

# The combining ability of popcorn $S_7$ lines for *Puccinia polysora* resistance purposes

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**ABSTRACT:** The present study resulted from the lack of information on the genotypic expression ruling the resistance to rust and on the main traits of economic importance to the herein addressed crop. The combining ability of 16 popcorn lines and hybrids was assessed in partial diallel cross in two representative locations in Rio de Janeiro State, Brazil, where forty simple hybrids were obtained. The experiments followed a completely randomized block design with four repetitions and were conducted under natural infestation conditions in order to test the southern rust severity in the entire plant (SSW) and in the leaf of the first ear (SFPE), as well as the morpho-agronomic traits of greater interest: grain yield (GY) and

popping expansion (PE). Additive gene effects prevailed in PE and the non-additive ones stood out in GY; however, both gene effects were important to the southern rust resistance characteristics (SSW and SFPE). Hybrids L65 × P10 and P7 × L70 presented appropriate specific combining ability (SCA) values in all traits; therefore, they were promising for the selection of superior genotypes. Lines L80, L76 and L77 expressed negative general combining ability (GCA) values in SSW and SFPE; line P10 recorded high and positive GCA values in GY and PE.

**Key words:** *Zea mays*, circular diallel, general and specific combining ability, southern rust.

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## INTRODUCTION

Southern rust has become one of the main diseases affecting corn crops in Brazil, mainly those subjected to high temperature and humidity conditions (Ramirez-Cabral et al. 2017; Casela and Ferreira 2002). Some factors such as the use of susceptible hybrids and monocropping in no-till systems in association with weather conditions favorable to inoculant outspread have increased the occurrence of this disease in Brazilian crops (Colombo et al. 2014; Costa et al. 2012). Pinho et al. (1999b) assessed the damages caused by corn rust and concluded that the reduced yield mostly resulted from southern rust. On the other hand, Pinho et al. (1999a) evaluated corn genetic control to resist southern and tropical rust. According to them, it is easier obtaining hybrids and lines resistant to southern rust than to tropical rust due to the smaller number of genes associated with the high heritability estimates recorded for southern rust.

However, studies about popcorn remain incipient when it comes to genotypic superiority investigations and southern rust heritability. Vieira et al. (2011) estimated the economic importance and the combining ability of popcorn rust-resistance traits via partial diallel. They concluded that the additive effects were more important to the popping-expansion expression and to southern rust severity. Vieira et al. (2012) also assessed the possibility of breeding popcorn to achieve blotch and *Cercospora* leaf spot resistance and found that the additive genes were also very important to the expression of these diseases.

Nevertheless, Kurosawa et al. (2016), in a similar study, discriminated popcorn accessions tolerant to *Puccinia polysora* from the germplasm bank of UENF. They conducted some diallel analyses to estimate the combining abilities aiming at identifying superior hybrids (Hallauer et al. 1988; Pinto et al. 2007). Diallels provide information about trait heritability and help breeding programs to properly develop superior gains (Hallauer et al. 2010; Cruz et al. 2012).

Although partial diallel crosses were suggested by Kempthorne and Curnow (1961) a few decades ago, they remain an option capable of providing greater flexibility to diallel crosses, despite their little application in plant breeding (Cruz et al. 2012; Vivas et al. 2014). However, Veiga et al. (2000) pointed out that the partial diallels are as efficient as the full diallels in classifying parents, the general and specific combining abilities and the magnitude of the estimates made for these parameters. Ferreira et al. (2004)

used information about a full diallel of 28 corn populations and about their ear production to simulate estimates on the general and specific combining abilities of partial diallels at variation ranging from 3 to 25 crosses per parent. One thousand partial diallels were generated and the GCA and SCA of partial diallels were almost completely similar to the GCA and SCA of the full diallel. Recently, Vivas et al. (2014) adopted the mixed modeling applied to a partial diallel in eight papaya progenies and confirmed that three crosses are enough to get good agreement (correlation greater than 0.8) between combining-ability estimates.

The general combining ability (GCA) and the specific combining ability (SCA) are estimated to characterize parents used in crosses. GCA is associated with additive effect genes, whereas SCA is estimated through cross behavior deviation when it comes to expectations about the association of parents' general combining ability with the non-additive genetic effects (Vencovsky 1987; Hallauer et al. 2010).

