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Forage potential of S₃ corn progenies in topcrosses and selection of testers of different genetic bases







Abstract – The objective of this work was to identify corn (*Zea mays*) genotypes with forage potential and to evaluate the efficiency of testers to discriminate forage traits in topcrosses, considering the contribution of additive and nonadditive genes. The experiment was carried out in the 2015/2016 and 2016/2017 crop seasons, in a randomized complete block design with three replicates. Thirty S₃ corn progenies were evaluated in topcrosses with the AG8025, P30B39, MLP102, 60.H23.1, and 70.H26.1 testers. The following traits were assessed: forage dry mass yield, neutral detergent fiber, acid detergent fiber, and forage dry mass degradability. Progenies 205.2, 159.6, and 199.2, in this order, presented the best performance for forage potential. Testers 60.H23.1 and 70.H26.1 better expressed the genetic variability between progenies. For all traits in both crop seasons, there is a predominance of the action of genes of nonadditive effects.

Index terms: *Zea mays*, additive effects, nonadditive effects, partial diallel.


Potencial forrageiro de progênies S₃ de milho em topcrosses e seleção de testadores de diferentes bases genéticas

Resumo – O objetivo deste trabalho foi identificar genótipos de milho (*Zea mays*) com potencial forrageiro e avaliar a eficiência dos testadores em discriminar características forrageiras em cruzamentos topcrosses, ao se considerar a contribuição de genes aditivos e não aditivos. O experimento foi conduzido nas safras de 2015/2016 e 2016/2017, no delineamento de blocos ao acaso, com três repetições. Avaliaram-se 30 progênies S₃ de milho em cruzamentos topcrosses com os testadores AG8025, P30B39, MLP102, 60.H23.1 e 70.H26.1. Foram avaliadas as seguintes características: produtividade de massa seca da forragem, fibra em detergente neutro, fibra em detergente ácido e degradabilidade da massa seca da forragem. As progênies 205.2, 159.6 e 199.2, nessa ordem, apresentaram o melhor desempenho para potencial forrageiro. Os testadores 60.H23.1 e 70.H26.1 melhor expressaram a variabilidade genética existente entre as progênies. Para todas as características nas duas safras, há o predomínio da ação de genes de efeitos não aditivos.

Termos para indexação: *Zea mays*, efeitos aditivos, efeitos não aditivos, dialelo parcial.

Jocimar Costa Rosa⁽¹⁾ ,
Marcos Ventura Faria⁽¹⁾ ,
Welton Luiz Zaluski⁽¹⁾ ,
Emanuel Gava⁽¹⁾ ,
Pedro Henrique Willemann Andreoli⁽¹⁾  and
Vitor Seiti Sagae⁽¹⁾ 

⁽¹⁾ Universidade Estadual do Centro-Oeste,
Departamento de Agronomia, Rua Simeão
Camargo Varela de Sá, nº 03, Vila Carli,
CEP 85040-080 Guarapuava, PR, Brazil.
E-mail: joce_jcosta@hotmail.com,
mfarria@unicentro.br,
luizzaluski@hotmail.com,
emanuel_gava2@hotmail.com,
pedrohwaandreoli@gmail.com,
vsagae@gmail.com

 Corresponding author

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Introduction

Corn (*Zea mays* L.) is one of the main crops used for forage production (Grignani et al., 2007). However, most corn breeding programs do not focus on the development of hybrids specifically for forage purposes (Nanavati, 2015), which would meet requirements such as high dry mass yield, good fiber quality, and high degradability, in order to obtain a good cost-benefit ratio and a high feed conversion ratio for the animals (Lei et al., 2018).

Topcrosses are one of the ways to improve the quality of progenies. However, studies on the selection of corn forage progenies and with more than three testers are still rare. This, in most cases, restricts a more accurate identification of the potential of different testers in discriminating forage traits and of the action of genes of additive and nonadditive effects, so far little discussed in the literature (Aslam et al., 2017).

In corn breeding programs, there is still not a consensus on how to select the best tester to discriminate the genetic potential of progenies (Lobato-Ortiz et al., 2010). Many authors concluded that the choice of the best tester should be based on genetic merit, with a high frequency of favorable alleles, as well as on genetic factors related to additive and nonadditive actions (Lobato-Ortiz et al., 2010; Pirondini et al., 2015; Seye et al., 2019). However, Hallauer et al. (2010) emphasized that the best tester is the one that simply classifies correctly the genetic merit of progenies based on estimates of genetic variance components, disregarding other information.

Because of the limited number of specific works related to the selection of appropriate testers for forage traits and the little information about the effects of gene action, estimates of genetic variance components, such as dry mass yield (DMY), neutral detergent fiber (NDF), acid detergent fiber (ADF), and dry mass degradability (DMD), have often also been neglected (Nanavati, 2015; Lei et al., 2018).

The fibrous portion represented by NDF and ADF is determinant in forage quality and its contents are inversely proportional to forage degradability (Lima et al., 2012). A higher NDF and ADF content in the corn forage used in cattle feed tends to limit voluntary consumption, resulting in a lower animal productive performance (Eastridge et al., 2017). Therefore, to assist in the selection of superior genotypes, it is important to carry out studies on the selection of

progenies and appropriate testers for forage traits, as well as to determine the action of the genes related to these traits.

