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The Role of a transcription factor in regulating rice response to drought stress

Diana Ha
*University of Nevada, Las Vegas*

Liyuan A. Zhang
*University of Nevada, Las Vegas*, liyuan.zhang@unlv.edu

Jeffery Shen
*University of Nevada, Las Vegas*, jeffery.shen@unlv.edu

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The Role of a Transcription Factor in Regulating Rice Response to Drought Stress

Diana Ha, Liyuan A. Zhang and Jeffery Q. Shen
School of Life Sciences, University of Nevada at Las Vegas

Abstract: The current water shortage is a major concern in regard to our global climate change crisis. A decrease in the availability of water will have direct effects on the development of plants. Some crops, such as Oryza sativa, or commonly known as rice, requires an abundant amount of water for adequate growth. With the water shortage crisis, it will become extremely difficult to harvest such crops to meet the world’s food demand. However, many plants have evolved mechanisms for overcoming and tolerating stresses such as drought. My research focuses on studying the proteins involved with these mechanisms. The WRKY superfamily is a family of transcription factors that up or down-regulate pathways in response to biotic and abiotic stresses in plants. We propose and hypothesize that OsWRKY70 plays a role in the abiotic stress of drought in rice. To identify the physiological role of this gene, we studied the phenotype of OsWRKY70 knockout mutants using an insertional transposon in comparison to its wildtype counterparts. This project aims to study the proteins involved with drought resistance in rice, which will pave the way for the production of genetically engineered crops that will be better at conserving water.

Introduction: WRKY transcription factors are proteins that bind to genes to turn them on or off (see Figure 1). They are the master switches in regulating plant responses to biotic (pathogen attack) and abiotic (cold, heat, drought) stress.

Figure 1: The WRKY Protein Binds to the W-box to Express or Repress Target Genes.

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Results:

Figure 2: OsWRKY70 Belongs to WRKY Group 1a.

OsWRKY70 belongs to the Group 1a WRKY superfamily of transcription factors - having two WRKY domains, each with a WRKY and zinc finger motif. Analysis of data from published papers suggest that OsWRKY70 is a protein that may be responsible in drought tolerance.

Methods:

• To test the physiological role of OsWRKY70, the expression of this gene was knocked out using transposon mutagenesis.
• PCR allowed for selection of mutants that are homozygous for the knockout trait. Quantitative real-time PCR (qRT-PCR) showed the expression levels of OsWRKY70 in the mutants.
• Seedling germination with growth on MS-media with 3% sucrose.

Figure 3: OsWRKY7 Knockout Mutants Were Created by the dSpm Transposon.

Insertion of this transposon causes a disruption in the transcription of the OsWRKY70 gene. Transposon mutants were obtained from University of California, Davis.

Figure 4: Homozygous Knockout Lines Were Confirmed by PCR.

Polymerase Chain Reaction (PCR) was utilized to select for homozygous knockout mutants. Panel A shows the different primers used for wildtype, homozygous, and heterozygous mutant lines. Panel B shows the possible band sizes for each primer pair. A band at 500 bp, using primer pair B and C, indicates both homozygous and heterozygous mutants. Additionally, homozygous mutants will not show a band with primer pair A and B. Panel C shows the actual results of the PCR after running on gel electrophoresis, indicating that both sample 1 and sample 2 are homozygous mutants. CT is the control sample from the wildtype line.

Figure 5: The Expression of OsWRKY70 in the Two Mutant Lines is Barely Detectable.

The expression of OsWRKY70 was analyzed by qRT-PCR for each mutant line and wildtype line. Results show that expression was barely detectable in both OsWRKY70 knockout mutants as compared to that of wildtype. Both knockout mutants were derived from the same line.

Figure 6: Knockout Mutants Germinated One Week Later Than Wildtype Seedlings.

Germination of seedlings on MS-media with 3% sucrose. OsWRKY70 knockout mutant seedlings did not germinate until one week after its wildtype counterpart.

Conclusions:

• PCR confirmed that the OsWRKY70 mutant lines we obtained were homozygous for the knockout of OsWRKY70 (see Figure 4).
• qRT-PCR analysis showed that mutant plants had a barely detectable amount of expression (see Figure 5). This indicates that the transposon was able to knockout the expression of OsWRKY70.
• Seedling germination showed that OsWRKY70 knockout mutants germinated one week after wildtype seedlings (see Figure 6), leading us to speculate that OsWRKY70 plays a role in germination in rice plants.
• Further analysis is ongoing to determine if OsWRKY70 plays a role in regulating responses to drought stress.

References:


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