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# Learning from Evolution: *Thellungiella* Generates New Knowledge on Essential and Critical Components of Abiotic Stress Tolerance in Plants

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**ABSTRACT** ***Thellungiella salsuginea*** **(*halophila*) is a close relative of *Arabidopsis thaliana* but, unlike *A. thaliana*, it grows well in extreme conditions of cold, salt, and drought as well as nitrogen limitation. Over the last decade, many laboratories have started to use *Thellungiella* to investigate the physiological, metabolic, and molecular mechanisms of abiotic stress tolerance in plants, and new knowledge has been gained in particular with respect to ion transport and gene expression. The advantage of *Thellungiella* over other extremophile model plants is that it can be directly compared with *Arabidopsis*, and therefore generate information on both essential and critical components of stress tolerance. *Thellungiella* research is supported by a growing body of technical resources comprising physiological and molecular protocols, ecotype collections, expressed sequence tags, cDNA-libraries, microarrays, and a pending genome sequence. This review summarizes the current state of knowledge on *Thellungiella* and re-evaluates its usefulness as a model for research into plant stress tolerance.**

**Key words: abiotic/environmental stress; ion channels; adaptation - evolutionary; comparative genomics; gene expression; Brassica.**

## INTRODUCTION

The sensitivity of crops to harsh climates and soil conditions is a major limitation for worldwide food production (Cakmak, 2002). However, tolerance to cold, drought, and salinity has evolved in many wild plant species, and these so-called extremophiles represent an important genetic reservoir that can be exploited for improving crop performance on marginal land.

In September 2004, a group of researchers from around the globe gathered in Paris to discuss the prospects of a small cruciferous plant to become a new model for molecular research into plant stress tolerance (Bressan et al., 2001; Zhu, 2001; Amtmann et al., 2005). The plant under question was *Thellungiella*, a member of the Brassica family and a close relative of the queen of plant models, *Arabidopsis thaliana* (Bressan et al., 2001; Zhu, 2001; Warwick et al., 2006). *Thellungiella*, or 'salt cress' as it is also referred to, resembles its famous cousin in many assets that make this plant so popular with geneticists and molecular biologists, including short lifecycle, small genome (approximately twice the size of the *Arabidopsis* genome), and copious seed production. In addition, *Thellungiella* is a real 'tough cookie', able to grow and reproduce under conditions of extreme cold, drought, and salinity.

Based on the experience gathered with *Thellungiella* in the individual laboratories, the Paris workshop participants decided to recommend *Thellungiella* as an extremophile plant model system and to promote its usage by sharing protocols, seed stocks, and BAC and cDNA libraries (www.thellungiella.org). Four years on, with some 40 experimental papers published and the full genome sequence pending, *Thellungiella* research is well underway. In this review, I will summarize the most important results obtained to date and reassess the usefulness of *Thellungiella* as a model species.

## PHYLOGENY AND ECOTYPES

The exact phylogenetic position of *Thellungiella* is still uncertain. Based on comparison of nuclear and chloroplast sequences, *Thellungiella* and *Arabidopsis* are considered to belong to different clades, despite their similar appearance and, within its clade, *Thellungiella* seems to be most closely related to Eutrema (Al-Shehbaz et al., 1999; Warwick et al., 2006; Sun et al., 2007). The *Thellungiella* genus contains four closely related species: *T. salsuginea* (Pallas) O.E. Schulz, *T. halophila*

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(C.A. Meyer) O.E. Schultz, *T. parvula* (Schrenk) Al-Shehbaz and O'Kane, and a recently described new species, *T. botschantzevii* (German, 2008). The Shandong ecotype from the North Eastern coast of China, which is most commonly investigated in the laboratories, has been cited as *T. halophila* but close inspection indicates that it belongs in fact to *T. salsuginea* and not to *T. halophila* (Figure 1). To avoid confusion, we will generally allude to the Shandong ecotype when talking about '*Thellungiella*' or otherwise specify the ecotype. In addition to the well characterized *T. salsuginea* ecotypes Shandong (from China) and Yukon (from Canada), several ecotypes from Russia and Kazakhstan belonging to *T. salsuginea* (Altai, Buriatia, Tuva, and Yakutsk), *T. halophila* (Bayanaul), and *T. botschantzevii* (Alei and Saratov) have been collected by Bert de Boer, Alexei Babakov and colleagues (Bert de Boer, personal communication). Other *Thellungiella* ecotypes have been found in the USA (Ray Bressan, personal communication). Phenotyping and genotyping of these collections will provide an essential resource for gene identification by QTL analysis and mutant mapping.

