Genetic control of sorghum resistance to leaf anthracnose


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A study was carried out to investigate the inheritance of resistance to anthracnose, caused by Colletotrichum sublineolum, in sorghum. Crosses between resistant and susceptible parents and backcrosses between F1 plants and the susceptible parents were carried out under field conditions. The F1 generations and the segregant populations were evaluated under artificial inoculation conditions in the greenhouse. In the F1 generation of all crosses with the respective isolates, all of the plants presented a resistance reaction except for the F1 plants resulting from the BR009 × SC283 cross. In the F2 generation, the frequencies of resistant and susceptible plants conformed to the hypothesis that one gene with two alleles controls host resistance, except in one cross. Out of the eight backcrosses, six presented segregation that corresponded to the hypothesis formulated. For most crosses, resistance was dominant, and the proportions of resistant and susceptible plants in the segregant populations conformed to the frequencies expected under the hypothesis of gene-for-gene resistance and dominant gene action.

Keywords: anthracnose, Colletotrichum sublineolum, inheritance of resistance, oligogenic resistance, Sorghum bicolor

Introduction

Anthracnose caused by Colletotrichum sublineolum is one of the most important phytosanitary problems affecting sorghum cultivation both in Brazil (Guimarães et al., 1998; Casela et al., 2001a,b; Wang et al., 2006) and in other parts of the world (Pande et al., 1994; Wharton & Julian, 1996; Nguigui et al., 2000; Marley et al., 2001; Wharton et al., 2001; Singh et al., 2006; Li & TeBeest, 2009; Perumal et al., 2009).

The disease thrives and becomes especially important economically when hot and humid environmental conditions coincide with abundant precipitation (Pande et al., 1994; Thakur & Mathur, 2000). The disease can develop any time throughout the culture cycle, and the pathogen can attack any aerial part of the plant, causing leaf blight, stalk rot and burning of the panicle and grains (Cardwell et al., 1989; Pande et al., 1994; Casela et al., 2001a). However, leaf anthracnose is the most common and severe form of the disease in susceptible cultivars during severe epidemics; it can reduce the production of grains and forage by 50% or more (Ferreira & Warren, 1982; Cardwell et al., 1989).

The main strategy for the management of anthracnose in sorghum is the use of genetic resistance (Wharton & Julian, 1996). Several lineages of sorghum have been selected that have shown increased resistance to leaf anthracnose in field and greenhouse studies (Ferreira & Warren, 1982; Pande et al., 1994; Singh et al., 2006). However, C. sublineolum is recognized for presenting a high level of pathogen variability; this variability results in the fast adaptation of the pathogen to the resistant cultivars in use, defeating their resistance very quickly (Cardwell et al., 1989; Pande et al., 1991; Casela & Frederiksen, 1994; Guimarães et al., 1999; Marley et al., 2001; Singh et al., 2006; Moore et al., 2008; Silva et al., 2008).

Attempts have been made to increase the durability of resistance to anthracnose in sorghum. Strategies such as the use of dilatory resistance, which reduces the rate of development of the disease (Guimarães et al., 1998), the identification and dissociation of virulence in the population of the pathogen (Casela et al., 2001a) and the use of mixtures of cultivars (Guimarães et al., 1998) have been adopted for this purpose. However, little of the basic information that would be necessary to support and provide strategies for increasing the durability of genetic resistance to anthracnose in sorghum is available.

The genetic variability that is present in the pathogen population indicates that there is potential for the identification of new host disease-resistance genes (Cardwell et al., 1989). In spite of the existing information both on the genetic variability of the virulence and host resistance observed in this pathosystem, as well as on the establishment of a differentiating series composed of a small number of lineages (Casela et al., 1996), little is known about...
the genetic control of resistance in this pathosystem. The present study was aimed at characterizing the genetic inheritance of resistance to leaf anthracnose in sorghum.

