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Cotton resistance to white mold (*Sclerotinia sclerotiorum*) evaluated by the oxalic acid method

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Abstract

The white mold (*Sclerotinia sclerotiorum*) is one of the most destructive cotton disease. Genetic resistance is one of the main strategies to control this disease. Thus, this study aimed to determine an appropriate period of exposition and concentration of oxalic acid to identify levels of physiological resistance to white mold in white fiber cotton genotypes. The study was implemented in two periods (August to October and November to January) in randomized complete block design, in a split-split-plot factorial in time, with five replications, where the plot factor was the concentration of oxalic acid (20; 40 mM) and the sub-plot factor was the 20 cotton genotypes, the sub-sub-plot factor was the time of exposure to oxalic acid (24h; 48h; 72h). The experimental units, or plots, were composed of test tubes with one cotton plant shoot partially immersed in oxalic acid solution. The biomass of the cotton shoots was evaluated at 24, 48, and 72 h. The loss of water caused by the exposure to oxalic acid indirectly indicates the level of cotton resistance to white mold. The results suggest that the most appropriate exposition time and concentration of oxalic acid for cotton evaluation of resistance to white mold were 20 mM at 48 hours, respectively. In these conditions, the cotton genotypes that presented the greatest average fresh mass were: UFU-14 A, UFU-14 B, UFU-14 F, UFU-14 H, and UFU-14 S, which could be used as sources of resistance to white mold in cotton breeding programs.

Keywords: *Gossypium hirsutum*; plant disease; sclerotia; ethanedioic acid; plant breeding.
Abbreviations: OA_oxalic acid.

Introduction

Cotton (*Gossypium hirsutum* L.) is an herbaceous plant mostly mainly grown to produce vegetable fiber, and is one of the main agricultural crops in the world. In Brazil, the 2018/19 harvest season presented an estimated cotton crop area of more than 1.61 million hectares, an area about 37% bigger than the previous harvest (CONAB, 2019). The rise of the cotton crop area, the establishment of modern agricultural systems (e.g. no-tillage soil management, improved harvesting systems), and the resurging of secondary cotton diseases highlighted the need for cotton crop improvements to minimize the losses of cotton production and quality due to biotic and abiotic stresses (Cia et al., 2008).

Among the cotton diseases, the white mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a big problem for cotton producers because it can negatively affect cotton production. After all, the pathogen produces resistant structures(*sclerotia*) of long-term survival, which) difficults the management of this phytopathogen (Reis et al., 2011; Garcia, 2012). The most common symptoms of white mold disease are wilting, tissue necrosis, wet rot of the stem, leaves, petioles and cotton balls (Charcar et al., 1999).

The plant infection can occur in two ways, through the *sclerotia* germination of mycelia (myceliogenic infection) or via the formation of apothecia that release ascospores (carpogenic infection). The mycelia and ascospores in contact with the susceptible host and under favorable conditions begin the the infection process and colonization of the plant tissue (Vieira et al., 2001; Görgen et al., 2009). Irrigated areas also provide favorable conditions for the development and increase of the severity of white mold; additionally, the extensive cotton flowering period, the high cost of fungicide applications and the lack of resistant cultivars hinder the management of this disease in cotton production areas (Guerra et al., 2002).

The plant disease control can be assessed through genetic improvement of the commercial cultivars. Genetic resistance is the most economical strategy; thus, the identification of

resistance of heritable nature is of primary importance, making the identification of resistant genotypes a research priority. For white mold plant disease, the methods most often used for the selection of resistant genotypes are the inoculation of detached leaves or excised petioles and the oxalic acid (OA) test (Rowe, 1993; Cunha, 2010; Jaccoud Filho et al., 2017).

The pathogenicity of the fungus is directly correlated to the primary factoroxalic acid (Godoy et al., 1990; Rowe, 1993; Dutton and Evans, 1996; Guimarães and Stotz, 2004; Chen et al., 2013; Jain et al., 2015). Oxalic acid is a strong organic dicarboxylic acid, synthesized by a broad range of pathogenic and non-pathogenic organisms, including bacteria, fungi, plants and some mammals (Liang et al., 2015). In addition to the pathogenic effect, the oxalic acid can also affect the growth and development of *S. sclerotiorum*, besides inhibiting the stomata closure in seedlings (Liang et al., 2015).