## DEVELOPMENT AND MORPHOLOGY

*Thellungiella* differs from *A. thaliana* in basic developmental programmes. In accordance with its extremophilic lifestyle, *Thellungiella* germinates and sets seeds over extended periods of time, which, in the laboratory, can create problems for comparative phenotypic characterization and needs to be taken into account in transformation protocols (e.g. through repetitive flower-dipping, see protocol by Bressan and colleagues, www.thellungiella.org). It also flowers later and requires vernalization to promote flowering. Fang and colleagues (2006, 2008) showed that ectopic expression of the *Thellungiella* FLC gene, a key regulator of the vernalization response pathway, caused a late-flowering phenotype in *A. thaliana* (Ler). Conversely, an RNAi construct silencing the endogenous ThFLC in *Thellungiella* produced an early flowering phenotype while retaining the same salt tolerance as wild-type. The authors propose that this transgenic line provides a better research model for plant salt tolerance studies than wild-type. Another way to obtain plants that are similar in growth rate and development (Figure 2) is to subject *Arabidopsis* and *Thellungiella* to different light regimes (10 h light at 200 $\mu$E m$^{-2}$ s$^{-1}$ for *A. thaliana* and 14 h light at 250 $\mu$E m$^{-2}$ s$^{-1}$ for *Thellungiella* deliver plants with large rosettes; Wang et al., 2006).

*Thellungiella* and *Arabidopsis* are very similar in form and shape. However, leaves of *Thellungiella* are often serrated (depending on growth conditions and ecotype) and appear slightly more succulent and waxy (Figure 2). In fact, one of the first experimental papers published on *Thellungiella* described a higher concentration and different composition of epicuticular waxes in leaves of *T. parvula* and *halophila* compared to *A. thaliana* (Teusink et al., 2002). At the tissue level, leaves have a second layer of palisade mesophyll cells while

**Figure 1.** The 'Real' *Thellungiella halophila* (Bayanaul) Growing in the Glasshouse of the Vrije University of Amsterdam.

Seeds supplied by D.A. German (Botanical Institute Barnaul, Russia). Photo by A.H. de Boer.

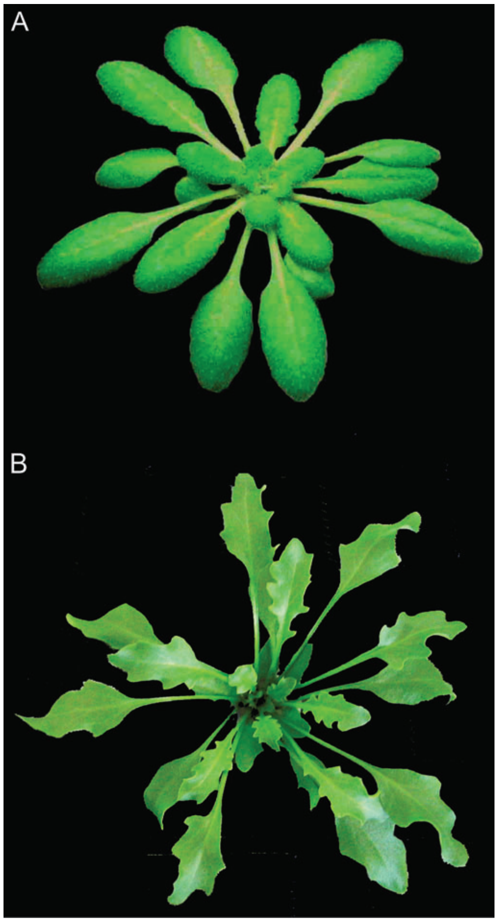

**Figure 2.** Typical Rosette of **(A)** *Arabidopsis thaliana* (Col0) and **(B)** *Thellungiella salsuginea* (Shandong) Grown in Low-Salt Hydroponic Culture with 10 and 14 h Daylight, Respectively.

Photos by V. Martínez, University of Glasgow.

roots develop both an extra endodermis and cortex cell layer compared to *Arabidopsis* (Inan et al., 2004). The same authors also observed that stomata in *Thellungiella* are present at higher density but are less open than in *Arabidopsis*, and respond to salt stress by closing more tightly. However, other laboratories reported that transpiration and stomatal conductance are less affected (decreased) by salt in *Thellungiella* than in *Arabidopsis* (Volkov et al., 2004; M'rah et al., 2006, 2007).

## STRESS TOLERANCE

Elizabeth Weretilnyk and colleagues from the Universities of McMaster and Waterloo in Canada are investigating the phenotypic plasticity of the Yukon ecotype in its natural environment. The alkaline salt flats in the Yukon Territory of Canada are characterized by a semiarid climate with a very short growing season. The permafrost soils are saturated with calcium carbonate, magnesium sulfate, and sodium chloride, and are deficient in essential macronutrients (Griffith et al., 2007). Depending on the climate conditions of a particular year, *Thellungiella* can be found as small, individual plants or as tall plants covering large areas (Figure 3). Profiling of transcriptome and metabolome from plants collected in different years is currently underway to correlate gene expression and metabolism with climatic data in the hope to reveal adaptive mechanisms.

