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Combining ability and genetic divergence in the selection of testers regarding grain yield and forage potencial in maize topcrosses[1]

Capacidade combinatória e divergencia genética na seleção de testadores para produtividade de grãos e potencial forrageiro em topcrosses de milho

Jocimar Costa Rosa[2]*, Marcos Ventura Faria[3], Welton Luiz Zaluski[3], Renan Santos Uhdre[2], Pedro Henrique Willemann Andreoli[3] and Vitor Seiti Sagae[3]

ABSTRACT - Genetic divergence analysis among testers and progenies associated with the combining ability and genetic parameters estmates can help in the selection of testers for the evaluation of grain yield and forage related traits in topcrosses, since testers suitable to both purposes are rare.The objective of this work was to select testers suitable for the evaluation of grain yield and forage traits is topcrosses with S3 progenies of maize based on the association between genetic divergence, general combining ability and genetic variance. The experiments were carried out in the 2015/16 and 2016/17crop seasons in Guarapuava-PR. We evaluated 150 topcrosses among 30 S_3 progenies and testers five testers (single hybrids AG8025 and P30B39, the elite inbred lines 60.H23.1 and 70.H26.1, and a mixture of inbred lines MLP102), The evaluated traits were plant height, ear height, grain yield, dry mass yield, neutral detergent fiber and acid detergent fiber andforage *in situ* digestibility. The testers 60.H23.1 and 70.H26.1 are the most recommended for discriminate the progenies regarding grain yield and forage traits. There was not linear correlation between genetic divergence, general combining ability and genetic variance.

Key words: *Zea mays* L. Topcross. Maize forage.

RESUMO - A análise da divergência genética entre testadores e progênies associada à capacidade de combinação e parâmetros genéticos pode auxiliar na seleção de testadores para a avaliação do rendimento de grãos e características forrageiras em cruzamentos topcrosses, uma vez que são raros os testadores adequados para ambos os propósitos. O objetivo deste trabalho foi selecionar testadores adequados para a avaliação do rendimento de grãos e de características forrageiras em topcrosses com progênies S_3 de milho com base na associação entre divergência genética, capacidade geral de combinação e variância genética. Os experimentos foram conduzidos nas safras 2015/16 e 2016/17 em Guarapuava-PR. Foram avaliados 150 cruzamentos topcrosses entre 30 progênies S_3 de milho e cinco testadores (os híbridos AG8025 e P30B39, as linhagens 60.H23.1 e 70.H26.1, e a mistura de linhagens MLP102). As características avaliadas foram altura de planta e de espiga, rendimento de grãos, rendimento de massa seca da forragem, teores de fibra em detergente neutro e fibra em detergente ácido e digestibilidade *in situ* da forragem. Os testadores 60.H23.1 e 70.H26.1 são os mais recomendados para discriminar as progênies quanto ao rendimento de grãos e características de forragem. Não houve correlação linear entre divergência genética, capacidade geral de combinação dos testadores e variância genética.

Palavras-chave: *Zea mays* L. Topcross. Milho forrageiro.

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*Author for correspondence
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[1]Part of the first author's Ph.D. thesis through Capes grant funding
[2]Programa de Pós-Graduação em Genética e Melhoramento, Universidade Estadual de Maringá, Maringá-PR, Brasil, joce_jcosta@hotmail.com (ORCID ID 0000-0002-3482-1283), renan_uhdre@hotmail.com (ORCID ID 0000-0003-2060-0241)
[3]Post-Graduate Program in Agronomy, Universidade Estadual do Centro-Oeste, Guarapuava-PR, Brasil, mfaria@unicentro.br (ORCID ID 0000-0001-8077-4708), luizzaluski@hotmail.com (ORCID ID 0000-0002-4497-4160), pedrohwandreoli@gmail.com (ORCI ID 0000-0002-5988-3966), vsagae@gmail.com (ORCID ID 0000-0001-5619-7576)

INTRODUCTION

The study of genetic divergence between genitors can help in the planning of crosses, besides directly contributing to the discrimination of heterotic groups (CRUZ; REGAZZI; CARNEIRO, 2013; MINGOTI, 2007). This technique allows determining the coefficients of genetic divergence among genotypes, contributing to the genetic breeding. In the majority of cases greater genetic divergence increases the chances of obtaining crosses with high combining ability (FAN *et al.*, 2016).

