



Combining ability and genetic divergence among tropical maize inbred lines using SSR markers

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ABSTRACT. Our objectives were to evaluate general and specific combining ability (SCA) and genetic divergence among tropical maize inbred lines using single sequence repeat (SSR) markers. Thirteen inbred lines were crossed based on a complete diallel scheme. Hybrids and three checks were evaluated in a lattice experimental design. Silk and anthesis flowering, average plant height, average ear height, white spot (*Pantoea ananatis*) and gray leaf spot (*Cercospora zea-maydis*) severity, and grain yield were evaluated. Significant differences ($p < 0.05$) for general and specific combining abilities were observed for all traits. Based on additive effects, inbred lines 1 (Flash) and 12 (SG 6015) were selected to reduce the flowering period and plant and ear height. Inbred lines 2 (CD 303) and 3 (AG 8080) were selected to reduce disease severity. For the simultaneous increase in grain yield and reduced severity of diseases, line 11 (AG 9090) as a parent or tester in topcross schemes is recommended. According to non-additive effects, crosses 2 (CD 303) × 13 (DKB 747) and 11 (AG 9090) × 12 (SG 6015) were selected for grain yield and future breeding programs. Six groups were identified using SSR markers; a major group contained six inbred lines. Because of the minor relationship between genetic divergence and SCA effects on grain yield limits, the use of the groups for future divergent crosses is recommended.

Keywords: *Zea mays* L.; diallel crosses; heterotic group.

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Introduction

Maize (*Zea mays* L.) is a major food crop and has substantial social and economic importance; it is directly used for human consumption as well as for animal feed and several industrial purposes (Grigolo, Azevedo, Krause, & Azevedo, 2011). Maize breeding programs typically focus on the selection of highly productive and disease resistant genotypes, having greater adaptability and stability (Gralak et al., 2015).

The diallel analysis is one of the most-used tools for obtaining genetic information in maize breeding programs. This controlled mating system enables the estimation of the general combining ability (GCA) and the specific combining ability (SCA), which are associated with additive and non-additive genetic effects, respectively (Griffing, 1956; Cruz, Regazzi, & Carneiro, 2012). According to the parental genetic basis, the diallel analysis results allow the selection of genotypes for the development of new breeding populations (Oliboni et al., 2013; Souza Neto et al., 2015; Bertagna et al., 2018), developing new hybrids for final trials (Aguar et al., 2004; Silva et al., 2010; Matias Jr., Kuki, Scapim, & Pinto, 2019), or heterotic group descriptions (Silva, Amaral Junior, Gonçalves, Freitas Junior, & Ribeiro, 2011; Gonçalves et al., 2014; Mendes, Miranda Filho, Oliveira, & Reis, 2015).

The development of single-cross hybrids depends on heterosis, which is related to genetic distance and the gene complementation effect (Lippman & Zamir, 2007; Schnable & Springer, 2013). Thus, selection of inbred lines based on genetic effects and heterotic groups is required to obtain superior single-cross hybrids. The identification of divergent parents is one of the first steps to obtain superior hybrids. This procedure has proved to be more reliable when molecular markers are used because they can be very useful in the identification of heterotic groups of genotypes as a consequence of the different allele frequencies of populations (Munhoz, Prioli, Amaral Junior, Scapim, & Simon, 2009; Ndhlela et al., 2015).

Several types of molecular markers are available for breeders and researchers, and new types of polymorphic markers are frequently being developed, enhancing the application of genetic sequencing in breeding programs (Bernardo, 2008; Idrees & Irshad, 2015). Some authors have already shown single sequence repeat (SSR) markers to be more suitable than random-amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), or single nucleotide polymorphisms (SNPs) for genetic divergency and germplasm characterization (Pejic et al., 1998; Vignal, Milan, SanCristobal, & Eggen, 2002; Ravi, Geethanjali, Sameeyafarheen, & Maheswaran, 2003; Varshney, Chabane, Hendre, Aggarwal, & Graner, 2007) primarily because of the reasonable cost-benefit, the high degree of polymorphism provided by a large number of alleles per locus (Vignal et al., 2002; Inghelandt, Melchinger, Lebreton, & Stich, 2010), and highly reproducible results (Jones et al., 1997).

