Selection of S3 progenies of forage maize based on topcrosses with different testers

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ABSTRACT. Maize forage is commonly used as silage in milk and beef cattle livestock production systems. Despite the wide variety of maize hybrids with high potential for grain production, few available hybrids have been developed exclusively for forage aptitude. The present study aimed to select S_3 maize progenies derived from the single hybrid AS1572 based on the combining ability of forage traits in topcrosses with testers AG8025, 70.H26.1, and MLP102. The 135 resulting topcross hybrids were assessed in partial diallel in Guarapuava and Rio Bonito do Iguaçu, Paraná State, Brazil. Were evaluated the contents of neutral detergent fiber (NDF, %DM) and acid detergent fiber (ADF, %DM), forage dry matter yield (DMY, t ha^{-1}), and *in situ* digestibility of forage dry matter (DIG, %DM). For all evaluated traits, the variability allowed us to select superior progenies. Progenies 3.1, 22.1, and 39.1stood out in terms of NDF, ADF, and DIG, whereas progenies 47.1, 73.1, 79.1, and 90.2 were efficient in increasing the forage dry matter yield. The testers AG8025 and 70.H26.1, of narrow genetic base, are the best to explore genetic variability among progenies.

Keywords: *Zea mays* L.; combining ability; digestibility; neutral detergent fiber; acid detergent fiber.

Introduction

Maize forage is commonly used as silage in livestock production systems for both milk and beef cattle (Neumann et al., 2009). Despite the importance of maize forage and the wide variety of maize hybrids with high potential for grain production, few available hybrids have been designed exclusively for forage use.

Genetic variability related to forage yield and quality is a premise for the success of maize-forage breeding programs (Guerrero et al., 2014). Gralak et al. (2017) reported the existence of variability in commercial hybrids regarding the quality of forage, and Barrière et al. (2010) showed that superior genotypes could be selected from already improved ones. Despite this variability, breeding programs have mainly focused on genotypes with high grain and dry matter yields. These programs have achieved significant gains for field traits (Barrière et al., 2004), such as maize stalk lodging resistance and increased lignin content, but have continued to ignore qualitative attributes (Barrière et al., 2005).

Maize breeding directed towards forage production should focus on highly digestible genotypes (Pereira, Von Pinho, Souza Filho, Fonseca, & Santos, 2011). These genotypes should be analyzed for an adequate fiber content and *in situ* dry matter digestibility (Jensen, Weisbjerg, Nergaard, & Hvelplund, 2005).

In maize breeding programs, the topcross methodology is an important strategy for evaluating the genetic merit of lines in partially inbred generations, in which it is possible to predict the ability of the partial inbred lines to generate valuable hybrids and keeping in the breeding program only the lines with better combining ability (Davis, 1927; Castellanos, Hallauer, & Cordova, 1998). The proper tester used in topcrosses should express efficiency in correctly classifying the genetic merit of the inbred lines. Few studies with a partial diallel design between maize inbred lines for characteristics related to forage maize can be found in the literature, and there is a lack of studies discussing the choice of inbred lines and testers for corn silage breeding programs (Figueiredo et al., 2018, Rosa et al., 2020).

Thus, the present study aimed to select S_3 maize progenies based on the combining ability of forage traits, in addition to verifying the efficiency of testers in the selection of promising progenies.

Material and methods

Genetic materials

Forty-five S_3 progenies obtained from maize single hybrid AS1572 by successive cycles of selfing were crossed by manual pollination with three testers (AG8025, 70.H26.1, and MLP102), resulting in 135 topcross hybrids. AG8025 hybrid was used as a commercial check. Tester AG8025 is a single-cross hybrid (narrow genetic base), and it was selected considering recommendations for grain and silage production. The elite line 70.H26.1 (narrow genetic base) was developed by the UEM maize-breeding program and was derived from the P30F53 hybrid, and MLP102 (wide genetic base) is a bulk of S_3 progenies derived from the P30B39 hybrid.

Agronomic trials

Topcross hybrids were evaluated under a partial diallel scheme in Guarapuava and Rio Bonito do Iguaçu, Paraná State, Brazil, during the 2014/2015 crop season. Guarapuava is located at 25° 21' S, 51° 31' W at an altitude of 1,050 m and exhibits dystroferric red oxisol soil and a Cfb climate. Rio Bonito do Iguaçu is located at 25° 37' S, 52° 33' W at an altitude of 560 m and exhibits eutrophic red latosol soil and a Cfa climate. At both locations, the annual precipitation ranges from 1,800 to 2,000 mm.

Six trials (three per location) were carried out, and each trial was used to assess the topcross hybrids obtained from a specific tester. The experiments were carried out in complete block design with randomized treatments, with two replications, arranged in contigous areas in each location. Each plot consisted of two 5 m-long rows spaced 0.8 m apart, with a stand density of 70,000 plants ha^{-1}.

The pre-sowing and cover fertilization procedures were identical in both locations. For pre-sowing fertilization, 450 kg ha^{-1} of N-P-K formula 8-20-20 + Zn fertilizer was applied. The first cover fertilization application was performed between stages V4 and V5 (90 kg N ha^{-1} + 45 kg K$_2$O ha^{-1}), and the second was applied between stages V7 and V8 (90 kg N ha^{-1}).

Forage bromatological trait evaluation

The plants from one row of each plot were cut at a standard height of 0.2 m to obtain forage when the kernels were at the ¾ milk line (R5 stage, corresponding to the phenological stage characterized as the pasty to farinaceous grain stage, when plants presented a total dry matter content of 30 - 35%). The cut plants were weighed to calculate the forage fresh matter yield (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 sample was dried in a forced air circulation oven at 55°C until it reaches constant weight. The pre-dried samples were processed in a knife mill and sieved (diameter < 1 mm), and 2.0 g (± 0.025 g) of the original sample was oven-dried at 105°C to determine the total dry matter content. We estimated the forage dry matter yield (DMY, t ha^{-1}) based on the fresh matter yield and final dry matter content.

