

Potential Application of TRAP (Targeted Region Amplified Polymorphism) Markers for Mapping and Tagging Disease Resistance Traits in Common Bean

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ABSTRACT

Genetic resistance is an important component of integrated strategies used to control problematic diseases in common bean (*Phaseolus vulgaris* L.). Molecular linkage maps have been used to identify, tag, and map disease resistance genes and QTL in common bean, leading to improved breeding strategies and implementation of marker-assisted selection. Most widely used marker types, random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphisms (AFLP), for linkage mapping in bean are located randomly throughout the genome and associate with particular traits by chance. We sought to determine the potential application of a new marker system, TRAP, which uses expressed sequence information and a bioinformatics approach to generate polymorphic markers around targeted candidate gene sequences. TRAP markers were amplified by fixed primers designed against sequenced expressed sequence tag (EST) associated with disease resistance in the Compositae Genomics database or against sequenced resistance gene analog (RGA) from common bean. Seventeen of 85 TRAP markers located in the BAT 93/Jalo EEP558 core mapping population mapped in the vicinity of R genes. Six of 21 TRAP markers generated in the Dorado/XAN 176 mapping population were linked with newly identified QTL, two conditioning resistance to ashy stem blight (14% and 16% of the phenotypic variation explained, R^2), and one each conferring resistance to *Bean golden yellow mosaic virus* (BGYMV) (15%) and common bacterial blight (30%). The TRAP marker system has potential for mapping regions of the common bean genome linked with disease resistance.

A GOAL of many crop-breeding programs is to incorporate marker-assisted selection (MAS) for disease resistance with traditional approaches to expedite germplasm enhancement and cultivar development (Miklas et al., 2006). For example, pyramiding genes for durable disease resistance against a hypervariable plant pathogen is facilitated by a combination of traditional greenhouse inoculation tests for detection of the epistatic gene followed by MAS of hypostatic genes (Miklas and Kelly, 2002; Kelly et al., 2003). Marker-assisted selection is also extremely useful for detection of individual resistance genes difficult to assay with the pathogen, such as *bgm-1* in common bean conditioning resistance to BGYMV (Urrea et al., 1996) and *Bct* for resistance to *Beet curly top virus* (Larsen and Miklas, 2004). Resistances to those and other diseases are dif-

ficult to assess by means of the pathogen because natural epidemics are unpredictable and the pathogen–vector is not amenable to ex situ disease screening methods.

RAPD has been the predominant marker system used to identify markers tightly linked with disease resistance traits in bean (Kelly and Miklas, 1998). Informative RAPD markers with utility for MAS are often converted to sequence-characterized amplified polymorphism (SCAR) markers to facilitate utilization across laboratories. RAPDs are arbitrary sequences of amplified DNA that are uniformly distributed across the genome. For this reason thousands of PCR reactions may be necessary to identify a single RAPD marker that is located in the specific genomic region of a targeted gene or trait. AFLP, also considered a random marker, generates more polymorphisms per PCR reaction for higher throughput; therefore, is replacing RAPD as the marker of choice for tagging traits and constructing genetic linkage maps. However, because the AFLP method also amplifies random sequences, the efficiency of both RAPD and AFLP for tagging specific traits can be low. Another shortcoming of both RAPDs and AFLPs is that they are primarily dominant markers systems that are unable to distinguish heterozygotes. Although microsatellite markers, also referred to as simple sequence repeats (SSR), are available in common bean only a few (<70) have been developed (Blair et al., 2003; Gómez et al., 2004). Thus, for common bean, SSRs are most applicable for map integration and genetic diversity studies. Generation and detection of RFLP markers is cumbersome and as with SSRs too few have been developed in common bean to be useful for gene tagging studies. A new random marker system called sequence related amplified polymorphism (SRAP) uses primers with AT- or GC-rich cores to amplify intragenic polymorphisms (Li and Quiros, 2001). SRAP has potential for candidate gene analysis of QTL (quantitative trait loci), and has been used to measure genetic diversity in pumpkin (*Cucurbita pepo* L.) germplasm (Ferriol et al., 2003) and to distinguish buffalograss [*Buchlōe dactyloides* (Nutt.) Engelm.] biotypes (Budak et al., 2004). The potential of SRAP for tagging specific traits in common bean has not been investigated.

