CROP PROTECTION

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: 19°28'03" S, longitude: 44°15'08" 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 Cg03.09 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 (10⁶ 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⁻¹of 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 (Cg05.07 and Cg03.09) of **C. graminicola**, the first (Cg05.07) being more aggressive than the second (Cg03.09) 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		
А	L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, BRS1010 , L13, L14, L15, L16, L17, L18, L19, L20, L21, L22, L23		
В	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		
С	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		
Е	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		
Н	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%.

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L7577.97B a80.47D aL7877.12B b83.80C aL9872.13C b77.97D aL10869.63C a65.47F aL6668.80C b88.80B aL9067.97C a70.47E aL15363.80D a56.30G bL8462.97D b79.63D aL16252.97F a46.30H bL15144.63G a47.13H aL15144.63G a47.13H aL18642.13G b47.13H aL18642.13G b41.301 aL20235.47H a38.801 aL20432.97H a41.301 aL20532.13L b38.801 aL20632.97I a32.801 aL20830.47I a33.801 aL21129.631 a32.971 aL22226.31 a32.971 aL23423.801 b39.471 aL20927.97I b32.971 aL21129.631 a22.971 bL22422.971 b32.971 aL22422.971 b32.801 bL23423.801 b29.63K aL22422.971 b32.801 bL23423.801 b29.63K aL22422.971 b32.80K aL22422.971 b			81.30C a
L7877.12B b83.80C aL9872.13C b77.97D aL10869.63C a65.47F aL6668.80C b88.80B aL9067.97C a70.47E aL15363.80D a56.30C bL8462.97D b79.63D aL16252.97F a46.30H bL15448.80F a49.63H aL18642.13G b47.13H aL18642.13G b47.13H aL18437.97H a41.301 aL20437.97H a38.801 aL20532.131 b38.801 aL20632.971 a32.133 aL20530.471 a33.801 aL20830.471 a32.977 aL21129.631 a32.977 aL23229.631 a32.977 aL23423.801 b32.871 aL22727.977 b32.971 aL23229.631 a32.971 aL23423.801 b29.63K aL22727.971 b32.971 aL23229.631 a32.971 aL23423.801 b29.63K aL22727.971 b32.971 aL23423.801 b29.63K aL22422.971 b32.971 aL23423.801 b29.63K aL22422.971 b32.971 aL23423.801 b29.63K aL23422.971 b32.971 aL23422.971 b32.971 aL23422.971 b32.931 aL23422.971 b32.133 aL23422.971	L102	80.47B a	71.30E b
L9872.13C b77.97D aL10869.63C a65.47F aL6668.80C b88.80B aL9067.97C a70.47E aL13363.80D a56.30G bL8462.97D b79.63D aL13657.13E a55.47G aL15148.80F a49.63H aL15144.63G a47.13H aL18642.13G b47.13H aL18340.47G a41.30I aL20437.97H a48.80F aL20532.13I b38.80I aL20532.13I b38.80I aL20530.47T a32.97J aL21129.63I a22.97L aL22226.3I a32.97J aL21429.63I a32.97J aL21529.63I a32.97J aL21632.97T b32.97J aL21727.97T b32.97J aL22823.80J b29.63K aL22423.80J b29.63K aL22423.80J b29.63K aL22423.80J b29.63K aL22422.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22533.80J b29.63K aL22623.80J b29.63K aL22727.97T b32.13J aL22822.97J b32.13J aL22422.97J b32.13J aL22523.80J b29.63K aL22623.80J b29.63K aL22723.80J b28.80K aL22822.97J	L75	77.97B a	80.47D a