The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using nonwoven fabric filter bags (density 100 g dm^{-3}) and applying neutral and acid detergent solutions, respectively (Van Soest, Robertson, & Lewis, 1991). For NDF, 0.5 mL of thermostable α-amylase was used for each sample. Moreover, the *in situ* digestibility of forage dry matter (DIG) was calculated for a 24-hour period as described by Pereira, Von Pinho, Bruno, and Calestine (2004).

Statistical analysis

The data were subjected to individual analysis of variance for each topcross in each location and then assessed for residual variance homogeneity. Finally, the joint analysis of variance was assessed when the ratio between the mean effective error less than or equal seven (Ramalho, Ferreira, & Oliveira, 2005). The analysis was performed considering all effects as random except for the means. The components of the genetic and phenotypic variance and the broad-sense heritability of traits were estimated for both locations.

Individual and joint analysis of partial diallel were performed with the F_1 combinations according to Geraldi and Miranda Filho (1988), adapted from Griffing's Method 4, in which the sums of squares of the topcross hybrids for the yield and bromatological traits of forage evaluated in both locations were partitioned in terms of the general combining ability of the progenies (Group I) and testers (Group II) and the specific combining ability.

For the individual analysis of partial diallel, the error degree of freedom consisted of the sum of the error degrees of freedom of the individual analysis of variance of the experiments with the topcross hybrids, and as

effective error we used the average of the mean square of error of individual analysis of variance of the experiments with the topcross hybrids. For the joint analysis of partial diallel, as combined error degree of freedom we used the sum of degrees of freedom of the mean effective error of individual partial diallel analysis. As mean square of the mean effective error we used the average of the mean effective errors of the individual analysis of the partial diallel.

The statistical software Genes (Cruz, 2016) was used to perform the analysis and estimate the genetic and phenotypic parameters.

Results and discussion

The interactions of topcrosses x locations, the GCA (general combining ability) of testers x locations, the GCA of progenies x locations, and the SCA (specific combining ability) x locations were significant ($p \leqslant 0.01$) for NDF, ADF, DMY, and DIG (Table 1). Such interactions demonstrated variation in the expression of additive and non-additive effect genes due to environmental variations. Therefore, progenies showing favorable combining ability estimates for each environment could be selected.

Regardless of the location, the sum of squares of the SCA (SS SCA) was higher than the sum of squares of the GCA (SS GCA) for NDF and ADF, with values of 54.52 and 59.23%, respectively, indicative of predominance of non-additive genetic effects. Additionally, the effects of SS GCA were greater for DMY and DIG, with 54.22 and 54.20%, respectively (Table 1), showing predominance of additive genetic effects. This fact allows the progenies selection in order to improve the germplasm.

Table 1. Summary of joint partial diallelic analysis involving 45 S_3 maize progenies and three testers for the combining ability of the forage traits acid detergent fiber (ADF, %DM), neutral detergent fiber (NDF, %DM), dry matter yield (DMY, t ha^{-1}) and *in situ* digestibility of dry matter (DIG, DM) evaluated in Guarapuava and Rio Bonito do Iguaçu, Paraná State, Brazil, in the 2014/15 crop season.

Source of Variation	DF	Mean Square							
		NDF		ADF		DMY		DIG	
Topcrosses	134	37.00		17.20		18.41	*	86.63	*
GCA Testers	2	300.90		186.63		152.48		1383.29	
GCA Progenies	44	37.58		12.87		22.90		80.13	
SCA	88	30.72		15.52		13.11		60.42	
Locations (L)	1	53.79	**	634.95	**	961.89	**	475.61	**
Topcrosses x Locations	134	35.18	**	13.57	**	13.25	**	61.79	**
GCA Testers x L	2	116.60	**	84.49	**	127.02	**	653.62	**
GCA Progenies x L	44	38.32	**	9.04	**	14.35	**	50.38	**
SCA x L	88	31.76	**	14.22	**	10.11	**	54.05	**
Mean effective error	264	6.12		1.56		1.12		5.35	
Coefficient of variation (%)		4.09		4.15		5.07		3.99	
Mean		60.73		29.97		20.88		58.18	
Mean Check (AG8025)		56.20		28.40		26.40		63.90	
SS$^{(1)}$ GCA Testers (%)		12.14		16.19		12.36		23.83	
SS GCA Progenies (%)		33.35		24.57		40.85		30.37	
SS GCA (%)		45.48		40.77		53.22		54.20	
SS SCA (%)		54.52		59.23		46.78		45.80	
SS GCA Testers x L (%)		4.95		9.29		14.31		15.79	
SS GCA Progenies x L (%)		35.77		21.88		35.56		26.77	
SS GCA x L (%)		40.71		31.17		49.87		42.56	
SS SCA x L (%)		59.29		68.83		50.13		57.44	

**, * Significant at the $p \leqslant 0.01$ and $p \leqslant 0.05$ probability levels according to the F test, respectively. $^{(1)}$Percentage of the sum of squares explained by GCA (G1 and G2) and SCA.

The SS GCA of the progenies was responsible for most of the observed trait variation and was twice as large as that of SS GCA from the testers for NDF and DMY (Table 1). This outcome indicates the variability of these traits within the studied population and supports the possibility of selecting promising progenies. Similarly, SS GCA progenies x locations was superior to SS GCA testers x locations, confirming that the largest variability for GCA effects occurred among progenies.

