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Next-generation sequencing from bulked-segregate analysis accelerates the simultaneous identification of two qualitative genes in soybean

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Next-generation sequencing (NGS)-based bulked-segregant analysis (BSA) approaches have been proven successful for rapidly mapping genes in plant species. However, most such methods are based on mutants and usually only one gene controlling the mutant phenotype is identified. In this study, NGS-based BSA was employed to map simultaneously two qualitative genes controlling cotyledon color of seed in soybean. Yellow-cotyledon (YC) and green-cotyledon (GC) bulks from progenies of a biparental population (Zhonghuang30 ´ Jiyu102) were sequenced. The SNP-index of each SNP locus in YC and GC bulks was calculated and Δ(SNP-index) was used to identify two genomic regions on chromosomes 1 and 11 harboring respectively loci *qCC1* and *qCC2*. These two BSA-seq-derived loci were further validated with SSR markers and fine-mapped. *qCC1* was mapped to a 30.7-kb region containing four annotated genes and *qCC2* was mapped to a 67.7-kb region with nine genes. These two regions contained respectively genes *D1* and *D2*, which had previously been identified by homology-based cloning as being associated with cotyledon color. Sequence analysis of the NGS data also identified a frameshift deletion in the coding region of *D1*. These results suggested that BSA-seq could accelerate the mapping of loci controlling qualitative traits, even if a trait is controlled by more than one locus.