Anthracnose

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib. is a serious seed-borne fungal disease of common bean. Relatively cool and humid conditions favor disease development (Pastor-Corrales et al., 1995). Bean breeders must pyramid genes for resistance to anthracnose due to the variability of the pathogen (Balardin et al., 1997). Pastor-Corrales (1991) proposed a set of differential lines to be used to characterize anthracnose races (Table 1). A binary value is assigned to each differential so that unique number can be generated for every anthracnose race.

Table 1. Binary Code, Resistance Genes and Gene Pool Origin of Differential Cultivars used to characterize Races of *Colletotrichum lindemuthianum*

Differential Cultivars	**Seed Type**	**Binary Code**	**Resistance Gene**	**Gene Pool** [a]
Michelite	1,P	1	*Co-11*	MA
Michigan Dark Red Kidney	6,K	2	*Co-1*	A
Perry Marrow	1,M	4	$Co\text{-}1^3$	A
Cornell 49-242	9,P	8	*Co-2*	MA
Widusa	1,M	16	$Co\text{-}1^5$	A
Kaboon	1,M	32	$Co\text{-}1^2$	A
Mexico 222	2,M	64	*Co-3*	MA
PI 207262	3,P	128	$Co\text{-}4^3$ *Co-9*	MA
TO	4,M	256	*Co-4*	MA
TU	8,P	512	*Co-5*	MA
AB 136	6,P	1024	*Co-6*	MA
G2333	6,P	2048	$Co\text{-}4^2$ $Co\text{-}5^2$ *Co-7*	MA

[a] MA= Middle American; A= Andean ; Source: Balardin et al. (1997);Awale et al., (2007).

Balardin et al. (1997) isolated the fungus from diseased leaves. Small pieces of infected tissue were surface sterilized and incubated on petri dishes containing a modification of Mathur's medium. The media contained dextrose (8 g liter^{-1}), $MGSO_4.7\ H_2O$ (2.5 g liter^{--1}), neopeptone (2.4 g liter^{-1}), yeast extract (2.0 g liter^{-1}) and agar (16 g liter^{-1}). For medium-term storage Pastor-Corrales et al. (1995) placed conidia harvested from sporulating colonies in a sterile solution of 10% peptone and 20% sucrose. The spore suspension was impregnated on sterile pieces of filter paper that was desiccated for 8 days over sterilized silica gel.

Preparation of bean pod agar media

- 500g of green bean pods
- 16g of Agar
- 1L distilled water

Pod and media preparation

1) Cut off the two ends of the bean pod
2) Wash the pods and either cook or autoclave them 15-20min.
3) Prepare the water Agar (16g of agar in 1L of distilled water) and autoclave for 20min.
4) Pour 3mL of water Agar in each tube (clean tubes)
5) Place the bean pod upright in each tube
6) Place cotton ball and cap
7) Autoclave them for 20 min.
8) Wait until media solidifies (30 min)

Inoculum preparation

Since the fungus is already grown in Petri dish, but not sporulating on artificial media bean pod agar may provide nutrients needed for sporulation.

1) Using sterile needle cut small pieces of agar with the fungus from the corner of the plates and place agar plugs in the tubes with sterile bean pods
2) Place the tubes in the incubator 25°C
3) Monitor after 3-4 days or wait until the fungus fully grows
4) Replate the fungus into new tubes containing media with pod
5) The fungus will be ready to inoculate in two weeks

Inoculation procedure

1) Add 10 drop of Tween20 in 1L of distilled water
2) Take spatula and remove the pods from the tubes
3) Place them in a beaker and add 200ml of distilled water
4) Stir using magnetic stir plate until spore suspension is formed
5) Place cheesecloth on the top of the funnel
6) Place the funnel with cheesecloth on the flask and pour the spore suspension .
7) Count the spore suspension and adjust the concentration of spores upto $1.2X10^6$spores/ml
8) Inoculate your plants. Re-isolate the fungus from inoculated

