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Identification of sources of resistance to anthracnose stalk rot in maize

Identificação de fontes de resistência à antracnose do colmo do milho

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ABSTRACT

Adoption of resistant cultivars is the primary measure used to control anthracnose stalk rot. The goal of this study was to identify maize-resistant genotypes to anthracnose stalk rot, which are similar to the hybrid 2B710. Experiments were performed at Embrapa Maize and Sorghum experimental fields in Brazil. The first experimental trial evaluated 234 maize lines as well as two commercials hybrids, BRS1010 (susceptible) and 2B710 (resistant). Artificial inoculations were performed with a strain at the blister (R2) phase, and evaluation of disease severity was performed after 30 days. The second experimental trial evaluated 48 maize lines and hybrids, inoculated with two ***Colletotrichum graminicola*** *strains. In the first trial, eight resistance groups were formed, and the last lines were more resistant, as was the hybrid 2B710, with values between 11.50% and 23.0% of severity. In the second trial, there was an interaction between the two factors, lines and isolates, and the lines often showed the same reaction features as those obtained in the first trial. However, the disease severity was higher for most lines, even when using other isolates. These lines with effective levels of resistance could be used in future studies of inheritance, in programs to develop hybrids, and to identify molecular markers associated with resistance to anthracnose stalk rot in maize.*

Key words: ***Zea mays****, germplasm bank,* ***Colletotrichum graminicola****.*

RESUMO

O uso de cultivares resistentes é a principal medida para o manejo da antracnose do colmo em milho. Neste trabalho, objetivou-se identificar linhagens com níveis de resistência à antracnose do colmo, similar ao híbrido 2B710, considerado resistente. Dois experimentos foram conduzidos na Embrapa Milho e Sorgo. No primeiro experimento, foram avaliados 234 linhagens e os híbridos BRS1010 (suscetível) e 2B710 (resistente). Foi realizada inoculação artificial com um isolado de ***C. graminicola****, na fase de pré-pendoamento e, após 30 dias, foi realizada a avaliação da severidade da antracnose no colmo. O segundo experimento foi conduzido com 48 linhagens e os híbridos inoculados com dois isolados de* ***C. graminicola****. No primeiro experimento, os genótipos formaram oito grupos com base na severidade da doença e as linhagens do último grupo foram consideradas as mais resistentes, incluindo o híbrido 2B710, em que os genótipos apresentaram valores de severidade entre 11,50 a 23%. No segundo experimento, houve interação entre os fatores linhagens e isolados e, de modo geral, as linhagens apresentaram a mesma tendência de reação obtida no primeiro experimento, no entanto, a severidade da doença foi maior para a maioria das linhagens, mesmo quando utilizado o outro isolado. Com isso, foi possível realizar a seleção de linhagens com bons níveis de resistência, as quais podem ser utilizadas em programas de melhoramento, em estudos de herança, desenvolvimento de híbridos e identificação de marcadores moleculares, associados com resistência à antracnose do colmo.*

Palavras-chave: ***Zea mays****, banco de germoplasma,* ***Colletotrichum graminicola****.*

INTRODUCTION

Anthracnose stalk rot, caused by ***Colletotrichum graminicola*** (Ces.) Wils., is found in maize worldwide. It is the principal disease of maize, capable of causing plant lodging, early plant death, and losses of approximately 35% in grain weight (BERGSTROM & NICHOLSON, 1999; DENTI & REIS, 2003; PALAVERSIC et al., 2009; COSTA et al., 2010a; JIRAK-PETERSON & ESKER, 2011; COTA et al., 2012).

Characteristic symptoms of anthracnose stalk rot are narrow and longitudinal lesions that

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have a wet aspect and are brown to reddish in color, while old lesions are typically dark brown to black in color. In the stalk tissues, it is possible to see typical dark-brown coloring, which corresponds to necrotic lesions that lead to plant lodging and early death (BERGSTROM & NICHOLSON, 1999; COTA et al., 2012).

This disease is one of the most important in maize crops and is very hard to control necessitating the use of integrated management practices. These include crop rotation, incorporation of residues in the soil, balanced fertilization (especially in the case of nitrogen and potassium), correct plant spacing, and control of stalk insect pests such as Sugarcane Borer (***Diatrea saccharalis*** Fabr.) and European corn borer (***Ostrinia nubilalis*** Hübner) (BERGSTROM & NICHOLSON, 1999; OLIVEIRA et al., 2004; COTA et al., 2015).

