## **PLANT BREEDING - Article**

# Identification of populations and hybrid combinations of maize for *in natura* consumption

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**ABSTRACT:** Brazil is one of the leading countries in the production of maize (*Zea mays*), with great potential for growing green maize, which has a superior commercial value in relation to maize marketed in the form of grains. Although important, the availability of cultivars recommended for the production of green maize is still very scarce. The objectives of the present study were to estimate genetic parameters and to identify promising hybrid combinations for the development of new green maize cultivars to farmers. In the summer crop of 2016/17, ten hybrid combinations obtained through a complete diallel of five maize populations, with attributes for *in natura* consumption, were evaluated in two sites of the state of São Paulo, Brazil: the Instituto Agronômico (IAC) in Campinas and Tatuí. A randomized block design was used

with two additional checks, with three replications, in plots with four five-meter rows spaced by 0.9 m in Campinas and 0.8 m in Tatuí, with 5 plants per meter. The following agronomic traits were assessed: grain yield, ear yield with straw and ear yield without straw, using Griffing's method 4. Significant effects of genotypes, environments and interaction genotypes × environments were detected for all traits. Estimates of the general combining ability led to the selection of populations P2, P4, and P5 as the ones with a higher concentration of favorable alleles for the characters evaluated. Estimates of specific combining ability and improved grain yield performance allowed P2xP3 to be selected as the most promising for production of green maize.

**Key words:** Zea mays, diallel, green maize, special corns, gene effects.

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#### INTRODUCTION

Maize (*Zea mays*) is often used in the human diet in a variety of ways – flours, baby corn, canned, *in natura*, among others – and it stands as one of the main sources of nutrients, with about 72% of its kernel weight composed of starch, 10% of protein, 4% percent of fat, and providing an amount of energy of 365 Kcal·100 g<sup>-1</sup> (Nuss and Tanumihardjo 2010; Sawazaki et al. 1979).

In Brazil, there are few cultivars indicated for *in natura* consumption available on the market. Only four of the 315 commercial cultivars, in addition to being suitable for grain and silage production, present suitability for green maize, representing about 1.27% of the registered cultivars (Pereira Filho and Borghi 2016). It is also worth noting the difficulty of obtaining these conventional commercial maize hybrids, as transgenics dominate the grain market. The importance of *in natura* maize for the preparation of specific foods increases the high value added of the product and the small number of cultivars available reinforces the importance of the development of common conventional maize cultivars specific for *in natura* consumption.

The alternatives of consumption of *in natura* corn require the selection of plants with not only agronomic characteristics but also with traits in line with the preferences of consumers, such as large and cylindrical ears, rows of straight grains and straw, longer post-harvesting longevity, yellow grain with fine pericarp and softness. Disease and pest resistance and uniformity in flowering and harvesting are desirable characteristics, which add higher quality to the product and increased ear yield.

Maize breeding uses diallel crosses extensively, a method that can use parents as inbred lines or pollinated varieties, in order to infer the type of gene action of the characters evaluated and to allow the selection of parental and/or hybrids (Hallauer et al. 2010). Diallel is also a crossover design that allows a detailed study of heterosis and the estimation of parameters, such as the general combining ability (GCA) and specific combining ability (SCA) parameters that are involved in the development stages of hybrids and maize varieties. Many methods can be applied to the study of combining capacity, especially those of Griffing (1956), Gardner and Eberhart (1966) and their modifications.

There are few studies that report the importance of diallel crosses in special corns, such as the evaluation of the performance *per se* of partially inbred lines and in relation

to their hybrids, aiming at grain yield, ear yields with and without straw and in the evaluation of new cultivars for the production of green maize (Rodrigues et al. 2009).

The objectives of the present study were to estimate genetic parameters, to identify the potential of maize populations in hybrid combinations and to evaluate promising hybrids to provide new green maize cultivars to farmers.

#### MATERIAL AND METHODS

In the summer crop of 2016/17, ten hybrid combinations of maize were obtained from a complete diallel of five populations, whose agronomic characteristics are presented in Table 1. For the selection of the five populations to compose the diallel, the following characteristics were considered in the stage of green corn: good straw, yellow and dent field grain.