**Materials and methods**

The experiments were conducted in an experimental field and a greenhouse at the Centro Nacional de Pesquisa de Milho e Sorgo (CNPMS) EMBRAPA, located in Sete Lagoas, Minas Gerais, Brazil. Five lineages of sorghum (BR008, BR005, BR013, BR009 and SC283) and seven isolates of *Colletotrichum sublineolum* (92.02, RB.04, 85.02, 84.02, 23.02, 201.01 and 204.01) were used. The lineages were selected because they presented contrasting phenotypes (resistance or susceptibility) when inoculated with the isolates of *C. sublineolum* (Table 1). Three combinations of lineages presenting differential interactions with different isolated pairs of *C. sublineolum* (i.e. a lineage characterized as resistant to one isolate and susceptible to another) were selected, with the second lineage presenting an inverse reaction to the same isolates (Table 1). Other combinations involving the lineages BR009 and SC283 and the lineages BR009 and BR013 were also selected.

The genetic analysis of the inheritance of resistance was carried out based on the phenotypes of the parents (P₁ and P₂), the F₁ and F₂ generations and the backcrosses (BC) between the F₁ generation and the susceptible parents. The evaluations were qualitative, and analysis of mycological nomenclature of CNPMS-EMBRAPA. The genetic control of resistance in this pathosystem. The present study was aimed at characterizing the genetic inheritance of resistance to leaf anthracnose in sorghum.

**Production of the F₁ and F₂ generations and BC**

The crosses were carried out in both field and greenhouse conditions to ensure that the number of F₂ seeds necessary for the inoculations and production of the subsequent generations, the F₂ and BC, would be obtained. For this production, five blocks composed of four rows of 5 m each were planted in the field, with a space of 0.9 m between the rows and 0.2 m between the plants. In both central rows of each block, the male-sterile lineage was sown (A) and in both lateral lines, the restorer lineage (R). The seeds collected in the six blocks formed the six simple hybrids (F₁). The two lines of the R lineages were sown with a time difference of 15 days to guarantee the coincidence of the flowering of the different lineages.

Before flowering, all of the panicles of each plant were covered with paper bags to promote the genetic isolation of the material during the crosses. During flowering, the pollen produced by the plants of the R lineages was transferred, with the use of the paper bags, to the male-sterile plants.

In the greenhouse, the lineages were sown in 23- × 18- × 18-cm plastic pots (top and base diameters, and height, respectively). Ten seeds were sown per pot, with thinning to five plants 15 days after emergence. For each lineage, six pots were used, totalling 30 plants per lineage. The bagging and crossing procedures were the same as those previously mentioned.

The F₂ generation was obtained by self-pollination of the F₁ plants. The F₁ seeds of each cross were sown in pots and maintained in the greenhouse. Five plants per pot and six pots per cross were used, totalling 30 plants for each F₁. Soon after the emergence of the panicles (before flowering), all of the panicles were covered with paper bags to ensure the genetic isolation of the material and the self-fertilization of the F₁ plants. The seeds collected formed the F₂ generation.

For the production of backcrosses, the F₁ plants of each cross were crossed with their respective susceptible parents. In the case of combinations where differential interactions were detected among the pairs of lineages and isolates, the F₁ plants were crossed with both parents, for study with both isolates. For all other combinations, only crosses between the F₁ plants and one of the susceptible parents were carried out. Fourteen blocks composed of four 5-m rows were sown in the field, using a spacing of 0.9 m between the rows and 0.2 m between the plants. The two central rows of each block were composed of F₁ plants, and the two lateral rows were composed of the respective parents. The two lineages formed by the parents were sown 15 days apart to guarantee the coincidence of flowering between the lineages and the F₁ plants to be crossed. Before flowering, all of the panicles of each parcel were covered with a paper bag and the crosses were carried out as previously described.

**Table 1** Types of resistance reactions and combinations of lineages of sorghum and isolates of *Colletotrichum sublineolum* selected for the study of resistance to anthracnose

