

Scientific Notes

Resistance of cotton genotypes to *Sclerotinia sclerotiorum* by the straw and oxalic acid tests

Leandro Henrique Mundim Aguiar⁽¹⁾, Larissa Barbosa de Sousa⁽¹⁾, Lísias Coelho⁽¹⁾, Ernane Miranda Lemes⁽¹⁾, Leonardo Humberto Silva e Castro⁽¹⁾ and Daniel Bonifácio Oliveira Cardoso⁽¹⁾

⁽¹⁾Universidade Federal de Uberlândia, Instituto de Ciências Agrárias, Campus Umuarama, Avenida Amazonas, s/nº, Bloco 2E, Sala 25, Umuarama, CEP 38400-902 Uberlândia, MG, Brazil. E-mail: leandroh@live.com, larissabsousa@hotmail.com, lisias@ufu.br, ernanelemes@yahoo.com.br, leonardohumbertoagro@hotmail.com, danieludia13@hotmail.com

Abstract – The objective of this work was to evaluate the efficiency of the straw and oxalic acid tests to identify resistance levels of white and colored cotton (*Gossypium hirsutum*) genotypes to white mold (*Sclerotinia sclerotiorum*). Ten genotypes were evaluated: five with colored fiber, four with white fiber, and a white-fiber susceptible genotype. The genotypes MAB-1 with white fiber and MAC-2 with colored fiber were the most resistant to white mold, according to the immersion in oxalic acid and straw tests, respectively. These genotypes can be recommended as resistance sources for breeding programs. Both assessed tests are complementary to each other; however, the straw test is more efficient in evaluating the resistance of cotton genotypes to white mold.

Index terms: *Gossypium hirsutum*, cotton resistance, ethanedioic acid, plant breeding, plant inoculation, white mold.

Resistência de genótipos de algodoeiro a *Sclerotinia sclerotiorum* pelos métodos straw test e imersão em ácido oxálico

Resumo – O objetivo deste trabalho foi avaliar a eficácia dos métodos “straw test” e imersão em ácido oxálico na identificação dos níveis de resistência do algodoeiro (*Gossypium hirsutum*) branco e colorido ao mofo-branco (*Sclerotinia sclerotiorum*). Foram avaliados dez genótipos: cinco de fibra colorida, quatro de fibra branca e um genótipo suscetível de fibra branca. Os genótipos MAB-1 de fibra branca e MAC-2 de fibra colorida foram os mais resistentes ao mofo-branco de acordo com os métodos imersão em ácido oxálico e straw test, respectivamente. Esses genótipos podem ser indicados como fontes de resistência para programas de melhoramento. Ambos os métodos analisados são complementares entre si; no entanto, o straw test é mais eficiente na avaliação da resistência dos genótipos de algodão ao mofo-branco.

Termos para indexação: *Gossypium hirsutum*, resistência do algodoeiro, ácido etanodioico, melhoramento de planta, inoculação de planta, mofo-branco.

Cotton (*Gossypium hirsutum* L. r. latifolium Hutch) is a very important crop worldwide, extensively used as a raw material in the textile industry (Fang et al., 2017). The color of its fiber is generally white, but may also be naturally colored (Carvalho, 2015). Among the diseases most harmful to cotton, is white mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. This disease can affect cotton plants at all phenological stages, forming sclerotia, stress-resistant structures that survive winter seasons (Schwartz & Singh, 2013).

The pathogenesis of *S. sclerotiorum* requires the secretion of oxalic acid, which is fundamental for

the virulence of the fungus (Liang et al., 2015; Uloth et al., 2015), as it participates in a series of complex reactions in the infectious process and is related to the modulation of programmed cell death (Williams et al., 2011).

Petzoldt & Dickson (1996) proposed a direct method for screening of resistance to white mold, in which the tip of the plant is exposed to the pathogen via an inoculated straw and the extension of the lesion on the plant apex is evaluated after eight days according to a scale of resistance (Singh et al., 2007). Kolkman & Kelly (2000) opted for an indirect method using oxalic

acid; its advantage is the possibility of evaluating a large number of genotypes in a short time, without the need to manipulate the fungus. In this case, the genotypes that present greater tolerance to oxalic acid are more resistant to the disease, since the acid is considered a primary factor of fungus pathogenicity (Uloth et al., 2015).

The objective of this work was to evaluate the efficiency of the straw and oxalic acid tests to identify resistance levels of white and colored cotton genotypes to white mold.

