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Biochemical and transcriptomic approach: the selection tools for afforestation of halomorphic environment

Vladislava Galović[1], Marko Kebert[1], Branislav Kovačević[1], Saša Orlović[1], László Szabados[2]

[1]University of Novi Sad, Institute of Lowland Forestry and Environment, Antona Čehova 13d, 21000 Novi Sad, Serbia; *e-mail: galovic@uns.ac.rs*

[2]Biological Research Centre, Hungarian Academy of Sciences, Hungary; *e-mail: szabados.laszlo@brc.mta.hu*

INTRODUCTION

As the climate change forest tree species is greatly affected by various environmental stresses. Soil salinity is one of the most severe abiotic stress that cause increasing environmental problems worldwide. Serbia has an increasing tendency of halomorphic soils in Vojvodina region. This made us interested in gathering knowledge of tolerance and response of various commercial poplar clones to salt stress using multidisciplinary approach.

MATERIAL AND METHODS

Seven candidate genes, two putative poplar homologues of GRAS family TFs (GRAS17 and GRAS16), DREB2 from ABA-independent pathway DREB family TFs and four abiotic stress inducible genes (RD29B, RD17, P5SC1, SOS1) were examined for their expression profiles. Furthermore, several biochemical parameters such as different radical scavenger capacities (estimated by DPPH and ABTS assays) and accumulation of total phenolic content and flavonoids as well as accumulation of two osmolytes, glycine betaine and proline, were quantified. Three perspective clones of *Populus deltoides* (B229, 182/81 and Pe19/66) and one of hybrid background *P.x euramericana* (clone M1) were subjected to NaCl induced salt stress in concentration range of 0-150-300mM hydroponically and the plant responses were tracked at different time points (3-8-24 hours) at the leaf level. Total RNA were isolated from all clones, that differs in tolerancy to salt stress. RNA was extracted and purified and reverse transcripted. Quantitative real time PCR (qPCR) was performed to test the expression of stress inducible genes on poplar and quantify the expression levels in salt sress induced poplar tissues. All expression efficiency of the genes were normalized by actin as a reference gene.

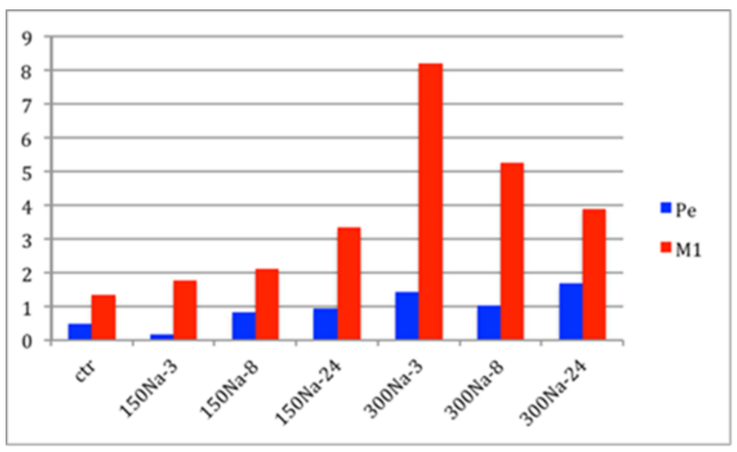

FIGURE 1: Differential PtRD17 gene expression in a salt tolerant (M1) and salt sensitive (Pe19/66) poplar clone, subjected to gradual increase of salt stress (0-150-300mM NaCl).

