

Program

British Council Synergy Symposium

September 3-4, 2018

George Evens Auditorium
Jacob Blaustein Institutes for Desert Research
Ben-Gurion University of the Negev
Midreshet Ben-Gurion, Israel



Sunday, September 2, 2018

20:30

Welcome dinner
for international speakers

Monday, September 3, 2018

08:00

Welcome remarks

08:10

Anne Visscher,
Royal Botanical Gardens, UK
Plant survival in challenging environments on Earth and in Space

08:50

Alexander Bowles,
University of Essex, UK
Evolutionary genomics: the emergence of drought tolerance in plants

09:30

Maheshi Dassanayake,
Louisiana State University, USA
*Insights into the genomic basis of extreme edaphic adaptations in *Schrenkiella parvula**

10:10

Coffee Break

10:40

Menachem Moshelion,
The Hebrew University, Israel
Whole-plant stress performance testing: A new tool for functional phenotyping

11:20

Zvi Peleg,
The Hebrew University, Israel
Phenotypic plasticity facilitates alterations in life-history strategies under combinations of environmental stresses

12:00

Vered Chalifa-Caspi, Ben-Gurion University of the Negev, Israel
NeatSeq-Flow: A platform for easy design and execution of complex high-throughput sequencing workflows for programmers and non-programmers alike

12:40

Lunch break

14:00

Simon Barak, Ben-Gurion University of the Negev, Israel
*Using *Arabidopsis* and its extremophyte relatives to identify genes involved in tolerance to abiotic stresses*

14:40

Stephen Chivasa, Durham University, UK
Gene discovery by signal interception - a tool for mining plant stress adaptation for biotechnology

15:20

Coffee break

15:50

Amy Marshall-Colon,
University of Illinois, USA
Predictive modeling to determine causal factors involved in long distance nitrogen signaling in plants

16:30

Esti Yeger-Lotem, Ben-Gurion
University of the Negev, Israel

Quantitative views into the tissue-selectivity of hereditary diseases

17:10

Closing remarks

Tuesday, September 4, 2018

08:30

Tour around Midreshet Ben-Gurion, Ben-Gurion's gravesite, view over the Wilderness of Zin

09:30

Brief refreshments

09:40

Shiri Freilich, Volcani Institute, Newe
Yaar, Israel

*What can a farmer do with NGS data?
Challenges in mapping quantitative traits
based on NGS data.*

10:20

Aaron Fait, Ben-Gurion University of
the Negev, Israel

*Correlation-based network analysis to
reduce complexity of metabolomics data
matrices*

11:00

Coffee break

11:20

Maxim Kapralov,
University of Newcastle, UK

*Exploring the natural diversity of
plant enzymes to increase efficiency
of photosynthesis in crops: Rubisco
with or without C_4 and CAM carbon
concentration mechanisms*

12:00

Philip Mullineaux,
University of Essex, UK

*Heat shock transcription factors- targets
for increasing yield in the face of stress
in the Brassicaceae. Can this strategy be
applied to all crop species?*

12:40

Ulrike Bechtold, University of Essex, UK

*Identification of genes important for heat
and drought tolerance in desert species*

13:20

Closing remarks



For more information
in.bgu.ac.il/en/bidr/FAAB/Pages/synergy-conference.aspx



Ben-Gurion University of the Negev
The Jacob Blaustein Institutes for Desert Research

Plant survival in challenging environments on Earth and in space

Anne Visscher

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Strategic Priority 1 of the Royal Botanic Gardens Kew is to document and conduct research into global plant and fungal diversity and its uses for humanity. One of Kew's main resources is its collections, with currently over 38,000 plant species represented as seed samples in the Millennium Seed Bank, as well as over 35,000 species stored as genomic DNA and silica-dried tissues. Genomics has a critical role to play in fulfilling Kew's mission, for example in building the correct tree of life through phylogenomics, molecularly describing the living and preserved collections, and linking intra- and inter-specific genetic variation to trait evolution and symbiotic relationships.