The objective of this work was to identify corn genotypes with forage potential and to evaluate the efficiency of testers to discriminate forage traits in topcrosses, considering the contribution of additive and nonadditive genes.

Materials and Methods

Thirty partially inbred S_3 lines from the corn breeding program of Universidade Estadual do Centro-Oeste, in the state of Paraná, Brazil, were obtained by the conventional method, in which the SG6015 commercial hybrid was self-fertilized during five successive cycles (Hallauer et al., 2010). These S_3 progenies were crossed with four testers of a narrow genetic base – AG8025, P30B39, LEM 2 (60.H23.1), and LEM 3 (70.H26.1) –, and a tester of a wide genetic base – MLP102. Testers AG8025 and P30B39 are single-cross hybrids recommended for grain and silage production; LEM 2 and LEM 3 are elite inbred lines from the corn breeding program of Universidade Estadual de Maringá, also located in the state of Paraná; and MLP102 is a mixture of partially inbred lines of population 102 derived from the Penta hybrid. A total of 150 topcross hybrids were obtained.

Two experiments were carried out in the 2015/2016 and 2016/2017 crop seasons, which were considered two environments, in the municipality of Guarapuava, in the state of Paraná, Brazil (25°21'S, 51°31'W, at an altitude of 1.050 m). The soil is characterized as a Latossolo Bruno distroférrico (Santos et al., 2018), an Oxisol. The climate is Cfb, with average temperatures between 17 and 18°C and annual precipitation between 1,800 and 2,000 mm (Nitsche et al., 2019).

The 150 topcross hybrids were arranged in the field in a randomized complete block design, with three replicates. The 30 S_3 progenies and the five testers were also arranged in a contiguous area, with three replicates. The experimental unit in both crop seasons consisted of two contiguous 5.0-m rows spaced 0.45 m apart, equivalent to a density of 60.000 plants per hectare.

Pre-planting fertilization was performed with 450 kg ha⁻¹ 08-20-20 N-P₂O₅-K₂O + Zn. Two cover fertilizations were applied in the V4 and V7 stages, respectively, each with 90 kg ha⁻¹ N + 45 kg ha⁻¹ K₂O.

Plants were cut when the grains presented 2/3 of the milk line at the R5 phenological stage. Five plants of the line were cut at 0.2 m from the soil and weighed to obtain fresh mass, which was subsequently extrapolated to fresh mass yield (FMY, kg ha⁻¹). Then, the plants were grounded in the EN 6600 stationary forage grinder (Nogueira Máquinas Agrícolas, São Paulo, SP, Brazil) with a particle size from 1.0 to 2.0 cm. Fodder homogenization was carried out to remove 0.25-kg samples to obtain the percentage of forage dry mass (DM).

To estimate DM, samples were dried in an oven with forced-air circulation, at 55°C, for 72 hours, and then weighed. DM was obtained through the relationship between initial and final weights (Gao et al., 2015; Miller et al., 2018). From the DM and FMY data, the forage dry mass yield (DMY, kg ha⁻¹) was obtained according to Grignani et al. (2007). The dried forage samples were milled in the EDB-5 Willey-type mill (De Leo Equipamentos Laboratoriais, Porto Alegre, RS, Brazil) with a particle size of 1.0 mm, for further use in the neutral detergent fiber (NDF), acid detergent (ADF), and DMD analyses.

DMD was determined through a rumen fistula placed into a bovine of the Jersey breed, which was adapted to a diet with 100% corn silage during the 15 days prior to the evaluation. Each sample consisted of three replicates. Only one animal was used, in order to reduce the influence of the different protein and bacteriological concentrations of ruminal fluid in the analyzed forage due to the specific and individual characteristics of each animal, aiming to minimize experimental error (Wang et al., 2017). For rumen incubation, 10x15-cm polyester/nylon tissue bags containing 6 g of the sample dried at 55°C were used. After 24 hours of incubation, the minimum period considered adequate to obtain accurate results (Rezaeenia et al., 2014; Du et al., 2016; Lei et al., 2018), all bags were removed at the same time and dipped in cold water. Then, they were washed under running water until it flowed clear. The sachets were taken to a forced-ventilation oven, at 55°C, until constant weight, after which each bag was weighed. Results were expressed as percentage of initial DM.

Samples in the rumen were incubated during a 24-hour period; 40 samples were evaluated per period. Fiber contents were determined in the TE-149 equipment (Tecnal, Piracicaba, SP, Brazil) for brand

detergent and also using a neutral detergent solution (adding 0.5 mL thermostable alpha-amylase per sample) for NDF and an acid detergent solution for ADF (Van Soest et al., 1991).

The data were subjected to Bartlett's homogeneity test. Accepting the hypothesis of homogeneous variances, the individual and joint analyses of variance were performed. The individual and joint partial diallelic analyses were carried out according to the model proposed by Geraldi & Miranda Filho (1988). The general combining ability of progenies and testers and the specific combining ability of the topcross hybrids were estimated based on the joint analysis of the partial diallel with parental and F₁ hybrid combinations, using the Genes statistical software (Cruz, 2013).

Results and Discussion

The dispersion of the DM values showed that the general average was 30% (Figure 1), which was considered a very good result according to Neumann (2010). Therefore, the obtained values were adequate for forage evaluations, with a greater accuracy in the estimates.

