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Fine-mapping and characteristic of a novel resistance gene conferring resistance to soybean mosaic virus

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Soybean mosaic virus (SMV) is one of the most popular virus diseases in China even all over the world, which affected the yield and quality of soybean seriously. In China, the infection range of soybean mosaic virus disease had reached 70 to 95 percent every year, leading to the loss of yields reaching 10 to 20 percent, even up to 50 percent in some areas. To identify the gene(s) involved in resistance to SMV G2 strain, the association of genotypic and phenotypic variations was examined in natural population with 440 soybean accessions, recombinant inbred lines (RILs) and F2 generation derived from a cross between soybean line 'Dongnong93-046' (immune to SMV G2 strain) and 'Hefeng25'(susceptible to SMV G2 strain). A total of 35,760 SNPs with minor allele frequencies (MAFs) > 0.05 were found among the 440 accessions by Specific Length Amplified Sequencing (SLAF-seq) and a single locus on Chr.13 was detected by genome wide association study on the resistance of soybean to SMV G2 strain. The locus was verified by association mapping through sequencing of resistant/susceptible gene pool from RILs based on Bulk separate analysis (BSA) and SLAF-seq method. A total of 141 markers were different between resistant/susceptible gene pool to SMV and three continuous candidate genomic regions associated with plant resistance to SMV G2 strain were generated, which were located in a range of 27.63Mbp-30.35Mbp on Chr.13. The segregation ratios of F2 individuals from the crosses of 'Dongnong93-046' and 'Hefeng25' were 760 resistant: 240 susceptible, which is consistent with the expected allelic frequency of a 3:1 ratio. Genetic analysis using graphical mapping indicated that resistance gene in 'Dongnong93-046' was co-segregated with SSR64 through screening 162 molecular markers in the interval of association mapping. Rsmv1 gene, which was approximately 2-kb region to SSR64, was cloned as a resistance candidate gene to SMV G2 strain. A total of four haplotypes were found in the coding region of Rsmv1 gene based on 20 resistant/susceptible soybean varieties and only one of them was resistant type with a nonsynonymous SNP making the change of amino acid type from serine to phenylalanine. The expression of Rsmv1 was up-regulated after inoculating SMV G2 strain in resistant soybean line 'Dongnong 93-046'. CRISPR/Cas9 based gene knockout vector was constructed for Rsmv1 and was transferred into resistant

soybean line 'Dongnong 93-046'. There were obvious SMV disease symptoms on the new leaves after inoculating SMV G2 strain to T2 generation mutants. Meanwhile, the overexpression vector pCAMBIA3301-Rsmv1 was constructed and was transferred into susceptible soybean line 'Dongnong50'. There were no obvious SMV disease symptoms on the new leaves after inoculating SMV N1 to T2 transgenic plants. The result showed that Rsmv1 gene might be a key factor participating in the SMV resistance reaction.