

## 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 <sup>a</sup>
Michelite	1,P	1	Co-11	MA
Michigan Dark Red Kidney	6,K	2	Co-1	A
Perry Marrow	1,M	4	Co-1 <sup>3</sup>	A
Cornell 49-242	9,P	8	Co-2	MA
Widusa	1,M	16	Co-1 <sup>5</sup>	A
Kaboon	1,M	32	Co-1 <sup>2</sup>	A
Mexico 222	2,M	64	Co-3	MA
PI 207262	3,P	128	Co-4 <sup>3</sup> 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-4 <sup>2</sup> Co-5 <sup>2</sup> Co-7	MA

<sup>a</sup> 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<sup>-1</sup>), MGSO<sub>4</sub>.7 H<sub>2</sub>O (2.5 g liter<sup>-1</sup>), neopeptone (2.4 g liter<sup>-1</sup>), yeast extract (2.0 g liter<sup>-1</sup>) and agar (16 g liter<sup>-1</sup>). 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  $1.2 \times 10^6$  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  $1.2 \times 10^6$  for greenhouse inoculations (Pastor-Corrales et al., 1995). Seven-day-old seedlings were inoculated and kept seven days in a chamber at  $21 \pm 1$  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 22-25° 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° 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 milliliter with a hemacytometer. Inglis et al. (1988) found the final concentration of field-collected dry leaf inoculum to be  $1.2 \times 10^4$  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.

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

Source: Inglis et al. (1988)



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Eleven 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.

<b>Gene Symbols</b>		<b>Genetic sources</b>	<b>Gene pool</b>	<b>Linked markers</b>	<b>Map location</b>
<b>New</b>	<b>Original</b>				
<i>Co-1</i>	A	MDRK	Andean	OF10 <sub>530</sub>	B1
<i>Co-1</i> <sup>2</sup>		Kaboon		SE <sub>ACT</sub> /M <sub>CCA</sub>	
<i>Co-1</i> <sup>3</sup>		Perry Marrow			
<i>Co-1</i> <sup>4</sup>		AND 277			
<i>Co-1</i> <sup>5</sup>		Widusa		OA18 <sub>1500</sub>	
<i>Co-2</i>	<i>Are</i>	Cornell 49242	MA	OQ4 <sub>1440</sub> OH20 <sub>450</sub> B355 <sub>1000</sub>	B11
<i>Co-3</i>	<i>Mexique 1</i>	Mexico 222	MA	NA	B4
<i>Co-3</i> <sup>2</sup>		Mexico 227			
<i>Co-4</i>	<i>Mexique 2</i>	TO	MA	SAS13, SH18	B8
<i>Co-4</i> <sup>2</sup>		SEL1308		SBB14, OC8	
<i>Co-4</i> <sup>3</sup>		PI 207262 <sup>y</sup>		OY20	
<i>Co-5</i>	<i>Mexique 3</i>	TU	MA	OAB3 <sub>450</sub>	B7
<i>Co-5</i> <sup>2</sup>		SEL1360		SAB3	
<i>Co-6</i>	Q	AB 136	MA	OAH1 <sub>780</sub> OAK20 <sub>890</sub>	B7
<i>Co-7</i>	NA <sup>z</sup>	HI G 2333 <sup>y</sup>	MA	NA	NA
<i>co-8</i>	NA	AB 136	MA	OPAZ20	NA
<i>Co-9</i>	NA	BAT 93	MA	SB12	B4
<i>Co-10</i>	NA	Ouro Negro	MA	F10	B4
<i>Co-11</i>	NA	Michelite	MA	NA	NA

<sup>z</sup> NA None available; MDRK – Michigan Dark Red Kidney; MA – Middle American;

<sup>y</sup> PI 207262 possesses 2-genes; G 2333 possesses 3-gene

Source: Kelly and Vallejo, 2004; Gonçalves-Vidigal and Kelly. 2006; Méndez-Vigo et al., 2005; Mahuku and Riascos, 2004; Rodríguez-Suárez et al., 2007.

Resistance to anthracnose in common bean is conditioned primarily by ten major independent genes, *Co-1* to *Co-11* as the *Co-3/Co-9* genes are allelic (Méndez-Vigo et al., 2005; Table 4). With the exception of the recessive *co-8* gene, all other nine 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-10* are Middle American in origin and *Co-1* is the only locus from the Andean gene pool. Seven 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; and *Co-9* and *Co-10* are located on B4 but do not appear to be linked. 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. The 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<sup>2</sup>* and *Co-4<sup>2</sup>* alleles (Awale et al., 2007).

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	<i>Co-1, Co-2</i>	Kelly et al. (2001) Kelly et al. (2000)
Newport	1 / White	<i>Co-1, Co-2</i>	Kelly et al. (1995)
Bayacora, Mestizo USPT-ANT-1	2M / Pinto	<i>Co-4<sup>2</sup></i>	Acosta-Gallegos et al. (2001b) Acosta-Gallegos et al. (2001a) Miklas et al. (2003)
	1 / Great Northern		
	7 / Red Mexican		
Catrachita	6 / Small red	<i>Co-6</i>	Young and Kelly (1996)
	5 / Pink		
	2R / Cranberry		
PC 50	6M / Red mottled		Balardin and Kelly (1998)
Red Hawk	6K / Dark red kidney	<i>Co-1, Co-2</i>	Kelly et al. (1998)
	1 / Snap		

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Photograph provided by Emmalea Ernest  
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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, 10/2007]