Tolerance of *Thellungiella* to high salt, drought, and cold has been confirmed in the laboratory. Unlike *Arabidopsis*, *Thellungiella* still thrives in high salt concentrations (100–500 mM; Inan et al., 2004; Taji et al., 2004; Gong et al., 2005), survives and re-generates after extended periods of drought (Arie Altman, personal communication), and withstands freezing ($LT_{50}$ values of −13°C for non-acclimated

**Figure 3.** *Thellungiella salsuginea* (Yukon) Growing in the Laboratory and its Natural Field Site in the Yukon Territory in Canada.

Upper left: Chamber-grown plant showing terminal flowers and prominent rosette. Photo by E.A. Weretilnyk, McMaster University. Upper right: Yukon field site in May 2006. Photo by J. Dedrick, McMaster University. Bottom: Yukon field site in July 2005. Photo by J. Dedrick, McMaster University.

plants and −19°C after acclimation (Griffith et al., 2007). Furthermore, Kant and colleagues showed that *Thellungiella* also grows better than *Arabidopsis* under moderate (1 mM nitrate) and severe (0.4 mM nitrate) nitrogen-limiting conditions (Kant et al., 2008). Higher salt tolerance of *Thellungiella* than *Arabidopsis* is reflected in lower concentrations of sodium ($Na^+$) and chloride ($Cl^-$) in the shoots and a higher potassium ($K^+$) to $Na^+$ ratio but not in a difference in leaf dehydration (Inan et al., 2004; Taji et al., 2004; Volkov et al., 2004; M'rah et al., 2007; Ghars et al., 2008). Several studies have linked superior ion homeostasis of *Thellungiella* under salt stress to the selectivity and regulation of individual ion transporters, and these will be described in more detail below. Probably due to the restriction of toxic $Na^+$ in the leaves, shoot growth reduction in high salt—albeit evident in *Thellungiella*—is much less pronounced than in *Arabidopsis*, at the level of both leaf initiation and leaf expansion (M'rah et al., 2007). Drought-stressed *Thellungiella* plants maintain constant water contents, thereby protecting shoot meristems from desiccation, which, in turn, allows fast re-growth after re-watering (Arie Altman, personal communication). Cold-treated *Thellungiella* plants lack endogenous ice nucleation or anti-freeze activity, indicating a potential for supercooling (Griffith et al., 2007). Finally, higher nitrogen-usage efficiency of *Thellungiella* is evident in higher nitrate uptake, and a higher content in total amino acids and total soluble protein (Kant et al., 2008).

## METABOLITES

Proline can act as a compatible solute and osmoprotectant in the cytoplasm (Munns and Tester, 2008). In response to salt treatment, proline levels seem to increase to higher levels in *Thellungiella* than in *Arabidopsis*, but there are conflicting data as to whether the mechanism of accumulation differs between the two species (Inan et al., 2004; Taji et al., 2004; Kant et al., 2006; Ghars et al., 2008). Proline accumulation was linked to constitutively high transcript levels of P5CS involved in proline biosynthesis (Taji et al., 2004), and to low expression of PDH required for proline catabolism (Kant et al., 2006).

Thioredoxin is a critical component of the defense system against oxidative damage and lipid peroxidation, and its abundance (CDSP32 thioredoxin) was found to be higher in *Thellungiella* than in *A. thaliana* under both control conditions and salt treatment (M'rah et al., 2007). Phytoalexins play an important, albeit not fully understood, role in pathogen defense (Rogers et al., 1996). Both *Arabidopsis* and *Thellungiella* increase phytoalexin biosynthesis also in response to abiotic stress, but differ in the type of compounds produced (e.g. camalexin by *A. thaliana*, wasalexins, and methoxybrassenin by *Thellungiella*; Pedras and Adio, 2008). Together with the above-mentioned findings on cuticular wax production, these studies emphasize the potential model function of *Thellungiella* not only for investigating stress tolerance, but also for deciphering specific biochemical pathways.