Resistance gene analogs (RGAs) are PCR-generated markers designed from conserved sequence motifs of cloned resistance (*R*) genes. Generally, RGAs occur in clusters and map close to resistance genes in bean (Creusot et al., 1999; Ferrier-Cana et al., 2003; Geffroy et al., 1999; Lopez et al., 2003; Rivkin et al., 1999) and other crops (Kanazin et al., 1996; Leister et al., 1996;

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Abbreviations: EST, expressed sequence tag; QTL, quantitative trait locus (loci); RGA, resistance gene analog; TRAP, targeted region amplified polymorphism.

Shen et al., 1998). RGAs have been found associated with R genes in bean conditioning resistance to anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib. (Creusot et al., 1999; Ferrier-Cana et al., 2003; Geffroy et al., 1999) and QTL conditioning partial resistance to anthracnose, angular leaf spot [*Phaeoisariopsis griseola* (Sacc.) Ferraris], and BGYMV (Lopez et al., 2003). Geffroy et al. (1999 and 2000) employed RGAs in the discovery, genomic characterization and evolutionary understanding of the R gene clusters conditioning resistance to bean anthracnose. RGAs provide a marker system that could be used to specifically tag disease resistance traits. However, RGAs are not yet amenable for high throughput gene tagging and marker-assisted selection in bean because too few have been developed for such purposes.

A simple and rapid PCR-based marker system, TRAP, was recently developed which uses EST information and a bioinformatics approach to generate polymorphic markers around targeted candidate gene sequences (Hu and Vick, 2003). TRAPs are amplified by one fixed primer designed from a target EST sequence in the database and a second primer of arbitrary sequence except for AT- or GC-rich cores that anneal with introns and exons, respectively. TRAPs were effectively used in assessing genetic diversity among wild sunflower (*Helianthus annuus* L.) accessions (Hu et al., 2003), in fingerprinting lettuce (*Lactuca sativa* L.) cultivars (Hu et al., 2005), in tagging a recessive branching gene in sunflower (Rojas-Barros et al., 2005), and in mapping QTL in a wheat (*Triticum aestivum* L.) intervarietal recombinant inbred population (Liu et al., 2005). Our objective was to determine potential application of the TRAP marker system for mapping and tagging disease resistance traits in common bean. Thus, the fixed primers used to generate TRAPs in this study were based on resistance gene sequences obtained from Compositae EST and *Phaseolus* sequence databases.

MATERIALS AND METHODS

Plant Materials

The recombinant inbred line (RIL) mapping population BAT 93/Jalo EEP 558 (BJ) was obtained from P. Gepts (University of California-Davis). The BJ population has been widely used to integrate common bean linkage maps (Freyre et al., 1998) and has become the core map for genomic placement of economically important traits (Kelly et al., 2003; Miklas et al., 2006). Only 70 of the BJ RILs were available for use in this study. The Dorado (syn. DOR 364)/XAN 176 (DX) population consisting of 79 RILs was obtained from J. Beaver (University of Puerto Rico-Mayaguez) and has been used previously to map loci conditioning resistance to diseases caused by bacterial, fungal, and viral pathogens in common bean (Miklas et al., 2000).

DNA Isolation and TRAP Primer Design

A composite leaf tissue sample (approximately 50 mg) was collected from the first emerging trifoliolate leaves from four plants of each RIL and the parents for both mapping populations. Genomic DNA was extracted with the FastDNA Kit

(Bio 101, Vista, CA) according to manufacturer instructions. The purified DNA was adjusted to 10 ng μL^{-1} with a fluorometer before all PCR reactions.

Fixed primers were either designed against the sequenced EST associated with disease resistance in the Compositae Genomics database: http://cgpdb.ucdavis.edu/database/php_my_admin/php_my_admin.php; verified 21 November 2005 (Michelmore, personal communication, 2002) or against sequenced RGAs from common bean in the National Center for Biotechnology Information database: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nucleotide&cmd=search&term=phaseolus+resistance+gene>; verified 6 December 2005. The fixed primers were selected by using the web-based PCR primer designing program "Primer 3" <http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi>; verified 21 November (Rozen and Skaletsky, 2000) with the following parameters: primer optimum, maximum, and minimum sizes set at 18 nt; and primer optimum, maximum, and minimum T_m at 53, 55, and 50°C, respectively.