Considering the mechanisms involved in the white mold pathogenicity, the evaluation of the plant reaction to immersion in oxalic acid solution could help identify physiological levels of resistance among genotypes (Kolkman and Kelly, 2000; Gonçalves, 2012). Therefore, the objective of this study was to determine the concentration and time of exposure to the oxalic acid solution to distinguish cotton genotypes resistance to white mold plant disease.

Results and discussion

The results present significant differences for OA concentration interacting with the time of exposition to OA solution in the first season (Table 1). In the second evaluation, there were significant differences for OA concentration, time of exposition to OA, and cotton genotypes. These results corroborate the expectation that the cotton genotypes within each concentration and time established would present genetic variability in their physiological response to the OA.

Oxalic acid concentration x time of exposition

The first season presented no significant differences between cotton genotypes. Still, the interaction between OA concentration and time of exposition to OA solution was significant, indicating that the concentrations and the exposure times to oxalic solution influence the fresh biomass (water content) of the plants used to estimate the genetic resistance to *S. sclerotiorum*.

The lack of differences among cotton genotypes in the first season demonstrated the great influence the environment during plant growth has over the plant resistance since the results were different in the second season. According to Soares (2015), these factors influence the plant biological processes such as the photosynthetic rate, accumulation of dry mass, increase of fresh biomass and water absorption, which can influence the plant reaction to the OA (Bergamin Filho et al., 1995).

Significant reductions in the cotton shoot fresh biomass were identified between the OA concentrations and among the periods of evaluation (Table 2).

According to Soares (2015), biotic and abiotic stresses, as the exposure to OA, directly influence the cotton physiological processes, reducing fresh biomass, decreasing water absorption and the accumulation of dry biomass. The presence of OA alters stomata functioning, inducing its opening through the accumulation of solutes; in addition, OA inhibits the abscisic acid, which controls the closing of the stomata (Guimarães and Stotz, 2004). These changes contribute to the water loss, significantly reducing the fresh plant biomass.

The loss of water directly affects cotton metabolism. Machado (2016) demonstrated that cotton genotypes showed average losses of 73.6% of fresh biomass when the soil reached 1/3 of the water hold field capacity, indicating that moderate water stress considerably reduces the cotton plant development. Oliveira et al. (2017) identified a direct correlation between the reductions in fresh plant weight as function of low water availability when studying the initial development and metabolism of cotton genotypes in conditions of water stress.

The variation of fresh biomass by exposure to oxalic acid

The genotype results for fresh biomass varied between the OA concentrations and among the periods of exposition to OA (Table 3), highlighting the variability of responses of cotton plants to OA. The genotype differentiation became more pronounced after 48 hours of exposition to the OA solution.

The results observed for the 20 mM OA concentration demonstrated great differentiation among the cotton genotypes (Table 3), being, therefore, indicated as appropriate OA concentration when the aim is to identify cotton genotypes with resistance to white mold. The data obtained after 48 hours of exposure of the plant to OA (20 mM) presented great variation among cotton genotype fresh biomass, indicating this period of exposition to OA solution as appropriate for identifying levels of resistance to the fungus.

Among the cotton genotypes studied, the UFU-14 A, UFU-14 B, UFU-14 F, UFU-14 H and UFU-14 S were those with the smallest reductions in fresh biomass after the exposition to OA solution. This result suggests that those genotypes have a genetic constitution responsible for mechanisms that mitigate the stress caused by OA exposition and, therefore, constitute potential sources of genes of resistance to white mold.

The source of resistance of these genotypes may be associated with great levels of lignin present in the stem. According to Yang et al. (2007), great levels of lignin in canola genotypes conferred greater partial resistance to white mold fungus. Similar results were also observed by Antônio (2012) in common bean, where cultivars more resistant to white mold presented more lignin in their tissues. Therefore, further studies are necessary to confirm that the results observed among cotton genotypes are caused by increased lignin accumulation in the cotton tissues.

Materials and methods

Plant growth and seasons

Two similar studies were implemented; the first study occured in the period from August to October (season 1), and the second, from November to January (season 2), which were periods with significant differences in temperature and photoperiod conditions. The cotton plants tested in this study were cultivated in plastic containers (experimental unit) filled with mixed substrate: sand, organic matter and soil (1:1:1). The mixture was sieved in a 2 mm screen.

