Evaluation and Expression Analysis of Alfalfa Genotypes in Response to Prolonged Salt Stress

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Abstract

We evaluated 12 alfalfa genotypes under long-term salinity stress. These genotypes were selected by screening160 individual plants from 15 populations for biomass under salinity and differences in Na, K/Na ratio and Cl concentration in shoots. Salt tolerance (ST) index of the 12 genotypes ranged from 0.39 to 1. The most salt-tolerant genotypes SISA14-1 (G03) and AZ-90ST (G10) that were the top performers for biomass, exhibited the least effect on shoot number and height. SISA14-1 (G03) was low accumulator of Na and Cl under salinity. There was a net reduction in shoot Ca, Mg, P, Fe, and Cu, while Mn and Zn increased under salinity. Salinity reduced foliar area and stomatal conductance; but, net photosynthetic rate and transpiration were not affected. Interestingly, salinity increased chlorophyll and antioxidant capacity in most genotypes; however they did not correlate well to ST index. Salt-tolerant genotypes showed upregulation of the *SOS1, SOS2, SOS3, HKT1, AKT1, NHX1, P5CS1, HSP90.7, HSP81.2, HSP71.1, HSPC025, OTS1, SGF29* and *SAL1*genes. Gene expression analyses allowed us to classify genotypes based on their ability to regulate different components of the salt tolerance mechanism. Our results indicate that pyramiding different components of the salt tolerance mechanism may lead to superior salt-tolerant alfalfa genotypes.

Effect of salinity on performance the alfalfa genotypes

Alfalfa is moderately tolerant to salinity and important to the dairy industry. Sodium tolerance/exclusion alone have not explained the variability in biomass accumulation. Specific gene expression in response to salinity stress can advance selection and breeding of alfalfa tolerant to salinity.

Figure 1. Evaluation of alfalfa genotypes under salinity stress. G01, SISA15; G02, Cuf101; G03, SISA14; G04, Cuf101; G05, SISA14; G06, SISA10; G07, SW9720; G08, SISA9; G09, SISA14; G10, AZ-90 ST; G11, SW9215; G12, Salado.

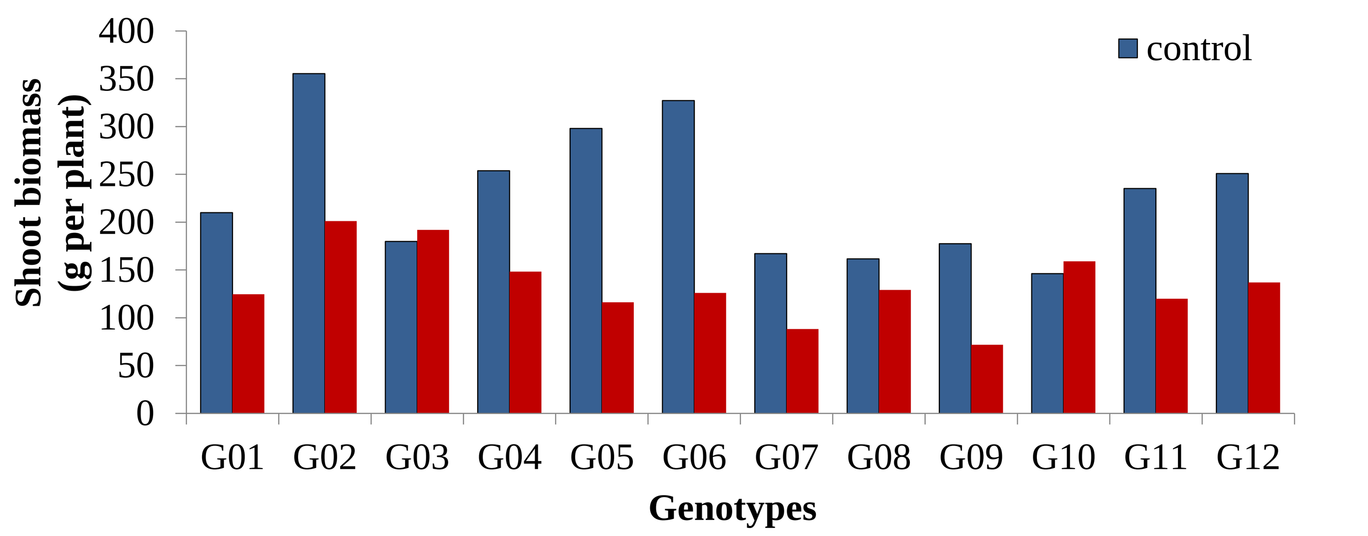

Figure 2. Accumulated shoot biomass per plant of twelve alfalfa genotypes. Different genotypes vary significantly for their performance under salinity.

