Confirmation of Molecular Markers and Flower Color Associated with QTL for Resistance to Common Bacterial Blight in Common Beans

Soon O. Park[1], Dermot P. Coyne[1], Nedim Mutlu[1], Geunhwa Jung[2], and James R. Steadman[1]; [1]University of Nebraska, Lincoln, NE 68583; [2]University of Wisconsin, Madison, WI 53706

Common bacterial blight (CBB), incited by *Xanthomonas campestris* pv. *phaseoli* (*Xcp*), is a serious disease of common bean (*Phaseolus vulgaris* L.). The expression of quantitative trait loci (QTL) may differ over environments or populations in various crops. Among 29 QTL affecting fruit size, soluble solids concentration or pH in a tomato cross, only four QTL were expressed in three environments (Paterson et al., 1991). Of seven QTL for seed size found in a common bean population, only one QTL was expressed in three environments (Park et al., 1998). No common genomic region associated with QTL affecting plant height was found in four maize populations (Beavis et al., 1991). Only one QTL affecting resistance to *Xcp* was consistently expressed in four common bean populations (Jung et al., 1998). The results show the importance of confirmation of the marker-QTL associations in a breeding program, particularly for traits like CBB resistance that have complex inheritance patterns, low narrow-sense heritabilities, and a number of genes involved.

Flower color (*V* gene) and RAPD markers have been reported to be associated with six QTL affecting leaf and pod resistance to *Xcp* in 70 F_6 recombinant inbred (RI) lines from the cross 'PC-50' x XAN-159 in greenhouse experiments (Jung et al., 1997). However, these marker-QTL associations have not been confirmed in other populations of the same cross and or a different cross, in different environments or with other *Xcp* strains. Therefore, our objective was to confirm significant associations of RAPD markers and the *V* gene with QTL for leaf and pod resistance to *Xcp* strains in a RI backcross population from the common bean cross BC_2F_6 'PC-50' [susceptible to *Xcp* (S)] x XAN-159 [resistant to *Xcp* (R)] in greenhouse and field experiments, and for leaf resistance to *Xcp* in an F_2 segregating population from the common bean cross pinto 'Chase' [moderate resistance to *Xcp* (MR)] x XAN-159 (R) in a greenhouse experiment.

Materials and Methods

PLANT MATERIALS. Sixty-four RI lines from the cross 'PC-50' (S) x XAN-159 (R), after two backcrosses to 'PC-50', were developed using the single-seed-descent breeding method. The parents and RI backcross lines were tested in experiments in greenhouse and field environments using a RCBD with three replications. Eighty-nine F_2 plants from the cross 'Chase' (MR) x XAN 159 (R) were also developed and grown in the greenhouse.

INOCULATION. Five *Xcp* strains (EK-11, DR-7, SC-4A, LB-2, and SX-114)(source: A.K. Vidaver, Dept. of Plant Pathology, UNL) were used in the RI backcross population. Two *Xcp* strains EK-11 and DR-7 were inoculated to the F_2 population. The multiple needle method was used to inoculate leaves in both populations. In the RI backcross population young pods were punctured by a dissecting needle and then 10 ul of a 10^7 CFU/ml bacterial suspension was introduced through the puncture inside the pod using a Pipetteman.

MARKERS. Total genomic DNA was extracted from lyophilized leaf tissue of the parents, 63 RI backcross lines, and 89 F_2 plants. Ten and seven primers were used for the RAPD analysis in the RI backcross and F_2 populations, respectively. Polymerase chain reactions (PCR) were performed in an air thermalcycler (model 1605; the Idaho Technology, Idaho Falls) in thin-walled glass

capillary tubes. The name of each RAPD marker is derived from the letter identifying the Operon kit or a "BC" prefix, the primer number and the approximate length of the marker.

STATISTICAL ANALYSIS. Single factor ANOVA for each pairwise combination of quantitative trait and marker locus was used to analyze the data for detection of QTL affecting resistance to the *Xcp* strains. Significant differences in trait associations were based on F-tests ($P<0.05$).

Results and Discussion

Jung et al. (1997) found RAPD markers and the *V* gene on linkage group (LG) 5 associated with a single QTL affecting leaf and pod resistance to *Xcp*. Marker BC437.1050 and the *V* gene were more associated with leaf and pod resistance to *Xcp* strains than other markers. The three most resistant lines and plants based on phenotypic evaluation in the RI backcross and F2 populations were identified by the marker BC437.1050 and the *V* gene. Marker BC420.900 was consistently associated only with leaf resistance to *Xcp* strains, and accounted for 8% to 30% of the variation for the traits.

RAPD markers on LG 1 associated with two QTL affecting leaf and pod resistance to *Xcp* were reported by Jung et al. (1997). Markers E4.1150 and E4.700 were significantly associated with a single QTL for pod resistance to two *Xcp* strains in the greenhouse in the RI backcross population and with leaf resistance to two *Xcp* strains in the F2 population. Markers AL7.650 and C7.900 were associated with another QTL only for pod resistance to two *Xcp* strains in the greenhouse-grown RI backcross population. However, the marker AL7.650 was not associated in the F2 population.

Molecular markers associated with two QTL for pod resistance to *Xcp* were found by Jung et al. (1997) on LGs 4 and 9. They also found an unassigned marker D13.1000 to be associated with leaf resistance to *Xcp*. Marker AP7.1800 was consistently associated with leaf and pod resistance to *Xcp* strains, except for pod resistance in the field. Unassigned marker D13.1000 was associated only with pod resistance to two *Xcp* strains. However, marker AL7.1050 on LG 4 was not associated in the RI backcross population. Therefore, among the six QTL previously detected, five in the RI backcross population and three in the F2 population were confirmed to be associated with resistance to *Xcp* in this study. The confirmed marker BC437.1050 and *V* gene on LG 5, along with other resistance genes from other germplasm, could be utilized to pyramid the different genes into a susceptible or partially resistant bean line or cultivar to enhance the level of resistance to *Xcp*.

Literature Cited

Beavis, W.D., D. Grant, M. Albertsen, and R. Fincher. 1991. Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. Theor. Appl. Genet. 83:141-145.

Jung, G., P.W. Skroch, D.P. Coyne, J. Nienhuis, E. Arnaud-Santana, H.M. Ariyarathne, S.M. Kaeppler, and M.J. Bassett. 1997. Molecular-marker-based genetic analysis of tepary bean-derived common bacterial blight resistance in different developmental stages of common bean. J. Amer. Soc. Hort. Sci. 122:329-337.

Jung, G., P.W. Skroch, J. Nienhuis, D.P. Coyne, E. Arnaud-Santana, and H.M. Ariyarathne. 1998. Confirmation of QTL associated with common bacterial blight resistance in four different genetic backgrounds in common bean. Crop Sci. 38:(accepted).

Park, S.O., D.P. Coyne, G. Jung, E. Arnaud-Santana, and H.M. Ariyarathne. 1998. Detection and mapping of RAPD markers associated with QTL affecting seed size and shape in common bean. Ann. Rpt. Bean Improv. Coop. 41:147-148.

Paterson, A.H., S. Damon, J.D. Hewitt, D. Zamir, H.D. Rabinowitch, S.E. Lincoln, E.S. Lander, and S.D. Tanksley. 1991. Mendelian factors underlying quantitative traits in tomato:comparison across species, generations, and environments. Genetics 127:181-197.