Maize inbred lines with a large number of alleles in common for a specific trait are considered to be poorly divergent, characterizing themselves as unsuitable for crosses of high heterotic potential, unlike inbred lines with high allelic divergence, which present greater potential for crosses. However, in topcross evaluations this concept cannot always be applied, because the contribution of different gametes of testers to the combining ability associated with the genetic parameters interferes in the real merit of the evaluated progenies, which can generate a misinterpretation of traits and genetic variability (HALLAUER; MIRANDA FILHO, 2010; LARIÈPE *et al.*, 2016).

Even though topcross is an efficient model, its still presents aspects that cause divergences, especially in the choice of the tester, it is not possible to determine a tester suitable for all the crosses and different traits. The problem tends to get worse when looking for testers that can be efficient to descriminate forage traits, little discussed in the literature so far. A single tester suitable for grain yield and forage traits is rarer yet (ROVARIS; PATERNIANI; SAWAZAKI, 2014).

Given this paradigm about choosing an efficient tester to accurately assess progenies genetic merit in terms of grain yield and forage traits, several studies are needed involving mainly more than one tester (NANAVATI, 2015). Estimates of combining ability, genetic divergence and genetic parameters can be used as important tools in the selection of an efficient tester, allowing greater efficiency in progeny selection. (VENCOVSKY; BARRIGA, 1992).

The objective of this work was to select efficient testers to discriminate the genetic merit of S_3 maize progenies regarding grain yield and forage traits based on the association between genetic divergence, general combining ability and genetic variance in topcrosses.

MATERIAL AND METHODS

Thirty S_3 maize progenies from the SG6015 hybrid were crossed with five testers: single hybrids AG8025 and P30B39B, elite inbred linees LEM 2 (60.H23.1) and LEM 3 (70.H26.1) and the mixture of inbred lines MLP102. The mixture of inbred lines is characterized for having a broad genetic basis, while the other testers have a narrow genetic basis.

The experiments were carried out in two consecutive years, in the 2015/16 (ENV 1) and 2016/17 (ENV 2) crop seasons. The soil is characterized as Bruno Distroferric Latosol, latitude 25º 21', longitude 51º 31' and altitude 1050 m. The climate is Cfb with average temperature between 17 and 18° C and precipitation between 1800 and 2000 mm annually (INSTITUTO AGRONÔMICO DO PARANÁ, 2019).

The 150 topcrosses hybrids were arranged in the field in a randomized complete block design, with three replications. The 30 S_3 progênies and 5 testers were also arranged and evaluated in contigous area, with three replicatios. The experimental unit in both crop seasons consisted of two contiguous 5m rows spaced 0.45m apart, equivalent to a density of 60.000 plants ha^{-1}. The progenies and the testers served as a comparison and reference factor for the estimation of the genetic parameters.

The height of plants (PH) and the height of ear insertion (EH) were evaluated. The grain yield (GY) was evaluated from the harvesting of all the ears of a plot line, with moisture correction to 13% and expressed in kg ha^{-1}.

The forage was obtained when the grains presented 2/3 of the milk line, in phenological stage R5. The plants of one row of the plot were cut at 0.2 m from the ground and weighed to obtain the weight of the green mass. The plants were then minced in a stationary forage harvester with a particles size of 1 to 2 cm. Samples of 0.25 kg were collected to obtain the dry mass content of the forage and, subsequently, the dry mass yield (DMY) was estimated in kg ha^{-1}.

The determination of the neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of forage was performed according to Van Soest, Robertson and Lewis (1991). The forage *in situ* digestibility (DIG) was performed in a rumen fistulated steer, Jersey, which was adapted to the diet with 100% maize silage, during the 15 days prior to the evaluation.

