ABSENCE OF DETECTION OF *Xanthomonas axonopodis* pv. *malvacearum* IN COTTON SEEDS USED IN THE NORTHEAST REGION OF MATO GROSSO DO SUL IN A THREE-BASED CROP SEASON STUDY

AUSÊNCIA DE DETECÇÃO DE Xanthomonas axonopodis *pv.* malvacearum *EM SEMENTES DE ALGODOEIRO USADAS NA REGIÃO NORDESTE DE MATO GROSSO DO SUL, EM UM ESTUDO DE TRÊS SAFRAS AGRÍCOLAS*

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ABSTRACT: The aim of this work was to evaluate the presence of the causal agent of angular leaf spot, *Xanthomonas axonopodis* pv. *malvacearum*, in 38 cotton seeds samples with or without linter used in the northeast region of Mato Grosso do Sul, Brazil, during the crop seasons of 2008/09, 2009/10 e 2010/11. It was used the semi selective culture media MSSXAN (beef extract - 3 g, peptone - 5 g, soluble starch - 10 g, sucrose - 5g; Tween 80 - 10 mL; $CaCl_2$ - 0.25 g; crystal violet solution at 1% - 150 mL; cephalexin - 50 mg; chlorothalonil - 10 mg; methyl thyophanate - 10 mg; agar – 15 g; sterile distilled water - 1000 ml) for the isolation of the bacteria and the suspect colonies of being *X. axonopodis* pv. *malvacearum* were purified and submitted to the Gram stain, potassium hydroxide and pathogenicity tests in the leaves of cotton plants of the susceptible genotype NC 53345. The presence of *X. a.* pv. *malvacearum* was not detected in the evaluated seed samples.

KEYWORDS: *Gossypium hirsutum*; Seed pathology. Dissemination.

INTRODUCTION

Nowadays the cotton (*Gossypium hirsutum*) growers located in northeast of Mato Grosso do Sul, Brazil, use high technology during the crop season (MELO FILHO; RICHETTI, 2003). Nevertheless, there are microorganisms pathogenic for cotton that decrease yield and are usually found associated with seeds.

Seed transmission of pathogens is certainly more liable to its establishment in a new locality than is aerial dissemination (BAKER; SMITH, 1966). Besides, seeds infested with bacterial pathogens are responsible for re-emergence of diseases of the past, movement of pathogens across international borders (GITAITIS; WALCOTT, 2007). For this reason, there are a need for detect these seed-borne microorganisms and semi-selective medium are commonly used, especially in Brazil (MEHTA et al., 2005; MARINGONI; CAMARA, 2006; HERBES et al., 2008; DEZORDI et al., 2009; TORRES et al., 2009).

There is a wide diversity of microorganisms which were found in cotton seed (SOAVE, 1985). Bueno et al. (2000) evaluated the presence of fungi in 15 lots of cotton seed obtained in different regions of Mato Grosso do Sul and founded various fungal species associated with a predominance of seed storage fungi. Theodoro et al. (2009) described the presence of several fungi genera in 18 cotton seed lots used in the northeastern of Mato Grosso do Sul State and among the plant pathogenic fungi observed were *Fusarium* spp. and *Colletotrichum* spp.

Angular leaf spot is caused by *Xanthomonas axonopodis* pv. *malvacearum* (Smith) Dye and some years ago was considered the most important disease affecting the cotton crop regarding the yield loss that it can cause (CHITARRA; ARAÚJO, 2003; CIA; SALGADO, 2005).

Evaluating the cotton seed health applied in Mato Grosso, Brazil, Mehta et al. (2005) and Dezordi et al. (2009) detected *Xanthomonas axonopodis* pv. *malvacearum* (Smith) Dye in some seed lots. They indicated routine tests to prevent the occurrence of angular leaf spots epidemics. Therefore, tolerance patterns of *Xanthomonas axonopodis* pv. *malvacearum* in cotton seed should be zero, since one of the main procedure to prevent and control this disease is the use of certified and healthy seeds (MACHADO, 2003). There are no papers reporting the presence or absence of

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Xanthomonas axonopodis pv. *malvacearum* in cotton seed used in the State of Mato Grosso do Sul.