White spot of corn is caused by the microbial complex *Phaeosphaeria maydis* (Rane, Payak, & Renfro, 1966) and *Pantoea ananatis* (Paccola-Meirelles, Ferreira, Meirelles, Marriel, & Casela, 2001; Gonçalves et al., 2013). This disease has occurred in Brazil since the 1990s. However, since 2010, the damage caused by the disease has increased, mainly in second crops and in regions with mild climates. With the increase in the second crop area, the occurrence of white spot has become a limiting factor for sustainable corn production (Cunha, Negreiros, Alves, & Torres, 2019).

The economic damage caused by white spot depends mainly on hybrid susceptibility associated with cultivation in regions with mild temperatures (< 25°C) and high relative air humidity (> 70%) (Fantin & Duarte, 2009). According to Carson (2005), for every 1% increase in the severity of white spot in the R5 maize stage, there was a reduction of 0.23% in grain yield and 0.16% in grain weight.

In São Paulo, Fantin and Duarte (2009) determined the correlation between corn yield and the severity level of this disease in the second crop. In more susceptible cultivars, the authors observed that severity above 25% caused an average decrease of 1,933 kg ha⁻¹ in grain yield. The more resistant hybrids exhibited a severity below 1% (Fantin & Duarte, 2009). According to Cota, Costa, Sabato, and Silva (2013), if not controlled, white spot could cause yield reductions of up to 60% in susceptible hybrids.

Maize cercosporiosis, caused by the fungus *Cercospora zae-maydis* and *C. zeina*, is one of the most important diseases in corn crops worldwide. In Brazil, the disease was first observed in the southwest of the Goiás State in 2000. Currently, it is present in almost all corn plantation areas in southcentral Brazil and occurs at high severity levels in susceptible cultivars, causing losses up to more than 80%.

High relative humidity, the presence of dew, and room temperature between 22 and 30°C are ideal conditions for the pathogen (Ward, Nowell, Stromberg, & Nutter, 1999; Paul & Munkvolk, 2005). Losses ranging from 20 to 60% in grain productivity because of cercosporiosis have been reported in several studies (Donahue, Stromberg, & Myers, 1991; Ward et al., 1999). The use of fungicides to control fungal diseases is especially recommended for special corn, such as sweet corn and popcorn, as well as seed corn production. In other cases, genetic resistance is highlighted as the most efficient alternative (Fantin, Duarte, & Pinto, 2003; Bradley & Ames, 2010).

The present study is justified because the genetic parameters of the inbred lines to be studied are not known and it is assumed there is genetic divergence between the lines because of their origin. Moreover, our goals were to evaluate the general combining ability (GCA), specific combining ability (SCA), and genetic divergence using SSR markers for the tropical maize available in our maize breeding program.

Material and methods

Field trial and statistical analysis

Thirteen inbred lines selected from different base populations were used as parents in a complete diallel design (Table 1). These genotypes denote the main core of the maize breeding program germplasm for the State University of Maringá and were obtained through several cycles of selfing and selection until a satisfactory level of homozygosity (S_7) was reached. The F_1 hybrids were obtained in the second growing season of 2017, at the Iguatemi Experimental Farm (23° 25' S, 51° 57' W, and an altitude of 550 m asl) located at Maringá, Paraná State, Brazil. The 13 inbred lines were grown pairwise using every possible cross in 10 m rows, spaced 0.9 m apart.

The trial area was prepared using a no-tillage system for desiccation of invasive plants using the non-selective contact herbicide Paraquat (4.0 L ha⁻¹). Basic fertilization consisted of 300 kg ha⁻¹ of a 08-20-20 formulation. Pest control was performed by the systemic application of an insecticide and contact based on

methomyl and chlorantraniliprole, according to needs. Two applications of atrazine and tembotrione were conducted for the control of invasive post-emergence plants. Nitrogen coating was applied 40 days after sowing with 300 kg ha⁻¹ of urea.

Table 1. Inbred lines, base population origin, cycle, type of grains, and the company of base population origin for the 13 inbred lines used in the single crosses.

Inbred line	Origin	Cycle	Type of grain	Company
1	FLASH	Very early	Flint	Syngenta
2	CD 303	Early	Semi dent	Coodetec/Corteva
3	AG 8080	Early	Semi flint	Bayer
4	AVANT	Early	Flint	Syngenta
5	AS1560	Early	Semi flint	Bayer
6	FORT	Early	Flint	Syngenta
7	GARRA	Early	Flint	Syngenta
8	A2560	Early	Flint	Bayer
9	DKB 350	Early	Semi flint	Bayer
10	30F33	Early	Flint	Corteva
11	AG 9090	Early	Semi dent	Bayer
12	SG 6015	Early	Semi flint	Limagrain
13	DKB 747	Early	Flint	Bayer

The 78 resulting single-cross hybrids were evaluated against three commercial checks (AS1633, P30F53, and DKB 290), for a total of 81 treatments. The trial was carried out during the 2017/2018 main growing season at the Iguatemi Experimental Farm in Maringá, Paraná State, Brazil, in a 9 × 9 lattice incomplete block design, with three replications. Each plot consisted of two 5 m long rows spaced 0.90 m apart, resulting in a total area of 9 m². Each plot was thinned at 30 days to a density of 5 plants m⁻¹, resulting in a population of approximately 55,500 plants ha⁻¹ at harvest time.

