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### **ACCEPTED MANUSCRIPT**

## Identification and Expression Profiling of All Hsp Family Member Genes under Salinity Stress in Different Poplar Clones

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#### ABSTRACT

Heat shock proteins (Hsps) play a key role for regulation of the changes during different stress conditions including salinity, drought, heavy metal and extreme temperature. Molecular based studies on the response mechanisms of forest trees to abiotic stresses started in 2006 when Populus trichocarpa genome sequence was completed as a model tree species. In recent years, bioinformatic analyzes have been carried out to determine functional gene regions of tree species. In this study, sHsp, Hsp40, Hsp60, Hsp90 and Hsp100 gene family memebers were identified in poplar genome. Some bioinformatics analyses were conducted, such as: identification of DNA/protein sequences, chromosomal localization, gene structure, calculation of genomic duplications, determination of phylogenetic groups, examination of protected motif regions, identification of gene ontology categories, modeling of protein 3D structure, determination of miRNA targeting genes, examination of sHsp, Hsp40, Hsp60, Hsp90 and Hsp100 gene family members in transcriptome data during salinity stress. As a result of bioinformatic analyzes made on P. trichocarpa genome; 60, 145, 49, 34, 12 and 90 genes belonging to members of sHsp, Hsp40, Hsp60, Hsp70, Hsp90 and Hsp100 protein families were firstly defined within the scope of this study. A total of 390 genes belonging to all Hsps gene families were characterized using different bioinformatics tools. In addition, salinity stress was applied to Populus tremula L. (Samsun) naturally grown in Turkey, Hybrid poplar species I-214 (Populus euramericana Dode. Guinier) and Black Poplar species (Populus nigra L.), Geyve and N.03.368.A clones. The expression levels of the selected Hsps genes were determined by the qRT-PCR method. After salt stress application in various poplar clones, expression levels of genes including PtsHsp-11, PtsHsp-21, PtsHsp-36, PtHsp40-113, PtHsp40-117, PtHsp60-31, PtHsp60-33, PtHsp60-38, PtHsp60-49, PtHsp70-09, PtHsp70-12, 33, PtHsp90-09, PtHsp90-12, PtHsp100-21, and PtHsp100-75 were increased. The role of the Hsps genes during salt stress has been revealed. Together with detailed bioinformatics analyses, gene expression analysis greatly contributes to understand functions of these gene family members. This research serves as a blueprint for future studies and offers a significant clue for the further study of the functions of this important gene family. Moreover, determined genes in this study can also be used for cloning studies in agricultural practices.

Keywords: Genome-wide analysis, Heat shock proteins, RNAseq analysis, Salt stress, Gene expression analysis

#### 1. Introduction

Because of high number of species and clones, poplar has an extended and diverse gene family that offers rich diversity. Its rapid growth provides good quality of forestation, high timber yield and unique landscaping for human beings. This particular genus includes species that differently react with response to drought and salt stresses. They may be considered as to be an option for plantations under stress conditions owing to their different stress response (Ceulemans and Universitaire Instelling, 1990). Poplar plantation is also useful for phytoremediation projects for landfill leachate in high salinity areas (Shannon et al., 1999; Shrive et al., 1994). The characteristic properties of poplar render its suitability for such practices such as rapid establishment, expansive biomass accumulation, extensive and deep root systems, high transpiration rates, convenience of asexual propogation, and extraordinary growth on marginal lands (Isebrands and Karnosky, 2002).

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