The objective of this study was to evaluate the presence of *Xanthomonas axonopodis* pv. *malvacearum* in cotton seeds used during the crop season of 2008/2009, 2009/2010 and 2010/2011 in the northeast of Mato Grosso do Sul.

MATERIAL AND METHODS

There were evaluated 33 samples of seeds without linter and 05 samples with linter of different cotton genotypes used in Chapadão do Sul, located in northeastern Mato Grosso do Sul, during the crop seasons of 2008/09, 2009/10 and 2010/11. Most of the seeds were sampled from market companies and were harvested by local farmers. As control, there were used cotton seeds naturally infected by *Xanthomonas axonopodis* pv. *malvacearum* from Piracicaba, SP.

Each sample of seeds was divided into five sub-samples of 100 g without linter and 120 g with linter. The seed samples without linters were macerated in 300 mL of sterile distilled water at 5 °C for 18 h, while the maceration of seeds with linter used 500 mL of sterile distilled water (DEZORDI et al., 2009).

The suspensions obtained from the seeds maceration were sowed with the aid of a platinum loop on the surface of the semi-selective medium MSSXAN developed by Dezordi et al. (2009) inside a Petri dish and prepared with the ingredients as follow: meat extract (3 g), peptone (5 g), soluble starch (10 g), sucrose (5 g), agar (15 g), Tween 80 (10 mL), calcium chloride (0.25 g) solution of 1% crystal violet (150 mL), cephalexin (50 mg), chlorothalonyl (10 mg), thiophanate methyl (10 mg) and sterile distilled water (1000 mL).

The Petri dishes were incubated at 28 °C for 72 h and after it was done the assess of the cultural characteristics of colonies formed and checked for hydrolysis of starch and lipolysis of Tween 80 in the culture medium. It was cultivated a previously identified strain of *Xanthomonas axonopodis* pv. *malvacearum* in the semi-selective media to serve as a positive pattern.

Five Petri dishes per sub-sample were used and each Petri dish was considered as two replications. The Petri dishes were scratched up in the bottom to provide two areas per plate. Each area was considered as a replication. For each sub-sample of seed cotton was obtained ten replications or a total of 50 representative areas per sample.

After the development of bacterial colonies suspects to be *Xanthomonas axonopodis* pv. *malvacearum* on the surface of the culture medium was done the purification by sown in Petri dish with the semi-selective medium, following by the Gram stain and differential solubility in potassium hydroxide. Thereafter, the colonies with bacterial cells Gram-negative were transferred for Petri dishes with the semi-selective medium MSSXAN and inoculated in cotton genotype NC 53345, susceptible to angular leaf spot. The plants were kept in 5 L pots inside a plastic tunnel and the inoculation was performed through leaf surface injection of a bacterial suspension in the concentration of 10^8 ufc.mL^{-1}.

The seed samples were considered infected after the observation of one or more colonies of *Xanthomonas axonopodis* pv. *malvacearum* with similar characteristic to the pattern isolate in at least one of the five sub-samples analyzed.

RESULTS AND DISCUSSION

Xanthomonas axonopodis pv. *malvacearum* was not associated in the seeds of all 38 samples assessed and used in northeast of Mato Grosso do Sul. The opposite was observed in the control seed infected sample (Table 1), which was observed the presence of a Gram-negative bacteria and bright, mucoid, convex and yellow colonies in accordance with the description of *Xanthomonas axonopodis* pv. *malvacearum* (SCHAAD et al., 2001). In addition, there were halos around these bacterial colonies developed in the semi-selective media culture, which indicates the presence of hydrolysis of starch and lipolysis of Tween 80. These isolates were pathogenic to the inoculated cotton seedlings.