Determining the tree of life for all plant and fungal genera using NGS sequencing data is one of Kew's key strategic outputs. This presents challenges as there are 24,000 genera, with no data yet available for ~43% of them. An Angiosperm-wide HybSeq bait design covering 353 single copy genes has just been released and will be used for the project. Another potential future project for which Kew is well-placed to contribute is the sequencing of all living species. Possible obstacles to its realization are sample collection/management, linking sequence to reference material, bioinformatics and international coordination.

Aside from these large-scale projects supported by genomics, there are several individual research groups at Kew that use sequencing to discover the drivers and processes underpinning global plant and fungal diversity. Regarding research on extremophytes, there is a particular focus on succulent desert plants, involving both vegetative and reproductive (seed) stages. One example is the study of global patterns in succulent plant diversity and the evolutionary processes underlying their adaptations and ecological success, using the genus *Aloe* (Asphodelaceae) as a model system. Another example is a research programme on the tolerance of seeds from succulent Aizoaceae species to extremely hot and dry desert conditions, which may also break seed dormancy. In addition, there is interest in developing a major project on halophytes, which to date have been researched at Kew mainly in the context of seed germination and salinity.

Although extreme environments away from Earth - in space - are not natural for life forms that have evolved on Earth, storage and transport of seeds through space has become a relevant area of study in the field of exobiology, especially in the context of human space exploration and long-term missions to other planets that require plant-based life support systems. GENESISS (Germination after Extreme Natural Exposure of Seeds on the International Space Station) is an ongoing project at Kew that was selected for flight by the European Space Agency in 2015. Its aim is to research how and why seeds from a diverse set of 24 plant species differ in their survival of outer space conditions such as vacuum (ultra-drying, anoxia), temperature fluctuations and ultra-violet radiation. It will involve analyses of seed viability and DNA mutation profiles following approximately 18 months of space exposure.

Evolutionary genomics: the emergence of drought tolerance in plants

Alexander Bowles

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Food security is an increasingly important global agenda. It has been estimated that by 2050 the world population will have risen to 9 billion people. With an expanding human population competing for environmental resources coupled with the detrimental impacts of climate change, abiotic stresses such as drought are having severe consequences for crop productivity. The improvements of crop yield by genetic methods have revealed much about the natural variation of genes that are involved in drought tolerance. Many of these traditional approaches have helped us understand the molecular basis of plant adaptations but the evolution of drought tolerance as a trait is an area of abiotic stress biology that is still poorly understood.

Genes important in drought tolerance are likely to have evolved across the plant tree of life influenced by major evolutionary innovations (e.g. transition of plants from water to land). By unravelling the evolutionary history of plants, the impacts of natural selection shaping plant genomes can be assessed. In the last decade, there has been a rapid increase in the quantity and quality of complete genomes from across the plant phylogeny which has allowed for large scale genomic comparisons. Inferences from these approaches will inform our understanding of the influences of speciation and duplication events on gene family innovation, expansion and reduction.

Using this evolutionary genomics approach, we have conducted a large scale all vs all BLAST of 208 genomes containing ~9 million protein coding genes. The outputs have been grouped using the clustering algorithm, MCL, and personalised scripts have been used to extract clusters of homologous genes. Evolutionarily conserved gene innovations, termed Novel Core, have been identified at every node in the plant phylogeny. Functional analysis of these groups has revealed the genomic changes that have accompanied the major plant developments including the evolution of the embryo, cell wall and seed. Amongst these outputs are important signalling pathways involved in the expression of drought tolerance such as ABA mediated responses.

By defining plants with a drought response status and incorporating these definitions into this comparative genomics pipeline, we are currently investigating the distribution of known drought response genes and identifying novel genes for experimental evaluation as potential targets to engineer drought resistance. By applying this genomic information, it is hoped that breeding targets for high yielding crop varieties with greater stress tolerance and water use efficiency will be identified.