**Table 1.** Description of the maize genotypes used to obtain the hybrids evaluated in this study at the green maize stage and the checks.

Populations					
Code	Genotypes	Origin	Stature	Usage	Туре
P1	F <sub>2</sub> CH <sub>1</sub> *	Agroceres	High	GM and S	TH
P2	F <sub>2</sub> CH <sub>2</sub> *	Biomatrix	Medium	GM and S	TH
P3	Cativerde	CATI	High	GM and S	V
P4	IAC 33	IAC	Short	G	V
P5	F <sub>2</sub> CH <sub>3</sub> *	Dow	Hight	GM	-
Checks					
Code	Genotypes	Origin	Stature	Usage	Туре
Check 1	AG1051	Agroceres	High	GM and S	DH
Check 2	Cativerde	CATI	High	GM and S	V

\*Generation F<sub>2</sub> of comercial hybrids (CH); GM: green maize, S: silage, G: grain; TH: triple hybrid; V: variety; DH: double hybrid.

The ten hybrids obtained and the two additional checks ('AG 1051' and 'Cativerde') were evaluated in the 2016/17 growing season at two sites in the state of São Paulo: at the Santa Elisa farm, Experimental Center of the Instituto Agronômico (IAC) in Campinas (lat 22°54' S; long 47°3' W and 600 m alt) and at the Regional Development Pole for the Southwest of São Paulo (APTA) in Tatuí (lat 23°21'20" S, long 47°51'25" W and 645 m alt). A randomized block design was used with three replicates, in plots consisting of four fivemeter rows, with 0.9 m between rows as spacing in Campinas

and 0.8 m between rows in Tatuí, with five plants per meter. The two central rows were considered when collecting the agronomic data. Fertilization was used, with 350 kg·ha<sup>-1</sup> of NPK 08-28-16 in the planting, with 250 kg·ha<sup>-1</sup> coverage of ammonium sulfate and the cultural treatments recommended by IAC 200 Bulletin.

The following agronomic traits were assessed: grain yield (GY), ear yield with straw (EYS), and ear yield without straw (EYW) of all plants of the plots, in physiological maturity phase and with the grain having a moisture content of 20%.

The individual and joint analyzes of variance were performed considering all fixed effects, and the Tukey test (p < 0.01) was used for the comparisons of means. Diallel analyzes were performed by the Griffing method 4 (Griffing 1956) considering only the F1s, according to the model:

$$\hat{Y}_{ij} = m + \hat{g}_i + \hat{g}_j + \hat{s}_{ij} + \bar{e}_{ij}$$

where  $\hat{Y}_{ij}$  observation of the hybrid combination involving the parents i and j; m: general mean;  $\hat{g}_i$  and  $\hat{g}_j$ : general combining ability (GCA) of i-th and j-th parents;  $s_{ij}$ : specific combining ability (SCA) effect of the cross among the i-th and j-th parents;  $\bar{e}_i$ : mean experimental error.

The biometric and diallel analyses were performed using the software package Genes (Cruz 2013).

#### RESULTS AND DISCUSSION

Table 2 summarizes the variance and diallel analyses, both joint and by environment. The joint analysis detected coefficient of variation (CV) estimates ranging from 8.4 to 9.9, indicating high experimental accuracy, according to Gomes (2000) and Fritsche Neto et al. (2012). Significant effect of genotypes (p < 0.01) was detected for the traits of grain yield (GY), ear yield with straw (EYS) and ear yield without straw (EYW), indicating that there are differences in performance between them, enabling the selection of superior genotypes of green maize. There was a pronounced environmental effect (p < 0.01) for all the characteristics (GY, EYS and EYW), and it could be stated that the environments influenced the evaluated traits. Significant effects in the interaction between genotypes  $\times$  environments (GxE) (p < 0.01) were observed for all traits, that is, the genotypes did not present coincident relative behavior in the different environments (Table 2). Therefore, the results were analyzed and discussed by environment.