Two experiments were carried out in 2016 – the straw test in a greenhouse and the immersion in oxalic acid test in a laboratory – at Universidade Federal de Uberlândia (UFU), in the municipality of Uberlândia, in the state of Minas Gerais, Brazil. Ten cotton genotypes from the germplasm bank of the cotton genetic improvement program of UFU were evaluated: five with colored fiber; four with white fiber; and a known susceptible commercial cotton cultivar, Delta Opal, also with white fiber. The evaluations started at the V3 phenological stage (Marur & Ruano, 2001), after inoculation of *S. sclerotiorum*.

The experimental design was completely randomized, with five replicates. In the first experiment, the experimental unit consisted of two 500-mL vessels filled with the commercial substrate, each with one plant. In the second, two 50-mL test tubes containing 30 mL oxalic acid (20 mmol L⁻¹) were used.

Isolates of *S. sclerotiorum* were obtained from sclerotia collected in soybean [*Glycine max* (L.) Merr.] fields in the municipality of São Miguel do Passa Quatro, in the state of Goiás, Brazil (17°03'31"S, 48°39'46"W). Disinfection was performed in 50% alcohol and 0.5% aqueous sodium hypochlorite for 30 to 60 s, respectively. Afterwards, the isolates were rinsed in sterile water and transferred to Petri dishes containing potato dextrose agar (PDA), incubated at 20±2°C, with 12 hours light for five days; the pathogen inoculum was transferred three times to plates with fresh PDA.

The plants were sectioned just below the first node above the cotyledon leaves, and inoculation was performed immediately after thinning. Unfiltered pipette plastic tips (200 µL) were used to cut the mycelium in the Petri dishes and to cover the tips of sectioned seedlings. The length of the lesion caused by the disease was measured three, five, and eight days

after inoculation. The data obtained were interpreted according to the scale presented by Petzoldt & Dickson (1996), according to the size of the lesion (cm), where: 0–2 cm, highly resistant; 2–3 cm, resistant; 3–5 cm, moderately resistant; 5–6 cm, susceptible; 6–7 cm, moderately susceptible; and >7 cm, highly susceptible.

The oxalic acid immersion test was carried out with a solution adjusted to pH 4 with 2 mol L⁻¹ sodium hydroxide (NaOH) – defined to simulate the pH of the plant metabolism during infection by the pathogen. At the V₃ stage, the base of the stem was cut and about 2 cm were immediately inserted into the test tube containing oxalic acid, with controlled temperature and humidity around 20°C and 70%, respectively. To check wilting development, 2-cm cotton shoots (control) were immersed in sterile distilled water, under the same conditions as the plants tested with oxalic acid (Carvalho et al., 2013).

Evaluations were performed after 12, 24, and 36 hours of immersion in oxalic acid. Wilt symptoms were classified according to the scale proposed by Kolkman & Kelly (2000): 1, no visible wilting symptoms; 2, one leaf with symptoms; 3, two leaves with symptoms; 4, three leaves with symptoms; 5, wilting of petioles; and 6, withering of any plant. Subsequently, the area under wilting progress curve (AUWPC) – indicating disease severity proportional to evaluation time, adapted from Campbell & Madden (1990) – was calculated to combine wilting symptom evolution into one result.

To test homogeneity and normality assumptions, respectively, Levene's and Shapiro-Wilk's tests were used, at 1% probability. After assumptions were met, the analysis of variance was carried out by the F-test, at 5% probability. Means were compared by the Scott-Knott test, at 5% probability, using the Sisvar software (Ferreira, 2003).

In the straw test, a significant interaction was observed between genotypes and evaluation times of average lesion length (cm) (Table 1). Typical disease symptoms, particularly stem necrosis, were observed three days after inoculation. The genotypes were divided into three, four, and six groups at three, five, and eight days after inoculation, respectively. The genotypes with better performance (more resistant) showed the smallest lesions. The MAC-2 genotype with colored fiber was the only one whose lesions did not increase over time, being classified as highly resistant (1.27 cm) on all three dates.

Most of the evaluations carried out on the eighth day allowed a better classification of genotypes according to differences in pathogen development by the straw test (Table 1). The MAB-1 (3.08 cm) and MAB-2 (4.25 cm) genotypes with white fiber, as well as the MAC-1 (3.66 cm) and LPC-07 (4.52 cm) genotypes with colored fiber, were classified as moderately resistant. The MAB-4 and MAC-3 genotypes, with white and colored fibers, respectively, were classified as susceptible, with mean lesion sizes of 5.85 and 5.13 cm. The MAB-3 and MAC-4 genotypes, with white and colored fibers, respectively, were moderately susceptible, with 6.75 and 6.57-cm lesions. The Delta Opal control was highly susceptible, showing a lesion of 7.38 cm, whereas the most resistant genotype to white mold was the MAC-2 genotype with colored fiber, which had a lesion of 1.27 cm.