Multi-ion salt stress adaptation explored using extremophyte genomics

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Schrenkilla parvula (Syn *Theilingiella parvula*), is an extremophyte relative of *Arabidopsis thaliana* and *Brassica* crops. *S. parvula* compared to *A. thaliana*, presents a unique system to identify naturally selected physiological traits, developmental responses, and genome reorganization behind adaptations to multi-ion salt stresses. Despite the stark differences in adaptations to extreme salt stresses, *S. parvula* and *A. thaliana* show extensive genome-wide macrosynteny. Genome structural variations, including tandem gene duplications, gene transpositions, and transposable element insertions interrupt the collinearity observed throughout the genomes and distinguish *S. parvula* from *A. thaliana* and other closely related non-halophytic species. Comparative physiological observations on *S. parvula* support the comparative transcriptomic studies between *S. parvula* and *A. thaliana* that highlight the stress-prepared responses of *S. parvula* even under stress neutral conditions. These studies have led to the identification of key physiological and developmental traits in *S. parvula*, and the gene families involved in ion transport and other metabolic pathways associated with salt stress responses. Genomic structural variants appear to have led the two species into two distinct lifestyle trajectories. We have observed tissue-specific isoform expression that may further add refinement to adaptive stress responses. The extremophyte genome of *S. parvula* acts as a repository of genetic changes that have enabled its successful niche adaptation to a hypersaline environment. Our genomic and transcriptomic dissection offers a framework to identify adjustments of genome architecture and expression that control a set of genes regulating fundamental physiological responses in salt stress adaptations.

Whole plant stress performance testing: A new tool for functional-phenotyping

Menachem Moshelion

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Many of the early components of a plant's response to biotic and abiotic stress are related to modifications in plant water relations and, therefore, can be used as characteristic markers at a very early stage of the stress. Here, I present a simple, yet effective experimental platform that is based on a gravimetric system combined with a unique irrigation-drainage system and soil and atmospheric probes. This system enables tight control on multiple soil water/salinity scenarios while continuously monitoring in a quantitative manner the soil-plant-atmosphere water relations. The system simultaneously monitors numerous plants for a variety of biotic and abiotic stresses at high resolution. Five Quantitative Physiological Traits (QPT) are determined concurrently: 1) whole-plant transpiration rate; 2) daily/periodically increase in plant biomass; 3) canopy conductance; 4) whole-plant water-use efficiency (WUE); and 5) Root influx. These QPTs are measured for single plants in an array, over time periods ranging from minutes to the entire growing season, under normal, stress and recovery conditions at different phenological stages. A supplemental algorithm that integrates these traits enables the calculation of additional important QPTs ; the root-to-shoot water flux ratios and whole-plant relative water content (RWC).

Use of this experimental platform for the comparative physiological characterization of several crop cultivars has revealed several plant stress-response strategies that we have classified according to their relative "conservative" (isohydric-like) or "risk-taking" (anisohydric-like) character. We describe a "calculated risk-taking" trait that can be used as a marker for the selection of abiotic stress tolerance and resilient plants.

Phenotypic plasticity facilitates alterations in life-history strategies under combination of environmental stresses

Lidor Shaar-Moshe¹, Ruchama Hayouka¹, Ute Roessner² and Zvi Peleg¹

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Climate change-driven stresses such as extreme temperatures, water deficit, and ion imbalance are projected to exacerbate and jeopardize global food security. Under field conditions, these stresses usually occur simultaneously and cause damage that exceeds single stresses. Plants have developed various reversible and non-reversible acclimation mechanisms to cope with the multifaceted nature of abiotic stress combinations. We hypothesized that in order to endure these stress combinations, plants elicit distinctive acclimation strategies through specific trade-offs between reproduction and defense. To investigate *Brachypodium distachyon* acclimation strategies to combinations of salinity, drought and heat, we applied a system biology approach, integrating physiological, metabolic and transcriptional analyses. We analyzed the trade-offs among functional and performance traits, and their effects on plant fitness. A combination of drought and heat resulted in escape strategy, while under a combination of salinity and heat, plants exhibited avoidance strategy. On the other hand, under combinations of salinity and drought, with or without heat stress, plant fitness was severely impaired. These results indicate that under combined stresses, plants' life-history strategies were shaped by the limits of phenotypic and metabolic plasticity and the trade-offs between traits, thereby giving rise to distinct acclimations. Our findings provide a mechanistic understanding of plant acclimation to combinations of abiotic stresses, and shed light on the different life-history strategies that can contribute to grass fitness and possibly to their dispersion under changing environments.