Gram negative saprophytic bacterial colonies were found, however, without the ability to hydrolysis starch (amylase production) and lipolysis Tween 80. In the used semi-selective medium some of these colonies had different cultural characteristics from those found in *Xanthomonas axonopodis* pv. *malvacearum*. Most of the saprophytic bacteria observed had slightly yellowish color and shaped like egg yolk, while minority colonies were white and mucoid beyond those brown and with a convex shape.

The absence of detection of *Xanthomonas axonopodis* pv. *malvacearum* in the evaluated seed samples can be explained by the use of resistant genotypes and absence of weather conditions for the occurrence of angular leaf spot in cotton grown in Chapadão do Sul.

Table 1. Detection of *Xanthomonas axonopodis* pv. *malvacearum* in cotton seeds used in the northeast region of Mato Grosso do Sul during some crop seasons using the semi-selective culture media MSSXAN.

Genotypes	Crop season	Classification [1]	City	Result[2]
Nuopal	2008/2009	WL	Chapadão do Sul	-
Nuopal	2008/2009	WL	Chapadão do Sul	-
DP 604 BG	2008/2009	WL	Chapadão do Sul	-
Delta Penta	2008/2009	WL	Chapadão do Sul	-
Delta Opal	2008/2009	WL	Chapadão do Sul	-
Sure-Grow	2008/2009	WL	Chapadão do Sul	-
DP-90 B	2008/2009	WL	Chapadão do Sul	-
Sicala 40	2008/2009	WL	Chapadão do Sul	-
Nuopal	2008/2009	WL	Chapadão do Sul	-
FM 993	2008/2009	WL	Chapadão do Sul	-
BRS Cedro	2008/2009	WL	Chapadão do Sul	-
FM 993	2008/2009	WL	Chapadão do Sul	-
FM 993	2008/2009	WL	Chapadão do Sul	-
FM 993	2008/2009	WL	Chapadão do Sul	-
BRS 293 Peneira 08	2008/2009	WL	Chapadão do Sul	-
FM 993	2008/2009	WL	Chapadão do Sul	-
FMT 701	2008/2009	WL	Chapadão do Sul	-
FMT 701	2008/2009	WL	Chapadão do Sul	-
FM 993	2009/2010	WL	Chapadão do Sul	-
FM 910	2009/2010	WL	Chapadão do Sul	-
FMT 701	2009/2010	WL	Chapadão do Sul	-
FM 993 Mida	2009/2010	WL	Chapadão do Sul	-
FMT 701	2009/2010	WL	Chapadão do Sul	-
FM 910	2009/2010	WL	Chapadão do Sul	-
FMT 701	2009/2010	WL	Chapadão do Sul	-
IPR Jataí IAPAR	2008/2009	WL	Chapadão do Sul	-
FMT 701	2009/2010	WL	Chapadão do Sul	-
DP-640 BG	2009/2010	WL	Chapadão do Sul	-
Nuopal	2009/2010	WL	Chapadão do Sul	-
FM 993	2009/2010	L	Chapadão do Sul	-
FM 910	2009/2010	L	Chapadão do Sul	-
FM 993	2009/2010	L	Chapadão do Sul	-
FMT 701	2009/2010	L	Chapadão do Sul	-
FM 910	2009/2010	L	Chapadão do Sul	-
FM 993	2010/2011	WL	Chapadão do Sul	-
FM 910	2010/2011	WL	Chapadão do Sul	-
FM 710	2010/2011	WL	Chapadão do Sul	-
P15 701	2010/2011	WL	Chapadão do Sul	-
CD 408 (Controle)	2004/2005	WL	Piracicaba-SP	+

[1] Classification: Without linter (L); With linter (L); [2]Presence (+) or absence (-) of *Xanthomonas axonopodis* pv. *malvacearum* pathogenic for cotton.

