Study of drought-related gene expression in drought stressed *in vitro* hop plants

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Climate change and drought causes made big fluctuations in yield and content of alpha acids in hops during last years. One of the ways to overcome drought issues is developing drought-tolerant varieties that can withstand water scarcity better together with high yield and stability of quantitative and qualitative parameters. For effective drought tolerance breeding is necessary to understand the gene regulatory mechanisms of response to drought stress (**Fig.1**). In this study, we simulated osmotic stress using two concentrations of sucrose (0.15 M and 0.4 M) in cultivation media for *in vitro* plants of three hop varieties (Saaz, Sládek and Saaz Shine). During the first three days of cultivation on media, we evaluated the expression levels of 40 genes involved in drought tolerance to find differences in their expression profiles. The most up-regulated genes have been dehydrins (**Fig.2**). From 5 studied gene homologs, dehydrin 18a Xero1 was the most up-regulated by osmotic stress from 500 to 30000 times (**Fig.3**). There were no significant differences between varieties and sucrose concentrations. Dehydrin Rab18b was also up-regulated by osmotic stress, highly for 0.4 M sucrose concentration (**Fig.4**). This gene was the most overexpressed for variety Saaz Shine (370 times). This variety is drought tolerant and we also found overexpression of dehydrin COR47 only for this variety (20 times) on 0.4 M sucrose concentration. Only this sucrose concentration activated up-regulation of dehydrin LEA (**Fig.5**). The expression of dehydrin COR410 was not influenced by osmotic stress. The other up-regulated gene was abscisic acid-intensive 5 (AI5). We studied 7 homologs of this bZIP transcription factor (**Fig.6**). Only one of them was up-regulated (from 15 to 250 times) independently on variety and sucrose concentration (**Fig.7**). The last up-regulated gene was transcription factor NAC2. We studied 4 homologs of this transcription factor (**Fig.8**). All of them were up-regulated by osmotic stress when 0.4 M sucrose concentration was more efficient for overexpression (**Fig.9, Fig.10, Fig.11, Fig.12**). These potential drought tolerance genes can be used as molecular markers for breeding in future. But still is necessary to make more deep study in glasshouse and field conditions under drought stress.

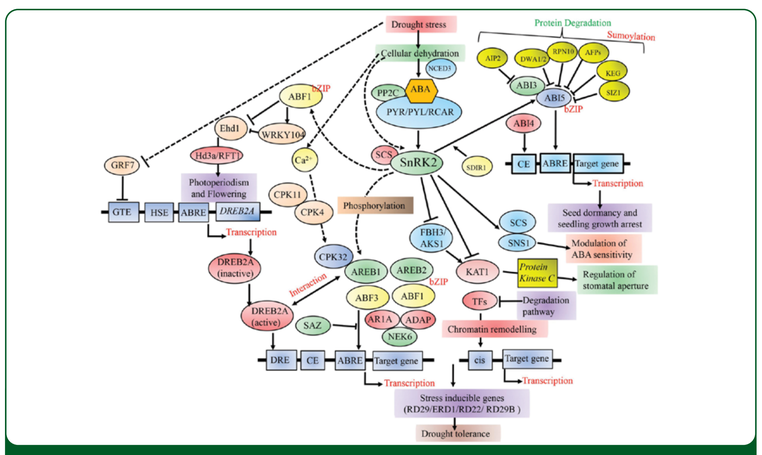

Figure 1. A schematic model of the transcriptional regulation of different TFs playing key roles in cellular dehydration in plants.

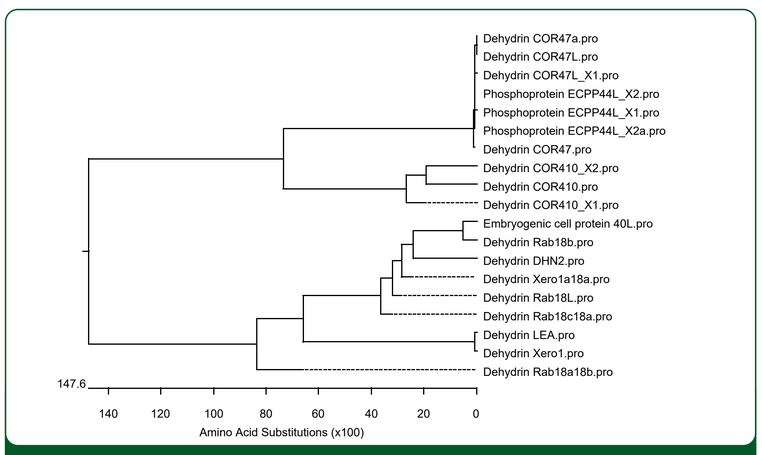

Figure 2. Phylogenetic analysis of protein sequences of dehydrin genes.

