

# Plant Science

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## SNP discovery and identification of loci associated with resistance to biotic and abiotic stresses using genotyping-by-sequencing in autotetraploid *Alfalfa*

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**A**lfalfa (*Medicago sativa* L.) is the world-wide forage crop. Changing trends to multipurpose uses increases demand for alfalfa. However, the production of alfalfa is challenged by endemic and emerging diseases and adverse environmental factors. Identification of genes/loci controlling biotic and abiotic stress resistance will facilitate breeding for improving alfalfa production. In the present investigation, we used an integrated framework of genome-wide association study (GWAS) with high-throughput genotyping by sequencing (GBS) to identify loci associated with resistance to Verticillium wilt and drought in alfalfa populations. Genotyping was done by GBS, followed by genotype calling using three bioinformatics pipelines including the TASSEL-GBS pipeline (TASSEL), the Universal Network Enabled Analysis Kit (UNEAK), and the haplotype-based FreeBayes pipeline (FreeBayes). The resulting numbers of SNPs, marker density, minor allele frequency (MAF) and heterozygosity were compared among the pipelines. The TASSEL pipeline generated more markers with the highest density and MAF, whereas the highest heterozygosity was obtained by the UNEAK pipeline (Figure 1). SNP markers generated from each pipeline were used for marker-trait association. We identified a group of SNP markers significantly associated with VW resistance and drought tolerance. Markers associated with VW resistance were located on chromosomes 1, 5, 6, 7 and 8. BLAST search using the flanking sequences of the resistance loci against *M. truncatula* genome identified candidate genes with disease resistance such as TIR-NBS-LRR protein and MDR-ABC transporter. Markers associated with drought tolerance were located in different chromosomal regions. Major loci identified in this work overlap with the reported QTLs in alfalfa. Validation of resistance loci in a broad range of alfalfa populations has been conducting using high throughput strategies. Functional markers closely linked to the resistance loci or derived from functional genes will be developed and used for genomics-assisted breeding for alfalfa with improved resistance to the disease and abiotic stress.

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