The aim of the present study was to assess the combining ability of 16 popcorn lines belonging to the germplasm collection of UENF through the partial diallel methodology suggested by Kempthorne and Curnow (1961) and outspread by Cruz et al. (2012) in order to identify genotypes resistant to *P. polysora*. Another objective was to identify the preponderant genetic effects on southern rust resistance as the initial step to establish a breeding program for the herein addressed crop in Northern and Northwestern Rio de Janeiro State.

## MATERIALS AND METHODS

Sixteen popcorn lines at the seventh self-pollination ( $S_7$ ) generation were crossed in partial diallel arrangement according to the methodology suggested by Kempthorne and Curnow (1961) ( $S = 5$ ), without the inclusion of reciprocal hybrids, which provided 40 hybrid combinations. In April 2014, four seeds were manually distributed per furrow, at depth 0.05 m, in 4-min rows spaced 0.90 m apart from each other and 0.20 m spacing between plants in order to obtain the hybrids. Ears were covered with plastic bags in the flowering period, before the stigma was apparent, in order to prevent contamination. The tassel of the line to be used as male parent, whose pollen had already been released, was covered with a Kraft paper bag when the stigma was ready for crossing. The cross was performed the day after the tassel was covered, and a paper bag was used to protect

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the ear after it was pollinated. Pollinations were performed in approximately 40 ears per cross in order to obtain a sufficient number of seeds to keep the process running.

Two experiments were implemented from September 2014 to January 2015, one at Antônio Sarlo State Agricultural College, (Campos dos Goytacazes, Northern Rio de Janeiro State, Brazil) and the other at the Experimental Station at UENF (Itaocara, Northwestern Rio de Janeiro State, Brazil) in order to assess the hybrids, parent lines and controls. Both experiments followed a completely randomized block design with four repetitions, according to the statistical model suggested by Hallauer et al. (2010), without any competition between lines and hybrids. The following treatments were set: 40 hybrids, three controls (hybrids IAC 125, L70 × L54, and L70 × P1), 16 parent lines and three varieties (UENF-14, UFV M-2 Barão de Viçosa, and BRS Angela). Two seeds were distributed per furrow, at depth 0.05 m, in 5-m rows spaced 0.90 m apart from each other, plants were spaced 0.20 m from each other in each line at each location.

The following morpho-agronomic traits were assessed: i) mean grain yield (GY) and ii) popping expansion (PE). GY was quantified by weighing the threshed grains; it was expressed in kg·ha<sup>-1</sup>. PE was determined through two repetitions per treatment and represented by 30 kernels per repetition. These kernels were subsequently subjected to expansion in microwave oven (Electrolux MEF41- 31 L) at maximum power (1,000 W) for 2 min 10 s. Next, the popcorn volume resulting from the expansion was quantified in a 1000-mL beaker, and the final-result was weighted at initial mass 30 g of grains; results were expressed in mL·g<sup>-1</sup>.

Southern rust severity in the field in the entire plant (SSW) was evaluated by means of visual assessments in order to quantify plant resistance to it; the descriptive scale suggested by Agrocere (1996) was adopted. Southern rust in the leaf of the first ear (SFPE) was also quantified through the diagrammatic scale by Cobb (Peterson et al. 1948), with modifications (Chester 1950; Pinho et al. 2000). Samples were collected from the middle portion of the adaxial surface of the leaf. Five plants per plot were subjected to both evaluation procedures, the first and the last five plants in the plot were excluded from the experiment. Thus, 15 central plants were considered the usable ones in the plots. The computer resources of the GENES software were employed for data analysis (Cruz 2013).

The method by Kempthorne and Curnow (1961) was used in the diallel analysis and expressed as follows:

$Y_{ij} = \mu + g_i + g_j + s_{ij} + \xi_{ij}$ , where in  $Y_{ij}$  = mean observation associated with the hybrid combination  $ij$  ( $i \neq j$ ), or with parent  $i$  ( $i = j$ );  $\mu$  = general mean;  $g_i$  and  $g_j$  = general combining ability (GCA) effects associated with parents  $i$  and  $j$ ;  $s_{ij}$  = specific combining ability (SCA) effects associated with parents  $i$  and  $j$ ; and  $\xi_{ij}$  = mean experimental error.