Fixed primers were derived from sunflower ESTs (Compositae Genetics database) that were selected for homology to disease resistance genes of different plant species. For example, EST contig A20101, has homology to: (i) LRR receptor-like protein kinase of *Nicotiana tabacum*, (ii) receptor-like protein kinase (EC 2.7.1.) of *Oryza sativa*, (iii) receptor protein kinase (TMK1), putative; protein id: At1g66150.1 of *Arabidopsis thaliana*, and (iv) receptor-like kinase RHG4 of *Glycine max*. Fixed primers derived from sunflower ESTs were also readily available for exploring the use of TRAP in bean, since the TRAP technique was originally developed with sunflower (Hu and Vick, 2003).

For development of arbitrary primers, the general principles of PCR primer design were upheld so as to avoid self-complementarity and improper GC content (40–60%). In addition, the following three parts were incorporated in each arbitrary primer: (i) the selective nucleotides were 3 to 4 nucleotides at the 3' end, (ii) the "core," consisted of 4 to 6 nucleotides with AT or GC rich regions, and (iii) a filler sequence makes up the 5' end (Li and Quiros 2001). The arbitrary primers used in the current study were selected from the SRAP primer list published by Li et al. (2003). The arbitrary primers were 5' end-labeled with IR dye 700 or IR dye 800 for autodetection by the Li-Cor Global DNA Sequencer (Li-Cor Biosciences, Lincoln, NE), or 5' end-labeled with 5-FAM dye for autodetection by the ABI Avant 3100 DNA sequencer (Applied Biosystems, Foster City, CA). The fixed and labeled primers were made by Qiagen-Operon (Alameda, CA). Table 1 lists primers used in this study.

PCR Protocols

For the Li-Cor Genotyper system PCR was conducted with a final reaction volume of 15 μL in 96-well microtiter plates in a GenAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA) with the following components: 2 μL of the 10 to 30 ng μL^{-1} DNA sample, 1.5 μL of 10 \times reaction buffer (Qiagen, Valencia, CA), 1.0 μL of 25 mM MgCl_2 , 1 μL of 5 mM dNTPs, 0.3 pmol each of 700- and 800-IR dye labeled arbitrary primers, 10 pmol of the fixed primer, and 1.5 units of *Taq* DNA polymerase (Qiagen, Valencia, CA). The PCR was performed by initially denaturing template DNA at 94°C for 2 min; then 5 cycles at 94°C for 45 s, 35°C for 45 s and 72°C for 1 min; followed by 35 cycles at 94°C for 45 s, 50°C for 45 s, and 72°C for 1 min; then a final extension step at 72°C for 7 min. Note that a lower annealing temperature was used the first five cycles to ensure amplification of multiple fragments, followed by the minimum annealing temperature of 50°C for the last 35 cycles.

Table 1. Four multiplex and three single-plex PCR used to generate TRAP markers for candidate disease resistance sequences in common bean mapping population BAT 93/Jalo EEP 558.

EST accession–source and fixed primer sequence	Arbitrary primer(s)	Sequence [5' filler, core (<i>italics</i>), selective nucleotides '3]	TRAPS/mapped	TRAP designation
A20I01a/Helianthus (LRR) 5'CCGAGTTGGTATGCTTGT'3	Sa12–700	5'TTCTAGGTAATCCAACAACA'3	poor rxn†	
B14G14b/Helianthus (NBS) AATCTCAAGGACAAAAGG	Ga5–800	GGAACCAAACACATGAAGA	12/9	PM1.(bp size)
A14H20a/Helianthus (kinase) CGAATCTCCACTAAACCC	Odd15–700	GCGAGGATGCTACTGGTT	2/2	PM2a.
B18F12a/Helianthus (NBS/LRR) GCTTCAGAGCATTGAAGT	Ga5–800	GGAACCAAACACATGAAGA	16/15	PM2b.
A21B09b/Helianthus (LRR) GAAAGACGAAGGAACAGG	Sa4–700	TTCTTCTTCCTGGACACAAA	5/5	PM3a.
AY237123a/Phaseolus (NBS/LRR) CAGAACTTGTGGTGGTG	Ga5–800	GGAACCAAACACATGAAGA	5/5	PM3b.
AF084026a/Phaseolus (NBS/RGA1) CCTAAATGGGAGGAAGTG	Odd26–700	CTATCTCTCGGGACCAAAC	7/5	PM4.
	Ga3–800	TCATCTCAAACCATCTACAC	poor rxn	
	Ga5–5 FAM	GGAACCAAACACATGAAGA	25/19	NG1.
	Ga5–5 FAM	GGAACCAAACACATGAAGA	9/7	NG2.
	Ga5–5 FAM	GGAACCAAACACATGAAGA	26/18	NG3.

