

## ***Cultivar and Germplasm Releases***

### **RELEASE OF FIVE COMMON BEAN GERmplasm LINES RESISTANT TO COMMON BACTERIAL BLIGHT: W-BB-11, W-BB-20-1, W-BB-35, W-BB-52, AND W-BB-11-56<sup>1,2</sup>**

Mildred Zapata<sup>3</sup>, George Freytag<sup>4</sup> and Robert Wilkinson<sup>5</sup>

J. Agric. Univ. P.R. 88(1-2):91-95 (2004)

Common bacterial blight is a major disease of dry beans worldwide. The disease is caused by the bacterium *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) = *X. axonopodis* pv. *phaseoli*. Few commercial common bean (*Phaseolus vulgaris* L.) cultivars are resistant to common bacterial blight caused by *Xcp*. Nebraska Great Northern No. 1 Sel. 27 has been used as a standard and is recognized as having a useful level of resistance to the bacterium. However, the resistance in this cultivar is not as great as that found in some tepary beans (*P. acutifolius* Gray) (McElroy, 1985; Freytag, 1989; Singh and Muñoz, 1999; Zapata, 1989).

The University of Puerto Rico, the Agricultural Research Service (ARS) of the U.S. Department of Agriculture, and Cornell University cooperatively announced the release of five common bean germplasm lines: Wilkinson (W)-Bacterial Blight (BB) -11; -20-1; -35; -52; and -11-56 in an "in-house" communication signed by administrators of the three institutions in 1990. These germplasm lines represent the culmination of more than 20 years of crossing and testing at Cornell to pyramid resistance for common bacterial blight disease caused by *Xcp* in the common bean (*P. vulgaris*) and of nearly 10 years of collaboration involving field testing, inoculation and selection of breeding lines between the UPR and ARS in Puerto Rico (PR). This cooperative work was supported in part by grants from the U.S. Agency for International Development (AID/CM/TA-C-73-26), AID/TA-C-1296, AID/DSAN/XII-G-0261 and CBA-UPR-18 (83-CRSP-2-2160), and the NY State Dry Bean Growers and Shippers fund.

### **Origin**

Bacterial blight resistance has been incorporated into three lines with determinate growth habits, which generally are more susceptible to common blight than indeterminate beans, and also two indeterminate bushy vine types. Superior levels of bacterial blight resistance were developed in lines W-BB-20-1 and W-BB-11-56, a bush and bushy vine type, respectively. The germplasm lines W-BB-20-1 and W-BB-11-56 (Zapata et al., 1991) have been used successfully as parental lines for breeding for resistance to common

<sup>1</sup>Manuscript submitted to Editorial Board 22 August 2003.

<sup>2</sup>This cooperative work was supported in part by grants from the U.S. Agency for International Development (AID/CM/TA-C-73-26), AID/TA-C-1296, AID/DSAN/XII-G-0261 and CBAG-UPR-18 (83-CRSP-2-2160), and the NY State Dry Bean Growers and Shippers fund.

<sup>3</sup>Plant Pathologist, Department of Crop Protection, University of Puerto Rico—Mayagüez, P.O. Box 9030, Mayagüez, PR 00681-9030.

<sup>4</sup>Plant Breeder (retired), USDA-ARS-TARS, Mayagüez, PR.

<sup>5</sup>Plant Breeder (retired), University of Cornell, Ithaca, NY.

bacterial blight of recently released lines USNA-CBB-1 and USNA-CBB-2, respectively (Miklas et al., 2001a; 2001b). The other lines are two bush or determinate types and one bushy vine or indeterminate type with resistance to common bacterial blight, superior to that of Great Northern No.1 Sel. 27. Another achievement was the development of a germplasm line, W-BB-52, having common blight resistance, bush type, and snap bean characteristics.

