Integration of Simple Sequence Repeat (SSR)Markers Into a Molecular Linkage Map ofCommon Bean (*Phaseolus vulgaris* L.)

K. Yu, S. J. Park, V. Poysa, and P. Gepts

Microsatellite or simple sequence repeat (SSR) markers have been successfully used for genomic mapping, DNA fingerprinting, and marker-assisted selection in many plant species. Here we report the first successful assignment of 15 SSR markers to the *Phaseolus vulgaris* molecular linkage map. A total of 37 SSR primer pairs were developed and tested for amplification and product-length polymorphism with BAT93 and Jalo EEP558, the parental lines of an *F* 7 recombinant inbred (RI) population previously used for the construction of a common bean molecular linkage map. Sixteen of the SSRs polymorphic to the parental lines were analyzed for segregation and 15 of them were assigned to seven different linkage groups, indicating a widespread distribution throughout the bean genome. Map positions for genes coding for DNAJ-like protein, pathogenesis-related protein 3, plastid-located glutamine synthetase, endochitinase, sn-glycerol-3 phosphate acyltransferase, NADP-dependent malic enzyme, and protein kinase were determined for the first time. Addition of three SSR loci to linkage group B4 brought two separated smaller linkage groups together to form a larger linkage group. Analysis of allele segregation in the *F* 7 RI population revealed that all 16 SSRs segregated in the expected 1:1 ratio. These SSR markers were stable and easy to assay by polymerase chain reaction (PCR). They should be useful markers for genetic mapping, genotype identification, and marker-assisted selection of common beans.

The development of genetic linkage maps is of special importance for crops such as the common bean (*Phaseolus vulgaris* L.), one of the most important protein sources for the world population. The identification of molecular markers linked to traits such as disease resistance will greatly facilitate marker-assisted breeding aimed at cultivar improvement. To date, most genetic linkage maps in common beans have been based primarily on restriction fragment length polymorphism (RFLP) and/or random amplified polymorphic DNA (RAPD) markers (Freyre et al. 1998).

The discovery of microsatellite or simple sequence repeat (SSR) markers has significantly increased the marker density of linkage maps for some mammals and plants (Cregan et al. 1999a; Love et al. 1990; Murray et al. 1994). Compared with other marker types, SSR markers have a number of advantages: (1) they are codominant and PCR based; (2) they are usually multiallelic and hypervariable; (3) they appear to be randomly and uniformly distributed throughout eukaryotic genomes (Hamada et al. 1982); and (4) they are accessible to other research laboratories via published primer sequences (Saghai-Maroof et al. 1994). Although SSRs are being used in developing genetic linkage maps for a number of plant species (Akagi et al. 1996; Cregan et al. 1999a; Milbourne et al. 1998; Senior et al. 1996), no SSRs have been developed and used for mapping common beans.

To develop SSR markers for common beans, we conducted a GenBank database search to identify SSR sequences present in common bean DNA sequences (Yu et al. 1999). Our results indicated that SSR sequences are fairly abundant in common bean genome. The goal of the present study was to integrate the SSRs identified from the GenBank database into a common bean molecular linkage map derived from the cross between BAT93 and Jalo EEP558 (Freyre et al. 1998; Nodari et al. 1993). The map locations (i.e., linkage group and approximate location within a linkage group) of 15 SSR markers were successfully determined. Their potential applications in common bean genetics and breeding were also discussed.
<table>
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<tr>
<th>GenBank accession number*&lt;sup&gt;a&lt;/sup&gt; (SSR name)</th>
<th>Name in GenBank (symbol)</th>
<th>Core motifs</th>
<th>Fragment size (bp)</th>
<th>Primer sequences&lt;sup&gt;+&lt;/sup&gt; (forward, reverse)</th>
<th>Trm&lt;sup&gt;+&lt;/sup&gt; and allele no.</th>
<th>Progeny segregation χ&lt;sup&gt;2&lt;/sup&gt; (3.84)&lt;sup&gt;+&lt;/sup&gt;</th>
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Figure 1. An integrated linkage map of common bean. Seven linkage groups with 15 SSR markers are shown with their names in italic. The gap between Core4a and Core4b in linkage group B4, which was filled by the addition of three SSR loci, is shown as well.