NeatSeq-Flow: A platform for easy design and execution of complex high-throughput sequencing workflows for programmers and non-programmers alike

Menachem Sklarz, Liron Levin, Michal Gordon and Vered Chalifa-Caspi

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Bioinformatics analysis of high-throughput sequencing (HTS) data typically requires the execution of a series of software packages, whose combination and set of parameters vary with the type of experiment and the research question at hand. Flexibility, modularity and reproducibility are therefore critical requirements for a system that manages these analyses. Moreover, the rapid emergence of new applications of HTS technology, together with inflation of new application-specific software tools, necessitates a generic system, which may be easily and rapidly adapted to new analysis workflows (WFs).

We have developed NeatSeq-Flow, a user-friendly, yet powerful platform for the design and execution of complex HTS analysis WFs. The platform suits skilled programmers, providing them with utmost flexibility to develop new WFs for any desired HTS analysis, as well as non-programmers who may use the platform through an intuitive graphical user interface (GUI), for existing as well as their own custom-made WFs.

NeatSeq-Flow and its GUI are intended to be installed locally, either on a computer cluster or on a single computer. A large repository of ready-to-use modules and WFs is available for a wide variety of HTS applications, including reference-alignment, *de novo* assembly, RNA-Seq, ChIP-Seq, SNP calling, metagenomics and others. A generic module enables threading together software tools for which pre-built modules are not yet available. Alternatively, users may build their own modules using a template, requiring only basic knowledge of python programming.

NeatSeq-Flow is designed with a clear separation between modules, WFs, sample information and actual execution. This setup enables the use of the same WF design on different sets of samples as well as using different WFs on the same set of samples, all of this without changing the individual elements. Moreover, this independency of elements enables easy sharing of WFs and modules between users.

NeatSeq-Flow enables efficient WF execution by parallelizing on both samples and WF steps. WF output files are neatly organized in an intuitive directory structure, relieving the user from the need to manage file location, or to transfer files between WF steps. NeatSeq-Flow operates by shell-script generation, thus allowing full transparency of the WF process and full control over the execution. NeatSeq-Flow offers real-time execution monitoring, detailed documentation and self-sustaining WF backups for reproducibility.

All of these features make NeatSeq-Flow an easy-to-use WF platform while not compromising on flexibility, reproducibility, transparency and efficiency. NeatSeq-Flow is freely available on GitHub at <http://neatseq-flow.readthedocs.io/en/latest/>

Using *Arabidopsis* and its extremophyte relatives to identify genes involved in tolerance to abiotic stresses

Simon Barak

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As challenges to food security increase, the demand for lead genes for improving plant tolerance to stress is growing. However, genetic screens of plant mutants typically yield very low frequencies of desired phenotypes. We therefore developed a systems biology approach for discovering new genes that regulate the response of plants to multiple abiotic stresses. By combining gene expression ranking, RNA co-expression analysis and screening of *Arabidopsis* mutants, we obtained a > 40-fold increase in gene discovery rate compared to classic mutant screens. Moreover, our gene ranking system can be used to prioritize mutants for screening.

One problem however with employing *Arabidopsis* to identify new stress tolerance determinants is that it is by no means a stress-tolerant species. Therefore, in the last decade and a half, naturally stress-tolerant extremophyte *Arabidopsis* relatives (*Brassicaceae*) have been used in comparative systems biology analyses to understand molecular adaptations to extreme habitats, and to search for novel stress tolerance determinants. Among the extremophyte *Arabidopsis* relatives that we are studying is *Anastatica hierochuntica* (True Rose of Jericho) from the Negev Desert in Israel, which is a member of the poorly investigated lineage III *Brassicaceae*. We previously showed that *A. hierochuntica* has a genome approximately 4.5-fold larger than *Arabidopsis*, divided into 22 diploid chromosomes. *A. hierochuntica* exhibits tolerance to heat, low N and salt stresses that are characteristic of its habitat, and displays common and species-specific metabolic stress tolerance strategies with its halophytic relative, *Eutrema salsugineum*. To further investigate molecular mechanisms of *A. hierochuntica* stress tolerance, we generated a high quality *A. hierochuntica* reference transcriptome, and performed a phylogenomic analysis based on 13,927 orthologous sequences of 17 *Brassicaceae* plant species including various crop plants, extremophytes and *A. thaliana*. We identified positively selected genes that may have been crucial for adaptation to abiotic stresses, and uncovered evidence suggesting that selection pressures leading to adaptation and speciation focus on common biological processes although the identity of the positively selected genes is specific to each species. We also identified positively selected genes that are specific to *A. hierochuntica*, and may underlie some of the morphological and life history characteristics that allow an opportunistic existence in the harsh desert environment.

