mathrm{r} = 2)]$ repetition nested with the *k*-th $[k = 1, 2, …, K\,(\mathrm{k} = 18)]$ block; r_j is the random effect of the *j*-th $[j = 1, 2, …., J\,(\mathrm{r} = 2)]$ repetition; ϵ_{ijk} is the residual effect, normally and independent distributed with mean equals zero and variance σ^2.

Considering each evaluated trait, estimations of the genetic and phenotypic parameters were performed according with the analysis of variance, considering the random

nature of the treatments. Sums of squares of the adjusted treatments were portioned into general combining ability (GCA) and specific combining ability (SCA) according to Griffing's (1956) diallel analysis method 1, which considers only F_1 hybrid combinations and adapted for partial diallel schemes, as described by Hallauer *et al.* (2010). The sum of the residual degrees of freedom from all four field trials, resulted from the analysis of variance, were considered for the residual degrees of freedom in the subsequent diallel analysis.

AMMI analysis (Zobel *et al.*, 1988) was performed when the P x T interaction was significant by the F test (*p* < 0.05), being each tester analogous as an environment in the genotype by environment analysis. Progeny and tester effects were considered as main additive effects, and the interaction among progeny and testers was considered as multiplicative effects. Using principal component analysis (PCA), the following model was fitted:

$$Y_{ij}=\mu+p_i+t_i+\sum\nolimits^{n}k\text{-}1\sqrt{\lambda_k y_{ik}\pm_{ik}}+r_{ij}+\epsilon_{ij}$$

where: Y_{ij} is the least square means of progeny *i*-th crossed with tester *j*-th; μ is the overall mean; p_i is the effect of the progeny *i*-th; t_i is the effect of the *t*-th tester; λ_k is the *k*-th eigenvalue from the PCA; y_{ik} is the eigenvector of the *i-th* progeny in the *k* axis of the PCA; α_{ik} is the eigenvector of the *j*-th tester in the *k* axis of the PCA; r_{ij} is the residual for the AMMI models when all axes are not used, and can be interpreted the proportion of the interaction that is not explained by the principal components used in the analysis; *n* is the number of retained axes in order to explain the interaction; and finally, ϵ_{ij} is the residual effect.

The number of used PCA axis was choosen based on the criteria established by Gauch & Zobel (1988), in which the sum of squares of the interaction (SQPxT) is restricted to the minor's degrees of freedom *n*-th axis. Analysis of variance, estimation of the phenotypic and genetic parameters, partial diallel analysis and AMMI-Biplot analysis were performed using R (R Development Core Team, 2022) and Genes (Cruz, 2013) software's.

RESULTS

Classical analysis

According with results of the analysis of variance (Table 2), significant differences (p < 0.05) were observed for treatments in all evaluated traits, a strong indication of genetic variation among evaluated treatments. Such vari-

ability represented by the parameters present in Table 1, can be assigned to the different topcross hybrids, enabling the selection of superior inbred lines and also for selecting the best tester and hybrid combination. Broad-sense heritability estimations, phenotypic and genetic parameters results are show in Table 1, according with the tester for each field trial. Associating these parameters with GCA and SCA estimations allows a better classification and selection of the progenies, as well as allelic contribution of the testers.

Broad-sense heritabilities for GY ranged from 12.3% to 58.9%, considering field trials with the inbred line tester 9.H3.33 and the commercial hybrid tester LG 6030, respectively. Considering FMY and DMY, higher values of broad-sense heritabilities were observed for tester LG 6030, with values ranging from 55.8% to 48.5%, respectively (Table 1).

Considering GY, the testers that showed the most efficient in releasing genetic variability of the progenies was the commercial hybrid LG 6030 (Table 1). Although, according with the least square means among the 316 topcrosses, the two best performing topcrosses for GY did not involve LG 6030 as a parent in the cross: 14 x 2B688 (6153.7 kg ha^{-1}) and 14 x 9.H3.33 (5859.4 kg ha^{-1}). Regarding DMY and FMY, commercial hybrid LG 6030 was the tester that provided the highest component of genetic variance among the topcross hybrids these traits (Table 1), indicating high efficiency of this tester for expressing genetic variability among progenies. For FMY, mean amplitude among the topcrosses was 19768.9 kg ha^{-1}, and for DMY mean amplitude was 8615.1 kg ha^{-1}.

For the diallel analysis results, mean squares of the general combining ability among testers (GCA I), general combining ability among S_2 progenies (GCA II) and the specific combining ability among progenies x testers (SCA) were significant (*p* < 0.05) for all evaluated traits (Table 2), indicating the presence of additive and non-additive effects on the genotypic variance of the traits, solely considering the genetic *pool* evaluated in these crosses.

