











# Combining ability and heterotic pattern in relation to F1 performance of tropical and temperate-adapted sweet corn lines

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**ABSTRACT:** The aims of this study were: to assess the genetic relationship of supersweet corn populations; and to establish the heterotic pattern of 49 supersweet (*sh2*) corn inbreds on F2S5 generation based on molecular marker data and specific combining ability. Forty-nine inbreds were evaluated using 20 SSR molecular markers, which were allocated into heterotic groups according to the discriminant principal component analysis. Twelve inbreds were crossed in a complete diallel scheme. The 81 entries (hybrids developed, parental lines and three commercial checks) were evaluated in a triple partial balanced lattice design (9 × 9) during the growing seasons of 2016/2017 and 2017. The general combining ability (GCA) and specific combining ability (SCA) were estimated. The SCA values were used to set the heterotic patterns of the parental lines as well. Commercial yield without husk (CYWH) and ear length (EL) were more informative to set the heterotic groups. Additive and non-additive effects were important on the genetic control of the evaluated traits. However, for five of the six traits, the non-additive/dominance genetic effects showed to be more important in both environments. In fact, the hybrids developed among tropical by temperate germplasm had better performance than those ones developed within the temperate germplasm itself. SSR based-genetic distance demonstrate to be a reliable predictor as significant correlation was obtained between genetic distance with hybrid performance (for length of ears, ear height and CYWH) and SCA for all observed traits. The non-additive genetic effect that predominantly controlled all traits was the feasible explanation for the good prediction.

**Key words:** shrunken, *Zea mays*, SSR genetic distance, hybrid performance.

## INTRODUCTION

In some countries like United States of America and Canada, the adoption of supersweet corn shrunken-2 (*sh2*) has revolutionized the industry and the fresh market, (Tracy et al. 2020). In Brazil, the sweet corn market is restricted to processing, and the main allele exploited is *sh2*. In 2012 only in Brazil, the total seed market was around U\$ 7 million dollars, and it was on the ninth position among the vegetable crops in terms of value (ABCSEM 2014).

As opposed to modern field corn, sweet corn breeding programs do not have well defined heterotic patterns in order to guide the development of most promising hybrids, populations and inbreds (Revilla and Tracy 1997). Some authors have reported significant heterotic effect with high magnitude for yield traits on sweet corn (Solomon et al. 2012a, 2012b, Yuwono et al. 2017, Demail et al. 2020).

Modern hybrids, then, are the result of crossing an inbred line from one heterotic pattern with an inbred from a different heterotic pattern. Nowadays, inbreds are classified into heterotic groups and are further sub-divided into families within a heterotic group (Lee and Tracy 2009). Classification of heterotic patterns is generally based upon several criteria such as pedigree, molecular marker-based associations, and performance in hybrid combinations (Laude and Carena 2015). Limited labor, cost, and time regarding hybrid formation and yield trials encourages corn breeders to identify potential F1 hybrids without crossing all possible combinations by line screening based on SSR-genetic distance (Dermail et al. 2020). The use of DNA marker data has been useful to complement pedigree information and assign diverse lines into heterotic groups (Senior et al. 1998, Barata and Carena 2006), but with limiting usefulness for predicting good heterotic combinations. Therefore, evaluating the performance of crosses among groups based upon genetically diverse parents has been considered essential to identify promising heterotic patterns (Laude and Carena 2015). Utilizing data from not only molecular information (e.g., marker data), but also from yield trials (e.g., testcrosses and mating designs) is an alternative (Barata and Carena 2006), as long as there is a good methodology to link both types of data.

In that sense, several authors have been studying the association of molecular data, mainly based on polymorphism observed with SSR markers, specific combining ability, heterosis and F<sub>1</sub> performance on sweet corn in order to define the heterotic pattern of the targeted germplasm (Solomon et al. 2012a, 2012b, Dermail et al. 2020). All of them have emphasized the importance of non-additive variance controlling the main yielding traits, but they did not find a great correlation between this effect with genetic distance obtained by molecular data yet.

The aims of this study were to assess the genetic relationship of supersweet corn populations adapted to Brazilian tropical growing condition; and to establish the heterotic pattern of 49 supersweet (*sh2*) corn inbreds on F<sub>2</sub>S<sub>5</sub> generation based on molecular marker data and specific combining ability among the selected parental lines.

## MATERIAL AND METHODS

A total of 49 supersweet (*sh2*) corn inbreds belonging to Sakata Seed Sudamerica Ltda. was used in this study. Fifteen inbreds were extracted from a base-population designated as PopTe1. This base-population, originated in Thailand, was developed crossing tropical by temperate germplasm and it is adapted to tropical growing conditions, keeping some superior cob and kernel quality traits typically from temperate supersweet corn. So, it will be referred as temperate background to distinguish from the other source used in this study. Twenty-four inbreds were extracted from a base-population designated as PopTe2, with the same origin of the previous one and quite similar behavior, some of the inbreds have the brachytic2 gene (*br2*). A third base-population was used in this study, and ten inbreds were extracted from it. This base-population designated as PopTr is coming from tropical field corn germplasm introgressed with shrunken-2.