Gene discovery by signal interception – a tool for mining plant stress adaptation for biotechnology

Steve Chivasa

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A fundamental property of biological systems, which spans from simple unicellular prokaryotes to complex multicellular eukaryotes, is collective “decision-making” between cells making up a colony or residing within the same tissue. In this process, neighbouring cells secrete signal molecules into a shared compartment, the extracellular matrix, for signal integration to enable cells in the same neighbourhood to synchronise their responses to internal or external cues. The integrated signal is sensed at the cell surface via receptors, which activate changes in gene expression to trigger a tissue-wide response. Our hypothesis is that the traffic of signals through the extracellular matrix is highly regulated and transiently surges during adaptive responses to stress. Therefore, we have taken the approach of intercepting these signals during plant stress responses as a way to identify new targets for manipulating crop tolerance to stress. As proof-of-concept, we have used *Arabidopsis* as a model system to identify key genes involved in drought stress and mycotoxin responses. Novel targets have been identified, which provide new insights into plant stress responses, and crop trials have commenced for some of these. We are extending this research using the sorghum model system to understand how this crop mounts an effective response enabling it to survive extreme drought and heat stress. Generation of large proteomics, metabolomics, and genomics datasets, and how mathematical modelling can help will be discussed.

Modeling dependence in nitrogen signaling cascades using dynamic transcriptome data

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Communication between organs is important for all biological organisms to report whole system status and maintain homeostasis. Organisms are able to respond dynamically to environmental perturbations through the coordinated action of signal transduction cascades among different tissues. Whole plant N-signaling likely involves several independent long-distance signal transduction pathways, reliant on many different signaling molecules (Takei et al., 2004, Osugi et al., 2017). Time series data are important for understanding the biological processes that are activated at different times and for inferring causality (Bar-Joseph et al., 2012). This presentation will explore causal gene-gene relationships in time series data from above- (shoot) and below- (root) ground organs, to understand the dependence structures in dynamic transcriptome data. Identification and validation of such causal relationships can help elucidate the underlying molecular and biochemical pathways involved in the N-signal response. Moreover, increased understanding of N-responsive biochemical pathways in different plant tissues can help predict emergent plant properties under N sufficiency and deficiency. We developed a new statistical model to identify causal interactions among genes from two different organs. This model predicted 3078 causal gene-gene interactions that include directional information about the tissue of origin for causal and target genes. Analysis of the 3078 gene interactions revealed that some gene pairs across organs are involved in the same or a linked biochemical pathway, and that several known nitrogen-responsive gene families, such as the bZIP, NLP, Cl-channel, SLAC/SLAH and NRT families, have roles as causal genes.

References

Bar-Joseph, Z., A. Gitter, and I. Simon. 2012. "Studying and Modelling Dynamic Biological Processes Using Time-Series Gene Expression Data." *Nature Reviews Genetics* 13 (8). Nature Publishing Group: 552–64.

Osugi A, Kojima M, Takebayashi Y, Ueda N, Kiba T, Sakakibara H. 2017. Systemic transport of trans-zeatin and its precursor have differing roles in Arabidopsis shoots. *Nat Plants*. 3:17112. doi: 10.1038/nplants.2017.112.

Takei K, Ueda N, Aoki K, Kuromori T, Hirayama T, Shinozaki K, Yamaya T, Sakakibara H. 2004. AtIPT3 is a key determinant of nitrate-dependent cytokinin biosynthesis in Arabidopsis. *Plant Cell Physiol*. 45(8):1053-62.

Quantitative views into the tissue-selectivity of hereditary diseases

Esti Yeger-Lotem

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A longstanding puzzle in human genetics is what limits the clinical manifestation of hundreds of hereditary diseases to certain tissues or cell types, while their causal genes are present and expressed throughout the human body. In recent years, we developed and applied several computational approaches to this enigma, which focus on the expression, interactions and paralogs of causal genes. In this talk, I will describe the approaches and tools that we developed toward better understanding of tissue-selective diseases and processes.

