

## From Systems Biology to Gene Function: Discovering New Determinants of Plant Tolerance to Abiotic Stresses

Simon Barak\*

French Associates Institutes for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel

Guaranteeing food security in an era of rising world population, global climate change, and pressure to use land situated in harsh environments for agriculture, is an increasing challenge. In the drive for improved crop performance, efforts have focused on identifying genes controlling many traits including greater tolerance to biotic and abiotic stresses. However, several problems in identifying new candidate genes can be distinguished. Classic forward and reverse genetic screens of plant mutants require the screening of up to hundreds of thousands of plants, yet the return is very low – typically <1%–3% of the mutant population display a desired phenotype [1-8]. Although computational methods have shown potential in identifying sets of candidate genes, very few studies have actually tested their predictions on mutant populations. Moreover, most of those studies that have screened mutants defective in candidate genes, have only screened a few mutants, making it difficult to robustly assess gene discovery rate [9-13].

We have been employing both systems biology approaches and classic molecular biology techniques, to identify and then characterize useful candidate genes for improving plant stress tolerance. In a recent report, we developed a systems biology-based screen for novel *Arabidopsis thaliana* abiotic stress regulatory genes that combines gene expression ranking and RNA co-expression analysis, followed by rigorous screening of over 120 T-DNA insertion lines [14,15]. We obtained a remarkable gene discovery rate of 62%, a 48-fold increase over classic genetic screens, and better than any other currently reported computational method. Our co-expression network of *Arabidopsis* regulatory genes can be inspected at <http://netbio.bgu.ac.il/arnet>.

Mutants of two genes that were identified in our screen exhibited greater tolerance to multiple abiotic stresses as well as enhanced expression of stress-responsive genes, suggesting that the two genes encode negative regulators of *Arabidopsis* abiotic stress responses [16]. We therefore designated the genes, *STRESS RESPONSE SUPPRESSOR (STRS) 1* and *STRS 2*. Both genes encode members of the DEAD-box RNA helicase family that possess RNA duplex unwinding activity, and can promote duplex formation as well as displacement of proteins from RNA [17]. DEAD-box RNA helicases are involved in virtually all aspects of RNA metabolism, and are often part of supramolecular complexes where they function in remodeling RNA and in assembly of ribonucleoprotein structures. In a recent study, we demonstrated that the STRS proteins are localized to the nucleolus, nucleoplasm and chromocenters (regions of heterochromatic, transcriptionally inactive DNA), and exhibit relocalization in response to abscisic acid (ABA) treatment and various abiotic stresses [18,19]. We also presented strong evidence suggesting that the STRSs are involved in RNA-directed DNA methylation-mediated epigenetic silencing of gene expression to bring about suppression of the *Arabidopsis* stress response.

Although *Arabidopsis thaliana* has provided a wealth of information on physiological and molecular mechanisms of stress tolerance, this species is actually sensitive to stress and is unlikely to possess stress tolerance mechanisms that are functional in naturally stress-tolerant plants. Therefore, we have also used both systems biology and molecular approaches to undertake comparative analyses of *Arabidopsis* and its naturally stress-tolerant relative, *Eutrema (Thellungiella) salsugineum*. *E. salsugineum* exhibits greater tolerance

than *Arabidopsis* to salt stress, low nitrogen stress, high boron levels, and heat stress [20-24]. We have shown that differential regulation of a basic set of stress tolerance genes might be a crucial component of *E. salsugineum* salt tolerance. For instance, we demonstrated that constitutive down-regulated expression of *E. salsugineum PDH*, encoding the proline catabolic enzyme, proline dehydrogenase, is correlated with increased levels of the osmoprotectant, proline, under control and salt-stress conditions in *E. salsugineum* shoots compared to *Arabidopsis* [21]. At the level of global primary metabolism, specific features of the *E. salsugineum* salt metabolome can be observed such as constitutively higher levels of TCA cycle intermediates, malate and citrate but constitutively lower levels of fumarate and the osmoprotectants, raffinose and galactinol, compared to *Arabidopsis* [25]. Interestingly, many metabolites are repressed in *E. salsugineum* when plants are grown *in vitro* on nutrient agar plates compared to soil-grown plants, yet the plants retain their salt tolerance under both growth conditions. This finding suggests that metabolic adaptive plasticity might allow the flexibility required for an extremophile lifestyle.

In conclusion, our combination of holistic systems biology and reductionist molecular biology approaches to investigate abiotic stress tolerance mechanisms in both temperate and extremophile plant model species, has facilitated: (i) the identification of novel genes regulating *Arabidopsis* responses to multiple abiotic stresses; (ii) the identification and characterization of DEAD-box RNA helicases that control epigenetic silencing of abiotic stress-responsive gene expression; (iii) differential expression of stress-tolerance genes and differential metabolic programming, in extremophile relatives of *Arabidopsis*.

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\*Corresponding author: Simon Barak, Ben-Gurion University of the Negev, French Associates Institutes for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institutes for Desert Research, Midreshet Ben-Gurion 8499000, Israel, Tel: +972-8-6596750; Fax: +972-8-6596752; E-mail: [simon@bgu.ac.il](mailto:simon@bgu.ac.il)

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