Material and Methods

Plant Materials
A recombinant inbred (RI) population of 75 F7 bean lines was used as the mapping population for this study. This RI population was derived from the BAT93 × Jalo EEP558 F2 population used for the construction of the original Davis map (Nodari et al. 1993), and was advanced to the F7 generation by single seed descent. Twelve common bean lines from a range of pedigrees (Yu et al. 1999) were used to evaluate allelic variation of the SSRs.

Database Search and SSR Development
The GenBank database search for SSR sequences in common beans was described by Yu et al. (1999). In total, 37 pairs of SSR primers were made and analyzed (Table 1). All of the primers were synthesized by GIBCO BRL. Genomic DNA isolation, PCR amplification, and PCR product separation with denaturing polyacrylamide sequencing gel were conducted according to the conditions described by Yu et al. (1999).

Data Analysis
Goodness-of-fit tests for a 1:1 segregation ratio were performed on SSR markers segregated in the RI population with Corel Quattro Pro 7 (Table 1). The integrated linkage map for the BAT93 × Jalo EEP558 RI population was constructed using MapMaker EXE 3.0 (Lincoln et al. 1992). Initially the Assign command was used to assign all SSR markers to linkage groups of the core map developed by Freyre et al. (1998) using LOD values greater than 2.0. Then the Try command was used to position each SSR marker on its linkage group. Only the 240 framework marker data were used here for analysis and development of the integrated linkage map (see links to individual linkage group maps at [http://agronomy.ucdavis.edu/gepts/geptslab2.htm](http://agronomy.ucdavis.edu/gepts/geptslab2.htm)).

Nomenclature
The SSR was named with the following naming system: PV stands for *Phaseolus vulgaris*, followed by the repeat motif in...
lowercase and an arbitrary number starting from 001 for each distinct repeat motif such as at001 or ctt001. A repeat motif of ag, ga, ct, and tc or att, tta, aat, taa, and tat, etc., is considered the same. For imperfect or compound repeats such as (GAA),TAG(GAT), the repeats with a higher number, in this case gat will be used. If two or more different repeats, for example, (ATCC),AG(TAC), have the same number of repeats, the repeat motif at the 5’ end will be used to designate the SSR name such as PV-atcc in this example. Therefore an SSR named PV-at001 or VA-ag001 means that the number one SSR found in Phaseolus vulgaris with an “at” repeat motif or the number one SSR found in Vigna aconitifolia with an “ag” repeat. This nomenclature is taking into consideration future expansion by other researchers with other technologies.

Results and Discussion

Allelic Variation of SSRs and Their Segregation

Twenty-four of the 37 SSR loci were polymorphic among the 12 bean genotypes with 2–10 alleles and 12 of them were monomorphic (Table 1). Among the 24 polymorphic loci, 13 (54%) of them are diallelic. When the 37 SSRs were tested with BAT93 and Jalo EEP558 for product length polymorphisms, 16 pairs of the primers could generate polymorphic bands between the parents, 14 pairs produced clear band(s), but without polymorphisms, 2 pairs amplified poorly, and 5 pairs failed to amplify. The frequency of polymorphic SSRs and of SSRs that could generate clear PCR products for this pair of the parental lines were 43% and 81%, respectively. Similar polymorphic frequencies were also observed when using the 37 SSRs in other genetic mapping populations (Pauls KP, personal communication). Poor amplification could be due to unsuitable primer sequences and/or improper PCR conditions (Akagi et al. 1996). The lack of amplification of an allele in the bean lines could be the result of divergence in the sequences flanking the SSRs, creating a null allele (Smulders et al. 1997). However, it could also result from the production of an undetectable amount of PCR products due to improper PCR conditions for that pair of primers (Lavi et al. 1994). In the latter case, optimization of PCR conditions would be necessary for individual lines or cultivars from which no fragments were amplified.