The high level of *Xcp* resistance in the W-BB lines was developed by pyramiding resistance from several sources, such as GN-1, Sel. 27, PI 207262, PI 180745, PI 180746, and 65859 (Table 1), primarily through a reciprocal backcross program. The reciprocal backcross process of pyramiding resistance began with crossing between plants that derive resistance from different sources. After screening the F<sub>3</sub> and F<sub>4</sub> generation for bacterial blight, the resistant plants were backcrossed to each parent and the resulting progenies were ultimately intercrossed. Typically one or more generations of selfing and screening for resistance followed this last cross. The progeny from the two backcrosses was screened for resistance for two generations. Additional *Xcp* resistance was combined through a parallel procedure. These pyramids of resistance were subsequently combined through a reciprocal backcross procedure. It was assumed that the progeny from the reciprocal backcross carried most of the resistance from each original source. This assumption was made because a high degree of recessiveness in the genes for resistance from many sources was observed. The reciprocal backcross procedure has the advantage of producing a higher percentage of homozygosity in which the recessive genes for resistance can be expressed. Also, it is suspected that some minor genes may be best expressed in the presence of certain other minor genes. If this is the case, it adds importance to the need for recovering "all" the resistance genes from both parents. Regardless of the real reason for why it works, experience has shown that the reciprocal backcross procedure is essential to accomplishing a satisfactory pyramiding of recessive genes for resistance.

Resistance to common blight was determined by screening promising families for their latent period (length of time between inoculation and symptom expression). 3M-152 (highly susceptible Puerto Rico line), Redkote (susceptible), and Sel. 27 (resistant) used as indicator lines, developed symptoms at three, four, and seven days after inoculation, respectively. The experiments were conducted in growth chambers at 29 °C. In the begin-

TABLE 1. Sources of resistance to *Xanthomonas campestris pv. phaseoli* (*Xcp*) used to develop the W-BB lines.

Lines	Progenitors with <i>Xcp</i> resistance					
	GN-1 <sup>1</sup>	Sel. 27 <sup>2</sup>	PI 207262 <sup>3</sup>	PI 180745 <sup>4</sup>	PI 180746 <sup>5</sup>	65859 <sup>6</sup>
W-BB-11	+ <sup>7</sup>	+	+	+		
W-BB-20-1	+	+	+	+	+	+
W-BB-35	+	+	+	+	+	+
W-BB-52	+	+	+			+
W-BB-II-56	+	+	+		+	

<sup>1</sup>GN-1 = Univ. of Idaho Great Northern #1.

<sup>2</sup>Sel. 27 = Great Northern Nebraska #1 Selection 27.

<sup>3</sup>PI 207262 = Plant Introduction from Colombia, SA.

<sup>4</sup>PI 180745 = Plant Introduction (*P. coccineus* × *P. vulgaris*) from Germany.

<sup>5</sup>PI 180746 = Plant Introduction (*P. coccineus* × *P. vulgaris*) from Germany.

<sup>6</sup>PI 65859 = (*P. vulgaris* × *P. coccineus*) from P.A. Lorz, Univ. of Florida.

<sup>7</sup>+ = Indicates source of resistance present in the line.

ning it was possible to conduct a test every week but as the incubation time increased the observation period also increased. Generally, an experiment in the growth chamber consisted of one or more related groups or families together with indicator lines. Also, when  $F_2$  populations were screened, one or both parents were included, especially when no obvious phenotypic markers were involved, to help confirm that a cross had been obtained to measure the effect of pyramiding genes for resistance. Plants that expressed resistance in the screening tests were transplanted to larger pots in the greenhouse and used for seed production and possible crossing. All crosses were made at Cornell whereas screening for *Xcp* resistance was conducted at Cornell and Puerto Rico using multi-needle wound inoculations (Zapata et al., 1985) of primary leaves on eight-day-old seedlings with  $10^6$ /CFU in a controlled growth chamber at about 29 °C and greenhouse conditions (Zapata et al., 1991). Symptom development was observed daily. Plants showing hypersensitive reactions were discarded. Susceptible plants showing chlorosis or progressive necrosis were discarded as soon as detected. Only plants showing no symptoms or having incubation periods longer than Sel. 27 were maintained.

From 1979 to 1985 the breeding lines were evaluated under tropical field conditions using inoculation with local strains of the pathogen at the UPR Fortuna Substation (Zapata et al., 1985). There was also some selection for resistance to ashy stem blight, as the fields had a high inoculum level of *Macrophomina phaseolina*. Seeds from plants selected for *Xcp* resistance were sent to Cornell for incorporation into the crossing program. The progenies of the crosses in the  $F_1$  generation were returned to Puerto Rico for evaluation.