## FUNCTIONAL CHARACTERIZATION OF *THELLUNGIELLA* GENES

Based on high sequence identity between *Thellungiella* and *Arabidopsis* (92% on average, Inan et al., 2004), several *Thellungiella* genes have now been cloned and functionally characterized, including TsVP, encoding a vacuolar pyrophosphatase (Gao et al., 2006; Duan et al., 2007; Li et al., 2008), ThCBL9, encoding a calcineurin-B-like protein (Sun et al., 2008), ThHSC70, encoding a heat-shock protein (Zhang et al., 2004), ThCYP1, encoding a cyclophilin (Chen et al., 2007), and ThZF1, encoding a Cys-2/His-2-type transcription factor (Xu et al., 2007). Overexpression of TsVP improved salt and drought tolerance in maize and cotton, which was correlated with higher vacuolar $H^+$-PPase activity (Li et al., 2008; Lv et al., 2008). Similarly, TsVP expression in tobacco improved growth on high salt and viability of msophyll protolasts under salt shock conditions (Gao et al., 2006; Duan et al., 2007). TsVP overexpressing tobacco plants accumulated more $Na^+$ in the leaves, indicating that the reason for improved salt tolerance was indeed due to efficient compartmentalization of $Na^+$ in the vacuoles (Gao et al., 2006). Overexpression of ThCBL9 (Sun et al., 2008) or ThHSC70 (Zhang et al., 2004) in *A. thaliana* enhanced tolerance to salt and osmotic stress or tolerance to high temperature and chilling, respectively. While the latter finding is in accordance with the function of HSC70 as a molecular chaperon, the role of CBL9 in salt tolerance remains to be further investigated. In *Arabidopsis*, this gene, in conjunction with the CBL-interacting protein kinase 23, activates the plasma membrane $K^+$-channel AKT1 (Li et al., 2006a; Xu et al., 2006; Lee et al., 2007), and therefore salt tolerance of CBL9 overexpressing lines could be related to improved $K^+$-uptake. Finally, heterologous expression of the ThCYP1 gene increased salt tolerance of both fission yeast and tobacco Bright Yellow 2 (BY-2) cells, suggesting that ThCYP1 may promote the appropriate folding of certain stress-related proteins (Chen et al., 2007). While all these studies provide evidence for a potential role of the respective *Thellungiella* genes in stress tolerance, they do not necessarily explain the difference of tolerance between *Thellungiella* and *A. thaliana*. Indeed, it has been shown that the homologous genes from *Arabidopsis* also endow the plant with enhanced tolerance when overexpressed (e.g. AtAVP1; Gaxiola et al., 2001). This observation not only provides evidence for an unrealized inherent potential for stress tolerance in the gene pool of *A. thaliana*, but raises two important questions: (1) are salt-tolerance genes differentially regulated in *Thellungiella* and *Arabidopsis*, and (2) does constitutively high expression create a disadvantage in non-stressed conditions? The above-mentioned studies report upregulation of the genes in *Thellungiella*, such as by heat and cold (Thhsc70), by salt, abscisic acid (ABA), $H_2O_2$ and heat shock (ThCYP1), by ABA, NaCl, and PEG (CBL9) and by salt and dought (ThZF1), but they do not provide comparative data from *Arabidopsis* or any other stress-sensitive species. Clearly, more detailed comparisons between *Arabidopsis* and *Thellungiella* genes are needed in the future.

## GENE EXPRESSION

Several approaches have been taken to investigate stress responses of *Thellungiella* at the level of gene expression including comparison of EST libraries obtained from plants grown under different stress conditions, microarrays, and quantitative polymerase chain reaction (qPCR). Wong and colleagues compared 6578 ESTs representing 3628 unique genes from cDNA libraries of cold-, drought-, and salinity-stressed plants of the Yukon ecotype and found very little overlap between gene expression in different conditions, suggesting that *Thellungiella* tailors responses that are very specific to the individual stress (Wong et al., 2005). A similar result was obtained when microarrays spotted with the ESTs were probed with mRNA obtained from stressed plants (Wong et al., 2006). Interestingly, drought stress induced down-regulation of many genes related to pathogen defense. The authors propose that the observed expression patterns reflect the capacity of *Thellungiella* to be more precise and economical in its stress response than sensitive species. EST libraries and microarrays constructed from the Yukon ecotype contained genes that have no homologs in the *Arabidopsis* and thus provide the opportunity for discovering new genes. However, their genome coverage to date is limited (approximately 10% of the genome). Other studies have used *A. thaliana* microarrays representing 7000 genes as full-length cDNAs (RIKEN array; Taji et al., 2004) or more than 25 000 genes as 70mers (‘Arizona’ array; Gong et al., 2005). Unlike arrays with shorter probes (Affymetrix ATH1; Volkov et al., 2004), these arrays produce good hybridization signals when probed with *Thellungiella* mRNA but, in contrast to the Yukon array, they only provide information on genes that are homologous to *Arabidopsis* genes. Nevertheless, these studies uncovered strikingly different responses of *Thellungiella* and *Arabidopsis* to salt stress. In general, *Thellungiella* shows much fewer transcript changes than *Arabidopsis* when exposed to the same stress. In response to a very short salt shock (2 h in 250 mM NaCl), the RIKEN array identified only six transcripts as up-regulated in *Thellungiella* compared to 40 in *Arabidopsis* (Taji et al., 2004). Similarly, Gong and colleagues found that fewer transcripts responded to a 150-mM NaCl treatment (for 3 and 24 h) in *Thellungiella* than in *Arabidopsis* but the number of responsive transcripts in *Thellungiella* increased with stress intensity (Gong et al., 2005). Thus, many transcripts showed a similar response in *Thellungiella* treated with 250 mM NaCl as in *Arabidopsis* treated with 150 mM NaCl. In addition to two large gene clusters representing this type of expression profile (one for up and one for down-regulated genes), they also identified smaller clusters containing genes that responded in only one of the species. Both studies (Taji et al., 2004; Gong et al., 2005) pointed to the phenomenon that transcript levels of some genes that are salt-stress inducible in *Arabidopsis* have already a higher transcript level in *Thellungiella* under low-salt conditions. Hence, comparative transcriptomics between *Thellungiella* and *Arabidopsis* have highlighted three important potential adaptations to extreme conditions: (1) specificity—*Thellungiella* regulates a specific set of genes in each stress situation, (2) anticipation—*Thellungiella* is constitutively prepared for stress, and (3) sensitivity—*Thellungiella* requires higher stress doses to induce transcriptional responses.