Among the 37 SSRs, 15 have dinucleotide repeats, 9 have trinucleotide repeats, 12 have tetranucleotide repeats, and 1 has a hexanucleotide repeat. Twelve (80%) of the 15 dinucleotide SSRs, 6 (66.7%) of the 9 trinucleotide SSRs, and 5 (41.7%) of the 12 tetranucleotide SSRs were polymorphic among the 12 bean lines (Table 1). Between the two parental lines, BAT93 and Jalo EEP558, 9 (60%) of the 15 dinucleotide SSRs, 2 (22%) of the trinucleotide SSRs, and 4 (33%) of the tetranucleotide SSRs showed product length polymorphism. In general, dinucleotide SSRs are more polymorphic. However, highly polymorphic trinucleotide SSRs were found in soybean genome (Rongwen et al. 1995). No clear relationship between total repeat length and the degree of polymorphism was observed in this study. Bell and Ecker (1994) also failed to detect a correlation between polymorphism information content and repeat length up to 50 nucleotides among Arabidopsis thaliana strains. The fact that polymorphisms were detected from shorter repeats such as (AT), and (GAAT), in this study (Table 1) demonstrated that shorter simple repetitive DNA sequences are also important sources for developing polymorphic SSR markers in common beans.

When a chi-squared test was performed at the 5% level of significance, no SSR markers deviated from the expected 1:1 segregation ratio (Table 1). No preferential transmission of maternal or paternal alleles was observed when all loci were considered. It is not unusual to find distorted segregation ratios in populations where a large number of markers have been analyzed. For example, 105 of 599 marker loci (18%) used for the core map showed a distorted segregation as determined by goodness-of-fit tests for a 1:1 ratio at P = .05 (Freyre et al. 1998). The non-skewed segregation results from this study may be due to the small number of SSRs tested. Skewed SSR markers were observed in other plant species (Kijas et al. 1997; Senior et al. 1996).

It should be noted that one of the SSR markers, VA-ag001, was identified from Vigna aconitifolia, a species distantly related to P. vulgaris. This SSR segregates normally in the RI population. In soybeans, up to 65% of the soybean SSR primer pairs amplified SSRs within Glycine and 85% of them were polymorphic within G. clandesina (Peakall et al. 1998). It should be interesting to test the applicability of the SSRs from P. vulgaris in Vigna species and vice versa.

Usually SSRs are codominant genetic markers following Mendelian inheritance (Beckman and Soller 1990). However, one SSR marker, PV-atgc001, demonstrated a dominant/recessive inheritance (data not shown). This is not unusual; occasionally dominant/recessive RFLP markers were also observed (Yu et al., unpublished data). The failed amplification in the Jalo line at the PV-atgc001 locus could be due to divergence in the sequences flanking the SSRs or other mutations such as deletion of the primer binding sites or a large insertion between the primers.

Distribution of SSRs on the Common Bean Map

The segregation information collected from the 16 polymorphic SSRs in this study was analyzed with MapMaker EXE 3.0 along with data from 240 framework markers for the core map (see links to individual linkage group maps at http://agronomy.ucdavis.edu/gepts/geptslab2.htm (Freyre et al. 1998). Because 13 markers were assigned to more than one linkage group and 11 markers including one SSR could not be assigned to any of the linkage groups, only 232 markers including 15 SSRs were placed on the integrated linkage map. No SSRs were assigned to linkage groups B1, B7, B8, and B10. Figure 1 shows the successful assignment of the 15 SSRs to seven bean linkage groups labeled with their names in italic letters.

SSR markers (PV-ct001, PV-gccacc001, and PV-gaat002) from genes coding for sn-glycerol-3-phosphate acyltransferase, DNAJ-like protein, and chalcone synthase, respectively, were mapped on linkage group B2. PV-gaat002 from the chalcone synthase promoter was mapped to the end of linkage group B2 with a map distance of 2.9 cM from the previously mapped RFLP locus using the chalcone synthase gene as a probe. The discrepancy between the map position of the SSR and RFLP markers could be due to experimental errors. The SSRs (VA-ag001 and PV-at008) from Vigna protein kinase gene and ribonuclease-like pathogenesis-related (PR) protein gene were mapped to linkage group B3. PV-at008 was mapped to the position as the previously mapped PR-1 gene in the Davis map (Freyre et al. 1998). As expected, two distinct SSRs (PV-gaat001 and PV-at003) from the small subunit of ribulose 1,5-bi-phosphate carboxylase/oxygenase (RbcS) gene were mapped to the position previously identified by the RbcS isozyme assay on linkage group B4. In addition, three dis-
tinct SSRs (PV-atgc001, PV-atgc002, and PV-ag004) from a family of genes coding for lectin or phytohemagglutinin (PHA) were mapped to a similar position as the previously mapped lectin gene (Lec) on B4, and PV-ag004 is the most robust locus with three alleles. Another SSR (PV-ctt001) from the protein kinase-1 gene was also mapped on linkage group B4. PV-at006 locus from an endochitinase gene, PV-at004 from a plastid-located glutamine synthetase gene, PV-at007 from a NADP-dependent malic enzyme gene, and PV-ag001 from the PR protein 3 gene were mapped on linkage groups B5, B6, B9, and B11, respectively. Only one SSR marker (PV-ag005) from the 5′ flanking sequence of a glutamine synthetase gene could not be positioned on any of the 11 bean linkage groups (Figure 1). Since seven of the genes were positioned to the bean linkage map for the first time, they could be used for identification of gene and trait relations.