Initially, nine inbreds were selected, three from each base-population, aiming to check whether any of the SSR molecular markers used in previous similar study (Lopes et al. 2015) would not set large polymorphic information and good distribution along the 20 maize chromosomes for the targeted germplasm. Sixteen seeds of each of the 49 inbreds were sown at trays, and leaf tissues were collected from eight germinated plantlets by inbred line. All SSR molecular markers used at this stage are available on <http://www.maizegdb.org> and were previously used in similar studies in field corn and sweet corn (Lopes et al. 2015). Twenty SSR molecular markers were selected to run this study, totalizing 7,840 DNA samples.

Genomic DNA was isolated according to the methodology described by Hoisington et al. (1994), with few modifications. Based on this data, Roger's genetic distance was obtained.

Genetic relationships among the inbreds were examined by applying the discriminant analysis of principal components (DAPC) on the 20 SSR markers using the Adegenet package of R software. The function DAPC was executed using the clusters identified by K-means (Legendre and Legendre, 1998). The number of clusters was assessed using the function `find.clusters`, evaluating a range from 1 to 40. The optimal number of clusters was chosen on the basis of the lowest associated Bayesian information criterion (BIC) (Jombart 2008).

Based on pedigree, data from previous characterization trials (data not shown) and the DAPC analyses, 12 out of 49 inbreds were selected to create a complete diallel design following the method II proposed by Griffing (1956). For that,

five inbreds from PopTe1, five inbreds from PopTe2, and two inbreds from PopTr (Table 1) were crossed generating 66 F1 combinations. Parental lines were also self-pollinated.

**Table 1.** Parental lines used in the diallel crosses, genetic group (measured by DAPC) and morphological traits.

Parental line	Source population	Group (DAPC)	Brachytic plant	Ear (length/format)
1	PopTe1	10	No	Medium/conic
2	PopTe1	15	No	Medium/conic
3	PopTe1	10	No	Medium/conic
4	PopTe1	4	No	Medium/conic
5	PopTe1	4	No	Medium/conic
6	PopTe2	6	Yes	Short/conic
7	PopTe2	6	Yes	Short/conic
8	PopTe2	2	No	Medium/conic
9	PopTe2	16	No	Medium/conic
10	PopTe2	13	Yes	Short/conic
11	PopTr	8	No	Long/cylindric
12	PopTr	11	No	Long/cylindric

DAPC: discriminant analysis of principal components.

The 66 diallel crosses developed with their 12 parental lines and three checks, Tropical Plus and Thunder Attribute (Syngenta Seeds) plus AF428 (Sakata Seed Sudamerica Ltda.), were evaluated in a triple lattice design  $9 \times 9$  at Bragança Paulista Research Station ( $23^{\circ}\text{S}$ ;  $47^{\circ}\text{W}$ ), owned by Sakata Seed Sudamerica Ltda., during the main growing season of 2016/2017, and second growing season of the year 2017. Standard agronomic practices were applied to both trials. Plots were three 5-m rows spaced of 0.9 m and containing 25 plants per row after thinning. Data was collected from the center row, and the following traits were evaluated: average of plant height (PH), measured from 10 plants from soil up to the top leaf insertion (m); average of ear height (EH), measured from the same ten plants from the soil up the insertion of the highest ear on the stem (m); total yield of all ears harvest at the middle row with husk converted to  $\text{t}\cdot\text{ha}^{-1}$  (TYWH); commercial yield of ears ( $\geq 15$  cm) without husk harvested at the middle row converted to  $\text{t}\cdot\text{ha}^{-1}$  (CYWH); average length (cm) of eight commercial ears (EL); and average number of kernel rows from eight commercial ears (KR).

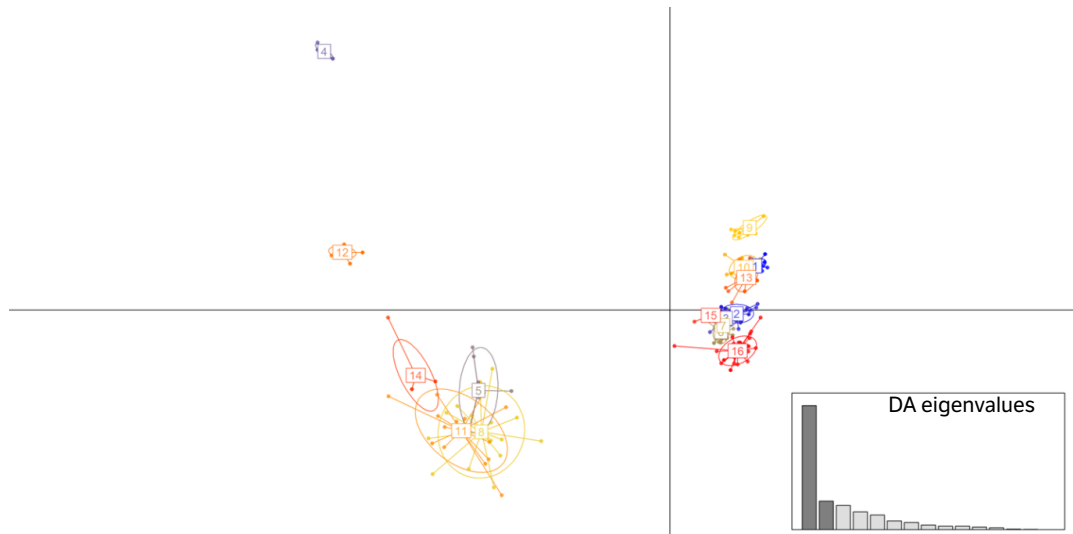
Intrablock analysis of variance recovering the interblock information according to Silva et al. (1999) was implemented in order to obtain the adjusted means by the software Genes (Cruz 2013). Before proceeding with the joint variance analysis of the two growing seasons evaluated (2016/2017 and 2017), the homogeneity of mean squares from the mean effective residual from individual variance analysis was checked. To run the joint analysis, a premise considering the ratio equal or below 4:1 between the highest and lowest residual variance according to Pimentel-Gomes (2000) was used.

