Charcoal Rot or Ashy Stem Blight

Photograph provided by H. F. Schwartz
AgImage - Colorado State University

Charcoal rot or Ashy stem blight, caused by *Macrophomina phaseolina* (Tassi) Goid, is favored by warm dry growing conditions and is often associated with lowland drought stress (Mayek-Pérez et al. 1997a; 1997b; 2002a, 2002c).

Abawi and Pastor-Corrales (1989; 1990) described screening procedures for ashy stem blight. Dried sclerotia are an effective form of inoculum to establish a high and uniform incidence of ashy stem blight. Sclerotia can be produced on an artificial medium consisting of 10 g peptone, 15 g dextrose, 0.25 g $MgSO_4$ and 0.5 g K_2HPO_4 per liter of water. The medium should be placed in a 1 cm layer in Petri plates. A 4 mm diameter disk of agar colonized with *M. phaseolina* was placed in the center of the Petri plate. After incubation for 15 days at 30^0 C, the mycelial-sclerotial mats of the pathogen were blended with distilled water, followed by centrifuging at 3000-5000 rpm. The pellets should be re-suspended in water and centrifuged again. The washed pellets should be spread on filter paper and allowed to dry for 48 hr at 30^0 C. The dried sclerotial masses were ground in a mortar and pestle and mixed in sterilized soil at a rate of 2 g of sclerotia per kg of soil. Greenhouse inoculations can be conducted by placing bean seed in pots on top of clean soil and then covering the seed with a layer of 2-3 cm of infested soil. Seedlings of a susceptible line will either fail to emerge or exhibit lesions after emergence. Lesions soon spread to the stem and can kill the seedling in 2-3 weeks.

Whole rice or sugar-beet seeds can also be used to prepare inoculum (Olaya et al., 1996; Pastor-Corrales and Abawi, 1988). For example, after autoclaving the rice seed with water (1 g rice seed to 1 ml of water), 7-day-old mycelial disks of agar infected with the pathogen are placed into containers of rice seed (Abawi and Pastor Corrales, 1990). The seed should be incubated at 30^0 C for 15 days. In the greenhouse, 2 to 3 rice seeds infected with *M. phaseolina* are placed around each bean seed and covered with clean soil. Field plots should be inoculated at a rate of 2 or more g of infected rice seeds per meter of row (Pastor-Corrales and Abawi, 1988). At planting, the infected rice seed and the bean seed should be placed in the open furrows and covered with soil. Most of the susceptible seedlings will show aboveground symptoms at emergence and can be evaluated using a 1-9 scale (Table 1). Spearman rank correlations between percentage germination and percentage of infection with ashy stem blight near senescence were positive and significant (Beaver et al., 1990; Mayek-Pérez et al., 2001b).

Echávez-Badel and Beaver (1987) inoculated bean plants in the greenhouse by inserting toothpicks infected with *M. phaseolina* into the stem just below the cotyledonary node. Inoculations were made 30 days after planting and the length of the lesion on the main stem was evaluated at 10, 20 and 30 days after inoculation. In Puerto Rico, uniform and severe levels of ashy stem blight infection were obtained when bean nurseries were planted after sorghum [*Sorghum bicolor* (L.) Moench]. Evaluation of reaction to charcoal rot disease in bean germplasm have been conducted through different countries such as Colombia (Pastor-Corrales and Abawi, 1988); México (Mayek-Pérez et al., 2001b; 2002b) or Kenya (Songa et al., 1997). Mayek-Pérez et al. (2001b; 2002b) showed that Mesoamerica germplasm (black beans) exhibited resistance to charcoal rot while Durango and Jalisco (pinto, bayo and flor de mayo beans) races exhibited susceptibility. Mayek-Pérez et al. (2001b, 2002b) and Pastor-Corrales and Abawi (1988) demonstrated that BAT 477 shows resistance to *M. phaseolina* under both greenhouse and field conditions. Close relationship between charcoal rot and drought resistance in bean has been found (Mayek-Pérez et al., 1997a, 1997b).