† Amplified products were not discernible probably because of problems with PCR or labeling of the arbitrary primer.

For the ABI DNA sequencer detection system PCR was conducted in a 15- μ L reaction mixture consisting of 20 ng DNA sample, 1.5 μ L 10 \times reaction buffer, 2.5 mM MgCl₂, 15 mM dNTPs, 0.75 pmol 5' (5-FAM) labeled arbitrary primer, 37.5 pmol fixed primer, 0.25 units of AmpliTaq DNA polymerase Stoffel Fragment (Applied Biosystems, Foster City, CA). Upon completing the PCR cycles, 7 μ L of 5 \times loading dye [containing 0.313 M Tris-HCl pH 6.8 at 25°C, 10% (w/v) SDS, 0.05% (w/v) bromophenol blue, and 50% (v/v) glycerol] were added to the reaction mixture. A 1- μ L aliquot was loaded onto a 6.5% (w/v) polyacrylamide sequencing gel in a Li-Cor Global DNA Sequencer using protocols recommended by the manufacturer. Electrophoresis was conducted at 1500 V for 3.5 h with polymorphic fragments visually scored from the printed images collected by the SAGA software. The sizes of the amplified fragments detected ranged from 40 to 1000 bp.

A total of 0.5 μ L of PCR product was added to 9.3 μ L deionized formamide and 0.2 μ L Rox labeled 500-bp size standard to resolve labeled amplified PCR product using the 3100 Avant Genetic Analyzer. Sequence analysis was conducted with a 36-cm capillary filled with POP4 at a constant temperature of 60°C. The injection protocol was 12 s at 15 A and the run protocol was 34 min at 13 A. Sequences were analyzed by the software package GeneScan and Genotyper (Applied Biosystems) and images were visually inspected for presence of polymorphic fragments. The sizes of the amplified fragments detected ranged from 40 to 600 bp.

Linkage Mapping and QTL Analysis

TRAP markers were integrated in the BJ and DX genetic linkage maps by MAPMAKER/EXP 3.0 (Lander et al., 1987). First TRAP markers were assigned to a linkage group by the GROUP command (LOD 3.0). Once grouped, the position of a TRAP within a linkage group relative to other framework markers was determined by the TRY command. Molecular marker data for the BJ population was kindly provided by P. Gepts (University of California-Davis, CA).

Association of TRAP markers with resistance to common bacterial blight (*Xanthomonas campestris* pv. *phaseoli* (Smith) Dye = *X. axonopodis* pv. *phaseoli* (Smith) Vauterin et al.), ashy stem blight [*Macrophomina phaseolina* (Tassi) Goid.], and BGYMV segregating in the DX population (Miklas et al., 2000) was determined by linear regression of disease score means on individual marker genotypes using PROC GLM (SAS Institute, 1987). *F* tests significant at *P* < 0.01 were used to indicate linkage between a TRAP and QTL.

RESULTS AND DISCUSSION

BJ Mapping Population

Eighty-five of 107 TRAP markers found segregating in the BJ population were placed on the core map (Table 1 and Fig. 1). Note that the linkage groups in Fig. 1 closely resemble the maps presented by Kelly et al. (2003) and Miklas et al. (2006), which correspond to the core map version of Freyre et al. (1998). The four multiplex PCR utilizing fixed primers from the Compositae Genomics database and visualized by the Li-Cor system detected 47 TRAP markers, 41 of which were mapped. Generally, one arbitrary primer reaction in the multiplex PCR had clearer band visualization than the partner arbitrary primer reaction, suggesting further optimization of the multiplex reaction is warranted for this particular common bean population. The three single-plex PCR visualized by the ABI 3100 DNA capillary sequencer generated 60 TRAP markers, of which 44 were placed on the BJ core map.

The fixed primers from sunflower ESTs were as efficient as fixed primers derived from common bean RGAs for generating TRAP markers, which is indicative of the conservative nature of resistance genes across species. The Ga5 primer was the most efficient arbitrary primer tested in the multiplex reactions for generating TRAPs and is the reason why it was used exclusively in this study with the single-plex reactions run on the ABI 3100 DNA capillary sequencer. Results indicate that both DNA sequence detection systems effectively generated TRAPs, but the capillary system which detected 20 TRAPs per single-plex PCR on average was more efficient than the polyacrylamide system which detected 12 TRAPs per multiplex PCR. The high level of polymorphism detected per fixed–arbitrary primer pair reaction is attributable to the core mapping population being derived from a wide cross between parents of diverse Middle American (BAT 93) and Andean (Jalo EEP558) origin.