However, currently, the main strategy to control anthracnose stalk rot remains the adoption of resistant genotypes: a practice considered both economically viable and environmentally friendly. Resistant cultivars carry genes with resistance to stalk rot infection, which are transferred via a quantitative inheritance mode, with the predominance of an additive genetic effect (CARSON & HOOKER, 1981; BADU-APRAKU et al., 1987; TOMAN & WHITE, 1993; BERGSTROM & NICHOLSON, 1999; PALAVERSIC et al., 2009; MATIELLO et al., 2012). Maize genotypes showing different levels of anthracnose stalk rot resistance have been described in germplasm banks; examples of such genotypes are the following: MP305, DE811ASR (JUNG et al., 1994, BROGLIE et al., 2006; FREY et al., 2011); DW1035 (TOMAM & WHITE, 1993); A556, A638, Oh43, R177 (CARSON & HOOKER, 1981); RD6502 (BADU-APRAKU et al., 1987); Bc19064 (PALAVERSIC et al., 2009); CML52 (CHUNG et al., 2011); Das2, Das64 (MATIELLO et al., 2012); H8664 (MATIELLO et al., 2013); 2B710 (GARDINGO, 2008; COSTA et al., 2010b; COTA et al., 2010; CARVALHO et al., 2013). However, in practice, the resistance levels of these genotypes remain weak, and information on effective resistance sources is lacking.

Thus, identifying sources of resistance to anthracnose stalk rot by genotype selection in a germplasm bank can help develop more resistant hybrids, inform studies of resistance inheritance, lead to identification of molecular markers for anthracnose stalk rot resistance genes, and have applications for maker-assisted selection. However, this kind of study is only possible with a germplasm bank, which offers a great range of genetic variability for testing. Hence, the objective of this study was to identify lines resistant to anthracnose stalk rot in the Embrapa Maize and Sorghum germplasm bank.

MATERIALS AND METHODS

Trials were performed in the experimental fields of Embrapa Maize and Sorghum, located in Sete Lagoas, state of Minas Gerais, Brazil (latitude: 19º28'03'' S, longitude: 44º15'08'' W; elevation: 732m).

The first experimental trial tested 234 maize genotypes from a germplasm bank (Banco de Germoplasma – BAG of Embrapa Maize and Sorghum). Also included in this trial were two positive controls, the commercial hybrids BRS1010 (Embrapa) and 2B710 (Dow Agroscience), which were used as susceptible and resistant genotypes, respectively. The experimental design consisted of randomized blocks with three replications, each formed by one row of 2m that was 0.8m from others rows, with five plants per meter.

The plant inoculations in the first experimental trial used the single spore strain *Cg*03.09 of ***C. graminicola***, following COSTA et al. (2014). The inoculations were performed at the pre-tassel stage using the methods of a sterile toothpick dipped in the spore suspension (10^6 conidiaml^{-1}). Before inoculation, the lower leaves from the healthier plants in the plots were removed, exposing the lower nodes, and thereafter, the superficial disinfestation of lower nodes was performed using a solution of 70% alcohol. Inoculation was made in the third internode, which was perforated using a sterilized manual perforator followed by insertion of the sterilized toothpick immersed in the spore solution. Toothpick was kept in the internode until the evaluation (COSTA et al., 2010b; COTA et al., 2010).

Crop fertilization at the time of planting was done by administering 300kg.ha^{-1}of NPK (8:28:16+0.4% Zn) and two urea applications (100kg.ha^{-1}) on the 15th and 30th day after planting. To control weeds, Atrazine (3L a.i.ha^{-1}) and Nicosulfuron (140g a.i.ha^{-1}) were applied 25 days after planting. The insecticide Spinosad (100mL.ha^{-1}) was applied 40 days after planting to control the fall armyworm (***Spodoptera frugiperda***). Whenever necessary, the trials were irrigated according to soil status demand.

In order to conduct the evaluation, the inoculated and non-inoculated stalks were harvested 30 days after inoculation. Stalks were longitudinally cut and the severity of stalk rot was evaluated by comparing the inoculated internode to a severity scale

developed by NICOLI et al. (2015). The severity data were first checked to meet ANOVA assumptions: data normality was checked using the Kolmogorov-Smirnov test and variance homogeneity using the Bartlett test (at 5% of probability, in the MINITAB 14 software program). Data normality was not met, and so values were subjected to angular transformation according to DINIZ et al. (2006). Subsequently, the ANOVA was performed, and the means were compared by the Scott-Knott test at a 5% level of probability using the GENES program (CRUZ, 2006).

The second experimental trial used 48 genotypes selected from the first trial and the same two commercial hybrids. Inoculation process was made using two single spore strains (*Cg*05.07 and *Cg*03.09) of ***C. graminicola***, the first (*Cg*05.07) being more aggressive than the second (*Cg*03.09) according to COSTA at al. (2014). This was done to ensure the resistance of a genotype to different strains, as there can be variation in severity related to different isolates (WHITE et al., 1987; COSTA at al., 2014).