Disease screening in the greenhouse can be conducted using an aqueous suspension of conidia cultured on an artificial medium (Schwartz et al., 1982). *C. lindemuthianum* can be grown on a perlite-cornmeal V-8 juice agar medium following the procedures described by Miles and Wilcoxson (1984). Inoculum for greenhouse screening can be prepared by suspending in water conidia collected from *C. lindemuthianum* cultures. A hemocytometer can be used to adjust the concentration of the inoculum (Inglis et al., 1988). CIAT researchers used a conidial concentration of 1.2×10^6 for greenhouse inoculations (Pastor-Corrales et al., 1995). Seven-day-old seedlings were inoculated and kept seven days in a chamber at 21±1 C at 95-100% relative humidity. Balardin and Kelly (1998) inoculated 7-10 day-old seedlings that had developed fully-expanded primary leaves. Inoculum was applied to the seedlings until there was runoff on the stem and the surface of the unifoliolate leaves. Plants were placed in a chamber with high humidity (> 95%) for 48 h at 22-25º C and evaluated for disease reaction at 7 d after inoculation. Six plants of each line were evaluated using a 1-9 scale to calculate a mean disease rating (Table 2).

Table 2. Evaluation scales for greenhouse screening for anthracnose reaction.

1-9 scale	Symptoms on 7-day-old seedlings
1	No symptoms (Resistant)
3	Very small lesions, mostly on primary leaves (Resistant)
5	Numerous enlarged lesions or sunken cancers on the lower sides of leaves or hypocotyls (Susceptible)
7	
9	

Source: Balardin et al. (1997)

Field screening for anthracnose resistance with a conidial suspension is more difficult due to the need to prepare large volumes of inoculum (Inglis et al., 1988). Bean lines screened in CIAT for resistance to anthracnose were inoculated four times with an aqueous suspension of conidia (Pastor-Corrales et al. 1995). The inoculations were initiated 3-4 weeks after planted and repeated at 10-day intervals. Because the conidial suspensions do not store well, it must be prepared immediately before inoculation. Inglis et al. (1988) noted that the use of dry inoculum is an efficient way to disseminate the anthracnose pathogen in field trials. In addition, the dry inoculum is simple to prepare and easy to store and transport. The inoculum can be prepared from diseased bean leaves collected from the field or from diseased leaves from greenhouse-inoculated plants. Inglis et al. (1988) dried diseased leaves without heat using an electric fan. The dried leaves were pulverized in a Wiley Mill and sifted in an 18-mesh sieve to < 1 mm. The inoculum can be stored in double plastic bags at 5-10^{o} C. The concentration of conidia per gram of dried inoculum can be estimated by diluting the inoculum 1:100 with water and counting the number of conidia per mililiter with a hemacytometer. Inglis et al. (1988) found the final concentration of field-collected dry leaf inoculum to be 1.2 x 10^4 conidia per gram. The field plots were inoculated 3-4 weeks after planting when the plants had two sets of fully-expanded trifoliolate leaves. The plots were inoculated early in the evening after overhead irrigation to provide moist conditions during the period of infection. Leaves of the plants receiving the dry inoculum were first wetted with an aqueous solution containing a sticking agent (12% potassium resinate and 2.5% potassium oleate a.i.) at a concentration of 250 mg/ml. Each 5-m row received 0.5 L of the solution with the sticking agent. The dry inoculum was shaken over the leaves so that the distribution would be as uniform as possible. Beginning at two weeks after inoculation and at weekly intervals, full-expanded leaflets from six plants were evaluated using a 1-5 scale to calculate a mean disease rating (Table 3).

Table 3. Evaluation scales for field screening for anthracnose reaction.

1-5 scale	1-9 scale	Percent of pods with lesions
1	1	1-10
2	3	11-25
3	5	26-50
4	7	> 50
5	9	Defoliation

Source: Inglis et al. (1988)

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

Thirteen specific genes for anthracnose resistance have been identified (Table 4). Conventional methods and marker-assisted selection has been used to develop bean cultivars with resistance to anthracnose (Table 5). The combination of resistance genes of Andean and Mesoamerican origin should provide more durable resistance (Balardin and Kelly, 1998; Pastor-Corrales et al., 1995).

Table 4. New and Original Anthracnose resistance gene symbols, genetic sources, gene pools, linked markers and map location for major genes conditioning resistance to anthracnose in common bean.

