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High-throughput SNP survey and screening of soybean mutants by DHPLC

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Targeted induced local lesion in genomes (TILLING) allows high-throughput detection of single nucleotide mutation as essential target for reverse genetics in soybean. Many practical applications of screening single nucleotide polymorphism (SNP) have been developed. Among them, denaturing high performance liquid chromatography (DHPLC) is known as precise, economical and suitable to high-throughput mutation screening. To optimize the conditions, 92 amplicons obtaining SNPs in soybean expressed sequence tag (EST), which previously identified between two soybean cultivars, Pureunkong and Jinpumkong 2 were screened by DHPLC. After all the amplicon sizes were checked on the agarose gel to determine buffer compositions, the whole sequences included primer sequence were used for set up the oven temperature of DHPLC. The amplicons over 1kb that produced blunt peaks were not suitable to DHPLC detection, because it would not distinguishable if mutation ratio is low in pooled genomes. Amplicons that containing intron with exon generally have uneven melting temperature then represent obscure peaks. Usually DHPLC could not identify the number of SNPs, but an amplicon with two SNP sites showed two kinds of heteroduplex peaks in one graph. With these selected primers, M_2 EMS mutant lines from Sinpaldalkong 2 and Jack will be screened.
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SNP discovery and mapping of genes related to soybean seed protein content

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Soybean is considered as an excellent source for seed protein among many crops. Improvement of the quality and quantity of soybean seed constituents is one of the most important targets in soybean breeding. The objective of this study was to identify single nucleotide polymorphisms (SNPs) of seed protein content related genes in soybean. A population of 90 recombinant inbred lines that was developed from a cross between Pureunkong and Jinpumkong 2 was used for mapping. With 76 primer sets designed from seed protein content related genes, a total of 18 SNPs were detected from nine amplicons. Five SNP markers were mapped on three linkage groups (LGs), AF162283 and AF271796 on LG G, TC225142 on LG M, and U59425 and X16469 on LG N. AF162283 and AF271796 markers significantly associated with previously known quantitative trait loci for soybean seed protein at the locus A245_2 and A235_1 on LG G. This study shows that SNPs for seed protein content as a quantitative trait may contribute for marker-assisted selection in cultivar improvement.