Sensitivity of the transcriptional responses could be linked to and should be separated from the degree of stress experience. In a recent study (B. Wang and A. Amtmann, unpublished results), we recorded transcriptional responses of membrane transporter-encoding genes of *Arabidopsis* and *Thellungiella* to salt stress using the AMT oligonucleotide array (Maathuis et al., 2003). Unlike other studies, this analysis was carried out separately for roots and shoots. We found that considerably fewer *Thellungiella* than *Arabidopsis* transcripts responded to the treatment (100 mM NaCl for 24 h) in the shoots but a similar number of transcripts (albeit different ones) responded in the roots of the two species. Considering that under identical salt treatment leaf cells are exposed to less $Na^+$ in *Thellungiella* than in *Arabidopsis* (see above) while root cells of both species experience the same concentration, this finding suggests that the number of stress-responsive transcripts is correlated to stress experience rather than stress sensitivity. However, two observations challenge this paradigm. First, differences in shoot $Na^+$ content will be very small after the short exposure times applied in some studies (e.g. 2 and 3 h), and, secondly, *Thellungiella* showed fewer transcript changes also in response to stresses that are directly sensed by the leaves (e.g. cold Wong et al., 2006) and ozone (Li et al., 2006b)). It will be interesting to test whether differential responsiveness of the two species to these stresses is already evident at the level of hormones and secondary messengers upstream of the transcriptional response.

While all studies agreed with respect to general trends in transcript regulation under stress, responses by individual genes are still under debate. For example, Taji and colleagues (2004) reported that the level of transcript encoding the plasma membrane $Na^+/H^+$ antiporter SOS1 was higher in *Thellungiella* than in *Arabidopsis* plants in control conditions and not further induced by salt. Based on qPCR experiments, Kant and colleagues (2006) measured a different and more detailed profile in which SOS1 was more strongly induced by salt in *Thellungiella* than in *Arabidopsis* as far as the shoots were concerned but constitutively higher in the roots (in control conditions). ThSOS1 induction in *Thellungiella* leaves during salt stress was confirmed at the protein level using an antibody against AtSOS1 (Vera-Estrella et al., 2005). The case of SOS1 exemplifies the need for detailed profiling of transcripts and proteins under a range of conditions covering a wide spectrum of stress doses and exposure times before species-specific regulation of genes that are potentially important for salt tolerance can be assessed.