For GY, the S_2 progenies (GCA II) with highest $\hat{g}_i$ values were: 63 ($\hat{g}i$ 761.0); 36 ($\hat{g}i$ 706.2); 79 ($\hat{g}i$ 626.2); 14 ($\hat{g}i$ 589.6); 29 ($\hat{g}i$ 550.1); 2 ($\hat{g}i$ 458.1); and 53 ($\hat{g}i$ 457.4). Regarding GCA II for DMY, eight progenies were selected based on the highest $\hat{g}i$ values: 14 ($\hat{g}i$ 1798.4); 36 ($\hat{g}i$ 1783.3); 38 (1303.3); 78 (1122.7); 5 ($\hat{g}i$ 1062.2); 27 ($\hat{g}i$ 1045.8); 51 ($\hat{g}i$ 1034.7); and 63 ($\hat{g}i$ 1013.0). For FMY, seven S_2 progenies were selected also based on their highest $\hat{g}i$ values: 63 (5523.7); 51 ($\hat{g}i$ 4856.5); 16 ($\hat{g}i$ 4690.3); 36 ($\hat{g}i$ 4630.4); 14 ($\hat{g}i$ 4547.1); 9 ($\hat{g}i$ 3495.5); and 5 (2825.3).

Testers 2B688 and LG 6030 showed higher and posi-

Table 1: Genetic variance ($\hat{\sigma}_g^2$), phenotypic variance ($\hat{\sigma}_f^2$), and broad-sense heritability (h^2) estimations for grain yield (GY), fresh matter yield (FMY), and dry matter yield (DMY), according with field trials divided by their testers: 2B688, LG 6030, 9.H3.33, and 53F.P37

Tester 2B688			
Parameters	**GY**	**FMY**	**DMY**
		$(kg\ ha^{-1})^2$	
$\hat{\sigma}_g^2$	217622.58	4418949.97	459285.13
$\hat{\sigma}_f^2$	403049.25	10116873.83	1230916.58
h2	0.5399	0.4368	0.3731
		$(kg\ ha^{-1})^2$	
General mean	5670.73	33380.97	10242.22
CV (%)[1]	10.74	10.11	12.13
Tester LG 6030			
Parameters	GY	FMY	DMY
		$(kg\ ha^{-1})^2$	
$\hat{\sigma}_g^2$	220440.62	8307038.03	1002093.22
$\hat{\sigma}_f^2$	373755.05	14878792.55	2064114.53
h^2	0.5898	0.5583	0.4855
		$(kg\ ha^{-1})^2$	
General mean	5304.79	36198.10	11131.47
CV (%)	10.44	10.02	13.09
Tester 9 H3.33			
Parameters	GY	FMY	DMY
		$(kg\ ha^{-1})^2$	
$\hat{\sigma}_g^2$	34772.57	5455867.09	273803.76
$\hat{\sigma}_f^2$	281932.67	14325071.08	1516549.30
h^2	0.1233	0.3808	0.1805
		$(kg\ ha^{-1})^2$	
General mean	4774.82	29060.95	10256.88
CV (%)	14.72	14.49	15.37
Tester 53F.P37			
Parameters	GY	FMY	DMY
		$(kg\ ha^{-1})^2$	
$\hat{\sigma}_g^2$	198071.17	5707084.76	488836.06
$\hat{\sigma}_f^2$	364010.98	11721897.21	1405873.10
h^2	0.5441	0.4868	0.3477
		$(kg\ ha^{-1})^2$	
General mean	4563.22	28502.81	8868.71
CV (%)	12.62	12.17	15.27

Table 2: Analysis of variance of a partial diallel among topcross hybrids among S_2 progenies and testers 2B688, LG 6030, 9 H3.33, and 53F.P37, for grain yield (GY), fresh matter yield (FMY), and dry matter yield (DMY)

Analysis of variance for the partial diallel

Source of variation	DF	Mean squares		
		GY	FMY	DMY
		$(kg\ ha^{-1})^2$		
Treatment	315	1035796 *	45579766 *	4366037 *
GCA (Group I)	3	40909288 *	2220631137 *	147843330 *
GCA (Group II)	78	829629 *	40229827 *	4100899 *
SCA	234	593320 *	19477805 *	2614965 *
Residual effects	256	375920	13576847	1996717

tive ĝi values, when compared with the other two testers, regarding GY. LG 6030 tester also stands out for its higher and positive ĝi values for FMY and DMY. These results show that tester LG 6030 could be used for increasing GY, FMY and DMY averages, especially due to higher favorable allele frequency for all traits, which are distinct and controlled by several genes (Table 3).

In this sense, considering GY, the topcrosses 14 x 9.H3.33, 14 x 2B688 and 63 x 9.H3.33 showed higher SCA estimations with at least one selected parent based on GCA value. For FMY, topcrosses 5 x LG 6030, 14 x 9.H3.33, 78 x LG 6030, 14 x 2B688, and 63 x 9.H3.33 showed higher SCA estimations with at least one selected parent based on GCA value. Finally, for DMY, topcrosses 63 x 9.H3.33, 14 x 9.H3.33, 36 x 53F.937, 14 x 2B688 and 51 x LG6030 showed higher non-additive effects estimations. Considering all SCA results, topcrosses 14 x 9.H3.33 and

14 x 2B688 were selected simultaneously for GY, DMY, and FMY, and should be used in future trials in the breeding program.

AMMI analysis

According with the AMMI analysis for the P x T interaction, the two first principal components (IPCA 1 and IPCA 2) were significant ($p < 0.05$) for the F test proposed by Gollob (1968), for all evaluated traits (Table 4). IPCA 1 was portioned with 78 degrees of freedom, whereas IPCA 2 was portioned with 76 degrees of freedom. Considering GY, IPCA 1 explained 40.9% of the sum of squares of the interaction (SQPxT), and IPCA 2 explained 32.9% of the SQPxT variation. Regarding FMY, IPCA 1 explained 41.3% of the variation, and IPCA 2 explained 31.9% of the variation. Finally, for DMY, IPCA 1 and IPCA 2 explained 41.8% and 34.3% of the variation from the interaction, respectively (Table 4).