The agronomic and forage data were submitted to the Bartlett and Shapiro-Wilk test (1937), accepting the hypothesis that the variances are homogeneous, and the errors have normal distribution, the statistics analyzes were performed using the statistical softwares GENES (CRUZ, 2013) and R (R CORE TEAM, 2015).

The genetic divergence between progenies and testers, based on the evaluated traits, was determined by the generalized distance of Mahalanobis (D^2). The genetic divergence matrix was used for the cluster analysis of the genotypes using the UPGMA method

(Unweighted Pair-Group Method using Arithmetic Avarages) (SILVA; PONIJALEKI; SUINAGA, 2012).

The dendrogram cut-off point and the number of groups were defined by the Mojema (1977) criterion, according to Silva, Ponijaleki and Suinaga (2012), based on the relative size of the dendrogram fusions (distances). The clustering consistency was verified using the cofenetic correlation coefficient. The value of the correlation between the two matrices was tested by the application of the aleatorization test of Mantel (1967) with 1000 resampling.

The genetic variance was estimated according to the expression $\sigma^2_G = (QMG - QMGA) / ra$, where QMG is the mean square of genotypes; QMGA is the mean square of the interaction genotypes x environments; r is the number of replications; a is the number of environments (crop seasons). The residual variance (σ^2_E) was estimated according to the expression $\sigma^2 = QMR / r$, where QMR is the mean square of the error; r is the number of replications. The average broad sense heritability (h_a^2) was estimated according to $h_a^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_E)$, where σ^2_G is the genetic variance; σ^2_E is the residual variance.

The diallel analysis was performed according to Method 2 adapted for partial diallel by Geraldi and Miranda Filho (1988) (genitors and F1s), in order to estimate the general combining ability (GCA) and specific combining ability (SCA) of the genitors from *pq* hybrid combinations, where *p* progenies (Group I) are crossed with *q* testers (Group II). Finally, Pearson's correlation coefficient (r) was calculated between general combining ability, genetic divergence and genetic variance, whose significance was verified by Student's T test, at 5% probability. The correlation was performed according to Pearson's proposal adapted for the use of genetic metrics (PEARSON, 1892).

RESULTS AND DISCUSSION

Acording to the diallel analysis, for plant height, there was significant effect of genotypes, general combining ability (GCA) of testers, GCA of progenies and specific combining ability (SCA) for the topcrosses evaluated. For ear height, there was no significant effect of the genotypes. For grain yield, there was a significant effect among the topcrosses, GCA of testers, GCA of progenies and SCA (Table 1).

Regarding to forage dry mass yield, there was significant effect only for the genotypes and GCA of testers. For neutral detergent fiber and acid detergent fiber there was a significant effect of genotypes and SCA. For forage *in situ* digestibility, there was significant effect of genotypes, GCA of progenies, SCA and GCA of testers (Table 1).

The significant effect of the GCA of testers and SCA are directly related to different contribution of the testers for the crossings and consequently to the efficiency in discriminating the variance present in progenies considering the evaluated traits. Testers with high GCA tend to be more efficient in expressing progenies variability and also present greater genetic divergence compared to the evaluated progenies (LARIÈPE *et al.*, 2016).

Table 1 - Significance of mean squares of the joint partial diallel analysis of the topcrosses among 30 S_3 progenies of maize and five testers evaluated in the 2015/16 and 2016/17 crop seasons

Sources of variation	Degrees of freedom	Mean square						
		PH	EH	GY	DMY	NDF	ADF	DIG
Genotypes	184	**		**	**	*	*	**
GCA of Testers	4	*		**	*			*
GCA of Progenies	29	**		**				**
SCA	150	**		**		*	*	**
Environments (E)	1							
Genotypes x Environments	184			**				
GCA of Testers x E	4							
GCA of Progenies x E	29							
SCA x E	150			**				
Error	736							

**, * significant at 1 and 5% of the F test, respectively. PH = plant height; EH = ear height; GH = grain yield; DMY = forage dry mass yield; NDF = neutral detergent fiber; ADF = acid detergent fiber; DIG = forage *in situ* digestibility; GCA = general combining ability; SCA = specific combining ability

It was decided to determine the genetic divergence matrices individually according to each environment, correlating them later, with the purpose of increasing the assertiveness in relation to the genetic divergence and the grouping, as done by Simon, Kamada and Moiteiro (2012).