L9872.13C b77.97D aL10869.63C a65.47F aL6668.80C b88.80B aL9067.97C a70.47E aL13363.80D a56.30G bL8462.97D b79.63D aL13657.13E a55.47G aL15148.80F a49.63H aL15144.63G a47.13H aL18642.13G b47.13H aL18340.47G a41.30I aL20437.97H a48.80F aL20532.13I b38.80I aL20532.13I b38.80I aL20530.47T a32.97J aL21129.63I a22.97L aL22226.3I a32.97J aL21429.63I a32.97J aL21529.63I a32.97J aL21632.97T b32.97J aL21727.97T b32.97J aL22823.80J b29.63K aL22423.80J b29.63K aL22423.80J b29.63K aL22423.80J b29.63K aL22422.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22533.80J b29.63K aL22623.80J b29.63K aL22727.97T b32.13J aL22822.97J b32.13J aL22422.97J b32.13J aL22523.80J b29.63K aL22623.80J b29.63K aL22723.80J b28.80K aL22822.97J	L78	77.12B b	83.80C 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 48.80I a L205 32.97I a 38.80I a L205 32.13I b 38.80I a L208 30.47I a 27.97K a L211 29.63I a 32.97J a L222 29.63I a 32.97J a L212 29.63I a 32.97J a L220 27.97T b 32.97J a L221 29.63I a 32.97J a L222 29.63I a 32.97J a L224 23.80J b 29.63K a L224 23.80J b 29.63K a L224 23.80J b 29.63K a L224 <			
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 48.80I a L205 32.97I a 38.80I a L205 32.13I b 38.80I a L208 30.47I a 27.97K a L211 29.63I a 32.97J a L222 29.63I a 32.97J a L212 29.63I a 32.97J a L220 27.97T b 32.97J a L221 29.63I a 32.97J a L222 29.63I a 32.97J a L224 23.80J b 29.63K a L224 23.80J b 29.63K a L224 23.80J b 29.63K a L224 <	L108	69.63C a	65.47F a
L15363.80D a56.30G bL8462.97D b79.63D aL13657.13E a55.47G aL16252.97F a46.30H bL15448.80F a49.63H aL15144.63G a47.13H aL18642.13G b47.13H aL20437.97H a41.30I aL20532.13I b38.80I aL20632.97I a32.13I aL20530.47I a33.80I aL21129.63I a32.97I aL23229.63I a32.97I aL21129.63I a32.97I aL22226.30I b31.30J aL22422.97I b32.97J aL22523.80J b29.63K aL21129.63I a32.97J aL22226.30I b31.30J aL22423.80J b29.63K aL22523.80J b29.63K aL22422.97J b27.97K aL22523.80J b28.80K aL22422.97J b27.97K aL22523.80J b28.80K aL22623.80J b28.80K aL22727.97K a21.31 aL22422.97J b27.97K aL22523.80J b28.80K aL22422.97J b22.131 aL22422.97J b22.131 aL22422.97J b22.131 aL22422.97J b22.131 aL22523.80J b28.80K aL22622.97J b22.131 aL22722.97J b22.131 aL22822.	L66	68.80C b	88.80B a
L8462.97D b79.63D aL13657.13E a55.47G aL16252.97F a46.30H bL15448.80F a49.63H aL15144.63G a47.13H aL18642.13G b47.13H aL18340.47G a41.30I aL20437.97H a41.30I aL20532.97I a32.13J aL20632.97I a38.80I aL20830.47I a38.80I aL21129.63I a22.97J bL22226.30I b31.47J aL21429.63I a30.47J aL22527.97I b32.97J aL22627.97I b32.97J aL21928.80I a30.47J aL22027.97I b32.97J aL23229.63I a30.47J aL22027.97I b32.97J aL22127.97I b32.97J aL22226.30I b31.30J aL22422.97J b27.97K aL22523.80J b29.63K aL22623.80J b28.80K aL22727.97J b32.80J bL22822.97J b37.13J aL22122.97J b37.13J aL22422.97J b37.13J aL22422.97J b37.13J aL22422.97J b37.13J aL22422.97J b37.13J aL22422.97J b37.13J aL22523.80J b37.13J aL22622.97J b37.13J a	L90	67.97C a	70.47E a
L13657.13E a55.47G aL16252.97F a46.30H bL15448.80F a49.63H aL15144.63G a47.13H aL18642.13G b47.13H aL18340.47G a41.30I aL20437.97H a41.30I aL20532.97I a32.13J aL20630.47I a33.80I aL19330.47I a22.97J kL21129.63I a22.97J aL22226.31 a30.47J aL23229.63I a30.47J aL21129.63I a30.47J aL22226.30 b31.30J aL22427.97I b32.97J aL22523.80J b29.63K aL22422.97J b27.97K aL22523.80J b29.63K aL22422.97J b27.97K aL22523.80J b28.80K aL22422.97J b27.97K aL22523.80J b28.80K aL22422.97J b32.13J aL22522.97J b32.13J aL22622.97J b32.13J aL22722.97J b32.13J aL22622.97J b32.13J aL22722.97J b32.13J aL22822.97J b32.13J aL22822.	L153	63.80D a	56.30G b
L16252.97F a46.30H bL15448.80F a49.63H aL15144.63G a47.13H aL18642.13G b47.13H aL18340.47G a41.30I aL20437.97H a41.30I aL20532.97I a32.13J aL20632.97I a33.80I aL20830.47I a33.80I aL21129.63I a22.97I aL23229.63I a22.97I aL21928.80I a30.47I aL22027.97I b32.97I aL22129.63I a22.97I aL22226.30I b31.30I aL22423.80J b29.63K aL22523.80J b29.63K aL22623.80J b29.63K aL22727.97I b32.97I aL22822.97J b31.30J aL22822.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22822.97J b32.13J aL2282	L84	62.97D b	79.63D a