Table 3: Estimates of general combining ability of testers for grain yield (GY), fresh matter yield (FMY), and dry matter yield (DMY)

Tester	GY	FMY	DMY
	$kg\ ha^{-1}$		
2B688	586.02	1582.50	99.46
LG 6030	243.72	4550.76	1036.14
9.H3.33	-293.02	-2800.53	166.66
53F.P37	-536.72	-3332.73	-1302.26
DP (Ĝi)	42.24	253.86	97.35
DP(Ĝi-Ĝi')	68.98	414.56	158.98

Table 4: Principal component analysis for additive main effects and multiplicative effects for grain yield (GY), fresh matter yield (FMY) and dry matter yield (DMY)

SV	DF	Mean squares								
		GY			FMY			DMY		
Prog. (P)	78	32355555			1568963255	**		159935076	**	
Test. (T)	3	61363932			3330946707	**		221764996	**	
P x T	234	69418533	**	%	2278903259	**	%	305950907	**	%
IPCA1	78	28429218	**	40.95	943444011	**	41.39	128115331	**	41.87
IPCA2	76	22895571	**	73.93	729244491	**	73.38	105344979	**	76.30
IPCA3	74	18093744	**	100.00	606214757		100.00	72490597		100.00
Residual	415	70087628			2300868591			308899832		

Considering GY, tester 2B688 presented the highest additive main effects (Figure 1). Regarding the P x T interaction, testers presented the most part of the contribution with higher scores for the multiplicative effects in the IPCA axis (Gauch & Zobel, 1988; Gauch, 2013). Progenies 36, 79, 14, and 29 showed a favorable performance for GY (Figure 1). These progenies also showed lower scores for the multiplicative effects (lower coordinates in the IPCA 1 axis, Figure 1), reflecting in small contributions for the P x T interaction. These results are similar with the classical approach, since these progenies also presented superior GCA and higher favorable allelic frequencies for grain yield.

According with IPCA 1 and IPCA 2 biplot (Figure 2),

which represents the multiplicative effects among the P x T interaction, progenies 14, 1, 4, and 75 showed lower contribution for the interaction and similar performance of the genetic merits among different testers, and can be considered the most stable progenies for GY.

Tester LG 6030 showed higher mean for FMY, considering the additive main effects (Figure 3). All testers showed higher scores for the P x T interaction (Gauch & Zobel, 1988; Gauch, 2013). Progenies 51, 16, and 14 showed higher mean values for the trait, among all progenies. These progenies also showed lower values for the multiplicative effects (Figure 3), indicating small deviations in the performance among testers and also small participation in the P x T interaction.
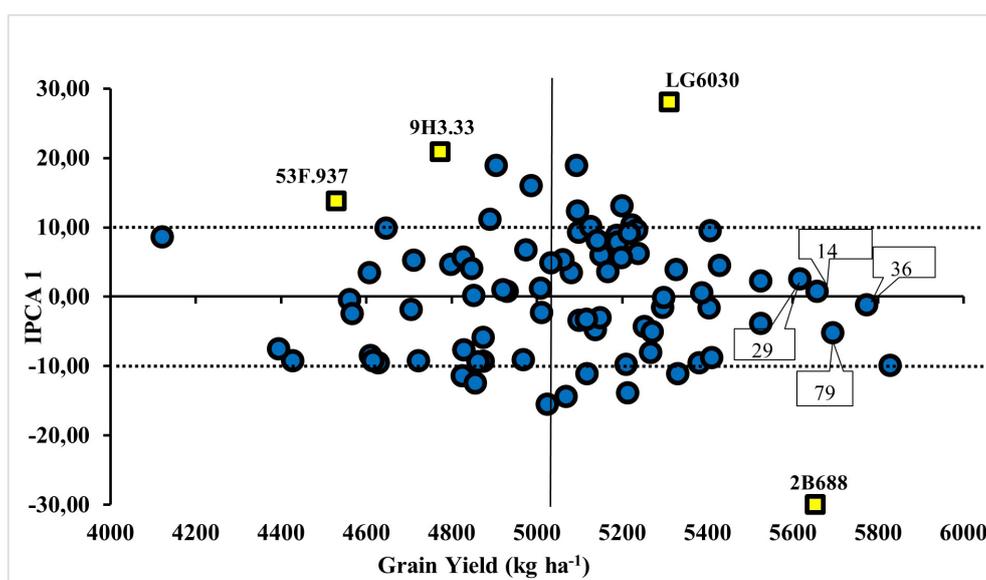