The most efficient testers in the expression of genetic variability between progenies were 60.H23.1 and 70.H26.1 for DMY, 70.H26.1 for NDF and ADF, and 60.H23.1 for DMD (Table 1). There was a greater amplitude of DMY averages for topcross hybrids with the 60.H23.1 and 70.H26.1 testers, and 16 out of the 20 topcrosses with higher averages were obtained from the crossing with these testers (Figure 2). However, testers 60.H23.1 and 70.H26.1 did not repeat this favorable result for NDF and ADF, since, for these traits, lower averages are desirable (Pirondini et al., 2015), as those obtained by the AG8025 and P30B39 testers. For

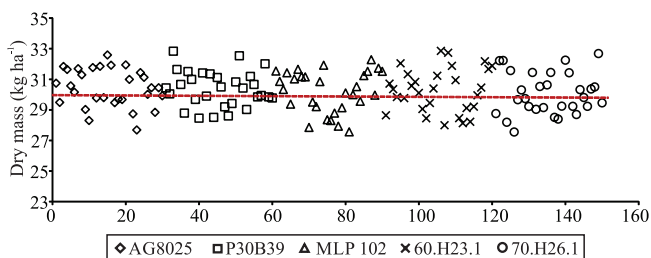


Figure 1. Dry mass averages used to calculate the evaluated corn (*Zea mays*) forage traits.

DMD, the 60.H23.1 tester provided the highest genetic variance and a median contribution to the increase of the averages in their respective crosses. These results show the importance of associating the estimates of genetic parameters with combining ability, as well as of the respective contribution of favorable alleles by the testers to correctly classify their merit (Hallauer et al., 2010; Lobato-Ortiz et al., 2010; Aslam et al., 2017).

In the diallel analysis, the contribution of the sum of squares of the specific combining ability (SCA) to DMY, NDF, ADF, and DMD was 57.94, 67.15, 82.40, and 74.79%, respectively (Table 2), showing a greater contribution of genes of nonadditive effects, differing from the expected performance of hybrids based on genitors with general combining ability (GCA). This result is contrary to that of the literature, which reports a greater contribution of additive genes to traits such as NDF, ADF, and DMD (Souza Filho et al., 2011; Gralak et al., 2014; Aslam et al., 2017).

Souza Filho et al. (2011) highlighted the action of genes of additive effect on NDF and ADF when evaluating corn hybrids from diallel crosses. Gralak et al. (2014) also found a greater contribution by genes of additive effect to traits such as NDF while studying

commercial hybrids in the central-southern region of Paraná. However, in the present work, the greatest contribution was from nonadditive genes, favoring hybrid exploration. Additive effects respond directly to the selection process, while nonadditive effects (dominance and epistasis) contribute to heterosis in certain combinations. Therefore, the predominance of genes of nonadditive effect in controlling forage traits may facilitate the process of corn breeding due to hybrid exploitation, which shows the importance of the results found in the present study (Abadi et al., 2011; Aslam et al., 2017).

The genotype x environment interaction was nonsignificant for all traits (Table 2). Likewise, a nonsignificant effect was observed for GCA testers x environments, GCA progenies x environments, and SCA x environments. These results are indicative of the consistency in the performance of the genotypes against environmental variations. In the literature, corn experimental hybrids in the central-southern region of Paraná showed significant interactions between combining ability estimates and different environments. This allows inferring that there was a differentiated response of the genotypes to

Table 1. Estimates of the components of variance and the genetic parameters of corn (*Zea mays*) forage quality evaluated in trials with topcross hybrids obtained with 30 S₃ corn progenies and five testers (AG8025, P30B39, MLP102, 60.H23.1, and 70.H26.1).

Genetic parameter	AG8025	P30B39	MLP102	60.H23.1	70.H26.1
Dry mass yield					
σ^2_G	26,822.83	16,252.80	8,154.69	29,463.12	35,028.65
σ^2	6,078.39	5,928.65	912.53	6,965.78	8,501.46
h_a^2	0.80	0.79	0.58	0.81	0.85
CVg	19.57	16.08	8.95	15.37	15.69
Neutral detergente fiber					
σ^2_G	2.91	0.87	1.61	1.38	15.13
σ^2	8.73	14.10	9.82	9.19	11.67
h_a^2	0.25	0.05	0.14	0.13	0.42
CVg	3.13	1.69	2.26	2.10	6.63
Acid detergente fiber					
σ^2_G	1.51	0.86	0.35	0.41	6.78
σ^2	5.23	8.37	9.04	6.07	5.15
h_a^2	0.22	0.09	0.03	0.07	0.56
CVg	4.26	3.10	2.00	2.18	8.71
Dry mass degradability					
σ^2_G	18.13	28.12	25.41	32.55	10.53
σ^2	14.34	16.15	18.55	13.41	17.44
h_a^2	0.55	0.63	0.57	0.71	0.03
CVg	8.35	10.35	10.06	11.61	1.51

⁽¹⁾ σ^2_G , genetic variance; σ^2 , residual variance; h_a^2 , broad-sense heritability; and CVg, coefficient of genetic variation.

environmental variations, disagreeing with the results obtained in the present work (Gralak et al., 2014; Marcondes et al., 2015).

As there is no significant effect on the genotype x environment interaction, it is possible to state that the results found in the present study are more consistent and that the genotypes are more promising for forage traits, given their consistent performance against environmental variations, combined with favorable averages (Vencovsky & Barriga, 1992; Ramalho et al., 2012).

