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Genetically modifying Arabidopsis thaliana with a gene from Drought-tolerant Xerophyte Larrea tridentata (Creosote Bush)

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

Larrea tridentata (Creosote Bush)
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Abstract: L. 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 in L. tridentata make it suitable to periods of severe water deficiency. Through the findings of Zou et al. [5,6], LWRKY21 synergistically works with abscisic acid (ABA) to transactivate both ABA-inducible DRE2 and DRE2 promoters. In addition, as ABA and gibberellic acid (GA) pathways are known to act antagonistically. Expectedly, the findings of Zou et al. suggest that LWRKY21 activates ABA signaling pathways and represses GA signaling pathways [5,6]. More importantly, the LWRKY21 transcription factor’s synergy with ABA is directly linked to some remarkable molecular adaptations of L. tridentata, some of which include stomatal closure to prevent transpiration, and slowing down gene expression to withstand dehydration [6]. To examine some of these mechanisms, the model plant Arabidopsis thaliana will be transformed with the LWRKY21 coding region via Agrobacterium-mediated transformation. Successful transformants will be selected and the subsequent generation of transgenic plants will be assayed.

Introduction

WRKY transcription factors have been identified as regulators and activators of pathways involved in stress (pathogen attack) and abiotic stress (heat, salinity, desiccation, and cold) [1]. By examining LWRKY21 expression in plants known to tolerate higher degrees of abiotic stress, plants can be genetically modified to conserve water. The issue of water conservation can be linked to global agriculture, on which global warming is having a negative effect. Many suffer from malnutrition, as the environment in which they live fails to sustain them. A major factor accelerating famine is drought. Drought shortages can impact crops, focusing on a plant that thrives in an extremely dry climate, crops can be engineered to acquire less water, improving the resiliency of regions of the world negatively impacted by drought. In this project, an Agrobacterium-mediated binary transgenic system will be engineered to carry the LWRKY21 coding region. The coding region, in addition to the drought-inducible DRE2 promoter and three copies of a hemagglutinin epitope tag (HA-tag), will be incorporated into the transfer DNA (T-DNA) of the binary vector. The 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 LWRKY21 will be transformed into Arabidopsis. Once transgenic plants are selected and subsequent generations are produced, the drought-tolerance of the genetically-modified plants will be experimentally determined by varying degrees of osmotic stress.

Methods and Materials

Genetic Constructs: The DRE2 promoter and terminase sequences were identified from the published literature [7,8]. In order to amplify LWRKY21 promoter and terminase, the genomic DNA of wheatgrass was extracted from 3 to 4-cm barley roots from two week old plants. The vector has been used in a previous project and is comprised of the plant transformation endogenous to maize (Zea Mays L) and is a binary construct that is utilized to for plant transformation. The vector contains the maize ubiquitin gene (UBI-motere from the maize ubiquitin gene.

Results

The LWRKY21 promoter was amplified from wheatgrass genomic DNA. The amplicons were purified from the transcriptional start site of the RD29A gene and sequenced. The 357 sequence was PCR amplified using two primers designed from the 5’ and 3’ end of the sequence. The primers were used in the RT-PCR reaction and the QRT-PCR reaction and the Southern Blot analysis were carried out to verify the fusion LWRKY21 promoter. The Southern Blot analysis was carried out as a result of the process of Agrobacterium-mediated transformation. The T-DNA containing LWRKY21 will be transformed into Arabidopsis. Once transgenic plants are selected and subsequent generations are produced, the drought-tolerance of the genetically-modified plants will be experimentally determined by varying degrees of osmotic stress.

Future Plans

All components of the experimental section of the T-DNA will be cloned into phiC31 SK- to simply cloning into the maize Agrobacterium binary T-vector (Fig. 6). The T-DNA section will be inserted into phiC31 SK- using restriction enzymes XmaII and NotI. The phiC31 SK- vector will be engineered to carry the 3xHA-tag, will be incorporated into the transfer DNA (T-DNA) of the binary vector. The 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 LWRKY21 will be transformed into Arabidopsis. Once transgenic plants are selected and subsequent generations are produced, the drought-tolerance of the genetically-modified plants will be experimentally determined by varying degrees of osmotic stress.

Acknowledgements

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References