Figure 1: Progeny and tester interaction biplot, considering IPCA 1 scores (y axis) and grain yield least square means (GY, x axis). ■ = testers; ● = progenies.
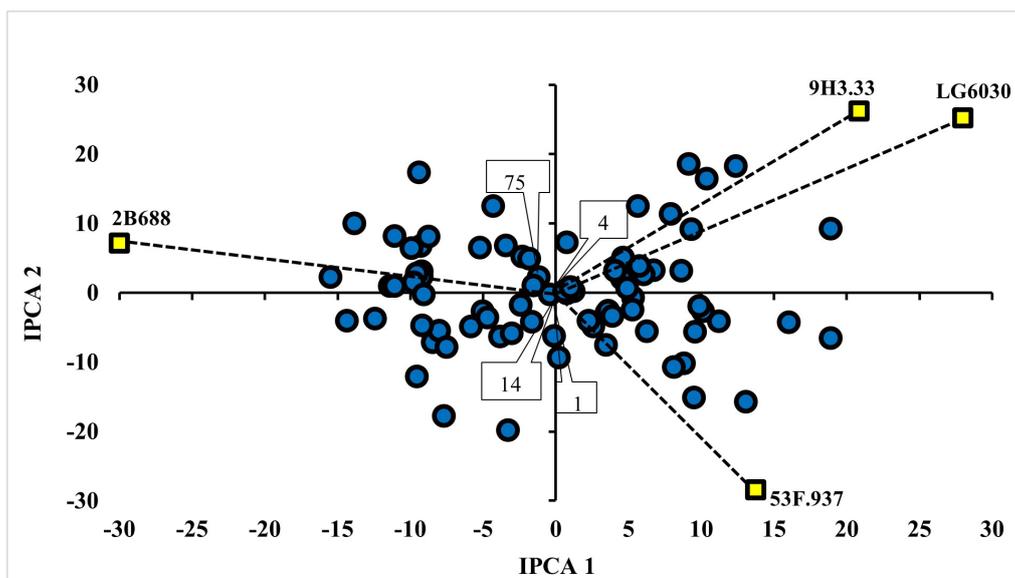
Figure 2: Progeny and tester interaction biplot, considering IPCA 1 scores (x axis) and IPCA 2 (y axis), for grain yield. ■ = testers; ● = progenies.

Progenies 49, 72, 65, 22, and 14 showed lower scores in the IPCA 1 and IPCA 2 biplot (Figure 4) revealing lower effects in the P x T, therefore, these inbred lines are considered more stable for their genetic merit regardless of the crossed tester. Tester LG 6030 was the tester with highest multiplicative effects in the P x T interaction, evidencing a large effect over the genetic merit of progenies.

Considering the results of the AMMI1 plot for DMY, tester LG 6030 showed highest mean value when compared with the other three testers (Figure 5), although, similar to the results for GY and FMY, this tester also showed the highest contributions for the P x T interaction. Progenies 36, 14, 51, and 27 showed highest mean values for the trait with small scores for IPCA 1 axis, indicating small effects in the interaction among testers (Figure 5).

For multiplicative effects of the interaction (Figure 6), progenies 31, 54, 14, 49, and 13 showed highest stability for their genetic merits when combined with the testers, in addition, these progenies also showed lower contributions for the P x T interaction. These results corroborate with the classical approach, since most of these inbred lines showed higher general combining abilities for the trait. Finally, testers 9H3.33 and LG 6030 showed highest contributions for the P x T interaction, resulting in a possible cofounding effect over the genetic merit of the progenies (Heinz *et al.*, 2019).

DISCUSSION

The results of variance analysis and genetic parameters are in concordance with other published papers regarding corn diallel analysis for forage traits and grain yield (Souza Neto *et al.*, 2015; Rosa *et al.*, 2022), highlighting the importance of genetic variability for a higher selection gain.

Associating these genetic parameters with GCA and SCA estimations allows a better classification and selection of the progenies, as well as allelic contribution of the testers. However, testers with higher positive allelic frequencies tends to mask the progenies performance, and should not be used for selecting future inbred lines (Hallauer *et al.*, 2010; Aslam *et al.*, 2017).

The choice of the best tester for topcrosses hybrids and progenies in corn breeding are mainly relied on classical approaches, such as estimating genetic and phenotypic variance, additive genetic effects and non-additive genetic effects, which are related with the combining ability of a tester (Hallauer *et al.*, 2010; De Almeida Filho *et al.*, 2016). In addition, the comparison of genetic variance components among topcrosses hybrids obtained with different testers are also used for selecting the most appropriate tester (Hallauer *et al.*, 2010; Guimarães *et al.*, 2012).

Broad-sense heritabilities for GY ranged from 12.3% to 58.9%, considering field trials with the inbred line tester 9.H3.33 and the commercial hybrid tester LG
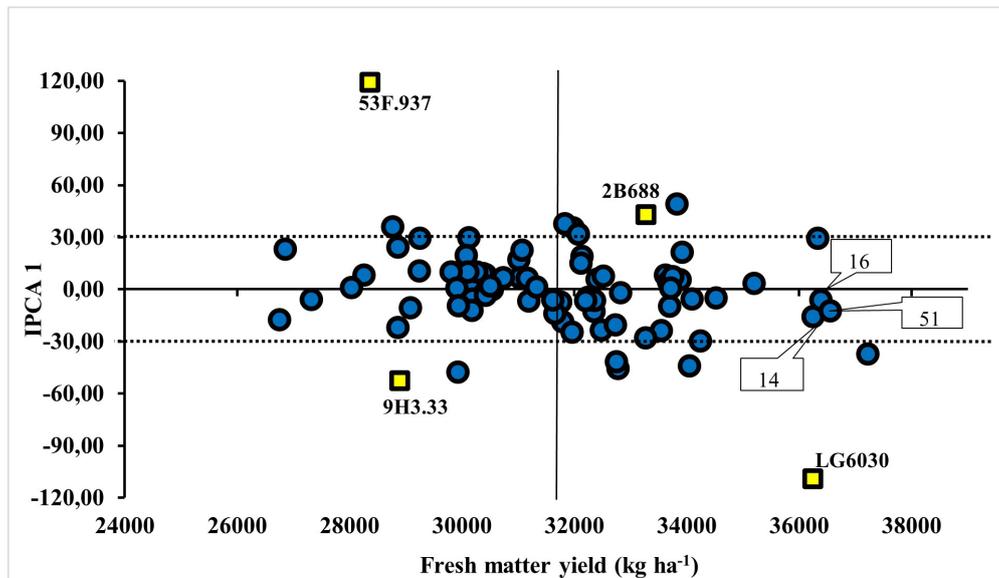
Figure 3: Progeny and tester interaction biplot, considering IPCA 1 scores (y axis) and fresh matter yield (x axis). ■ = testers; ● = progenies.