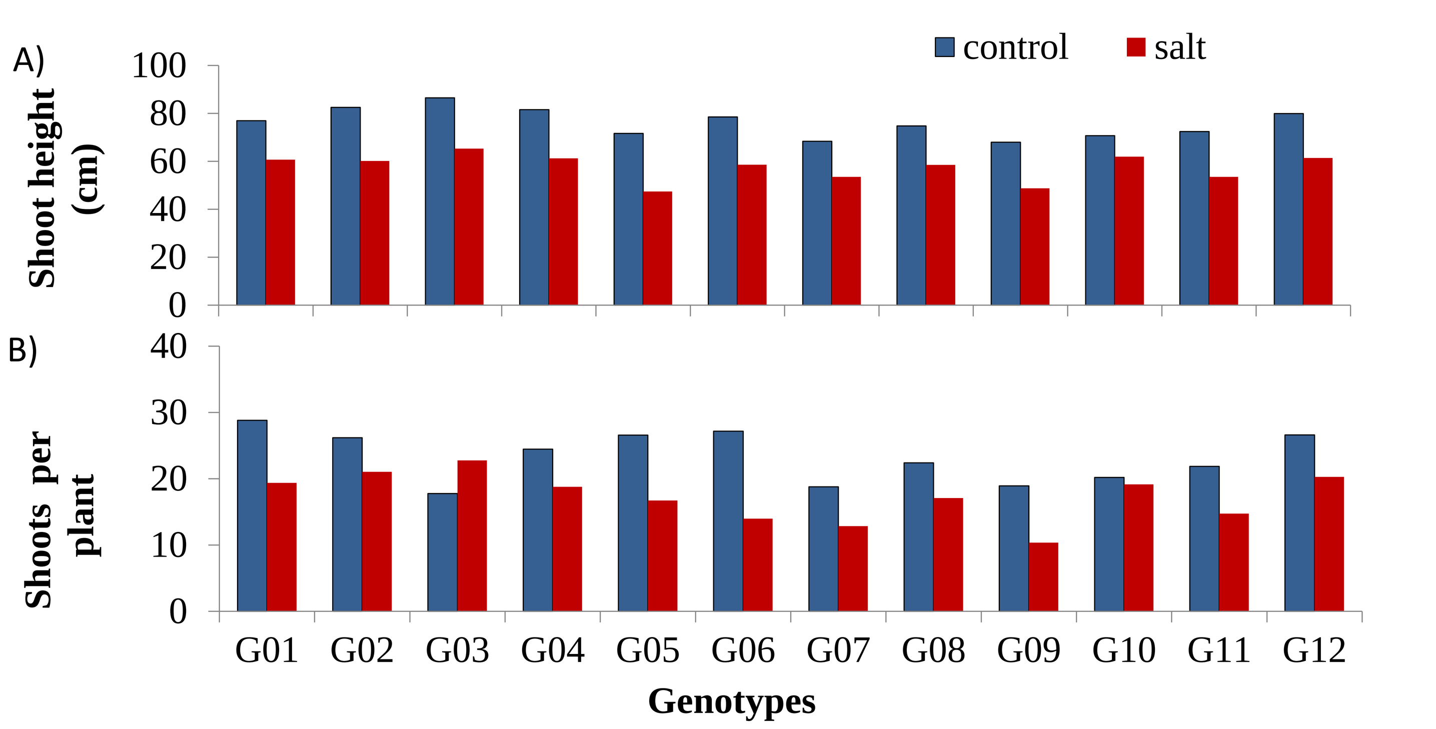

Figure 4. Shoot height (A) and shoots per plant (B) of twelve alfalfa genotypes. Salinity decreased the shoot height in different genotypes from 12 % to 34 % compared to control. Under salinity stress, G03 had no decrease in biomass, was the tallest , and had increased shoot number, while G10 showed the least salinity effect on height and minimal decrease (~5%) in shoot number. G09, the lowest in biomass with low ST index, was one of the shortest with the lowest shoot number.