## ION TRANSPORT

The fact that, under salt stress, *Thellungiella* accumulates less $Na^+$ in its shoot than *Arabidopsis* has been confirmed by many studies (see above). This observation suggests that transport processes are important for salt tolerance in *Thellungiella*. Lower net $Na^+$ uptake into the shoot can be due to lower net $Na^+$ uptake into the root (either through lower unidirectional influx or through higher unidirectional efflux), lower root–shoot transport of $Na^+$ (either through lower xylem loading or higher recovery from the xylem), and higher $Na^+$ recycling from the shoots into the roots (higher phloem loading). Our study with radioactive $^{22}Na$ (Wang et al., 2006) showed that in 100 mM NaCl, the steady-state unidirectional $Na^+$ influx into the roots was significantly smaller in *Thellungiella* than in *A. thaliana* (approx 50%). Surprisingly, unidirectional efflux was also slightly smaller in *Thellungiella*. $Na^+$ influx was inhibited in both species by micromolar external $Ca^{2+}$, which inhibits voltage-independent, non-selective channels in other species (Davenport and Tester, 2000), but not by $Cs^+$ and $TEA^+$, two well known inhibitors of voltage-gated $K^+$-selective channels (Véry and Sentenac, 2002). In a subsequent electrophysiological study (Volkov and Amtmann, 2006), we confirmed that $Na^+$ uptake across the plasma membrane of root cells generates an electric current that changes instantaneously when a voltage step is applied, indicating that the open probability of the underlying channel is not voltage-dependent. Unfortunately, the genes encoding this type of voltage-independent channel (VIC) have not yet been identified. Cyclic nucleotide-gated channels have been suggested as one candidate system mediating instantaneous current into *A. thaliana* roots (Maathuis and Sanders, 2001; Demidchik and Maathuis, 2007) but the $Na^+$ current recorded in *Thellungiella* roots was not altered by addition of cyclic nucleotides. In accordance with the radiotracer data, $Na^+$ inward current was significantly smaller in *Thellungiella* than in *Arabidopsis*, and this difference could be attributed to a higher selectivity of the *Thellungiella* VIC for $K^+$ over $Na^+$ (Volkov and Amtmann, 2006). The difference in $Na^+$ permeability between *Thellungiella* and *Arabidopsis* was reflected in the membrane potential. Whereas *Arabidopsis* showed a large depolarization after addition of $Na^+$ to the external medium, *Thellungiella* showed only a small and transient depolarization (Volkov and Amtmann, 2006). This is an important observation because membrane depolarization decreases $K^+$ uptake through inward-rectifying $K^+$-selective channels (Amtmann et al., 1999; Amtmann and Sanders, 1999). Our findings therefore explain not only the lower $Na^+$ content of *Thellungiella*, but also the relatively higher $K^+$ content compared with *Arabidopsis* (Wang et al., 2006). Furthermore, it was recently shown that expression of the high-affinity uptake system HAK5 depends on the membrane potential with HAK5-transcript levels increasing at more negative membrane potentials (Nieves-Cordones et al., 2008). Such regulation provides the basis for HAK5-induction under $K^+$-starvation (which hyperpolarizes the membrane). More importantly, considering the difference in membrane potential between the two species (Volkov et al., 2006), it could explain higher HAK5 expression (and high-affinity $K^+$-uptake) under salt stress in *Thellungiella* compared to *Arabidopsis* (Alemán et al., 2008). The combined evidence exemplifies how a structural feature of one transporter ($K^+$/$Na^+$-selectivity of VIC) can have a number of knock-on effects on the regulation of other transporters. Another important consequence of decreased $Na^+$ permeability is related to energy conservation. Quantitative comparison of $Na^+$ inward current at the respective resting membrane potentials with net $Na^+$ accumulation in the shoots showed that the observed difference in $Na^+$ influx into root cells was sufficient to explain the difference in net shoot uptake between *Thellungiella* and *Arabidopsis*. The calculations even predicted a reduced necessity for $Na^+$ efflux in *Thellungiella*. Since $Na^+$ efflux is an energy-consuming process, this finding raises the possibility that salt tolerance in *Thellungiella* is linked to its overall energy balance in high salt conditions.

The fact that $Na^+$ influx into root cells is sufficient to explain the difference in shoot $Na^+$ accumulation between *Thellungiella* and *Arabidopsis* does not exclude an involvement of other transport processes in salt tolerance. A recent study by the Bohnert lab showed that knock-down of SOS1 by RNAi transforms *Thellungiella* into a salt-sensitive species (Oh et al., 2007, 2008). Detailed analysis of the RNAi lines provided evidence that SOS1 is required to avoid $Na^+$ influx from the root tip into the root elongation zone during early stages of salt stress, and to recover $Na^+$ from the xylem during prolonged salt stress. These findings prove that SOS1 is required for salt tolerance but do not necessarily explain the difference in salt sensitivity between *Arabidopsis* and *Thellungiella*. However, previous studies strongly suggest that differential regulation of this transport system exists between the two species (Taji et al., 2004; Kant et al., 2006). It has recently been shown that the stability of the *Arabidopsis* AtSOS1 mRNA is increased during salt stress in a ROS-dependent manner (Chung et al., 2008), raising the possibility that structural differences between ThSOS1 and AtSOS1 enhance ThSOS1 stability in the absence of salt stress and/or enhance stress-induced induction (Oh et al., 2008). This type of study should bring us closer to answering the question of whether SOS1 contributes to the difference in salt tolerance between *Arabidopsis* and *Thellungiella*. To summarize the current state of knowledge: (1) SOS1 function is essential for growth on high salt of both *Thellungiella* and *Arabidopsis*; (2) lower $Na^+$ permeability of VICs is not sufficient for salt tolerance but critical for the difference in shoot $Na^+$ accumulation between *Thellungiella* and *Arabidopsis*. The functions of both systems in restricting shoot $Na^+$ accumulation are summarized in Figure 4.