6030, respectively (Table 1). These heritability values are similar when compared with other topcrosses for grain yield (Figueiredo *et al.*, 2018), highlighting that GY is a quantitative inherited trait, with low to medium values and highly influenced by environmental conditions.

Considering FMY and DMY, higher values of broad-sense heritabilities were observed for tester LG 6030 (Table 1). These observed values suggest that these traits, similar to GY, are strongly influenced by a large number of genes and by environmental effects (Guimarães *et al.*, 2012).

The genetic variance estimation among topcrosses hybrids ($\hat{\sigma}_g^2$) is highly related with the ability of a tester for expressing the genetic merit of the progenies and also with genetic variability among progenies (Miranda Filho & Gorgulho, 2001). According with Hallauer *et al.* (2010), selecting a tester with low-frequencies of favorable alleles, especially for quantitative traits that are influenced by non-additive effects, allows a correct evaluation and selection of the progenies based on their genetic merits. 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 (Hallauer *et al.*, 2010; Guimarães *et al.*, 2012; Aslam *et al.*, 2017).

Significant effects for SCA shows greater contribution of genes with nonadditive effects, differing from the expected performance of topcrosses based on the general combining ability of progenies and testers. This result is similar when compared with other published studies for forage corn breeding, in which some authors reported the contribution of additive and non-additive genetic effects for FMY, DMY and GY (Souza Neto *et al.*, 2015; Rosa *et al.*, 2020).

According with Singh & Chaudhary (1979), choosing a progeny with higher favorable allele frequencies, based on the general combining ability ($\hat{g}_i$), the contrast among two randomly choosen progenies should be as high as possible. For GY, the S_2 progenies (GCA II) with highest $\hat{g}_i$ values were: 63 ($\hat{g}i$ 761.0); 36 ($\hat{g}i$ 706.2); 79 ($\hat{g}i$ 626.2); 14 ($\hat{g}i$ 589.6); 29 ($\hat{g}i$ 550.1); 2 ($\hat{g}i$ 458.1); and 53 ($\hat{g}i$ 457.4).

Progenies 63, 36 and 14 showed higher CGA estimations for all evaluated traits simultaneously, standing out with higher favorable allele frequency with additive effects and should be used for future selfing and crosses for improving genetic gains in the breeding program. Topcrosses involving these progenies showed higher values of fresh matter, dry matter, and grain yield. These results corroborate with Marcondes *et al.* (2016) and Mendes *et al.* (2015), where the authors were able to select inbred lines with higher favorable allele frequencies for most of the traits related with grain yield, forage yield and quality.

It is important to highlight that testers with higher frequencies of favorable alleles could mask the genetic merit of the progenies, misleading the interpretation of the results and selection of the best progenies (Duarte & Vencovsky, 1999). An efficient tester can be defined as one that can generate different hybrids with itself, minimizing the influence over the average performance of these hybrids (Hallauer *et al.*, 2010).
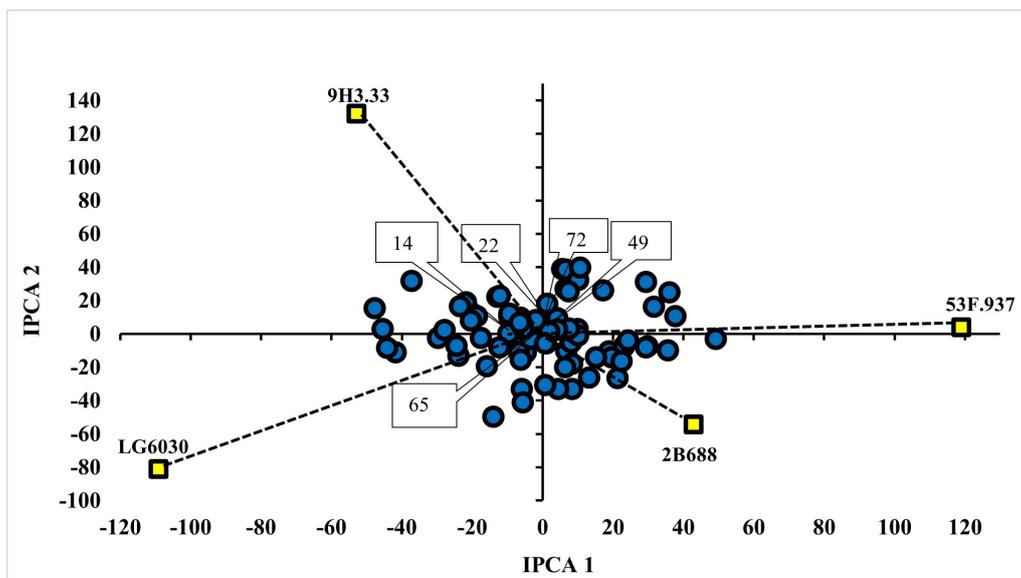
Figure 4: Progeny and tester interaction biplot, considering IPCA 1 scores (x axis) vs IPCA 2 (y axis), for fresh matter yield. ■ = testers; ● = progenies.