The following traits were evaluated: days to anthesis (AT); days to silking (SI); average plant height (PH, m), and average ear height (EH, m) of six competitive plants. Additionally, white spot (WS) and gray leaf spot (GLS) severity were evaluated 25 days after flowering using the diagrammatic scale proposed by Agrocerec (1996) under natural disease infestation, as well as grain yield (GY, kg plant⁻¹) standardized to 13% moisture content.

A two-step analysis was performed for each evaluated trait. The first step consisted of an intrablock analysis of variance with the recovery of interblock information, which is a usual procedure for a lattice experimental design. The following model was used:

$$Y_{ijk} = \mu + r_j + b(r)_{j/r} + t_i + \varepsilon_{ijk}$$

where: Y_{ijk} is the vector from observed data, μ is the overall mean, r_j is the replication effect, $b(r)_{j/r}$ is the nested effect of blocks within replications, t_i is the treatment effect, and ε_{ijk} is the residual effect. Adjusted treatments were considered as fixed effects.

The second step consisted of a diallel analysis, considering model IV proposed by Griffing (1956), where only the F₁ crosses are used in the analysis. The sums of squares of the F₁ adjusted treatments were partitioned into GCA and SCA, according to the model:

$$Y = \mu + g_i + g_j + s_{ij} + \varepsilon_{ijk}$$

where: Y is the vector with the adjusted means for each F₁ cross, μ is the overall mean, g_i and g_j are the GCA effect for the parents in each cross, s_{ij} is the SCA effect related to each specific diallel cross, and ε_{ijk} is the residual effect. Effects were considered significant when $p < 0.05$. All analyses were performed using the Genes (Cruz, 2013) software.

DNA extraction and genetic divergence

The youngest leaves of five plants were sampled from each inbred line approximately 30 days after germination, immediately frozen in liquid nitrogen, and transferred to -80°C freezers. The DNA was extracted using a protocol described by Hoisington, Khairallah, and González-de-Léon (1994), with slight adaptations.

DNA quality was evaluated on 1% agarose gel and quantified using a Picodrop microliter UV/Vis spectrophotometer, and the DNA concentration was adjusted to 10 ng μL^{-1} for amplification.

DNA amplification was performed in a thermal cycler using the Touchdown PCR methodology (Don, Cox, Wainwright, Baker, & Mattick, 1991) and separated using 4% agarose gel (50% agarose and 50% agarose metaphor) in TBE buffer X 0.5 (44.5 mM Tris, 44.5 mM boric acid, and 1 mM EDTA). The gels were exposed to an electric field of 60 volts for approximately 4 hours, stained with 0.5 $\mu\text{g mL}^{-1}$ ethidium bromide solution, and photographed under a UV light. The alleles that were amplified were differentiated using a 100 pb DNA ladder from Invitrogen.

The SSR marker profile for each inbred line was determined by numerical codes related to each allele, where presence/absence was scored as 1 and 0, respectively, according to the multiallelism of each SSR marker (Cruz et al., 2012). Heterozygosity, number of polymorphic loci, and the total number of alleles were assessed using GenAIEx software version 6.5 (Peakall & Smouse, 2012). The polymorphism of each primer (PIC) was evaluated using Power Maker software (Liu & Muse, 2005). Modified Rodger's distance and cophenetic correlation were performed using Genes software (Cruz, 2013).

Results and discussion

Least-square means of the treatments resulted in significant differences ($p < 0.05$) for all evaluated traits (Table 2), indicating differences among the least-square means of the crosses. The experimental coefficients of variation were considered to be of low to medium magnitude for all traits when compared to other reported studies of diallel crosses using inbred lines (Durães et al., 2002; Silva et al., 2010; Conrado et al., 2014; Werle et al., 2014) and also when compared with the reference values proposed by Fritsche Neto, Vieira, Scapim, Miranda, and Rezende (2012) for maize, indicating excellent experimental precision.