L15448.80F a49.63H aL15144.63G a47.13H aL18642.13G b47.13H aL18340.47G a41.301 aL20437.97H a41.301 aL20235.47H a38.801 aL20632.971 a32.13J aL20532.131 b38.01 aL20830.471 a33.801 aL21129.631 a22.971 aL22229.631 a32.971 aL22027.97K a12.211L22027.97I b32.971 aL22129.631 a32.971 aL22229.631 a32.971 aL22329.631 a32.971 aL22423.80J b29.63K aL22523.80J b29.63K aL22422.97J b32.80J bL22422.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22822.97J b32.13J aL22822.97J b32.13J aL22822.97J b32.13J aL22822.97J b37.13J a	L136	57.13E a	55.47G a
L15144.63G a47.13H aL18642.13G b47.13H aL18340.47G a41.30I aL20437.97H a41.30I aL20235.47H a38.80I aL20632.97I a32.13J aL20532.13I b38.80I aL19330.47I a33.80J aL20830.47I a32.977K aL21129.63I a22.97L bL22226.30I a30.47J aL23229.63I a22.97L bL21928.80I a30.47J aL22027.97I a29.63K aL22129.63I b31.30J aL22226.30I b31.30J aL22423.80J b29.63K aL22523.80J b28.80K aL22422.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22523.80J b28.80K aL22422.97J b32.13J aL22523.80J b28.80K aL22622.97J b32.13J aL22722.97J b32.13J aL22822.97J b32.13J aL22422.97J b32.13J aL22523.80J b32.13J aL22622.97J b32.13J aL22722.97J b32.13J aL22822.97J b32.13J aL22822.97J b37.13J a	L162	52.97F a	46.30H b
L18642.13G b47.13H aL18340.47G a41.30I aL20437.97H a41.30I aL20235.47H a38.80I aL20632.97I a32.13J aL20532.13I b38.80I aL19330.47I a33.80J aL20830.47I a32.97J aL21129.63I a22.97J bL22229.63I a22.97J bL21928.80I a30.47J aL22027.97I a29.63K aL22127.97I b31.30J aL22226.30I b31.30J aL23423.80J b29.63K aL22422.97J b23.80J bL22422.97J b32.13J aL22422.97J b32.13J aL22422.97J b32.13J aL22822.97J b32.13J aL22822.97J b32.13J aL22822.97J b37.13J a	L154	48.80F a	49.63H a
L18340.47G a41.30I aL20437.97H a41.30I aL20235.47H a38.80I aL20632.97I a32.13J aL20532.13I b38.80I aL19330.47I a33.80U aL20830.47I a27.97K aL21129.63I a22.97J aL22229.63I a30.47J aL20927.97I a29.63K aL21229.63I a30.47J aL22027.97I b32.97J aL22128.80I a30.47J aL22226.30I b31.30J aL22423.80J b29.63K aL22523.80J b29.63K aL22422.97J b32.97J aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b32.13J aL22822.97J b32.13J aL22822.97J b37.13J a	L151	44.63G a	47.13H a
L20437.97H a41.30I aL20235.47H a38.80I aL20632.97I a32.13J aL20532.13I b38.80I aL19330.47I a33.80J aL20830.47I a27.97K aL21129.63I a22.97L bL21229.63I a30.47J aL22027.97K a30.47J aL21129.63I a30.47J aL22027.97I a29.63K aL22126.30I b31.30J aL22226.30I b31.30J aL23423.80J b29.63K aL20923.80J b29.63K aL22523.80J b28.80K aL22422.97J b27.97J bL22122.97J b32.13J aL22122.97J b32.13J aL22822.97J b32.13J aL22822.97J b37.13J a	L186	42.13G b	47.13H a
L20235.47H a38.80I aL20632.97I a32.13J aL20532.13I b38.80I aL19330.47I a33.80J aL20830.47I a27.97K aL21129.63I a22.97J aL23229.63I a20.47J aL21928.80I a30.47J aL22027.97I a29.63K aL22127.97I b32.97J aL22226.30I b31.30J aL22423.80J b29.63K aL22523.80J b29.63K aL22422.97J b27.97K aL22122.97J b27.97K aL22122.97J b27.97K aL22322.97J b27.97K aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b28.80K a2B71022.13J b37.13J a	L183	40.47G a	41.30I a
L20632.971 a32.13J aL20532.131 b38.801 aL19330.471 a33.80J aL20830.471 a27.97K aL21129.631 a32.97J aL23229.631 a30.471 aL21928.801 a30.471 aL22027.97I a29.63K aL22727.97I b32.97J aL22226.301 b31.30J aL22329.63V b29.63K aL22423.80J b29.63K aL22523.80J b29.63K aL22422.97J b27.97K aL22122.97J b32.13J aL22226.7J b32.13J aL22422.97J b28.80K aL22122.97J b32.13J aL22822.97J b37.13J a	L204	37.97H a	41.30I a