All the primer pairs amplified TRAPs of different sizes that mapped to the exact same locations (e.g., PM1.479 and PM1.825 on B6; NG1.161 and NG1.342 on B7), which is characteristic of amplified template DNA possessing repeated sequences. Repeated DNA sequences are commonly found in R-gene clusters

Eighty-five TRAP markers were positioned across all 11 linkage groups (Fig. 1). Many of the remaining unmapped TRAP markers were linked with one another (data not shown). Only eight TRAPs were completely unlinked with another marker. Linkage group B8 had the most TRAPs with 11 and B10 the least with three. TRAP markers tended to cluster with one another, with clusters of TRAPs most pronounced on B1, B4, B6, B8, and B11. It will be interesting to see if the TRAP makers not associated with disease resistance traits on the current core map, such as those clustered toward the end of B6, are tagging nonfunctional R genes or resistance genes yet to be mapped.

Seventeen TRAP markers mapped to 11 genomic regions containing major R genes that primarily controlled resistance to specific pathotypes of the hypervariable pathogens causing anthracnose and rust diseases (Table 2). Most of the 17 R-gene-associated TRAPs (PM3b.475, NG1.122, NG1.391, PM2b.396, PM1.522, NG3.325, PM2b.570, PM3a.366, PM2b.852, NG1.514, and PM4.719) mapped near or within R-gene clusters on B4, B7, B8, and B11 (Fig. 1). There were also many TRAP markers that mapped near QTL conditioning disease resistance. The collocation of QTL with R-gene clusters and TRAP markers on B4, B7, B8, and B11, provide further support for the assumption that certain quantitative resistance is under similar mechanisms of control as R genes (Lefebvre and Chèvre, 1995). Associations of RGA and/or R genes with QTL conditioning disease resistance have been observed in common bean (Geffroy et al., 2000; Lopez et al., 2003), pepper (*Capsicum annuum* L.) (Pflieger et al., 1999) and potato (*Solanum tuberosum* L.) (Gebhardt and Valkonen, 2001).

Two TRAP markers, NG3.81 and NG3.325, generated by a fixed primer designed from the published DNA sequence for RGA1 in bean were linked with R genes conditioning resistance to bean rust on linkage group B11 in the BJ core map (Fig. 1). Lopez et al. (2003) previously mapped RGA1 (A and B variants) to B11 but in the vicinity of anthracnose resistance gene *Co-2*. Molecular characterization of the genomic region possessing *Co-2* revealed the presence of conserved se-

quence motifs belonging to the NBS-LRR disease resistance gene family (Creusot et al., 1999). It is not surprising that TRAP markers generated from a fixed primer (RGA1) associated with anthracnose resistance would map in the vicinity of rust resistance because R genes for anthracnose and rust collocate (Miklas et al., 2002; Kelly and Vallejo, 2004) on linkage groups B1, B4, and B11. Collocation of R genes, as in this example for anthracnose and rust, suggests they may derive from a common ancestral R gene that underwent duplication and divergence (Geffroy et al., 1999; Hulbert et al., 2001; Michelmore and Myers, 1998).

DX Mapping Population

There were only 21 TRAP markers generated by eight multiplex PCR in the DX population. The low frequency of 1.3 TRAPs generated per primer pair is attributable to derivation of the DX population from a narrow cross between parents (Dorado and XAN 176) of similar Race Mesoamerican origin within the Middle American gene pool. Similarly, Haley et al. (1994) using RAPDs observed less polymorphism among bean genotypes compared within a race or gene pool and more polymorphism among genotypes compared across gene pools and races.

Eight of the 21 TRAPs were unlinked, four integrated into existing linkage groups, and nine formed new partial linkage groups consisting of 2, 3, and 4 TRAP markers. None of the 21 TRAP markers were associated with any of the existing eight QTL or two R genes for rust resistance previously detected and mapped in the population (Miklas et al., 2000). However, four new putative QTL ($P < 0.01$) for resistance to ashy stem blight (2), BGYMV, and common bacterial blight in the greenhouse leaf-inoculation assay, were detected by TRAP markers (Table 3).