The evaluated seed lots were of genotypes with some resistance to angular leaf spot, such as cultivars DP 604 BG, 701 FMT, Fiber Max 993, Max 910 and Fiber NuOPAL (AGUIAR, 2011; BAYER, 2011) and according Cia & Salgado (2005), the use of resistant cultivars is an efficient method to control this disease.

The occurrence of a plant disease depends of some specific environmental conditions and the same happens in the transmission of pathogens to leaves and reproductive tissues from seeds (AGRIOS, 2005). Improving the research about the epidemiological behavior of bacterial blight of onion, Roumagnac et al. (2000) developed a semi-selective medium to detect *Xanthomonas* spp. in onion seeds and could detect this pathogen only in seeds produced in fields that had diseased plants. Silva et al. (2010) did not observed cotton plants

with symptoms of angular leaf spot in some northeast cities of Mato Grosso do Sul, during the crop seasons of 2007/08 and 2008/09. This field research was done by evaluation of 7.540 cotton plants and the fact was attributed mainly to unfavorable climatic factors to the disease. Theodoro et al. (2011) evaluated the cotton seed-to-plant transmission of *Xanthomonas axonopodis* pv. *malvacearum*, in the field conditions of Mato Grosso do Sul in the 2009/10 and 2010/11crop seasons. It was used artificially infected seeds to obtain different lots of contaminated seeds and they evaluated the incidence of plants with symptoms of angular leaf spot periodically until the 45 days after sown and was verified a lack of transmission of *Xanthomonas axonopodis* pv. *malvacearum* in emerged cotton plants. The authors analyzed climatic data and attributed this fact specially to the unfavorable climatic conditions to the disease in both crop seasons.

Through the use of the semi-selective culture medium MSSXAN, Dezordi et al. (2009) showed that *Xanthomonas axonopodis* pv. *malvacearum* was present in 19 of the 25 cotton seed samples evaluated, indicating its high platting efficiency. Nevertheless, Mehta et al. (2005) detected a very small percentage of infected seeds with *Xanthomonas axonopodis* pv. *malvacearum* through the use of a semi-selective culture media without starch, Tween 80 and crystal violet and reported that epidemics of angular leaf spot can happen even from a low percentage of seed infection.

There are some reports about incidence of plant pathogenic bacteria associated with seeds of cultivated plants (MING et al., 1991; FRANKEN et al., 1993; MOURA; ROMEIRO, 1993). Herbes et al. (2009) pointed out that the use of bean seeds infected with *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* was the primary source of inoculum of bacterial wilt in the state of Santa Catarina. Analyzing 38 seed lots of bean used in the State of Paraná, Torres et al. (2009) founded that 50% had *Xanthomonas axonopodis* pv. *phaseoli*. These data indicated that this pathogen has spread widely in Paraná, probably associated with the use of infected seeds, susceptible genotypes and favorable climatic conditions to the development of epidemics.

The saprophytic microbial diversity observed did not influence negatively the evaluations because there were a reduced number of their colonies and there was a discolored and clear halo surrounding the colonies of *Xanthomonas axonopodis* pv. *malvacearum*. These microorganisms were present even in seeds that have suffered the chemical delintering with sulfuric acid. This information is according to those obtained by Mehta et al. (2005) and Dezordi et al. (2009) that noticed the presence of bacterial flora during the detection of *Xanthomonas axonopodis* pv. *malvacearum* in cotton seeds using semi-selective culture medias. However, it is common the development of non-target organisms in semi-selective culture medias because they were prepared to give a high recovering efficiency and increasing their selectivity may decrease the recovery efficiency of all or some strains of the target organism (GITAITIS; WALCOTT, 2007).

The results obtained in this study showed that *Xanthomonas axonopodis* pv. *malvacearum* was not associated with seed cotton used in three consecutive crop seasons in northeast Mato Grosso do Sul.