The magnitudes of interactions SSGCA x locations and SS SCA x locations were similar for DMY. On the other hand, for traits NDF, ADF, and DIG there was greater participation of SS SCA x environment in the total variance (Table 1), due to greater contribution of genes with non-additive effects for the interaction.

All testers were efficient in positioning the topcrosses hybrids among the genotypes with the lowest mean NDF values and those originating from progenies with a favorable GCA in both locations (Figure 1). Testers AG8025 in Guarapuava and 70.H26.1 in Rio Bonito do Iguaçu positioned a greater number of topcross hybrids in the quadrant with the lowest NDF means that originated from progenies with a favorable GCA (smaller than twice the standard deviation) (Figure 1). However, tester 70.H26.1 was more efficient for exploring the genetic variability among progenies in both locations, showing higher genetic variance and high heritability (h^2) for topcrosses (Table 2). Rosa et al. (2020) also reported the good performance of tester 70.H26.1 in exploring the genetic variability among forage maize progenies. In this context, the tester that provides the highest estimates of genetic variance among topcross hybrids is the most appropriate, allowing the expression of the genetic variability of the progenies and highlighting the ones with greatest genetic merit (Guimarães et al., 2012; Bolson, Scapim, Clovis, Amaral Junior, & Freitas, 2016; Miotto et al., 2016; Figueiredo et al., 2018).

Progeny 39.1 was placed in the favorable quadrant for selection by both MLP102 and 70.H26.1 in Guarapuava, and progeny 88.4 was highlighted by all three testers in Rio Bonito do Iguaçu, Paraná State, Brazil (Figure 1).
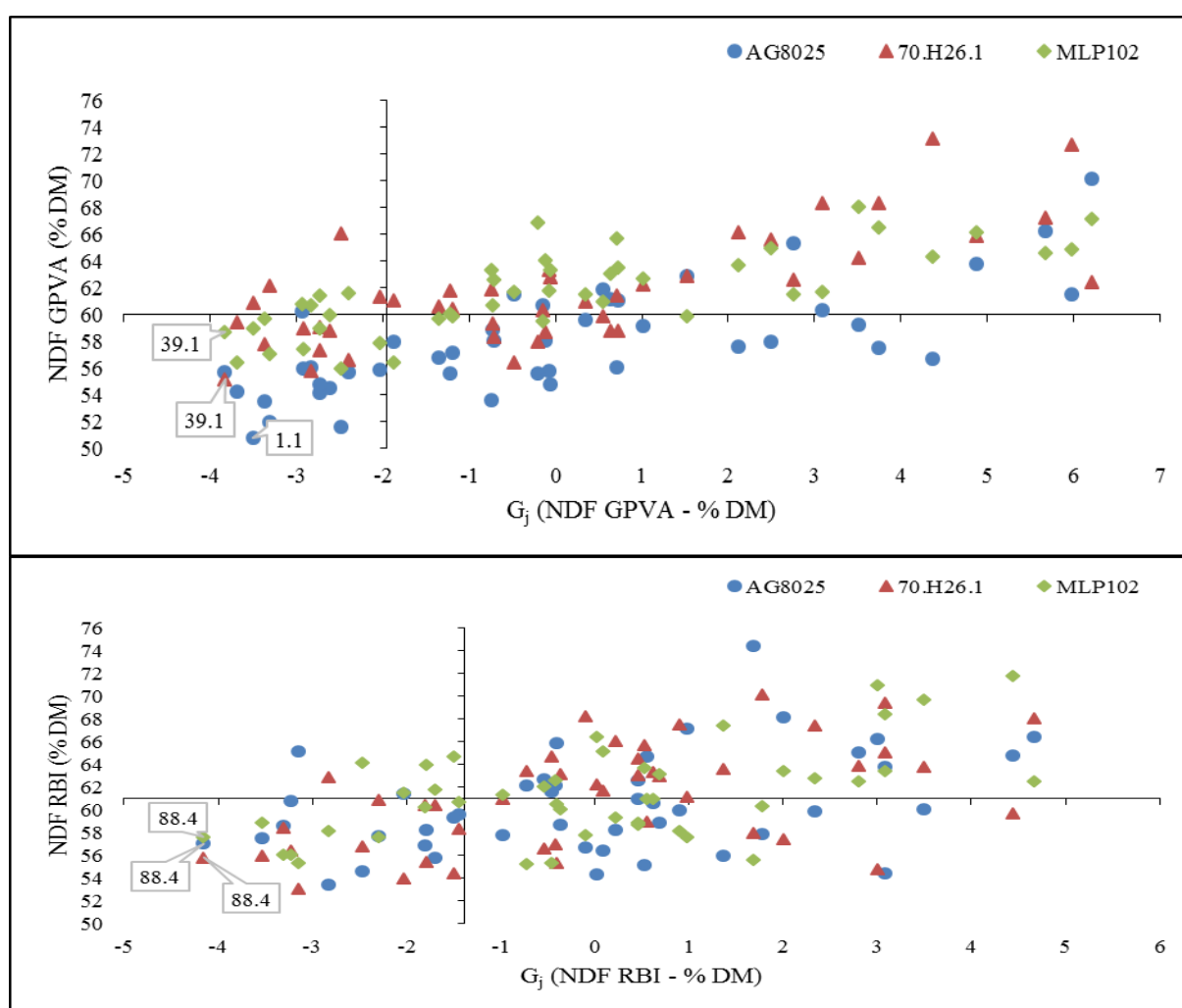

Figure 1. Dispersion among the means of neutral detergent fiber (NDF) values of 135 topcross hybrids from the crossing of 45 S_3 progenies with testers AG8025, 70.H26.1 and MLP102 and estimates of the general combining ability (G_j) evaluated in Guarapuava (GPVA) and Rio Bonito do Iguaçu (RBI), Paraná State, Brazil, during the 2014/15 crop season. *The X-axis crosses the maximum acceptable NDF value (Neumann, 2011), and the Y-axis crosses twice the standard deviation of G_j.

