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Technical Note

Virulence of *Sclerotinia sclerotiorum* in common bean using inoculation on leaf axils method

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Abstract

Considered one of the most important diseases in common bean (*Phaseolus vulgaris L.*), white mold caused by *Sclerotinia sclerotiorum* (LIB. DE BARY), Ss, is the topic of studies in various parts of the world. The present work aimed to use the method of inoculation on leaf axils, to evaluate the virulence of three Ss isolates in common bean plantlets, in a greenhouse. The severity of the disease was evaluated with the help of a scale of grades from 1 to 9, based on the length of the lesions observed seven days after inoculation of the fungus. Joint analysis of the data revealed that isolates CEN1147 and CEN969 were the most virulent. The results obtained in this work allowed us to select isolates with good levels of pathogenicity for use in experiments that aim to control white mold on the common bean crop. However, due to high variation on the length of the lesions observed the method of leaf axils and scale of grades resorted were not considered appropriate in assessing the virulence of the pathogen isolates in question.

Key words: White mold, *Phaseolus vulgaris L.*, variety BRS Estilo

Virulência de *Sclerotinia sclerotiorum* ao feijoeiro comum utilizando o método de inoculação nas axilas foliares

Resumo

Considerado como uma das doenças mais importantes para o feijão comum (*Phaseolus vulgaris L.*), o mofo branco causado por *Sclerotinia sclerotiorum* (LIB. DE BARY), Ss, é tema de estudos em várias partes do mundo. O presente trabalho objetivou utilizar o método de inoculação das axilas foliares para avaliar a virulência de três isolados de Ss a plântulas de feijoeiro comum, em casa de vegetação. A severidade da doença foi avaliada com auxílio de escala de notas de 1-9, baseada no comprimento das lesões observadas, sete dias após a inoculação do fungo. A análise conjunta dos dados revelou que os isolados CEN1147 e CEN969 foram os mais virulentos. Os resultados obtidos neste trabalho permitiram selecionar isolados com bons níveis de patogenicidade para uso em experimentos que visam controlar o mofo branco na cultura do feijoeiro. Entretanto, devido à alta variação no comprimento das lesões observadas, o método de inoculação nas axilas foliares e a escala de notas empregada não foram considerados adequados na avaliação da virulência dos isolados do patógeno em questão.

Palavras chave: Mofo-branco, *Phaseolus vulgaris L.*, variedade BRS Estilo

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Virulencia de *Sclerotinia sclerotiorum* al frijol común utilizando el método de inoculación en las axilas foliares

Resumen

Considerada como una de las enfermedades más importantes para el frijol común (*Phaseolus vulgaris* L.), el moho blanco causado por *Sclerotinia sclerotiorum* (LIB. DE BARY), Ss, es sujeto de estudios en diversas partes del mundo. Este estudio tuvo como objetivo utilizar el método de inoculación de las axilas de las hojas para evaluar la virulencia de tres aislados de Ss a las plántulas de frijol, en invernadero. La severidad de la enfermedad fue evaluada con una escala de notas de 1 a 9, en base a la longitud de las lesiones, siete días después de la inoculación del hongo. El análisis conjunta de los datos reveló que los aislados CEN1147 y CEN969 fueron los más virulentos. Los resultados obtenidos en este estudio permitieran seleccionar aislados con buenos niveles de patogenicidad para su uso en experimentos que tienen como objetivo controlar el moho blanco en el cultivo de frijol. Sin embargo, debido a la alta variación en la longitud de las lesiones, el método de inoculación en axilas foliares y la escala de las no fueran considerados apropiados en la evaluación de la virulencia de los aislados del patógeno en cuestión.

