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Gene structure and characterization of lipoxygenase-2 gene (Lx_2) in soybean

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Objectives

The objectives of this study are to discover variation of lipoxygenase-2 gene at the nucleotide level and to elucidate its expression pattern between Pureunkong and Jinpumkong 2.

Materials and Methods

○ Soybean genotypes: Pureunkong (Lx_2 dominant allele), Jinpumkong 2 (Lx_2 null allele), 90 RILs (single-seed descent from Pureunkong x Jinpumkong 2, NPJ3-NPJ92), and other 5 soybean cultivars.

○ Isolation of 5'-flanking sequences of Lx_2 by chromosome walking DNA Walking SpeedUP™ Premix Kit (Seegene, Seoul, South Korea).

○ Confirmation of the result of chromosome walking by PCR.

○ Co-segregation test of the 175 bp insertion with Lx_2 gene among the 90 RILs.

○ Total RNA extraction from young leaves and mid-maturation seeds 35 days after anthesis.

○ RT-PCR: SuperScript™ One-Step RT-PCR (Invitrogen Corporation, Carlsbad, CA, USA).

Results and discussion

The amplified length of Pureunkong and Jinpumkong 2 by chromosome walking was 1,713 bp and 1,891 bp, respectively. Five SNPs and one large 175 bp insertion were identified in promoter sequence of Jinpumkong 2, compared to that of Pureunkong (Fig. 1). With primer designed to amplify genomic DNA around the 175 bp insertion, only Jinpumkong 2 and NPJ15 showed the insertion (Fig. 2).

90 RILs (from NPJ3 to NPJ92) were screened with the primer and their band patterns were also compared with the SNP T/C marker previouisly developed for co-segregation of Lx_2. And the insertion was found to be co-segregated with Lx_2, suggesting that the primer can be used as a marker for screening of lipoxygenase-2 in soybean (Table 1). The band pattern of NPJ38 was discordant with the result of SNP T/C marker and it might be caused by the SNapShot error or segregation between the SNP site and the insertion site.

The expression patterns of Lx_2 were assessed using reverse transcription (RT)-PCR (Fig. 3). Lipoxygenase-2 was preferentially expressed in seeds in both cultivars, even though Jinpumkong 2 lacks the enzyme in seeds. Since gene expression is a multi-step process, further study is needed to clarify which step is responsible for the lack of the enzyme and how it works.

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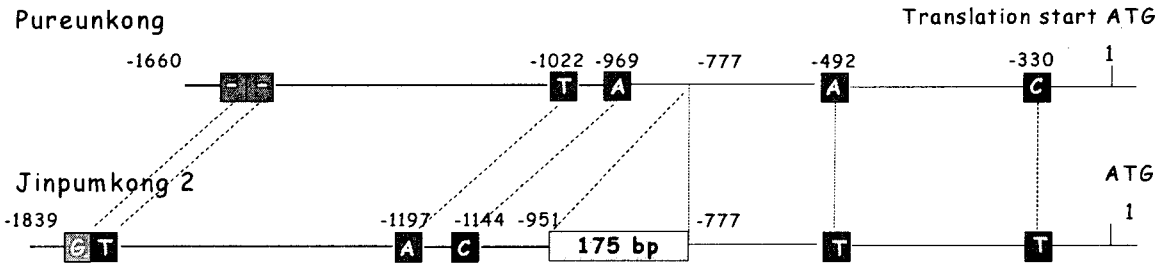

Fig. 1. Diagrammatic representation of the differences in 5'-flanking sequences of the Lx_2 gene between Pureunkong and Jinpumkong 2.

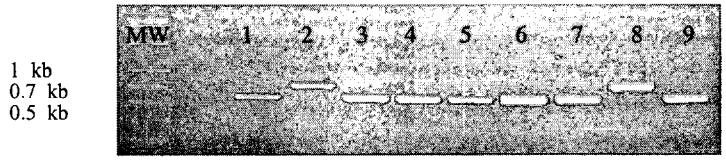

Fig. 2. Confirmation of the 175 bp insertion by PCR. Lane 1: Pureunkong, lane 2: Jinpumkong 2, lane 3: SS2-2,. lane 4: PI96188, lane 6 : Danbaekong, lane 7: Pungsannamulkong, lane 8: NPJ 15, lane 9: NPJ 17.

Table 1. Co-segregation test of the 175 bp insertion with Lx_2 gene.

Cultivar	Purenkong	Jinpumkong 2	NPJ3	...	NPJ38	...	NPJ58	...	NPJ92
SNP T/C marker	1	3	3	...	1	...	2	...	1
175 bp	1*	3*	3	...	3	...	2*	...	1

1* = homozygote for the Pureunkong band.

2* = heterozygote for parental bands.

3* = homozygote for the Jinpumkong 2 band.

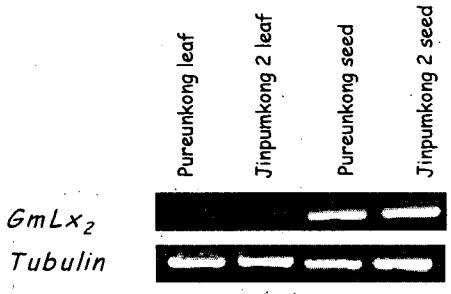

Fig. 3. Expression patterns of the $GmLx_2$ gene. A soybean tubulin gene was used as a control for equal loading.