The balanced diallel and its joint analysis were run according to method II proposed by Griffing (1956). Specific combining ability effect was considered as genetic distance in a  $(12 \times 12)$  matrix. In order to transform all effects to positive magnitude, a constant was added following the study conducted by Pinto et al. (2001). Aiming to identify which traits contributed to explain the largest variance of the data set, a principal component analysis with the respective specific combining ability effects according to the significance from the joint diallel analysis was run. Based on the selected genetic distance matrix, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method was performed. Aiming to check the association among the genetic distance obtained by molecular data, the genetic distance obtained from the specific combining ability effects and the mean for different traits evaluated, Pearson's correlation coefficients was calculated (Pearson 1896). Also, a correlation network among all the variables studied was established.

All analyses described were done using GENES statistic software (Cruz 2013), except the principal component analysis, that was performed through the "rgl" package; and the correlation network that was performed through "qgraph" package installed at R statistic program. The R statistic program is available at <http://www.r-project.org/>.

## RESULTS AND DISCUSSION

The forty-nine *sh2* inbreds evaluated in this study were distributed in 16 groups according to the DAPC performed using 20 SSR molecular markers (Fig. 1).



**Figure 1.** Scatterplots with the distribution of 49 supersweet corn inbred lines obtained from three base populations based on discriminant analysis of principal components analysis done with genetic information from 20 SSR molecular markers.

Inbreds from PopTe1 were allocated into the groups 1, 3, 9, 10 and 15. In groups 2, 6, 7, 13 and 16, the inbreds were placed coming from PopTe2, and in groups 4, 5, 8, 11, 12 and 14 from PopTr. There was no group containing inbreds coming from different base-population. Also, it was possible to identify three main clusters: the first one at the upper right, containing the major part of the inbreds coming from PopTe1, except the group 13, which grouped inbreds from PopTe2; the second one at downright containing the major part of the inbreds coming from PopTe2, except the groups 3 and 15, which grouped inbreds from PopTe1; and the third cluster at down left containing the major part of the inbreds coming from PopTr. Groups 4 and 12 were the farthest groups at this study, but inbreds coming from them were dropped due the lack of fitting between silks and tassels flowering to produce seeds on F1 crosses. According to the distribution obtained in Fig. 1, inbreds located into the first and second clusters (PopTe1 and PopTe2) showed to be genetically closer than those ones located into the third cluster comprised by inbreds from PopTr.

There was significant difference ( $p < 0.05$ ) among the genotypes studied for all traits evaluated, as well as for the environments where trials were located (Table 2). Genotypes by environments interaction was significant only for EH and TYWH. Unfolding genotypes degrees of freedom, it was possible to identify significant difference among the hybrids developed within PopTe1 and PopTe2, as well as among the populations PopTe1  $\times$  PopTe2, PopTr  $\times$  PopTe1, and PopTr  $\times$  PopTe2 for most of the traits evaluated.

Regarding the orthogonal linear contrast applied, the most important ones were: contrast two (PopTe1  $\times$  PopTe2 + PopTe1 + PopTe2) vs. (PopTr  $\times$  PopTe1 + PopTr  $\times$  PopTe2), contrast four ((PopTe1  $\times$  PopTe2) vs. (PopTe1 + PopTe2)), and contrast five (PopTe1 vs. PopTe2). Contrast two aiming to check whether there was a great contribution in crosses among inbreds coming from the pure tropical population (PopTr), with the inbreds from the temperate background (PopTe1 and PopTe2) against the crosses among inbreds coming from the two temperate populations. For this contrast, significant difference for all traits evaluated was identified, except for KR (Table 2). Looking at the negative contrast estimative magnitude, it was possible to assume that hybrids developed among tropical by temperate germplasm were superior than those ones developed within the temperate germplasm itself. For TYWH, the contrast estimative was positive under growing season 2016/2017 and negative under growing season 2017, even though the estimative from growing season 2017 was greater than estimative obtained on growing season 2016/2017, demonstrating the superiority of hybrids developed among tropical by temperate germplasm.