Gene Symbols		**Genetic sources**	**Gene pool**	**Linked markers**	**Map location**
New	**Original**				
Co-1	*A*	MDRK	Andean	$OF10_{530}$	B1
Co-1[2]		Kaboon		SE_{ACT}/M_{CCA}	
Co-1[3]		Perry Marrow			
Co-1[4]		AND 277			
Co-1[5]		Widusa		$OA18_{1500}$	
Co-2	*Are*	Cornell 49242	MA	$OQ4_{1440}$	B11
				$OH20_{450}$	
				$B355_{1000}$	
Co-3	*Mexique 1*	Mexico 222	MA	NA	B4
Co-3[2]		Mexico 227			
Co-3[3]	*Co-9*	BAT 93			
Co-4	*Mexique 2*	TO	MA	SAS13, SH18	B8
Co-4[2]		SEL1308		SBB14, OC8	
Co-4[3]		PI 207262[y]		OY20	
Co-5	*Mexique 3*	TU	MA	$OAB3_{450}$	B7
Co-5[2]		SEL1360		SAB3	
Co-6	Q	AB 136	MA	$OAH1_{780}$	B7
				$OAK20_{890}$	
Co-7	NA[z]	HI, MSU 7-1	MA	NA	NA
		G 2333[y]			
co-8	NA	AB 136	MA	OPAZ20	NA
Co-9	NA	BAT 93	MA	SB12	B4
Co-10	NA	Ouro Negro	MA	F10	B4
Co-11	NA	Michelite	MA	NA	NA
Co-12	NA	Jalo Vermelho	Andean	NA	NA
Co-13	NA	Jalo Listras Pretas	Andean	OPV20 700	B3

[z] NA None available; MDRK – Michigan Dark Red Kidney; MA – Middle American;
[y] PI 207262 possesses 2-genes; G 2333 possesses 3-genes
Source: Kelly and Vallejo, 2004; Gonçalves-Vidigal and Kelly. 2006; Gonçalves-Vidigal et al., 2007, 2008, 2009; Méndez-Vigo et al., 2005; Mahuku and Riascos, 2004; Rodríguez-Suárez et al., 2007; Souza et al., 2007; Vallejo and Kelly, 2008, 2009.

Resistance to anthracnose in common bean is conditioned primarily by eleven major independent genes, *Co-1* to *Co-13* as the *Co-9/ Co-3*[3] and *Co-7/ Co-3* genes are allelic (Méndez-Vigo et al., 2005; Sousa et al., 2009; Table 4). With the exception of the recessive *co-8* gene, all other genes are dominant genes and multiple alleles exist at the *Co-1, Co-3, Co-4* and *Co-5* loci. A reverse of dominance at the *Co-1* locus suggests that an order of dominance exists among individual alleles at this locus. The nine resistance genes *Co-2* to *Co-11* are Middle American in origin and *Co-1, Co-12* and *Co-13* loci come from the Andean gene pool. Eight resistance loci have been mapped to the integrated bean linkage map and *Co-1* resides on linkage group B1; *Co-2* on B11, *Co-3* on B4; *Co-4* on B8; *Co-6* on B7; *Co-13* on B3; and *Co-9/ Co-3*[3] and *Co-10* are located on B4 but do not appear to be linked. The three independent genes in G2333 are allelic to previously mapped genes at the *Co-3, Co-4* and *Co-5* loci. *Co-7* appears to be another allele at *Co-3* locus (Sousa et al., 2009); *Co-4*[2] is the most broadly resistance allele at the *Co-4* locus (Young et al., 1998); and *Co-5*[2] is a second allele at *Co-5* locus that has a more restricted resistance pattern than the original *Co-5* allele in TU (Vallejo and Kelly, 2009).

Three *Co*-genes map to linkage groups B1, B4 and B11 where clusters with genes for rust resistance are located. In addition, there is co-localization with major resistance genes and QTL that condition partial resistance to anthracnose. Other QTL for resistance may provide putative map locations for the major resistance loci still to be mapped. Molecular markers linked to the majority of major *Co*-genes have been reported and these provide the opportunity to enhance disease resistance through marker-assisted selection and gene pyramiding. Ten *Co*-genes are represented in the anthracnose differential cultivars, but are present as part of a multi-allelic series or in combination with other *Co*-genes, making the characterization of more complex races difficult. Although the *Co*-genes behave as major Mendelian factors, they most likely exist as resistance gene clusters as has been demonstrated on the molecular level at the *Co-2* locus. Conventional methods and marker-assisted selection has been used to develop bean cultivars with resistance to anthracnose (Tables 4, 5). The combination of resistance genes of Andean and Mesoamerican origin should provide more durable resistance (Balardin and Kelly, 1998; Kelly and Vallejo, 2004; Pastor-Corrales et al., 1995) and the most effective pyramid combines the *Co-1*[2] *and Co-4*[2] alleles (Awale et al., 2007). Recently race 105 was detected in Manitoba (Conner, R.L. pc) that overcomes the *Co-1*[2] allele in Kaboon.