Palabras clave: moho blanco; *Phaseolus vulgaris L.*; variedad BRS Estilo

Introduction

The fungus *Sclerotinia sclerotiorum* [(LIB.) DE BARY], (Ss), causes the disease known as white mold of common bean (*Phaseolus vulgaris* L.). As well as infecting bean, this soil-borne fungus infects about 408 other botanical species, including soybean. In the bean plant, the symptoms are most visible in the aerial parts, once flowering starts, in the form of lesions on the stalks, leaves and pods, mainly in the area closest to the soil. Under ideal conditions for disease development (low temperatures and high humidity), the lesions evolve to soft rot, covered by the white mycelia of the fungus, forming numerous sclerotia, which are the fungal resistance structures (BOLAND and HALL, 1994).

Virulence is a term used to express varying degrees of pathogenicity of a organism, which can cause more or less damage to the host in a specified time (BLUM, 2006). Various inoculation methods have been used in studies of Ss in bean plants, conducted under controlled conditions or in the field. Among these methods are: inoculation of sclerotia in the roots (SCHWARTZ et al., 1987); using of excised stems (MIKLAS et al., 1992; KULL et al., 2003); straw test on the apex of the main stalk (PETZOLDT and DICKSON, 1996; FERREIRA et al., 2013), among others.

The leaf axils method has been described for evaluation of germoplasm resistance to *Ss* (TOLÊDO-SOUZA and COSTA, 2003) but not in studies of assessment of virulence. The present study aimed to evaluate the inoculating mycelial discs on the leaf axils to assess the virulence of three isolates of *Ss* on common bean plantlets, under controlled conditions.

The experiments were conducted three times in a greenhouse at Embrapa Cenargen (Phytopathology Laboratory), using seeds from common bean of the commercial category Carioca (variety BRS Estilo), each one during the second half of the months of April, May and June 2014. The *Ss* isolates that were used belong to the Collection of Fungi for Biological Control of Plant Pathogens and Weeds, at Embrapa Cenargen, namely: CEN217 – obtained from *Cyperus rotundus* L., in the Federal District and collected in 2012; CEN960 – isolated from the soil of native forest in Rio Grande do Sul state, in 2011, and CEN1147 – originating from soil cultivated with bean, in the state of Goiás, collected in 2012.

The experiments were arranged in an entirely randomized design including four treatments (three strains of *Ss* and one control without pathogens). The experimental unit consisted of a plastic cup of 300 mL, filled with autoclaved soil containing one plantlet of bean each. The seeds were superficially disinfected by immersing them sequentially in alcohol 70% (30 s), sodium hypochloride at 1% (3 min) and twice-sterilized distilled water. They were then sown in the soil-filled cups. To obtain the inoculum, the three *Ss* isolates were placed in potato-dextrose-agar (PDA) culture medium for seven days at 24° C and with a 12-hour photoperiod. When the bean seedlings were 11 days old, BDA discs (5 mm in diameter) containing fungal mycelia were removed from the culture and

placed on the axils of the primary leaves, leaving the back of the colony in contact with the plant tissue. Blank controls received discs of uncolonized medium. Seven days after inoculation, the disease severity was assessed with a scale from 1-9, based on the length of the lesions in accordance with TOLÊDO-SOUZA and COSTA (2003): 1 = absence of symptoms; 2 = circular lesion beginning to develop; 3 = lesion up to 1.0 cm in length; 4 = lesion between 1.0 and 1.5 cm; 5 = lesion greater than 1.5 cm; 6 = lesions on the stalk, presence of mycelia and wilt on one of the leaves; 7 = lesions on the stalk and presence of mycelia and wilt on both leaves; 8 = symptoms of general wilt; 9 = dead plantlet.

The comparison of the virulence isolates was performed by mixed ANOVA considering the months of evaluation as random effect. The model residues were subjected to Boxcox transformation. The analyzes were developed in statistical by computer language R program, free access in http://www.r-project.org/ site, and the significance level was 5%.

The joint analysis of the means of the three experiments showed that isolates CEN969 and CEN1147 were more virulent than the third isolate CEN217 (Figure 1). In the Tolêdo-Souza and Costa (2003) experiments they founded that the best method to differentiate the genotypes for resistance to white mold were the leaf axil with similar results as well as the inoculation of flowers with ascospores. Similar virulence was observed here for isolate CEN1147 originating from soil cultivated with bean soil cultivated with bean indicating a probable adaptation to the host.

