Development of SCAR markers linked to common bacterial blight resistance genes (QTL) in common bean

G. Jung1, S. Beebe2, J. Nienhuis1, S. Park3, D. Coyne4, J. Marita1, F. Pedraza2, and J. Tohme2
1Dept. of Horticulture, Univ. of Wisconsin, Madison, WI 53706; 2CIAT, Cali, Colombia; 3Agriculture and Agri-Food Canada Greenhouse & Processing Crops Research Center, Ontario; 4Dept. of Horticulture, Univ. of Nebraska, Lincoln, NE 68583

Common bacterial blight (CBB), incited by Xanthomonas campestris pv. phaseoli (Smith) Dye (Xcp), is one of severe seed-borne diseases of common bean (Phaseolus vulgaris L.). Breeding for resistant cultivars to Xcp is the most effective measures to control CBB. A germplasm line XAN 159 derived from an interspecific cross between P. vulgaris and P. acutifolius PI 319443 might have recovered majority of the resistant genes from the accession (McElroy, 1985). CBB resistance is inherited quantitatively with differential leaf and pod reactions to CBB and low heritability including XAN 159 (Arnaud-Santana et al., 1994).

Use of tightly linked RAPD markers to genes of interest for marker assisted selection (MAS) will be difficult because of multiple bands in RAPD amplified products and sensitivity in the reaction conditions. Therefore, Paran and Michelmore (1993) reported advantages of Sequence Characterized Amplified Region (SCAR) of PCR-based genetic marker over RAPD markers. Since then, several studies on the development of SCAR linked to genes for disease resistance and other agronomic traits have been reported (Melotto et al. 1996; and Naqvi and Chattoo, 1996). SCARs are useful as highly reproducible genetic markers for marker-assisted selection, especially for quantitatively inherited disease resistance. The objective of this study was to convert RAPD markers linked to genes for CBB resistance into more reliable, specific, and easily recognizable PCR markers (SCAR) in order to saturate the chromosomal regions linked to CBB resistance physically in the common bean cross "PC 50" x XAN 159 and to examine efficiency of SCAR markers.

Materials and Methods: Three sources of recombinant inbred lines (RI) were used. (1) Seventy F6 RI lines derived from the cross "PC 50" x XAN 159 that previously used to construct a RAPD linkage map and identify RAPD marker locus-QTL associations for resistance to Xcp in common bean (Jung et al. 1997), (2) 100 RI lines derived from the cross SEL1309 x DOR476 which was used to tag QTL for CBB resistance. The SEL1309 was developed for leaf resistance to CBB at CIAT (Steve Beebe, personal communication), and (3) Five navy bean lines, HR45, HR52, HR53B, HR67, and HR71 which were selections for resistance to CBB at AAFC-GPCRC, Harrow, Ontario (S.J. Park, personal communication). HR45 was derived from the cross of HR13-621*2 x (XAN 159 x HR13-621).

RAPD marker Y5 mapped in linkage group 5 and linked to a major QTL for CBB resistance in the "PC 50" x XAN 159 population was converted into a SCAR marker (SCARLG5). Another RAPD marker linked to CBB resistance gene tagged in the SEL1309 x DOR476 population was also converted into a SCAR marker (SCARLG1). For each cloned RAPD amplification product, two oligonucleotides were designed to be used as SCAR primers. Sequences of each primer containing the original 10 bases of the RAPD primer plus the next 10-14 bases extended internally from each primer were designed.

Results and Discussion: Of the three chromosomal regions associated with CBB resistance derived from the cross "PC 50" x XAN 159 (Jung et al, 1997), most significant gene effects for CBB resistance were associated with linkage group 5 and with two regions on linkage group 1. One RAPD marker linked to resistance to CBB was identified from the SEL 1309 x DOR 476 population. This RAPD marker was converted into a SCAR marker (SCARLG1). Then the SCAR marker (SCARLG1) was tested against 70 RI lines in the mapping population "PC 50" x XAN 159 and mapped to the
same chromosomal region which was previously detected to be associated with CBB resistance in linkage group 1 in the "PC 50" x XAN 159 population.

The amplified fragments of two RAPD markers linked to CBB resistance were cloned. The identities of the cloned fragments were verified by mapping the SCAR marker using an original segregating population in which the particular RAPD marker segregated and by comparing sequences of two cloned fragments with understanding the possibility of size difference. As evidence of the authenticity of the cloned fragments, no recombinations were observed between RAPD and SCAR markers. In addition, identical nucleotide sequences were obtained of three independent amplified fragments sequenced in 200 base pairs or more which included the decamer sequence used for amplifying the original RAPD fragment. This step is critical to avoid identifying contaminating sequences of similar size bands in the gels used for fractioning the products of the RAPD reaction. Both SCARs in linkage group 1 and 5 could be utilized as tools for molecular marker-assisted selection (MAS) for CBB resistance in hybrid populations derived from XAN 159 and also for pyramiding different resistant genes.

The efficiency of the SCAR markers was tested with five CBB resistant HR-lines. Ten plants each of the HR-lines were inoculated with Xcp on a single plant basis and the individual genomic DNAs for PCR amplification were examined with the pairs of two SCAR primers. The SCAR marker (SCARLG1) in linkage group 1 was amplified in all of the HR lines. The SCAR marker (SCARLG5) in linkage group 5 amplified in HR45, HR52 and HR67 lines but did not amplify in HR53B and HR71 lines. HR53B and HR71 lines were moderately susceptible in the greenhouse inoculation test though they were resistant in the field. Results indicated that the susceptibility of those two lines might be due to the absence of a major QTL in linkage group 5.

SCARs posses a number of relevant advantages over RFLP and RAPD markers for use in high resolution mapping and MAS for traits. Advantages of the use of SCARs is detecting specificity of a single locus, less sensitive to PCR reaction conditions, and convertible into codominant markers. This study was the first attempt to apply SCAR-based indirect selection of a disease resistance gene (QTL) in common bean. Availability of these SCARs for other sources of CBB resistance genes shall be useful in gene pyramiding and fine-mapping of the loci for positional approaches for CBB.

References cited