When comparing the sum of squares of the GCA of testers and progenies, a greater contribution by progenies was observed regarding the assessed traits. Similar results were reported by Abadi et al. (2011) while evaluating corn hybrids with forage potential, which proves the effective merit of progenies and testers. This is desirable because the genetic merit of progenies allows the tester to proportionally influence all progenies; therefore, a high GCA is required for a tester to be considered adequate (Vencovsky & Barriga, 1992). Considering the GCA, it is possible to state that there are favorable genes for forage traits in testers AG8025 and P30B89, showing that these are genotypes with forage predisposition (Rezaenia et al., 2014).

The significance of the GCA and SCA estimates was based on 1.5 times standard deviation values, corresponding to a procedure similar to that of Rodovalho et al. (2012), who used values of twice the standard deviation as a criterion for significance. For NDF and ADF, negative estimates are desired to reduce averages, whereas, in contrast, for DMY and DMD, positive ones are preferred.

Tester 60.H23.1 presented higher GCA for DMY values, followed by tester P30B39 (Figure 3). AG8025 and P30B39 were favorable in reducing the average NDF and ADF contents, which directly reflects degradability and, consequently, animal conversion rate (Miller et al., 2018). AG8025 and P30B39 also contributed to positive GCA estimates for DMD. The P30B39 tester stands out for its high GCA in all forage traits, showing that it has favorable genes for increasing DMY and DMD averages and reducing NDF and ADF contents, which are distinct and controlled by several genes (Gao et al., 2015).

Regarding the GCA for DMY, progenies 226.3, 230.3, 205.2, and 16.5 were, in this order, those that contributed the most to the combining ability. In relation to NDF, only progeny 159.6 presented a favorable result for its reduction (Figure 4).

Table 2. Summary of the joint diallel analysis of the cycle conjunction between 30 S₃ corn (*Zea mays*) progenies and five testers evaluated in the 2015/2016 and 2016/2017 crop seasons⁽¹⁾.

Source of variation	Degree of freedom	Medium square			
		DMY	NDF	ADF	DMD
Genotypes	184	214.41**	61.24*	30.81*	172.07**
GCA testers	4	689.74	455.23	38.17	411.93*
GCA progenies	29	140.34	64.20	27.85	167.52**
SCA	150	152.39	50.44**	31.14*	157.85**
Environments (E)	1	0.8812	0.0756	0.0165	0.0064
Genotypes x environments	184	124.28	46.11	22.16	21.26
GCA testers x E	4	273.16	247.21	38.32	39.02
GCA progenies x E	29	98.64	34.70	16.41	10.74
SCA x E	150	123.22	34.04	22.06	22.86
Error	736	25.68	30.40	18.39	47.49
SSGCA testers (%)		6.99	16.16	2.69	5.2
SSGCA progenies (%)		10.32	16.52	14.25	15.34
SSGCA (%)		17.31	32.68	16.94	20.55
SSSCA (%)		57.94	67.15	82.40	74.79
Group (%)		24.75	0.17	0.66	4.66
Mean		28,653.47	56.35	28.68	49.18

⁽¹⁾GCA, general combining ability; SCA, specific combining ability; SSGCA, sum of squares of the general combining ability; SSSCA, sum of squares of the specific combining ability; DMY, forage dry mass yield; NDF, neutral detergent fiber; ADF, acid detergent fiber; and DMD, forage dry mass degradability. ** and *Significant by the F-test, at 1 and 5% probability, respectively.

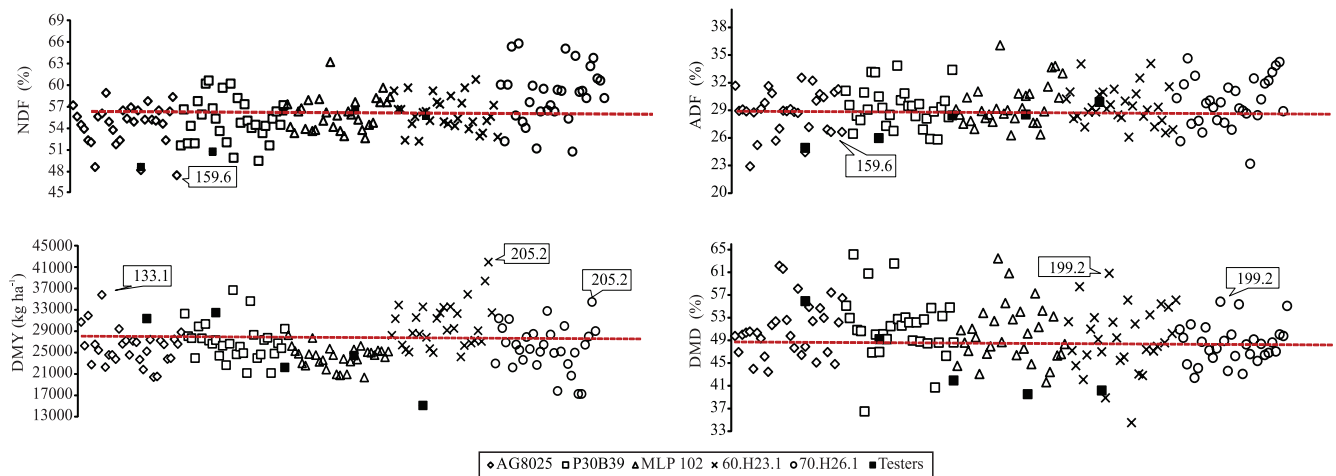


Figure 2. Averages of the S₃ corn (*Zea mays*) progenies for dry mass yield (DMY), neutral detergent fiber (NDF), acid detergent fiber (ADF), and dry mass degradability (DMD), evaluated in the 2015/2016 and 2016/2017 crop seasons. The horizontal lines indicate general averages.

