Physiological & Transcriptional Analyses of Two Alfalfa Cultivars With Different Salt Tolerance

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Alfalfa (*Medicago sativa* L.) production decreases under salt stress. Understanding possible morphological, physiological, and molecular changes of alfalfa in response to salt stress is of great interest. The responses of two alfalfa cultivars (salt tolerant 'Halo', salt intolerant 'Vernal') were studied for 12 weeks in five gradients of salt stress in a sand based hydroponic system in the greenhouse. The accumulation of elements and organic compounds in different tissues of alfalfa under salt stress were evaluated using synchrotron beamlines. In addition, an RNA-Seq technique was applied to identify the differentially expressed genes (DEGs) associated with salt stress in the two alfalfa cultivars at 0 h, 3 h, and 27 h under 12 dS m^{-1} salt stress maintained by NaCl. 'Halo' showed significantly greater germination percentage and seed vigor than 'Vernal' at 16 dS m^{-1}. The pattern of chlorine accumulation for 'Halo' was: root > stem ≥ leaf at 8 dS m^{-1}, and root ≥leaf > stem at 12 dS m^{-1}, potentially preventing toxic ion accumulation in leaf tissues. In contrast, for 'Vernal', it was leaf > stem ≥ root at 8 dS m^{-1} and leaf > root ≥stem at 12 dS m^{-1}. Amide concentration in the leaf and stem tissues was greater for 'Halo' than 'Vernal' at all salt gradients. RNA-Seq study identified a total of 237 DEGs in leaves and 295 DEGs in roots of the two alfalfa cultivars. In leaf tissue, the two cultivars had a similar number of DEGs at 3 h and 27 h of salt stress, however, in root tissue, 'Halo' maintained 55 and 56 DEGs at 3 h and 27 h, respectively, while the number of DEGs decreased from 42 to 10 for 'Vernal'. This differential expression pattern highlights different genetic responses of the two cultivars to salt stress at different time points. Interestingly, 28 (leaf) and 31 (root) salt responsive candidate genes were highly expressed in 'Halo' under salt stress, of which 13 candidate genes were common for leaf and root tissues. About 60% of DEGs were assigned to known gene ontology (GO) categories. The genes were involved in transmembrane protein function, photosynthesis, carbohydrate metabolism, defense against oxidative damage, cell wall modification and protection against lipid peroxidation. The identified DEGs and other trait differences between the two cultivars are significant for understanding the genetic basis of salt tolerance in alfalfa.