Common Bacterial Blight

Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* [synonymous with *Xanthomonas campestris* pv. *phaseoli* (Smith) Dowson] is a serious seed-borne disease of beans in both temperate and tropical production zones. Management of common blight includes the use of disease-free seed, crop rotation and resistant varieties (Saettler, 1989).

Figure 1. Typical symptoms of CBB infection of dry bean leaves in the field. Note lemon yellow border encompassing necrotic tissue with greasy water-soaked appearance. Also note concentrated infection occurring at the leaf margins (Photo by P. Miklas).

Screening and inoculum preparation

Screening for resistance to CBB is conducted in the greenhouse using artificial techniques (multiple needle, razor blade) and in the field using natural epidemics or high pressure delivery of inocula produced on petri-plates or collected from infected leaves.

[updated by P Miklas, April 2011]

Zapata et al. (1985) prepared inoculum for greenhouse screening using cell suspensions of *Xanthomonas campestris* cultures grown at 48 h at 26° C. All concentrations were adjusted to 10^9 cfu/ml using standard turbidometric and dilution plating techniques. Inoculum for field inoculation was prepared by collecting leaves from plants at the R6-R7 stage of development with typical common bacterial blight symptoms. The infected leaves were macerated in a high-speed blender with 10 ml of 0.01 M phosphate buffer (pH 7) per 1 g of infected tissue. In the greenhouse, trifoliolate leaves of plants at initiation of flowering (R1 stage of development) were inoculated by placing the bean leaf over a plastic sponge saturated with inoculum and perforating it with a multiple-needled florist frog (2 cm square metal base supporting rows of needles 3 mm apart and 12 mm in length). After inoculation, high humidity was maintained by mist irrigation every few minutes. In the field, a pneumatic sandblasting pistol connected by an air hose to an air compressor was used to inoculate bean plants at the R1 stage of development. The bacterial suspension from the field-infected leaves and 28.4 g of fine silica sand was placed in the reservoir of the pistol. The mixture was sprayed twice over one-half of each row at 345 kPa (50 psi) with the pistol held 30-60 cm from the plant. This method differs from the technique reported by Webster et al. (1983b) who sprayed a suspension of 5 x 10^7 cfu/ml onto plants using a sprayer nozzle at 3 kg/cm^2 at weekly intervals beginning 3 weeks after planting.

Figure 2. Field CBB infection under natural epidemic (Photo by P. Miklas).

Gilbertson et al. (1988) prepared dry leaf inoculum from diseased bean leaves collected 14 days after greenhouse plants had been razor-blade-inoculated. Diseased leaves were dried at 28-30º C for 24-48 h, ground thoroughly in a Waring blender, sifted through a 32 mesh sieve (< 500 µm) and stored in plastic bags at 4 or -20º C. Pathogenic strains of *Xanthomonas campestris* pv. *phaseoli* were recovered from dry-leaf inoculum stored up to 6 years. Dry leaf inoculum can also be prepared using leaves collected from field from plants expressing common bacterial blight symptoms (Saettler, 1989). To prepare the suspension, 4 g of dry leaf inoculum was mixed with 1.9 L of water plus three drops of sticker