Table 5. Sources of resistance to anthracnose in different bean seed classes.

Name or number	Seed color / type	Resistance genes	Reference
Jaguar Phantom	9 / Black	*Co-1*,*Co-2*	Kelly et al. (2001) Kelly et al. (2000)
Newport	1 / White	*Co-1*,*Co-2*	Kelly et al. (1995)
Bayacora, Mestizo USPT-ANT-1	2M / Pinto	*Co-4*^{2}	Acosta-Gallegos et al. (2001b) Acosta-Gallegos et al. (2001a) Miklas et al. (2003)
	1 / Great Northern		
	7 / Red Mexican		
Catrachita	6 / Small red	*Co-6*	Young and Kelly (1996)
	5 / Pink		
	2R / Cranberry		
PC 50	6M / Red mottled		Balardin and Kelly (1998)
Red Hawk	6K / Dark red kidney	*Co-1*, *Co-2*	Kelly et al. (1998)
	1 / Snap		

Photograph provided by Emmalea Ernest
University of Delaware

Twelve 12 Differential Bean Varieties listed in Table 1.
Left to Right
Top Row: Mexico 222, PI 207262, TO, TU, AB136, G2333.
Bottom Row: Michelite, MDRK, Perry Marrow, Cornell 49242, Widusa, Kaboon.

[Updated; J.D. Kelly, 01/2010]

REFERENCES

Acosta-Gallegos, J.A., F.J. Ibarra-Pérez, R. Rosales-Serna, B. Cázares-Enríquez, P. Fernández-Hernandez, A. Castillo-Rosales and J.D. Kelly. 2001a. Registration of Bayacora pinto bean. Crop Sci. 41:1645-1646.

Acosta-Gallegos, J.A., F.J. Ibarra-Pérez, R. Rosales-Serna, P. Fernández-Hernandez, A. Castillo-Rosales and J.D. Kelly. 2001b. Registration of Mestizo pinto bean. Crop Sci. 41:1650-1651.

Awale, H., E. Falconi, J.C. Villatoro and J. D. Kelly. 2007. Control and characterization of *Colletotrichum lindemuthianum* Isolates from Ecuador and Guatemala. Annu. Rept. Bean Improv. Coop. 50: 85:86.

Balardin, R.S. and J.D. Kelly. 1998. Interaction among races of *Colletotrichum lindemuthianum* and diversity in Phaseolus vulgaris. J. Amer. Soc. Hort. Sci. 123:1038-1047.

Balardin, R.S., A.M. Jarosz and J.D. Kelly. 1997. Virulence and molecular diversity in *Colletotrichum linemuthianum* from South, Central and North America. Phytopathology 87:1184-1191.

Bassett, M.J. 2004. List of genes – *Phaseolus vulgaris* L. Annu. Rept. Bean Improv. Coop. 47:1-24.

Campa, A., C. Rodríguez-Suárez, A. Pañeda, R. Giradles, & J. J. Ferreira, 2005. The bean anthracnose resístance gene Co-5 is located in linkage group B7. Annu. Rept. Bean Improv. Coop. 48: 68-69.

Gonçalves-Vidigal, M.C., and J.D. Kelly. 2006. Inheritance of anthracnose resistance in the common bean cultivar Widusa. Euphytica151:411-419.

Gonçalves-Vidigal, M.C., C.R. Silva, P.S, Vidigal Filho, A. Gonela, and M.V. Kvitschal. 2007. Allelic relationships of anthracnose (*Colletotrichum lindemuthianum*) resistance in the common bean *(Phaseolus vulgaris* L.) cultivar Michelite and the proposal of a new anthracnose resistance gene, *Co-11*. Gen. Mol. Biol. 30:589-593.

Gonçalves-Vidigal, M.C., G.F. Lacanallo, P.S. Vidigal Filho. 2008. A new Andean gene conferring resistance to anthracnose in common bean (*Phaseolus vulgaris* L.) cultivar Jalo Vermelho. Plant Breeding 127:592-596.

Gonçalves-Vidigal, M.C., P.S. Vidigal Filho, A.F. Medeiros, and M.A. Pastor-Corrales. 2009. Common Bean Landrace Jalo Listras Pretas is the Source of a New Andean Anthracnose Resistance Gene. Crop Sci. 49:133-138.

Inglis, D.A., D.J. Hagedorn and R.E. Rand. 1988. Use of dry inoculum to evaluate beans for resistance to anthracnose and angular leaf spot. Plant Disease 72:771-774.