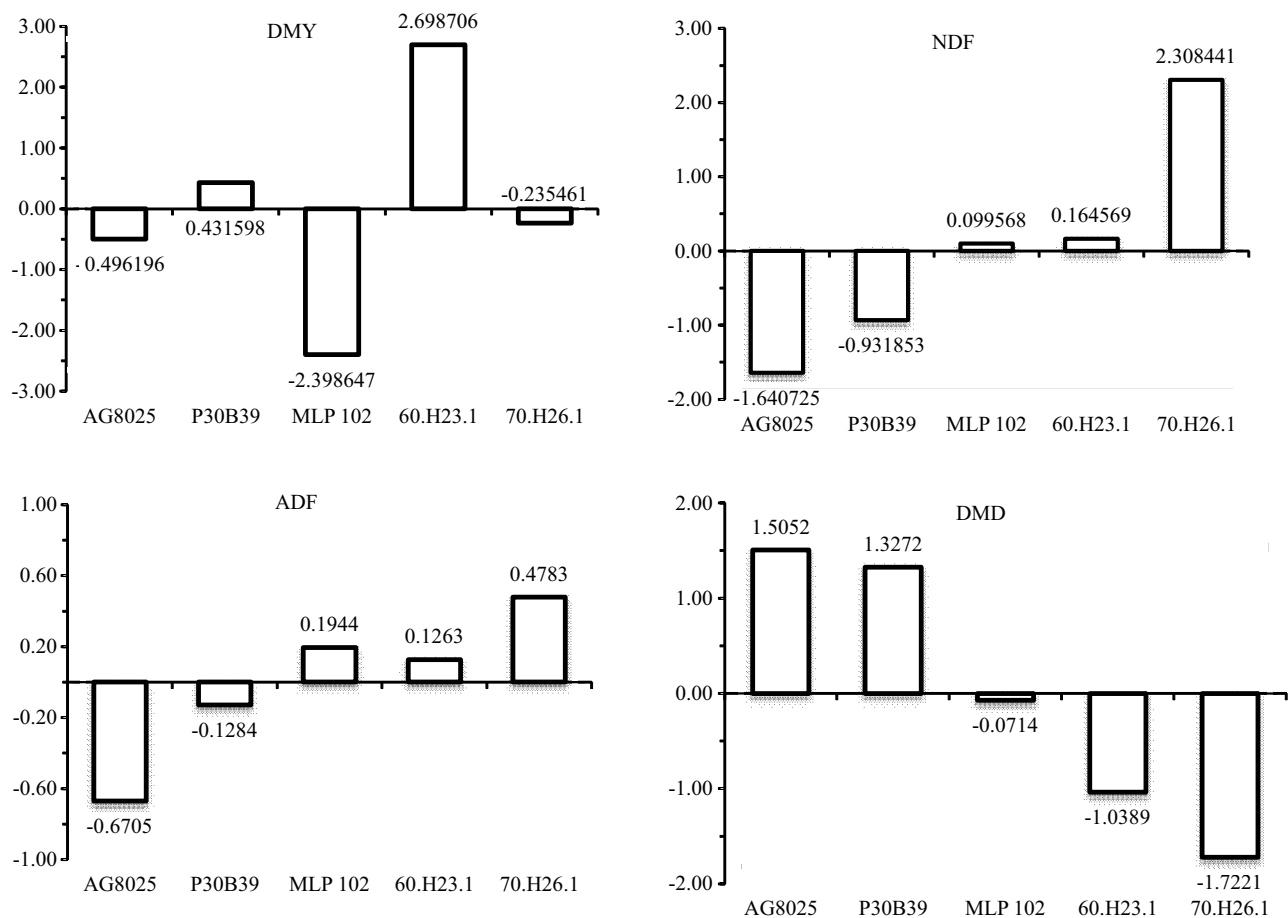


Figure 3. Averages of the general combining ability (GCA) of the tester for the traits forage dry mass yield (DMY), neutral detergent fiber (NDF), acid detergent fiber (ADF), and dry mass degradability (DMD) of corn (*Zea mays*) evaluated in the 2015/2016 and 2016/2017 crop seasons.

For ADF, once again, progeny 159.6 presented a favorable contribution to the GCA above the selection line. Progenies 172.1, 216.2, 195.2, and 41.3 also showed a favorable weight of genes that contribute to the reduction of the ADF content. Therefore, it is worth highlighting the favorable contribution of progeny 159.6 to both NDF and ADF in the crosses it participated in, since it is the most indicated progeny for these traits based on the GCA.

Regarding DMD, progeny 110.3 stood out because it contributed with the most genes of additive effects to increase this characteristic. Progeny 199.2 also contributed positively to the GCA, but with less relevance. These results favor future crossings that aim to increase DMD levels, mainly due to a high GCA coupled with high averages.

To find the best tester for each trait, the performances of the hybrids for both the GCA of the progenies that originated them and the SCA in the cross-section with each

tester were analyzed, seeking to express the best topcrosses and the greatest complementarity between the genitors.