This trial was conducted in a randomized block design, and the treatments were applied in a factorial 50 x 2 arrangement (50 genotypes x 2 strains), with three replicates. The plots were formed by one row of 2m that was spaced 0.8m apart from other rows, with five plants per meter. The crop management, inoculation, and subsequent evaluation of stalk rot severity followed the procedures described for the first trial. The data were subjected to ANOVA and, where necessary, the means were compared using the Scott-Knott test using a 5% level of probability in the GENES program (CRUZ, 2006).

RESULTS AND DISCUSSION

In the first trial, a significant difference was observed among the lines ($P<0.05$), which formed eight groups according to a means test (Table 1). The genotypes in the first group (A) were the most susceptible, whereas the genotypes in the last group (H) were considered the most resistant because they had the lowest disease severity values. There were 22 genotypes in the group A, showing severity values between 82% and 92%, including the commercial hybrid BRS1010. For the other groups, there were 29 genotypes in B, 33 in C, 23 in D, 41 in E, 20 in F, 31 in G, and 35 lines in H, which included the resistant hybrid 2B710 (Table 1). The most resistant lines and the resistant hybrid 2B710 had severity values between 11.5% and 23.0% to form the last group, H.

In the second trial, there was a significant interaction among lines and the ***C. graminicola*** strain ($P<0.05$), indicating that the lines showed severity levels that were dependent on one specific strain (Table 2). According to COSTA at al. (2014), there are different races, pathotypes, and haplotypes of ***C. graminicola*** in maize that are spread across the regions of Brazil. In general, the strains tended to show the same reaction as that obtained in the first

Table 1 - Groups of severity levels of the anthracnose stalk rot in maize formed by Scott-Knott test ($P<0.05$), containing 234 lines and two hybrids (BRS 1010 and 2B710).

Groups[a]	Genotypes
A	L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, **BRS1010**, L13, L14, L15, L16, L17, L18, L19, L20, L21, L22, L23
B	L24, L25, L26, L27, L28, L29, L30, L31, L32, L33, L34, L35, L36, L37, L38, L39, L40, L41, L42, L43, L44, L45, L46, L47, L48, L49, L50, L51, L52
C	L53, L54, L55, L56, L57, L58, L59, L60, L61, L62, L63, L64, L65, L66, L67, L68, L69, L70, L71, L72, L73, L74, L75, L76, L77, L78, L79, L80, L81, L82, L83, L84, L85
D	L86, L87, L88, L89, L90, L91, L92, L93, L94, L95, L96, L97, L98, L99, L100, L101, L102, L103, L104, L105, L106, L107, L108
E	L109, L110, L111, L112, L113, L114, L115, L116, L117, L118, L119, L120, L121, L122, L123, L124, L125, L126, L127, L128, L129, L130, L131, L132, L133, L134, L135, L136, L137, L138, L139, L140, L141, L142, L143, L144, L145, L146, L147, L148, L149
F	L150, L151, L152, L153, L154, L155, L156, L157, L158, L159, L160, L161, L162, L163, L164, L165, L166, L167, L168, L169
G	L170, L171, L172, L173, L174, L175, L176, L177, L178, L179, L180, L181, L182, L183, L184, L185, L186, L187, L188, L189, L190, L191, L192, L193, L194, L195, L196, L197, L198, L199, L200
H	L201, L202, L203, L204, L205, L206, L207, L208, L209, L210, L211, L212, L213, L214, L215, L216, L217, L218, L219, L220, L221, L222, L223, L224, L225, L226, L227, L228, L229, L230, L231, L232, L233, L234, **2B710**, L236

[a]A – genotypes with average severity levels of 82.13 to 92.13%; B – 72.13 to 80.47%; C – 62.47 to 71.30%; D – 52.97 to 62.13%; E – 41.30 to 51.13%; F – 34.63 to 40.47%; G – 23.80 to 32.97%; H – 11.43 to 22.97%.

Table 2 - Severity levels of anthracnose stalk rot in 48 maize lines and two hybrids (BRS1010 and 2B710), inoculated with two ***Colletotrichum graminicola*** strains (*Cg*03.09 and *Cg*05.07).

