Original Article

IDENTIFICATION OF FUNGI ON DISEASED SOYBEAN SEEDS HARVESTED DURING A HIGH RAINFALL PERIOD IN MATO GROSSO DO SUL, BRAZIL

IDENTIFICAÇÃO DE FUNGOS EM SEMENTES DOENTES DE SOJA COLHIDAS DURANTE UM PERÍODO DE INTENSA PRECIPITAÇÃO PLUVIOMÉTRICA EM MATO GROSSO DO SUL, BRASIL

Josiane Vogel CORTINA[1]; Gustavo de Faria THEODORO[2]; David Russell WALKER[3]

1. Biologist, Campus of Chapadão do Sul, Federal University of Mato Grosso do Sul, Chapadão do Sul, MS, Brazil; 2. Agronomist, Teacher, Campus of Chapadão do Sul, Federal University of Mato Grosso do Sul, Chapadão do Sul, MS, Brazil. gustavo.theodoro@ufms.br ; 3. Researcher of the United States Department of Agriculture-Agricultural Research Service and Teacher of the University of Illinois. USA.

ABSTRACT: The aim of this work was to evaluate the incidence of several genera of fungi on soybean seeds harvested duringa period of high rainfall in Mato Grosso do Sul, Brazil. Agronomic trait means from 110 plants were determined from data obtained at the time of harvest. From the seeds obtained, 800 were selected that showed discoloration of the tegument, with or without visible fungal colonies. Half of the seeds were superficially disinfected by immersion in a 1% sodium hypochlorite solution for 3 minutes, and all 800 seeds were then incubated to stimulate fungal growth. A modified blotter-test method was used in which 25 seeds were deposited on filter paper placed in a germination box, and a saturated NaCl solution (-1,0MPa) was used to inhibit germination of the seeds. After incubation for 7 days at 25ºC, fungal growth was inspected using optical and stereoscopic microscopes to identify the genera of the fungi present on the basis of their morphologies. On average, there were 50,3 pods per plant, 2,0 seeds per pod, and 31,7 visibly diseased seeds per soybean plant. The mean weight of 100 seeds was 14,72 g and there were 15,30 g of seed per plant, of which 4,58 g were visibly diseased on average. Among the fungi observed were *Fusarium* spp. (80-90%), *Phomopsis* spp. (39-45 %), *Cercospora* spp. (22-30 %), *Colletotrichum*spp. (5-10 %), *Rhizoctonia*spp. (< 2%) and *Penicillium*spp. These results showed that there is a need to breed new soybean genotypes with resistance to the most common seed diseases.

KEYWORDS: *Glycine max.* Seed decay. Purple seed stain.

INTRODUCTION

Brazil is one of the world's largest producers of soybeans and in the 2009/2010 season production was around 68 million metric tons. The midwestern region accounted for 46.0% of the total, and the state of Mato Grosso do Sulranked fifth in production nationaly (AGRIANUAL, 2010).Seed yields are often reduced by diseases caused by fungi, bacteria, viruses and nematodes. According to Almeida et al. (2005), annual production losses due to diseases vary by region and depend on climatic conditions, but average about 15 to 20%. Diseases caused by pathogens able to colonize seed tissue can also reduce the physiological and health quality of seeds, resulting in decreased market value and viability (BRANCÃO et al., 2002; GALLI et al., 2007; HAMAWAKI et al., 2002). Some of the most important seed-transmitted fungal pathogens are *Phomopsis* spp., *Cercosporakikuchii*, *Colletotrichum truncatum* and *Cercosporasojae*, and various fungi associated with disease that develops during storage (GOULART, 2005).

According to Correa e Ramos (2010), 25% of the potential revenue from soybean produced in Brazil is spent on internal transportation costs. Additional losses arecaused by microorganisms on seeds or grains. In Brazil the cultivation of early and medium maturation genotypes in order to be able to harvest seeds between February and March can increase the incidence of diseased seeds because of the high temperature and humidity at harvest (HAMAWAKI et al., 2002).