Table 2. Results of the diallel analysis of variance of the seven analyzed traits in the diallel crosses conducted at Maringá, Paraná State, Brazil, during the 2017/2018 main growing season.

S.V. ¹	D.F. ²	Mean square						
		PH	EH	WS	GLS	AT	SI	GY
Treat.	77	0.072 [*]	0.06 [*]	0.77 [*]	1.32 [*]	9.29	11.36 [*]	1.55 [*]
GCA	12	0.36 [*]	0.31 [*]	2.76 [*]	6.29 [*]	41.68 [*]	56.57 [*]	3.01 [*]
SCA	65	0.019 [*]	0.015 [*]	0.40 [*]	0.41 [*]	3.31 [*]	3.01 [*]	1.28 [*]
Residual	136	0.003	0.003	0.992	0.078	2.05	1.86	0.39
Mean	-	2.086	1.054	1.615	1.903	60.190	60.538	6.722
CV (%)	-	2.961	5.412	19.683	19.683	2.383	2.260	9.172
$\hat{\phi}^2$ GCA	-	0.0104	0.009	0.080	0.188	1.20	1.52	0.079
$\hat{\phi}$ SCA	-	0.0053	0.0038	0.1002	0.11	0.42	0.53	0.30

^{*}significant at 5% probability; ^{ns} non-significant at 5% probability; ¹S.V.: Source of variation; ²D.F.: Degrees of freedom; ³ $\hat{\phi}^2$: quadratic component. average plant height (PH, m); average ear height (EH, m); white spot (WS) severity; gray leaf spot (GLS) severity, days to anthesis (AT); days to silking (SI); grain yield (GY, kg plant⁻¹).

The data presented in Table 2 illustrated that the severity values for both diseases were relatively low based on the Agroceres scale. Despite differences among susceptibility levels, the significance of the GCA and SCA effects indicated that environmental conditions did not favor the occurrence of the diseases evaluated.

Diallel analysis indicated significant differences in GCA and SCA for all evaluated traits (Table 2). This was an important indication of different genetic contributions among inbred lines for the additive effects, and also a direct result of the differential performance of the single-cross hybrid combinations compared to that expected from the GCA of their parents. According to quadratic component magnitudes, the contribution of the GCA effect was higher for AN, SI, PH, EH, and GLS severity, which was an indication of additive effects that controlled these traits (Table 3). Similar results were also observed by Freitas Jr., Amaral Jr., Pereira, Cruz, and Scapim (2006) and Kuki et al. (2017), who also observed higher importance for the additive effects for PH and EH, as well as flowering period. The contribution of the non-additive effects was higher only for WS severity and GY. Higher importance of non-additive effects was already expected for GY (Pfann et al., 2009; Oliboni et al., 2013; Senhorinho, Pinto, Scapim, Milani, & Nihei, 2015; Bertagna et al., 2018).

According to Cruz et al. (2012), GCA significance was attributed to additive effects, showing there was variability among the evaluated parents for the occurrence of favorable alleles, which could be selected based on genetic effects for the formation superior hybrids and testers in topcross schemes. SCA significance expressed the presence of non-additive gene effects in the related loci that affected the trait because, in the absence of dominance, SCA does not indicate significance in the diallel analysis (Vencovsky & Barriga, 1992).

Table 3. General combining ability estimations (\hat{g}_i) for the evaluated traits at Maringá, Paraná State, Brazil, during the 2017/2018 growing season.

Inbred line	\hat{g}_i						
	PH	EH	WS	GLS	AT	SI	GY
1	-0.198	-0.149	0.1031	0.256	-2.298	-2.328	0.193
2	0.129	0.120	-0.047	0.141	-0.147	0.622	0.397
3	-0.021	-0.011	-0.013	0.551	-0.324	-0.227	0.276
4	-0.023	0.025	-0.104	0.307	-0.622	-1.300	-0.017
5	-0.039	-0.058	0.410	-0.185	0.307	-0.170	0.080
6	-0.086	-0.068	0.174	0.297	0.20	0.357	0.063
7	0.123	0.162	-0.475	-0.185	2.190	2.164	0.039
8	0.075	0.030	-0.182	-0.675	0.617	0.568	-0.75
9	-0.037	0.004	-0.139	-0.163	-0.59	-1.122	0.056
10	-0.063	-0.119	0.364	0.515	-0.023	0.589	-0.133
11	0.177	0.124	-0.508	-0.944	1.579	1.905	0.276
12	-0.078	-0.088	0.322	0.088	-1.075	-1.542	-0.346
13	0.041	0.029	0.096	-0.003	0.195	0.484	-0.125
DP (\hat{g}_i)	0.010	0.0095	0.052	0.046	0.239	0.228	0.104
DP ($\hat{g}_i - \hat{g}_i$)	0.015	0.014	0.077	0.069	0.353	0.336	0.153

