Developing Rapid Technologies to Access Root Cell-Type Specific Gene Regulation in Rice (Oryza sativa L.)

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Environmental stress factors are increasingly experienced in agricultural systems worldwide, leading to dramatic losses of yield for many farmers. In rice, these suboptimal growth conditions invoke responses at the organ-, tissue-, and cell-specific levels that enable metabolic to developmental processes relevant to survival. This study serves to develop technologies to analyze the cell-type specific gene regulation mechanisms in rice in response to stress and apply these genetic mechanisms to the creation of stress-tolerant crop varieties. The INTACT (Isolation of Nuclei TAgged in specific Cell Types) technology allows for rapid purification of cell nuclei for analysis of the transcriptome (in our case, via qRT-PCR). The seedling-Agroinoculation method of rice root transformation seeks to improve upon the current method of calli-induced transformation in efficiently producing transgenic plants for examination of genes involved in the stress-tolerance mechanism. Nuclei was successfully isolated via INTACT, with RNA transcripts retrieved and analyzed. An estimated of 25,000 nuclei was captured within our tissue sample with a success rate of 24.39%, which is expected to increase with a larger sample size. GUS reporter gene assays conducted on transformed plants localized the expression of cell-type specific promoters in the rice root and identified nine genes that may play a role in the stress-tolerance mechanism. Results of the new transformation method were observed within 3 weeks post-transformation, in relation to the 4-6 months needed for the current method. The development of our technologies paves the path for the genetic engineering of stress-tolerant crop varieties in areas of need, helping the agricultural industry sustain the growing world population.

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