According with Mendes *et al.* (2015), in diallel crosses the best hybrids should be those for which at least one of the parents was selected based on its higher $_i$ estimation, thereby, presenting a higher frequency of favorable alleles than the average frequency of the parents involved in the crosses.

In topcrosses schemes, it is known that testers with higher favorable allele frequencies tends to affect the genetic merit of progenies, affecting their ranking and selection. Since classical approaches relies in additive effects for choosing testers, Charcosset *et al.* (1993) proposed partitioning the interaction among tester and progenies into additive main effects and multiplicative effects, presenting the results in a biplot format and allowing an easier interpretation of the genetic effects.

Progeny x tester interaction was significant for all evaluated traits (Table 1), a common result reported in the literature for corn breeding (Guimarães *et al.*, 2012; Tamirat *et al.*, 2014; Marcondes *et al.*, 2016; Rosa *et al.*, 2020; Rosa *et al.*, 2022). This result can be interpreted as variation of the progeny performance among the different crossed testers, but limits the interpretation regarding the magnitude of the interaction among each topcross. In this sense, the AMMI model represents an alternative approach for decomposing the P x T interaction into additive main effects and multiplicative effects (Charcosset *et al.*, 1993; Duarte & Vencovsky, 1999).

Since the first two principal components explains more than 70% of the variation of the P x T interaction, both principal components were used for evaluating the performance of progenies and testers (Duarte & Vencovsky, 1999). Oliveira *et al.* (2010) reported that the first two principal components explained 81% of the G x E interaction in a multi trial corn breeding program.

AMMI biplot models (Figure 1) are described with the trait value on the x-axis and the progeny x tester interaction (IPCA1) scores in the y-axis (Gauch, 2013). Progenies and testers with values close to zero show small deviations among crosses and are considered more stable than those more distant. Distant values of progenies and testers are considered to have greater specific combining ability and higher contribution for P x T interaction (Shah *et al.*, 2015).

The additive main effects from the P x T interaction are show in the horizontal line (y-axis), and the variation related with the multiplicative effects are presented in the vertical line (x-axis) of the AMMI 1 biplot. AMMI 2 biplot represents the multiplicative effects in both principal components. Biplot results allows the identification of specific crosses by the magnitude and the signal of the scores (Gauch, 2013). The main objective of the analysis is to select testers that have their scores closer to zero in both axes, representing, in this sense, testers that have low favorable allele frequency for the most important loci and allowing progenies to express their genetic merit.
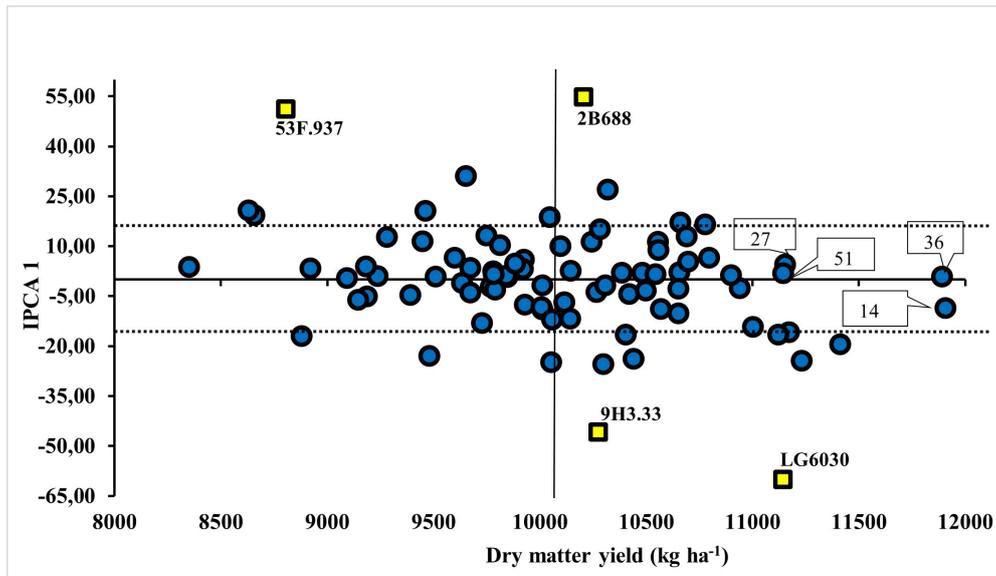
Figure 5: Progeny and tester interaction biplot, considering IPCA 1 scores (y axis) and dry matter yield (x axis). ■ = testers; ● = progenies.