**Table 2.** Joint variance analyses for 12 parental lines, their 66 F1s combinations and three checks evaluated during growing seasons 2016/2017 and 2017 for plant height (PH), ear height (EH), total yield of ears with husk (TYWH), commercial yield of ears without husk (CYWH), ear length (EL) and number of kernel rows (KR).

Source of variation	DF	Mean square					
		PH (m)	EH (m)	TYWH (t·ha <sup>-1</sup> )	CYWH (t·ha <sup>-1</sup> )	EL (cm)	KR
Genotypes	(80)	0.411*	0.186*	78.711*	40.320*	12.176*	5.274*
Hybrids (F1)	(65)	0.247*	0.131*	26.367*	14.789*	4.522*	3.817*
PopTe1	9	0.037ns	0.018*	41.508*	11.927*	6.518*	1.570*
PopTe2	9	0.135*	0.054*	39.827*	14.476*	0.974*	3.505*
PopTe1 × PopTe2	24	0.209*	0.040*	21.152*	16.898*	2.172*	3.716*
PopTr × PopTe1	9	0.051ns	0.019*	23.723*	9.299*	2.699*	5.711*
PopTr × PopTe2	9	0.019ns	0.017*	11.106*	5.152ns	1.681*	4.442*
Contrast one (C1)	1	0.248*	0.037*	0.166ns	6.966ns	2.381*	17.101*
Contrast two (C2)	1	3.832*	2.736*	20.678*	53.541*	116.221*	0.006ns
Contrast three (C3)	1	0.393*	0.586*	8.947ns	43.551*	3.693*	0.013ns
Contrast four (C4)	1	0.478*	0.099*	96.961*	83.832*	10.825*	2.633*
Contrast five (C5)	1	3.917*	3.119*	33.999*	0.186ns	1.853*	2.100*
Parental lines (P)	11	0.494*	0.196*	10.227*	4.558ns	4.668*	6.010*
Checks (C)	2	0.230*	0.323*	34.021*	50.172*	13.977*	9.567*
F1 vs. C	1	0.219*	0.136*	51.119*	13.358*	15.199*	38.063*
P vs. (F1+C)	1	10.658*	3.430*	4,351.354*	2,100.471*	585.592*	50.559*
Environments (E)	1	4.618*	3.799*	2,325.465*	322.470*	819.500*	47.515*
Genotypes × E	(80)	0.061ns	0.012*	8.366*	3.678ns	0.559ns	0.565ns
Hybrids (F1) × E	(65)	0.061ns	0.009ns	8.526*	4.095*	0.577ns	0.591*
PopTe1 × E	9	0.011ns	0.016*	6.444ns	4.694ns	0.248ns	0.858*
PopTe2 × E	9	0.011ns	0.012ns	5.907ns	1.740ns	0.577ns	0.500ns
(PopTe1 × PopTe2) × E	24	0.142*	0.007ns	12.294*	3.099ns	0.511ns	0.454ns
(PopTr × PopTe1) × E	9	0.016ns	0.017*	3.275ns	5.664*	0.677ns	0.717ns
(PopTr × PopTe2) × E	9	0.002ns	0.005ns	4.062ns	4.086ns	0.666ns	0.419ns
Contrast one (C1) × E	1	0.001ns	0.001ns	2.792ns	4.442ns	0.879ns	0.198ns
Contrast two (C2) × E	1	0.131ns	0.004ns	29.549*	16.655*	0.227ns	0.283ns
Contrast three (C3) × E	1	0.008ns	0.004ns	25.961*	11.418*	1.868*	0.434ns
Contrast four (C4) × E	1	0.028ns	0.001ns	17.847*	3.283ns	0.149ns	1.826*
Contrast five (C5) × E	1	0.003ns	0.003ns	5.777ns	10.347ns	2.628*	2.322*
P × E	11	0.061ns	0.015*	7.810ns	2.296ns	0.412ns	0.466ns
C × E	2	0.020ns	0.013ns	2.815ns	0.211ns	0.858ns	0.317ns
(F1 vs. C) × E	1	0.157ns	0.058*	23.134*	0.392ns	0.879ns	0.185ns
[P vs. (F1+C)] × E	1	0.031ns	0.038*	0.396ns	1.991ns	0.113ns	0.870ns
Mean effective error	272	0.050	0.008	4.656	2.948	0.452	0.431
Contrast estimative		Mean environment(m)/growing season 2016/2017(1)/growing season 2017(2)					
Contrast one (C1)		13.34m	5.18m	-10.89m	-70.57m	41.26m	-110.5m
Contrast two (C2)		-38.66m	-32.7m	17.541	-63.911	-212.9m	-1.54m
				-197.152	-225.092		
Contrast three (C3)		1.14m	1.39m	3.841	-5.881	-1.011	0.21m
				-14.762	-18.222	-6.012	
Contrast four (C4)		8.47m	3.85m	68.861	112.14m	40.29m	3.331
				172.342			36.422
Contrast five (C5)		3.61m	3.22m	10.64m	-0.79m	5.441	-5.431
					-0.472	0.142	

DF: degrees of freedom; \*significant at 5% level; ns: no significant; C1: PopTr vs. (PopTe1 × PopTe2 + PopTe1 + PopTe2 + PopTr × PopTe1 + PopTr × PopTe2); C2: (PopTe1 × PopTe2 + PopTe1 + PopTe2) vs. (PopTr × PopTe1 + PopTr × PopTe2); C3: (PopTr × PopTe1) vs. (PopTr × PopTe2); C4: (PopTe1 × PopTe2) vs. (PopTe1 + PopTe2); C5: PopTe1 vs. PopTe2.