Mihail and Taylor (1995), Mayek-Pérez et al. (1997c) and Mayek-Pérez et al. (2001c) found a high level of pathogenic variability among isolates of *M. phaseolina,* although isolates from the same location and species had related pathotypes. Jones et al. (1998), Mayek-Pérez et al. (2001c), Vandemark et al. (2000); Su et al. (2001) and Almeida et al. (2003) confirmed the high genetic diversity of *M. phaseolina* when isolates from different host or geographical origins were compared using AFLPs, RAPDs or RFLPs marker methodologies. Reyes-Franco et al. (2005b) found Mexican isolates of *M. phaseolina* are more aggressive in common bean that those from other countries such as Japan, Brazil, Australia, USA or Italy. In addition, Reyes-Franco et al (2005a) demonstrated that Mexican isolates are genetically different that isolates from Asia, per example, but similar to American (USA, Brazil, Argentina, Colombia) isolates. In order to establish a method for the characterization of pathogenicity patterns of *M. phaseolina*, Mayek-Pérez et al. (2001c) proposed a set of bean differential cultivars and assigned a binary value to each cultivar, similar to the system used for anthracnose. The 12 cultivars and their binary value are described on Table 2.

BAT 477, Negro 8025 and TLP 19 had resistance to the greatest number (≥ 70%) of the isolates from Mexico (Mayek-Pérez et al., 2001c). Resistance to a highly resistant strain of *M. phaseolina* was reported to be conferred by two complementary dominant genes in TLP 19 and BAT 477 (Olaya et al., 1996; Mayek-Pérez et al., 2001a). Molecular markers linked to the resistance to charcoal rot have been identified in BAT 477 (Olaya et al., 1996) (Table 2). Miklas et al. (1998) have identified variable number of QTLs conditioning resistance to charcoal rot in XAN 176 under field conditions in Puerto Rico.

Table 1. Rating scale (1-9) used to evaluate beans for aboveground infections caused by *Macrophomina phaseolina*.

Root rot score	Evaluation of stem rot caused by *Macrophomina phaseolina*
1	No visible symptoms.
3	Lesions are limited to cotyledonary tissues.
5	Lesions have progressed from cotyledons to about 2 cm of stem tissues.
7	Lesions are extensive on stems and branches. The foliage exhibits chlorosis and necrosis.
9	Most of the stem, petioles and growing point are infected. A considerable amount of pycnidia and sclerotia is produced.

Source: Abawi and Pastor-Corrales (1990).

Table 2. List of bean cultivars used and their assigned binary value. The sum of the assigned numbers of each cultivar infected by an isolate of *M. phaseolina* gives a unique number which describes the pathotype of the isolate.

Cultivar	Race	Assigned value
Bayo Durango	Durango	1
Pinto UI-114	Durango	2
Pinto Villa	Durango	4
Bayo Mecentral	Jalisco	8
G 4523	Nueva Granada	16
Rio Tibagí	Mesoamerica	32
Azufrado Tapatío	Jalisco	64
G 19428	Perú	128
SEQ 12	Mesoamerica	256
BAT 477	Mesoamerica	512
Negro 8025	Mesoamerica	1024
TLP 19	Mesoamerica	2048

Source: Mayek-Pérez et al. (2001c)

Table 3. Sources of resistance to ashy stem blight in different seed classes.