In the TOLÊDO-SOUZA and COSTA (2003) they founded that the best method to differentiate the genotypes for resistance to white mold were the leaf axil with similar results as well as the inoculation of flowers with ascospores. The isolates UnB 1541 was more aggressive than UnB 1547, with averages of 5.2 and 3.6, respectively. The same virulence was observed here for isolate CEN1147 originating from soil cultivated with bean soil cultivated with bean indicating a probable adaptation to the host.

Nevertheless differences in virulence isolates to individual plants and in susceptibility of some plants to the isolates of *Ss* have been reported in the literature with differing results depending on the inoculation method used. MIKLAS et al. (1992) used excised stems of bean to measure the virulence of isolates *Ss* and did not differ statistically for virulence assessed by the length of the injury sustained in stem. Otherwise, KULL et al. (2003) verify that the cut-stem was the most efficient method to identify susceptible and resistant white mold genotypes in dry bean. According to FERREIRA et al. (2013) the straw test was the best method to differentiate the genotypes for sources of resistance to the disease.

The method of inoculation of *Ss* used allowed distinguish the isolates in terms of disease severity (Figure 2). On the other hand, it was observed that the lesions did not develop evenly among the treatments which led to a large variability in the data.

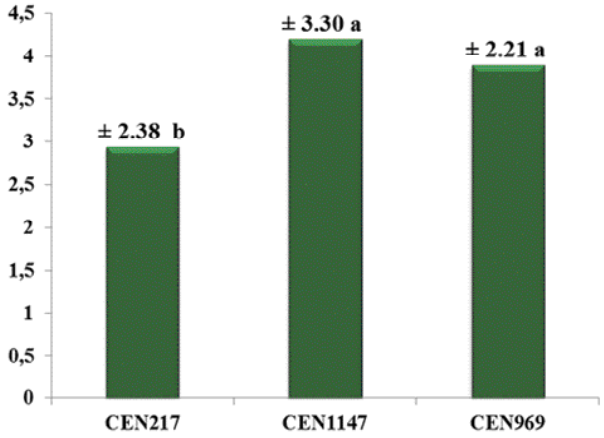

Figure 1. Virulence of *Sclerotinia sclerotiorum* isolates using the method of inoculating mycelial discs on the leaf axils of common bean plantlets. X-axis: isolates of *S. sclerotiorum*, and y-axis: averages with Boxcox transformation. Different letters indicate that the isolates differ according to average comparisons obtained under the mixed model (P <0.05), the random effect variance (months) = 0.715, variance of the fixed effect (isolated) = 6.679. Brasília-DF, 2014.

