Molecular Genetic Analysis of Osmoregulation, Osmotic Adjustment and Growth in Arabidopsis

DOE Grant No. DE-FG02-04ER15615

09/01/2004 - 08/31/2008

The molecular mechanism by which plants control cellular solute and water content, called osmoregulation, is critical for cell expansion and survival, particularly in response to cellular water deficit or low water potential. Two loci, *lwr1* and *lwr2* (*low water potential response*), that affect osmoregulation were isolated as part of a larger screen to identify mutants with alterations in low water potential-induced proline accumulation. When seedlings of *lwr2* were exposed to a steady low water potential stress over a period of several days using PEG-infused agar plates, the mutants had lesser proline accumulation and osmotic adjustment than the wild type, Ben. A mapping population was raised but it was not possible to reliably identify the mutants in the population and thus could not be used for mapping. The mutant *lwr1* was mapped and the gene identified. The mutant *lwr1* had greater accumulation of proline, higher total solute content, greater osmotic adjustment at low water potential, altered abscisic acid content, and increased sensitivity to applied abscisic acid with respect to Pro content than the wild type, Ben, when the water potential was decreased over a period of several days using PEG-infused agar plates. *lwr1* also had altered growth and morphology, including defects in trichome branching with the majority of the trichomes having a single point, shortened siliques which were crooked, and significantly lengthened time to flowering. Using bulk segregant analysis, the *lwr1* loci was mapped to the lower arm of chromosome II, near the marker nga168. Further detailed fine mapping located the mutation to the gene *PKL*, At2g25170, which was previously identified as a gene involved in altered root development. *PKL* encodes a chromatin remodeling factor. The mutation in *lwr1* introduced a stop codon in the 14th exon of At2g25170. The mutant was not complemented by 4 other known mutants having a disrupted *PKL* gene confirming the placement of this mutation in *lwr1*. In response to ABA treatment, *lwr1* and 5 different mutants in At2g25170, in two different genetic backgrounds, had

elevated proline content compared to the wild type. Proline levels of *lwr1* in response to water-deficit were not the same as in the originally published mutant; *lwr1* and other mutants in At2g25170 had lower proline content than wild type in response to water deficit. A series of microarray hybridizations have been completed comparing control and PEG-induced stress in *lwr1*, *lwr2* and the wild type. Analyses of these data are ongoing and are expected to shed light on the gene mutated in *lwr2* and to indicate the extent of changed of gene expression that are involved in the response of *lwr1* to water deficit stress. In addition, microarray experiments were completed on ABA treatments and ABA and stress treatments combined for *lwr1* in order to further explore the role of ABA in plant water-deficit stress.