## RESULTS AND DISCUSSION

Significance was detected at 1% probability in all GCA environments in almost all assessed traits, and it allowed assuming that additive effects are relevant to their expression. These traits are of greatest importance to popcorn breeding and the significances express the variability in the parents and hybrids group. Such variability influences the selection of superior parents or segregants in advanced generations.

Vieira et al. (2011) evaluated the combining ability of popcorn lines through circulating diallel and found that SCA had no effect on southern rust severity. They assumed that the effects from additive genes are more important to the differentiation of genotypes expressing resistance. Similar to results recorded by Vieira et al. (2011), the present study showed the significant effect of SCA on SFPE in places other than the Campos dos Goytacazes environment (Tables 1 and 2). Therefore, it was not possible to accurately confirm the variability expression activity between genotypes.

**Table 1.** Mean square estimates of popcorn genotypes (parents and F<sub>1s</sub>), of general and specific combining abilities (GCA and SCA), and the residual; mean squares of combining ability effects on morpho-agronomic traits and on two traits related to southern rust resistance in partial diallel. Campos dos Goytacazes, Rio de Janeiro, Brazil.

SV	d.f.	Mean squares <sup>1</sup>			
		GY	PE	SSW	SFPE
Genotype	55	388698548.98**	59.89**	89.63**	102.19**
GCA	15	1743352.60**	219.44**	220.98**	298.43**
SCA	40	4690847.85**	0.0016 <sup>n.s.</sup>	40.38**	28.59 <sup>n.s.</sup>
Residual	165	492600.59	13.03	24.89	20.49
Mean squares of effects					
GCA		52114.67	8.60	8.17	11.58
SCA		1049561.8	-3.26	3.87	2.03

<sup>1</sup>GY = grain yield; PE = popping expansion; SSW = southern rust severity in the entire plant; SFPE = southern rust severity in the leaf of the first ear. <sup>n.s.</sup> = not significant at 5% probability level in the F test; \* = significant at 5% probability level in the F test; and \*\* = significant at 1% probability level in the F test.

**Table 2.** Mean square estimates of popcorn genotypes (parents and  $F_{1s}$ ), of general and specific combining abilities (GCA and SCA), and the residual; the mean squares of the combining ability effects on the morpho-agronomic traits and on traits related to the southern rust resistance evaluated through partial diallel. Itaocara, Rio de Janeiro, Brazil.

SV	d.f.	Mean squares <sup>1</sup>			
		GY	PE	SSW	SFPE
Genotype	55	2185915.64**	64.76**	336.71**	0.13**
GCA	15	3646176.64**	237.34**	397.38**	0.13**
SCA	40	1638317.76**	0.00679 <sup>ns</sup>	313.96**	0.12**
Residual	165	8539489458.00	10.19	112.29	0.04
Mean squares of effects					
GCA		116342.82	9.46	11.88	0.00
SCA		196092.2	-2.54	50.42	0.02

<sup>1</sup>GY = grain yield; PE = popping expansion; SSW = southern rust severity in the entire plant; SFPE = southern rust severity in the leaf of the first ear. <sup>ns</sup> = not significant at 5% probability level in the F test; \* = significant at 5% probability level in the F test; and \*\* = significant at 1% probability level in the F test.

With regard to the mean squares of the effects, GCA values were higher than those of SCA in the CE, SSW and SFPE traits analyzed in Campos dos Goytacazes (Table 1). These results consolidate additivity superiority in grain popping expansion (Pereira and Amaral Junior 2001; Rodovalho et al. 2012) and reveal the great importance of additive effects to the expression of southern rust resistance. Therefore, the use of parents in intra-population breeding programs is the best alternative to get gains through selection processes.

