

POLYPLOIDY AND STRESS: DOES CHROMOSOME DOUBLING AFFECT STRESS TOLERANCE IN ALFALFA?

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Chromosome doubling is known to affect stress tolerance in plants, but different species and genotypes exhibit diverse responses. Depending on geographical area and season, climate changes are exacerbating the occurrence of drought, flooding, salinization of agricultural soils, and increased UV exposure. Polyploidization can be one of the tools that breeders can use to cope with abiotic stress. We subjected full-sib diploid ($2n=2x=16$) and tetraploid ($2n=4x=32$) alfalfa plants obtained through sexual polyploidization to four abiotic stress types. Three $2x$ and three $4x$ genotypes with 5 biological replicates were used. Plants were reared in pots either in a growth chamber or in a greenhouse (flooding stress) for 3 weeks after being transplanted into 3 L pots filled with a mix of soil, sand and peat moss (1:1:1 ratio). Drought stress was applied by suspending irrigation and monitoring pot soil water content until 9-9.5% soil water content was reached in each pot. Salinity stress was induced by irrigating with 0, 100 or 200 mM NaCl twice, with a seven-day interval between each

irrigation. Flooding stress was obtained by submerging pots for 0, or 20 days. UV stress was applied at 3,6 KJ/m² UVB or 14,4 KJ/m² UVB for 0 or 3 days. Leaf tissue was sampled and immediately frozen in liquid nitrogen. Leaf relative water content, chlorophyll content, leaf gas exchange parameters, physiological traits and metabolites correlated with stress responses were measured using established protocols on stressed and control plants. The results obtained so far show that, for drought stress, there was a significant effect of stress treatments on all plants, but ploidy was significant for only a few traits; the ploidy x stress interaction (that would reveal a differential response between 2x and 4x plants) was only significant for stomatal conductance and photosynthetic efficiency, but with higher susceptibility of 4x plants. UV or flooding stress responses were not consistently affected by ploidy or ploidy x stress interaction. On the contrary, 4x plants exhibited better tolerance to salt stress with respect to 2x plants, as indicated by a lower decrease in stomatal conductance, a lower increase in proline content, and particularly by lower visible damage. Further molecular studies including transcriptomics, proteomics, metabolomics, and mobilomics will unravel the intricate mechanisms underlying the contribution of genome duplication to enhance tolerance to salinity stress in *M. sativa*.