Based on the genetic divergence among progenies and testers determined by the generalized distance of Mahalanobis in the 2015/16 crop season (ENV 1), the grouping between the genotypes by the UPGMA method was confirmed by the coefficient of cofenetic correlation with value of 0.7513, which indicated an adequate adjustment between the graphical representation of the cluster and its original matrix. The diagnosis showed low collinearity, whose value of 37.33 is considered adequate for this type of procedure (CRUZ; REGAZZI; CARNEIRO, 2013).

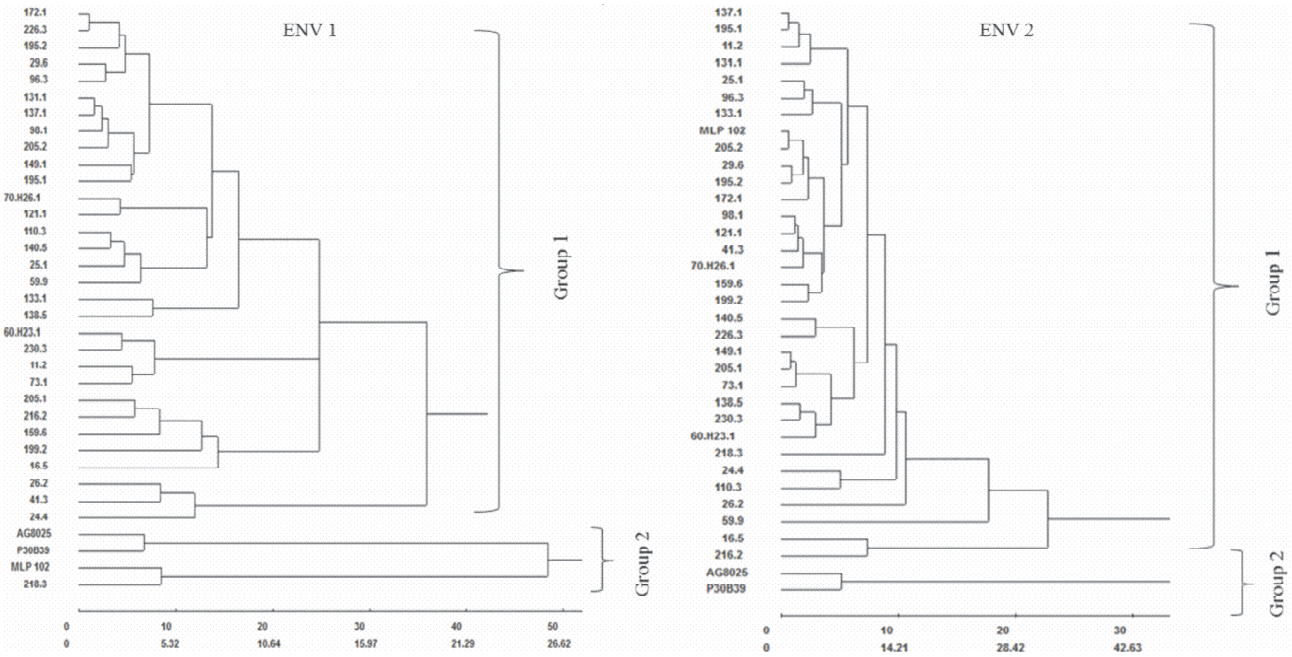
Two distinct groups were formed in ENV 1. Three of the five testers made up the divergent group compared to that of the majority of progenies. Progeny 218.3 was the only one that was distinguished from the others, included in the group with the AG8025, P30B39 and MLP102 testers. Testers 60.H23.1 and 70.H26.1 remained in the group composed by the majority of the progenies, a justifiable fact because they are elite inbred lines, which, according to the traits evaluated, approximates them to the progenies (SZARESKI *et al.*, 2018) (Figure 1).