The "cerrado" region of west-central Brazil has become a major center of soybean production due in large part to the abundance of precipitation during the principal growing season and the generally predictable pattern of rainfall, combined with a mild climate that allows continuous cropping throughout the year. In the 2010/2011 growing season, however, soybean seed yields and market values in Mato Grosso do Sul and neighboring states dropped sharply due to the occurrence of high rainfall and high temperatures at the time of harvest. The problem was exacerbated by delays in planting at the beginning of the growing season that were also caused by excessive rainfall, which was thought to have been influenced by the "La Niña" weather patterns that affected South America in 2010. The effect of the late season rains on delaying harvest

Received: 01/12/11
Accepted: 26/05/12

and maintaining damp conditions in seed pods left in the field increased both the incidence and severity of seed diseases relative to the levels observed in most years. The aim of this work was to evaluate the incidence of major pathogenic fungi associated with soybean seeds harvested under these conditions, and to estimate some agronomical characteristics of the plants.

MATERIALS AND METHODS

The cultivar FMT Anta 82 RR was planted at a density of 282.667 plants ha^{-1}at the Federal University of Mato Grosso do Sul research area near the city of Chapadão do Sul, in the northeastern part of the state of Mato Grosso do Sul, Brazil. In the 2010/2011 growing season, FMT Anta 82 RR was one of the most popular cultivars grown in the cerrado regions, and it was therefore considered a good representative of the cultivars currently grown in this region.FMT Anta 82 RR is an earlier maturity group cultivar marketed by the Mato Grosso Foundation. It is resistant to *Phomopsisphaseoli* f. sp. *meridionalis*, *Cercosporasojina* and *Heteroder a glycines* race three (FUNDAÇÃO MATO GROSSO, 2011).During the growing season, the plants had received four applications of the mixed fungicide pyraclostrobin + epoxiconazole(25 + 66,5 gha^{-1}) between the R1 and R6 developmental stages(RITCHIE et al., 1994), using an application rate of 130 Lha^{-1}. Pests and weeds were managed according to technical recommendations for the crop(COSTA, 1996).

Seeds from 110 plants of the cultivar FMT Anta 82 RR were harvested and those obtained from each plant were put in individual paper bags. A total of 800 seeds were selected for having discoloration of the tegument, with or without visible fungal colonies at the time of harvest. These seeds were chosen to insure the presence of fungi, and to estimate the amount of seed weight contaminated by fungi. They were separated into two groups of 400 seeds each, and one of the seeds was immersed in a1% sodium hypochlorite solution for 3 minutes to kill superficial microorganisms (BRASIL, 2009). Subsequently, 25 seeds were deposited on two pieces of filter paper placed in a germination box (11 x 11 cm), and a saturated salt (NaCl) solution (-1,0MPa) was used to inhibit germination (MACHADO et al., 2003). The seeds were incubated at 25 °C for 7 days under a photoperiod of 12 hours to promote fungal growth. The genera of fungi growing out of the seeds or on the surface of the seed coats were subsequently identified on the basis of morphological structures with the aid of optical and stereoscopic microscopes (BARNETT; HUNTER, 1998). The experimental design was completely randomized design with two treatments represented by the group of seeds that was disinfected and the untreated group, and with 16 replications. The mean incidences for each fungus identified in the treatments were compared using the Tukey Test at a 5% probability level. The following components of seed yield were also evaluated: mass of 100 seeds(mix of healthy and diseased); number of pods per plant; number of seeds per pod; mass of seeds per plant; and the number and mass of diseased seeds per plant.

RESULTS AND DISCUSSION

During the 2010/2011 growing season, soybean seed yieldsin the west-central region of Brazil seriously reduced due to a combination of seed diseases and precocious germination of seeds in the pod prior to harvest (BRASIL, 2011).Precipitation data obtained from a meteorological station showed that the municipality of Chapadão do Sul received 200 mm of rain per month more than it does in a typical yearduring the period of the soybean harvest, and this precipitation and was the major cause of the loss.

The genera and incidence of fungi growing from incubated seeds are shown in Table 1. As expected,the numerical mean incidence of most of the fungal genera was higher on the non-immersed seeds than on the disinfected ones, though the difference was only statistically significant for*Fusarium* spp. and *Rhizoctonia* spp. The incidence of *Phomopsis* spp. was unique in being numerically higher on the disinfected seeds, though the difference was not statistically significant. Noun identified fungi or visible bacterial colonies were observed on or near the evaluated seeds.