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RESUMO: O objetivo deste trabalho foi avaliar a presença do agente causal da mancha-angular, *Xanthomonas axonopodis* pv. *malvacearum*, em 38 amostras de sementes de algodoeiro com ou sem línter utilizadas na região nordeste de Mato Grosso do Sul nas safras 2008/09, 2009/10 e 2010/11. Foi empregado o meio de cultura semi-seletivo MSSXAN (extrato de carne - 3 g; peptona - 5 g; amido solúvel - 10 g; sacarose - 5g; Tween 80 - 10 mL; $CaCl_2$ - 0,25 g; solução de cristal violeta a 1 % - 150 µL; cefalexina - 50 mg; clorothalonil - 10 mg; tiofanato metílico - 10 mg; ágar - 15 g; água destilada esterilizada - 1.000 mL) para o isolamento da bactéria nas sementes dos diferentes lotes e colônias suspeitas de serem *X. axonopodis* pv. *malvacearum* foram purificadas e posteriormente submetidas à coloração diferencial de Gram, teste de KOH e patogenicidade em folhas de plântulas suscetíveis de algodoeiro, genótipo NC 53345. Não foi constatada a presença de *X. a.* pv. *malvacearum* nas amostras de sementes analisadas.

PALAVRAS-CHAVE: Gossypium hirsutum. Patologia de sementes. Disseminação.

REFERENCES

AGRIOS, G. N. **Plant pathology**, 5 ed., San Diego:Elsevier, 2005. 922 p.

AGUIAR, P. H. **Variedade de algodão da FMT reduz custos no Mato Grosso**. Disponível em: < http://www.portaldoagronegocio.com.br/conteudo.php?id=15514>. Acesso em 07.08.2012.

BAKER, K. F.; SMITH, S. H. Dynamics of seed transmission of plant pathogens. **Annual Review of Phytopathology**, n. 4, p. 311-332, 1996.

BAYER. **Sementes Fibermax.** Disponível em: <http:// http://www.certificacaofibermax.com.br>. Acesso em 07.08.2012.

BUENO, Y. R. M., PAIVA, F. A., BACCHI, L. M. A. Qualidade sanitária de sementes de algodão (*Gossypium hirsutum* L.) produzidas no Mato Grosso do Sul. **Summa Phytopathologica**, Jaboticabal, v. 26, n. 4, p. 463-466, 2000.

CHITARRA, L. G.; ARAÚJO, A. E. Mancha angular causada por *Xanthomonas axonopodis* pv. *malvacearum* em algodoeiro. **Fitopatologia Brasileira**, Brasília, v. 28, p. 173-175, 2003.

CIA, E.; SALGADO, C. L. Doenças do algodoeiro. In: KIMATI, H.; AMORIM, L.; REZENDE, J. A. M.; BERGAMIN FILHO, A.; CAMARGO, L. E. A. **Manual de fitopatologia**: doenças das plantas cultivadas, v. 2, 4 ed., São Paulo: Agronômica Ceres, 2005. p. 41-52.

DEZORDI, C.; MARINGONI, A. C.; MENTEN, J. O. M.; CAMARA, R. C. Semi-selective culture medium for *Xanthomonas axonopodis* pv. *malvacearum* detection in cotton seeds (*Gossypium hirsutum* L.). **Asian Journal of Plant Pathology**, p. 1-11, 2009.

FRANKEN, A.; KAMMINGA, G. C.; SNIJDERS, W.; VAN DE ZOUWEN, P. S.; BIRNBAUN, Y. E. Detection of *Clavibacter michiganensis* subsp. m*ichiganensis* in tomato seeds by immunofluorescence microscopy and dilution planting. **European Journal of Plant Pathology**, Netherlands, v. 99, p. 125-137, 1993.

GITAITIS, R.; WALCOTT, R. The epidemiology and management of seedborne bacterial diseases. **Annual Review of Phytopathology**, n. 45, p. 371-397, 2007.

HERBES, D. H., THEODORO, G. F., MARINGONI, A. C.; DAL PIVA, C. A.; ABREU, L. Detecção de *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* em sementes de feijoeiro produzidas em Santa Catarina. **Tropical Plant Pathology**, Lavras, v. 33, n. 2, p. 153-156, 2008.