Two groups were also formed in 2016/17 crop season (ENV 2) and testers AG8025 and P30B39 again made up the divergent group in relation to the progenies. Unlike ENV 1, progeny 218.3 and the MLP102 tester remained in the group with the other progenies, as well as testers 60.H23.1 and 70.H26.1 (Figure 1). Arnhold, Silva and Viana (2010) and Simon, Kamada and Moiteiro (2012) also showed differences in the values of genetic divergence and genotype allocation in relation to the evaluation environments, thus justifying the analysis according to each environment. The cofenetic correlation coefficient in ENV 2 (0.9452) indicated an adequate adjustment between the cluster and its original matrix. The collinearity was within the desirable standards considered low, with a value of 80.43 (Figure 1).

A correlation analysis between matrices in the two environments was also carried out. The value of the correlation was 0.84, and significant by the T test, confirming the efficiency of the clustering in each environment, despite the variation of the genetic divergence values (ALENCAR; BARROSO; ABREU, 2013; CRUZ; REGAZZI; CARNEIRO, 2013).

The amplitude of Mahalanobis generalized distances in ENV 1 ranged from 2.24 (between progeny 121.1 and tester 70.H26.1) to 135.01 (between progeny 26.2 and tester AG8025). In ENV 2, the distances ranged from 0.83

Figure 1 - Dendogram illustrating the genetic divergence established by the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method, considering the generalized distance of Mahalanobis (D^2), based on seven traits of 30 S_3 maize progênies and five testers in ENV 1 (2015/16 crop season) and ENV 2 (2016/17 crop season)



(between progeny 205.2 and tester MLP102) to 226.28 (between progeny 24.4 and tester P30B39).

The relative contribution of each character highlighted the traits DMY, GY, PH and DIG in ENV 1, with greater contribution to genetic divergence among the genotypes with values of 34.49%, 30.11%, 13.40% and 6.60% respectively, totaling 84.62% (Table 2). In ENV 2, the same traits were highlighted, however with different values. The highest contribution to the genetic divergence was evidenced by GY (41.97%), followed by PH (33.27), DMY (11.24%) and DIG (5.87%), totaling 92.36% (Table 2).

The relevance of the traits GY, PH, DMY and DIG in the contribution to genetic divergence is emphasized because they stand out in both environments, justifying the selection and analysis based on these traits, as was done by Simon, Kamada and Moiteiro (2012) and Alves *et al.* (2014), also evaluating the genetic divergence among maize genotypes.

Considering genetic variance (σ^2_G), tester 70.H26.1 was the best to promote the expression of the variability among progenies for traits PH, GY and DMY. Tester 60.H23.1 provided higher σ^2_G for DIG, being more efficient in expressing the genetic variability among progenies, considering this importante trait for forage purpose (Table 3) (CLOVIS *et al.*, 2015; MARCONDES *et al.*, 2016).

An efficiente tester is the one who simply correctly classifies the genetic merit of progenies, with information based on estimates of genetic variance components disregarding the other information (HALLAUER; MIRANDA FILHO, 2010). However, several studies confirm that this statement may not always be considered, due to the different behavior of testers in relation to different progenies evaluated and the incorrect discrimination of the traits attributed to the low ability of the tester to combine and the effects related to genetic divergence of the tester, promoting a non-existent genetic variance (ALY, 2013; ASLAM *et al.*, 2017; ORTIZ *et al.*, 2010).

In general, the most efficient testers, based on genetic variance of topcrosses (Table 3), presented low values of genetic divergence in relation to the other genotypes, remaining in the group formed with the progenies (Figure 1), making it possible to state that greater genetic divergence did not reflect better performance by the testers in discriminating genetic variability among S_3 progenies (ORTIZ *et al.*, 2010; SZARESKI *et al.*, 2018).