During five summer seasons (1986 to 1990) nurseries were planted at the UPR Fortuna Substation; individual plant selections for *Xcp* resistance in the  $F_3$  generation were made from heterogenous lines inoculated with local *Xcp* strains. Plant rows ( $F_{3,4}$ ) from resistant plants were grown in nurseries during the winter season at the USDA-ARS Isabela Research farm and selected for agronomic traits and seed yield potential. Resistance to *Xcp* in foliage of individual plants at flowering was confirmed three times by using multi-needle inoculations under controlled greenhouse environments at Mayagüez, using four pure strains from the American Type Culture Collection (ATCC) and from two local sources of *Xcp* (Table 2).

TABLE 2. *Reaction of individual bean lines to inoculation with X. campestris pv. phaseoli under greenhouse and field conditions.*

Identity	Greenhouse <i>Xcp</i> pathovar/origin					Field
	<i>phaseoli</i> ATCC 9563	<i>phaseoli</i> PR 820	<i>fuscans</i> ATCC 11766	<i>vignicola</i> ATCC 11648	<i>glycines</i> ATCC 17915	<i>phaseoli</i> field strains PR
W-BB-11	I	I	T	I	I	T
W-BB-20-1	I	I	I	I	I	R
W-BB-35	I	I	S	I	I	T
W-BB-52	I	I	R	I	I	S
W-BB-II-56	I	I	I	I	I	R

I = Immune, no lesions; R = Resistance, very small (1 to 3 mm), chlorotic but non-progressive lesions; T = Tolerant slow disease development, takes 8 to 10 days under controlled growth conditions and 44 days after inoculation under field conditions to develop 25% chlorotic lesions and less than 25% of necrotic lesions; S = Susceptible under controlled growth conditions, takes six days to show symptoms and 44 days to develop necrosis on 50% of the inoculated tissue under field conditions.

### Botanical Description

Line W-BB-11 (from Cornell line 84-4216-1) has a bushy vine (Type III) plant habit, a height of 70 cm, straight pods 5 to 7 cm long, and small gray to black seed weighing 0.27 g/seed. W-BB-11 is earlier than Arroyo Loro (2W-33-2), which matures about 75 days after planting.

Line W-BB-20-1 (from Cornell line 84-4446-1) has a determinate bush (Type I) plant habit, a height of 40 cm, slightly curved pods 5 to 8 cm long and white seed weighing 0.24 g/seed. This line has the I gene resistance to Bean Common Mosaic Virus (BCMV). W-BB-20-1 is earlier than Arroyo Loro (2W-33-2), which matures about 75 days after planting.

Line W-BB-35 (from Cornell line 84-4454-1) has a determinate bush (Type I) plant habit, a height of 30 cm, late season, broad straight pods 5 to 8 cm long, and a large rounded, yellowish brown seed weighing 0.43 g/seed. This line has a protected (probably by bc2-2) I gene resistance to BCMV. W-BB-35 is later than Arroyo Loro (2W-33-2), which matures about 75 days after planting.

Line W-BB-52 (from Cornell line 84-4610-3) has a determinate bush (Type I) plant habit, a height of 20 cm, early season, flat curved pods 7 to 9 cm long with some snap characteristics and long cream colored seeds weighing 0.30 g/seed. This line does not have the I gene for BCMV resistance. W-BB-52 is earlier than Arroyo Loro (2W-33-2), which matures about 75 days after planting.

Line W-BB-11-56 (from Cornell line 85-8250-1) has a bushy vine (Type III) plant habit, a height of 70 to 120 cm, late season, curved pods 7 to 10 cm long with some snap characteristics and a good seed set in Puerto Rico of a pinto seed type weighing 0.25 g/seed. This line has protected (probably by bc2-2) I gene resistance to BCMV. W-BB-11-56 is later than Arroyo Loro (2W-33-2), which matures about 75 days after planting.