<table>
<thead>
<tr>
<th>Lineages</th>
<th>Isolates*</th>
<th>Type of reactionb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR008</td>
<td>92.02</td>
<td>R</td>
</tr>
<tr>
<td>BR005</td>
<td>92.02</td>
<td>S</td>
</tr>
<tr>
<td>BR008</td>
<td>RB.04</td>
<td>S</td>
</tr>
<tr>
<td>BR005</td>
<td>RB.04</td>
<td>R</td>
</tr>
<tr>
<td>Combination 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR008</td>
<td>85.02</td>
<td>R</td>
</tr>
<tr>
<td>BR013</td>
<td>85.02</td>
<td>S</td>
</tr>
<tr>
<td>BR008</td>
<td>84.02</td>
<td>S</td>
</tr>
<tr>
<td>BR013</td>
<td>84.02</td>
<td>S</td>
</tr>
<tr>
<td>Combination 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR008</td>
<td>23.02</td>
<td>R</td>
</tr>
<tr>
<td>BR009</td>
<td>23.02</td>
<td>S</td>
</tr>
<tr>
<td>BR008</td>
<td>201.01</td>
<td>S</td>
</tr>
<tr>
<td>BR009</td>
<td>201.01</td>
<td>R</td>
</tr>
<tr>
<td>Combination 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR009</td>
<td>204.01</td>
<td>R</td>
</tr>
<tr>
<td>SC283</td>
<td>204.01</td>
<td>R</td>
</tr>
<tr>
<td>Combination 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR009</td>
<td>RB.04</td>
<td>R</td>
</tr>
<tr>
<td>BR013</td>
<td>RB.04</td>
<td>S</td>
</tr>
</tbody>
</table>

* Isolates of *C. sublineolum* were classified according to the mycological nomenclature of CNPMS-EMBRAPA.

b R: resistant; S: susceptible.

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Inoculation of the P1, P2, F1 and F2 generations and BC

To assess the type of reaction and the proportions of resistant and susceptible plants in the P1, P2, F1 and F2 generations and BC1, inoculations were carried out in the greenhouse. In the case of the P1, P2 and F1 generations and BC1, seeds were sown in 23 × 18 × 18-cm plastic pots. Five pots were used for each generation with four seeds sown per pot. For the F2 generation, 75 pots were used and four seeds were sown per pot, totalling 300 F2 plants. The pots were placed on counters in a greenhouse, at a temperature of approximately 25–28°C.

Monosporic cultures of the selected isolates of *C. sublineolum* (Table 1) were preserved in test tubes containing mineral oil. Isolates were transferred from test tubes to Petri plates containing oatmeal agar medium (OAM; 60 g oatmeal and 20 g agar L⁻¹) and tetracycline (3 g L⁻¹). After transplantation, the plates were maintained in a growth chamber under fluorescent light (12 h per day), with the temperature between 25 and 28°C, for 10 days, to achieve sporulation. After abundant sporulation was obtained a superficial scraping was performed, with a spatula, for the liberation of the spores. The suspensions were filtered through two layers of gauze to eliminate fragments of mycelium and culture medium. After filtering, the suspensions were adjusted to a concentration of 10⁶ conidia mL⁻¹ using a haemocytometer.

The inoculations were carried out on 30-day-old greenhouse grown plants by spraying with a suspension of conidia, at approximately 20 mL per pot, to the point of runoff. After the inoculations, the plants were maintained in a dark, humid chamber. After 18 h, the humid chambers were opened and the plants were placed on counters in a greenhouse, at a temperature of about 25°C, until the time for the evaluations.

Individual evaluations of the degree of resistance or susceptibility of the plants were carried out 10 days after the inoculations, using grades from 1 to 5 as follows (Casela & Ferreira, 1987): 1, absence of symptoms; 2, presence of a small number of elongated lesions without sporulation, or those of a hypersensitivity reaction (mild infection); 3, presence of elongated lesions without sporulation, or those of a hypersensitivity reaction, with up to 20% of the leaf area affected; 4, severe infection with sporulating lesions and some coalescence, with 21–40% of leaf area affected; 5, very severe infection, with sporulating and coalesced lesions, more than 40% of leaf area affected and abundant sporulation. Grades 1, 2 and 3 were considered indicative of resistance and grades 4 and 5 as indicative of susceptibility.

Data analysis

After the evaluations, counting of the resistant and susceptible plants from each generation of the different crosses was carried out. Because the resistance expected in this study was of the vertical type, which is usually controlled by one or few genes, the analysis was carried out based on Mendelian genetics. The following hypotheses were tested:

- **General hypothesis:**
  The resistance to each anthracnose race is controlled by vertical resistance genes with two alleles and complete dominance.

- **Specific hypotheses:**
  1. The F1 generation will present only resistant plants.
  2. In the F2 generation, there will be segregation with a ratio that is close to three resistant: one susceptible.
  3. In the BC1, there will be segregation in the ratio of one resistant: one susceptible.