In case of contrast four, it was significant for all traits evaluated. Considering the positive contrast estimative, it was possible to notice the superiority of hybrids developed among the two temperate populations despite of the behavior from hybrids developed within each temperate population. Lastly, for contrast five, it was not observed significant difference only for CYWH. The contrast estimative showed the superiority of hybrids developed within PopTe1 when compared to hybrids developed within PopTe2, except for EL on growing season 2017 and KR on growing season 2016/2017 with negative magnitude.

GCA was significant for all traits, except for CYWH. Also,  $GCA \times E$  was significant for all evaluated traits (Table 3). Regarding SCA, effect was significant for all the traits, whereas  $SCA \times E$  was only significant for TYWH. Additive and non-additive variance were important in controlling the traits evaluated. However, considering the quadratic components, the SCA effect showed to be more important for all traits and in both environments of this study. One exception was KR, in which the magnitude of GCA and SCA quadratic components effects were quite similar.

**Table 3.** Joint diallel variance analyses for 12 parental lines and their 66 F1s combinations evaluated during growing seasons 2016/2017 and 2017 for plant height (PH), ear height (EH), total yield of ears with husk (TYWH), commercial yield of ears without husk (CYWH), ear length (EL) and number of kernel rows (KR).

Source of variation	DF	Mean square					
		PH (m)	EH (m)	TYWH (t·ha <sup>-1</sup> )	CYWH (t·ha <sup>-1</sup> )	EL (cm)	KR
Genotypes	(77)	0.42*	0.18*	80.85*	40.59*	11.84*	4.83*
GCA	11	1.32*	60.24*	60.24*	11.79ns	14.76*	21.34*
SCA	66	0.27*	84.28*	84.28*	45.39*	11.35*	2.08*
Environments (E)	1	4.76ns	3.47ns	2,328.45nss	315.56ns	778.78ns	4708ns
Genotypes × E	(77)	0.06ns	0.01*	8.31*	3.81ns	0.55ns	0.58ns
$GCA \times E$	11	0.13*	0.03*	14.06*	7.50*	1.44*	0.86*
$SCA \times E$	66	0.05ns	0.01ns	7.36*	3.19ns	0.40ns	0.53ns
Error	272	0.050	0.008	4.656	2.948	0.452	0.431
Quadratic component of effects							
GCA <sup>1</sup>	--	0.012	0.008	0.745	0.043	0.165	0.277
SCA <sup>1</sup>	--	0.039	0.011	13.206	6.025	1.741	0.249
GCA <sup>2</sup>	--	0.020	0.010	0.802	0.276	0.184	0.232
SCA <sup>2</sup>	--	0.033	0.014	14.239	8.204	1.851	0.331
Ratio GCA/SCA1	--	0.308	0.727	0.056	0.007	0.0945	1.112
Ratio GCA/SCA2	--	0.606	0.714	0.056	0.034	0.099	0.701

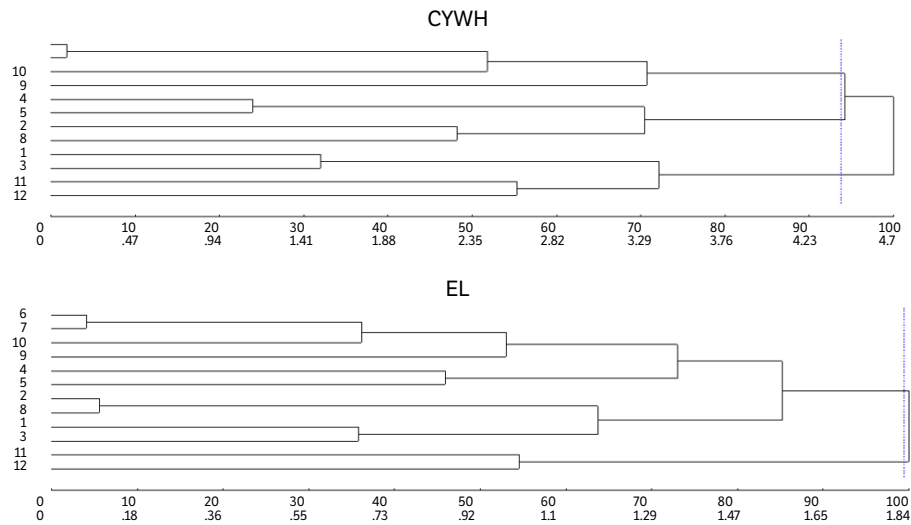
DF: degrees of freedom; GCA: general combining ability; SCA: specific combining ability; \*significant at 5% level; ns: no significant; <sup>1</sup>growing season 2016/2017; <sup>2</sup>growing season 2017.