In Campinas, the genotypes showed a significant difference for GY and EYS. The hybrid combinations presented significant difference only for EYS. The effects of GCA and SCA were not significant and thus there were no conditions for the selection of hybrids and parameter estimates. In Tatuí, the effects of genotypes, hybrid and checks showed a highly significant difference for all traits (Table 2), indicating that there were better conditions to discriminate the genotypes and the hybrid combinations. In addition, the effects of GCA and SCA were significant for all traits, indicating the expression of additive, dominant and overdominance effects in the expression of the evaluated traits.

The means for GY, EYS and EYW of green maize hybrids in both locations are presented in Fig. 1, along with their groupings by the Tukey test (p < 0.05). It was observed that the performance of the genotypes in the Tatuí environment, which obtained grain yield of 8,060 kg·ha<sup>-1</sup> on average, was higher than the performance in Campinas, 6,617 kg·ha<sup>-1</sup> on average. For GY, it was observed the formation of two groups in Campinas (a and b) and three groups in Tatuí (a, b and c) among the hybrids and the checks, with averages varying from 5,639 to 7,517 kg·ha<sup>-1</sup> in Campinas and from 6,458 to 9,571 kg·ha-1 in Tatuí (Fig. 1). For EYS in Campinas, the genotypes evaluated were distinguished in two groups (a and b), while the formation of four groups (a, b, c and d) was observed in Tatuí, with averages ranging from 9,249 to 14,010 kg·ha<sup>-1</sup>. In Campinas, the treatments did not present statistical difference among each other for EYW, being grouped together (a). In Tatuí, in turn, the genotypes were separated into three groups (a, b and c), but the hybrids and the checks showed statistically similar performances.

The hybrid P1xP2 kept a similar average of productivity in the two evaluated environments, producing little more than 6,400 kg·ha<sup>-1</sup>. The hybrid combination P2xP5 produced on average 7,649 kg·ha<sup>-1</sup>. The hybrid P2xP3 stood out in Tatuí with the highest yield among the hybrid combinations, 9,229 kg·ha<sup>-1</sup>. Although not statistically different from some hybrids and checks, the hybrids P2xP3 and P2xP5 presented high grain yield and high ear yield with and without straw and could be used in maize breeding programs that seek the aptitude of *in natura* consumption.

The joint variance analysis showed no significance for the general combining ability (GCA) and specific

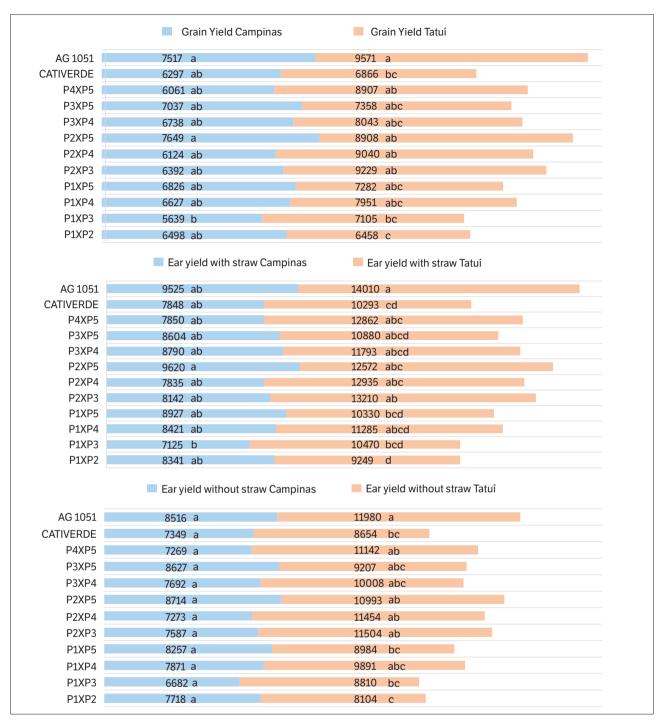
**Table 2.** Means square in the joining and individual analyses of variance and complete diallel analysis for grain yield, ear yield with straw and ear yield without straw of green maize hybrids. Campinas and Tatuí, SP, Season 2016/17.