Considering the association of the GCA and SCA with selection criteria greater than 1.5 times the standard deviation for DMY, progenies 205.2 and 133.1 had positive estimates for both of these combining abilities, respectively, with testers 70.H26.1 and 60.H23.1 and with tester AG8025 (Figure 5). In relation to genitors, tester 60.H23.1 and progeny 205.2 presented a high positive GCA. This is recommended because at least one of the parents should have a high GCA so that there is a real genetic gain in the performed crossings and also high averages (Bouvet et al., 2016).

Topcross 205.2 x 60.H23.1 presented the highest estimate for SCA (Figure 5), showing high complementarity among genitors. This crossbreeding generates a simple and prominent hybrid, besides presenting adequate averages for the desirable traits.

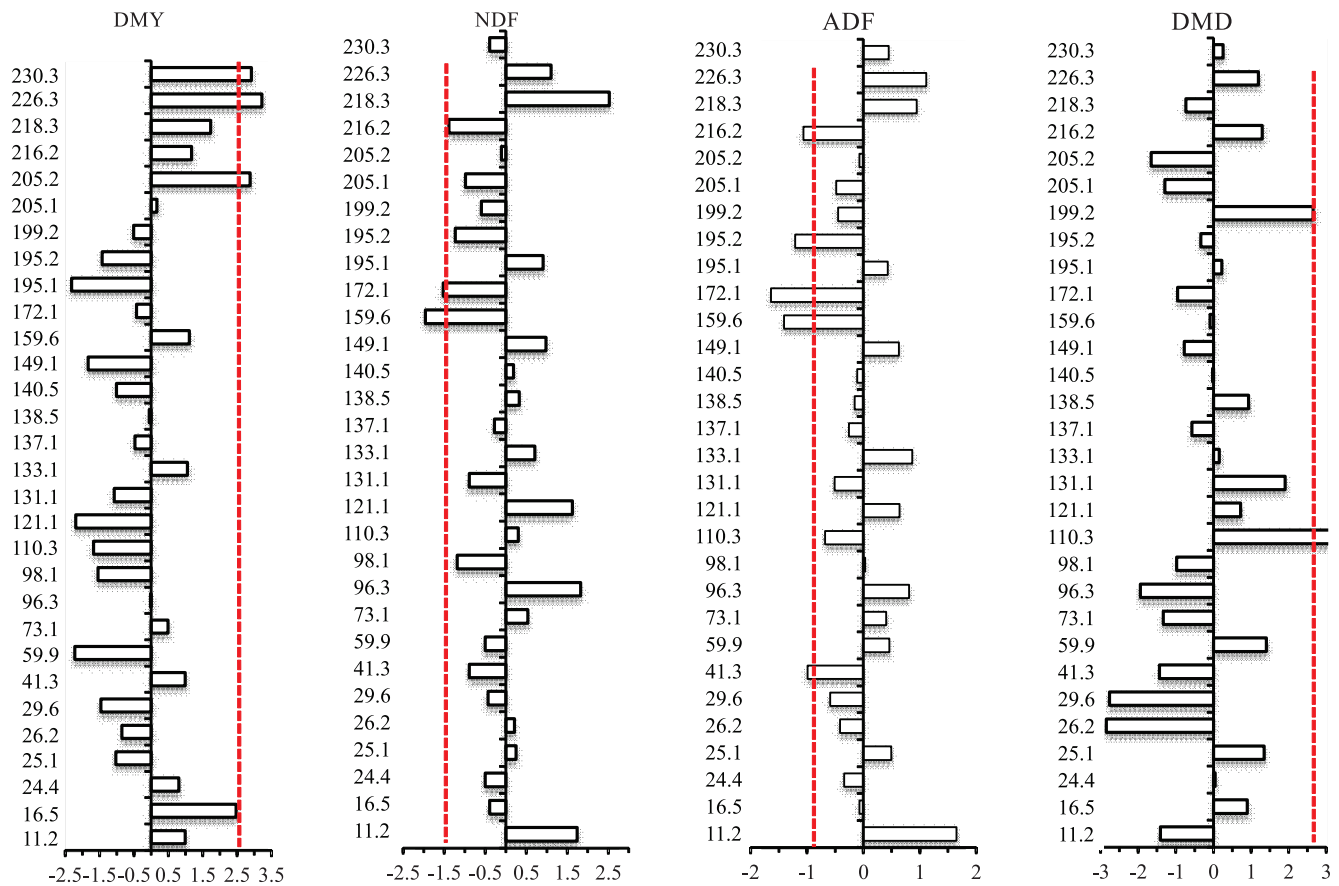


Figure 4. Estimates of the general combining ability (GCA) of the S₃ corn (*Zea mays*) progenies for dry mass yield (DMY), neutral detergent fiber (NDF), acid detergent fiber (ADF), and dry mass degradability (DMD), evaluated in the 2015/2016 and 2016/2017 crop seasons. The vertical lines indicate 1.5 times the standard deviation of the GCA.

This shows that, even in a single topcross, it is possible to have more than one goal during the breeding process, taking into account the performance of the genotype (Nanavati, 2015), in this case a potential hybrid.