Park and Dhanvantari (1987) prepared inoculum of four local strains, two of *X. campestris* pv *phaseoli* var. fuscans no. 12 and no. 118 and two of non-fuscans no. 18 and no. 98 that were grown on yeast salts-agar (Dye 1968) for 48 h at 25° C. A sterile distilled water suspension of bacterial growth was made, adjusting the concentration to 15 Klett units against a red filter in a Klett-Summerson colorimeter. Suspensions of the four strains were mixed in equal volume and 7 L were added to 190 L of water in a spray tank. The inoculum, containing 10^6 colony-forming units mL^{-1}, was applied at about 1333 kPa to ensure visible water-soaking injury using a spray boom. Bean plants were inoculated at about 3 wk old in a field screening nursery. Assessment for blight was made twice, 3 and 6 wk after inoculation. Leaf blight was rated visually as: 1 = no visible symptoms, 2 = small flecks or lesions on less than 5 % of leaf surface, 3 = small lesions covering 5-20 % of leaf surface, 4 = lesions of various sizes covering 20-40% of leaf surface with some chlorosis, 5 = lesions coalescing to cover 40-60 % of leaf surface and surrounded by orange-yellow chlorosis, 6 = large necrotic lesions with spreading chlorosis covering 60-80 % of leaf surface, and 7 = lesions and chlorosis spreading, leaf wilting and defoliation. Leaf blight was scored on 10 single plants per row. Pod blight was rated as: 1 = no visible symptoms, 2 = a few small lesions, 3 = lesions of moderate size, 4 = large lesions, 5 = large coalescing lesions. Percent pod infection was recorded as number of infected pods out of total number of pods from plants per row. We also inoculated bean plants by the multiple needle method (Andrus, 1948) under controlled environmental condition in growth rooms/greenhouses.

Zapata et al. (1985) prepared inoculum for the evaluation of pods using cell suspensions of *Xanthomonas campestris* cultures grown at 48 h at 26° C. All concentrations were adjusted to 10^9 cfu/ml using standard turbidometric and dilution plating techniques. Pods in mid-pod-fill were harvested from plants, disinfected in a dilute sodium hypochlorite solution (0.02% Cl) and washed three times in tap water. The pods were placed on moistened paper towels in trays. Two needles mounted 2 mm apart were used to make 4-5 mm long scratches through the epidermis of the pods. A 0.01 mm drop of inoculum was deposited over the distal scratch marks. The pods were incubated in an environmental chamber at 27º C with fluorescent lighting (3,220 lux) for 12 h. Readings were taken daily. A 1-5 scale was used to evaluate the pods, where 1 = no water soaking, 2 = 1mm wide water soaked area, 3 = 2-3 mm wide water soaked area,

4 = water soaked region coalescing over scratches and 5 = water soaked area reached the pod suture. Ariyarathne et al (1998) observed similar symptoms for detached and attached pods inoculated with *Xcp* and suggested using the former to reduce environmental variance.

Figure 3. Pods showing CBB infection (Photo from M. Silbernagel collection).

Aggour et al. (1988a) evaluated pod wall and pedicel inoculation techniques to screen beans for resistance to common bacterial blight. The edge of a razor blade that had been immersed in a 48-hour culture of *Xcp* was used to inoculate bean pods (R7-R8). The pods were evaluated 15 days after inoculation using a 1-9 scale, where 1 = no visible lesion, 2 = < 0.5 - < 1.0 mm, 3 = 1.0 mm, 4 = 2 mm, 5 = 3 mm, 6 = 4 mm, 7 = 5 mm, 8 = 6 mm and 9 = ≥ 7 mm sized lesions. The method was successful in detecting a differential pod reaction on bean lines. The pedicels of flower buds (24 to 36 hours before anthesis) and small pods (2-3 days old) were injected with about 0.01 ml of *Xcp* inoculum (10^8 cfu in a PO_4 buffer, pH 7.1) using a syringe with a 30 gage x 1.77 cm needle. When the pedicel was too thin, a surface scratch was made on the pedicel and a drop of inoculum was placed over the scratch. The technique was found to be useful to select for pedicel resistance of *Xcp*.

Aggour et al. (1988b) described different seed inoculation methods to select beans for resistance to common bacterial blight. A concentration of 10^6 cfu in a PO_4 buffer (pH 7.1) was used for all inoculation methods. Soaking dry seed in a bacterial cell suspension for 6 hours to inoculate the embryo produced the greatest number of seedlings with visible symptoms. There was good agreement between results of the seed inoculation methods and leaf inoculation of seedlings using the multi-needle method.