Because the bean molecular map is reasonably saturated, the overall map coverage of the bean genome was not improved with addition of the 15 SSR markers. However, there was a gap present in linkage group B4 on the core map (see links to individual linkage group maps at http://agronomy.ucdavis.edu/gepts/geptslab2.htm). The integration of 3 SSR loci on that linkage group generated a new framework and brought the two separated smaller linkage groups together to form a larger linkage group (Figure 1; B4). As a result, the map positions for some previously mapped markers are slightly changed (Figure 1; B4, Core4a, and Core 4b). With 13 SSRs from 13 distinct genes (PV-gaat001 and PV-at003 are from the same Rbsc gene; PV-atgc001, PV-atgc002, and PV-ag004 are from the PHA gene family) mapped to 13 different locations on 7 different linkage groups, it may be reasonable to predict that SSR markers may be dispersed randomly throughout the bean genome. Clearly, more bean SSR loci will have to be identified and mapped before the question of randomness of their distribution can be answered with certainty. Although random distribution of SSRs was reported in several plant species (Wu and Tanksley 1993; Bell and Ecker 1994; Senior et al. 1996), clustering of SSR loci was observed in soybeans (Cregan et al. 1999a). Likewise, mapping of ISSRs in common beans also suggests clustering of microsatellite sequence-based markers.

Previous studies in other plant species indicated that all SSRs mapped thus far appeared to be single locus markers (Akikaya et al. 1995; Senior et al. 1996; Thomas and Scott 1993). None of the SSRs in this study produced more than one set of bands that segregate independently. This is in contrast with the large number of amplification products typically found when using RAPDs (Williams et al. 1990) or AFLPs (Vos et al. 1995). Although SSRs produce less information per marker, the problems of repeatability typically associated with RAPDs are minimized. Furthermore, SSR markers avoid the difficulties of genetically dissecting multiple-banding patterns with respect to the multiple genetic loci that often occur with AFLP markers. Because SSRs are selected as single-locus markers, they map to reproducible positions in the genome without the complications that are associated with other types of markers. As a result, SSR markers can be used as dependable, comparable, and highly informative points of reference across a wide range of bean populations for genomic map integration and comparative genomic analysis.

**Application of SSRs**

Plant breeders have become increasingly interested in marker-assisted selection for efficient and precise transfer of genes conditioning important agronomic traits (Lee 1995). The successful use of marker technology depends on the availability of a large number of highly polymorphic markers, close linkage between the marker locus and the gene of interest, and the ease of using the markers. In common beans, most of the molecular markers used today are RAPD markers which are dominant/recessive, less polymorphic, and not reproducible. Because SSR markers are codominant, more polymorphic and stable, and much easier to assay compared with AFLP or RFLP markers, they should be very useful for genomic mapping and gene (or QTL) tagging if a large number of SSRs are developed. In addition, polymorphisms of SSRs can be detected by PCR and automated DNA sequencing, thus a great number of DNA samples can be analyzed at the seedling stage for marker-assisted selection in practical plant breeding programs (Cregan et al. 1999b). Furthermore, highly polymorphic SSR loci are valuable tools for identification of bean lines/cultivars and for genetic diversity analysis among bean germplasm. Although the sample size in this study is small, it demonstrates the potential of developing a large quantity of SSRs in this crop. The introduction of a codominant, informative, and easy to detect marker class, along with 37 pairs of SSR primer sequences, will be useful for the bean research community. Currently work is under way to generate additional bean SSR markers through screening small insert genomic DNA libraries.

**References**


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