Name or number	Seed color / type	Resistance genes	Reference
XAN 176 TLP 19, TLP20 Negro Tacaná Negro Perla Jamada, Negro 8025	9 / Black	*Unknown*	Miklas et al. (1998) Mayek-Pérez et al. (2001b, 2002b); Frahm et al. (2004), Abawi and Pastor-Corrales (1988; 1990)
	1 / Navy		
BAT 477 SEQ 12 Manzano, Bayo Zacatecas, Bayo Baranda	2 / Cream Cream	*Mp-1*, *Mp-2* *Derived from BAT 477 x Negro 8025 (probably Mp1 and Mp2)*	Mayek-Pérez et al. (2001b, 2002b), Olaya et al. (1996), Abawi and Pastor-Corrales (1988) Mayek-Pérez et al. (2001b, 2002b)
PT 91084	2M / Pinto		Mayek-Pérez et al. (2002b)
	1 / Great Northern		
	7 / Purple		
	6 / Small red		
	5/ Pink		
	2R / Cranberry		
ICA Palmar (G 4523)	6M / Red mottled		Mayek-Pérez et al. (2002b)
	6K / Dark red kidney		
	1 / Snap		
Carioca	"Ojo de cabra"		Mayek-Pérez et al. (2001b)
Amarillo de Calpan	Yellow		Mayek-Pérez et al. (2002b)
Landraces ("Pastilla de Teocaltiche", Michoacán 9-1-A, "Colorados" from Teopisca, Chiapas) and *P. coccineus* ("ballacote" from Querétaro, México)	Miscellaneous		Mayek-Pérez et al. (2002b)

Photograph provided by H. F. Schwartz
AgImage - Colorado State University

References

Abawi, G.S. and M.A. Pastor Corrales. 1990. Root rots of beans in Latin America and Africa; diagnosis, research methodologies and management strategies. CIAT, Cali, Colombia. 114 p.

Abawi, G.S. and M.A. Pastor Corrales. 1989. Ashy stem blight screening procedures and virulence of *Macrophomina phaseolina* isolates on dry edible beans. Turrialba 39: 200-2007.

Almeida, A.M.R., R. V. Abdelnoor, C.A. Arrabal-Arias, V.P. Calvalho, S.S. Jacoud-Filho, S.R.R. Marín, L.C. Benato, M.C. Pinto, and C.G.P. Carvalho. 2003. Genotypic diversity among Brazilian isolates of *Macrophomina phaseolina* revealed by RAPD. Fitopatol. Bras. 28: 279-285.

Beaver, J.S., M. Martínez and G. Godoy. 1990. Evaluation of dry beans for resistance to ashy stem blight. J. of Agric. of the Univ. of Puerto Rico. 74:349-355.

Echávez-Badel, R. and J.S. Beaver. 1987. Resistance and susceptibility of beans (*Phaseolus vulgaris* L.) to ashy stem blight [*Macrophomina phaseolina* (Tassi) Goid. J. Agric. Univ. Puerto Rico 71:403-405.

Frahm, M.A., J. C. Rosas, N. Mayek-Pérez, E. López-Salinas, J.A. Acosta-Gallegos, and J. D. Kelly. 2004. Breeding beans for resistance to terminal drought in the lowland tropics. Euphytica 136: 223-232.

Jones, R.W., S. Canada, and H. Wang. 1998. Highly variable minichromosomes and highly conserved endoglucanase genes in the phytopathogenic fungus *Macrophomina phaseolina*. Can. J. Bot. 76: 694-698.

Mayek-Pérez, N., C. López-Castañeda, J.A. Acosta-Gallegos. 1997a. Efecto del déficit hídrico y altas temperaturas en el desarrollo y rendimiento de frijol inoculado con *Macrophomina phaseolina*. Revista Mexicana de Fitopatología 15: 111-125.

Mayek-Pérez, N., J.A. Acosta-Gallegos, C. López-Castañeda, E. López-Salinas, J. Cumpeán-Gutiérrez, and E. Acosta-Díaz. 1997b. Resistance to *Macrophomina phaseolina* in common beans Ander field conditions. Ann. Rep. Bean Improv. Coop. 40: 99-100.

Mayek, N., C. López, y J.A. Acosta. 1997c. Variación en características culturales in Vitro de aislamientos de *Macrophomina phaseolina* y su virulencia en frijol común. Agrociencia 31: 187-195.