Breeding

Varela et al. (1996) evaluated two common bean populations for CBB resistance in the field in the F_3 and F_4 generations and in the greenhouse in the F_5 generation. A randomized complete block design with three replications was used. Experimental units were 1 m row lengths in the field and single pots in the greenhouse. The lines were inoculated in the field at the R6 stage of development with a backpack sprayer using a bacterial concentration of 3×10^7 cfu/ml (Schuster and Coyne, 1981). In the greenhouse, the lines were inoculated at 14 days after planting using the multiple needle technique. The lines were evaluated 14 days after inoculation for CBB reaction using the CIAT 1-9 scale and percentage of leaf area affected. Selection in the F_4 and F_5 generations was more effective because of higher heritabilities. CBB evaluations using the CIAT 1-9 scale were better than estimates of percentage of leaf area infected due to higher heritabilities and greater precision to detect differences among lines.

Figure 4. Leaves inoculated by the multiple needle method exhibiting disease score ratings of 1 (far left), 2 (second from left), 3 (third from left), 5 (third from right), 8 (second from right) and 9 (far right) (Photo by P. Miklas).

Singh and Muñoz (1999) developed dry beans at CIAT in Colombia with high levels of resistance to common bacterial blight by field screening advanced generation ($\geq F_5$) lines in unreplicated single row plots. Susceptible and resistant checks having known CBB reactions were planted throughout the nursery. Beginning about 3 weeks after planting, the rows were inoculated two to four times at 7-10 day intervals. The leaves in the rows were inoculated with a backpack sprayer using a bacterial concentration between 10^7 and 10^8 cfu/mL. The most CBB resistant lines were subsequently evaluated in replicated trials. Three leaflets of the first or second trifoliolate leaf of the resistant lines were inoculated with twin surgical blades about 3 weeks after planting. The lines were also inoculated 3-5 times, at weekly intervals, by spraying the canopy. At mid-pod-fill the pods were inoculated with multiple needles using a florist frog. The trifoliolate leaves were evaluated 7 to 15 days after inoculation and the pods were evaluated about 1 week after inoculation.

Singh and Muñoz (1999) noted several problems related to breeding for CBB resistance. Single plant selection under high disease pressure is recommended to maintain high levels of CBB resistance. Differential CBB reaction of leaves and pods requires that both plant organs must be screened for resistance. Because resistance to specific strains of *Xcp* has been observed, lines should be inoculated with strains from the target region. Finally, plants should be evaluated for CBB reaction at the same stage of development to avoid selecting plants having later maturity.

Michaels et al. (2005) developed CBB resistant navy bean cv. OAC Rex, tested as OAC 95-4, which was derived from the cross HR20-728/MBE 7 made in 1988. MBE 7 was a selection from the cross ICA Pijao/PI 440795//Ex Rico 23 and was used to provide resistance to common bacterial blight, transferred through an interspecific cross, and subsequent embryo rescue, from the *Phaseolus acutifolius* A. Gray line PI 440795 (Parker, 1985). F_1 plants were grown in the field in 1988 at the University of Guelph Elora Research Station (ERS), Elora, Ontario. F_6 plants were screened in an indoor growth room in the winter of 1991 for CBB resistance using multiple-pin techniques (Andrus, 1948). Resistant selections were grown in the field at ERS in 1991 and 1992 as F_6 and F_7 generations, respectively and assessed for CBB resistance using the dry leaf inoculum method (Gilbertson et al., 1988). OAC 95-4 was tested in Ontario coop cultivar registration and performance trials during 1995-1999. OAC Rex was registered (registration no. 5491) on May 24, 2002 by Variety Registration Office, Canadian Food Inspection Agency, AAFC.

Breeding and Marker-Assisted Selection

Miklas et al. (2005) and Mutlu et al. (2005a and b) used field screening and multiple needle inoculations in the greenhouse, combined with MAS for the SCAR markers SAP6 and SU91, to develop CBB resistant dark red kidney (USDK-CBB-15), white kidney (USWK-CBB-17), cranberry (USCR-CBB-20 and pinto (ABCP-8) germplasm lines (Table 1).