	Sources	Degree	Means square			
	of		Grain yield	Ear yield with straw	Ear yield without straw	
	Variance	Freedom	kg·ha <sup>·1</sup>			
	Genotypes (G)	11	2,724,064.75**	4,969,604.68**	3,621,649.72**	
	Hybrids	9	1,964,834.01ns	3,418,086.79**	2,682,825.26**	
	Checks	1	11,555,200.02**	21,814,685.02**	15,137,591.07**	
	Hybrids vs Checks	1	726,006.0803ns	2,088,185.344ns	555,128.5747ns	
	GCA	4	3,034,816.24ns	5,527,368.23ns	4,441,060.32ns	
Joining	SCA	5	1,108,848.24ns	1,730,661.64ns	1,276,237.20ns	
Johning	Environments (E)	1	37,467,351.85**	188,761,802.33**	92,322,080.8**	
	Genotypes × E	11	1,597,806.62**	3,020,164.81**	2,749,481.78**	
	GCA × E	4	1,955,425.63**	4,006,766.82**	3,868,956.57**	
	SCA × E	5	1,607,712.50**	2,777,738.31**	2,244,053.42**	
	Error	44	467,325.86	765,522.12	796,782.3	
	CV (%)		9.31	8.71	9.99	
	Genotypes (G)	11	1,033,103.5*	1,593,404.03*	1,178,873.90ns	
	Hybrids	9	947,181.24ns	1,421,737.8*	1,199,093.23ns	
	Checks	1	2,233,210.04*	4,215,978.37*	2,041,900.00ns	
Oi	Hybrids vs Checks	1	606,297.45ns	515,825.78ns	133,873.88ns	
Campinas	GCA	4	761,201.21ns	994,845.67ns	1,183,862.62ns	
	SCA	5	1,095,965.25ns	1,763,251.50*	1,211,277.72ns	
	Error	22	391,839.29	630,582.44	723,270.37	
	CV (%)		9.45	9.43	10.9	
	Genotypes (G)	11	3,288,767.86**	6,396,365.45**	5,192,257.60**	
Tatuí –	Hybrids	9	2,779,904.46**	5,320,322.19**	4,449,964.63**	
	Checks	1	10,975,537.5**	20,720,416.66**	16,592,083.62**	
	Hybrids vs Checks	1	181,768.89ns	1,756,703.53ns	473,068.3ns	
	GCA	4	4,229,040.668**	8,539,289.38**	7,126,154.26**	
	SCA	5	1,620,595.50*	2,745,148.44**	2,309,012.90**	
	Error	22	542,812.43	900,461.79	870,294.28	
	CV (%)		9.14	8.1	9.27	

ns, \*\* and \*: no significant, significant at 5% and 1% probability by test F.

combination ability (SCA) of the genetic parameters, with genetic divergence among the genotypes in Campinas not having been observed (Table 4). However, when evaluating the genotype combining ability in Tatuí, it was observed that the effects of the general combining ability (GCA) were significant (p < 0.01) for YG, EYS and EYW, whereas the effects of the specific combining ability (SCA) were significant (p < 0.06) for GY and EYS, evidencing that the additive and nonadditive

genetic effects are important. Thus, the number of favorable alleles donated by the parents and the allelic complementation obtained in the specific crosses were fundamental for the formation of superior hybrids. These results corroborate the ones found in Rodrigues et al. (2009), which evaluated eight lines and 28 hybrids of green maize for the same traits and verified that the nonadditive effects were more important in the selection of superior hybrids (Table 2).



**Figure 1.** Means for grain yield (GY), ear yield with straw (EYS) and ear yield without straw (EYW) of green maize hybrids. Campinas and Tatuí-SP, Season 2016/17. Means followed by the same letter do not differ by the Tukey test (p < 0.05). Hybrid combinations and controls between the same locations.

The interaction of the effects of GCA  $\times$  E was significant (p < 0.01) for the evaluated traits, showing that the environment may influence the expression of the favorable alleles of the parents (Table 2). The significant effects of SCA  $\times$  E suggest the need to select different hybrid

combinations in specific environments. Previous research has shown that both GCA and SCA can interact with environments (Pixley and Bjarnason 1993).

