Genomics-Assisted Breeding to Enhancing Alfalfa Resistance to Biotic & Abiotic Stresses

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Alfalfa production is challenged by endemic and emerging diseases, and adverse environmental factors. Developing alfalfa varieties with resistance to these stressors is imperative for sustainable alfalfa production. Conventional breeding procedures to develop alfalfa cultivars are time consuming and cost ineffective. Genomics strategies would help to accelerate the breeding process. The objectives of this research are to: 1) Identify DNA markers associated with resistance to soil borne diseases in alfalfa to clearly define the genetic basis of resistance to disease and accelerate breeding programs. 2) Identify alfalfa DNA markers and germplasm associated with drought and salt tolerance to clearly define the genetic basis of resistance to these stressors and accelerate breeding programs. Verticillium wilt (VW) is a soil-born fungus disease and causes severe yield loss in alfalfa. However, the genetic controlling to VW resistance in alfalfa is still not clear. Here we report two linked resistance (R) genes, MsVR38 and MsVR39 involved in VW resistance in alfalfa. We found that MsVR39 plays a positive role in VW resistance, whereas negative regulation was found in MsVR38. The sequence analysis of MsVR38 showed one nucleotide missing in the resistant plant but not the susceptible line, indicating the normal expression of MsVR38 affected the resistance gene MsVR39 activation. Further investigation on knockout mutants of MtVR130 and MtVR140 corresponding to MsVR38 and MsVR39 respectively, demonstrated that the knockout of MtVR130 conferred greater resistance to VW than the wildtype in M. truncatula. Bioassay of stem infection from M. truncatula mutants and wildtype also demonstrated that Mtvr130 is more resistant to V. alfalfae than Mtvr140 and the wildtype. A possible mechanism is that when a pair of highly identical TIR-NBS-LRR genes are both normally expressed, they are more likely to form heterodimers, which inhibits the expression of the resistance gene, MsVR39, thus causing susceptibility to the VW disease. Markers derived from the resistance gene have been developed and used in MAS.

Developing resistance alfalfa is an important breeding target for its sustainable production in arid and semi-arid regions. To understand the genetic base of abiotic stress resilience, two alfalfa populations were used for evaluating drought and salt resistance in the field. Genome-wide association by next-generation sequencing were conducted for mapping resistance loci. Twenty-eight markers were associated with yield under drought. Most of the markers were identified across different harvest periods under water deficit. The loci associated with biomass yield under water deficit located throughout all chromosomes in the alfalfa genome. Our results suggest that biomass yield under drought may involve a different mechanism compared to that of non-stress. Several stress-responsive genes were identified near to the drought resistance loci, including leucine-rich repeat receptor-like kinase, B3 DNA-binding domain protein, translation initiation factor IF2 and phospholipase-like protein. Marker-trait association identified a total of 42 markers significantly associated with five traits associated with salt tolerance, including fresh and dry weights, plant height, relative water content, stomatal conductance in another population. They were located on all chromosomes except chromosome 2. Of those identified, 13 were associated with multiple traits. Several loci identified in the present study were also identified in the previous reports while most loci are new. BLAST search revealed that 19 putative candidate genes were closely located with 24 significant markers. Among them, B3 DNA-binding protein, Thiamine pyrophosphokinase and IQ calmodulin-binding motif protein were identified among multiple traits in the present and previous studies. With further investigation, the markers closely linked to drought and salt resistance loci can be used for MAS and for the development of new alfalfa cultivars with improved resistance to drought and high salinity.

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