

# Transcription strength and halophytic lifestyle

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When plants experience elevated concentrations of NaCl only a few species survive and set seeds. However, survival specialists – termed halophytes or, generally, extremophiles – can be found in essentially every plant family, seemingly identifying genes determining the halophytic lifestyle as a legacy of all plants. We have indicated the importance of transcription control leading to ‘copy number variation’ (CNV), specifically strength of transcription and/or stability, as a crucial characteristic that distinguishes halophytes from their salt-sensitive (glycophytic) relatives [1,2]. Interspecies comparisons of expressed sequence tags (ESTs) over the years have shown it is probable that the tolerance phenotype is not, or not to a significant degree, based on global differences in protein/enzyme complement or protein folding or stability characteristics, although examples in these categories are equally likely to exist. Stress-dependent gene expression programs can be particularly useful in defining differences. For example, comparisons by microarray hybridizations of the salt-sensitive *Arabidopsis thaliana* (*Arabidopsis*) with its relative *Thellungiella salsuginea* (previously *T. halophila*), an extremely salt-tolerant species, showed stress-specific, stress intensity-dependent, distinct responses in the halophyte. The *Arabidopsis* response, even at low stress levels, might best be characterized as chaotic and all-embracing [3]. Obviously, *T. salsuginea* is tolerant to higher concentrations of sodium ions, whereas *Arabidopsis* perceives a much lower concentration as stressful. However, if it is not the nature of regulated genes and proteins that determine plant salinity stress tolerance, the question is how more organized transcription programs might perform that service.

One aspect distinguishing plant species, apart from gene copy number, appears to be the strength of transcript expression that is recorded as CNV [4,5]. In a comparison between *Arabidopsis* and *T. salsuginea* under control and salt stress conditions, we have shown that a fundamental difference exists in the steady-state amounts of *SOS1* mRNAs in both conditions, and in the levels of induction during salt stress [1]. *SOS1* has been well-studied and is known as a crucial component of the salt extrusion and intra-plant distribution systems [6]. Moreover, RNA-interference with the *SOS1* gene in *T. salsuginea*, which reduced transcript amounts, converted the halophyte into a salt-sensitive species [1].

These observations have now been extended by including genome sequences and gene expression characteristics

of a third related species. Similar to the juxtaposition of *A. thaliana* Col0 and *T. salsuginea*, *SOS1* gene expression shows the same behaviour when the halophytic *Thellungiella parvula* is included ([7]; see the [Supplementary Data Figure S1](#)). We observed a fundamental difference between *Arabidopsis* and the halophytes at both the basal, non-stress level and upon induction by high salt [7].

Given the importance implied by high expression of *SOS1* for halophytic lifestyle, we compared 5'-untranslated potentially regulatory regions of available *SOS1* sequences – including eight from halophytic species. Two sequence features in the 5'-UTR stand out [7]: (i) compared to glycophyte *SOS1*, the nucleotide topology is more pyrimidine-rich in halophytes (where at least 100 bp of 5'-UTR sequences were available for comparison); and (ii) a stable stem-loop structure is predicted close to the initiation codon for all halophyte *SOS1* sequences, and only for the halophytes (Table 1).

Untranslated leader sequences are known to affect translation efficiency, subcellular localization and/or mRNA stability [8]. For example, stem-loop structures in the 5'-UTR have been related to translation initiation or mRNA stability in bacteria, archaea, chloroplasts, animals and plants [9]. Similarly, stretches of (CT)<sub>n</sub> and (CTT)<sub>n</sub> are widespread among plant genomes, often enriched in 5'-UTR regions of genes [10,11]. Short (CTT)<sub>n</sub> tracts in *Arabidopsis* are associated with salicylic acid signalling [10], and 5'-UTR poly-pyrimidine tracts have been correlated with enhanced basal level expression [12].