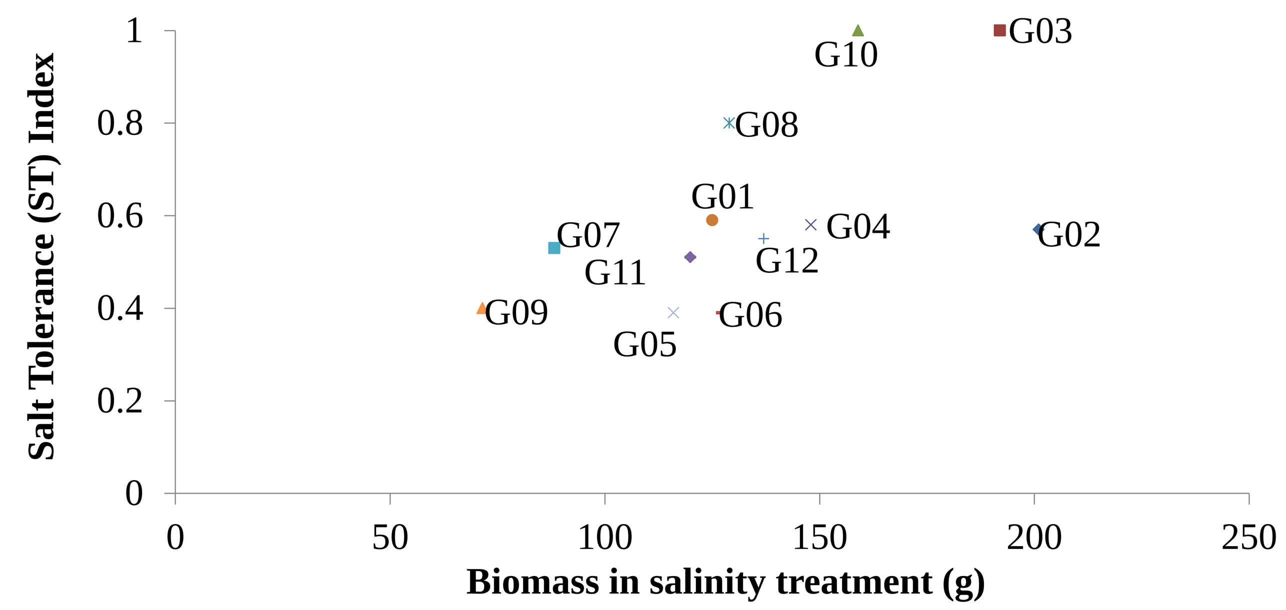

Figure 3. Relationship between accumulated biomass and salt tolerance (ST) index of alfalfa genotypes. ST index is the ratio of performance under salinity to performance under control. The genotypes had ST indexes ranging from 1 to 0.39. The most salt-tolerant clones G03, G10 and G08 were among top biomass yielders under salinity.