1 (FLASH), 2 (CD 303), 3 (AG8080), 4 (AVANT), 5 (AS1560), 6 (FORT), 7 (GARRA), 8 (A2560) 9 (DKB350), 10 (30F33), 11 (AG9090), 12 (SG6015), and 13 (DKB747). average plant height (PH, m); average ear height (EH, m); white spot (WS) severity; gray leaf spot (GLS) severity; days to anthesis (AT); days to silking (SI); grain yield (GY, kg plant⁻¹).

Maize breeding programs seek hybrids that combine high grain yield, an early cycle, higher disease resistance, and lower estimates of plant and EH. Therefore, GCA enables the best parents to be selected based on the additive genetic effects to form superior single-cross hybrids with a higher frequency of favorable alleles (Cruz et al., 2012).

Considering \hat{g}_i estimations for PH and EH, inbred lines 1, 6, 10, and 12 could be recommended for future crosses with lower plant and ear height, according to their lower \hat{g}_i values compared with other inbred lines (Table 3). Inbred line 11 exhibited the lowest \hat{g}_i values for WS and GLS severity. Additionally, and inbred line 7 could be selected only for WS and inbred 8 for GLS only.

Negative \hat{g}_i values for AT and SI, expressed in days from sowing until the flowering period, basically express how early a genotype flower, which is desirable for breeding programs and farmers. In this scenario, inbred lines 1, 4, 9, and 12 can be used as genitors or testers for reducing both traits in future crosses. Regarding the GY trait, promising genotypes should be selected based on the highest GCA estimations. Considering this, inbred lines 2, 3, and 11 were superior in terms of frequency of favorable genes with additive effects. Furthermore, inbred line 11 was superior for WS, GLS, and GY, and inbred line 1 and 11 were superior for PH, EH, AT, and SI; however, none of the inbred lines used in the diallel scheme was simultaneously superior for all traits (Table 3).

It is important to select hybrid combinations that exhibit favorable \hat{s}_{ij} estimations involving at least one parent with a favorable \hat{g}_i effect on the trait. Thus, the best hybrids would be those for which at least one of the parents was selected based on its \hat{g}_i estimation, thereby presenting a higher frequency of favorable alleles than the average frequency of the parents involved in the crosses (Vencovsky & Barriga, 1992; Cruz et al., 2012).

Tables 4 and 5 show the SCA estimators (\hat{s}_{ij}) for GY and maize genetic resistance against the two diseases analyzed in our study: WS and GLS.

According to Cruz et al. (2012), the effect of SCA is interpreted as the deviation of the hybrid from what would be expected based on the GCA of its genitors. Thus, low values of \hat{s}_{ij} indicate that hybrids perform as expected based on their GCA (\hat{g}_i) values, whereas high absolute values of \hat{s}_{ij} indicate better or poorer performance than expected. SCA estimates highlight the importance of genes with non-additive effects.

Considering the best-inbred lines selected based on their additive effects, the crosses 2 × 13 and 11 × 12 were the most promising for higher GY because these crosses presented higher and positive \hat{s}_{ij} values, apart from the superiority of inbred lines 2 and 11, which could be selected for their GCA based on their highest additive effects.

Considering the results for the genetic divergence using SSR markers, 89 out of 221 primers were polymorphic for all 13 inbred lines, representing 40.27% of the total. After primer selection, 38 markers were used for the genetic divergence analysis. The number of alleles per locus for the lines ranged from two to six, totaling 114 alleles (Table 6). These results were higher than those described by Dandolini et al. (2008), who reported 27.4% of polymorphic markers using tropical popcorn inbred lines and the number of alleles ranged from two to five.

Table 4. Estimates of the specific combining ability effects (\hat{s}_{ij}) for grain yield (GY) and the maize genetic resistance against two important diseases: white spot (WS) and gray leaf spot (GLS).

