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Abstract

Transcriptional Down-Regulation of Various Genes in Alfalfa Enhances Tolerance to Abiotic Stresses †

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Abstract: Alfalfa (*Medicago sativa* L.) is a perennial legume esteemed for its yield, adaptability and superior nutritional quality as a forage crop. However, alfalfa production is often impacted by various environmental challenges such as drought and poor drainage throughout the growing season, which lead to a decline in farmers' profitability. These factors are anticipated to become more problematic in the coming years due to global warming scenarios, and as such, there is a need for the development of alfalfa cultivars with enhanced abiotic stress resilience. In this study, five gene homologs (*CBF2*, *ACBP3*, *TAC1*, *FAO3* and *HB2*) negatively regulating various abiotic stresses in other closely related crop species were identified in alfalfa, and RNAi genotypes exhibiting down-regulation of each gene, respectively, were generated. The RNAi genotypes were subjected to drought and flooding treatments, respectively, to assess their responses to abiotic stresses. Preliminary results demonstrated that alfalfa genotypes with reduced expression of *TAC1* exhibited increased tolerance to drought, while the down-regulation of *ACBP3* and *HB2* in alfalfa led to enhanced tolerance to flooding. Further experiments are underway to unravel the mechanisms driving increased abiotic stress tolerance in these genotypes. Our aim is to use the knowledge gained in this study to produce transgene-free highly adaptable alfalfa germplasm using advanced molecular breeding platforms such as genome editing via CRISPR/Cas, which could reduce production costs and enhance biomass production by minimizing forage crop losses under extreme weather conditions.

Keywords: abiotic stress tolerance; alfalfa; crop improvement; forage; plant biotechnology

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