For PH, the most interesting hybrids were:  $6 \times 7$  (-0,23),  $4 \times 5$  (-0,21) and  $2 \times 7$  (-0,11). For EH, also  $6 \times 7$  (-0,12) and  $4 \times 5$  (-0,11) were the hybrids with best SCA estimates. Hybrids  $3 \times 6$  (0,84),  $4 \times 7$  (0,83),  $4 \times 12$  (0,8),  $2 \times 12$  (0,78) and  $10 \times 12$  (0,74) highlighted due their SCA for KR. On the opposite, hybrids  $11 \times 12$  (-1,08) and  $4 \times 5$  (-0,85) had the lowest SCA estimates. Among the hybrids evaluated,  $8 \times 9$  (0,55) and  $7 \times 8$  (0,46) were developed using inbreds coming from the same base-population and highlighted for their SCA.

Specific combining ability for EL, TYWH and CYWH were estimated. Some hybrids had large SCA estimates for most of these traits, like:  $1 \times 10$ ,  $2 \times 11$ ,  $3 \times 8$ ,  $4 \times 6$ ,  $5 \times 6$ ,  $5 \times 7$ ,  $5 \times 10$ ,  $7 \times 11$ ,  $7 \times 12$ , and  $8 \times 12$ . On the other hand,  $2 \times 4$ ,  $3 \times 5$ ,  $4 \times 5$ ,  $6 \times 7$ ,  $7 \times 9$ ,  $7 \times 10$ , and  $8 \times 10$  had the lowest SCA estimates. Out of the hybrids mentioned, it was worth to point out hybrids  $1 \times 11$ ,  $1 \times 12$ ,  $6 \times 12$  and  $9 \times 12$  with good SCA for EL;  $2 \times 7$  and  $4 \times 10$  with good SCA estimates for TYWH on growing season 2016/2017, and  $2 \times 8$  also with high SCA for TYWH under growing season 2017.

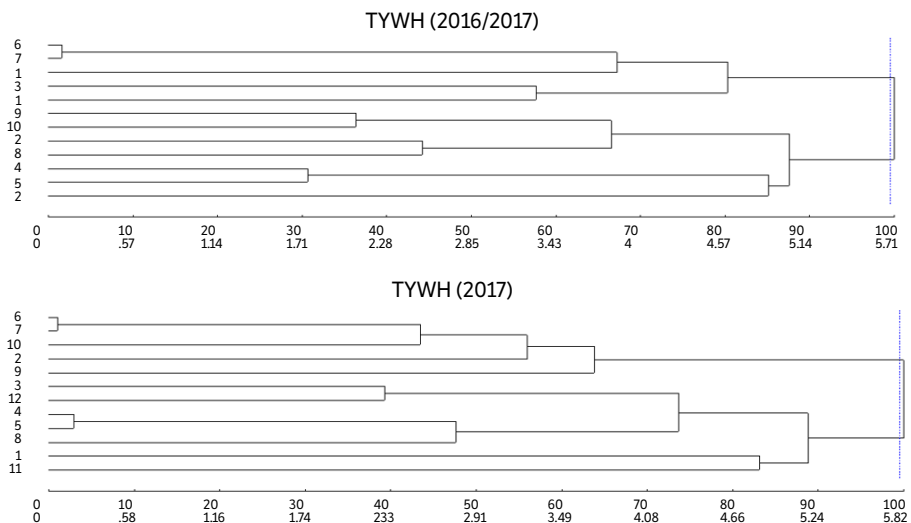
Based on the discriminant analysis of principal components run with SCA effects (data not shown), three traits that conferred the largest part of the total variance were selected. SCA of EL, CYWH and TYWH were considered as genetic distance to run UPGMA hierarchical analysis among the 12 *sh2* inbreds used as parental line in a complete diallel crossing block scheme. In Fig. 2, it is presented the association for CYWH and EL. Three groups were established concerning CYWH:

group 1 with parental lines 6, 7, 9 and 10; group 2 with parental lines 2, 4, 5 and 8; and group 3 with parental lines 1, 3, 11 and 12. There was a tendency to allocate the parental lines coming from the common base population into the same group: PopTe2, PopTe1 plus PopTe2 and PopTr, at groups 1, 2 and 3, respectively. Two groups were established concerning EL: group 1 with parental lines 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10; and group 2 with parental lines 11 and 12. Again, groups formed allocated the most divergent inbreds, according to what is expected based on its background (PopTr), from the rest of the inbreds which are coming from most similar background (PopTe1 and PopTe2).