Few authors have reported lower NDF means than those obtained here in diallel assessments (Gralak et al., 2014; Mendes, Von Pinho, Pereira, Faria Filho, & Souza Filho, 2008) and topcross evaluations (Marcondes et al., 2015; Marcondes et al., 2016), although some have reported similar estimates (Assis et al., 2014; Mendes, Pereira, & Souza, 2015). According to Neumann (2011), NDF values lower than 42% are considered very good, while those between 42.1 and 53% are good, and values between 53.1 and 65% are average.

Table 2. Estimates of variance components and genetic and phenotypic parameters related to neutral detergent fiber (NDF, %DM), acid detergent fiber (ADF, %DM), dry matter yield of forage (DMY, t ha^{-1}), and *in situ* forage digestibility (DIG) of topcrosses of S$_3$ maize progenies with testers AG8025, 70.H26.1, and MLP102 evaluated in Guarapuava and Rio Bonito do Iguaçu (RBI), Paraná State, Brazil, during the 2014/15 crop season.

Parameter	NDF (%, DM)					
	Guarapuava			Rio Bonito do Iguaçu		
	AG8025	70.H26.1	MLP102	AG8025	70.H26.1	MLP102
σ^2_F	15.64	18.66	9.33	19.08	20.62	17.1
σ^2_G	12.72	16.04	6.65	16.42	17.33	13.64
h^2_a	0.81	0.86	0.71	0.86	0.84	0.80
CV_G	6.15	6.52	4.18	6.7	6.81	6.01
CV_G/CV_E	1.47	1.75	1.11	1.76	1.62	1.4
Parameter	**ADF (%, DM)**					
	Guarapuava			Rio Bonito do Iguaçu		
	AG8025	70.H26.1	MLP102	AG8025	70.H26.1	MLP102
σ^2_F	6.31	7.19	3.23	7.16	8.62	8.18
σ^2_G	5.87	6.38	2.49	6.50	7.69	7.15
h^2	0.93	0.89	0.77	0.91	0.89	0.87
CV_G	8.70	8.53	5.42	8.52	9.10	8.18
CV_G/CV_E	2.60	1.99	1.30	2.22	2.03	1.86
Parameter	**DMY (t ha^{-1})**					
	Guarapuava			Rio Bonito do Iguaçu		
	AG8025	70.H26.1	MLP102	AG8025	70.H26.1	MLP102
σ^2_F	8.74	4.54	5.45	11.20	5.21	6.71
σ^2_G	7.79	4.02	5.07	10.61	4.69	6.32
h^2	0.89	0.89	0.93	0.95	0.90	0.94
CV_G	12.28	9.04	10.25	15.87	12.28	12.15
CV_G/CV_E	2.02	1.96	2.60	3.00	2.12	2.86
Parameter	**DIG (%, DM)**					
	Guarapuava			Rio Bonito do Iguaçu		
	AG8025	70.H26.1	MLP102	AG8025	70.H26.1	MLP102
σ^2_F	24.93	41.36	31.10	32.11	23.94	26.28
σ^2_G	21.58	39.27	29.46	29.98	22.49	23.68
h^2	0.87	0.95	0.95	0.93	0.94	0.90
CV_G	7.54	11.61	9.66	9.21	8.43	7.89
CV_G/CV_E	1.80	3.06	3.00	0.15	2.78	2.13

σ^2_F = phenotypic variance, σ^2_G = genetic variance, σ^2 = residue variance, h^2 = broad-sense heritability, CV_G = coefficient of genetic variation, CV_G/CV_E = ratio between the genetic and experimental coefficient of variation.

In both locations, the testers originated topcross hybrids with ADF contents of less than 30%, which is considered very good (Neumann, 2011), and they produced progenies with a favorable GCA (Figure 2). Tester MLP102 was the least efficient in discriminating the variability among progenies in Guarapuava, presenting the lowest genetic variance estimation among the topcrosses (Table 2). In both locations, the mean ADF values of the topcrosses (Figure 2) were close to those reported by other authors who have assessed maize inbred lines in topcrosses (Marcondes et al., 2015; Marcondes et al., 2016) and in diallels (Gralak et al., 2014). Mendes et al. (2008) evaluated crosses between inbred lines with high and low digestibility of forage dry matter and identified hybrids with lower ADF values. Assis et al. (2014) obtained higher values when assessing commercial maize genotypes used for grain production and whole-plant ensiling. Such diversity shows the variability in ADF contents, as reported by Barrière et al. (2010).