However, the mean squares of SCA effects were higher than those of GCA in SSW in Itaocara; the effects of GCA and SCA on SFPE were equivalent (Table 2). These results may indicate that hybridization-based breeding aiming at exploiting the heterotic effect is the best alternative to provide gains more resistant to southern rust. Therefore, it is possible confirming the great influence of the dominance effect on the expression of southern rust severity in popcorn. However, results in the present study do not corroborate the observations by Vieira et al. (2011), thus indicating the greatest prevalence of additive effects on southern rust severity. According to Nihei and Ferreira (2012), the heritability of the gene expressing southern rust resistance in corn has non-additive effect, but such statement did not fit SSW in the present study, because of the divergent results of the studied environments. Accordingly, the real impact of

gene effects on the expression of southern rust resistance in popcorn remains uncertain.

Colombo et al. (2014) quantified the effects of general (GCA) and specific (SCA) combining abilities, as well as the reciprocal effect associated with southern rust resistance in eight commercial common-corn hybrids. They found that the higher the mean square of GCA in SCA in these parents, the greater the additive gene action to control southern rust resistance. Silva et al. (2001) and Vieira et al. (2011) stressed that the additive gene effects were important to control the resistance to *P. polysora*; however, they also observed that some hybrid combinations were promising. Thus, it is possible inferring that the non-additive gene effects also contributed to the resistance to the disease.

With respect to the popping expansion (PE), similar to the assertions made by Freitas Junior et al. (2006) and Schwantes et al. (2017), the superiority of GCA effects confirms the great importance of additivity to the expression of this trait. The estimates of the  $\hat{g}_i$  effects on ER allowed classifying P1, L70, P8 and P10 as the most promising lines since they recorded the highest positive  $\hat{g}_i$  magnitudes in Campos dos Goytacazes environment (Table 3). Lines P10, L54, and P8, in their turn, stood out in Itaocara environment (Table 3).

Only P10, out of the aforementioned lines, displayed high  $\hat{g}_i$  value in the GY and PE traits in the two cultivation areas (Table 3). It is common finding lines presenting high GCA values in GY and low GCA in PE, or vice-versa, because these traits are negatively correlated to each other, as mentioned by Freitas et al. (2013). However, after analyzing the herein addressed locations, it was possible to observe that this line showed high values in both traits; therefore, it is of great value for future breeding programs focused on simultaneously exploiting heterosis to improve PE grain yield and additivity.

Only lines L80, L61, L77, L65, and L76 showed low and negative values in both southern rust resistance traits (SSW and SFPE) in Campos dos Goytacazes environment (Table 3). Lines L80, L63, L70, L76, L61, and L77 showed the lowest SSW and SFPE negative values in Itaocara. Lines L80, L76, and L77 expressed the best values in both locations and in the studied traits, thus demonstrating their importance to the development of popcorn breeding programs aimed at enhancing gains from plants more resistant to southern rust.

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**Table 3.** General combining ability effect estimates ( $\hat{g}_i$ ) of the two morpho-agronomic traits and of the two traits related to the southern rust resistance evaluated in partial diallel among 16 popcorn lines. Campos dos Goytacazes and Itaocara, Rio de Janeiro, Brazil.

Line	Traits <sup>1</sup>							
	GY		PE		SSW		SFPE	
	Campos	Itaocara	Campos	Itaocara	Campos	Itaocara	Campos	Itaocara
L55	-576.22	29.84	0.26	1.78	3.76	2.61	4.91	0.06
P1	-75.78	-417.33	3.14	1.03	1.12	0.49	-1.02	-0.03
L65	-293.13	-437.42	-1.63	-1.06	-1.60	1.08	-0.77	0.01
P6	342.00	407.74	0.85	-0.48	1.88	2.94	2.02	0.06
L63	97.91	-348.86	-0.89	-1.66	2.42	-3.51	-3.89	-0.08
L54	163.57	629.98	0.91	3.42	0.72	3.25	0.76	0.04
P3	31.73	429.91	-2.53	-0.50	1.62	1.49	1.73	0.01
P7	327.78	258.90	1.55	1.82	-0.72	2.93	0.64	0.06
P10	105.85	224.74	2.51	3.90	-0.23	4.17	2.99	0.09
L80	122.73	-196.86	-4.27	-4.55	-8.11	-7.44	-5.50	-0.13
L76	315.42	-17.20	0.78	1.90	-0.28	-3.75	-3.73	-0.08
L88	1.19	-280.72	-5.57	-4.42	-0.85	3.65	3.44	0.07
P8	-59.81	-174.31	2.72	2.56	0.77	1.22	3.05	0.04
L61	-313.96	-123.31	-0.34	-2.27	-0.92	-2.05	-2.11	-0.04
L70	15.25	229.55	2.81	0.98	1.09	-4.41	-1.51	-0.06
L77	-204.53	-214.64	-0.30	-2.45	-0.65	-2.66	-1.01	-0.03

<sup>1</sup>GY = grain yield; PE = popping expansion; SSW = southern rust severity in the entire plant; SFPE = southern rust severity in the leaf of the first ear.