Data for traits affecting seed yield and appearance with regard to plant health are presented in Table 2. On average there were 50,3 pods per plant, 2,0 seeds per pod, 14,72 g for each 100 seeds, 15,3 g of seed per plant, and 4,58 g of diseased seeds per plant. There were 31,7 seeds per plant, with typical changes caused by fungi that accounted for approximately 31% of diseased seeds produced per plant.

The data from the surface-disinfected seeds indicate that fungi from the genera *Fusarium*, *Phomoposis*, *Cercospora* and *Colletotrichum* had colonized the seed internal tissue, and were thus largely protected from the sodium hypochlorite treatment. With regard to the genera *Rhizoctonia*, *Aspergillus*, *Mucor*, *Penicillium* and *Rhizopus*, the lack of detection or very low levels of detection on disinfected seeds suggest that these fungi did not colonize the seed tissue, or at least did so to a very limited extent. Spores attached superficially to the seed coat on some seeds may have either survived exposure to the 1% sodium hypochlorite solution, or may have escaped contact with it if they were protected by pockets of air or other physical barriers.

Table 1. Genera and incidence (% of seeds infected) off ungi associated with seeds harvested during a period of high rainfall at the end of the 2010/2011 cropin Chapadão do Sul, Mato Grosso do Sul, Brazil .

	Seed treatment*		
Fungi	Immersed**	Non-immersed	Prob. > F
*Fusarium*spp.	80,00 a	90,50 b	0,0024
*Phomopsis*spp.	44,75	39,00	0,1486
*Cercospora*spp.	22,50	29,75	0,1437
*Colletotrichum*spp.	4,75	10,00	0,1653
*Rhizoctonia*spp.	0,25 a	2,00 b	0,0356
*Aspergillus*spp.	0,25	1,50	0,1169
*Mucor*spp.	0,25	0,25	< 0,010
*Penicillium*spp.	0,00	1,25	0,0968
Rhizopus spp.	0,00	0,50	0,1535

* 800 selected seeds with discoloration and with or without visible fungal colonies; ** 400seeds immersed in an aqueous solution of1% hypochlorite for 3minutes.

Table 2. Agronomic trait means for 110 soybean cv. FMT Anta RR81 plants grown inChapadão do Sul, MatoGrosso do Sul, Brazil, during the 2010/2011 crop season.

Agronomictraits	Average values
Number ofpods per plant	50,23 ± 7,61
Number ofseedsper pod	2,07 ± 0,06
Number of diseased seedsper plant	31,73 ± 13,59
Mass of100seeds(g)	14,72 ± 1,05
Mass of seeds per plant (g)	15,25 ± 2,29
Mass of diseased seeds per plant (g)	4,58 ± 2,00

The first five pathogens listed in Table 1 can cause important diseases and economic losses in soybeans, and can be spread to areas recently cleared, as well as surviving as saprophytes on crop residues (ALMEIDA et al., 2001; REIS et al., 2011).In addition, the pathogens are effectively transmitted via seeds from infected plants if rainfall is abundant during seed formation (SINCLAIR, 1993; SHAH; BERGSTRON, 2000).

The data from this experiment agree with those obtained by Brancão et al. (2002), who reported finding only 12 genera of fungi, especially *Fusarium* spp., *Cercosporakikuchii*, *Phomopsissojae*, *Cladosporium* spp., *Aspergillus* spp. and *Penicillium* spp. Through the identification of fungi in soybean seeds from seven municipalities of Mato Grosso do Sul, in the 1992/93 season, Goulart et al. (1997) found *Fusarium semitectum*, *Aspergillus* spp., *Penicillium* spp. *Phomopsis* spp., *Cercosporakikuchii*, *Cladosporium* spp., *Colletotrichum truncatum* and *Alternaria* spp. to be prevalent in samples. Henning e Yuyama (1999) evaluated the sanitary quality of soybean seeds produced in different regions of Brazil between the 1992/93 and 1996/97 seasons, and found that *Cercosporakikuchii* was the most frequent pathogen in most places,with the highest average incidence. Unlike the observations made in this work, the authors found that *Colletotrichum truncatum* had the lowest average incidence.