Mayek-Pérez, N., C. Lopez-Castañeda, E. López-Salinas y J.A. Acosta-Gallegos. 2001a. Herencia de la resistencia genética a *Macrophomina phaseolina* (Tassi) Goid. en frijol. Agrociencia 35:637-648.

Mayek-Pérez, N., C. López-Castañeda, E. López-Salinas, J. Cumpián-Gutiérrez, J.A. Acosta-Gallegos. 2001b. Resistencia a *Macrophomina phaseolina* (Tassi) Goid. en frijol común en condiciones de campo en México. Agrociencia 35: 649-661.

Mayek-Pérez, N., C. López-Castañeda, M. González-Chavira, R. García-Espinosa, J. Acosta-Gallegos, O. Martínez de la Vega, and J. Simpson. 2001c. Variability of Mexican isolates of *Macrophomina phaseolina* based on pathogenesis and AFLP genotype. Physiol. Mol. Plant Pathol. 59: 257.264.

Mayek-Pérez, N., Padilla-Ramírez, J.S., Acosta-Gallegos, J.A. and C. López-Castañeda. 2002a. Leaf morphology and dry matter partitioning of bean in response to *Macrophomina phaseolina* and drought stress. Scientiae Naturae 4:33-44.

Mayek-Pérez, N., C. López-Castañeda, J.A. Acosta-Gallegos. 2002b. Reacción de germoplasma de *Phaseolus* sp. a *Macrophomina phaseolina*. Rev. Fitotec. Mex. 25: 35-42.

Mayek-Pérez, N., R. García-Espinosa, C. López-Castañeda, J. A. Acosta-Gallegos, and J. Simpson. 2002c. Water relations, histopathology and growth of common bean (*Phaseolus vulgaris* L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. Physiol. Mol. Plant Pathol. 60: 185-195.

Mihail, J.D., and S.J. Taylor. 1995. Interpreting variability among isolates of *Macrophomina phaseolina* in pathogenicity, pycnidium production, and chlorate utilization. Can. J. Bot. 73: 1596-1603.

Miklas, P.N., V. Stone, C.A. Urrea, E. Johnson and J.S. Beaver. 1998. Inheritance and QTL analysis of field resistance to ashy stem blight in common bean. Crop Sci. 38:916-921.

Olaya, G., G.S. Abawi, and N.F. Weeden. 1996. Inheritance of the resistance to *Macrophomina phaseolina* and identification of RAPD markers linked to the resistance genes in beans. Phytopathology 86: 674-679.

Pastor-Corrales, M.A. and G.S. Abawi. 1988. Reactions of selected bean accessions to infection by *Macrophomina phaseolina*. Plant Dis. 72: 39-41.

Reyes-Franco, M.C., S. Hernández-Delgado, M. Medina-Fernández, N. Mayek-Pérez. 2005. Genetic variability of *Macrophomina phaseolina*, the causal agent of charcoal rot in common beans. Ann. Rep. Bean Improv. Coop. 48: 112-113.

Reyes-Franco, M.C., R. Beas-Fernández, S. Hernández-Delgado, N. Mayek-Pérez. 2005. Pathogenicity of *Macrophomina phaseolina* isolates from México and other countries on common beans. Ann. Rep. Bean Improv. Coop. 48: 114-115.

Songa, W., J. Hillocks, A.W. Mwango'mbe, R. Buruchara, and W.K. Ronno. 1997. Screening common bean accessions for resistance to charcoal rot (*Macrophomina phaseolina*) in Eastern Kenya. Expl. Agric. 33: 459-468.

Su, G., S.O. Suh, R.W. Schneider, and J.S. Russin. 2001. Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*. Phytopathology 91:120-126.

Vandemark, G., O. Martínez, V. Pecina, and M.J. Alvarado. 2000. Assessment of genetic relationships among isolates of *Macrophomina phaseolina* using a simplified AFLP technique and two different methods of analysis. Mycologia 92: 656-664.