### Identification of Resistance Using Genetic Markers

A portion of the resistance in these lines to common bacterial blight was derived from GN#1 Sel. 27 as indicated by the presence of the sequence characterized amplified region (SCAR) marker linked with a quantitative trait locus for resistance to common bacterial blight on linkage group B10 SAP-6-820 (Miklas et al., 2000) (Table 3). Suitable markers to identify the other sources of resistance have not yet been developed.

TABLE 3. *Identification of bacterial blight resistance using four genetic markers (SCARS).*

Lines	SCARS Markers			
	XAN 159 B-8 <sup>1</sup> Su 91	OAC 88 B-8 R-7313	GN#1 Sel 27 B-10 SAP-6	XAN 159 B-6 BC 420
W-BB-11	— <sup>2</sup>	—	+	—
W-BB-20-1	—	—	+	—
W-BB-35	—	—	+	—
W-BB-52	—	—	+	—
W-BB-11-56	—	—	+	—

<sup>1</sup>Indicates the linkage group.

<sup>2</sup>— Indicates absence and + presence of the marker for resistance.

### Seed Availability

Small quantities of seed of the germplasm lines are available upon request from the Bean Program, Tropical Agricultural Research Station, Agricultural Research Service, U.S. Department of Agriculture, 2200 Pedro Albizu Campos Ave. Suite 201, Mayagüez, PR, 00680-5470, or from Dr. Mildred Zapata, Crop Protection Department, University of Puerto Rico, P.O. Box 9030, Mayagüez, PR 00681-9030. We ask that appropriate recognition of source be given when this germplasm contributes to a new cultivar.

### LITERATURE CITED

- Freytag, G. F., 1989. Inheritance of resistance to three strains of common bacterial blight (*Xanthomonas campestris*) in the cultivated tepary bean (*Phaseolus acutifolius* var. *latifolius*). *Ann. Rep. Bean Improv. Coop.* 32:101-102.
- McElroy, J. B., 1985. Breeding for dry beans *Phaseolus vulgaris* L. for common bacterial blight resistance derived from *Phaseolus acutifolius* A. Gray. Ph.D. Thesis Cornell Univ.
- Miklas, P. N., J. R. Smith, R. Riley, K. F. Grafton, S. P. Singh, G. Jung and D. P. Coyne, 2000. Marker-assisted breeding for pyramided resistance to common bacterial blight in common bean. *Ann. Rep. Bean Improv. Coop.* 43:39-40.
- Miklas P. N., J. R. Smith, K. F. Grafton, D. P. Coyne and M. A. Brick, 2001a. Release of Pinto and Great Northern Bean Germplasm lines USPT-CBB-1, USPT-CBB-2, USPT-CBB-3, and ISGN-CBB-4 with erectness and resistance to common bacterial blight, rust and mosaic. *Ann. Rep. Bean Improv. Coop.* 44:183-184.
- Miklas P. N., J. R. Smith, A. N. Hang, K. F. Grafton and J. D. Kelly, 2001b. Release of Navy and Black Bean Germplasm lines USNA-CBB-1, USNA-CBB-2, USNA-CBB-3, USNA-CBB-4, and USNA-CBB-5 with resistance to common bacterial blight. *Ann. Rep. Bean Improv. Coop.* 44:181-182.
- Singh, S. P. and C. G. Muñoz, 1999. Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. *Crop Sci.* 39:80-89.
- Zapata, M., G. F. Freytag and R. E. Wilkinson, 1985. Evaluation for bacterial blight resistance in beans. *Phytopathology* 75:1032-39.
- Zapata, M., 1989. Host-pathogen interactions of the tepary bean, *Phaseolus acutifolius*, with the common bean blight pathogen, *Xanthomonas campestris* pv. *phaseoli*. Ph.D. Thesis. Nebraska Univ.
- Zapata, M., R. Wilkinson, G. F. Freytag, H. Vélez, F. H. Ortiz and J. López-Rosa, 1991. Incorporating resistance to *Xanthomonas campestris* pv. *phaseoli* into bean using the latent period as a resistance marker. *J. Agric. Univ. P.R.* 75(4):345-352.