Over the last 30 years, numerous individual genes putatively responsible for the halophyte/glycophyte dichotomy have been reported based mainly on transcriptome and genome studies. Some of these might be real, but there are also many marginal or simply conjectured stress-tolerance entities. The rationale associating either the genes and their proteins with salt stress, and how they bring about ‘tolerance’ is frequently only weakly supported. However, a number of expression studies that achieved overexpression of putatively beneficial proteins in precisely targeted tissues have achieved protection [13]. If overexpression is achieved, increased expression strength is a result. In the model presented here, the *SOS1* genes (Table 1), which constitute a crucial component of plant salinity tolerance, show increased transcription in the *Thellungiella* species irrespective of the condition compared to *Arabidopsis*. Although this is at least in part a consequence of the significantly different promoter structure observed before [1,7], preferential transcript stability or preferential translation seem to provide additional, and possible fundamental,

Table 1. <sup>a</sup> 5'-UTR CT% and hairpin structures in *SOS1* sequences<sup>b</sup>

Species	CT content (%)	Predicted stable hairpin structures (MFE in kcal/mol)
<i>Thellungiella salsuginea</i> <sup>c</sup>	80	-4.4
<i>Thellungiella parvula</i> <sup>c</sup>	72	-6.2
<i>Salicornia brachiata</i> <sup>c</sup>	70	-6.5
<i>Chenopodium quinoa</i> <sup>c</sup>	69	-4
<i>Rhizophora mangle</i> <sup>c,d</sup>	-	-25.12
<i>Mesembryanthemum crystallinum</i> <sup>c,d</sup>	-	-7.8
<i>Mimulus guttatus</i> <sup>e</sup> , mgf024507m	71	-6.5
<i>Brachypodium distachyon</i> <sup>f</sup> , Bradi4g00290	67	-16.4
<i>Oryza sativa</i> , LOC_Os12g44360	68	None
<i>Manihot esculenta</i> , cassava18086.valid.m1	62	None
<i>Manihot esculenta</i> , cassava39868.m1	60	None (-1.5)
<i>Ricinus communis</i> , 29780.t000067	58	None
<i>Zea mays</i> , GRMZM2G098494	56	None
<i>Manihot esculenta</i> , cassava20295.m1	55	None (-2.2)
<i>Manihot esculenta</i> , cassava39869.m1	55	None
<i>Medicago truncatula</i> , Medtr2g043140	49	None
<i>Vitis vinifera</i> , GSVIVT00030673001	48	None
<i>Cucumis sativus</i> , Cucsa.026500	47	None
<i>Sorghum bicolor</i> , Sb08g023290	41	None
<i>Cucumis sativus</i> , Cucsa.026490	41	None
<i>Populus trichocarpa</i> , POPTR_0010s11130	41	None
<i>Carica papaya</i> evm., TU.supercontig_2.68	40	None
<i>Arabidopsis thaliana</i>	-	None (-3.26+ two bulges in the hairpin structure)
<i>Arabidopsis lyrata</i> 484121	-	None (-2.1)
<i>Selaginella moellendorffii</i> 74518	54	None
<i>Selaginella moellendorffii</i> 75049	52	None
<i>Physcomitrella patens</i>	50	None
<i>Chlamydomonas reinhardtii</i> , Au9.Cre24.g769600	48	None

<sup>a</sup>The table has been modified after [7].

<sup>b</sup>Sequences were selected only when the sequence similarity to *Arabidopsis SOS1* was significantly higher than the similarity to *Arabidopsis NHX8* (an antiporter with high sequence similarity to *SOS1*). The CT content as a percentage is calculated for the first 100 nucleotides upstream of the initiation codon. A value is not calculated if the 5'-UTR sequence predicted or available is shorter than 100 bp. Species with a predicted stable hairpin structures are indicated with predicted minimum free energies (MFE). If a weak structure was predicted the MFE is given in parentheses. A minimum free energy of -3.6 kcal/mol or less was considered to represent a stable stem-loop structure. Minimum free energy was not calculated if no stem-loop structure was predicted when the stem-loop prediction varied with sequence length increments or multi-loop structures were predicted. Zuker's algorithm, version 1.8.2 of Vienna RNA Package RNAfold (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>) was used for stem-loop predictions.

<sup>c</sup>Known halophytes.

<sup>d</sup>The 5'-UTR is too short to provide for %CT calculations.

<sup>e</sup>*Mimulus guttatus* is known for salt tolerance.

<sup>f</sup>*Brachypodium distachyon* is known for tolerance to several abiotic stresses.

elements that enable the halophytic lifestyle. In the latter functions the structure of the 5'-UTR region could play a role. The forthcoming availability of more plant genome sequences and complete transcriptome analyses based on next-generation DNA sequencing tools will help us scrutinize a hypothesis that assumes CNV, in the form of gene duplications or by altered transcript expression or stability, as a part of the mechanisms that guided plant evolutionary adaptations to different stressful environments.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.tplants.2010.10.006](https://doi.org/10.1016/j.tplants.2010.10.006).

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