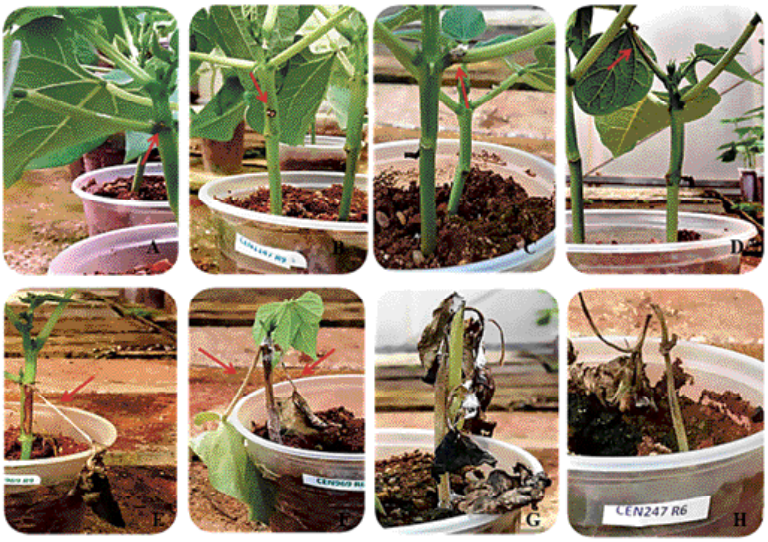

Figure 2. Symptoms observed and associated with grades used in the evaluation of the virulence of the *Scletorinia sclerotiorum* isolates (indicated by red arrows): A- circular lesion beginning to develop; B- lesion up to 1.0 cm in length; C- lesion between 1.0 and 1.5 cm; D- lesion greater than 1.5 cm; E- lesions on the stalk, presence of mycelia and wilt on one of the leaves; F- lesions on the stalk and presence of mycelia and wilt on both leaves; G- plantlet with symptoms of general wilt, due to intense mycelial development and H- dead plantlet. Brasília-DF, 2014.

Conclusions

The results obtained in this work allowed us to select isolates with good levels of pathogenicity for use in experiments that aim to control white mold on the common bean crop. The evaluation using scale of grades and the inoculating mycelium discs in the leaf axils exhibited variation and was not considered appropriate to assess the virulence of *S. sclerotiorum* isolates in question and another method must be employed to further studies.

Acknowledgments

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References

BLUM, L.E.B. Fitopatologia: o estudo das doenças de plantas. Brasília: Otimismo, 2006. 265p.

BOLAND, G.J.; HALL, R. Index of plants of hostes *Sclerotinia sclerotiorum*. Canadian Journal Plant Pathology, Quebec, v.16, n.1, p.93-108, 1994. doi: 10.1080/07060669409500766

FERREIRA, L. U.; SOUZA, T. L. P. O.; LOBO JÚNIOR, M.; PEREIRA, H. S.; MELO, L. C.; FARIA, L. C., WENDLAND, A., RIBEIRO, V. A. Avaliação de Métodos de Inoculação Artificial e Caracterização de Cultivares de Feijoeiro Comum Quanto à Reação ao Mofo Branco. In: 7º Congresso Brasileiro de Melhoramento de Plantas, Uberlândia, 2013.

KULL, L.S.; T.D.; VUONG; K.S.; POWERS, K.M.; ESKRIDGE, J.R.; STEADMAN; HARTMAN, G.L. Evaluation of resistance screening methods for Sclerotinia stem rot of soybean and dry bean. Plant Disease, Iowa, v.87, p.1471-1476, 2003. doi:10.1094/PDIS.2003.87.12.1471

MIKLAS, P. N., GRAFTON, K. F.; NELSON, B. D. Screening for partial physiological resistance to white mold in dry bean using excised stems. Journal of the American Society for Horticultural Science, Alexandria, v.117, n.2, p.321-327, 1992.

PETZOLDT, R.; DICKSON, M. H. Straw test for resistance to white mold in beans. Annual Report of the Bean Improvement Cooperative, Michigan, v.39, p.142-143, 1996.

SCHWARTZ, H. F.; CASCIANO, D. H.; ASENGA, J. A.; WOOD D. R. Field measurement of white mold effects upon dry beans with genetic resistance or upright plant architecture. Crop Science, Madison, v.27, n.4, p.699-702, 1987. doi: 10.2135/cropsci1987.0011183X002700040018x

STEADMAN, J., K. ESKRIDGE, J. COSTA, K. GRAFTON, J. KELLY, K. KMIECIK, J. KOLKMAN, J. MYERS, AND P. MIKLAS. 2001. Evaluation of sources of resistance to *Sclerotinia sclerotiorum* in common bean with five test methods at multiple locations. Annual Report of the Bean Improvement Cooperative, Michigan, v.44, p.89-90.

TOLÊDO-SOUZA, E. D.; COSTA, J. L. S. Métodos de inoculação de plântulas de feijoeiro para avaliação de germoplasma quanto a resistência a *Sclerotinia sclerotiorum* (Lib.) De Bary. Pesquisa Agropecuária Tropical, Goiânia, v.33, n.2, p.57-63, 2003.