

Linkage analysis and QTL mapping of fall dormancy and winter-hardiness in alfalfa using GBS SNPs

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Fall dormancy (FD) reduces growth and yield of certain alfalfa (*Medicago sativa* L.) genotypes in response to decreased temperature and day length. Similarly, winter-hardiness (WH) is associated with survival and ecological distribution of alfalfa. Therefore, understating the genetic basis of the two traits is crucial for developing cold tolerant non-dormant alfalfa cultivars. The objective of this study was to identify quantitative trait loci (QTLs) associated with FD and WH in alfalfa using a pseudotestcross F1 mapping population. The F1 population was developed by crossing two cultivars with contrasting fall dormancy, 3010 (FD= 2) x CW1010 (FD=10). The F1 progenies, parents, and checks were planted in a RCBD design with three replications at Athens and Blairsville, GA. The FD was measured as regrowth height after four weeks of autumn clipping on 21st September. Dormancy was also assessed using regrowth height measured in early winter. SNP markers were developed using genotyping by sequencing (GBS) method. FD levels of parents and F1 were assigned based on a regression equation derived using standard check's regrowth and their known fall dormancy rating. Similarly, winter-hardiness was assessed as winter damage using scores (1-5) using visual rating with 1 being winter-hardy and 5 winter-susceptible. A moderate positive correlation was observed between fall dormancy and winter-hardy scores, especially when dormancy was assessed in early winter. 5,348 single dose allele (SDA) SNPs for maternal parent, 3010, and 2327 SDA for paternal parent, CW1010 were obtained using Tassel 3.0 UNEAK and GBS-SNP-CROP pipelines. Of the filtered SNPs, maternal linkage map retained 1837 SNPs and paternal parent retained 1377 SNPs in 32 linkage groups (LG). For each of the parental genetic map, average density was 1.5 cM/marker. 32 LGs for each parent were assigned to four corresponding homologs using the eight chromosomes of *M. truncatula* as reference. 45 significant ($P \leq 0.05$) QTLs for dormancy and 35 QTLs for winter-hardiness were detected using composite interval mapping with WinQTL Cartographer 2.5. More than 70% of the dormancy QTLs detected for both parents did not share genomic regions with winter-hardiness QTLs. These results suggest independent inheritance of fall dormancy and winter hardiness in alfalfa and therefore the simultaneous genetic improvement of the two traits to develop non-dormant winter hardy alfalfa cultivars.