Resistant lines derived from the cross Tio Canela 75/VAX 6 were selected in Honduras for resistance to virulent isolates of Xcp using the multiple needle inoculation technique and marker-assisted selection (MAS) for the presence of the SCAR markers SAP6 and SU91. (Beaver and Rosas, unpublished).

Park and Dhanvantari (1993) developed common bean germplasm line HR45 (Reg. no. GP-114, PI 570661) at Agriculture and Agri-Food Canada Greenhouse & Processing Crops Research Centre, Harrow, ON, and released in 1993. It was selected for small white navy bean type with improved resistance to common bacterial blight incited by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye and for upright plant type. HR45 was derived from the cross of HR13-621*2//XAN159/HR13-621, of which the original single cross between XAN159 and HR13-621 was made in 1986.

Table 1. Sources of resistance to common bacterial blight in different seed classes.

Name or number	Seed color / type	QTL marker & Resistance sources	Reference
VAX 5	9 / Black	SAP6, SU91	Singh et al. (1999)
ICB-3		SAP6, P. coccineus	Miklas et al. (1999)
USBK-CBB-5		SAP6	Miklas et al. (2001a)
XAN 112		SAP6, PI207262	CIAT
ICB-8	1 / White	SAP6, P. coccineus	Miklas et al. (1999)
USNA-CBB-1 to 4		SAP6	Miklas et al. (2001a)
Wilk-2		SAP6, SU91, & BC420	Cornell
CBB-Teebus		SU91, BC420	Fourie and Herselman (2002)
HR-45		BC420	Park & Dhanvantari (1993)
OAC Rex		BC73	Michaels et al. (2005?)
USPT-CBB-1 to 3	2M / Pinto	SAP6, P. coccineus	Miklas et al. (2001b)
Chase		SAP6	Coyne et al.(1994)
ABCP-8		SAP6, SU91	Mutlu et al (2005)
USGN-CBB-4	1 / Great Northern	SAP6	Miklas et al. (2001b)
Weihing			Coyne et al. (2000)
BelNeb-RR-1		SAP6	Stavely et al. (1989)
ABC Weihing		SU91	Mutlu et al. (2008)
VAX 6	7 / Purple	SAP6, SU91	Singh and Muñoz (1999)
VAX 3	6 / Small red	SAP6, SU91	Singh et al. (2001)
ICB-6		SAP6, P. coccineus	Miklas et al. (1999)
XAN 309		SAP6, SU91	CIAT
	5 / Pink		
USCR-CBB-12, 13	2R / Cranberry	SAP6	Miklas et al. (2001c)
USCR-CBB-20		SAP6, SU91	Miklas et al. (2011)
Pomjor 17	6M / Red mottled		Beaver et al. (1992)
USWK-CBB-17	1K/White kidney	SAP6, SU91	Miklas et al. (2006b)
PR9443-4	5K / Light red kidney		Beaver et al. (1999c)
USLK-CBB-9 & 10			Miklas et al. (2001c)
USDK-CBB-11	6K / Dark red kidney	SAP6	
Montcalm			MSU (Adams, Saettler)
USDK-CBB-15		SAP6, SU91	Miklas et al. (2006a)
	1 / Snap		
W-BB -11,-20-1, -35, -52, -11-56	Miscellaneous	SAP6	Zapata et al. 2004

Molecular marker UBC420 for HR45 (Yu et al., 2000 and 2004)
Molecular marker BC73 for OAC Rex (Bai et al., 1997; Miklas, et al. 2000)

Note that Vandemark et al. (2008) showed that BC420 QTL was not effective in the absence of SU91 QTL. Both QTL in combination had a disease rating of 1, SU91 only a rating of 3, and BC420 or none had a rating of 9. It is also well known that to date BC420 QTL can only be deployed in white and black beans due to tight linkage with the *V* seed/flower color locus on chromosome 6. Miklas et al. (2003) showed that SAP6 QTL derived from GN landrace not tepary bean.

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