For PH, the highest σ^2_G estimate was presented in the topcross with tester 70.H26.1 (Table 3), whose GCA was -0.11 (Figure 2). For GY and DMY, the highest σ^2_G estimates occurred in the topcrosses with tester 70.H26.1, whose GCA estimate was negative (-1216,04 and -2355,46 respectively). For DIG, the highest σ^2_G was presented in the topcross with tester 60.H23.1 (Table 3), which presented a negative contribution of GCA with an estimate of -1.04 (Figure 2).

In the present study, testers with negative GCA showed greater σ^2_G estimates in topcrosses, in this case it is possible to infer that testers with negative GCA were more efficient in allowing the expression of variability among the progenies, since the tester does not provide favorable alleles with additive effects on the performance of some progenies in topcrosses, which justifies the greater efficiency of the use of inbred lines LEM 2 (60.H23.1) and LEM 3 (70.H26.1) as testers (FAN *et al.*, 2016; VENCOVSKY; BARRIGA, 1992).

There are reports in the literature that, in topcrosses, greater complementarity between testers and progenies coming from genitors with high genetic divergence may favor the exploration of variability by the tester (HALLAUER; MIRANDA FILHO, 2010; SIMON; KAMADA; MOITEIRO, 2012). Rovaris, Paterniani and Sawazaki (2014) and Tamirat

Table 2 - Estimates of the relative contribution of each trait (S.j) to the genetic divergence among 30 S_3 maize progenies and testers AG8025, P30B39, MLP102, 60.H23.1 and 70.H26.1 according to the generalized distance of Mahalanobis (D^2) in the 2015/16 (Environment 1) and 2016/17 (Environment 2) crop seasons

Variable	Environment 1		Environment 2	
	S.j	(%)	S.j	(%)
Plant height	1604.812	13.403	5565.435	33.279
Ear height	326.564	2.728	210.926	1.261
Grain yield	3605.870	30.116	7019.310	41.973
Forage dry mass yield	4129.989	34.494	1879.931	11.241
Neutral detergent fiber	739.229	6.174	795.772	4.758
Acid detergent fiber	775.417	6.476	270.318	1.616
Forage in situdigestibility	791.316	6.609	981.908	5.871

Table 3 - Estimates of the variance components and heritability of the analysis of the evaluated traits in topcrosses in the 2015/16 and 2016/17 crop seasons in Guarapuava-PR

Genetic parameters	AG8025	P30B39	MLP102	60.H23.1	70.H26.1
			Plant height (PH)		
σ^2_F	65.0772	108.0006	200.9643	77.9244	198.1309
σ^2_G	20.3433	62.6596	33.2869	23.4532	158.4577
h_a^2	0.31	0.58	0.16	0.30	0.79
			Grain yield (GY)		
σ^2_F	1925271	2111130	1527899	2501719	2658750
σ^2_G	1209256	1252114	1369816	1516600	1770489
h_a^2	0.62	0.59	0.89	0.60	0.66
			Forage dry mass yield (DMY)		
σ^2_F	3347122	2048145	1402722	3642890	4113001
σ^2_G	2682283	1625280	815469	2946312	3502865
h_a^2	0.80	0.79	0.58	0.81	0.85
			Forage in situ digestibility (DIG)		
σ^2_F	32.4852	44.2862	43.9666	45.9713	27.9771
σ^2_G	18.1354	28.1269	25.4112	32.5581	10.5311
h_a^2	0.55	0.63	0.57	0.71	0.37

σ^2_G = genetic variance, σ^2_F = phenotypic variance, h_a^2 = broad sense heritability

Figure 2 - Estimates of the general combining ability (GCA) of the testers for the traits evaluated in the 2015/16 and 2016/17 crop seasons in Guarapuava-PR

