PA11

SNP Marker Development and SNP-based Genetic Map Construction in Soybean

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Objective

The objectives of this study were to develop SNP markers by comparing aligned sequenced DNA fragments generated by PCR between two soybean genotypes and map these SNPs to the SSR frame map of soybean.

Materials and Methods

- λ Plant materails: 'Pureunkong', 'Jinpumkong' and 90 RIL population derived from F_2 progeny of Pureunkong × Jinpumkong 2 as mapping population.
- λ Primers were designed from Tentative Consensus sequences (TCs) (http://www.tigr.org) from roots of 7 day old 'Bragg' supernodulating mutant.
- λ SNPs survey: Direct sequencing of PCR product and Seqscape software for SNPs survey.
- λ SNP genotyping: AcyclPrime FP SNP Detection Kit (PerkinElmer Life Sciences, MA, USA).

Results and Discussion

Approximately 66% of the primer sets produced a single PCR product were able to obtain reliable sequence data and 15% of these had at least one SNP (Table 1). Overall, a total of 237kbp, consisting of 132 kbp of coding sequence and 105 kbp of non-coding sequence, were surveyed in each of two soybean genotypes. A set of 118 TC-SNP markers was developed between Pureunkong and Jinpumkong 2 from the analysis of 767 unigenes. Until now, 69 SNP loci were mapped into the RILs. The genetic map comprised of 19 linkage groups (LGs) span 984.2 cM of Kosambi map distance (Fig. 1). A total of 10 mapped SNP markers were located to be linked with QTL for soybean seed protein content. This study demonstrates that these TC-SNPs greatly facilitated the development of a genetic map, and provided putative candidate genes for some important agronomic traits related to nitrogen metabolism which could be valuable for linkage disequilibrium studies for candidate gene approach in soybean. In addition, these SNPs may be promising for marker-assisted selection in plant improvement and for filling in gaps of pre-existing SSR-based maps.

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Table 1. Number of PCR primers designed and results of sequence analysis.

Primers designed	795	
Primers produced a single band	687	86%
Primers produced multiple bands (determined by agarose analysis)	44	5.5%
and multiple amplicons (determined via sequence analysis)	165	20%
Primers produced no band	64	8%
Single amplicon verified with sequence analysis	522	66%
Fragments with at least 1 SNP	118	15%

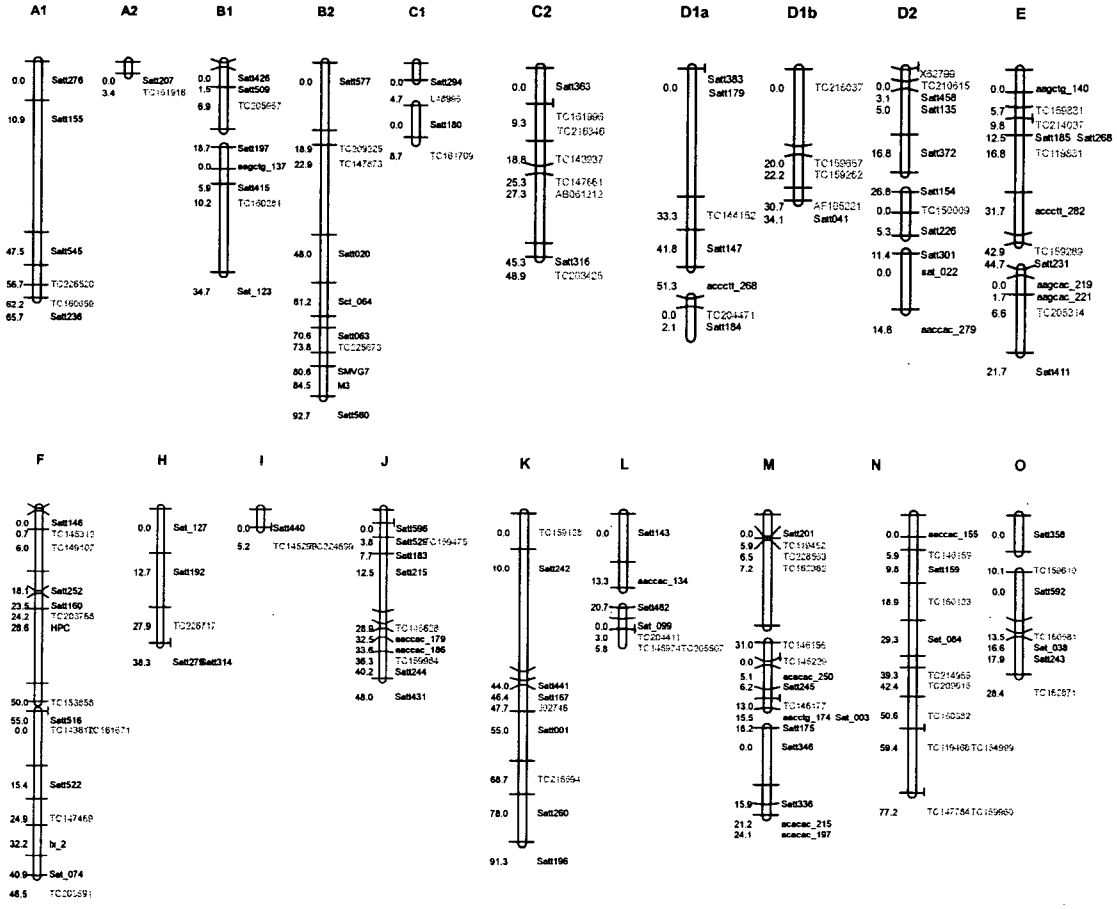

Fig.1 Genetic linkage map of the RIL population of Pureunkong x Jinpumkong 2. The left and right-hand sides show marker names or estimated map distance (cM), respectively. LGs were designated according to Cregan et al. (1999)