To test the hypotheses formulated, the statistical chi-square test (χ²) was employed with a P-value cutoff of 5%.

Results

In the F1 generation of all crosses with the respective isolates, all of the plants presented a resistance reaction except for the F1 plants resulting from the BR009 × SC283 cross inoculated with isolate 204.01 (Table 2). In this cross, 31 susceptible plants and no resistant plants were observed.

In the F2 generation, the frequencies of resistant and susceptible plants conformed to the hypothesis that one gene with two alleles controls host resistance to a specific race of *C. sublineolum* (Table 2). However, in the BR008 × BR009 inoculated cross with isolate 201.01, the frequencies did not match the hypothesis tested. In this case, of the 269 plants inoculated, only 30 were observed to be susceptible, while 67 were expected (Table 2).

Out of the eight backcrosses, six presented segregation that corresponded to the hypothesis formulated (Table 2). In the other backcrosses, the number of susceptible plants observed was significantly lower than the values expected. In the F1 (BR008 × BR013) × BR008 backcross, the values of the resistant and susceptible plants observed were exactly those expected (Table 2).

The analyses of the types of resistance reactions and the proportions of resistant and susceptible plants in the parental lineages, F1 and F2 generations and BC1 allowed for the formation of inferences about the genetic composition of the lineages used. Because the pathosystem studied conforms to the gene-for-gene resistance and susceptibility model, the analysis of the genetic composition of the pathogen must also be considered in an analysis of the genetic composition of the lineages (Casela *et al.*, 1995). In this case, the denomination *Cs* (*C. sublineolum*) was used for resistance genes and *Avr* (*Avirulence*) for the avirulence genes. The proportions of resistant and susceptible plants in the F1 and F2 generations and BC1 in the crosses involving BR008, BR005, BR013 and BR009 (Table 2), and of the type of reaction of the isolates 92.02, R8.04, 85.02, 84.02, 23.02 and 201.02, in the same lineages (Table 3), can be explained by the presence of a single dominant resistance gene in each lineage. This gene was given the following
denominations: \(Cs_1\) (BR008), \(Cs_2\) (BR005), \(Cs_3\) (BR013) and \(Cs_4\) (BR009), considering the genetic composition of the isolates and the theoretical crosses according to Tables 4 and 5.

In the generations of the \(BR009 \times SC283\) cross inoculated with RB.04 (Tables 1 and 2), a recessive resistance gene in the lineage \(SC283\) was detected, called \(cs_5\), and a dominant susceptibility gene in the lineage \(BR009\).

### Discussion

Reports in the literature of the inheritance of sorghum resistance to leaf anthracnose are few, and they present different descriptions of the type of genetic control of the resistance in this pathosystem. There is evidence that resistance to the anthracnose stages of leaf and stalk rot is under independent genetic control (LeBeau & Coleman, 1950; Casela et al., 1997).

In this study, the results of the crosses supported the hypothesis of vertical genes with two alleles controlling sorghum resistance to specific races of \(C. sublineolum\). As for the type of gene action, there was variation in the dominance or recessiveness to resistance in the lineages used. Out of the eight combinations analysed (pairs of lineages and isolates), only \(SC283\) versus 204.01 exhibited recessive resistance. Variation in the dominance or recessiveness of the resistance, resulting from the use of different isolates of \(C. sublineolum\), has been observed by several authors. LeBeau & Coleman (1950), working with lineages of sorghum that varied from extreme susceptibility to high resistance to anthracnose, verified that resistance is controlled by a recessive gene. Singh et al.
analysed the genetic inheritance of resistance to anthracnose by means of a cross between the cultivars HC136 (highly susceptible) × G73 (resistant) and, using RAPD + SCAR markers, observed the susceptibility of the F1 generation and corroborated that the resistance was controlled by a recessive gene. Similar results were previously obtained by Boora et al. (1998) in field assays with the use of RAPD molecular markers, where the resistance to leaf anthracnose in the lineage SC326-6 appeared to be controlled by a recessive gene when

Table 4  Theoretical genetic composition of four lineages of sorghum and six isolates of Colletotrichum sublineolum, according to the type of resistance reaction presented in the Table 3