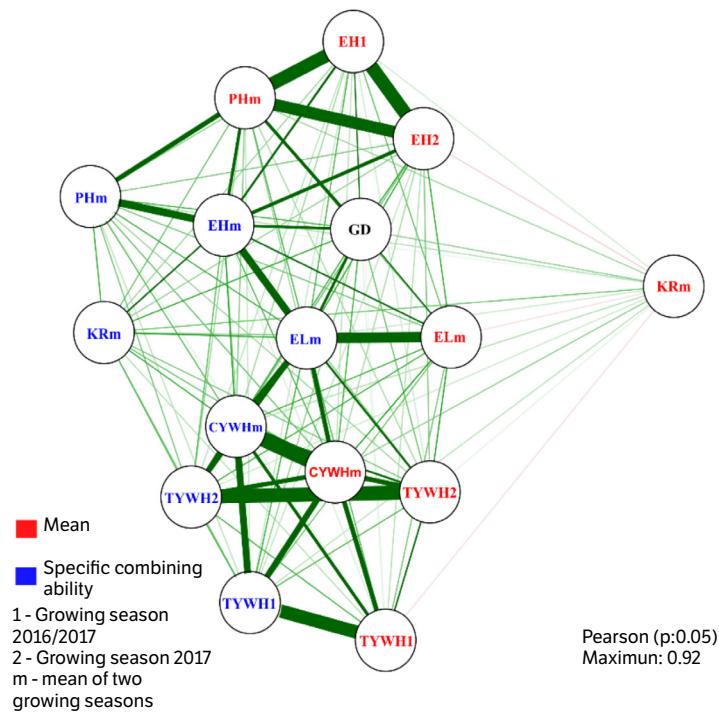
**Figure 2.** Association among 12 parental supersweet corn (*sh2*) inbreds revealed by Unweighted Pair Group Method with Arithmetic Mean based on mean specific combining ability on growing seasons 2016/2017 and 2017 for commercial yield of ears without husk (CYWH) and ear length (EL).

In Fig. 3, it is presented the association for TYWH growing season 2016/2017 and TYWH growing season 2017. Two groups were established in both cases. Concerning the growing season 2016/2017, group 1 allocated parental lines 1, 3, 6, 7 and 11; group 2, parental lines 2, 4, 5, 8, 9, 10 and 12. For the growing season 2017, group 1 allocated parental lines 2, 6, 7, 9 and 10; and group 2, parental lines 1, 3, 4, 5, 8, 11 and 12. For this trait, the distinguishment among the different groups has low evidence to be strong correlated to what is expected based on pedigree information.



**Figure 3.** Association among 12 parental supersweet corn (*sh2*) inbreds revealed by Unweighted Pair Group Method with Arithmetic Mean based on specific combining ability on growing seasons 2016/2017 and 2017 for total yield of ears with husk (TYWH).

The association level among the mean values for PH, EH, TYWH, CYWH, EL and KR with their respective SCA estimates, and Roger's genetic distance obtained through 20 SSR molecular markers are presented in Fig. 4. Correlation was significant for most of the associations studied, except for among GD with SCA and mean of TYWH measured in the growing season 2016/2017. Also, there was no significant correlation between GD and mean for KR mean environment. Despite of the correlation significance in most of the cases, it was possible to identify the greater correlation value for SCA and its respective means for TYWH, CYWH and EL. When GD was associated with SCA and mean, the correlation values were lower than those ones obtained by the association among SCA and their respective means.



**Figure 4.** Correlation network of 66 supersweet corn (*sh2*) hybrids based on Pearson's correlation ( $p = 0.05$ ). Red and green lines represent negative and positive correlation, respectively. Line width is proportional to the correlation strength. Variables evaluated were genetic distance (GD), plant height (PH), ear height (EH), total yield of ears with husk (TYWH), commercial yield of ears without husk (CYWH), ear length (EL) and number of kernel rows (KR).

Some efforts have been made on tropical sweet corn breeding programs in order to diversify the germplasm pool by crossing tropical by temperate background to generate heterotic groups (Solomon et al. 2012b). These heterotic groups have been defined with the purpose of commercial sweet corn hybrid development, but inbreds used to develop these hybrids have not had their genetic effects extensively studied in sweet corn breeding programs.

In this context, the identification of heterotic groups and the knowledge about the genetic relationship among populations of tropical and temperate sweet corn constitute the first step to create better complementary breeding populations. Many researchers indicate that multivariate techniques (i.e., cluster, PCA, PCoA or multidimensional scaling) have been considered as a useful tool for classify the specialty corn germplasm (Laude and Carena, 2015). In this study, DAPC and UPGMA clustering methods generated based on molecular markers and phenotypic traits, respectively, represented well the relationship among the inbreds that was successfully discriminated according to the temperate (Thailand) and tropical (Brazil) base-population origin. This result was important to define the inbreds used to design the complete diallel evaluated and consequently identify the heterotic patterns of these populations. Furthermore, our clustering results were comparable to similar studies conducted in sweet corn, in which the lines were clustered on the basis of origin (Solomon et al. 2012a, Roy and Kim 2016). The last is very useful considering that the heterotic patterns in sweet corn are poorly defined, and the



genetic relationship of the inbred lines that generates these heterotic groups have been scarcely studied (Revilla and Tracy 1997, Solomon et al. 2012a).