The combinations showing the highest estimates of the  $\hat{g}_i$  effects on the GY trait in Campos dos Goytacazes environment were P1 × L76, L63 × L76, L65 × P10, P1 × L80, L65 × L80, L63 × L61, and P7 × L77 (Table 4). All these combinations have at least one parent presenting high  $\hat{g}_i$  (Table 3). Combinations L54 × L70 and L54 × L61 displayed the highest SCA in Itaocara environment (Table 4), and such result corroborates the  $\hat{g}_i$  results expressed by line L54 (Table 3), which recorded one of the highest values for this trait.

Combinations P6 × L61 and L65 × L88 in SSW were better in Campos dos Goytacazes environment (Table 4). The remarkable performance of P6 × L61 was already expected since it contained the parents showing the best  $\hat{g}_i$  estimates (Table 3). The best SSW hybrids in Itaocara environment were P7 × L70 and L55 × L80 (Table 4), and lines L80 and L70 were among the parents presenting the most prominent  $\hat{g}_i$  values (Table 3). Hybrids presenting the best SSW value in Campos dos Goytacazes (Table 4) showed high values in Itaocara, and it suggests that the environment directly influences the southern rust levels in the entire plant.

The best SCA values were recorded for hybrids P6 × L61 and L80 × L77 in SFPE in Campos dos Goytacazes (Table 4), both hybrids had at least one of the parents presenting high  $\hat{g}_i$  estimate (Table 3). The hybrids evidencing the lowest SCA in Itaocara were L55 × P3 and L54 × L77 (Table 4). Only line P7 showed negative  $\hat{g}_i$  value among the parents (Table 3). The best hybrids in both environments showed negative or null values; therefore, it is possible inferring that there was no environment influence on this trait.

Parents can be used as good hybrid-performance predictors when the heterotic effects are not markedly important either to phenotypic or genotypic traits. Line tracking or evaluation *per se* is crucial for corn breeding programs (Vieira et al. 2011). It was not possible to accurately infer the gene effects responsible for southern rust resistance because the GCA and SCA values were discrepant in SSW. This result corroborates those recorded for popcorn blotch by Vieira et al. (2009), who reported that the resistance to this disease can be provided by the combined action of additive and non-additive effect genes.

The hybrids standing out in the combined analysis of the assessed traits were L65 × P10 and P7 × L70.

**Table 4.** Specific combining ability effect estimates ( $\hat{s}_{ij}$ ) of the two morpho-agronomic traits and of the two traits related the southern rust resistance evaluated through partial diallel among 16 popcorn lines. Campos dos Goytacazes and Itaocara, Rio de Janeiro, Brazil.