For NDF, progeny 159.6 presented favorable estimates for the GCA, remaining above the selection criterion, in the same way that topcross 159.6 x

AG8025 had a low SCA. For ADF, once again, progeny 159.6 presented a favorable GCA value, whereas topcross 159.6 x AG8025 had one for SCA (Figure 5). Therefore, progeny 159.6 has the potential of reducing fibrous portions and of increasing digestibility percentage in future crossings, which is an important result due to the difficulty of obtaining genotypes that

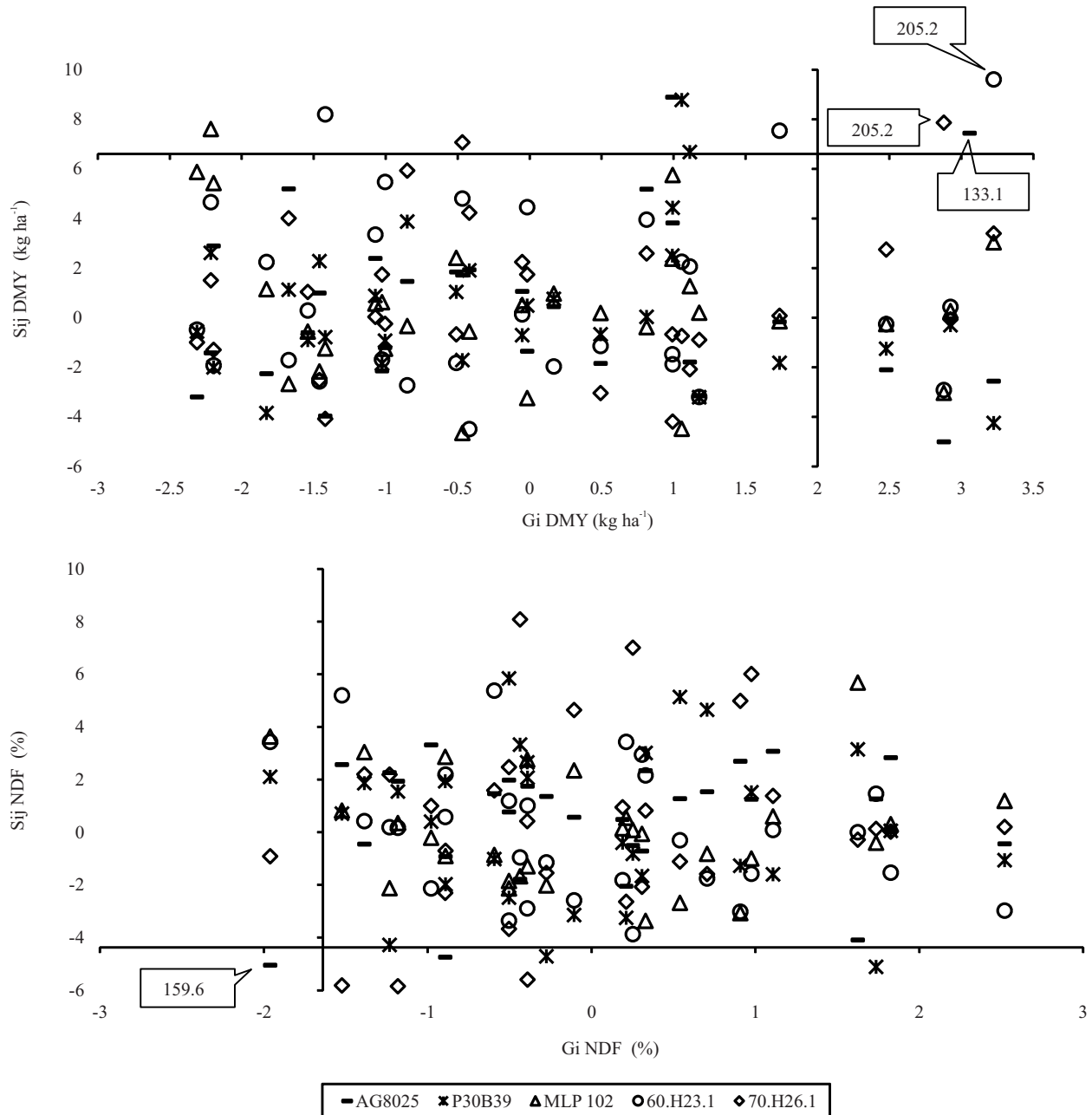


Figure 5. Dispersion of the estimates of the general combining ability (GCA) of corn (*Zea mays*) progenies (Gi) and of the specific combining ability (SCA) of the respective progenies with testers (Sij) in the 2015/2016 and 2016/2017 crop seasons, for the traits dry mass yield (DMY) and neutral detergent fiber (NDF). The vertical and horizontal axes pass through the values of 1.5 times the standard deviation for GCA and SCA, respectively.

favor the reduction of the fiber contents (Pirondini et al., 2015; Seye et al., 2019), which evidences a higher concentration of genes that allow reducing NDF and ADF contents.