The TRAP H64.245, forming a partial linkage group consisting of three TRAP markers, was linked with QTL conditioning resistance to common bacterial blight expressed in greenhouse leaf inoculation assays (19 and 30% of phenotypic variation explained, R^2) (Table 3). The G64.680 marker, also a component of a partial linkage group comprised of three TRAPs, detected a QTL conferring resistance to ashy stem blight in the field (14%). The unlinked TRAP G64.345 detected a QTL for resistance to ashy stem blight (15.8%) in a different field environment. G64.700 detected a QTL for BGYMV (13.4%) in one of three test environments. These putative QTL expressed in individual environments require further proof of expression in additional environments and populations to confirm their existence.

The same genomic regions possessing H64.245, G64.680, and G64.345 also detected QTL of minor effect ($P < 0.03$) against different pathogens BGYMV (8.2%), CBB (14.5%), and BGYMV (9.8%), respectively (data not shown). The genomic regions in common bean that possess QTL conferring resistance to multiple pathogens (see review by Miklas et al., 2006) was observed by Miklas et al. (2000) in the same Dorado/XAN 176 population and in a different popu-

Table 2. Genomic association of 17 TRAP markers with major disease resistance R genes in the BAT 93/Jalo EEP 558 mapping population (see Fig. 1 for explanation of gene symbols).

TRAP marker(s)	Linkage group	R genes
PM3b.708, NG1.385	B1	<i>Co-1, Co-x, Co-w, Ur-9</i>
PM1.45	B2	<i>I, Pse-3, Co-u</i>
NG1.365	B3	<i>bc-1²</i>
PM3b.475	B4	<i>Co-10, Co-(Rvi), Ur- Ouro Negro</i>
NG1.391, NG1.122	B4	<i>Co-9, Co-3, Co-y, Co-z, Ur-5, Ur-Dorado, Pse-1</i>
PM3b.606	B6	<i>Ur-4</i>
PM2b.216	B7	<i>Bct, Co-v</i>
PM1.522	B8	<i>Ur-13, Phg-2</i>
NG3.81	B11	<i>Ur-6</i>
NG3.325, PM2b.570, PM3a.366, PM2b.852	B11	<i>Ur-7, Ur-BAC6</i>
NG1.514, PM4.719	B11	<i>Co-2, Ur-3, Ur-11, Ur-Dorado</i>

Table 3. TRAP markers in the Dorado/XAN 176 RIL population associated with QTL conditioning resistance to common bacterial blight (CBB), Bean golden yellow mosaic virus (BGYMV), and ash stem blight (ASB).

TRAP markers	Linkage between markers	CBB GH-leaf Year 1	CBB GH-leaf Year 2	BGYMV Environ-1	ASB Year 1	ASB Year 2
				<i>R</i> ² / <i>P</i> value†		
H64.245	5.6 cM	19/0.006	30/0.007			
E64.800	8.5 cM	15.6/0.000	18/0.006			
A64.725		10.2/0.004				
AT5a	21 cM					
F64.405	19 cM					
G64.680					14/0.000	
G64.345	unlinked					15.8/0.006
G64.700	unlinked			13.4/0.001		

† Phenotypic variation explained (*R*²), and probability (*P*) as determined by an *F* test.

lation by Lopez et al. (2003). Whether the TRAP markers are detecting individual QTL with pleiotropic effect against multiple pathogens or a cluster of linked QTL each with specificity for resistance to a different pathogen could not be determined in this study.

CONCLUSIONS

As observed in earlier studies, the TRAP technique detected numerous polymorphic markers that were reproducible and heritable as either dominant or co-dominant markers. TRAP markers in the BJ core mapping population mapped to 11 genomic regions with known R genes and to regions possessing QTL controlling disease resistance. TRAP markers newly identified four putative QTL conditioning resistance to bacterial, fungal, and viral diseases in the Dorado/XAN 176 population. TRAP markers, with fixed primers designed against sequences associated with disease resistance show promise for mapping regions of the *P. vulgaris* genome linked to resistance.