Kelly, J.D. and V.A. Vallejo, 2004. A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. HortScience 39:1196-1207.

Kelly, J.D., G.L. Hosfield, G.V. Varner, M.A. Uebersax and J. Taylor. 2001. Registration of Jaguar black bean. Crop Sci. 41:1649-1650.

Kelly, J.D., G.L. Hosfield, G.V. Varner, M.A. Uebersax and J. Taylor. 2000. Registration of Phantom black bean. Crop Sci. 40:572.

Kelly, J.D., G.L. Hosfield, G.V. Varner, M.A. Uebersax, R.A. Long and J. Taylor. 1998. Registration of Red Hawk dark red kidney bean. Crop Sci. 38:280-281.

Kelly, J.D., G.L. Hosfield, G.V. Varner, M.A. Uebersax, L.K. Afanador and J. Taylor. 1995. Registration of Newport navy bean. Crop Sci. 35:1710-1711.

Mahuku, G.S., and J.J. Riascos. 2004. Virulence and molecular diversity within *Colletotrichum lindemuthianum* isolates from Andean and Mesoamerican bean varieties and regions. Eur. J. Plant Path. 110:253-263.

Melotto, M., R.S. Balardin and J.D. Kelly. 2000. Host-pathogen interaction and variability of Colletotrichum lindemuthianum. In: Colletotrichum Host specificity, pathology, and host-pathogen interaction. (D. Prusky, S. Freeman, and M.B. Dickman, Eds), pp 346-361. APS press St. Paul, MN.

Méndez-Vigo, B., C. Rodríguez-Suárez, A. Pañeda, J. J. Ferreira and R. Giraldez, 2005. Molecular markers and allelic relationships of anthracnose resistance gene cluster B4 in common bean. Euphytica 141:237-245.

Miklas, P.N., J.D. Kelly and S.P. Singh. 2003. Registration of anthracnose resistant pinto bean germplasm line USPT-ANT-1. Crop Sci.43:1889-1990.

Miles, M.R. and R.D. Wilcoxson. 1984. Production of fungal inoculum using a substrate of perlite, cornmeal, and potato-dextrose agar. Plant Dis. 68:310.

Rodríguez-Suárez C., B. Méndez-Vigo, A. Pañeda, J.J. Ferreira, and R. Giraldez. 2007. A genetic linkage map of *Phaseolus vulgaris* L. and localization of genes for specific resistance to six races of anthracnose (*Colletotrichum lindemuthianum*). Theor. Appl. Genet. 114: 713-722

Pathania, A., P.N. Sharma, O.P. Sharma, R.K. Chahota, B. Ahmad, and P. Sharma. 2006. Evaluation of resistance sources and inheritance of resistance in kidney bean to Indian virulences of *Colletotrichum lindemuthianum*. Euphytica 149:97-103.

Pastor-Corrales, M.A. 1991. Estandarización de variedades diferenciales y de designación de razas de Colletotrichum lindemuthianum. Phytopathology 81:694 (abstract).

Pastor-Corrales, M.A., M.A. Otoya, A. Molina and S.P. Singh. 1995. Resistance to *Colletotrichum lindemuthianum* isolates from Middle America and Andean South America in different common bean races. Plant Disease 79:63-67.

Schwartz, H.F., M.A. Pastor Corrales and S.P. Singh. 1982. New sources of resistance to anthracnose and angular leaf spot of beans (*Phaseolus vulgaris* L.). Euphytica 31:741-754.

Sousa, L.L., M.C. Gonçalves-Vidigal, P.S. Vidigal Filho, A.S.Cruz, and T.R. Costa. 2009. Allelic relationships of anthracnose resistance in MSU 7-1. Line. Annu. Rep. Bean Improv. Coop. 52:48-49.

Vallejo, V.A., and J. D. Kelly. 2008. Molecular tagging and genetic characterization of alleles at the *Co-1* anthracnose resistance locus in common bean. ICFAI Univ. J. Genetics & Evolution 1:7-20.

Vallejo, V., and J. D. Kelly. 2009. New insights into the anthracnose resistance of common bean landrace G 2333. The Open Horticulture J. 2:29-33.

Young, R.A., M. Melotto, R.O. Nodari, and J.D. Kelly. 1998. Marker assisted dissection of the oligogenic anthracnose resistance in common bean cultivar, G 2333. Theor. Appl. Genet. 96:87-94.