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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 xerophytes C5 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 the plants are known to alter under periods of severe water deficiency. Through the findings of Zou et al. [5,6], LwWRKY21 synergistically works with abscisic acid (ABA) to transactivate both ABA-inducible rd29a and rd29b promoters. In addition, as ABA and gibberellic acid (GA) pathways are known to act antagonistically. Expectedly, the findings of Zou et al. suggest that LwWRKY21 activates ABA signaling pathways and represses GA signaling pathways [5,6]. More importantly, the LwWRKY21 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 plants’ arabidopsis thaliana will be transformed with the LwWRKY21 coding region via Agrobacterium-mediated transformation. Successful transfectants will be selected and the subsequent generation of transgenic plants will be assayed. Both phenotypic (screening) and genotypic (qRT-PCR and Southern Blot) examination will allow the function and expression patterns of LwWRKY21 to be elucidated under simulated drought. In order for LwWRKY21 to be successfully transformed into Arabidopsis, a tumor-inducing (T) plasmid must be engineered to carry LwWRKY21.

Introduction
WRKY transcription factors have been identified as both repressors and activators of pathways involved in biotic stress (pathogen attack) and abiotic stress (heat, salinity, dehydration, and cold)[3]. By examining phenotype in transgenic tobacco 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, on which global warming is having a negative effect. Many suffer from malnutrition, as the environment in which they live fails to sustain viable crops. A major factor accounting for famine is drought. Stress responses when the plant has already been damaged, meaning that efforts focused on a plant that thrives in an extremely and climate, crops can be engineered to require less water, improving the sustainability of regions of the world negatively impacted by drought. In this project, an arabidopsis thaliana (Arabidopsis) transgenic strain will be engineered to carry the LwWRKY21 coding region. The coding region, in addition to the drought-inducible rd29a promoter and three copies of a heat-shock-skin tag (Hsp10), will be incorporated into the transgenic 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 transgenic transformation. The T-DNA containing LwWRKY21 will be transformed into Arabidopsis. Once transgenic plants are selected and subsequent generations are produced, the drought resistance of the genetically-modified plants will be experimentally determined by varying degrees of osmotic stress.

Methods and Materials
Genetic Material Extraction: The total DNA of L. tridentata was extracted from 3-4 rosette leaves of two week old plants. The rosette leaves were frozen in liquid nitrogen and ground into powder. DNA was extracted using the CTAB method.[1] DNA concentration was measured using a Nanodrop 1000. The DNA vector. This section of the binary vector will be subject to horizontal gene transfer as a result of the transgenic transformation. The T-DNA containing LwWRKY21 will be transformed into Arabidopsis. Once transgenic plants are selected and subsequent generations are produced, the drought resistance of the genetically-modified plants will be experimentally determined by varying degrees of osmotic stress.

Results

Discussion:

The rd29a promoter was amplified from arabidopsis thaliana genomic DNA. Strips were taken upstream from the transcription start sites of the RD29A gene. Both the forward and reverse primers for PCR amplification using T7 and T3 primers were designed to have a single restriction enzyme site (SacI) and a single sacI cloning site. The PCR products of 337 bp were digested with SacI, purified, and ligated into pBlueScript SK+. The ligated products were transformed into Escherichia coli, and the ligated plasmid was recovered.

Rationale

The LwWRKY21 coding region was amplified from PCR amplicons as described by the authors of [5,6]. The LwWRKY21 coding region, was cloned in frame to the N terminal of the 3xHA-Tag. PCR, using T7 and T3, primers was used to verify the location of the LwWRKY21 coding region.

Future Plans

The rd29a promoter-driven T-DNA construct will be transformed into Arabidopsis. The transgenic plants will be selected and the subsequent generation of transgenic plants will be assayed. Both phenotypic (screening) and genotypic (qRT-PCR and Southern Blot) examination will allow the function and expression patterns of LwWRKY21 to be elucidated under simulated drought. In order for LwWRKY21 to be successfully transformed into Arabidopsis, a tumor-inducing (T) plasmid must be engineered to carry LwWRKY21.

References

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