The finding of significant differences between disinfected and untreated seeds for only *Fusarium* spp. and *Rhizoctonia* spp. differs from the data obtained by Galli et al. (2007), who evaluated the transmission of plant pathogens via seed in four soybean genotypes and found that seed decontamination with 1% sodium hypochlorite for 3 minutes reduced the incidence of *Fusarium* spp., *Phomopsis* spp. and *Colletotrichum dematium* var. *truncata*. This discrepancy could have resulted from a variety of causes, including differences in the resistance of the soybean genotypes used, different types or ratios of pathogens present at the different locations, differences in the length of time between initial contact with the pathogens and harvest of the seed, and/or environmental differences, particularly in temperature and the timing, intensity and frequency of precipitation events.

The incidence of some fungi did not suffer a deleterious effect (P> 0.05) from exposure to sodium hypochlorite, possibly because some of these fungi produced conidiomata that were protected from the action of the chemical. The level of colonization of soybean seed by *Phomopsis* spp. varies by genotype and fungicide application programs (WRATHER et al., 2004), and infections can be internal and asymptomatic, which may explain the absence of any reduction in the incidence of this microorganism in or on seeds treated with sodium hypochlorite (SINCLAIR, 1993; XUE et al., 2008). *Penicillium* spp. and *Rhizopus* spp. were eradicated from seeds by the effect of sodium hypochlorite, while others were found in a lower or equal number of seeds. This fact can be explained by internal colonization of seeds by fungi, favored by variable organic substances of seeds (XUE et al., 2008). The results of this study indicate that there is a possible need for larger studies to guide adjustments in the official Brazilian methodology (BRASIL, 2009), since exposure to 1% sodium hypochlorite for three minutes was not effective in eradicating some storage fungi from the seeds.

The agronomic trait means data shown in Table 2 indicate that despite the high rainfall which occurred during the harvest period of the crop, these trait values were relatively high (NAVARRO; COSTA, 2002). The high mass of diseased seeds per plant showed that approximately 30% of the mass of seeds produced per plant was reduced by fungi. This is according to the estimated losses for Mato Grosso do Sul during the 2010/11 season (BRASIL, 2011). These seeds can cause depreciation in seed value or the elimination of soybean seed lots by reducing some physiological characteristics (PATHAN et al., 1989). Furthermore, sub-optimal storage and transportation could exacerbate the development of diseases on seeds that became infected with a pathogen prior to harvest, further decreasing the value of seeds by the time they are sold.

The results of this study provide some guidance to soybean breeders regarding the need to develop cultivars with resistance to the most frequently encountered seed fungi. Since the high amounts of precipitation seen during the 2010/11 growing season were not predicted or anticipated, there had been no specific effort to apply fungicides to protect the seeds prior to maturity, and application of fungicides to plants that have reached the R8 stage of development is unlikely to provide effective protection. Development of cultivars with genetic resistance to major seed diseases is therefore an important goal for reducing economic losses when rainfall delays harvesting(ORTH; SHUH, 1994; UPCHURCH; RAMIREZ, 2010).