REFERENCES

- Blair, M.W., F. Pedraza, H.F. Buendia, E. Gaitán-Solís, S.E. Beebe, P. Gepts, and J. Tohme. 2003. Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). *Theor Appl Genet* 107:1362–1374.
- Budak, H., R.C. Shearman, I. Parmaksiz, and I. Dweikat. 2004. Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. *Theor. Appl. Genet.* 109:280–288.
- Creusot, F., C. Macadre, E. Ferrier-Cana, C. Riou, V. Geffroy, M. Seignac, M. Dron, and T. Langin. 1999. Cloning and molecular characterization of three members of the NBS-LRR subfamily located in the vicinity of the *Co-2* locus for anthracnose resistance in *Phaseolus vulgaris*. *Genome* 42:254–264.
- Ferrier-Cana, E., V. Geffroy, C. Macadré, F. Creusot, P. Imbert-Bolloré, M. Sévignac, and T. Langin. 2003. Characterization of expressed NBS-LRR resistance gene candidates from common bean. *Theor. Appl. Genet.* 106:251–261.
- Ferriol, M., B. Picó, and F. Nuez. 2003. Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. *Theor. Appl. Genet.* 107:271–282.
- Freyre, R., P.W. Skroch, V. Geffroy, A.F. Adam-Blondon, A. Shirmohamadali, W.C. Johnson, V. Llaca, R.O. Nodari, P.A. Pereira, S.M. Tsai, J. Thome, M. Dron, J. Nienhuis, C.E. Vallejos, and P. Gepts. 1998. Towards an integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. *Theor. Appl. Genet.* 97:847–856.
- Gebhardt, C., and J.P. Valkonen. 2001. Organization of genes controlling disease resistance in the potato genome. *Annu. Rev. Phytopathol.* 39:79–102.
- Geffroy, V., M. Seignac, J.C.F. de Oliveira, G. Fouilloux, P. Skroch, P. Toguét, P. Gepts, T. Langin, and M. Dron. 2000. Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localization of quantitative trait loci with genes involved in specific resistance. *Mol. Plant Microbe Interact.* 13:287–296.
- Geffroy, V., D. Sicard, J.C.F. de Oliveira, M. Seignac, S. Cohen, P. Gepts, C. Neema, T. Langin, and M. Dron. 1999. Identification of an ancestral resistance gene cluster involved in the coevolution process between *Phaseolus vulgaris* and its fungal pathogen *Colletotrichum lindemuthianum*. *Mol. Plant Microbe Interact.* 12:774–784.
- Gómez, O.J., M.W. Blair, B.E. Frankow-Lindberg, and U. Gullberg. 2004. Molecular and phenotypic diversity of common bean landraces from Nicaragua. *Crop Sci.* 44:1412–1418.
- Haley, S.D., P.N. Miklas, L.K. Afanador, and J.D. Kelly. 1994. RAPD-marker variability between and within gene pools in common bean. *J. Am. Soc. Hortic. Sci.* 119:122–125.
- Hu, J., O.E. Ochoa, M.J. Truco, and B.A. Vick. 2005. Application of the TRAP technique to lettuce (*Lactuca sativa* L.) genotyping. *Euphytica* 144:225–235.
- Hu, J., G.J. Seiler, C.C. Jan, and B.A. Vick. 2003. Assessing genetic variability among sixteen perennial *Helianthus* species using PCR-based TRAP markers. Proc. 25th Sunflower Research Forum, Fargo, ND. <http://www.sunflowerusa.com/research/research-workshop/documents/88.PDF>; verified 21 November 2005.
- Hu, J., and B.A. Vick. 2003. Target region amplification polymorphism: A novel marker technique for plant genotyping. *Plant Mol. Biol. Reporter* 21:289–294.
- Hulbert, S.H., C.A. Webb, S.M. Smith, and Q. Sun. 2001. Resistance gene complexes: Evolution and utilization. *Annu. Rev. Phytopathol.* 39:285–312.
- Kanazin, V., L.F. Marek, and R.C. Shoemaker. 1996. Resistance gene analogs are conserved and clustered in soybean. *Proc. Natl. Acad. Sci. USA* 93:11746–11750.
- Kelly, J.D., P. Gepts, P.N. Miklas, and D.P. Coyne. 2003. Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. *Field Crops Res.* 82:135–154.
- Kelly, J.D., and P.N. Miklas. 1998. The role of RAPD markers in breeding for disease resistance in common bean. *Mol. Breed.* 4: 1–11.
- Kelly, J.D., and V.A. Vallejo. 2004. A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. *HortScience* 39:1196–1207.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Ann. Eugen.* 12:172–175.
- Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daley, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181.
- Larsen, R.C., and P.N. Miklas. 2004. Generation and molecular mapping of a SCAR marker linked with the *Bct* gene for resistance to *Beet curly top virus* in common bean. *Phytopathology* 94: 320–325.
- Lefebvre, V., and A.-M. Chèvre. 1995. Tools for marking plant disease and pest resistance genes: A review. *Agronomie* 15:3–19.
- Leister, D., A. Ballvora, F. Samini, and C. Gebhardt. 1996. A PCR-based approach for isolating pathogen resistance genes from po-