RESULTS

The most significant induction of the salt-related genes was after 3 and 8 hours after salt treatment predominantly in lower salt concentrations (150mM). P5CS gene showed no or low induction by salt in B229 clone, but stronger activation in M1 and in 182/81clones. RD17 gene was induced in B229 in low level concentration (150mM). In M1 the expression is significantly stronger than in Pe19/66 clone suggesting the different tolerance to salt stress. GRAS16 gene is clearly repressed by salt in M1 clone while GRAS17 was repressed in B229 clone. GRAS17 induction in M1 and Pe19/66 clones highly varied but slight activation in clone Pe19/66 after 8hrs of salt treatment. In clone 182/81 3 hours after salt treatment (150mM, 300mM) lead to very high transcript levels. DPPH radical antioxidant power is increasing in 8 hours of salt treatment with the same pattern throughout the clones while Pe19/66 had higher reaction to stress in comparison to other clones. Total phenols were relatively stable in all clones and slightly differ through the time point. Total flavonoids are increasing in the late phases of the stress. Proline showed increasing tendency by the hour of the stress. Clones Pe19/66 and B229 react more dynamically.

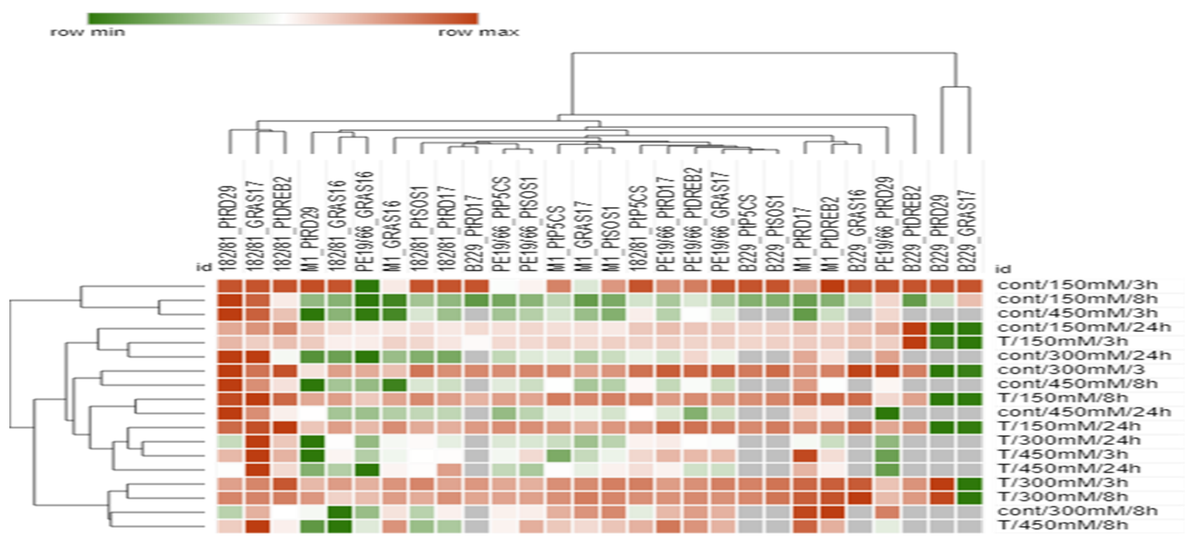

FIGURE 2: Heat map analyses of salt stress differential expression data

CONCLUSIONS

The most important segment of this study represents getting successful strategy for approaching the climate change problem with biochemical and functional genomic tools for testing gene expression in order to obtain the first applicable insights into the future protection of poplar species in the area of Srem, Vojvodina halomorphic region, Serbia. Different level and pattern of expression have been occurred for different stress induced genes. The clone specific expression was noticed in TF coding genes, two homologues, GRAS16 and GRAS17 where clear repression of GRAS17 was noticed in B229 clone while GRAS16 in M1. Biochemical and the functional genomic data were in accordance.

Acknowledgments

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REFERENCES

Joseph Benjamin, Luigi Lucini, Saranya Jothiramshekara Ajay Parida (2019). Metabolomic insights into the mechanisms underlying tolerance to salinity in different halophytes. Plant Physiology and Biochemistry. Volume 135: 528-545.doi.org/10.1016/j.plaphy.2018.11.006

Galovic V., Orlovic S., Fladung M. (2015): Characterization of two poplar homologs of the GRAS/SCL gene, which encodes a transcription factor putatively associated with salt tolerance. iForest-Biogeosciences and Forestry. Vol, 8, pp. 780-785. doi: 10.3832/ifor1330-008