

The pecan basic leucine zipper transcription factor *CibZIP43* involved in stress resistance study

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Abstract: The pecan, scientifically known as *Carya illinoensis* (Wangenh.) K. Koch, is indigenous to the region of the Mississippi River Valley and is commonly referred to as the American pecan. It is a member of the Juglandaceae family and the *Carya* genus. Pecan is a valuable tree species that is visually appealing and economically advantageous, making it a popular choice for landscaping and forestry purposes. The pecan industry significantly contributes to increasing farmers' revenue and improving the agricultural composition in Anhui Province. In recent times, due to shifts in agricultural and forestry practices as well as the effects of global warming, the challenges of soil salinization and extreme high temperatures have become more pronounced, impeding the progress of the pecan industry. Hence, there is an urgent need for comprehensive mechanistic investigations to elucidate the response mechanisms of pecan trees to stress triggered by elevated levels of salt and heat, with the aim of effectively overcoming these challenges. The basic leucine zipper (bZIP) is a transcription factor broadly found in higher plants and is involved in numerous crucial physiological processes: growth and development, stress responses, etc. The functional mechanism of this family in pecans' response to adversity remains to be elucidated. This research project discovered 77 bZIP transcription factors in pecans. In accordance with the pecan genome, a total of 77 bZIP transcription factors were identified and categorized into 12 distinct subfamilies (S, B, A, D, F, E, G, I, J, H, K, and C). Examine the evolutionary lineage, the phylogenetic tree, promoter cis-acting elements, conserved motifs, gene structure, and *Ka/Ks* of *CibZIPs*, along with other relevant characteristics, using bioinformatics tools and techniques. The S1 subfamily is believed to have a significant impact on the response to abiotic stress. Examination of the observed expression profile indicated that S1-bZIPs exhibit responsiveness to various abiotic stressors including salt, heat stress, drought, and submergence stress, with particular emphasis on *CibZIP38*, *CibZIP43*, and *CibZIP55*. The results of subcellular localization and transcriptional self-activation experiments indicated that *CibZIP38*, *CibZIP43*, and *CibZIP55* are proteins localized within the nucleus and possess self-activating properties. The findings from the experiments involving bimolecular fluorescence complementation (BiFC) and [luciferase complementation imaging](#) (LCI) assays demonstrate the potential interactions between *CibZIP38*, *CibZIP43*, and *CibZIP55* with *CibZIP27* and *CibZIP63* within plant cellular environments. The findings from dual-luciferase reporter assays and yeast one-hybrid experiments suggest that *CibZIP38*, *CibZIP43*, and *CibZIP55* have the ability to interact with the G-box element. The utilization of GUS staining and GUS activity assay revealed that the *CibZIP43* promoter was activated in response to both salt and heat stress conditions. The overexpression of *CibZIP43* in *Arabidopsis thaliana* resulted in enhanced tolerance to salt and heat stress in seeds, seedlings, and adult plants of the transgenic lines.

Overexpression of *CibZIP43* resulted in elevated levels of chlorophyll, relative water content, and reactive oxygen species scavenging capacity, while decreasing membrane damage, and enhancing proline accumulation in transgenic lines exposed to salt and heat stress conditions. The Na^+/K^+ balance was maintained during exposure to salinity stress. The *CibZIP43* gene potentially enhanced the salt tolerance of the overexpression lines by modulating the expression of genes associated with Na^+/K^+ equilibrium and the removal of reactive oxygen species. In conditions of heat stress, the *CibZIP43* gene potentially enhances the heat tolerance of the overexpressed lines primarily through the modulation of the expression of heat-responsive transcription factors and proteins. This research investigated and examined the bZIP gene family in pecan by utilizing whole-genome data. Furthermore, it elucidated the role of *CibZIP43* in response to salt and heat stress using a dependable heterologous genetic transformation system. These findings not only contribute to the advancement of the *CibZIP* gene family theory in relation to salt and heat stress responses but also provide valuable genetic materials for the development of pecan varieties with enhanced tolerance to salt and heat stress.

Keywords: pecan, bZIP transcription factor, *CibZIP43*, salt stress, heat stress