Table 3 shows the estimates of the general combining ability for GY, EYS and EYW of the green maize breeders

**Table 3.** Estimates of general combining ability (GCA) and standard deviations (SD) for grain yield (GY), ear yield with straw (EYS) and ear yield without straw (EYW) of parents green maize. Tatuí, SP, Season 2016/17.

Parents	GY	EYS	EYW
Parents		(kg·ha⁻¹)	
P1	-1,105.33	-1,633.34	-1,416.75
P2	507.33	577.09	672.00
P3	-125.78	39.59	-169.89
P4	609.56	879.85	818.75
P5	114.22	136.81	95.89
SD (g <sub>i</sub> )	240.27	308.69	304.53
$SD(g_i-g_j)$	379.90	488.08	481.51

**Table 4.** Estimate of specific combining ability and standard deviations (SD) for grain yield (GY), ear yield with straw (EYS) and ear yield without straw (EYW) of hybrids combination of green maize. Tatuí, SP, Season 2016/17.

Hybrids	GY	EYS kg·ha⁻¹	EYW
P1xP2	-972.17	-1,253.11	-1,160.92
P1xP3	308.28	505.19	386.90
P1xP4	418.61	479.52	479.10
P1xP5	245.28	268.40	294.92
P2xP3	818.94	1,034.37	992.18
P2xP4	-104.72	-80.90	-46.46
P2xP5	257.94	299.64	215.20
P3xP4	-468.94	-685.07	-650.80
P3xP5	-658.28	-854.49	-728.28
P4xP5	155.06	286.44	218.16
$SD(s_{ij})$	329.00	422.69	417.00
$SD(s_{ij}-s_{ik})$	537.26	690.24	680.96
$SD(s_{ij}-s_{kl})$	379.90	488.08	481.51

in Tatuí. The following populations stand out, due to the expressive and positive values of GCA for the three traits: P2, from a commercial hybrid of Biomatrix, P4, a variety of IAC, and P5, from a hybrid of Dow AgroSciences. High GCA estimates indicate additive gene effects for the observed traits, that is, these populations show a high frequency of favorable alleles. It is important to emphasize that the general combining ability (GCA) results from the behavior of a given parent upon crosses in which it participated, reflecting the effect of additive genes. Moreover, the specific combining ability (SCA)

is the deviation of a hybrid from the expected, based on the GCA of its parents, expressing the effect of nonadditive genes. Thus, for plant breeding, the ideal hybrid combination is the one which simultaneously has a high SCA estimate and the presence of at least one parent with high GCA estimate (Cruz et al. 2012).

Populations P4 (609.56 kg.ha<sup>-1</sup>) e P2 (507.33 kg·ha<sup>-1</sup>) showed high values of GCA for GY. Similar results were obtained by Machado et al. (2008), who report that the variety 'BRS 106' presented GCA for GY of 731.36 kg·ha<sup>-1</sup>, evidencing the presence of favorable alleles in this variety. According to Morello et al. (2002), selection based on the effect of GCA indicates varieties with potential for use in the synthesis of new compounds (Table 3).

The hybrid P2xP3, as already mentioned, presented one of the highest average grain yields among the intervarietal hybrids, considering the yield traits of straw and no straw, by the Tukey test and presented the highest SCA, due to the high GCA level of P2 and complementarity between these two populations. The parents P2 and P3 showed high values of GCA, but with opposite signs. This may explain the greater SCA, due to the greater complementarity in relation to the characteristics of grain weight and weight of ears with straw, also reported by Murtadha et al. (2018). The population P1, despite its negative GCA, shows good combination with the populations P3, P4 and P5, with reasonable SCA values for the hybrids P1xP3, P1xP4 and P1xP5 (Table 4).

### CONCLUSION

The GCA estimates allowed the selection of the populations P2, P4 and P5, with a higher concentration of favorable alleles for GY, EYS and EYW, and potential for use in the formation of new hybrids and recurrent intrapopulation selection in green maize breeding programs. The SCA estimates and a better performance in grain and ear productivity allowed the selection of the P2xP3 hybrid as the most promising and heterotic for the production of green maize.

There was a predominance of additive and no additive effects in the expression of grain yield, ear with straw and ear without straw traits in Tatuí, allowing for the identification of populations and promising hybrids.