RESUMO: O objetivo deste trabalho foi avaliar a incidência de gêneros de fungos em sementes de soja colhidas durante um período de intensa pluviosidade em Mato Grosso do Sul, Brasil. Foram determinadas algumas características agronômicas em 110 plantas, no momento da colheita. A partir das sementes colhidas, foram selecionadas 800 com descoloração do tegumento, com ou sem colônias de fungos visíveis. Metade das sementes foram desinfestadas superficialmente por imersão em uma solução de hipoclorito de sódio a 1% por 3 minutos e todas os 800 sementes foram incubadas para estimular o crescimento de fungos. Foi utilizado o método 'blotter-teste' modificado, em que 25 sementes foram depositadas em papel de filtro colocado em uma caixa de germinação. Foi empregada uma solução saturada de NaCl (-1,0 MPa) para inibir a germinação das sementes. Após a incubação por sete dias a 25 ºC, o crescimento de fungos foi avaliado utilizando microscópios ópticos e estereoscópicos para identificar morfologicamente os gêneros. Em média, houve 50,3 vagens por planta, 2,0 sementes por vagem, e 31,7 sementes visivelmente doentes por planta soja. O peso médio de 100 sementes foi 14,72 g e houve 15,30 g de sementes por planta, dos quais 4,58 g estavam visivelmente doente. Entre os fungos observados, detectou-se *Fusarium* spp. (80-90%), *Phomopsis* spp. (39-45%), *Cercospora* spp. (22-30%), *Colletotrichum* spp. (5-10%), *Rhizoctonia* spp. (<2%) e *Penicillium* spp. Estes resultados mostraram que há necessidade de serem desenvolvidos genótipos de soja resistentes às doenças mais comuns que incidem nas sementes.

PALAVRAS-CHAVE: *Glycinemax.* Podridão de sementes. Mancha púrpura das sementes.

REFERENCES

AGRIANUAL. China determina o preço da soja mas o câmbio preocupa. **Agrianual 2011 - anuário da agricultura brasileira**, FNP, São Paulo, Brazil, p. 419-450. 2010.

ALMEIDA, A.M.R.; SARAIVA, O.F.; FARIAS, J.R.B.; GAUDÊNCIO, C.A.; TORRES, E. Survival of pathogens on soybean debris under no-tillage and conventional tillage systems. **Pesquisa Agropecuária Brasileira**, Brasília, v. 36, n. 10, p. 1231-1238, out. 2001.

ALMEIDA, A. M. R; FERREIRA, L. P.; YORINORI, J. T.; SILVA J. F.; HENNING, C. V.; GODOY, C. V.; COSTAMILAN, L. M. ; MEYER, M. C. Doenças da soja. In: KIMATI, H.; AMORIM, L.; REZENDE, J. A. M.; BERGAMIM FILHO, A. L.; CAMARGO, L. E. A.(Ed.) **Manual de Fitopatologia**. São Paulo; Agronômica Ceres, 2005. v.2 Doenças das Plantas Cultivadas, cap. 64, p. 569-588.

BARNETT, H. L.; HUNTER, B. B. **Illustrated genera of imperfect fungi**. St. Paul: APS, 4. ed., 1998. 218 p.

BRANCÃO, N.; NUNES, C. D. M.; GASTAL, M. F. C.; RAUPP, A. A. A.; PORTO, M. P.; WENDT, W. **Ocorrência de fungos em sementes de sorgo, milho, soja e trigo**. Pelotas, RS, 2002. (Embrapa Comunicado Técnico, n. 76).

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes.** Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. – Brasília: Mapa/ACS, 2009. 399 p.

BRASIL. **MS perde parte da safra de soja por causa das chuvas**. Disponível em <http://www.brasil.gov.br/noticias/arquivos/2011/03/11/ms-perde-parte-da-safra-de-soja-por-causa-das-chuvas>. Acesso em 19.09.2011

CORREA, V. H. C.; RAMOS, P. A. Precariedade do transporte rodoviário brasileiro para o escoamento da produção de soja do Centro-Oeste: situação e perspectivas. **Revista de Economia e Sociologia Rural**, Brasília; v. 48, n. 2, p. 447-472, 2010.

COSTA, A. J. **Cultura da soja**. Porto Alegre: Cinco Continentes, 1996. 233p.

FUNDAÇÃO MATO GROSSO. **Anta 82 RR**. Disponível em <http://www.tmg.agr.br/cultivares/soja/?cultivar=anta82>. Acesso em 19.09.2011

GALLI, J. A.; PANIZZI R. C.; VIEIRA, R. D. Efeito de *Colletotrichumdematium*var.*truncata*e *Phomopsissojae* na qualidade sanitária e fisiológica de sementes de soja. **Summa Phytopathologica**, Botucatu, v. 33, n. 1, p. 40-46, 2007.

GOULART, A. C. P. **Fungos em sementes de soja**: detecção e importância. Dourados, EMBRAPA- CPAO, 1997. 58 p.