Parents	Hybrid	WS	GLS	GY	Parents	Hybrid	WS	GLS	GY
1 x 2	1	-0.32139	0.051723	-0.38346	3 x 5	25	0.468122	-0.68063	-0.05293
1 x 3	2	0.305982	0.795696	-0.03669	3 x 6	26	-0.29045	-0.22714	-0.8573
1 x 4	3	0.063106	0.212853	-0.37354	3 x 7	27	0.277526	-0.31595	0.534317
1 x 5	4	-0.04762	0.278532	0.110866	3 x 8	28	-0.46901	0.094341	0.530041
1 x 6	5	0.270927	-0.09236	0.160329	3 x 9	29	0.018644	-0.33889	-0.18636
1 x 7	6	-0.10687	0.104848	0.050014	3 x 10	30	-0.33288	-0.12743	0.414741
1 x 8	7	-0.31809	-0.45332	0.881338	3 x 11	31	-0.09434	-0.19245	0.107314
1 x 9	8	-0.0767	0.081906	0.632602	3 x 12	32	-0.15988	-0.24081	-0.01067
1 x 10	9	0.320536	0.324909	0.309205	3 x 13	33	0.131941	0.197274	0.101435
1 x 11	10	-0.30958	-0.46774	-0.39422	4 x 5	34	-0.17376	0.022541	-0.88012
1 x 12	11	-0.16168	-0.32684	-0.68274	4 x 6	35	0.128367	0.142705	1.428311
1 x 13	12	0.381377	-0.49021	-0.2737	4 x 7	36	-0.23301	-0.13537	-0.84617
2 x 3	13	0.374955	0.640349	-0.13686	4 x 8	37	-0.04522	-0.42329	0.310487
2 x 4	14	0.251975	0.298329	0.631487	4 x 9	38	0.092683	-0.111	0.595851
2 x 5	15	0.436779	0.172623	0.354263	4 x 10	39	0.107334	0.195094	-0.42098
2 x 6	16	-0.17403	-0.54737	-0.94287	4 x 11	40	0.198106	-0.39252	0.225026
2 x 7	17	-0.2563	0.395359	-0.06129	4 x 12	41	0.116152	-0.44088	-0.04149
2 x 8	18	-0.28195	-0.09972	0.258102	4 x 13	42	-0.27512	0.235904	-0.22182
2 x 9	19	-0.52663	-0.35734	-0.12303	5 x 6	43	0.04551	0.158947	0.664754
2 x 10	20	0.133297	-0.71791	0.666169	5 x 7	44	-0.20094	-0.11913	0.479472
2 x 11	21	0.306159	-0.17935	-0.90339	5 x 8	45	-0.11166	0.275388	0.521329
2 x 12	22	0.191369	-0.06999	-0.47397	5 x 9	46	0.627238	0.382648	-0.98554
2 x 13	23	-0.13423	0.433301	1.114863	5 x 10	47	0.040895	-0.32703	-0.64237
3 x 4	24	-0.23061	0.395636	-0.40704	5 x 11	48	-0.25092	0.195757	0.083635

Table 5. Estimates of the specific combining ability effects (\hat{s}_{ij}) for grain yield (GY) and the maize genetic resistance against two important diseases: white spot (WS) and gray leaf spot (GLS).

Parents	Hybrid	WS	GLS	GY	Parents	Hybrid	WS	GLS	GY
5 x 12	49	-0.49706	0.210481	-0.70368	8 x 9	64	0.635879	0.123957	-0.58507
5 x 13	50	-0.33659	-0.57012	1.050323	8 x 10	65	0.814712	0.382732	-0.4509
6 x 7	51	-0.49484	-0.28498	0.781569	8 x 11	66	0.654241	0.716258	0.131808
6 x 8	52	-0.22496	-0.16071	-0.09944	8 x 12	67	-0.20434	-0.04609	-0.26391
6 x 9	53	-0.16915	0.230445	0.689257	8 x 13	68	-0.36078	-0.31986	-0.81144
6 x 10	54	-0.58634	0.426132	0.370493	9 x 10	69	-0.42003	-0.19245	-0.0039
6 x 11	55	0.46663	0.728114	-0.13573	9 x 11	70	-0.14866	0.046447	-0.03373
6 x 12	56	0.614531	-0.033602	0.404351	9 x 12	71	0.086301	0.155803	0.060423
6 x 13	57	0.413801	-0.03776	-2.46371	9 x 13	72	0.044784	0.357309	0.23026
7 x 8	58	-0.08882	-0.08968	-0.42226	10 x 11	73	-0.40664	-0.4582	-0.26906
7 x 9	59	-0.16435	-0.37884	-0.29076	10 x 12	74	0.440765	0.444	0.13336
7 x 10	60	-0.11687	0.006107	-0.30032	10 x 13	75	0.005211	0.044049	0.193563
7 x 11	61	0.302271	0.100932	-0.51525	11 x 12	76	-0.31573	0.016228	1.186799
7 x 12	62	0.220317	0.543621	0.209435	11 x 13	77	-0.40153	-0.11348	0.516802
7 x 13	63	0.861885	0.173092	0.381338	12 x 13	78	-0.33075	0.090509	0.516802
DP (\hat{S}_{ij})	-	0.166069	0.147879	0.329239	DP (\hat{S}_{ij})	-	0.166069	0.147879	0.329239
DP ($\hat{S}_{ij} - \hat{S}_{ik}$)	-	0.24530	0.218432	0.486318	DP ($\hat{S}_{ij} - \hat{S}_{ik}$)	-	0.24530	0.218432	0.486318
DP ($\hat{S}_{ij} - \hat{S}_{kl}$)	-	0.232712	0.207223	0.461362	DP ($\hat{S}_{ij} - \hat{S}_{kl}$)	-	0.232712	0.207223	0.461362