The plants (one per plastic container) were cultivated under

Table 1. Analysis of variance for fresh biomass of 20 cotton genotypes of white fiber for the factors OA concentration and time of exposition in two evaluation periods.

Source of variation	df	Square mean	
		First season	Second season
Block	4	12.39073	1.981705
Concentration	1	408.09388*	291.152581*
Error 1	4	8.508707	1.766864
Genotype	19	1.875754	6.181884*
Concentration*Genotype	19	2.543341	4.032656*
Error 2	152	1.601170	1.554322
Time	3	31.792072*	27.870237*
Time*Concentration	3	0.651170*	0.889824*
Time*Genotype	57	0.029352	0.075677*
Time*Genotype*Concentration	57	0.050964	0.043839*
Error 3	480	0.047749	0.029788
Total corrected	799	-	-
C.V. 1 (%)	-	109.19	54.08
C.V. 2 (%)	-	47.37	50.72
C.V. 3 (%)	-	8.18	7.02
General average		2.6714	2.4579

*: significant by the *F* test (p<0.05).

Table 2. Average fresh weight loss resulted from the exposure of the cotton shoots to the oxalic acid solution in the first season.

Time	Concentration	
	20 mM	40 mM
Initial	3.7802 aA	2.4493 bA
24 hours	3.5877 aB	2.1403 bB
48 hours	3.2873 aC	1.7053 bC
72 hours	2.8871 aD	1.5336 bD

Averages followed by the same uppercase and lowercase letters in column and in line, respectively, do not differ by the Scott-Knott test (p<0.05).

similar management conditions in both seasons until the V4 phenological stage (Marur and Ruano, 2004). The plants were sectioned two centimeters above the substrate level for posterior immersion in oxalic acid solution.

Twenty white fiber cotton genotypes were evaluated, being 16 genotypes part of the Cotton Program of Genetic Improvement (PROMALG - UFU), and four commercial cultivars (Table 4).

Experimental development

Both experiments were carried out in two phases; the first phase was conducted in a greenhouse for cotton plant growth under the conditions of each season; the second phase was conducted in the laboratory for determination of the cotton genotypes susceptibility. All experiments were carried out at the Federal University of Uberlândia, Campus Umuarama (18°53'04.6" S and 48°15'36.6" W), in Minas Gerais state, Brazil.

The laboratory experiment with the excised cotton plants was set as a randomized block design in a split split-plot parcel scheme, with the concentration of oxalic acid solution settled in the plot factor, the cotton genotypes in the subplot, and the time of exposition to the oxalic acid solution in the sub-sub-plot, with five replications. The experimental plots were composed by test tube containing one cotton shoot about 2 cm dipped in oxalic acid solution.

Oxalic acid solution

The test tubes were filled with 40 mL of OA solution (20 or 40 mM) following indications found in Kolkman and Kelly (2000). The pH of the OA solutions was calibrated to 4.0 with a sodium hydroxide solution (NaOH, 5 M).

Biomass evaluations

The cotton shoot fresh biomass was evaluated as soon as the shoot was sectioned, and its base (about 2 cm) was immediately immersed in a solution of oxalic acid in test tubes for 24 hours. After this period, the fresh biomass was again evaluated and at 48 and 72 hours after immersion in oxalic acid solution. At each plant weight evaluation, the cotton shoot had its base gently dried with an absorbent paper, then was immediately weighed and returned to the tubes. These assessments were intended to determine which immersion period was the most appropriated to the assess white mold effects on cotton genotypes.

The differences between fresh biomasses of each period of evaluation are a result of the physiological processes that the oxalic acid accelerated and are used to assess the level of resistance of each cotton genotypes to white mold, which means that the cotton shoot that loses more water will be severely affected by wilting and consequently is a genotype more susceptible to *S. sclerotiorum*.

Statistical evaluation

The results of variation of fresh biomass between cotton genotypes were initially submitted to the presuppositions of the ANOVA model (normality of residue and homogeneity of variances, p<0.01). After presuppositions attendance, the results were submitted to ANOVA (F test, p<0.05), and the averages of biomasses from the genotypes were compared

Table 3. Fresh mass of cotton plants exposed to oxalic acid solutions during growing periods.