- tato with potential for wide application in plants. *Nat. Genet.* 14: 421–429.
- Li, G., M. Gao, B. Yang, and C.F. Quiros. 2003. Gene to gene alignment between the *Brassica* and *Arabidopsis* genomes by transcriptional mapping. *Theor. Appl. Genet.* 107:168–180.
- Li, G., and C.F. Quiros. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in *Brassica*. *Theor. Appl. Genet.* 103:455–461.
- Liu, Z., J.A. Anderson, J. Hu, T.L. Friesen, J.B. Rasmussen, and J.D. Faris. 2005. A wheat intervarietal linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. *Theor. Appl. Genet.* 111:782–794.
- Lopez, C.E., I.F. Acosta, C. Jara, P. Pedraza, E. Gaitan-Solis, G. Gallego, S. Beebe, and J. Thome. 2003. Identifying resistance gene analogs associated with resistances to different pathogens in common bean. *Phytopathology* 93:88–95.
- Michelmore, R.W., and Meyers, B.C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. *Genome Research* 8:1113–1130.
- Miklas, P.N., and J.D. Kelly. 2002. Registration of two cranberry bean germplasm lines resistant to bean common mosaic and necrosis potyviruses: USCR-7 and USCR-9. *Crop Sci.* 42:673–674.
- Miklas, P.N., J.D. Kelly, S.E. Beebe, and M.W. Blair. 2006. Common bean breeding for resistance to biotic and abiotic stresses: From classical to MAS. *Euphytica* (in press).
- Miklas, P.N., M.A. Pastor-Corrales, G. Jung, D.P. Coyne, J.D. Kelly, P.E. McClean, and P. Gepts. 2002. Comprehensive linkage map of bean rust resistance genes. *Annu. Rpt. Bean. Improv. Coop.* 45:125–129.
- Miklas, P.N., V. Stone, M.J. Daly, J.R. Stavely, J.R. Steadman, M.J. Bassett, R. Delorme, and J.S. Beaver. 2000. Bacterial, fungal, and viral disease resistance loci mapped in a recombinant inbred common bean population ('Dorado'/XAN 176). *J. Am. Soc. Hortic. Sci.* 125:476–481.
- Pflieger, S., V. Lefebvre, C. Caranta, A. Blattes, B. Goffinet, and A. Palloix. 1999. Disease resistance gene analogs as candidates for QTLs involved in pepper-pathogen interactions. *Genome* 42: 1100–1110.
- Rivkin, M.I., C.E. Vallejos, and P.E. McClean. 1999. Disease-resistance related sequences in common bean. *Genome* 42:41–47.
- Rojas-Barros, P., C.C. Jan, and J. Hu. 2005. Mapping of a recessive branching gene in RHA 271 using molecular markers. *Proc. 27th Sunflower Research Forum, January 2005, Fargo, ND.* http://www.sunflowernsa.com/research/research-workshop/documents/Rojas_MappingGene_05.pdf; verified 21 November 2005.
- Rozen, S., and H.J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. p. 365–386. *In* S. Kravetz and S. Misener (ed.) *Bioinformatics methods and protocols: Methods in molecular biology.* Human Press, Totowa, NJ.
- SAS Institute. 1987. *SAS/STAT guide for personal computers, Version 6.* SAS Institute, Cary, NC.
- Shen, K.A., B.C. Meyers, M.N. Islam-Faridi, D.B. Chin, D.M. Stelly, and R.W. Michelmore. 1998. Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. *Mol. Plant Microbe Interact.* 11:815–823.
- Urrea, C.A., P.N. Miklas, J.S. Beaver, and R.H. Riley. 1996. A co-dominant randomly amplified polymorphic DNA (RAPD) marker useful for indirect selection of BGMV resistance in common bean. *J. Am. Soc. Hortic. Sci.* 121:1035–1039.