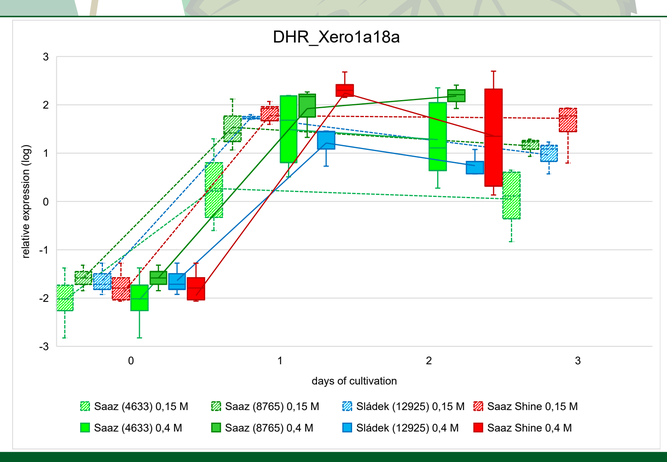

Figure 3. The relative expression of dehydrin 18a Xero1 gene (relative to reference genes) in different hop varieties under osmotic stress.

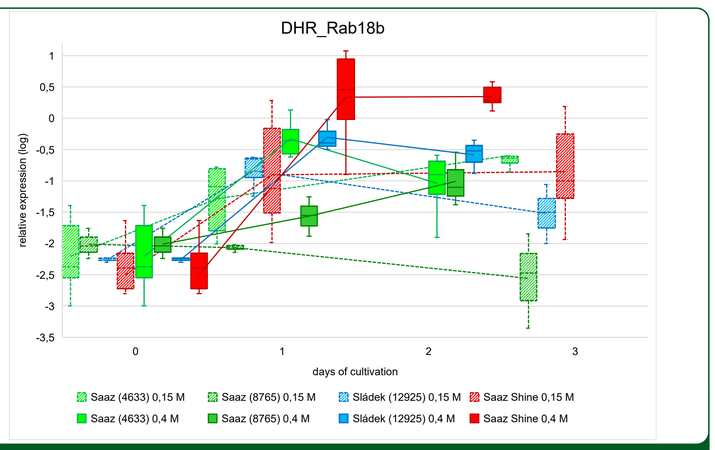

Figure 4. The relative expression of dehydrin Rab18b gene (relative to reference genes) in different hop varieties under osmotic stress.

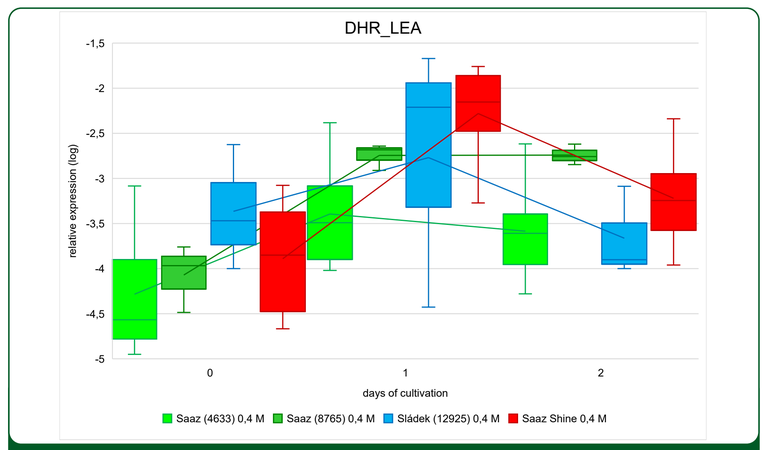

Figure 5. The relative expression of dehydrin LEA gene (relative to reference genes) in different hop varieties under osmotic stress.