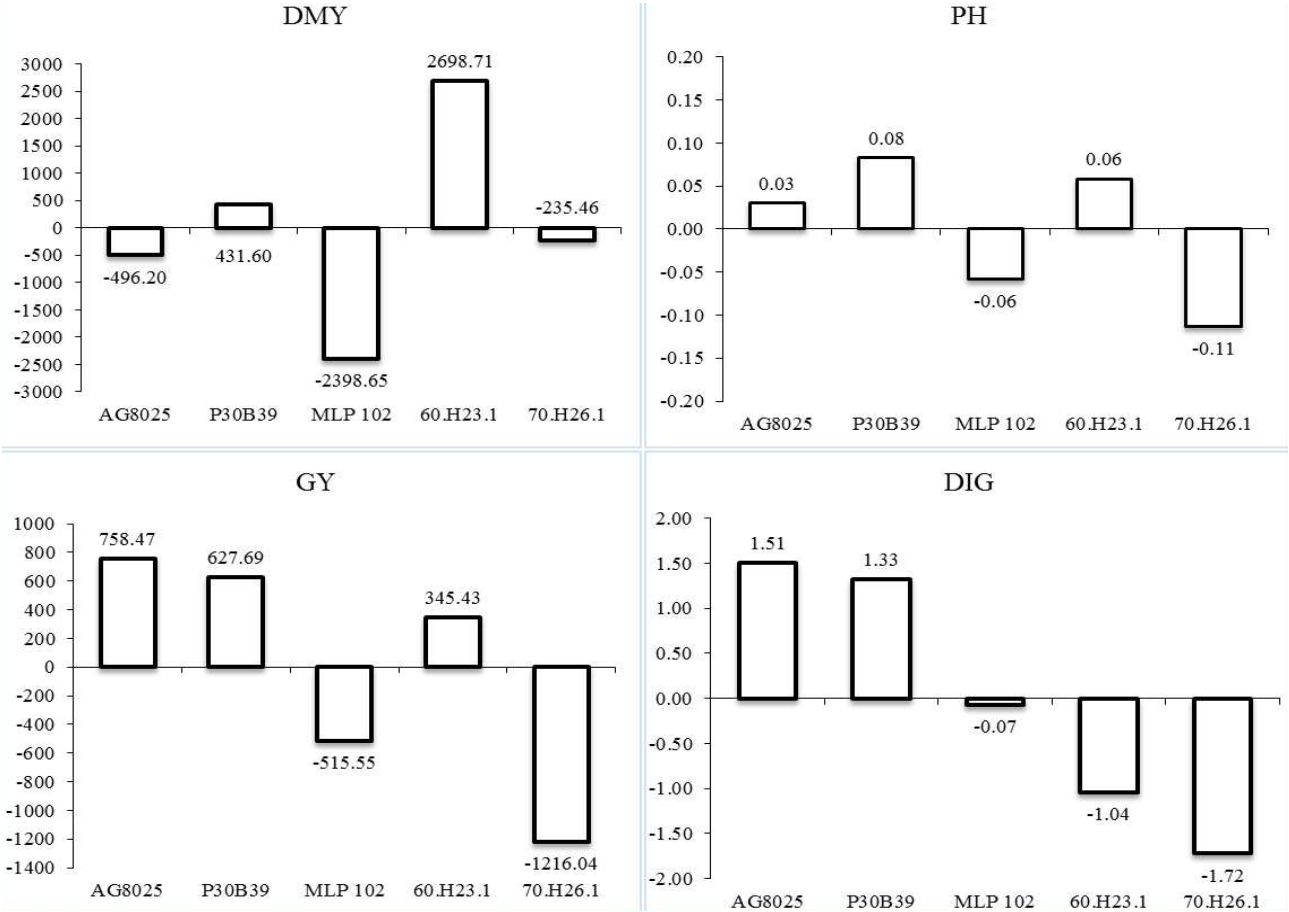
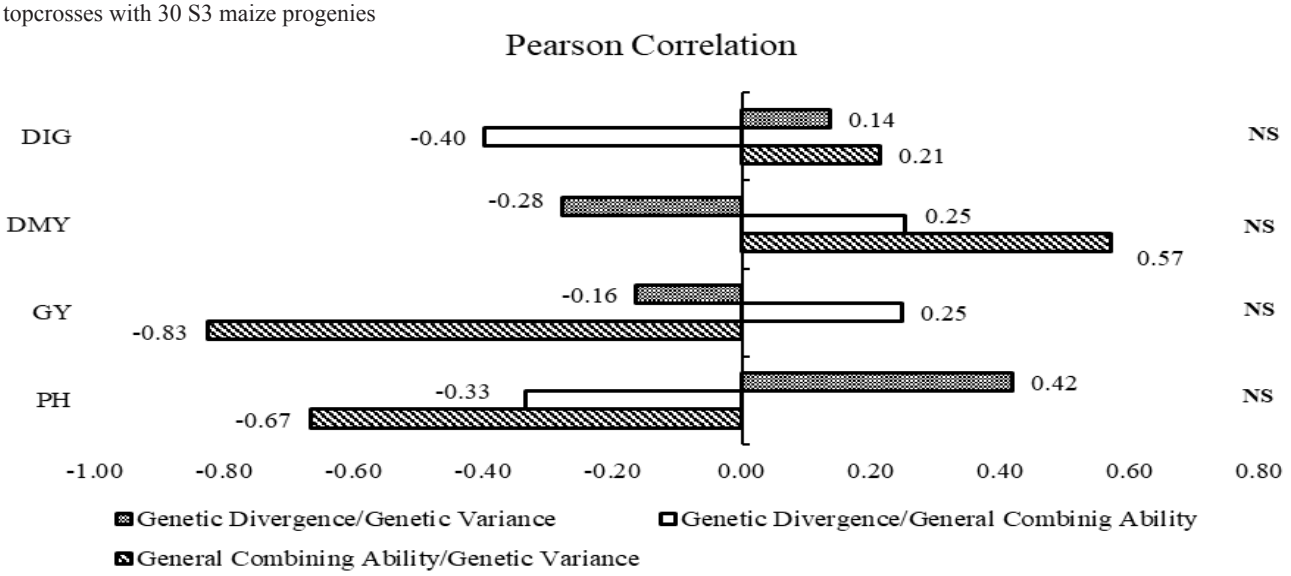