Sweet corn breeding programs focuses mainly on the use of hybrid vigor, which is dependent on the genetic diversity between parental lines. Here, the temperate and tropical populations studied showed significant difference for all the evaluated traits. Also, for most of the evaluated traits, significant differences were observed among the hybrids developed within PopTe1 and PopTe2, as well as among the populations PopTe1 × PopTe2, PopTr × PopTe1 and PopTr × PopTe2. These results demonstrate the presence of enough genetic variance to exploit different combinations among inbreds studied and improve the selection gain. In addition, the contrast estimates contributed to differentiate the best combinations for all the traits. The hybrids developed among tropical by temperate germplasm had better performance than those ones developed within the temperate germplasm itself, except for number of kernel rows trait (KR). This superiority was also observed by different researchers who crossed tropical maize with temperate USA germplasm demonstrating the substantially greater utility of tropical hybrids and inbreds as breeding parents (Goodman 2004). According to our results, for total yield measured as TYWH, the contrast estimates demonstrate the superiority of hybrids developed among tropical by temperate germplasm. Consequently, the diversification of the Brazilian tropical maize germplasm may be used in hybrid combinations without yield penalty in tropical environments. Further, in our study, the comparison between the temperate populations showed the superiority of hybrids developed within PopTe1 when compared to hybrids developed within PopTe2, except for EL and number of KR on growing seasons 2017 and 2016/2017, respectively.

This study showed that additive and non-additive effects were important into the genetic control of the evaluated traits. However, for five of the six traits evaluated, the non-additive/dominance genetic effects showed to be more important in both environments. According to Falconer and Mackay (1996), heterosis is largely a function of non-additive/dominance genetic effects. In sweet corn, on the basis of GCA/SCA ratio, Dickert and Tracy (2002) reported that traits with high heterosis had very low GCA/SCA ratios and vice versa. In our study, we showed that the yield component traits (TYWH, CYWH and EL) showed the lowest values for the GCA/SCA ratio, suggesting that heterosis level is a function of non-additive genetic effects. These results are in agreement with Solomon et al. (2012a), who reported considerable level of heterosis in most economically important traits in sweet corn.

In this study, the correlation between molecular marker distance (GD) and F1 performance was significant, and the strength of association was moderate for EL, EH and CYWH ( $r > 0.5$ ). In field corn, moderate to strong association ( $r > 0.60$ ) between F1 yield traits and SSR-based distance in tropical maize (Reif et al. 2003) and European maize (Phumichai et al. 2008) was also reported. However, different studies conducted in sweet corn showed that correlation between GD with F1 performance were in most of the cases insignificant, suggesting that the potential of markers to predict hybrid performance and heterosis represented a controversial result (Solomon et al. 2012a, 2012b, Demail et al. 2020). On the other hand, it seems that correlation between GD with F1 performance depend on the type of germplasm and markers. In sweet corn, Solomon et al. (2012a) reported the absence of association between GD and F1 performance suggesting that the observed genetic diversity may not guarantee the development of improved hybrids. In a recent study, Demail et al. (2020) mentioned that the gene effects and germplasm type were suspected as responsible factors, affecting poor correlation between GD and F1 performance. On the other hand, as suggested by Bernardo (1992), effective prediction of hybrid performance and heterosis based on molecular markers would be feasible when dominance effect are strong. This probably confirm our findings, because in our study non-additive genetic effect predominantly controlled all observed traits, and SSR based-genetic distance showed moderate correlation with important economically traits (TYWH, EL and CYWH).

## CONCLUSION

Additive and non-additive effects were important into the genetic control of the evaluated traits. However, for five of the six traits, the non-additive/dominance genetic effects showed to be more important in both environments. In fact, the hybrids developed among tropical by temperate germplasm were superior than those ones developed within the temperate germplasm itself, demonstrating to be the heterotic patterns to be exploited. SSR based-genetic distance demonstrated to

be a reliable predictor as a significant correlation was obtained between GD with hybrid performance (for EL, EH, and CYWH) and SCA for all observed traits. The non-additive genetic effect that predominantly controlled all traits was the feasible explanation for the good prediction.

## AUTHORS' CONTRIBUTION

**Conceptualization:** Souza Neto, I. L., Figueiredo, A. S. T., Scapim, C. A., Conteras-Soto, R. I., Zanutto, M. D. and Uhdre, R. S. **Methodology:** Souza Neto, I. L., Scapim, C. A. and Zanutto, M. D. **Investigation:** Souza Neto, I. L., Figueiredo, A. S. T., Conteras-Soto, R. I., Zanutto, M. D. and Scapim, C. A. **Writing – Original Draft:** Souza Neto, I. L., Scapim, C. A. and Uhdre, R. S. **Writing – Review and Editing:** Souza Neto, I. L., Scapim, C. A., Uhdre, R. S. and Zanutto, M. D. **Resources:** Souza Neto, I. L., Figueiredo, A. S. T., Conteras-Soto, R. I., Zanutto, M. D. and Uhdre, R. S. **Supervision:** Zanutto, M. D.

## DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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