Decoding Climate Resilience: Functional Profiling of Protein Phosphatase 2C Family Genes for Abiotic Stress Tolerance in Rice

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Rice (Oryza sativa L.) is a staple food, providing 20% of the caloric needs for half of the global population. Rice production is adversely affected by abiotic stresses such as drought, salinity, and extreme temperatures, which are exacerbated by climate change. To adapt to abiotic stress responses, rice has intricate signaling pathways, particularly those mediated by the phytohormone abscisic acid (ABA). When rice plants undergo stress, ABA signaling pathways are activated, which causes a series of gene expressions and physiological responses resulting in stress tolerance. Clade A subfamily of the Protein Phosphatase 2C (PP2C) gene family is known to be a negative regulator of the ABA signaling pathway. While the role of PP2C genes in stress response is known, there is an existing gap in understanding the specific genes that significantly contribute to stress signaling in rice. In the present study, using bioinformatics, we were able to identify PP2C genes to be knocked out. Then we induced abiotic stress treatments followed by gene expression profiling of selected clade A subfamily PP2C genes which revealed differential expression at different time points. Further, we performed knockouts of the selected PP2C genes using CRISPR/Cas9 gene-editing technology in rice protoplasts, and were able to observe the changes in gene expression levels, validated through real-time PCR. The localization of the sgRNA/Cas9-GFP complex in isolated rice protoplasts was visualized by high-resolution microscopy. This characterization of PP2C genes will assist in the development of stress-tolerant rice varieties, ensuring sustainable rice production for the global population.