The oxalic acid test also allowed differentiating the cotton genotypes according to the progress of wilt (Table 2). The obtained coefficient of variation of 13.55% was below those found by Kolkman & Kelly

(2000) when evaluating common bean (*Phaseolus vulgaris* L.), indicating high experimental precision. The susceptible cultivar Delta Opal and the LPC-07 genotype with colored fiber did not present wilting responses after 36 hours immersed in oxalic acid solution; therefore, they were not included in Table 2. These results are indicative that oxalic acid may be not an appropriate test for all cotton genotypes.

The MAB-1 genotype with white fiber showed a greater tolerance to wilt, presenting an AUWPC about 62% lower than that of the most affected genotypes, i.e., MAB-3, MAC-2, and MAC-3 (Table 2). The MAB-1, MAB-3, and MAC-3 genotypes had similar responses in both tests. However, MAC-2, classified as highly resistant by the straw test, was considered susceptible by the oxalic acid test, indicating genetic but not physiological resistance since the resistance genes were not expressed during immersion in oxalic acid; this result is indicative that different resistance mechanisms are activated by each test.

The efficacy of the second test was confirmed by comparing the plants immersed in oxalic acid solution for 36 hours with the control immersed in sterile distilled water, which did not show any symptoms. The obtained results indicate that the method proposed by Kolkman & Kelly (2000) can be adapted and used in cotton plants. The MAB-1 genotype presented resistance in both tests, being a possible source of

Table 1. Average lesion length measured three times after artificial inoculation (3, 5, and 8 days) with *Sclerotinia sclerotiorum* of white and colored cotton (*Gossypium hirsutum*) genotypes by the straw test, at the V3 phenological stage⁽¹⁾.

Genotype	Average lesion length (cm)			Classification ⁽²⁾
	3	5	8	
White cotton				
MAB-1	0.71Aa	1.46Ba	3.08Cb	MR
MAB-2	1.17Ab	2.50Bb	4.25Cc	MR
MAB-3	1.63Ab	3.81Bc	6.75Cf	MS
MAB-4	0.65Aa	3.14Bc	5.85Ce	S
Delta Opal	1.96Ab	4.55Bd	7.38Cg	HS
Colored cotton				
MAC-1	1.30Ab	3.54Bc	3.66Bb	MR
MAC-2	0.58Aa	0.89Aa	1.27Aa	HR
MAC-3	1.27Ab	3.45Bc	5.13Cd	S
MAC-4	1.03Ab	4.59Bd	6.57Cf	MS
LPC-07	0.25Aa	2.41Bb	4.52Cc	MR

⁽¹⁾Means followed by equal letters, lowercase in the columns and uppercase in the rows, belong to the same group by the Scott-Knott test, at 5% probability. ⁽²⁾Classification of cotton genotypes for resistance to *S. sclerotiorum* based on average lesion length (cm) at eight days after inoculation: MR, moderately resistant with 3–5-cm lesions; MS, moderately susceptible with 6–7-cm lesions; S, susceptible with 5–6-cm lesions; HS, highly susceptible with > 7-cm lesions; and HR, highly resistant with 0–2-cm lesions.

Table 2. Area under wilting progress curve (AUWPC) 36 hours after immersion in oxalic acid solution of white and colored cotton (*Gossypium hirsutum*) genotypes, at the V3 phenological stage⁽¹⁾.

Genotype	AUWPC
White cotton	
MAB-1	32.4a
MAB-2	73.2b
MAB-3	83.4c
MAB-4	73.8b
Colored cotton	
MAC-1	70.8b
MAC-2	84.0c
MAC-3	85.2c
MAC-4	75.6b

⁽¹⁾Means followed by equal letters belong to the same group by the Scott-Knott test, at 5% probability.

resistance genes, which may be related to the ability to degrade oxalic acid (Dutton & Evans, 1996).

It should be noted that field tests are required to confirm the results obtained by the two assessed test. The MAB-1 and MAC-2 genotypes, with white and colored fibers, respectively, are the more resistant to *S. sclerotiorum* and are indicated as sources of resistance. The cultivar Delta Opal is considered as a susceptible control, especially in the straw test. Both the straw and immersion in oxalic acid solution tests are practical for the evaluation of resistance of white and colored cotton genotypes to white mold and can be considered complementary for the study of the interaction between *S. sclerotiorum* and cotton. However, the straw test, which shows the direct interaction between plant host and pathogen, is still more efficient to evaluate plant resistance.

Acknowledgments

To Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig, process number CAG-APQ-02926-14) and to the cotton breeding program of Universidade Federal de Uberlândia (UFU), for financial support and assistance.

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Received on April 18, 2017 and accepted on February 16, 2018