Cotton genotype	20 mM				40 mM			
	Initial fresh biomass (g)	Fresh biomass 24 h (g)	Fresh biomass 48 h (g)	Fresh biomass 72 h (g)	Initial fresh biomass (g)	Fresh biomass 24 h (g)	Fresh biomass 48 h (g)	Fresh biomass 72 h (g)
DP-555	3.496aB	3.494aB	3.130bC	2.614cB	2.310aB	2.024aA	1.670bA	1.524bA
UFU14-A	4.112aA	4.144aA	3.878aA	3.428bA	2.344aB	2.154aA	1.858bA	1.690bA
UFU14-B	4.450aA	4.408aA	3.938bA	3.334cA	2.226aB	2.096aA	1.780bA	1.542bA
UFU14-C	3.082aC	3.070aC	2.694bD	2.420bC	2.230aB	2.120aA	1.818bA	1.612bA
UFU14-D	2.986aC	3.014aC	2.758aD	2.378bC	2.098aC	1.958aB	1.664bA	1.454bA
DP-1227	2.820aC	2.836aC	2.474bD	2.146bC	1.884aC	1.682aB	1.280bB	1.170bB
UFU14-E	3.624aB	3.624aD	3.180bC	2.626cB	2.606aA	2.460aA	2.052bA	1.786bA
UFU14-F	4.210aA	4.116aA	3.654bA	3.036cA	2.264aB	2.086aA	1.630bA	1.424bA
FMT-705	2.106aD	2.088aD	1.872aE	1.568bD	1.672aC	1.560aB	1.282bB	1.096bB
UFU14-G	2.074aD	2.046aD	1.806bE	1.528bD	2.166aB	1.860aB	1.446bB	1.344bB
UFU14-H	4.036aA	3.906aB	3.574bA	3.036bA	1.954aC	1.754aB	1.328bB	1.222bB
UFU14-J	2.940aC	2.976aC	2.700aD	2.370bC	2.574aA	2.334aA	1.774bA	1.596bA
UFU14-K	2.644aC	2.636aC	2.430aD	2.132bC	2.356aB	2.196aA	1.796bA	1.572bA
UFU14-L	3.896aB	3.780aB	3.412bB	2.862cB	2.442aB	2.216aA	1.700bA	1.520bA
UFU14-M	4.264aA	4.086aA	3.456bB	2.760cB	2.402aB	2.160aA	1.656bA	1.492cA
UFU14-N	3.588aB	3.582aB	3.032bC	2.564cB	2.322aB	2.154aA	1.618bA	1.434bA
UFU14-OB	2.874aC	2.838aC	2.514bD	2.166bC	2.844aA	2.526aA	2.028bA	1.750bA
UFU14-P	3.820aB	3.798aB	3.436bB	3.124bA	2.320aB	1.858bB	1.512cB	1.418cA
UFU14-S	4.406aA	4.198aA	3.620bA	3.042cA	2.396aB	2.030bA	1.578cA	1.484cA
TMG-81	2.172aD	2.240aD	2.054aE	1.666aD	1.940aC	1.660aB	1.322bB	1.208bB

Averages followed by the same uppercase and lowercase letters in the column and in the line, respectively, do not differ by the Scott-Knott test at 0.05 significance level.

Table 4. The white fiber cotton genotypes evaluated.

PROMALG-UFU[1]				Commercials
UFU14-A	UFU14-E	UFU14-J	UFU14-N	DP-555
UFU14-B	UFU14-F	UFU14-K	UFU14-OB	DP-1227
UFU14-C	UFU14-G	UFU14-L	UFU14-P	FMT-705
UFU14-D	UFU14-H	UFU14-M	UFU14-S	TMG-81WS

[1]Program of Genetic Improvement of Cotton – Federal University of Uberlândia.

by the Scott-Knott test ($p<0.05$). The data were analyzed using the R statistical program integrated into the Genes software (Cruz, 2016).

Conclusions

The use of oxalic acid solution at the concentration of 20 mM presented better differentiation of cotton genotypes after 48 hours of plant immersion in the oxalic acid solution. The genotypes that showed greater levels of resistance to oxalic acid, and therefore to white mold, were UFU-14 A, UFU-14 B, UFU-14 F, UFU-14 H, and UFU-14 S.

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