Polymorphism values (PIC) ranged from 0.23 (UMC1714, with two alleles) to 0.72 (MMC0501, with six alleles), with an average value of 0.46 (Table 6). Similar results were also reported by Lopes, Scapim, Mangolin, and Machado (2014) using 15 sweet corn inbred lines in a divergence genetic study, where the authors found 15 out of 100 polymorphic SSR markers with an average PIC of 0.41. The PIC can be used to differentiate markers based on their polymorphisms because the allele loci number and relative frequency of the alleles are used for estimating PIC. According to Botstein, White, Skolnick, and Davis (1980), values higher than 0.5 are considered highly informative, whereas values lower than 0.25 are considered low informative markers.

Table 6. Number of alleles at each of the 38 loci and their polymorphism values (PIC).

Loci	Total number of alleles	PIC
UMC1029	4	0.6300
UMC2080	2	0.3475
BNLG1367	4	0.4822
UMC1585	2	0.3712
UMC1068	4	0.5681
UMC1318	2	0.3648
BNLG1175	5	0.6630
UMC2198	2	0.3161
UMC2025	2	0.3745
UMC1250	2	0.3573
UMC1230	3	0.5818
UMC2257	3	0.4792
UMC1227	2	0.3203
BNLG1297	3	0.5298
UMC2071	3	0.3901
UMC2115	3	0.4184
UMC2164	2	0.3712
UMC2319	2	0.3281
UMC1069	3	0.4204
UMC1714	2	0.2342
UMC2343	3	0.4090
UMC1506	2	0.3729
MMC0501	6	0.7210
UMC2172	2	0.3447
UMC2214	4	0.6533
BNLG1927	5	0.6639
UMC2165	3	0.4958
UMC1399	2	0.3749
UMC1287	3	0.3188
UMC2357	4	0.5839
UMC2047	2	0.3745
UMC1590	5	0.5963
UMC2350	3	0.5876
UMC1656	3	0.4473
UMC1357	3	0.5583
BNLG1046	4	0.6460
UMC1702	2	0.3749
UMC2281	3	0.5709

Genetic divergence between inbred lines was calculated using Roger's modified distance (Goodman & Stuber, 1983) and the 38 polymorphic SSR markers. The dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) clustering method. Groups were divided with a cut-off value of 0.6868, as suggested by Mojena (1977), with $k = 1.25$, thereby avoiding a possible confounding factor for group separation.

The UPGMA dendrogram clustered the 13 inbred lines into six different groups (Figure 1), with a higher distance (0.83) between inbred lines 5 and 13 and the lowest distance (0.55) between inbred lines 5 and 6. Group 1 encompassed inbred lines 5, 6, 3, 4, 8, and 7, and was the largest reported group in this study. Group 3 originated by clustering inbred lines 1 and 9, whereas Group 4 included inbred lines 11 and 13. The other inbred lines were allocated solely in different groups. Concerning the main groups 1, 3, and 4, most of that inbred lines were obtained from base populations that originated from Syngenta and Bayer hybrids (Table 1), which suggests a certain similarity between the germplasm of these companies.

The estimated cophenetic correlation coefficient (r) was 0.58, which was similar to that observed by Guimarães et al. (2007) ($r = 0.57$) and by Alves, Filho, Burin, Toebe, and Silva (2015) ($r = 0.58$). Ferreira (2008) suggested a value close to 1 provided a better adjustment among distances, although Patto, Satovic, Pêgo, and Fevereiro (2004) recommended a value higher than 0.56 for a good adjustment considering maize inbred lines.