Effect of salinity on the ion composition of the alfalfa genotypes

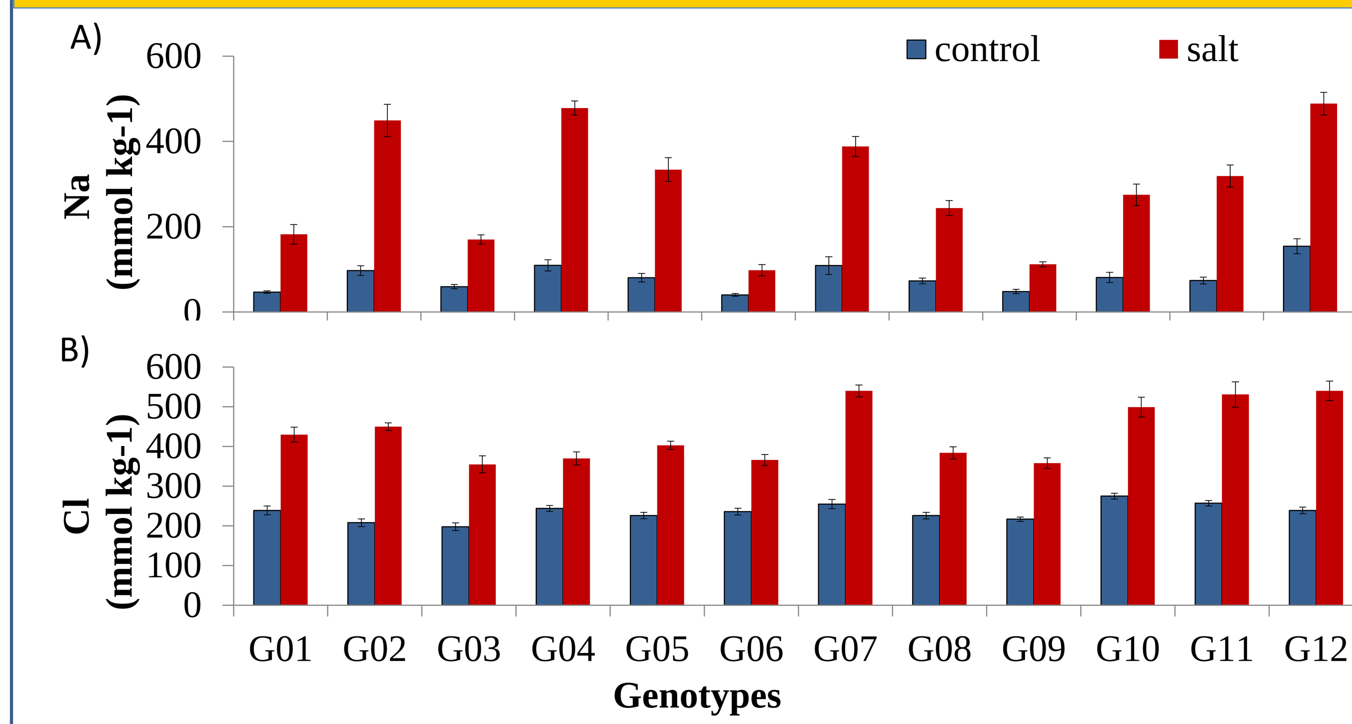

Figure 5. Shoot Na (A) and Cl (B) concentrations of twelve alfalfa genotypes under salinity. All genotypes exhibited increased Na and Cl concentrations under salinity. G03, which showed high ST index, had only 2.9-fold increase in shoot Na concentration. G03 stored the least amount of Cl under both the control and the salt treatment.

