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KASP Marker Validation for Novel SNPs of *Sg-1* Gene that Determines Soyasaponin Derivatives

Sang-Beom Lee[1], Joo-Mi Jeon[1], Jung-Kyung Moon[1], Dool-Yi Kim[1], Mi-Suk Seo[1], Yu-Na Kim[1], Hyeon-Jung Kang[1], Soo-Kwon Park[1]*

[1]National Institute of Crop Science, RDA, Wanju 55365, Republic of Korea

[Introduction]

Soyasaponin is oleanane-triterpenes and are classified into group A saponins and 2,3-dehydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one DDMP saponins by chemical structure. However, the proportion of DDMP saponins in soybeans is much smaller than that of group A saponins, and their chemical structure is unstable, making extraction and analysis difficult in soybeans. Therefore, we performed genome-scale profiling of group A soybean saponins, which not only induce astringency and bitterness, but also play a role in reducing the number of inflammatory cells infiltrating the liver. As a result of the last study, 13 novel SNPs were discovered by analyzing the genomic sequence variation of the candidate gene Sg-1 involved in group A saponin biosynthesis. In this study, 13 novel SNPs were converted into KASP markers to verify markers that discriminate group A saponin derivatives in soybean varieties.

[Materials and Methods]

In the genomic sequence of Glyma.07g254600, thirteen novel SNPs closely associated with Group A saponin content of 328 accessions were converted to KASP (Kompetitive Allele Specific PCR) SNP genotyping. The 328 resources were clustered based on the calculated genetic distance using the 131,625 SNPs that were included in the 130K SoySNP. KASP uses two fluorophores to distinguish genotypes. In this study, two 5' fluorescently labeled oligos, FAM and HEX, were used. These oligo sequences are designed to interact with the sequences of the tails of the allele-specific primers.

[Results and Discussion]

Thirteen SNPs variants were identified in the genomic sequence of Glyma.07g254600, of which six significant variants were converted to KASP markers. The Six KASP markers (3, 4, 5, 6, 7 and 13 SSA) genotypes were classified into reference and alternative alleles by fluorescence signals (FAM and HEX), and these genotypes discriminated 98.5 - 99.4% of the Aa and Ab derivatives. In SSA3, there was a 6bp deletion in the 5'UTR, and amino acid substitutions were made in 4, 5, 6, 7 and 13 SSA, which are non-synonymous SNPs. In addition, the contents of Aa and Ab derivatives by genotype of these six markers were significant at $p < .001$. This result is an important basis for discriminating group A saponin derivatives, and can be utilized as important data to identify the function of the derivatives in the future .

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*Corresponding author: E-mail. sookwonpark@korea.kr Tel. +82-063-238-5324