

Expressed sequence tags from the Yukon ecotype of *Thellungiella* reveal that gene expression in response to cold, drought and salinity shows little overlap

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Received 8 January 2005; accepted in revised form 22 April 2005

Key words: abiotic stress, acclimation, *Arabidopsis*, Brassicaceae, canola, desiccation, EST, freezing, salt, tolerance

Abstract

Thellungiella salsuginea (also known as *T. halophila*) is a close relative of *Arabidopsis* that is very tolerant of drought, freezing, and salinity and may be an appropriate model to identify the molecular mechanisms underlying abiotic stress tolerance in plants. We produced 6578 ESTs, which represented 3628 unique genes (unigenes), from cDNA libraries of cold-, drought-, and salinity-stressed plants from the Yukon ecotype of *Thellungiella*. Among the unigenes, 94.1% encoded products that were most similar in amino acid sequence to *Arabidopsis* and 1.5% had no match with a member of the family Brassicaceae. Unigenes from the cold library were more similar to *Arabidopsis* sequences than either drought- or salinity-induced sequences, indicating that latter responses may be more divergent between *Thellungiella* and *Arabidopsis*. Analysis of gene ontology using the best matched *Arabidopsis* locus showed that the *Thellungiella* unigenes represented all biological processes and all cellular components, with the highest number of sequences attributed to the chloroplast and mitochondria. Only 140 of the unigenes were found in all three abiotic stress cDNA libraries. Of these common unigenes, 70% have no known function, which demonstrates that *Thellungiella* can be a rich resource of genetic information about environmental responses. Some of the ESTs in this collection have low sequence similarity with those in Genbank suggesting that they may encode functions that may contribute to *Thellungiella*'s high degree of stress tolerance when compared with *Arabidopsis*. Moreover, *Thellungiella* is a closer relative of agriculturally important *Brassica* spp. than *Arabidopsis*, which may prove valuable in transferring information to crop improvement programs.

Introduction

Plants have a remarkable ability to cope with a wide range of abiotic stresses. Nevertheless, major abiotic stresses such as drought, freezing temperatures, and salinity are primarily responsible for the discrepancy that exists between maximal and actual crop yield worldwide. The yields of most

major crop plants are reduced by more than 50% (Bray *et al.*, 2000), representing an economic hardship for farmers.

Numerous efforts have been made to understand and manipulate abiotic stress responses (for review, see Wang *et al.*, 2003). To this end, *Arabidopsis thaliana* has been the model organism of choice due to its small genome, rapid life cycle,

and availability of genetic tools. This is exemplified by identification in *Arabidopsis* of the C-repeat/dehydration-responsive element binding factor (CBF) gene family as key transcriptional activators and the associated downstream cold-regulated (COR) genes (Thomashow, 1999), as well as the salt overly sensitive (SOS) family, components of a signal transduction pathway that regulates ion homeostasis and salt tolerance (Zhu, 2001). However, it is becoming increasingly clear that it is difficult to study the genetics of abiotic stress tolerance using *Arabidopsis* as a model system owing to the fact that *Arabidopsis* has a limited capacity to survive saline, drought or freezing conditions (Bressan *et al.*, 2001).

Recently, *Thellungiella salsuginea* (previously classified as *T. halophila* and hereafter referred to as *Thellungiella*), another member of the family Brassicaceae (Al-Shehbaz *et al.*, 1999), has been identified as a potential model system for studies of abiotic stress tolerance (Bressan *et al.*, 2001; Inan *et al.*, 2004; Volkov *et al.*, 2004; Griffith *et al.*, unpublished). Several ecotypes of *Thellungiella* have been identified, including the Shandong ecotype from maritime habitats in China (Inan *et al.*, 2004) and the Yukon ecotype from saline meadows in subarctic Canada (Cody, 2000; Griffith *et al.*, unpublished). Not only does *Thellungiella* share many features that make *Arabidopsis* an excellent model system, but it is also an 'extremophile' that can tolerate salinity as high as 500 mM NaCl (Inan *et al.*, 2004). In addition, the Yukon ecotype of *Thellungiella* survives freezing to temperatures as low as -19°C (Griffith *et al.*, unpublished). These conditions are far more extreme than those tolerated by *Arabidopsis*.

Expressed sequence tags (ESTs) are obtained by single-pass sequencing of cDNA clones and provide information on the transcribed regions of a genome. Because cDNA libraries are typically generated from specific tissues or developmental stages or other experimental conditions and are randomly selected for sequencing, EST representations provide a dynamic view of genome content and expression. Here we present a set of EST data from an ongoing functional genomics project that is designed to identify the molecular mechanisms underlying abiotic stress tolerance in the Yukon ecotype of *Thellungiella*. In this study, we collected and analyzed 6578 ESTs from cDNA libraries

prepared from leaves cold-, drought-, and salt-stressed *Thellungiella*. Recent EST and microarray studies have focused on early time points, frequently only hours or days after imposing an abiotic stress, in order to identify genes involved in signaling pathways and transcriptional regulation of abiotic stress-induced genes in plants (Fowler and Thomashow, 2002; Kreps *et al.*, 2002; Seki *et al.*, 2002; Wang *et al.*, 2003). In contrast, our libraries included cDNAs from plants exposed for days to weeks to an abiotic stress in order to identify genes that may be mechanistically involved in acclimation or stress resistance in the steady state. Our primary goal was to survey the mRNA populations under the various stresses to determine whether the close relationship between *Arabidopsis* and *Thellungiella* could be exploited to quickly characterize responses to abiotic stress and whether the responses to different abiotic stresses were similar to each other. The genetic information obtained from this remarkable abiotic stress-tolerant crucifer is a key step in the discovery of genes involved in abiotic stress tolerance.

Materials and methods

Plant materials and stress treatments

Plants of the Yukon ecotype of *Thellungiella salsuginea* (Pall.) O.E. Schulz (Al-Shehbaz *et al.*, 1999; Cody, 2000) were grown in controlled environments with an irradiance of $250\ \mu\text{mol photons m}^{-2}\text{ s}^{-1}$; a 21-h daylength, and a day/night temperature regime of $22/10^{\circ}\text{C}$. When the plants were 4-weeks-old, they were subjected to stress treatments as described below in order to provide material for the construction of abiotic stress-induced cDNA libraries as well as subtracted libraries.

For cold treatment, plants were shifted to a day/night temperature regime of $5/4^{\circ}\text{C}$ and leaves were sampled at 24 h, 1 week and 3 week time points. Freezing stress was conducted by transferring 1-month-old plants, which were acclimated at 5°C for 2 weeks, to a chamber with a day/night temperature regime of $5/-4^{\circ}\text{C}$ for 2 weeks. For the drought treatment, water was withheld from 1-month-old plants until they wilted (about 3 days), and for the drought plus re-watering treatment, drought-treated plants were re-watered