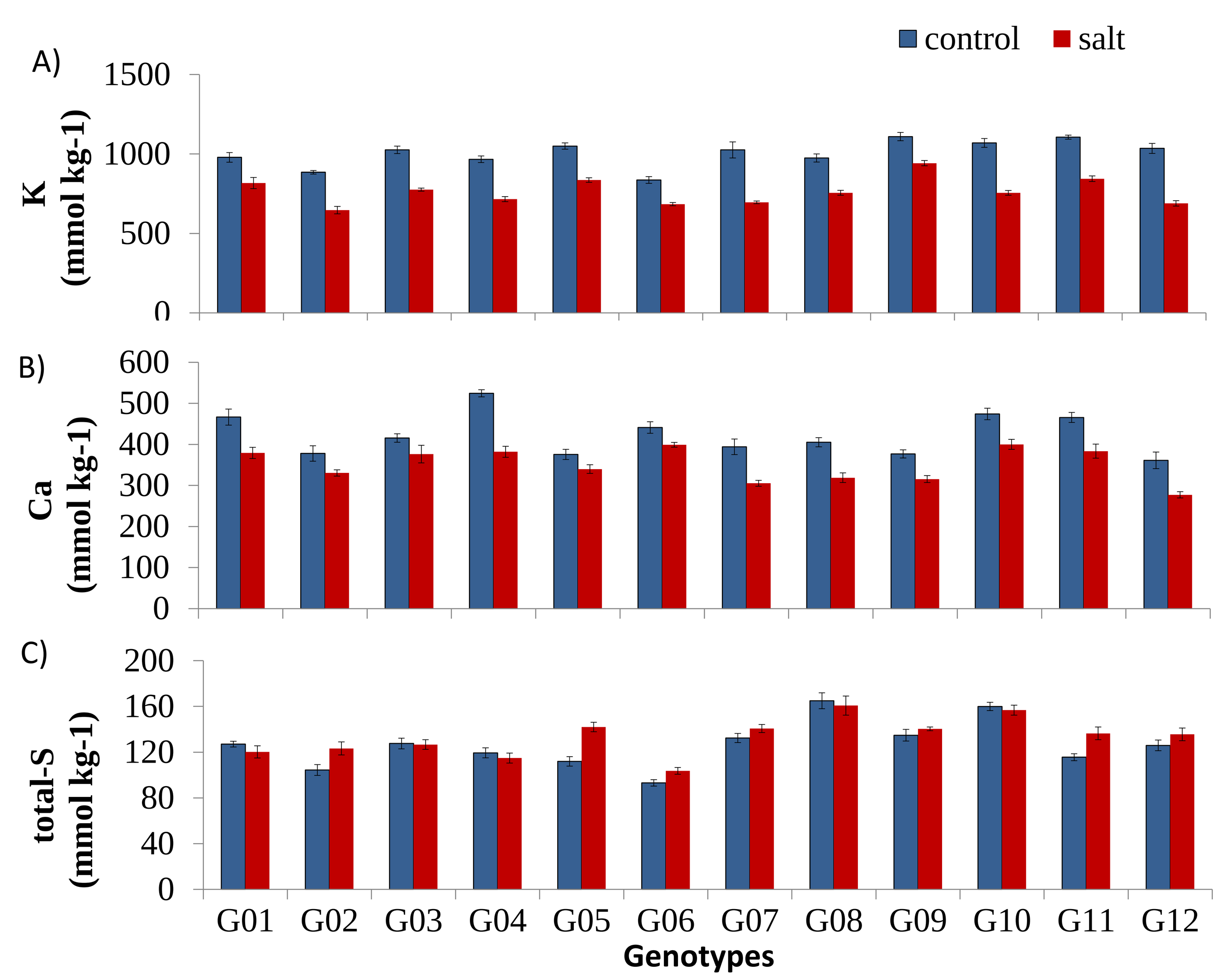

Figure 6. Shoot K (A), shoot Ca (B) and shoot S (C) concentrations of twelve alfalfa genotypes under salinity. On average, shoot K concentration decreased by 24.1 % compared to control. G09 maintained the highest level of K and showed the least (15.1 %) decrease in K. Plants under salinity showed significant reduction in average shoot Ca (-17.2 %). G10 had the highest Ca concentration (400 mmol kg^{-1}) under salinity. There was little effect of salinity on total-S (-6 %). The highest S concentrations were observed in G08 and G10 under both treatments.

Effect of salinity on the photosynthetic rate, transpiration, and stomatal conductance of the alfalfa genotypes

Net photosynthesis rate (**Pn**) and transpiration (**Tr**) did not show any significant change in the salt treatment as compared to the control in most genotypes. However, there was considerable variation among genotypes. Stomatal conductance (**gs**) showed consistent decrease in all genotypes in the salt treatment**;** although only few were statistically significant**.** Salinity increased the Chlorophyll content in all genotypes.

Expression analysis of genes involved in salt tolerance in roots and leaves of control and salinity-stressed alfalfa genotypes