(\$ij)	Traits <sup>1</sup>							
	GY		PE		SSW		SFPE	
	Campos	Itaocara	Campos	Itaocara	Campos	Itaocara	Campos	Itaocara
L55XP7	-153.38	-205.29	-0.0095	0.0000	3.30	20.88	-0.32	0.32
L55XL80	394.74	116.03	0.0005	-0.0001	3.94	-5.65	1.78	-0.12
P1XP10	66.99	-659.5	-0.0105	0.0058	0.20	3.45	-1.08	-0.02
P1XL76	1277.25	425.77	0.028	-0.0051	2.00	-3.85	-2.72	-0.05
L65XL80	1062.40	792.19	-0.004	-0.0042	0.80	4.23	-1.02	0.01
L65XL88	-13.78	-268.39	0.0025	-0.0102	-4.21	12.17	3.07	0.24
P6XL76	252.51	498.48	0.0012	-0.003	-1.5	5.45	0.49	0.06
P6XP8	-590.42	-381.08	-0.0012	-0.0086	-0.06	9.01	-0.44	0.17
L63XL88	802.61	156.38	-0.0049	0.077	1.52	3.3	0.31	-0.02
L63XL61	1049.05	-381.02	0.0035	0.0808	2.59	-2.83	2.11	-0.06
L54XP8	606.11	72.24	0.0023	-0.0038	1.86	8.25	1.35	0.13
L54XL70	705.61	1088.38	0.0024	-0.0098	-2.72	3.28	-0.84	0.01
P3XL61	855.76	-434.25	-0.002	-0.0083	-3.11	12.47	-3.53	0.24
P3XL77	412.57	-408.47	-0.0064	0.0021	3.62	-0.99	3.19	-0.01
P7XL61	109.02	392.32	0.0051	-0.0122	-0.77	-4.26	0.95	-0.06
P7XL77	1025.99	-88.57	-0.0635	-0.0018	-0.78	11.42	-0.82	0.34
P10XL70	778.28	172.51	-0.001	-0.0152	2.73	3.28	-2.01	0.07
L55XP3	181.83	189.26	-0.003	0.0039	2.21	-4.78	-0.05	-0.18
L55XP10	-350.56	2.21	0.0285	0.0036	-3.94	13.21	-0.19	0.49
L55XL76	888.88	193.99	-0.0066	-0.0073	3.86	0.53	2.96	0.01
P1XP7	-556.07	153.01	0.00	0.0023	0.69	8.97	0.31	0.13
P1XL80	1090.17	394.32	-0.0007	0.0021	0.58	-1.69	0.78	0
P1XL88	626.13	-374.04	0.0007	-0.0139	1.32	4.35	-0.82	0.15
L65XP10	1147.1	696.14	0.0212	0.0095	0.43	1.62	-0.85	-0.03
L65XL76	192.77	-341.92	-0.0038	-0.0114	2.98	2.71	5.26	-0.01
L65XP8	-85.5	-1270.4	-0.0058	-0.0071	-3.25	9.34	-0.37	0.14
P6XL80	880.47	11.47	0.0051	0.0042	4.7	-2.13	4.67	-0.06
P6XL88	913.4	-334.68	-0.0035	-0.0018	4.81	0.88	1.02	0.03
P6XL61	732.97	842.37	0.0005	-0.008	-4.62	2.75	-4.38	-0.03
L63XL76	1160.04	828.41	0.0058	0.0758	1.95	7.62	2.91	0.03
L63XP8	904.45	-327.81	0.0038	0.0801	-1.35	1.67	1.48	-0.01
L63XL70	-493.64	-921.67	-0.0007	0.0742	-0.18	2.48	-3.21	0.09
L54XL88	855.89	880.87	0.002	-0.0069	3.97	-0.75	2.16	0.1
L54XL61	423.94	944.58	-0.004	-0.0032	2.04	5.44	-0.94	0.03
L54XL77	569.53	121.46	0.0035	0.0072	-1.97	-5.47	-2.41	-0.15
P3XP8	280.94	250.08	0.0016	-0.0089	-0.8	-4.35	0.51	-0.06
P3XL70	-125.53	303.99	0.0005	-0.0049	-2.12	-3.92	-1.48	0
P7XL70	722	418.34	0.025	-0.0088	-2.53	-6.53	0.88	-0.11
P10XL77	295.03	94.48	0.0024	0.0018	4.48	4.38	4.71	0.01
L80XL77	589.41	284.97	-0.0064	-0.0019	-1.64	3.77	-4.23	0

<sup>1</sup>GY = grain yield; PE = popping expansion; SSW = southern rust severity in the entire plant; SFPE = southern rust severity in the leaf of the first ear.



## CONCLUSION

The superior hybrids were L65 × P10 and P7 × L70, since they presented the best GY and PE values. They allowed the simultaneous selection of both characteristics, and the constantly negative SCA values for southern rust resistance.

Lines L80, L76 and L77 were promising for the selection of southern rust resistant genotypes, because they expressed negative GCA values. The P10 line was promising for the selection based on GY and PE characteristics.

Additive effects prevailed in popping expansion, and non-additive effects stood out in grain yield.

Additivity and dominance were not exclusionary in the expression of southern rust resistance.

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