Figure 3 - Perason's correlation between genetic divergence, genetic variance and general combining ability of five testers used in topcrosses with 30 S3 maize progenies



ns: Not statistically significant at 5% alpha level by T-test

et al. (2014) described that testers with favorable estimates of combining ability were more efficient in discriminating variability among progenies. Disagreeing with the literature, in the present work this statement was not evidenced for DMY, GY and DIG, that high genetic divergence among genitors does not reflect on greater efficiency of the testers in expressing the existing variability among progenies (FAN *et al.*, 2016; SZARESKI *et al.*, 2018).

The absence of association can be confirmed by Pearson's correlation analysis adapted to genetic metrics, which did not express a significant effect on the linear correlation between genetic divergence and general combining ability, nor between general combining ability and genetic variance for any of the traits analyzed (Figure 3).

An absolute rule for the choice of the best tester was not evidenced, being necessary the analysis and choice based on several phenotypic and genotypic parameters, appropriate to each case. In a similar way, it was noticed that the contribution of favorable values of GCA by the testers not always favor the expression of variability among progenies. The genetic divergence among genitors is an important condition for good complementarity between them, but it is not characterized as a condition for a tester to be more efficient at discriminating the variability among progenies (SZARESKI *et al.*, 2018; VENCOVSKY; BARRIGA, 1992).

CONCLUSIONS

1. There was not a single suitable tester for discriminate the genetic potential among progenies for both grain yield and forage traits;
2. Greater genetic divergence between tester and progenies did not characterize the best tester;
3. There was no significant linear correlation between genetic divergence, general combining ability and genetic variance;
4. The testers 60.H23.1 and 70.H26.1 are the most recommended to discriminate the genetic potential among progenies regarding grain yield and forage traits.

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