What can a farmer do with NGS data? Challenges in mapping quantitative traits based on NGS data

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Until recent years, genetic mapping was mostly based on single-copy, low-throughput DNA markers where whole-genome projects relied on a maximum of several hundreds of markers. The post-genomic era, where reference genomes are available, and genotyping is moving towards sequence-based methods, calls for a methodological shift and implementation of suitable tools. Next generation breeding is transforming into a process of continuous molecular marker-guided assembly of plant genomes using pre-defined desired alleles. NGS enhances the ability to effectively dissect traits and identify tightly-linked markers or causative genetic factors that can be used for Marker-Assisted Selection (MAS). The challenge remains in selection of markers associated with the determination of complex polygenic traits, where multiple QTLs interact with each other and with the genetic background. Typically, results from association studies provide a list of discrete candidate loci per trait. High-resolution genetic mapping provides the necessary empirical data to address the effect of epistatic (gene-by-gene) interactions more thoroughly than was possible in the past. In an ongoing project, we test the relevance of several approaches for simulating the phenotypic effects of multiple-loci data. For different traits, we consider all the effect of pairwise combinations between selected QTLs and construct QTL-traits networks. I will discuss our ongoing attempts to develop and applying pipelines that will allow researchers to handle the raw sequence data from their studies and provide a start-to-end user-friendly solution for Genotype-by-Sequencing (GBS)-based genetic studies, starting from the raw data, to the construction of QTL interactions networks, and marker selection.

Correlation-based network analysis to reduce complexity of metabolomics data matrices

Aaron Fait

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The holistic description of plant metabolism is a non-trivial undertaking. Sensitive analytical platforms, which are capable of accurately quantifying the levels of a plethora of chemical features, can assist in tackling this task. However, the ever-increasing amount of high-throughput data, often from multiple technologies, requires significant computational efforts for integrative analysis. Network analysis has been introduced in the recent years to study plant metabolism and investigate the interactions between metabolites. Hence, network analysis can help interpret complex data sets through the identification of key network components. The relationship between structural and biological roles of network components can be evaluated and utilized to aid metabolic engineering. By employing correlation-based network analysis, we shed light on the regulation of amino acid metabolism in tomato seeds.

Exploring the natural diversity of plant enzymes to increase efficiency of photosynthesis in crops: Rubisco with or without C₄ and CAM carbon concentration mechanisms

Maxim Kapralov

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The major enzyme responsible for assimilation of inorganic carbon into organic biomass in plants, algae and bacteria is ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), whose performance is often one of the limiting steps in photosynthesis and can greatly affect plant survival and crop yield alike. Composed of eight large and eight small subunits, plant Rubisco is one of the largest and most abundant enzymes that command a suite of assembly and catalytic chaperones. As the performance of Rubisco can greatly affect crop yields, substantial efforts have been made to study its structure and function using directed mutagenesis, with the aim to artificially improve Rubisco performance. However, neither the practical problem of delivering better enzymes for crops nor the fundamental questions about Rubisco evolution in different groups of plants have been resolved so far. We analyse results of the Rubisco mutagenesis experiment performed by nature during evolution of C₃, C₄ and CAM plants. Using Maximum likelihood and Bayesian analyses of the *rbcL* genes, which encode large subunits of Rubisco, we found that positive Darwinian selection affects Rubisco evolution in most lineages of plants, and a small number of amino acid replacements is responsible for the fine tuning of Rubisco kinetics in different lineages. We further pinpointed residues under directional selection in c. 7000 plant species, and checked whether amino acid replacements at these sites correlate with climate parameters such as temperature and precipitation experienced by studied species. All sites under selection are located in functionally important regions of the Rubisco enzyme. Our results suggest that Rubisco properties are being adjusted by natural selection to better fit the environmental conditions and that the currently predominant “one size fits all” model for Rubisco kinetics is incorrect. We discuss evolutionary aspects of Rubisco performance in plants and their biotechnological implications for recombinant Rubisco bioengineering in crops.

Heat shock transcription factors- targets for increasing yield in the face of stress in the Brassicaceae. Can this strategy be applied to all crop species?

