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Understanding of Genome Duplication for SNP Marker Development in Soybean

Chunmei Cai, Kyujung Van, Moon Young Kim and Suk-Ha Lee*
School of Plant Science, Seoul National University, Seoul 151-921, Korea

Objective

The objective of this study was to achieve a comprehensive understanding of the effects of gene duplication in SNP development, identify duplicated genes and investigated the divergence of homoeologous regions in the soybean genome.

Materials and Methods

After primers amplified with genomic DNA of 'Pureunkong' and 'Jinpumkong 2' produced double PCR products and poor quality sequence were selected, they were excised and cloned into TA vector. Ten independent clones were chosen randomly for sequencing. In addition, two pairs of duplicated genes with SNPs were mapped using RILs derived from the cross of Pureunkong × Jinpumkong 2.

Results and Discussion

With eight primer sets, sequences were separated into two or three groups in two genotypes, even though they were produced from the same primer (Table 1 and 2). The comparison of sequences between direct sequencing and TA cloning showed complete consistency. Producing multiple products leaded to poor sequencing results in direct sequencing, indicating the amplification of members of a gene family. A comparison of the paralogous sequences revealed that the sequence similarities between duplicated regions were conserved. Divergence resulted from a combination of point mutation and insertion/deletion events (Table 1 and 2). Two pairs of duplicated genes with SNPs were mapped to confirm that they are present in different positions. Two paralogous of TC144678 and TC159079 were mapped in linkage group (LG) c1, k and H,F respectively (fig.1). Conclusive evidence of gene duplication was provided at the DNA sequence level in soybean. An understanding of gene duplication in a species should provide valuable insights into its evolutionary history and genome structure. The information presented here should be useful to complement chromosome walking and positional cloning in soybean.

* Corresponding author : Suk-Ha Lee Tel : 02-880- 4545 E-mail : sukhalee@snu.ac.kr

Table 1. Sequence comparison between paralogous sequences produced from a single band in Pureunkong.

PCR primer	Group 1		Group 2		No. of Indels	No. of mutations	Identity (%)	
	No.of clones	Length(bp)	No.of clones	Length(bp)			Coding	Noncoding
TC119773	5	1347	5	1292	25	80	98	42
TC144678	3	1368	7	1360	29	92	94	85
TC151260	5	1434	5	1429	65	85	95	85
TC218232	3	1698	7	1698	104	80	98	84

Table 2. Sequence comparison between paralogous sequences produced from two bands in Pureunkong.

Primer	Upper band				Lower band		No. of Indels	No. of mutations	Identity (%)	
	Group 1		Group 2							
	No.of clones	Length (bp)	No.of clones	Length (bp)	No.of clones	Length (bp)			Coding	Noncoding
TC119115	4	981	6	975	2	778	4	59	90	Not available
TC159079	2	159			2	1139	5	37	99	Not available
TC204444	2	537			2	300	3	25	54	Not available
TC204932	2	985			2	704	98	10	88	Not available

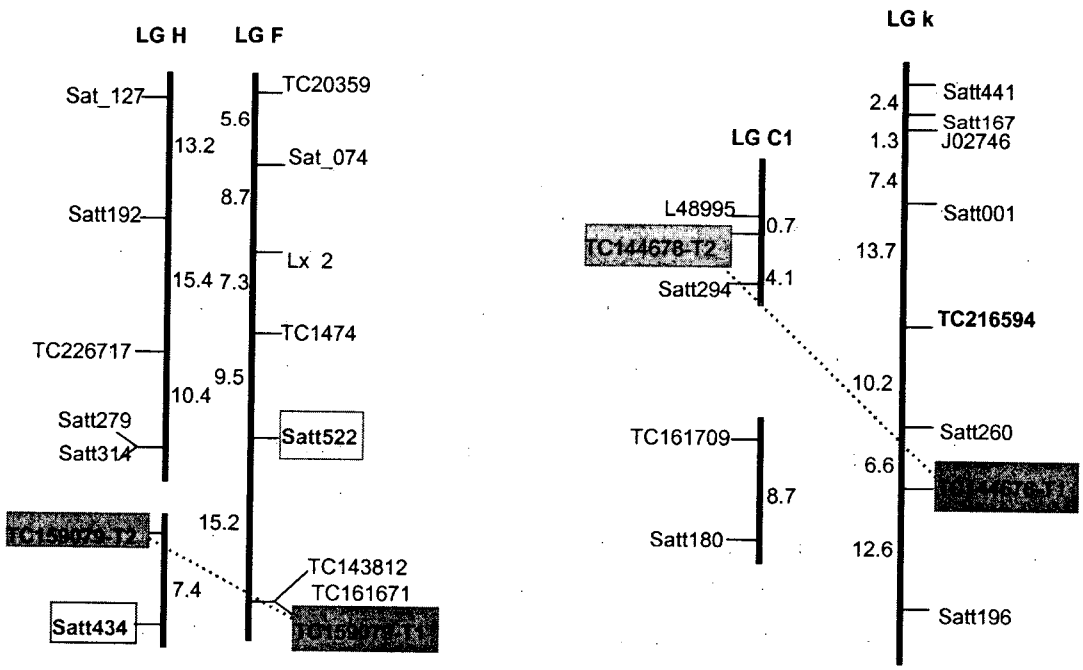

Fig. 1. Paralogous mapping of TC159079 and TC144678 on soybean SSR-based frame map. The right and left-hand sides show marker names and estimated map distance (cM), respectively. LGs were designated according to Cregan et al. (1999).