GOULART, A. C. P. Tratamento de sementes de soja com fungicidas. In: ZAMBOLIM, L. (Org.) **Sementes: qualidade fitossanitária**.Viçosa: UFV, 2005, cap. 16, p. 451-479.

HAMAWAKI, O. T., JULIATTI, F. C., GOMES, G.M; RODRIGUES, F. A. Avaliação da qualidade fisiológica e sanitária de sementes de genótipos de soja do ciclo precoce/médio em Uberlândia, Minas Gerais. **Fitopatologia Brasileira**, Brasília, v. 27, n. 2, p. 201-205, 2002.

HENNING, A. A.; YUYAMA, M. M. Levantamento da qualidade sanitária de sementes de soja produzidas em diversas regiões do Brasil, entre as safras 1992/93 e 1996/97. **Revista Brasileira de Sementes**, Londrina, v. 21, n. 1, p. 18-26, 1999.

MACHADO, J. C.; OLIVEIRA, J. A.; VIEIRA, M. G.; ALVES, M. C. Controle de germinação de sementes de soja em testes de sanidade pelo uso da restrição hídrica. **Revista Brasileira de Sementes**, Londrina, v. 25, n. 2, p 77-81, 2003.

NAVARRO, J. R., H. M.; COSTA, J. A. Contribuição relativa dos componentes do rendimento para produção de grãos em soja. **Pesquisa Agropecuária Brasileira**, Brasília, v. 37, n. 3, p. 269-274, 2002.

ORTH, C. E.; SHUH, W. Resistance of 17 soybean cultivars to foliar, latent, and seed infection by *Cercosporakikuchii*. **Plant Disease**, St. Paul, v. 78, n. 7, p. 661-664, 1994.

PATHAN, M. A.; SINCLAIR J. B.; MCCLARY, R. D. Effects of *Cercosporakikuchii* on soybean seed germination and quality. **Plant Disease**, St. Paul, v.73, n.9, p. 720-723, 1989.

REIS, E. M.; BARUFFI, D.; REMOR, L; ZANATTA, M. Decomposition of corn and soybean residues under field conditions and their role as inoculum source. **Summa Phytopathologica**, Botucatu,v. 37, n. 1, p. 65-67, 2011.

RITCHIE, M. A.; HANWAY, J. J.; THOMPSON, H. E.; BENSON, G. O. **How a soybean plant develops**. Ames: Iowa State University of Science and Technology, Cooperative Extension Service, 1994. 20p. (Special Report, 53).

SHAH, D. A.; BERGSTROM, G. C. Epidemiologia e manejo de patógenos transmitidos por sementes, com ênfase nos fungos que formam picnídios. **Revisão Anual de Patologia de Plantas**, Passo Fundo, v. 8, p. 339-364, 2000.

SINCLAIR, J. B. *Phomopsis* seed decay of soybeans – a prototype for studying seed disease.**Plant Disease**. Plant Disease, v. 77, n. 4, p. 329-334, 1993.

UPCHURCH, R. G.; RAMIREZ, M. E. Defense-related gene expression in soybean leaves and seeds inoculated with *Cercosporakikuchii* and *Diaporthephaseolorum* var. *meridionalis*. **Physiological and Molecular Plant Pathology**, v. 75, p. 64-70, 2010.

XUE, H.Q.; UPCHURCH, R.G.; KWANYUEN, P. Relationships between oleic and linoleic acid content and seed colonization by *Cercosporakikuchii* and *Diaporthephaseolorum*. **Plant Disease**, v. 92, n.7, p.1038-1042, 2008.

WRATHER, J. A.; SHANNON, J. G.; STEVENS, W. E.; SLEPER, D. A.; ARELLI, A. P. Soybean cultivar and foliar fungicide effects on *Phomopsis* sp. seed infection. **Plant Disease**, v. 88, n.7, p. 721-723, 2004.

XUE, H.Q.; UPCHURCH, R. G.; KWANYUEN, P. Relationships between oleic and linoleic acid content and seed colonization by *Cercosporakikuchii* and *Diaporthephaseolorum*. **Plant Disease**, v. 92, n. 7, p. 1038-1042, 2008.