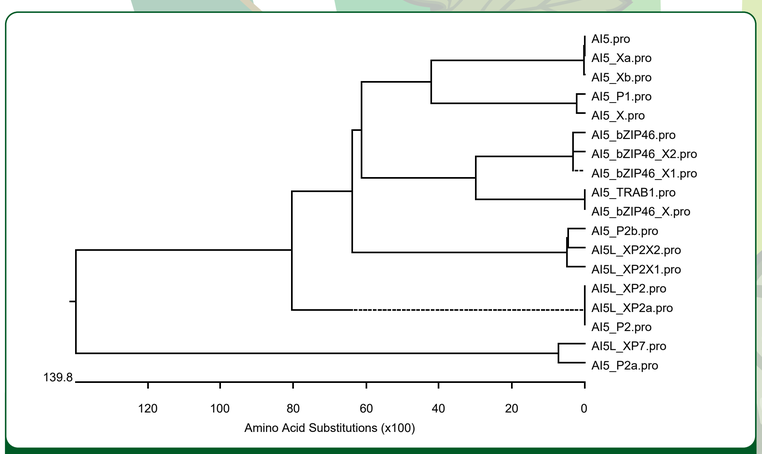

Figure 6. Phylogenetic analysis of protein sequences of abscisic acid-intensive 5 genes.

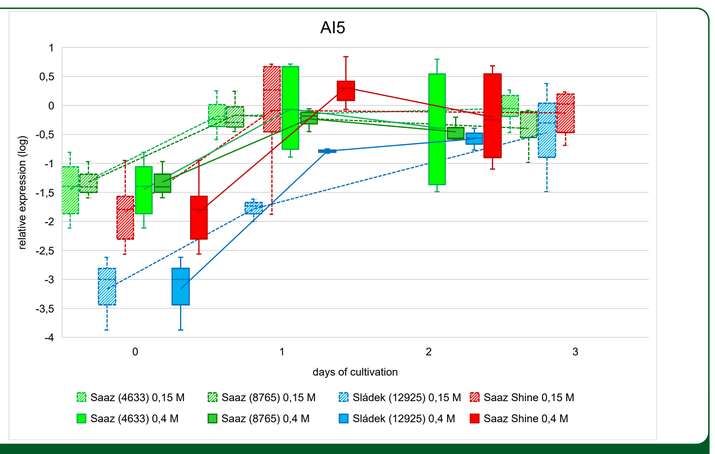

Figure 7. The relative expression of AI5 gene (relative to reference genes) in different hop varieties under osmotic stress.

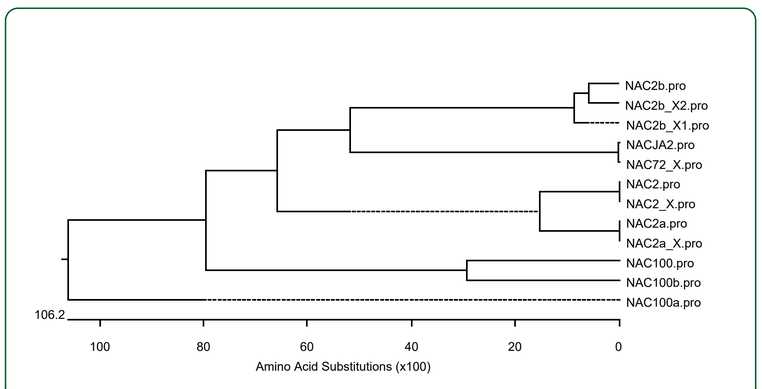

Figure 8. Phylogenetic analysis of protein sequences of NAC2 genes.

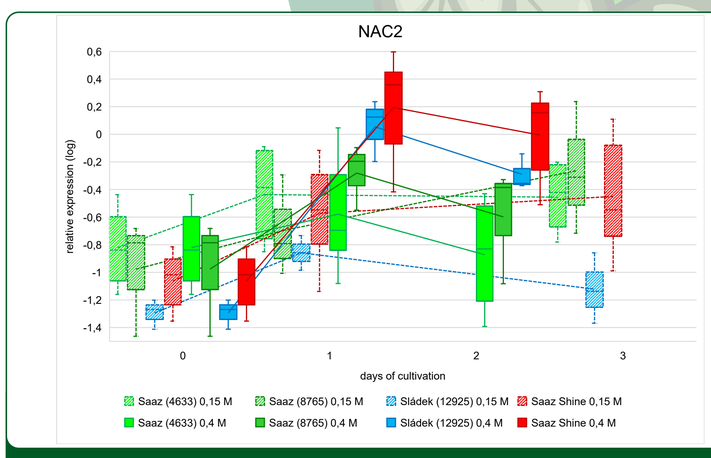

Figure 9. The relative expression of NAC2 gene (relative to reference genes) in different hop varieties under osmotic stress.