MACHADO, A. Q. Padrões de tolerância de patógenos em sementes de algodão. **Fitopatologia Brasileira**, Brasília, v. 28, p. 172-173, 2003.

MARINGONI, A. C.; CAMARA, R. C. *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* detection in bean seeds using a semi-selective culture medium. **Brazilian Journal of Microbiology**, São Paulo, v. 37, n. 4, p.451-455, 2006.

MEHTA, Y. R.; BOMFETI, C.; BOLOGNINI, V. A semi-selective agar medium to detect the presence of *Xanthomonas axonopodis* pv. *malvacearum* in naturally infected cotton seed. **Fitopatologia Brasileira**, Brasília,v. 30, n. 5, p. 489-496, 2005.

MELO FILHO, G. A.; RICHETTI, A. **Cadeia produtiva do algodão de Mato Grosso do Sul: eficiência econômica e competitividade.** Dourados : Embrapa Agropecuária Oeste, 2003. 72p. (Série Documentos n. 54).

MING, D.; YE, H.; SCHAAD, N. W., ROTH, D. A. Selective recovery of *Xanthomonas* spp. from rice seed. **Phytopathology**, St. Paul, v. 81, n. 11, p. 1358-1363, 1991.

MOURA, A. B., ROMEIRO, R. S. Detecção e quantificação de *Pseudomonas syringae* pv. *lachrymans* em lotes de sementes de pepino. **Revista Brasileira de Sementes**, v. 15, p. 183-186, 1993.

ROUMAGNAC, P.; GAGVENIN, L.; PRUVOST, O. Detection of *Xanthomonas* sp., the causal agent of onion bacterial blight in onion seeds using a newly developed semi-selective isolation media. **European Journal of Plant Pathology**, Netherlands, v. 106, p. 867-877, 2000.

SCHAAD, N. W., JONES, J. B., LACY, G. H. *Xanthomonas*. In: SCHAAD, N. W., JONES, J. B., CHUN, W. (Ed.) **Plant pathogenic bacteria**. 3ed., St.Paul:APS Press, 2001. p. 175-200.

SILVA, R. R.; THEODORO, G. F.; STAUDT, R. C. Avaliação da incidência de doenças em algodoeiros cultivados na região de Chapadão do Sul. **Revista Brasileira de Oleaginosas e Fibrosas**, Campina Grande, v. 14, n. 2, p. 91-95, 2010.

SOAVE, J. Diagnóstico da patologia de sementes de algodoeiro no Brasil. **Revista Brasileira de Sementes**, v. 7, n. 1, p. 195-200, 1985.

THEODORO, G. F.; CORREIA, H. C.; CHUMPATI, A. A. Inexistência de transmissão de *Xanthomonas axonopodis* pv. *malvacearum* via semente-algodoeiro em condições de campo, no Cerrado sul-mato-grossense. **Bioscience Journal**, Uberlândia, v. 27, p. 701-705, 2011.

THEODORO, G. F.; CHUMPATI, A. A.; CORREIA, H. C.; THEODORO, J. V. C.; CASSETARI NETO, D. ; MACHADO, A. Q. ; MARINGONI, A. C. . Identificação de fungos associados às sementes de algodoeiro empregadas na região de Chapadão do Sul. In: VII Congresso Brasileiro do Algodão, 2009, Foz do Iguaçú. **Anais**. Campina Grande : Embrapa Algodão, 2009. p. 1108-1114.

TORRES, J. P.; SILVA JÚNIOR, T. A. F.; MARINGONI, A. C. Detecção de *Xanthomonas axonopodis* pv. *phaseoli* em sementes de feijoeiro provenientes do Estado do Paraná, Brasil. **Summa Phytopathologica**, Botucatu, v. 35, n. 2, p. 136-139, 2009.