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Isolate</th>
<th>92.02</th>
<th>RB.04</th>
<th>85.02</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>92.02</td>
<td>RB.04</td>
<td>85.02</td>
<td></td>
</tr>
<tr>
<td>BR008</td>
<td>Cs1, cs2, cs3, cs4, cs5, cs6, cs7, cs8</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>BR005</td>
<td>cs1, cs2, cs3, cs4, cs5, cs6, cs7, cs8</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>BR013</td>
<td>cs1, cs2, cs3, cs4, cs5, cs6, cs7, cs8</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>BR009</td>
<td>cs1, cs2, cs3, cs4, cs5, cs6, cs7, cs8</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>
crossed with the lineage BT×623 (BR009), which was highly susceptible to anthracnose. These previous results are in accordance with those obtained in this work for the BR009 × SC283 cross, confirming the existence of recessive genes controlling resistance to anthracnose in the lineage SC283 and, therefore, the existence of dominant susceptibility genes in the lineage BR009 (Table 3). The presence of dominant susceptibility genes in BR009 may be one of the factors that explains the extreme susceptibility of this lineage under both field and greenhouse conditions. Mehta et al. (2005), characterizing the inheritance and stability of resistance in this pathosystem at different locations, also concluded that resistance is controlled by a single locus with dominant or recessive gene action. However, these authors also considered that additional genes may be present in the germplasm.

Based on the types of resistance reactions in the lineages BR008, BR005, BR013 and BR009 to the six isolates tested (Table 3), in the context of the hypothesis of genes of vertical resistance with two alleles, which has been supported by this work and the gene-for-gene model of resistance and susceptibility, the theoretical genotypes of the lineages and isolates were defined, taking into consideration the presence of a resistance gene in each lineage and its corresponding avirulence gene in the isolates (Table 4). Only one avirulence gene was detected in each of isolates 92.02 and 84.03, namely Avr1 and Avr3, respectively, while three were detected in isolate 23.02: Avr1, Avr2 and Avr3. In the other isolates, two avirulence genes were detected. This information enabled the definition of the possible genotypes of the F2 in each cross and the proportions expected for plants resistant and susceptible to each isolate (Table 5). The 3:1 ratio between resistant and susceptible plants that was observed for these crosses in the assays conducted was confirmed by this theoretical analysis.

In the gene-for-gene model, eliciting molecules produced by the avirulence genes are recognized by receptor molecules produced by the corresponding resistance genes; the resistance process is based on this recognition (Flor, 1946). Therefore, although there are several virulence genes, the presence of one avirulence gene in the pathogen is enough to activate the mechanisms of resistance in the plant, provided that its genotype includes the corresponding resistance gene (Table 5).

In other works, the inheritance of resistance was reported to be determined by a larger number of genes with partial dominance or an additive effect; by a dominant gene without cytoplasmic influence; or by a single locus with multiple alleles (Murty & Thomas, 1989; Tenkouano & Miller, 1993). Sources of dilatory resistance, inherited as a polygenic trait, have also been identified (Casela et al., 1993).

Further research is necessary to confirm the cause of these inconsistencies observed in the F2 generation (BR008 × BR009, isolate 201.01) and the BC1 plants F1 (BR009 × SC283) × BR009 and F1 (BR009 × BR013) × BR013. Some possible reasons for this inconsistency are: that resistance to anthracnose in sorghum can be encoded by two closely linked dominant genes or by a single genetic locus with multiple allelic forms. Genetic modifiers present in the lineages for a quantitative trait such as cuticle thickness could affect the segregation and reaction to the disease (Coleman & Stokes, 1954; Jones, 1979; Tenkouano, 1993; Mehta et al., 2005). Additionally, failures in inoculations could occur, especially where deviations observed are related to a larger number of resistant plants. Singh et al. (2006) and Mehta et al. (2005) also observed escape when they evaluated the inheritance of resistance to anthracnose in sorghum. For these authors, the evaluation of the disease is subject to escape and that is why susceptible plants are classified as resistant.

For the purpose of genetic breeding, it must be considered that sources of dominant resistance present more advantages than recessive sources, because resistance will be expressed in hybrids when the dominant allele is present in at least one of the parents (Mehta et al., 2005).

Based on the results of this work, it is possible to conclude that sorghum resistance to leaf anthracnose is of the gene-for-gene type, with a predominant mode of inheritance of complete dominance.

Acknowledgements

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