Lines	*Cg*03.09 Severity (%)*	*Cg*05.07 Severity (%)*
L16	92.97A a	87.97B b
L11	92.97A a	90.47B a
L1	92.97A a	93.80A a
L9	92.13A a	82.13C b
L22	92.13A a	84.63C b
BRS 1010	90.47A a	92.97A a
L50	90.47A a	93.80A a
L32	83.80B a	75.47D b
L28	82.13B b	91.30B a
L67	81.30B a	84.63C a
L54	81.30B a	85.47C a
L47	81.30B a	83.80C a
L55	80.47B a	81.30C a
L102	80.47B a	71.30E b
L75	77.97B a	80.47D a
L78	77.12B b	83.80C a
L98	72.13C b	77.97D a
L108	69.63C a	65.47F a
L66	68.80C b	88.80B a
L90	67.97C a	70.47E a
L153	63.80D a	56.30G b
L84	62.97D b	79.63D a
L136	57.13E a	55.47G a
L162	52.97F a	46.30H b
L154	48.80F a	49.63H a
L151	44.63G a	47.13H a
L186	42.13G b	47.13H a
L183	40.47G a	41.30I a
L204	37.97H a	41.30I a
L202	35.47H a	38.80I a
L206	32.97I a	32.13J a
L205	32.13I b	38.80I a
L193	30.47I a	33.80J a
L208	30.47I a	27.97K a
L211	29.63I a	32.97J a
L232	29.63I a	22.97L b
L219	28.80I a	30.47J a
L220	27.97I a	29.63K a
L227	27.97I b	32.97J a
L222	26.30I b	31.30J a
L234	23.80J b	29.63K a
L209	23.80J b	29.63K a
L225	23.80J b	28.80K a
L224	22.97J b	27.97K a
L221	22.97J b	32.13J a
L228	22.97J b	28.80K a
2B710	22.13J b	37.13J a
L216	22.13J b	30.47J a
L231	21.30J b	27.97K a
L236	19.63J a	22.97L a
Average	53.13**	55.55**

*Means followed by the same capital letter in the column, and the same lower case letter in the line, does not differ by the Scott-Knott test at 5% probability. **Average severity for each strain.

experimental trial. However, the disease severity was greater in the second experimental trial for most of the strains, even for the aggressive isolate *Cg*03.09. For example, the most resistant lines in the first trial (Table 1) showed values 23% above the severity in the second trial (Table 2), in which higher severity values were observed when using the most aggressive strain *Cg*05.07. As expected, the hybrid BRS1010 was considered susceptible, showing severity above 90% for both strains. By contrast, the hybrid 2B710 was considered resistant, showing a severity of 22.1% (Table 2) when inoculated with the strain *Cg*03.09, and 37.1% when inoculated with the strain *Cg*05.07. In a joint analysis of the two trials, it was possible to detect nine lines showing resistance features, namely, L234, L209, L225, L224, L221, L228, L216, L231, and L236. These lines showed the same resistance features as those of the resistant hybrid 2B710.

The evaluation method adopted in the present study was efficient to classify the lines to form different resistance categories to anthracnose stalk rot, as done in many reports (CARSON & HOOKER, 1981; BADU-APRAKU et al., 1987; TOMAN & WHITE, 1993; COTA et al., 2010; MATIELLO et al., 2012; COSTA at al., 2014).

According to our results, there are resistance sources in the lines from the germplasm bank with high levels of resistance, which are similar to the levels reported in the commercial resistant hybrid 2B710. In a study performed with landraces, the varieties "branco oito carreiras", "oito carreiras branco", "branco duro canguçú", and "sabuguinho caboroxo" were all effective resistance sources to anthracnose stalk rot, being similar to the resistant hybrid 2B710 (GARDINGO, 2008). Many genotypes showing red pigmentation in their tissues are generally considered resistant to anthracnose stalk rot. The red mark is a kind of background to resistance; though there are some red susceptible genotypes. The simple hybrid 2B710 has an effective resistance level and showed red pigmentation in the stalk surface and leaves veins, which appears in some genotypes due to the production of carotenoids and flavonoids (GARDINGO, 2008; COSTA et al., 2010b; COTA et al., 2010; COTA et al., 2012; CARVALHO et al., 2013). This genotype has been used as a positive control in studies investigating anthracnose stalk rot resistance, as well as for genotype selection in germplasm banks; in addition, this hybrid has shown good agronomic features in many studies (COTA et al., 2012; CARVALHO et al., 2013; ZUCARELI et al., 2013, COSTA et al., 2014).

In the present study, the lines L234, L209, L225, L224, L221, L228, L216, L231, and L236 showed effective resistance levels. Therefore, they can be recommended for introduction and use in anthracnose stalk rot-breeding programs. Moreover, these lines can be used in studies of resistance inheritance and crop yield losses, and for molecular marker identification, associated with the enhancement of maize resistance to anthracnose stalk rot.

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