In both locations, tester AG8025 was more efficient in discriminating progenies based on DMY, positioning 10 topcross hybrids that originated from progenies with favorable estimates of GCA of DMY (more than twice the standard deviation) in Guarapuava and 12 topcrosses in Rio Bonito do Iguaçu (Figure 4). Additionally, this tester allowed increased genetic variability exploration and provided elevated estimates of h^2 (Table 2). Progenies 88.1 and 39.1 were in the favorable quadrant for selection by all three testers in Guarapuava and Rio Bonito do Iguaçu, Paraná State, Brazil, respectively (Figure 3), which confirmed the superiority of these progenies for DMY. The DMY averages (Figure 4) were similar to those reported by Marcondes et al. (2016) in topcrosses with S$_4$ maize progenies, while other papers have reported inferior values (Assis et al., 2014; Marcondes et al., 2015; Mendes et al., 2008; Mendes et al., 2015).
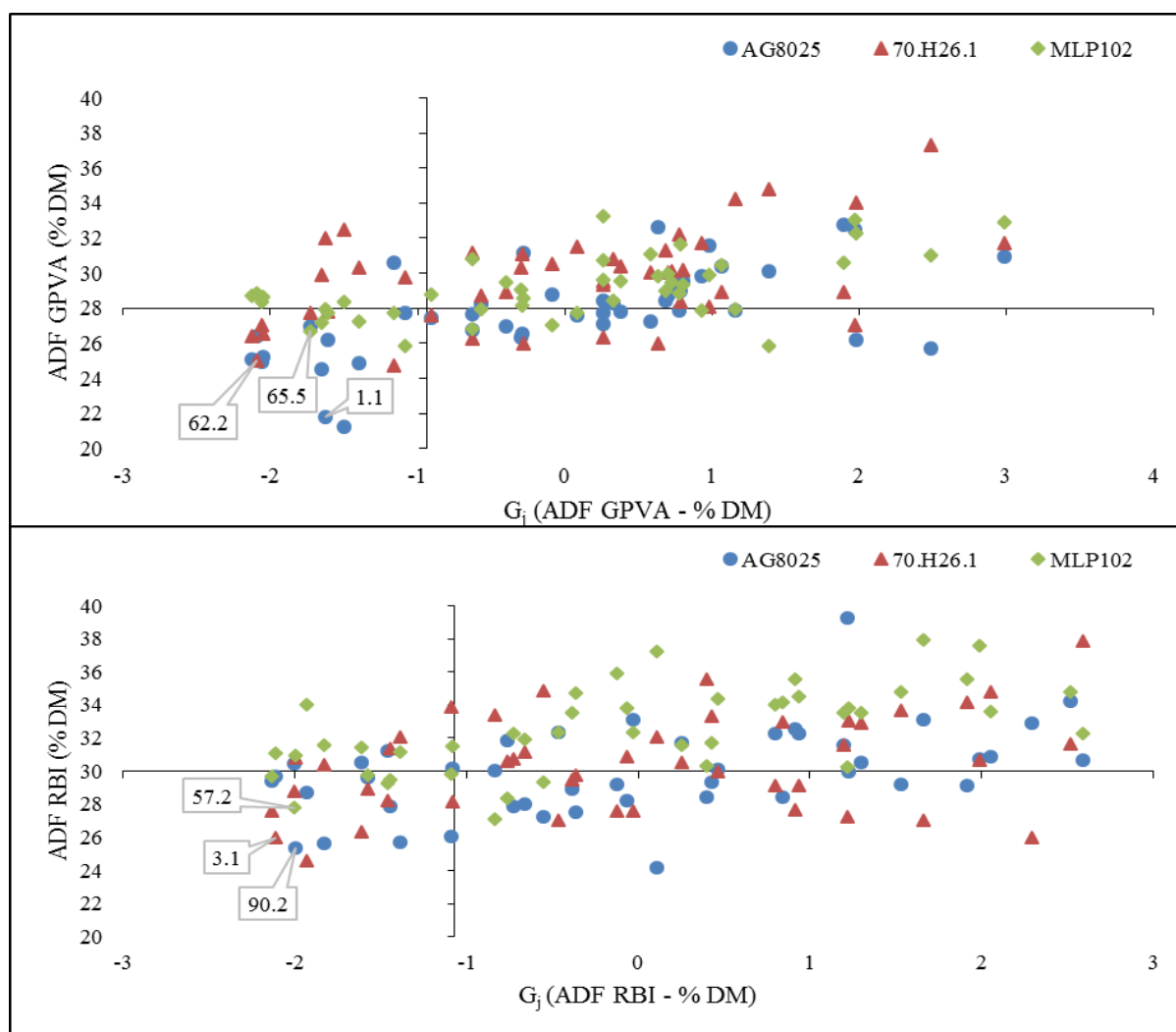
Figure 2. Dispersion among the means of acid detergent fiber (ADF) values of 135 topcross hybrids from the crossing of 45 S_3 progenies with testers AG8025, 70.H26.1 and MLP102 and estimates of the general combining ability (G_j). The experiment was conducted in Guarapuava (GPVA) and Rio Bonito do Iguaçu (RBI), Paraná State, Brazil, during the 2014/15 crop season. *The X-axis crosses the maximum acceptable ADF value, and the Y-axis crosses twice the standard deviation of G_j.

These disparities show the plasticity of these traits under genotypic and environmental effects. In both locations, all testers efficiently discriminated the progenies in terms of DIG. However, tester AG8025 stood out since it positioned the greatest number of topcross hybrids in the quadrant favorable for selection; i.e., it resulted in progenies with the highest means and favorable GCA values for DIG (twice the standard deviation) (Figure 4). Progeny 3.1 was positioned in the quadrant favorable for selection by tester AG8025 in both locations and by tester MLP102 in Guarapuava, while progeny 4.1 was chosen by testers MLP102 and 70.H26.1 in Rio Bonito do Iguaçu, Paraná State, Brazil (Figure 4).

Genotypes exhibiting DIG values higher than 60% have been reported in both locations and are considered good (Neumann, 2011); therefore, the variability among genotypes allows the selection of superior progenies. Tres et al. (2014) reported dry matter digestibility (*in vitro*) values of maize genotypes varying from 62.63% to 80.75%, while Mendes et al. (2015) reported an average of 62.98%.