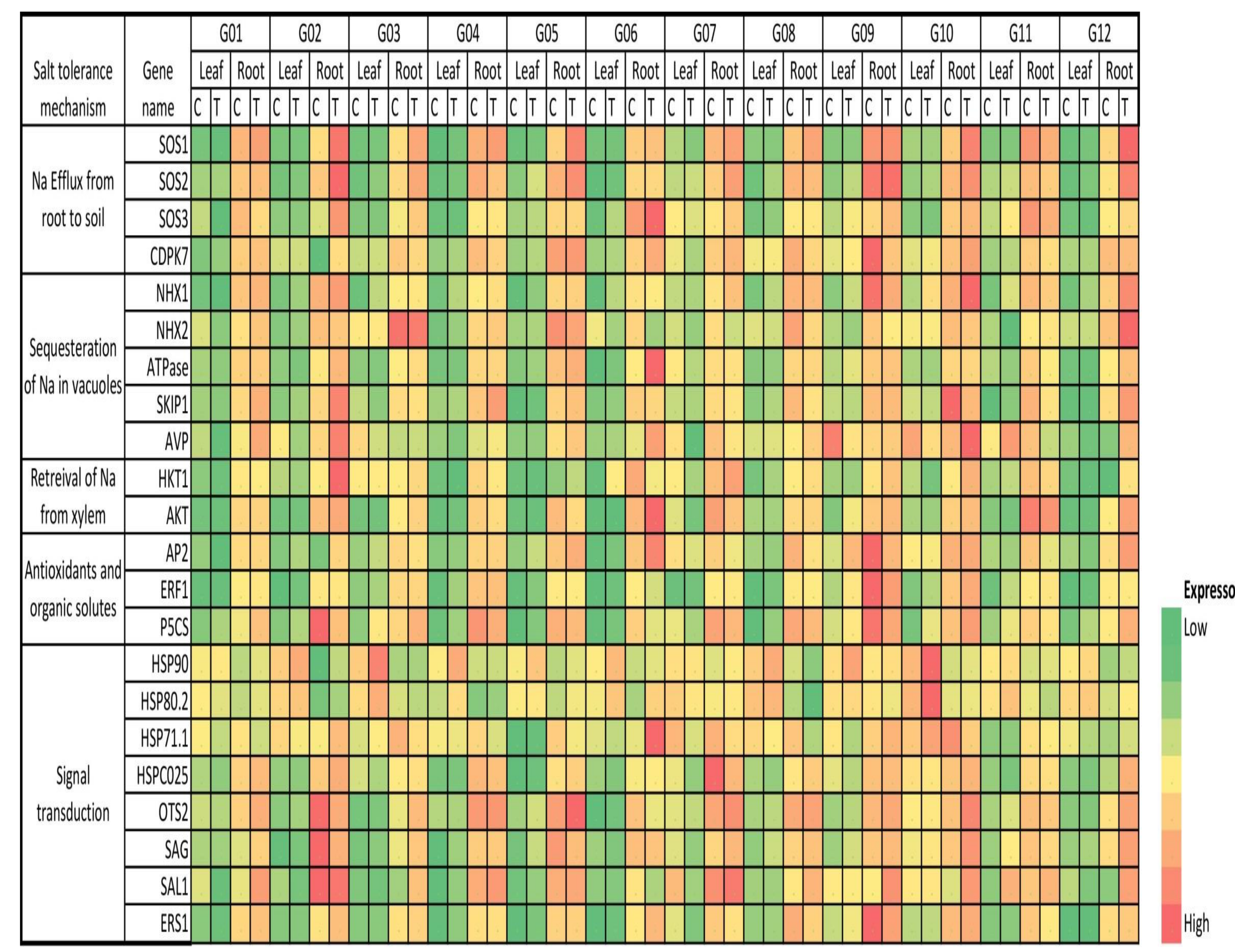

Figure 7. Heat map presenting relative expression of 22 genes known to play important role in different mechanisms of salt tolerance in leaves and roots of 12 alfalfa genotypes. C, Control; T, Treatment (salt); G01 to G12, 12 alfalfa genotypes. The identified genes were classified into five groups based on their different mechanisms of salt tolerance and their involvement in: i) Na efflux from root to soil. ii) Sequestration of Na in vacuoles. iii) Retrieval of Na from xylem. iv) Antioxidants and organic solutes. v) Signal Transduction. Genotypes G02, G03, G08, G10 and G12 showed significant upregulation of several genes under salinity vs. control. This analysis shows that pyramiding of genes involved in different mechanisms of salt tolerance may help develop better salt-tolerant genotypes.

Summary

In this investigation we are able to differentiate genotypes based on different components of salt tolerance mechanism. Combining attributes from different genotypes by crossing and selecting for lines with multiple components of salt tolerance mechanism may help in developing alfalfa lines with enhanced salt tolerance. In addition, this knowledge may help in isolating genes or quantitative trail loci important in salt tolerance.

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