With regard to DMD, progeny 199.2 presented positive GCA estimates, as well as of SCA in crossbreedings with the elite test lineages. The 199.2

x 60.H23.1 and 199.2 x 70.H26.1 topcrosses stood out, reaffirming the merit and good complementarity of these progenies in the generation of promising hybrids (Figure 6). For DMD, it can be concluded that progeny 199.2 contributes to favorable genes for high degradability due to its high GCA and SCA at crosses, allowing the further exploration of this trait in hybrids. It

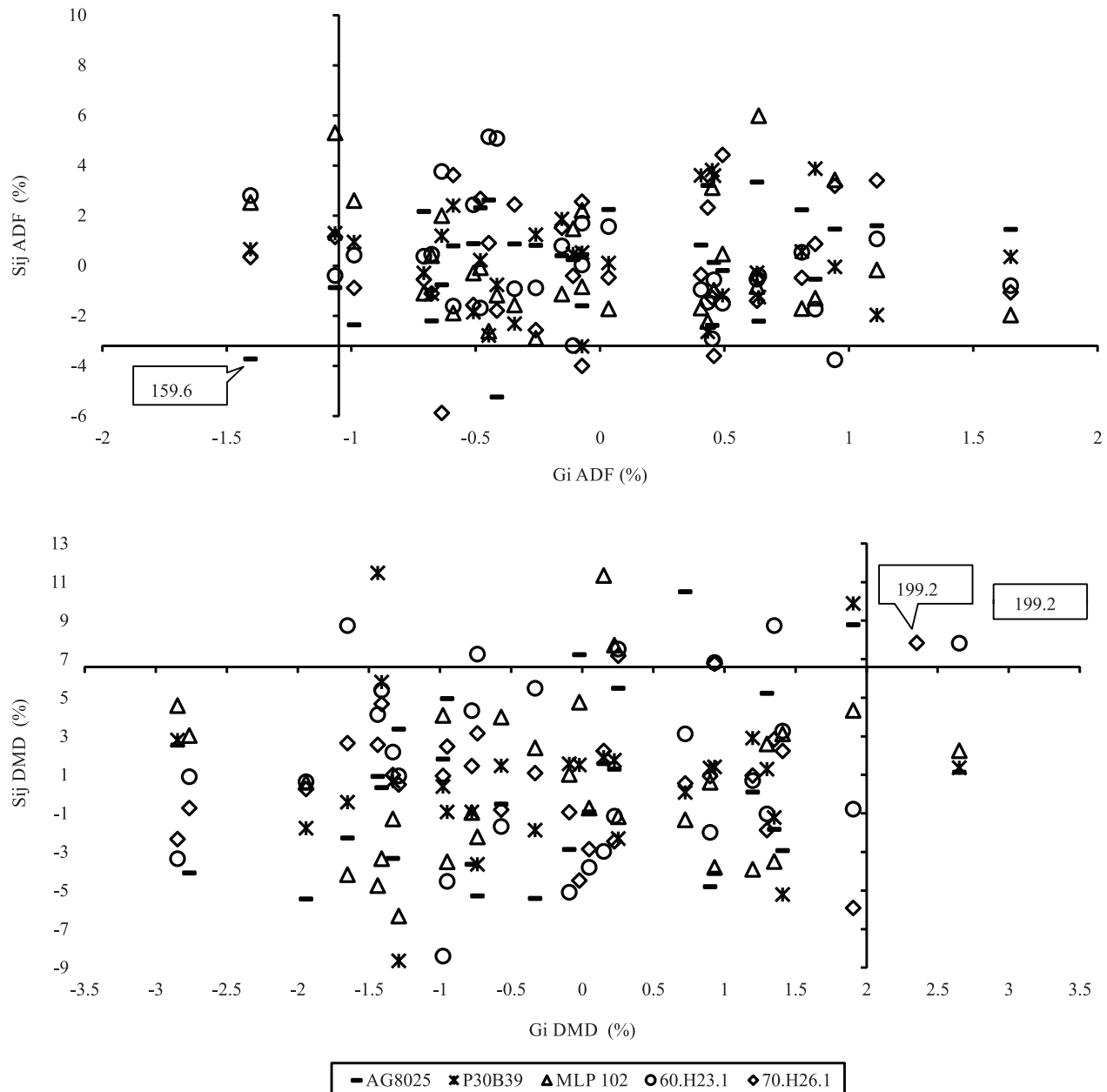


Figure 6. Dispersion of the estimates of the general combining ability (GCA) of corn (*Zea mays*) progenies (Gi) and of the specific combining ability (SCA) of the respective progenies with testers (Sij) in the 2015/2016 and 2016/2017 crop seasons, for the traits acid detergent fiber (ADF) and dry mass degradability (DMD). The vertical and horizontal axes pass through the values of 1.5 times the standard deviation for GCA and SCA, respectively.

is worth mentioning a possible recombination between progenies 159.6 and 199.2 to combine favorable genes for the reduction of ADF and NDF through progeny 159.6 and for high degradability through progeny 199.2, considered the favorable averages for both (Figure 3). Abadi et al. (2011), Bouvet et al. (2016), and Li et al. (2017) reported similar results, while evaluating combining ability.

Significant variations were observed regarding the traits expressed by the evaluated topcrosses. This result can be attributed to the genetic variability between progenies and testers, pointing out the great potential of using progenies to generate promising hybrids. Even genotypes that did not present a high DMY were favorable to other traits of interest in breeding, such as low NDF and ADF or high DMD contents, which could serve as a source of variability for the generation of new forage hybrids (Abadi et al., 2011; Seye et al., 2019).

Conclusions

1. Testers 60.H23.1 and 70.H26.1 better express the genetic variability between corn (*Zea mays*) progenies.
2. Progenies 205.2, 159.6, and 199.2 show the best performance for forage traits.
3. For all the traits, there is a predominance of genes of nonadditive effects.

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