In general, the traits presented little influence of the location, since the heritability estimates were high for all of them (Table 2), allowing greater gains to be achieved via selection.

In terms of the GCA estimates of the testers, AG8025 stood out for all traits in both locations, except for DIG in Rio Bonito do Iguaçu. Tester 70.H26.1 showed a favorable GCA only for ADF in Rio Bonito do Iguaçu, and tester MLP102 presented a favorable GCA for DMY and DIG in Rio Bonito do Iguaçu (Figure 5).

The above evidence showed that a single tester was not enough to determine the best progenies for all locations, making it necessary to use two or more testers when selecting progenies.
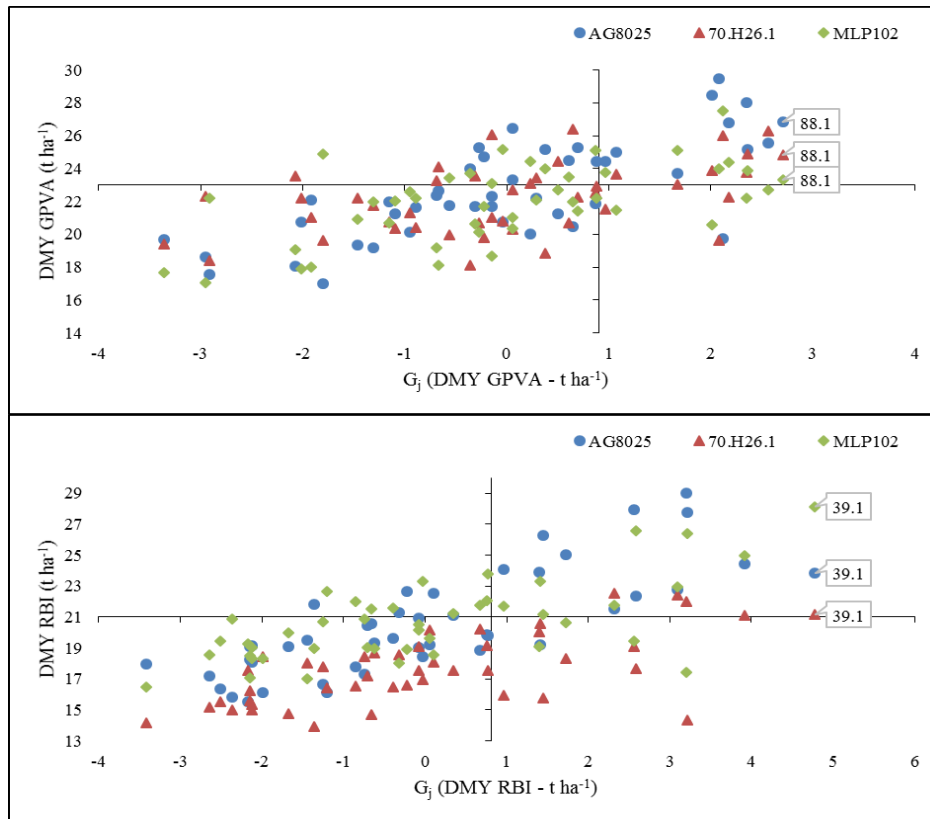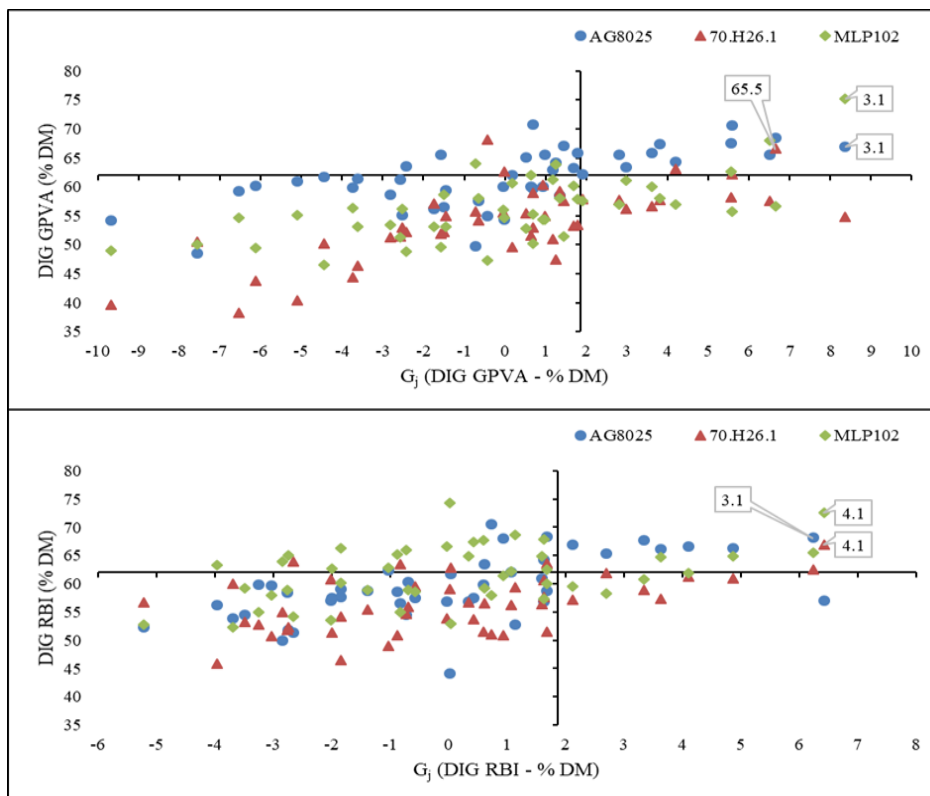
Figure 3. Dispersion among the means of forage dry matter yield (DMY, t ha^{-1}) of 135 topcross hybrids from the crossing of the 45 S$_3$ progenies with testers AG8025, 70.H26.1 and MLP102 and estimates of the general combining ability (G$_j$) evaluated in Guarapuava (GPVA) and Rio Bonito do Iguaçu (RBI), Paraná State, Brazil, during the 2014/15 crop season. *The X-axis crosses the maximum acceptable DMY value, and the Y-axis crosses twice the standard deviation of G$_j$.


