

## Genetic analysis of tropical rust resistance in popcorn lines

### Análise genética da resistência à ferrugem tropical em linhagens de milho-pipoca

Rafael Egea Sanches<sup>I</sup> Carlos Alberto Scapim<sup>I</sup> Dauri José Tessmann<sup>II</sup> Rafael Augusto Vieira\*  
Marcos de Araújo Rodovalho<sup>I</sup> Klayton Flávio Milani<sup>I</sup>

#### ABSTRACT

The objectives of this study were to identify promising popcorn lines and hybrids for genetic resistance to tropical rust (*Tr*; *Physopella zea*) in diallel crosses of nine lines derived from a large part of Brazilian popcorn germplasm and to estimate genetic parameters for *Tr* resistance. Crosses and checks were tested in a random block design, with artificial inoculations under greenhouse conditions. Plots consisted of a single 14-L pot, with four plants. *Tr* was measured by severity and area under the disease progress curve. Data was analyzed by Griffing's diallel model, and genetic parameters were estimated. Heritability estimates to *Tr* resistance was medium. Specific combining ability-SCA had significant effects ( $P < 0.10$ ) for resistance, and was eight to nine times larger than general combining ability-GCA ( $P > 0.10$ ). This indicated the major importance of non additive gene effects. In general, breeding possibilities for resistance was restricted. The line 9 ( $L_{7,4}$ ) had the most outstanding GCA for resistance to *Tr*, and hybrids  $1 \times 7$  and  $3 \times 6$  had outstanding SCA and were recommended for breeding.

**Key words:** *Zea mays* L., *Physopella zea*, genetic parameters, general and specific combining ability, artificial inoculation.

#### RESUMO

Os objetivos deste estudo foram identificar linhagens e híbridos de milho-pipoca promissores quanto à resistência genética à ferrugem tropical (*Ftrop*, *Physopella zea*) em cruzamentos dialélicos, envolvendo nove linhagens derivadas de uma grande parte do germoplasma brasileiro de milho-pipoca, e estimar parâmetros genéticos envolvendo a resistência à *Ftrop*. Cruzamentos e testemunhas foram testados

em delineamento de blocos ao acaso, sob condições de casa-de-vegetação e inoculação artificial. As parcelas consistiram de um vaso de 14 litros, com quatro plantas. A *Ftrop* foi avaliada pela severidade e pela área abaixo da curva de progresso da doença. Os dados foram analisados pelo modelo dialélico de Griffing e parâmetros genéticos foram estimados. As estimativas de herdabilidade foram médias. A capacidade específica de combinação-CEC teve efeito significativo ( $P < 0,10$ ) para a resistência e foi oito a nove vezes maior que a capacidade geral de combinação-CGC ( $P > 0,10$ ). Isso indicou que os efeitos gênicos não aditivos foram mais importantes. Em geral, as possibilidades de melhoramento foram restritas. A linhagem 9 ( $L_{7,4}$ ) teve a mais destacada CGC para a resistência e os híbridos  $1 \times 7$  e  $3 \times 6$  tiveram destacada CEC e foram recomendados para melhoramento.

**Palavras-chave:** *Zea mays* L., *Physopella zea*, parâmetros genéticos, capacidades geral e específica de combinação, inoculação artificial.

#### INTRODUCTION

Tropical rust in maize, caused by the fungus *Physopella zea* (Mains) Cummins & Ramachar (syn. *Angiospora zea* Mains) has been a major disease of corn in the middle-west and southeast regions of Brazil (KIMATI et al., 1997; VON PINHO et al., 1999), especially in low-altitude areas and late planting dates. Losses associated with tropical rust are up to 60% in susceptible maize hybrids (VON PINHO et al., 2000; CASELA et al., 2006), and frequently occur when

<sup>I</sup>Programa de Pós-graduação em Genética e Melhoramento, Departamento de Agronomia, Universidade Estadual de Maringá (UEM), 87020-900, Maringá, PR, Brasil. E-mail: rfavieira@gmail.com. \*Autor para correspondência.

<sup>II</sup>Programa de Pós-graduação em Agronomia, UEM, Maringá, PR, Brasil.

photosynthetic leaf tissue is rendered nonfunctional due to lesions. In this sense, genetic resistance is indubitably the most cost-effective way for controlling the tropical rust and to prevent its yield losses.