Philip M. Mullineaux

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Heat Shock Factors (HSFs) are a highly conserved class of transcription factors (TFs) found across the Eukarya. They are well known as key determinants of cellular resistance to heat stress as well as a range of other environmental stresses, such as heavy metal exposure, drought stress, infection by some pathogens and oxidative stress. However, what is less appreciated is that HSFs are also essential for cell function under non-stress conditions. In yeast and fruitfly, loss of function of their single HSF genes is lethal or results in embryo abnormalities, respectively. In vertebrates, the number of HSF genes expands to 4-5. Nevertheless, each of these genes still has roles in development as well as in induction of stress tolerance. Higher plants, in contrast, have >20 HSF genes, divided into 3 structurally distinct classes, A, B and C. Classes A and C are transcription activators, while B class HSFs are transcription repressors. This increased number and complexity of plant HSF gene families has extended their roles, for example, in disease resistance, and their patterns of expression, such as seed-specific expression of *Arabidopsis AtHSFA9*. However, the complexity does not end there. With an expanded number of HSF genes in plants has come the concept of a subset of HSF “master regulator” genes that control the expression of other HSF genes; an arrangement not seen anywhere other than in higher plants. The concept was first proposed from studies on the tomato *HSF* family, such that *SIHSFA1* appears to have this dominant role at least as far as conferring acquired thermotolerance. In contrast, in *Arabidopsis*, the master regulator role is shared between the clade A1 class of *AtHSFs*. The 4 paralogous genes (*AtHSFA1a, b, d* and *e*) influence the expression of many, if not all, other *HSFs*. We recently conducted detailed genome-wide studies showing that *HSFA1b* regulates >900 protein encoding genes and >400 long non-coding antisense RNAs. In addition, *HSFA1b* is at the top of a hierarchical network of 27 TF genes and through these, controls the expression indirectly of a further >1700 genes. There is evidence from other recent studies that *HSFA1a* exerts similar overlapping patterns of control. This understanding of TF gene family composition may be crucial in exploiting these and other gene classes to confer stress tolerance to heterologous species. The problem is illustrated as follows: Over-expression of *AtHSFA1b* in *Arabidopsis* and *Brassica napus* confers tolerance to abiotic and biotic stress in lab and field situations and subtle developmental effects lead to increased seed yield though redistribution of biomass in favour of reproductive structures. However, over-expression of *AtHSFA1b* in tobacco has no major effects, implying that the use of a master regulator TF gene to increase stress tolerance will be confined to species of the same family. In complete contrast, wheat *TaHSFA4a* confers cadmium (Cd) tolerance to a *yeast* Cd-hypersensitive strain! We suggest that we have to develop methodology that can recognise master regulator HSFs since simply identifying orthologs of these genes in different species may not suffice. The implications of these observations for exploitation of extremophile genes in crop species will be considered further.

Identification of genes important for heat and drought tolerance in desert species

Ulrike Bechtold

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Many studies of drought stress responses in resurrection plants and other xerophytes have identified genes, proteins and metabolites that respond to severe drought stress and/or desiccation. This has provided insight into drought resistance mechanisms that allow xerophytes to persist under the most extreme environmental conditions. The C3 species, *Rhazya stricta* (*R. stricta*), does not undergo a dormant state but is able to withstand periods of low rainfall, leaf temperatures of up to 43°C, and light intensities of greater than 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. *R. stricta*, commonly grows in arid zones and overcomes water restriction by accessing water in underground river beds, which allows it to grow and maintain high photosynthetic rates despite the extreme temperature and light conditions. Gene families specific to *R. stricta*, such as photosynthesis- and respiration-associated genes responded to diurnal temperature changes and high vapour pressure deficits (VPD), and by combining transcriptomics and evolutionary genomics, we showed that some of these genes have diverged from their homologs in other species. Therefore, *R. stricta* could provide alternative gene targets responsible for maintaining photosynthesis and growth under harsh environmental conditions. We are presently investigating the response of *R. stricta* to reduced water availability by comparing plants grown inside and outside of known underground river beds. A significant reduction in stomatal conductance and leaf water potential was observed in plants outside of the river bed, and genes that uniquely respond to water limitation in *R. stricta* compared to other well-studied xerophytes are currently being identified.