Hybrid Proline and Glycine-Rich Proteins from Rice Protect Cells Against Freezing Damage

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As the earth's population continues to grow exponentially, the importance of reliable crop harvests becomes increasingly vital to survival. Weather can be unpredictable, and patterns of rain, drought, freezing, heat, pathogen attack and other stresses can lead to substantial decreases in crop yield. One agricultural crop of importance to humans is rice. Rice is the second largest grain crop in the world and accounts for more than a fifth of the calories consumed by the human species. In addition to feeding humans, rice is also a key ingredient in the feed of many domesticated animals including dogs, cats, and livestock animals.

Research into stress-responsive genes can identify proteins that help plants to withstand stresses that would normally kill them. Rice is normally grown in subtropical climates, but in temperate climates, occasional freezing poses a substantial threat to crop viability, especially at the delicate seedling stage when a late frost is most likely. For these reasons, research into making rice more robust and tolerant to cold stress is of great importance.

A set of genes found in *Oryza sativa* (rice) were identified to have homology to the previously identified cold-induced gene, EARLI1, from *Arabidopsis thaliana*. One of these genes was shown to improve low-temperature germinability of rice. The genes code for proteins composed of a proline-rich or glycine-rich region flanked by a signal domain at the amino terminus and an 8-cystine motif at the carboxyl terminus. Previous work has shown that the expression of EARLI1-like genes enhances the survival of yeast subjected to freezing at -20º C for a short time. Because of the homology of the new rice genes to EARLI1, it has been hypothesized that, when expressed in the yeast *Saccharomyces cerevisiae*, the hybrid proline-rich proteins (HyPRPs) and hybrid glycine-rich proteins (HyGRPs) of rice will also enhance freezing survival of the yeast.

Seven genes were amplified from rice genomic DNA and restriction sites were introduced for cloning purposes. Os03g0103100, a gene coding for a HyPRP, was cloned into the pESC-URA expression vector and introduced into *S. cerevisiae*. The yeast were subjected to a freezing assay. The freezing survival of the yeast expressing the putative cold protein was compared to that of the control yeast containing an empty pESC-URA vector. It was found that while the control yeast showed only a 10.47 +/- 3.71% survival rate after freezing, the yeast expressing the Os03g0103100 protein had a 21.86 +/- 3.99% survival rate. The expression of this protein significantly enhanced the survival of the Os03g0103100 yeast over the control yeast ($p < 0.02$; 2-tailed t-test, paired, $n=2$) by more than doubling the survival. Once the other six rice genes are cloned into yeast, any differences between the HyPRPs and HyGRPs ability to confer freezing tolerance to cells will be illuminated.