In fact, the maize germplasm available for popcorn breeding programmes has a narrow genetic base. The popcorn germplasm often performs worst than dent corn (ZIEGLER, 2001), possibly as consequence of the small number of popcorn lines developed from flint corn, modified by selection to maximize popping expansion and quality (KANTETY et al., 1995). Frequently this leads to poorer agronomic traits including susceptibility to diseases (ZIEGLER, 2001; ARNHOLD, 2008). In this regard, screenings and genetic studies of foliar diseases resistance, such as tropical rust, are strongly encouraged to supply information about the most promising parental genotypes and advantageous breeding techniques. In general, scientific informations about genetic resistance to *P. zeae* are incipient in common maize, and even unpublished for popcorn germplasm. Moreover, popcorn demands has increased in Brazil, and the needs of breeding programmes for developing superior cultivars also correspondingly increases.

In the present study, the objectives were to identify promising popcorn lines and hybrids for genetic resistance to tropical rust (*P. zeae*) in diallel crosses of inbred lines derived from a large part of Brazilian popcorn germplasm, and to estimate genetic parameters of tropical rust resistance. This study intended to provide scientific information about tropical rust resistance for popcorn breeding programmes in Brazil, especially those conducted by public universities and governmental research centers.

## MATERIALS AND METHODS

### Germplasm, experimental design, and inoculations

Nine popcorn lines (in the 8<sup>th</sup> endogamic stage) derived from several Brazilian breeding

populations of popcorn were crossed in a complete diallel matching scheme (Table 1). Crosses were manually performed in 2008/09, and 36 hybrids were obtained. Hybrids from the diallel and four check hybrids ('Zélia', 'Jade', 'IAC112' and 'IAC125'), completing 40 entries, were tested in a random block design with three replications. Both 'IAC 112' and 'IAC 125' are intermediate resistant cultivars, whereas the reaction of 'Zélia' and 'Jade' are not designed (CRUZ & PEREIRA FILHO, 2010). A single experiment was conducted during December 2009 to February 2010, under greenhouse controlled conditions, in the Universidade Estadual de Maringá, PR. Experimental plots consisted of a single 14L-pot with four plants after thinning. Pots contained a mixture of soil and *Pinus* bark in a ratio 2:1.

Inoculum production was previously conducted by inoculating tropical rust in plants of the hybrid 'Zélia'. About 10 single-uredia isolates were obtained, and mixed to form a pool. Isolates of the pool were collected in maize production areas in Northeast Paraná, Brazil. After 14 days inoculations on 'Zélia', inoculum was prepared by collecting urediniospores from the surface of the affected leaves. For this, the leaves were washed with distilled water. Inoculum maintenance was performed as described until the experiment reached the V7-V8 grown stage, when the inoculations were performed.

Inoculum suspension for the experiment was adjusted to  $1.5 \times 10^5$  urediniospores per mL. One inoculation was carried out using a 4.7 L-pulverizator of previous compression, and about 25mL of suspension were applied to each pot. Inoculation was performed after 7 p.m, and whole plants were inoculated. Inoculated plants were kept under high humidity conditions (90 to 100%) and 22 to 25°C of air temperature during the first 14h. After this, humidity varied from 65 to 90%, and temperature from 20 to 35°C.

### Disease evaluations and data analyses

Severity assessments were conducted every seven days from the 7<sup>th</sup> to the 21<sup>st</sup> days after the

Table 1 - Origin of the popcorn lines used in this study.

Inbred line	Origin
1 (L <sub>1,1</sub> 'Zélia')	hybrid 'Zélia'/Pioneer Hi-Bred
2 (L <sub>3,2</sub> CMS 42)	yellow grains open pollinated variety CMS 42/Embrapa
3 (L <sub>4,4</sub> CMS 43)	white grains open pollinated variety CMS 43/Embrapa
4 (L <sub>7,1</sub> UEM-M2)	open pollinated variety UEM-M2/UEM
5 (L <sub>8,4</sub> 'Zaeli')	'Zaeli', a material with unknown genealogy
6 (L <sub>9,1</sub> 'IAC 112')	Hybrid 'IAC112/IAC'
7 (L <sub>9,2</sub> 'IAC 112')	Hybrid 'IAC112/IAC'
8 (L <sub>9,3</sub> 'IAC 112')	Hybrid 'IAC112/IAC'
9 (L <sub>7,4</sub> UEM-M2)	open pollinated variety UEM-M2/UEM

inoculation. Severity was estimated for all leaves of plants in the plots. Yellow halos were opportunely included as affected area. The mean of plots was used to estimate the area under the disease progress curve (AUDPC), as described by CAMPBELL & MADDEN (1990). Both severity at 14<sup>th</sup> and 21<sup>th</sup> days, and AUDPC (which integrates all the evaluations) was used in statistical data analyses.