The observation that *Thellungiella* effectively restricts $Na^+$ accumulation in the shoots suggests that $Na^+$ compartmentalization during salt stress is less important here than in *Arabidopsis* or other salt-sensitive species. Nevertheless, Vera-Estrella and colleagues (2005) measured increased $Na^+$/$H^+$ antiport activity across the tonoplast of *Thellungiella* leaf cells under salt stress. Surprisingly, an antibody against the *Arabidopsis* vacuolar $Na^+$/$H^+$ antiporter AtNHX1 (also recognizing AtNHX2 and AtNHX3) did not detect the protein in *Thellungiella*, suggesting either low similarity of antigenicity in

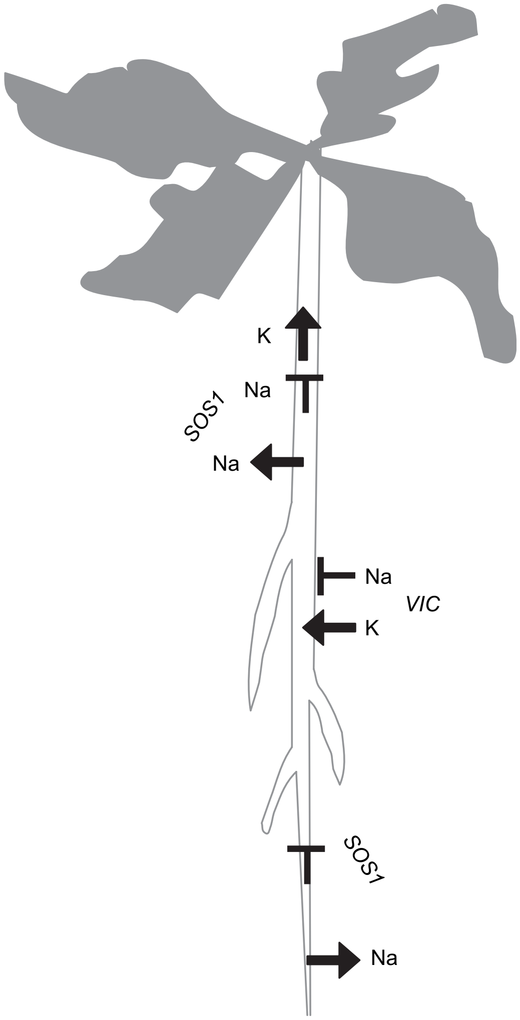

**Figure 4.** Effective Exclusion of $Na^+$ from the Shoot of *Thellungiella* (Shandong) Is Achieved through the Combined Action of a Voltage-Independent Channel (VIC) and a $Na^+/H^+$ Antiporter (SOS1).

VIC has higher selectivity for $K^+$ over $Na^+$ than the respective system in *Arabidopsis*, thereby limiting $Na^+$ influx into root cells and maintaining a negative membrane potential that activates $K^+$ inward rectifying channels and high-affinity transport systems. SOS1 exports $Na^+$ from the root tip and xylem, thereby limiting its transport into the root elongation zone and the shoot. For details and references, see text.

NHX-type transporters between the two species or the involvement of other transporters in $Na^+/H^+$ antiport.

## TECHNIQUES, TOOLS, AND RESOURCES

One of the advantages of *Thellungiella* as a model species resides in its amenability to a wide range of physiological and molecular techniques (Figure 5). The above reviewed studies provide evidence for applicability of physiological and electrophysiological techniques (patch clamp, tracer flux analysis, and ion exchange assays), as well as transcriptomics and metabolomics. In my own lab, we have shown that