Figure 4. Dispersion of the means of *in situ* digestibility of forage dry matter (DIG, t ha^{-1}) among 135 topcross hybrids from the crossing of 45 S$_3$ progenies with testers AG8025, 70.H26.1 and MLP102 and estimates of the general combining ability (G$_j$) evaluated in Guarapuava (GPVA) and Rio Bonito do Iguaçu (RBI), Paraná State, Brazil, during the 2014/15 crop season. *The X-axis crosses the maximum acceptable DIG value, and the Y-axis crosses twice the standard deviation of G$_j$.
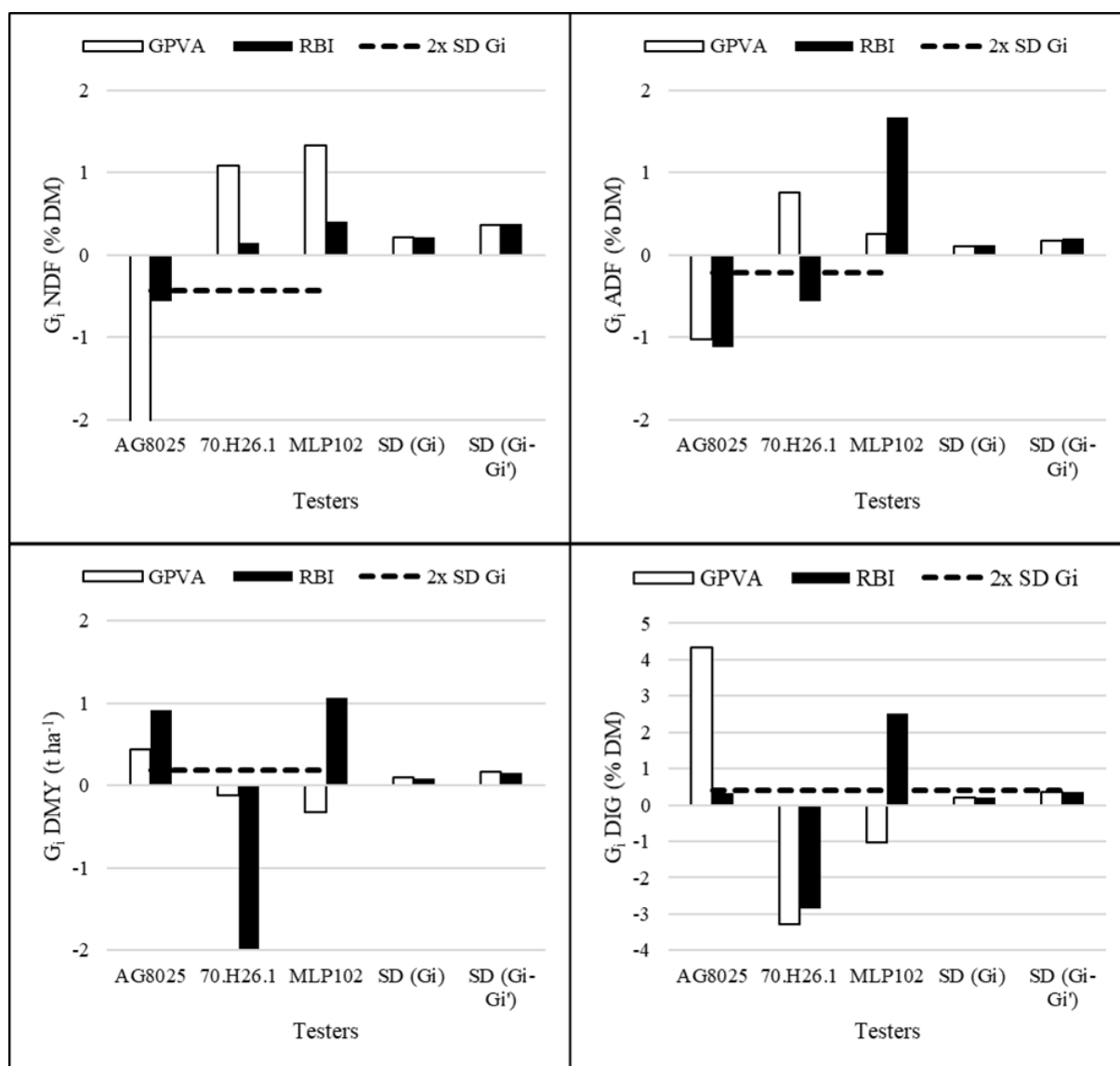
Figure 5. Estimates of the general combining ability (G_i) for testers AG8025, 70.H26.1 and MLP102 in crosses with 45 S$_3$ maize progenies for neutral detergent fiber (NDF) and acid detergent fiber (NDF) contents, dry matter yield (DMY, t ha^{-1}), and *in situ* digestibility of forage dry matter (DIG) evaluated in Guarapuava (GPVA) and in Rio Bonito do Iguaçu (RBI), Paraná State, Brazil, during the 2014/15 crop season.