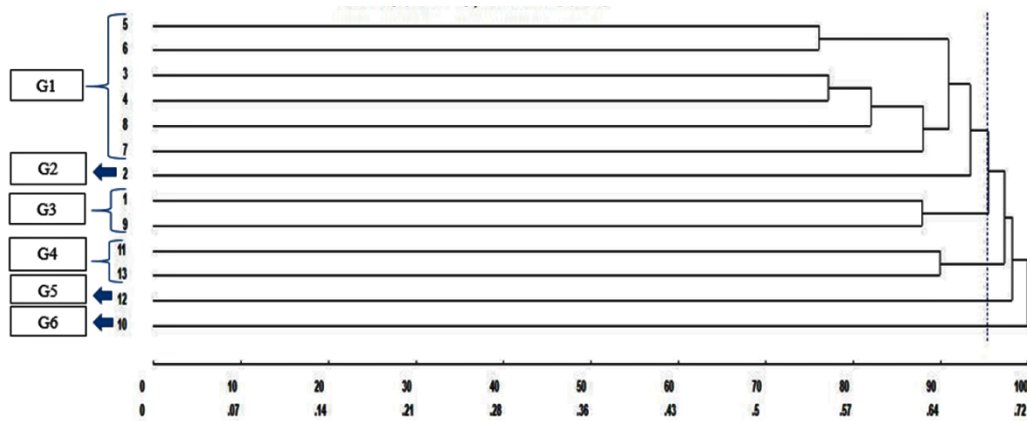


Figure 1. Genetic distance among the 13 tropical maize inbred lines using Rogers's modified distance and clustered using UPGMA. 1 (FLASH), 2 (CD 303), 3 (AG8080), 4 (AVANT), 5 (AS1560), 6 (FORT), 7 (GARRA), 8 (A2560) 9 (DKB350), 10 (30F33), 11 (AG9090), 12 (SG6015), and 13 (DKB747).

Taking into account the 39 hybrids from these companies, for those with positive SCA for GY, 29 had parents from different genetic groups (Figure 1). This indicated that genetic divergence among parents might explain the expression of non-additive effects in hybrids. However, this was not a consistent result because the hybrid with the highest SCA estimation (4×6) both had inbred lines clustered in the closest genetic groups. Some authors reported a good concordance among non-additive effects and genetic distances estimated using molecular markers for the flowering period and plant and EH (Lanza, Souza Junior, Ottoboni, Vieira, & Souza, 1997; Sun, William, Liu, Kasha, & Pauls, 2001; Souza et al., 2008), but low or almost no relationship among genetic divergence using SSR markers and phenotypic data for GY, a complex quantitative trait (Guimarães et al., 2007; Paterniani et al., 2008; Munhoz et al., 2009; Fernandes, Schuster, Scapim, Vieira, & Coan, 2015).

The lack of correlation among genetic divergence and SCA for GY observed in this study could be mainly explained by the random choice of SSR markers (Table 7). Thus, the SSR markers used herein were not necessarily associated with QTLs previously identified for any trait. Low genetic map resolution, the complex genetic architecture of traits, and a small number of polymorphic SSR markers available could also have contributed to the low correlation observed. A higher number of polymorphic markers and field trials in different years/seasons should improve these correlations for complex traits (Fernandes et al., 2015), but the costs for a large SSR-mapping panel might limit this analysis.

Table 7. Pearson's correlation estimates of genetic divergence, specific combining ability for grain yield and average grain yield.

Variables	Correlation	Probability (%)
x1 x x2	-0.0969	40.3349
x1 x x3	-0.0735	53.0005
x2 x x3	0.8359	0.0**

** significant at 1% and 5% probability; *non-significant at 5% probability. x1: genetic distance; x2: specific combining ability for grain yield; x3: average grain yield.

Conclusion

Inbred lines 2 (CD 303), 3 (AG 8080), and 11 (AG 9090) were selected based on additive effects and should be used in future hybrid combinations and as topcross testers. Single-cross hybrids 2 (CD 303) \times 13 (DKB 747) and 11 (AG 9090) \times 12 (SG 6015) were selected based on the non-additive effects and could be used for future breeding programs. Six groups were identified using SSR markers, with the major group containing six inbred lines. The low relationship between genetic divergence and SCA effects for GY limited the use of the groups for future divergent crosses.

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