L20532.131 b38.801 aL19330.471 a33.80J aL20830.471 a27.97K aL21129.631 a32.97J aL23229.631 a22.97L bL21928.801 a30.47J aL22027.971 a29.63K aL22727.97I b32.97J aL22226.301 b31.30J aL23423.80J b29.63K aL20923.80J b29.63K aL22422.97J b28.80K aL22523.80J b28.80K aL22422.97J b32.13J aL22822.97J b32.13J aL21122.97J b37.13J a	L202	35.47H a	38.80I a
L19330.471 a33.80J aL20830.471 a27.97K aL21129.631 a32.97J aL23229.631 a22.97L bL21928.801 a30.47J aL22027.97I a29.63K aL22727.97I b32.97J aL22226.301 b31.30J aL22423.80J b29.63K aL2523.80J b29.63K aL22422.97J b27.97K aL22122.97J b27.97K aL22122.97J b32.13J aL22822.97J b37.13J a	L206	32.97I a	32.13J a
L20830.471 a27.97K aL21129.631 a32.97J aL23229.631 a22.97L bL21928.801 a30.47J aL22027.971 a29.63K aL22727.97I b32.97J aL22226.30I b31.30J aL23423.80J b29.63K aL20923.80J b29.63K aL22523.80J b29.63K aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b37.13J a	L205	32.13I b	38.80I a
L21129.631 a32.97J aL23229.631 a22.97L bL21928.801 a30.47J aL22027.971 a29.63K aL22727.97I b32.97J aL22226.301 b31.30J aL22423.80J b29.63K aL22523.80J b29.63K aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b37.13J a	L193	30.47I a	33.80J a
L23229.631 a22.97L bL21928.801 a30.47J aL22027.971 a29.63K aL22727.971 b32.97J aL22226.301 b31.30J aL23423.80J b29.63K aL20923.80J b29.63K aL22523.80J b29.63K aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b37.13J a	L208	30.47I a	27.97K a
L21928.80I a30.47J aL22027.97I a29.63K aL22727.97I b32.97J aL22226.30I b31.30J aL23423.80J b29.63K aL20923.80J b29.63K aL22523.80J b28.80K aL22422.97J b32.13J aL22122.97J b32.13J aL22822.97J b37.13J a	L211	29.63I a	32.97J a
L22027.971 a29.63K aL22727.971 b32.97J aL22226.301 b31.30J aL23423.80J b29.63K aL20923.80J b29.63K aL22523.80J b28.80K aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b28.80K a2B71022.13J b37.13J a	L232	29.63I a	22.97L b
L22727.971 b32.97J aL22226.301 b31.30J aL23423.80J b29.63K aL20923.80J b29.63K aL22523.80J b28.80K aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b28.80K a2B71022.13J b37.13J a	L219		30.47J a
L22226.301 b31.30J aL23423.80J b29.63K aL20923.80J b29.63K aL22523.80J b28.80K aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b28.80K a2B71022.13J b37.13J a	L220	27.97I a	29.63K a
L23423.80J b29.63K aL20923.80J b29.63K aL22523.80J b28.80K aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b28.80K a2B71022.13J b37.13J a	L227	27.97I b	32.97J a
L20923.80J b29.63K aL22523.80J b28.80K aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b28.80K a2B71022.13J b37.13J a		26.30I b	
L22523.80J b28.80K aL22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b28.80K a2B71022.13J b37.13J a		23.80J b	29.63K a
L22422.97J b27.97K aL22122.97J b32.13J aL22822.97J b28.80K a2B71022.13J b37.13J a			
L22122.97J b32.13J aL22822.97J b28.80K a2B71022.13J b37.13J a		23.80J b	28.80K a
L228 22.97J b 28.80K a 2B710 22.13J b 37.13J a			
2B710 22.13J b 37.13J a			
1216 22 131b 20 471 c			
	L216	22.13J b	30.47J a
L231 21.30J b 27.97K a			
L236 19.63J a 22.97L a	L236	19.63J a	
Average 53.13** 55.55**	Average	53.13**	55.55**

Table 2 - Severity levels of anthracnose stalk rot in 48 maize lines and two hybrids (BRS1010 and 2B710), inoculated with two *Colletotrichum graminicola* strains (Cg03.09 and Cg05.07).

 * 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 Cg03.09. 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 Cg05.07. 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 Cg03.09, and 37.1% when inoculated with the strain Cg05.07. 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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