Analysis of variance (ANOVA) was previously performed in a random block design. Next, GRIFFING's (1956) diallel analysis was carried out using diallel means and mean square error (MSE) for each variable. Degrees of freedom for diallel consisted of 70 (diallel hybrids - 1 \* blocks - 1). Hybrids and blocks had random effects. Finally, genetic parameters from diallel analyses were estimated according to CRUZ & REGAZZI (1994) and VENCOVSKY & BARRIGA (1992). Statistical procedures were carried out using the program Genes (Cruz, 2006).

## RESULTS AND DISCUSSION

Hybrids means varied from 26% (3×6) to 46% (1×5) for tropical rust severity at 14<sup>th</sup> days after the first inoculation. At 21 days, hybrids ranged from 33% (7×9) to 48% (1×5), and from 227 (3×6) to 443 (1×5) for the area under the disease progress curve (AUDPC). These means suggested that some hybrids and its parental lines may differ in respect to tropical rust resistance. In fact, previous ANOVA indicated (P<0.10) that the hybrids from the diallel crosses had different tropical rust severity levels at 14<sup>th</sup> days and AUDPC, but not for severity at 21<sup>st</sup> days (data not shown).

Diallel analyses were carried out for the variables with significant effects in ANOVA, as well as

genetic parameters were estimated (Table 2). Specific combining ability (SCA) had significant effects (P<0.10) for tropical rust resistance, evaluated by severity at 14<sup>th</sup> and AUDPC. General combining ability (GCA) was not significant (P>0.10). In this sense, it was inferred that non additive gene effects play essential role in the regulation of hybrids tropical rust resistance. The variance components from SCA were eight (14<sup>th</sup>-severity) and nine times (AUDPC) larger than those for GCA. This suggested that breeding possibilities are larger using non additive genes effects from SCA. On contrary, percents of GCA and SCA mean squares were respectively 55% and 45%. Heritability estimates were 0.41 using 14<sup>th</sup>-severity, and 0.40 using AUDPC. Such estimates are medium, and shown that only a partition of phenotypic differences among hybrids came from genetic causes.

Although GCA had not significant effect, general combining ability estimates were studied. According to the general combining ability estimates ( $\hat{g}_i$ ), negative and relevant values are desirable, since they indicated that such parental contributed to increase the resistance level on their offsprings (VIEIRA et al., 2009). In this sense, the line 9 (L<sub>7,4</sub>-UEM-M2) had outstanding  $\hat{g}_i$  for both 14<sup>th</sup>-severity and AUDPC (Tables 3), and is the most appropriated for popcorn breeding programmes for tropical rust. This line can be recommended to obtain hybrids with high genetic resistance for tropical rust. Specific combining ability estimates ( $\hat{s}_{ij}$ ) measures the effects of non additive genes (CRUZ & REGAZZI, 1994), and some of them had outstanding negative values both 14<sup>th</sup>-severity and AUDPC (Table 3). Such estimates were detected for two hybrid combinations (1×7 and 3×6). These hybrid combinations had outstanding performance compared

Table 2 - Diallel analysis for resistance to tropical rust (*P. zeae*) in popcorn lines, and estimates of genetic parameters.

Sources of variation	df	-----Mean square-----	
		14 <sup>th</sup> -severity	AUDPC
Hybrids	35	67.09*	4,860.35*
General combining ability (GCA)	8	76.71 <sup>ns</sup>	5,855.23 <sup>ns</sup>
Specific combining ability (SCA)	27	64.24*	4,565.57*
Error	70	39.62	2904.92
Mean		34	310
Cve (%)		18	17
Genetic variance		9	651
-----Variance components for-----			
GCA		1	61
SCA		8	553
Broad-sense heritability		0.41	0.40

\* P<0.10 by F-test. <sup>ns</sup> P>0.10 by F-test.

Table 3 - General ( $\hat{g}_i$ ) and specific combining ability ( $\hat{s}_{ij}$ ) estimates for resistance to tropical rust (*P. zeae*) in popcorn lines, measured by severity at 14<sup>th</sup> days after inoculation (above the diagonal) and the area under the disease progress curve (AUDPC, below the diagonal).