Considering the GCA for NDF, progenies 3.1 and 39.1 could be selected because of their favorable GCA estimates in both locations. On the other hand, progenies 19.1, 62.2, 88.1, and 88.4 presented favorable GCA estimates for ADF in both locations (Figure 6) and could be used to improve these traits in the crosses in which they integrate.

Progenies 17.2, 47.1, 73.1, 79.1, and 90.2 could be selected to increase DMY since they presented favorable GCA estimates for DMY in both locations. Progenies 3.1, 22.1, and 51.1 are indicated for use in increasing DIG, as they presented favorable GCA estimates of DIG in both locations (Figure 6). Only progeny 3.1 stood out regarding more than one trait in both locations (NDF and DIG); therefore, it should be maintained in the breeding program and used in crosses to improve digestibility and reduce fiber content in forage of the resulting hybrids. The other progenies are equally relevant and can be used in crosses with complementary desirable traits.

Furthermore, in Guarapuava, progeny 22.1 stood out because of its favorable GCA estimates for all traits, while progeny 29.1 had favorable estimates for NDF, ADF, and DIG (Figure 6).

In Rio Bonito do Iguaçu, progenies 4.1, 39.2, and 47.1 had favorable estimates of GCA for increasing DMY and DIG (Figure 6), showing positive attributes for improving hybrid forage productivity and/or quality.
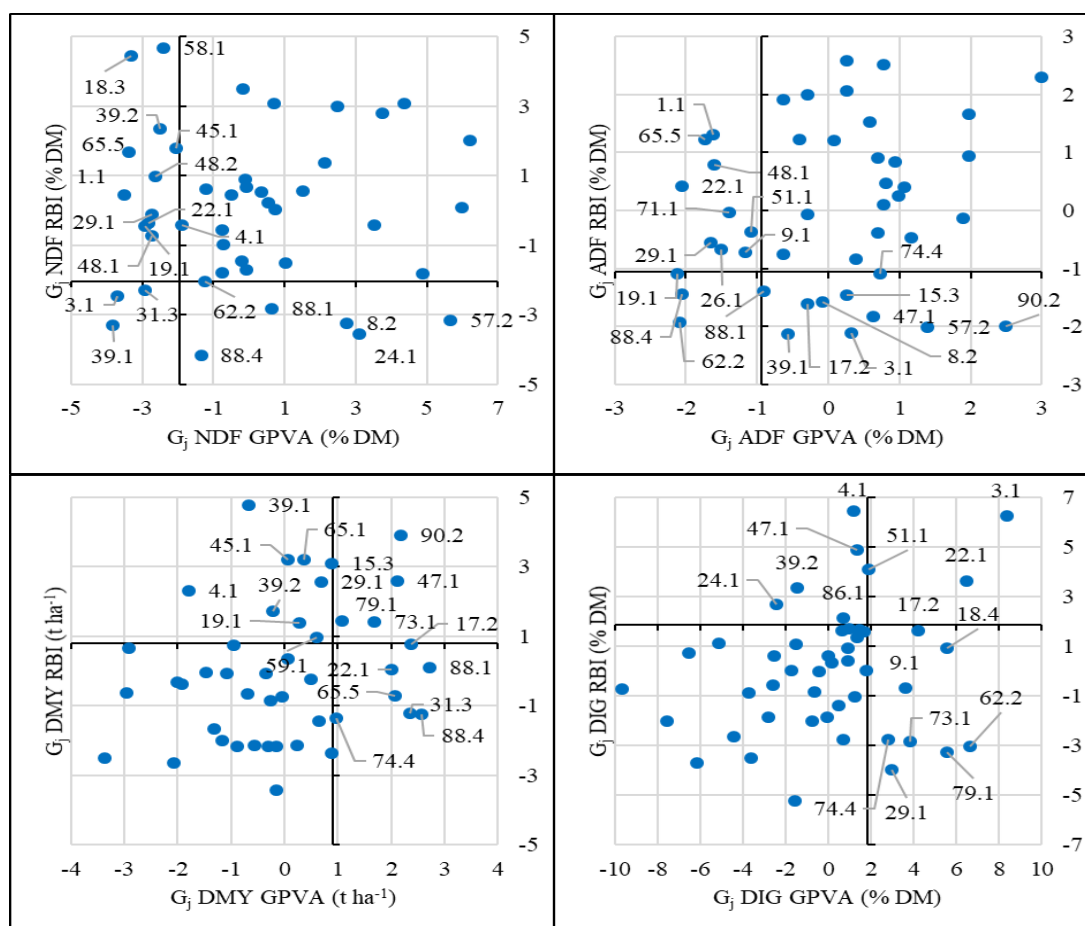
Figure 6. Estimates of the general combining ability (G$_j$) for the neutral detergent fiber (NDF) and acid detergent fiber (ADF) content, dry matter yield (DMY, t ha^{-1}), and *in situ* digestibility of forage dry matter (DIG) of 45 S$_3$ progenies in crosses with testers AG8025, 70.H26.1 and MLP102, evaluated in Guarapuava (GPVA) and Rio Bonito do Iguaçu (RBI), Paraná State, Brazil, during the 2014/15 crop season.

Conclusion

Progenies 3.1, 22.1 and 39.1 stood out in terms of NDF, ADF, and DIG, whereas progenies 47.1, 73.1, 79.1, and 90.2 stood out in terms of increased forage dry matter yield. These progenies should continue to undergo inbreeding into advanced generations and should be tested in crosses with other genotypes with the aim of complementing positive forage production traits.

More than one tester was necessary to correctly identify the assessed progenies. The testers AG8025 and 70.H26.1, of narrow genetic base, were the best to explore genetic variability among progenies and discriminate them.

Acknowledgements

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