Even though AMMI biplots are highly informative about how testers behave when crossed with progenies, classical approaches such as genetic variance and combining ability should be the main part of the results for choosing the best tester in a breeding program.

Testers 2B688 and LG 6030 were the testers that showed higher multiplicative effects and main participation in the P x T interaction (Figure 2), suggesting greater influence over the genetic merit of the progenies. Considering it, both hybrids are not appropriate for using as testers of progenies selected from this population, contradicting the higher GCA results for both testers obtained in the classical analysis (Shah *et al.*, 2015; Heinz *et al.*, 2019).

Progenies with higher general combining abilities in the classical approach presented score values closely grouped to the axis intersection in the AMMI2 biplot, for all evaluated traits. This tendency, however, was not observed for testers since those with higher combining abilities also showed higher scores in the AMMI2 biplot, consequently, higher contributions for the multiplicative effects in the P x T interaction.
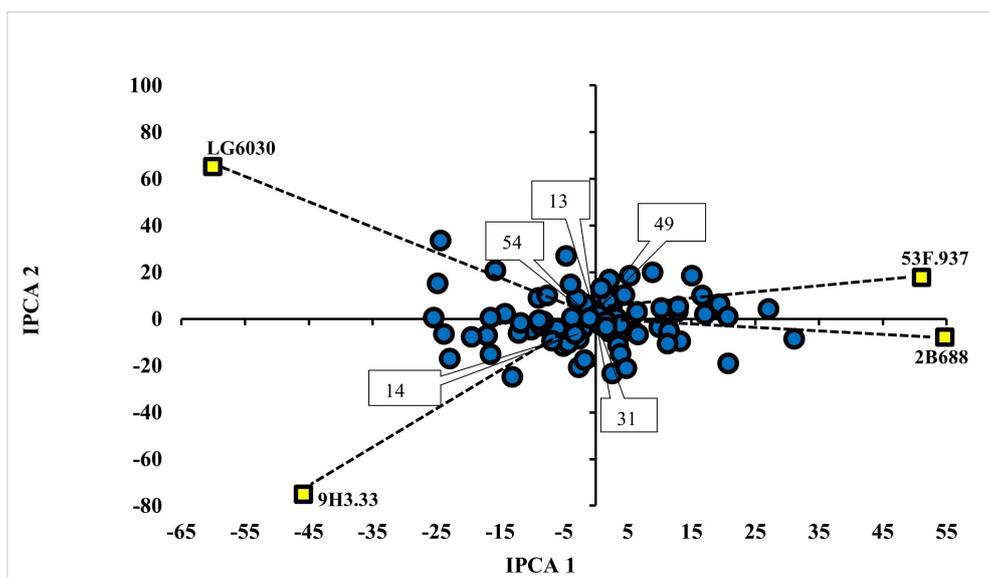

Figure 6: Progeny and tester interaction biplot, considering IPCA 1 scores (x axis) vs IPCA 2 (y axis), for dry matter yield. ■ = testers; ● = progenies.

Tester LG 6030 showed great performance for maximizing topcrosses performance and efficiently discriminates tested progenies, considering only the results for the classical approach. Surprisingly, AMMI analysis revealed that LG 6030 was the tester with higher contribution for the P x T interaction in all evaluated traits, revealing influence over the progenies performance due to its higher favorable allele frequencies. Classical approaches tend to rely their tester selection on the highest general and specific combing ability, which might result in selecting testers that overestimate the genetic merit of a progeny due to tester effects.

CONCLUSIONS

Testers LG 6030 and 2B688 showed higher general combining ability and genetic parameters, and were the best testers according the classical approach.

All testers showed higher effects in the P x T interaction, and also higher scores of main additive effects and multiplicative effects.

AMMI analysis was complementary to the classical approach, especially for progeny selection and for identifying additive and multiplicative effects in the topcrosses.

Progeny 14 was selected due to its higher general combining ability and also in the AMMI analysis for all evaluated traits, and should be used for future crosses in the forage breeding program.

Topcrosses 14 x 9.H3.33 and 14 x 2B688 were selected due to their higher specific combining ability and also in the AMMI analysis for all evaluated traits, and should be used in future trials in the forage breeding program.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in carrying the research and publishing the manuscript.

REFERENCES

Aslam M, Sohail Q, Maqbool MA, Ahmad S & Shahzad R (2017) Combining ability analysis for yield traits in diallel crosses of maize. Journal of Animal and Plant Sciences, 27:136-143.

AOAC - Association of Official Analytical Chemists (1990) Official methods of analysis. 15º ed. Arlington, AOAC International. 1117p.

Charcosset A, Denis JB, Lefort-Buson M & Gallais A (1993) Modelling interaction from top-cross design data and prediction of F1 hybrid value. Agronomie Sciences, 13:597-608.

Cruz CD (2013) Genes - a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum Agronomy, 35:271-276.

Davis RL (1927) Agricultural experimental station. Report of the Plant Breeder, 1:14-15.

De Almeida Filho JE, Guimarães JFR, Silva FFE, de Resende MDV, Muñoz P, Kirst M & Resende Junior MFR (2016) The contribution of dominance to phenotype prediction in a pine breeding and simulated population. Heredity, 117:33-41.

