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Norris Lam  
*University of Nevada, Las Vegas*

Liyuan A. Zhang  
*University of Nevada, Las Vegas*

Lingkun Gu  
*University of Nevada, Las Vegas*

Qingxi J. Shen  
*University of Nevada Las Vegas, School of Life Sciences, Mentor*

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Genetically Modifying Arabidopsis thaliana with a Gene from Drought-Tolerant Xerophyte

Larrea tridentata (Creosote Bush)

Norris Lam, Liyuan A. Zhang, Lingkun Gu, and Qingxi J. Shen
School of Life Sciences, University of Nevada, Las Vegas 89154

Abstract: Larrea tridentata, or desert creosote bush, is a xerophytic C₃ plant native to the American Southwest, and is known to have evolutionarily developed sophisticated cellular mechanisms to deal with periods of intense abiotic stress. Particularly, complex signaling pathways are involved in the development of drought tolerance. In this study, we are focusing on a plant that thrives in extremely arid conditions, providing an opportunity to understand the molecular mechanisms underlying drought tolerance. Using a combination of molecular biology and functional analysis, we identified a gene in Larrea tridentata (LtWRKY21) that functions as a transcription factor, which could be activated by abscisic acid (ABA). The gene was subsequently expressed in Arabidopsis thaliana (A. thaliana) under the control of a drought-inducible promoter, and the resulting transgenic plants were found to be more tolerant to drought stress.

Introduction

WRKY transcription factors have been identified as repressors and activators of pathways involved in stress response (pathogen attack) and abiotic stress (heat, salinity, desiccation, and cold) [1]. The impact of WRKY transcription factors in plants known to tolerate high degrees of abiotic stress, plants can be genetically modified to conserve water. The issue of water conservation can be linked to global agriculture, as which is globally warming is having a negative effect. Many suffer from malnutrition, as the environment in which they live fails to sustain them. A major factor accounting for drought is climate change. Changes in climate and rainfall patterns, the increase in desertification, focusing on a plant that thrives in an extremely arid and climate, crops can be engineered to acquire drought, improving the resistance of regions of the world negatively impacted by drought. In this project, a xerophytic Larrea tridentata binary virus (LtWRKY21) vector will be engineered to carry the LtWRKY21 coding region. The coding region, in addition to the drought-inducible RD29A promoter and three copies of a hemagglutinin epitope-tag (HA-tag), will be incorporated into the transgenic DNA (T-DNA) of the binary vector. This section of the binary vector will be subject to horizontal gene transfer as a result of the process of Agrobacterium-mediated transformation. The T-DNA containing LtWRKY21 will be transformed into Arabidopsis. Once mature plants are selected and subsequent generations are produced, the drought tolerance of the genetically-modified plants will be experimentally determined by reducing varying levels of intrinsic stress.

Methods and Materials

Genetic DNA Extraction

The genomic DNA from H9262 and H11002 was extracted from 3-month-old plants. The leaves were ground in liquid nitrogen in the presence of phenol. The resulting DNA was treated with RNase and purified using standard procedures.

Gene Cloning

LtWRKY21 was amplified from Larrea genomic DNA. Strips were taken from the restriction enzyme site of the RD29A promoter. The 658bp sequence was PCR amplified using two primers designed from the 5’ and 3’ end of the sequence. Strips and Self-restriction enzymes were also included in the primers to facilitate cloning. To verify the ligations into pRUB120, the restriction enzyme EcoRI was used.

Rationale

Fig. 1: LtWRKY21 and the RD29A Promoter

The LtWRKY21 gene was expressed in the “native” and “mutated” promoter. The 3xHA-tag protein was determined to be an effective transactivation factor in transgenic plants. The HA-tag was used to verify the insertion into the pRUB120 vector.

Fig. 2: RD29A Promoter

The RD29A promoter was amplified from Arabidopsis genomic DNA. Strips were taken from the restriction enzyme site of the RD29A promoter. The 658bp sequence was PCR amplified using two primers designed from the 5’ and 7’ end of the sequence. Strips and Self-restriction enzymes were also included in the primers to facilitate cloning. To verify the ligations into pRUB120, the restriction enzyme EcoRI was used.

Fig. 3: LtWRKY21 Coding Region

The LtWRKY21 coding region was amplified from the PCR template used by Zou et al. [5]. The template had been previously amplified from a c. tridentata cDNA clone obtained from Dr. John Cushman (University of Nevada, Reno). The 658bp sequence was PCR amplified using two primers containing PstI restriction enzyme sites. The stop codon from the coding region was excluded during amplifications in order to produce a fusion protein with the HA-tag. To verify the ligations into pRUB120, the restriction enzyme EcoRI was used.

Fig. 4: 3xHA-Tag

After section cut of pDT01020104, the T-DNA section will be ligated into the pRUB120 vector. The T-DNA section will be the cloned into the pRUB120 vector. The T-DNA section will be used for transgenic plant transformation.

Fig. 5: RD29A Terminator

The RD29A terminator was amplified from Arabidopsis genomic DNA. Strips were taken from the restriction enzyme site of the RD29A promoter. The 251bp sequence was PCR amplified using two primers designed from the 5’ and 7’ end of the sequence. Strips and Self-restriction enzymes were also included in the primers to facilitate cloning. To verify the ligations into pRUB120, the restriction enzyme EcoRI was used.

Fig. 6: pDT01012004/BlueScript SK + with T-DNA Insertion

All components of the ropedational section of the T-DNA will be cloned into the pRUB120 vector to simplify cloning into the main Arabidopsis binary T-vector plasmid. The T-DNA section will be the cloned into the pRUB120 vector. The T-DNA section will be used for transgenic plant transformation.

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