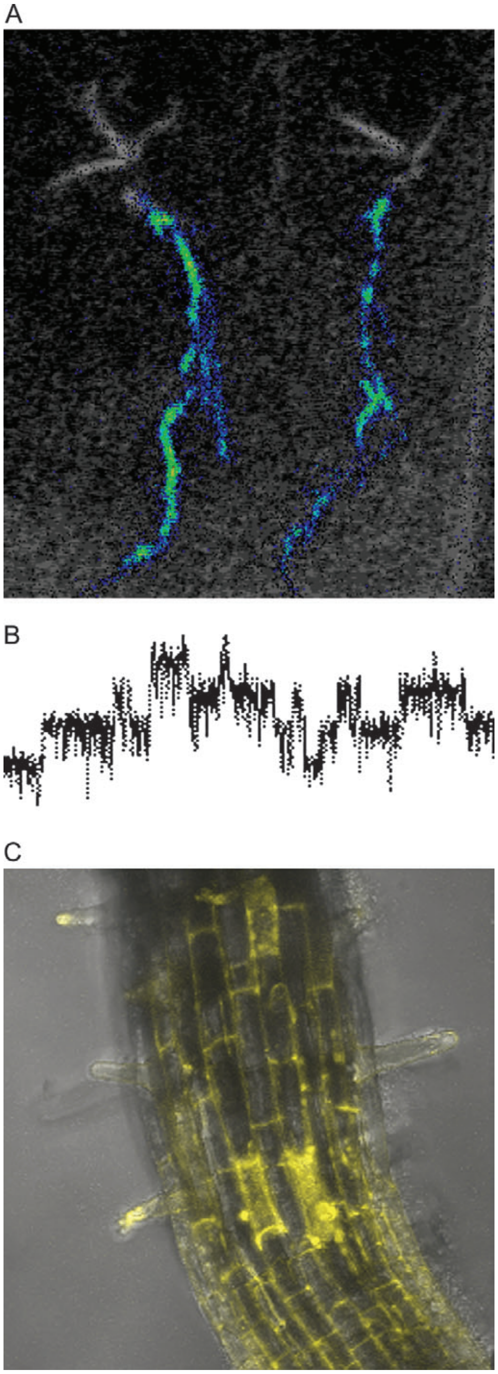

**Figure 5.** Technical Approaches to Study the Physiology and Molecular Biology of *Thellungiella* (Shandong).

**(A)** Micro-grafted hybrid plants comprising shoots of wild-type *Thellungiella* and roots of luciferase-transformed *Arabidopsis thaliana* plants (graft and photo by G. Littlejohn, University of Glasgow).

**(B)** Patch clamp recordings of picoAmpere-currents passing through distinctly opening and closing single ion channels in the plasma membrane of *Thellungiella* root protoplasts (recording by V. Volkov, University of Glasgow).

**(C)** Visualization of the epidermal cells of *Thellungiella* roots transiently expressing Yellow Fluorescent Protein (YFP) linked to the ER marker gene HDEL after transfection with *Agrobacterium rhizogenes* (photo by P. Camapanoni, University of Glasgow).

micrografting techniques can be used to investigate hybrid plants between *Thellungiella* and *Arabidopsis* (G. Littlejohn and A. Amtmann, unpublished results). Efficient transformation of *Thellungiella* can be achieved by flower-dipping (protocol available at www.thellungiella.org) and leaf disc transformation (Li et al., 2007). On the basis of an improved transformation protocol, Bressan and colleagues have created a T-DNA knockout collection, which is available to the research community for a modest fee to cover seed divisions and handling (contact Ray Bressan at bressan@purdue.edu). A transient root transformation protocol has also been shown to work in *Thellungiella* (Campanoni et al., 2007). Several cDNA libraries have been constructed from *Thellungiella* plants grown under various stress conditions (Wong et al., 2006; Liu et al., 2007; Ni et al., 2007). One cDNA library has been used to transform fission yeast (*Saccharomyces pombe*) and several transformants selected on high salt are now under investigation (Liu et al., 2007). Another cDNA library has been shuttled into the binary vector pCB406 and the resulting population of *A. thaliana* transformants will be available for functional gene mining (Ni et al., 2007). Most importantly, K. Schumaker, R. Wing (University of Arizona) and T. Mitchell-Olds (Duke University) have launched a sequencing program at JGI, which is expected to deliver a full genome sequence of *Thellungiella* over the next couple of years. The sequence will provide an invaluable tool both for comparative genomics within the Brassicaceae (Schranz et al., 2006) and for the identification of novel genes.

## CONCLUSIONS

Research into *Thellungiella* has made enormous progress over the last few years and is likely to gain further momentum over the years to come, considering the wealth of available tools and a pending genome sequence. The studies reviewed here are evidence that *Thellungiella* has already yielded new information on how plants can achieve a high level of resistance against abiotic stresses. The most detailed knowledge gain to date concerns transport properties of *Thellungiella*, with $K^+/Na^+$ selectivity of root ion channels and function of SOS1 in $Na^+$ exclusion from the root tip and xylem emerging as crucial mechanisms for limiting the accumulation of toxic $Na^+$ in the shoot. The particular advantage of *Thellungiella* for stress tolerance research resides in its high similarity to *Arabidopsis* with respect to morphological appearance, metabolic pathways, and cDNA sequence and yet a striking difference in stress tolerance. Research into *Thellungiella* can therefore build on the enormous wealth of knowledge that has been accumulated for *Arabidopsis* and transfer it to comparative physiology and genomics. Direct comparison between the two species has already delivered exciting new results. The emerging picture is that *Thellungiella* uses similar molecular entities to *Arabidopsis* to deal with stress but maintains a state of stress anticipation in non-stressful conditions, and operates more specific regulation under stress. The comparative aspect is critical for future success with this species because so many of the essential components for stress tolerance already exist in *Arabidopsis* (and crops), and can improve stress tolerance when overexpressed. Real understanding and agricultural benefits will only be gained if we continue to investigate the species-specific regulation and the cost/benefit balance of individual stress tolerance mechanisms. Clearly, such understanding will be invaluable for avoiding unwanted side effects and ensuring sustainability of future efforts to improve crop performance on marginal and irrigated land through genetic modification. Let evolution be our teacher!

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