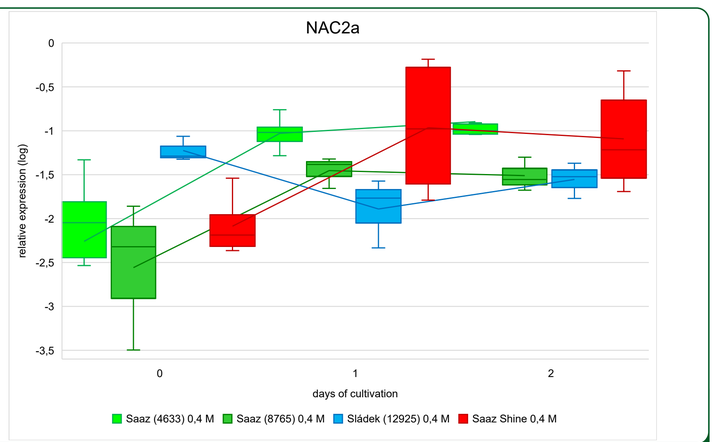

Figure 10. The relative expression of NAC2a gene (relative to reference genes) in different hop varieties under osmotic stress.

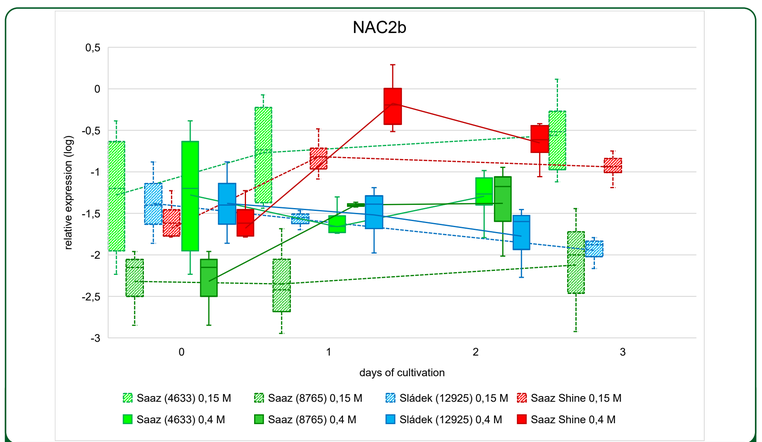

Figure 11. The relative expression of NAC2b gene (relative to reference genes) in different hop varieties under osmotic stress.

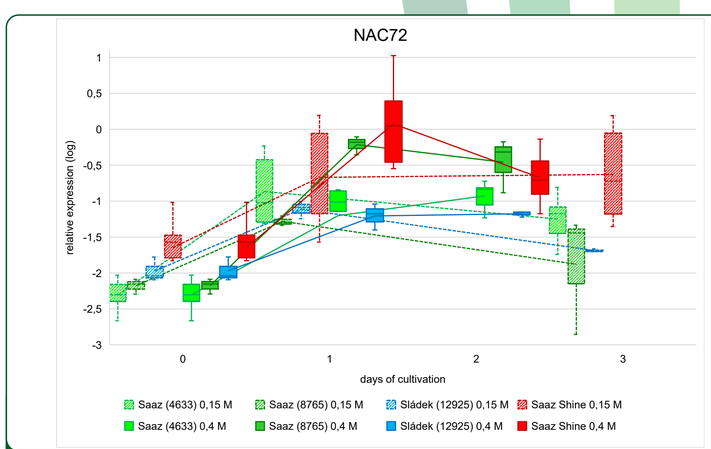

Figure 12. The relative expression of NAC72 gene (relative to reference genes) in different hop varieties under osmotic stress.

This work was supported by the National Agency for Agricultural Research of the Ministry of Agriculture of CR in project QL24010353.