De Oliveira RL, Von Pinho RG, Balestre M & Ferreira DV (2010) Evaluation of maize hybrids and environmental Evaluation of maize hybrids and environmental stratification by the methods AMMI and GGE biplot stratification by the methods AMMI and GGE biplot. Crop Breeding and Applied Biotechnology, 10:247-253.

Duarte JB & Venkovsky R (1999) Interação genótipos x ambientes: uma introdução à análise "AMMI". Ribeirão Preto, FUNPEC. 60p.

Dunière L, Sindou J, Chaucheyras-Durand F, Chevallier I & Thévenot-Sergentet D (2013) Silage processing and strategies to prevent persistence of undesirable microorganisms. Animal Feed Science and Technology, 182:01-04.

Figueiredo AST, Pinto RJB, Scapim CA, Rizzardi DA, Conteras-Soto RI, Matsuzaki RA, Jobim CC & Faria MV (2018) Topcrosses in the selection of testers and inbred lines S_3 for the yield and bromatological quality of silage maize. Maydica, 63:01-13.

Gauch HG (2013) A Simple Protocol for AMMI Analysis of Yield Trials. Crop Science, 53:1860-1869.

Gauch HG & Zobel RW (1988) Predictive and postdictive success of statistical Analysis of yield trials. Theorical applied genetics, 76:01-10.

Gollob HF (1968) A statistical model which combines features of factor analitic and analysis of 271 variance techniques. Psychometrika, 33:73-115.

Griffing B (1956) Concept of general and specific combining ability in relation to diallel crossing system. Australian Journal of Biological Sciences, 9:463-493.

Guimarães LJM, Miranda GV, de Lima RO, Maia C, Oliveira LR & Souza LV (2012) Performance of testers with different genetic structure for evaluation of maize inbred lines. Ciência Rural, 42:770-776.

Hallauer AR, Carena JM & Miranda Filho JB (2010) Quantitative genetics in maize breeding. New York, Springer. 663p.

Heinz R, Ribeiro LP, Gonçalves MC, Bhering LL & Teodoro PE (2019) Selection of maize top-crosses for different nitrogen levels through specific combining ability. Bragantia, 78:208-214.

Marcondes MM, Faria MV, Mendes MC, Gabriel A, Neiverth V & Zocche JC (2016) Breeding potential of S_4 maize lines in topcrosses for agronomic and forage traits. Acta Scientiarum.Agronomy, 38:307-315.

Miranda Filho JB & Gorgulho EP (2001) Cruzamentos com testadores e dialelos. In: Nass LL, Valois ACC, Melo IS & Valadares-Inglis MC (Eds.) Recursos Genéticos e Melhoramento de Plantas. Rondonópolis, Fundação MT. p.649-671.

Mendes MHS, Pereira CH & Souza JC (2015) Diallel analysis of maize hybrids for agronomic and bromatological forage traits. Acta Scientiarum.Agronomy, 37:141-146.

Nanavati JI (2015) Combining ability analysis for quantitative characters in forage maize (Zea mays L.). Forage Research, 41:30-33.

Nitsche PR, Caramori PH, Ricce W da S & Pinto LFD (2019) Climatic Atlas of the State of Parana. Londrina, Instituto Agronômico do Paraná. Available at: <https://www.idrparana.pr.gov.br/Pagina/Atlas-Climatico> Accessed on: November 19th, 2021.

R Development Core Team (2022) R: A language and environment for statistical computing. Vienna, R Foundation for Statistical Computing. Available at: <https://www.R-project.org/>. Accessed on: March 10th, 2022.

Rosa JC, Faria MV, Zaluski WL, Gava E, Andreoli PHW & Sagae VS (2020) Forage potential of S_3 corn progenies in topcrosses and selection of testers of different genetic bases. Pesquisa Agropecuária Brasileira, 55:e01283.

Rosa JC, Scapim CA, Faria MV, Uhdre RS, Zaluski WL & Sagae VS (2022) Maize topcrosses for yield performance by additive main effects and multiplicative interaction analysis. Ciência Rural, 52:e20210286.

Shah L, Rahman HU, Ali A, Bazai NA & Tahir M (2015) Combining ability estimates from line × tester mating design in maize (*Zea mays* L.). Journal of Agricultural Science and Research, 3:71-75.

Singh RK & Chaudhary BD (1979) Biometrical methods in quantitative genetic analysis. New Delhi, Kalyani. 304p.

Silva RR & Benin G (2012) Análises Biplot: conceitos, interpretações e aplicações. Ciência Rural, 42:1404-1412.

Sprague GF & Tatum LA (1942) General vs. specific combining ability in sigle crosses of corn. Jornal American Society of Agronomy, 34:923-932.

Souza Neto IL, Pinto RJB, Scapim CA, Jobim CC, Figueiredo AST & Bignotto LS (2015) Diallel analysis and inbreeding depression of hybrid forage corn for agronomic traits and chemical quality. Bragantia, 74:42-49.

Tamirat T, Alamerew S, Wegary D & Menamo T (2014) Test Cross Performance and Combining Ability Study of Elite Lowland Maize (*Zea mays* L.) Inbred Lines at Melkassa, Etihopia. Advances in Crop Science and Technology, 2:01-09.

Zobel RW, Wright MJ & Gauch HG (1988) Statistical analysis of a yield trial. Agronomy Journal, 80:388-393.