

ROOTED TRIFOLIATE LEAVES OF COMMON BEANS FOR EXTENDED EVALUATION OF THE REACTION TO COMMON BACTERIAL BLIGHT

Mohamed F. Mohamed, E. Arnaud-Santana, and Dermot P. Coyne
Department of Horticulture, University of Nebraska, Lincoln, NE 68583-0724

Common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye (*Xcp*) is a serious disease affecting common bean (*Phaseolus vulgaris* L.). Mohamed et al. (Euphytica 65:161-166, 1993) developed a method to evaluate the reaction of bean germplasm to *Xcp* using detached rooted trifoliolate leaves. The detached leaves were inoculated with *Xcp* at time of planting in the rooting medium or 1 week later. There was no significant difference in the amount of CBB disease on the inoculated attached versus the detached leaves. The rooted trifoliolate leaves remained viable and green for 2 to 3 months growing in potting medium in the greenhouse. Extended time for inoculation would offer a convenience for bean breeders when evaluating the disease reaction of large bean germplasm collections with several *Xcp* strains and to evaluate leaves of plants to *Xcp* at different stages of the development of the plant. In this study the reaction of 5-week-old rooted trifoliolate leaves of common bean to *Xcp* was determined.

Seeds of four bean cultivars/lines 'Charlevoix' (MSU, East Lansing, MI), 'PC-50' (Dominican Republic [DR]), 'Venezuela 44' (Venezuela), and XAN-159 (Centro Internacional de Agricultura Tropical, Cali, Colombia) were planted in 15 cm clay pots (1.8 liter) containing a mixture of the following parts by volume, 2 soil:2 sand:2 sphagnum peat:1 vermiculite, in the greenhouse, Lincoln, NE. The four bean entries were arranged in a RCBD with 4 replicates. Seeds were planted August 14, 1992 (Aug., Sept. mean day lengths = 13.3, 12.31 hrs, respectively) and the planting was repeated November 18, 1992 (Nov., Dec. mean day lengths = 9.5, 9.05 hrs, respectively). Ten-day-old seedlings were fertilized at 3-day intervals with 200 ppm N from a 9:3.5:16.5 NPK fertilizer containing trace elements. During August the mean day/night temperature was 32±5C and 24±1C, respectively, and during November, 1992 was 26±2C and 21±1C, respectively.

The first fully expanded trifoliolate leaves excised from 17-day-old plants were incubated for root induction following the procedure described by Mohamed et al. (1993). The cultures of the trifoliolate leaf cuttings were arranged in a RCBD with 4 replicates corresponding to the same replication used for seed planting. The inoculations with *Xcp* were conducted 1, 3, and 5 weeks after the week of incubation to induce rooting on petioles of trifoliolate leaf cuttings. These inoculation-time-treatments were applied at random within each bean entry and replicate. The experiment was arranged as a split-plot in a RCBD. The whole-plots were the bean entries and the sub-plots were the inoculation times. Each sub-plot unit was a set of five 0.4 liter pots (one leaf cutting/pot).

The *Xcp* inoculum was prepared by suspending 48 hour-old cultures of *Xcp* strain V₄S₁ (DR), grown on MXP medium (Claflin et al., Phytopathology 77:730-734, 1987), in standard phosphate buffer (pH 7.1) with 10 mM magnesium sulphate. *Xcp* inoculum concentration used was 1.5 x 10⁷ CFU (colony-forming units)/ml. The

multiple needle method (Andrus, *Phytopathology* 38:757-759, 1948) was used for inoculation. Two leaflets of each trifoliolate were inoculated with Xcp and the third leaflet was inoculated with only the solution of the phosphate buffer (control). The % of the inoculated area developing the common blight disease symptoms based on visual determination was recorded 12 days after the inoculation. The ANOV was performed using arc sine transformed data.

The disease reactions of the bean entries were similar for the 3 different inoculation times within each experiment. The four bean entries in the August planting date differentiated into two groups ($P < 0.05$) based on their reaction to Xcp. XAN-159 (9% of inoculated area with CBB symptoms) expressed a high level of resistance (R) to Xcp while 'Charlevoix' (67%), 'PC-50' (67%), and 'Venezuela 44' (66%) were highly susceptible (S) and not significantly different from each other. XAN-159 also exhibited a high level of R (3%, $P < 0.05$) to Xcp in the experiment planted in November while 'Venezuela 44' (71%) was slightly more S ($P < 0.05$) than 'Charlevoix' (60%) and 'PC-50' (63%).

The rooted trifoliolate leaves can be used to evaluate common blight disease reaction for an extended period of time offering the bean breeder flexibility in screening large bean germplasm collections. It is known that differential flowering and maturity due to photoperiod x temperature interactions may affect the expression of common blight reaction (Coyne et al., *Proc. Amer. Soc. Hort. Sci.* 98:94-99, 1973). This method could be used to overcome the latter problem so that leaves of a large and diverse germplasm could be detached and rooted in the early vegetative stages and screened for reaction to Xcp when time and resources permit. Also, the method could be used to test leaves for their reaction to Xcp at different stages of development of the plant. Detached leaves from young bean plants and later from the same plants during the pod development stage could be rooted and tested at the same time for their reaction to Xcp under the same temperature and photoperiod conditions. The former leaves would become mature or senescent if they remained attached to the plant at the time of pod development.