Inbred lines	$\hat{s}_{ij}$ 1,3									$\hat{g}_i$ (14 <sup>th</sup> sev.) <sup>2</sup>
	1	2	3	4	5	6	7	8	9	
1		-5.7	-1.4	4.2	8.0	0.6	<b>-7.6</b>	0.9	0.9	1.5
2	-51.8		0.9	2.1	6.6	5.2	-3.3	-0.8	-4.8	0.5
3	-10.3	16.5		0.5	0.3	-8.1	4.7	-0.1	3.2	0.8
4	36.0	16.7	-10.8		-6.2	0.7	-4.4	3.7	-0.6	-1.4
5	68.4	61.4	-2.5	-43.1		-3.1	3.8	-4.7	-4.7	2.1
6	8.1	38.1	-64.2	7.7	-30.7		5.4	-2.8	2.2	-1.1
7	-62.7	-34.8	37.5	-26.5	29.0	48.2		0.7	0.7	-1.3
8	8.6	-10.6	1.0	32.6	-43.3	-29.9	11.2		3.2	2.2
9	3.6	-35.6	32.7	-12.6	-39.3	22.6	-1.8	30.4		-3.4
$\hat{g}_i$ (AUDPC) <sup>4</sup>	13.7	4.6	7.4	-6.3	18.0	-9.0	-12.4	16.4	-32.5	

Relevant negative estimates (twice respective standard deviation-SD) are highlighted in bold. Check hybrids means (14<sup>th</sup> severity – AUDPC) IAC112: 35.0 – 349.5 ; IAC125: 38.7 – 382.3 ; Zélia: 32.0 – 323.0; Jade: 29.7 – 295.8 . <sup>1</sup>SD: 3.1 (14<sup>th</sup> severity); <sup>2</sup>SD: 1.3 (14<sup>th</sup> severity); <sup>3</sup>SD 29.0 (AUDPC). <sup>4</sup>SD: 11.9 (AUDPC).

to the expected based on its GCA, and indicates that some non additive genes for tropical rust are composing the genome of these lines. Once no favorable  $\hat{g}_i$  lines were included in these hybrids, they may be cautiously exploited by breeding.

In this study, tropical rust resistance levels have been conditioned by major non additive genes in popcorn Brazilian germplasm. On contrary, additive genes effects has played a major role for tropical rust in common maize (VON PINHO et al., 1999; LIMA et al., 2000). Since the most advantageous breeding methods are defined based on the genes effects predominance, and the inheritance pattern for resistance seems different in popcorn compared to common maize, this study increases in relevance to guide popcorn breeding programs on tropical rust resistance information. In this part of Brazilian germplasm, tropical rust resistance has exhibited a different inheritance pattern, compared to gray leaf spot (GLS, *Cercospora* spp.) and northern leaf blight (NLB, *Exserohilum turcicum*) resistance, which both additive and non additive effects had precious breeding possibilities, but the additive effect was major (VIEIRA et al., 2009). Likewise, tropical rust resistance sources are also supposed to be narrow than those for the two mentioned diseases.

Even though breeding possibilities for resistance to tropical rust seems quite restricted in popcorn germplasm, since it was found that just a single inbred line (L<sub>7.4</sub>) contributes for tropical rust resistance, in fact, this line is promising as source of resistance genes in popcorn. This is reinforced when it's taking

into account the check hybrid means, with known tropical rust resistance level. They suggested that best new hybrids had an intermediate resistant to susceptible pattern of tropical rust resistance (see footnotes, Table 3). Additionally, not very promising hybrid combinations were found from the diallel. The most promising ones do not include the L<sub>7.4</sub> in its composition. Additionally, as according to previous studies, L<sub>7.4</sub> is susceptible to NLB (VIEIRA et al., 2009). In fact, this reinforces the difficulty to aggregate multiple diseases resistance in popcorn lines, possibly as a consequence of popcorn narrow genetic base.

Genetic studies of resistance to *P. zeae* are quite rare in maize germplasm (VON PINHO et al., 1999; LIMA et al., 2000), even though the disease is important in Latin American and Caribbean countries. For breeding programmes, the lack of background studies leads to the ignorance in respect to genetic control potential and promising resistance sources. Especially in popcorn, such ignorance is indubitably a problem, once germplasm base is narrow worldwide (ZIEGLER, 2001). The present study provided new insights about breeding possibilities in popcorn, possible sources of resistance and gene effects controlling the maize resistance to tropical rust, which is an important support for popcorn breeders in breeding programs.

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