#### **AUTHORS' CONTRIBUTION**

Conceptualization, Paterniani M. E. A. G. Z. and Sawazaki E.; Methodology, Paterniani M. E. A. G. Z. and Sawazaki E.; Investigation, Rocha D. S, Ticelli M. and Sawazaki E.; Writing – Original Draft, Rocha D. S., Rovaris S. R. S. and Rodrigues C. S.; Writing – Review and Editing, Rovaris S. R. S. and Rodrigues C. S.; Funding Acquisition, Sawazaki E. and S Paterniani M. E. A. G. Z.; Resources, Rocha D. S., M. Ticelli M., Eduardo Sawazaki and Paterniani M. E. A. G. Z.; Supervision, Eduardo Sawazaki and Paterniani M. E. A. G. Z.

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# **REFERÊNCIAS**

Cruz, C. D. (2013). GENES – a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum. 35, 271-276. https://doi.org/10.4025/actasciagron.v35i3.21251

Cruz, C. D., Regazzi, A. J. and Carneiro, P. C. S. (2012). Modelos biométricos aplicados ao melhoramento genético. Viçosa: UFV.

Fritsche-Neto, F., Vieira, R.A., Scapim, C.A., Miranda, G.V. and Rezende, L.M. (2012). Updating the ranking of the coefficients of variation from maize experiments. Acta Scientiarum Agronomy, 34, 99-101. https://doi.org/10.1590/S1807-86212012000100014

Gomes, F. P. (2000). Curso de estatística experimental. 14. ed. Piracicaba: Degaspari.

Gardner, C. O. and Eberhart, S. A. (1966). Analysis and interpretation of the variety cross diallel and related populations. Biometrics, Tucson, 22, 439-452.

Griffing, B. A. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. Australian Journal Biology Science, East Melbourn, 9, 463-493.

Machado, A. T., Nass, L. L. A. and Pacheco, C. P. (2008). Cruzamentos intervarietais de milho avaliados em esquema dialélico parcial. Revista Brasileira de Milho e Sorgo, 7, 291-394. https://doi.org/10.18512/1980-6477/rbms.v7n3p291-304

Morello, C. L., Ferreira, J. M. and Miranda Filho, J. B. M. (2002). Heterosis and combining ability among varieties of maize in acid soil 1. Pesquisa Agropecuária Tropical, 32, 89-95.

Murtadha, M. A., Ariyo, O. J. and Alghamdi, S. S. (2018). Analysis of combining ability over environments in diallel crosses of maize (*Zea mays*). Journal of the Saudi Society of Agricultural Sciences, 17, 69-78. https://doi.org/10.1016/j.jssas.2016.01.004

Nuss, E. T. and Tanumihardjo, S. A. (2010). Maize: a paramount staple crop in the context of global nutrition. Comprehensive Reviews in Food Science and Food Safety, 9, 417-436. https://doi.org/10.1111/j.1541-4337.2010.00117.x

Pereira Filho, I. A. and Borghi, E. (2016). Mercado de Sementes de Milho no Brasil: Safra 2016/2017. Documentos, 202. Sete Lagoas: Embrapa Milho e Sorgo. [Accessed 2018 July 16]. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1060346/mercado-de-sementes-de-milho-no-brasil-safra-20162017

Pixley, K. V. and Bjarnason, M. S. (1993). Combining ability for yield and protein quality among modified-endosperm opaque-2 tropical maize inbreds. Crop Science, 33, 1229-1234. https://doi.org/10.2135/cropsci1993.0011183X003300060023x

Rodrigues, F., Pinho, R., Albuquerque, C., Filho, E. and Goulart, J. (2009). Combining Ability of Inbred Lines of Sweet Corn. Bragantia, 68, 75-84. https://doi.org/10.1590/S0006-87052009000100009

Sawazaki, E., Pommer, C. V. and Ishimura, I. (1979). Avaliação de cultivares de milho para utilização no estádio de verde. Ciência e Cultura, 31, 1297-1305. https://doi.org/10.21664/2238-8869.2017v6i1. p232-251