◎ 유전·육종·생명공학·품질·가공 분야

A01

Conversion of Single Nucleotide Polymorphism (SNP) into Allele - Specific PCR Marker for Supernodulation in Soybean

Moon Young Kim, Kyujung Van, and Suk-Ha Lee*

School of Plant Science, Seoul National University, Seoul 151-744, Korea

Objective

The present study was performed to identify single nucleotide polymorphism (SNP) of the gene *GmNARK* (*Glycine max* nodule autoregulation receptor kinase) between Sinpaldalkong 2 and supernodulating mutant SS2-2, isolated form M_2 families mutagenized by ethymethane sulfonate. Furthermore, the identified SNP for *GmNARK* was converted into a allele-specific PCR marker.

Materials and Methods

Plant materials: A F_2 population of SS2-2 x Sinpaldalkong 2, six normal-nodulating cultivars, and two super-/hypernodulating mutants

SNP discovery for the *GmNARK* gene : Sequencing of PCR products corresponding to *GmNARK* (Genbank Acc. No. AY16665) of Sinpaldalkong 2 and SS2-2 and aligning of the sequences using Seqscape software (Applied Biosystems)

Design of allele-specific primers for SNP sites on GmNARK using SNAPER program

Allele-specific PCR cycling condition: 5 min at 95°C followed by 28 cycles of 30 s at 95°C and 1 min at 64°C

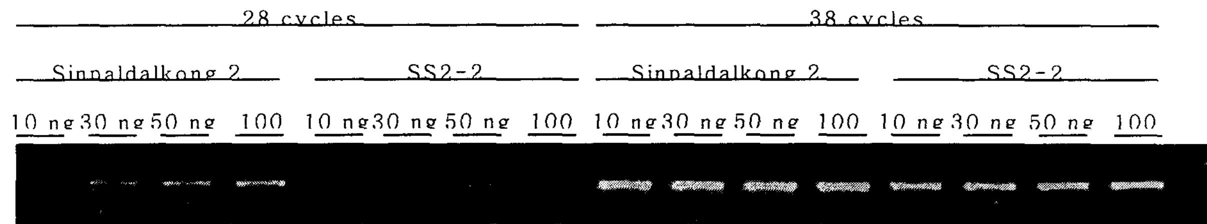
Results and Discussion

The *GmNARK* gene was found to have a SNP between wild type Sinpaldalkong 2 and its supernodulating mutant SS2-2. Transversion of A to T at 959 bp of the *GmNARK* sequences to change Lysine (AAG) to stop codon (TAG), terminating its translation in SS2-2. Base on the identified SNP of *GmNARK*, five primer pairs specific to each allele were designed using the Web Snaper program to develop a single nucleotide amplified polymorphism (SNAP) marker for supernodulation. One A-specific primer pair produced a band present in only Sinpaldalkong 2 and two T-specific pairs showed a band in only SS2-2. Both complementary PCRs using each allele-specific primer pair were performed to genotype supernodulation against F_2 progeny of Sinpaldalkong 2 x SS2-2. Among 28 individuals with normal phenotype, 8 individuals having only A allele specific band were homozygous and 20 individuals were found to be heterozygous to the SNP A/T, having both bands. Twelve supernodulating individuals showed an only band specific to the T allele.

This allele-specific PCR marker, called as SNAP marker for supernodulation could be analyzed only through simple PCR and agarose gel electrophoresis. The marker might therefore be faster and cheaper than other genotyping methods such as a cleaved amplified polymorphic sequence marker with demand of restriction enzymes.

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

(A)



(B)

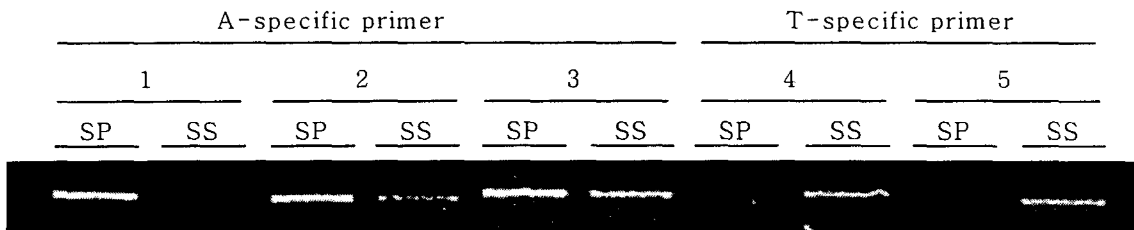


Fig 1. (A) Analysis of PCR specificity for a SNAP primer pairs according to template DNA concentrations and amplification cycles. The primer pairs specific to the A allele for the SNP site of *GmNARK* was used. (B) Specificity of five allele-specific primer pairs for each allele. The numbers of 1 to 5 represent 5 primer pairs, respectively. SP, Sinpaldalkong 2; SS, SS2-2.

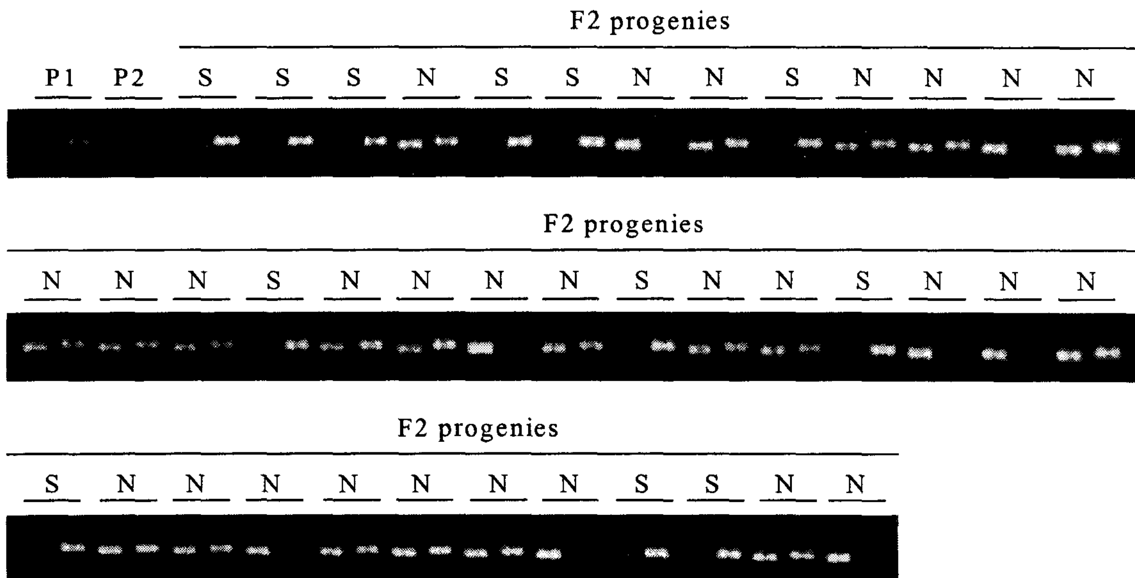


Fig 2. Genotyping of the SNAP marker for supernodulation in the F2 progeny of SS2-2 x Sinpaldalkong 2. Band patterns of the SNAP markers were scored as normal (N) and supernodulating (S). For each line the left and right lanes show the amplification products obtained with the A and T-specific primers, respectively. The lines showing both bands specific to each allele were heterozygous.