

# Investigating the Impact of Neo-Autotetraploidy on Water Stress Response in Alfalfa

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Increased drought periods are one of the many consequences of climate change. Polyploidy, the presence of more than two complete sets of chromosomes in a cell, has long been recognized as a force of genome evolution and speciation in plants. Whole genome duplication (WGD) can generate novelty at both cellular and whole plant levels with the potential to increase stress tolerance. This study investigated the influence of WGD on tolerance to water shortage using full-sib alfalfa plants with either  $2n=2x=16$  or  $2n=4x=32$ , obtained by crossing two meiotic mutant  $2x$  parental plants producing both  $n$  and  $2n$  gametes.

Plants were arranged in randomized blocks in a growth chamber ( $23\pm 1^\circ\text{C}$  temperatures, 80 % humidity,  $324 \mu\text{mol m}^{-2} \text{s}^{-1}$  light provided by fluorescent tubes and halogen bulbs, with 16/8 photoperiod) 3 weeks after being clipped and 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 individual pots until 9-9.5% soil water content was reached. At complete soil water saturation (baseline) and stress time points physiological parameters associated with water stress were evaluated: photosynthetic rate, stomatal conductance, transpiration rate, intracellular  $\text{CO}_2$  concentration, chlorophyll content, leaf relative water content. Proline and malondialdehyde accumulation were measured in leaf tissue samples collected at stress time point and baseline. An ANOVA was conducted using a linear mixed effects model to assess the impact of genotype, ploidy, treatment, and block. There was a significant effect of stress treatments on all genotypes for most variables and different response among genotypes for some traits. Physiological and biochemical analyses demonstrated a clear influence of ploidy on photosynthetic rate, stomatal conductance, chlorophyll content, leaf relative water content, and proline concentration. However, no consistent effects of the ploidy x stress treatment interaction was observed, indicating that in our genotypes responses to water stress are not improved by WGD.

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