

***EvoNet*: A Phylogenomic and Systems Biology approach to identify genes underlying plant survival in marginal, low-Nitrogen soils**

Kranthi Varala^{1*} (kranthi.varala@nyu.edu), Rodrigo Gutiérrez,² Dennis Stevenson,³ Rob DeSalle,⁴ W. Richard McCombie,⁵ Jean-Michel Ané,⁶ Heidi Kaeppler⁶ and **Gloria Coruzzi**¹

¹New York University, New York; ²Pontificia Universidad Católica de Chile, Santiago, Chile; ³New York Botanical Garden, New York; ⁴American Museum of Natural History, New York; ⁵Cold Spring Harbor Laboratory, New York and ⁶University of Wisconsin, Madison

<http://evonet.org>

Project Goals: This DOE BER sustainability project aims to identify the key genes and gene regulatory networks that enable “extreme survivor” plants to adapt and grow in marginal, extremely nitrogen (N) -poor soils in the arid Chilean Andes. These extreme survivor species cover the main branches in flowering plants and include 7 grass species of particular interest for biofuels. We focus on 24 “extreme survivor” Chilean species that are relevant to biofuels and compare their genomes to 24 Californian “sister” species that live in a N replete arid environment. Exploiting a novel phylogenomic pipeline we have developed, we use a “paired species” sampling strategy to identify the genes that support the evolutionary divergence of the extreme survivors in the deserts of the Chilean Andes from their sister species in California. The genes thus identified will help to discover the mechanisms underlying physiological and developmental processes that allow efficient assimilation of nitrogen in nitrogen-poor, dry soils. We will test selected genes in the model grass *Brachypodium* for their ability to confer enhanced growth on low N and/or dry environments. The genes and network modules so uncovered can potentially be translated to biofuel crops to greatly increase biomass and nitrogen use efficiency in marginal, low-fertility soils.

This collaborative project exploits the genomes of “extreme survivor” plants adapted to thrive in marginal, extremely Nitrogen (N) poor soils in the arid Chilean Andes. It uses a previously validated phylogenomic pipeline we developed called *BigPlant* [1], and a “paired species” sampling strategy, to identify the genes that distinguish these “extreme survivors” in Chile from their related species adapted to similarly dry regions in California (CA) that are not constrained by N. These “extreme survivor” species and paired species samplings represent multiple independent origins of the traits (adaptation to low N and drought), and therefore offer diverse genomic backgrounds within which the survival traits repeatedly arose. We apply our *BigPlant* phylogenomic approach [1,2] to the study of “marginal survivor” strategies as follows:

Aim 1. Sample transcriptomes of 24 Chilean “extreme survivors” & paired CA species (NYU, NYBG, Chile). ***Progress:*** Completed RNA-seq analysis of all 24 Chilean species and collected 11/24 sister species from CA & their RNA-Seq library preparations are in progress. ***Aim 2.*** Phylogenomic analysis of 48 “paired species” to identify genes that repeatedly support nodes that distinguish the extreme survivors in Chile from their sister species in CA (AMNH, NYU). ***Progress:*** We enhanced the *BigPlant* phylogenomic pipeline to build phylogeny from 32

paired species with ~20-30K orthologs per species. This phylogenomic analysis of 70+ species translates to ~1.7 million orthologs and ~2 million informative characters, building the largest phylogenomic tree ever constructed to date. This phylogenomic analysis identified candidate genes that provide support for the evolution of low-N adaptation in the Chilean species.

NEW We compared 32 Chilean species we sequenced to their closest related species whose transcriptome is already available. In this analysis (*EvoNet v1*), we captured 19 evolutionarily independent origins of low-N/Drought adaptation (see Figure 1). Our *BigPlant* phylogenomic

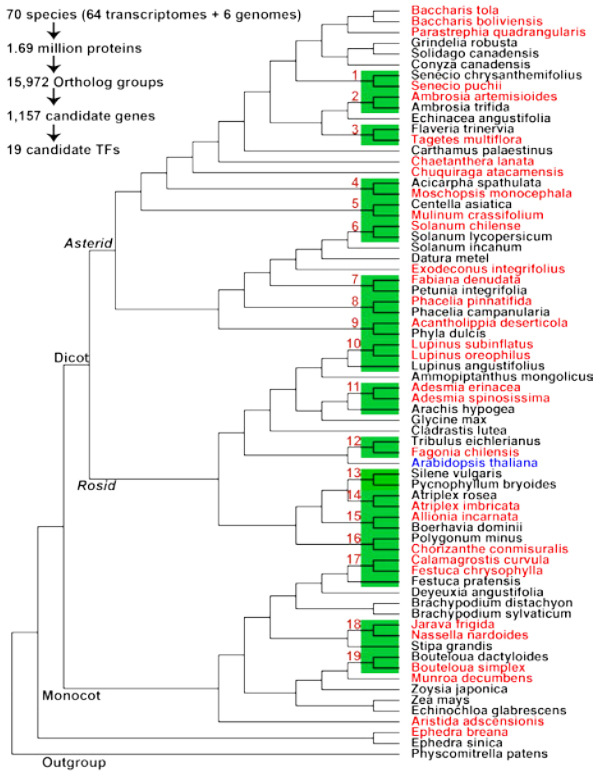


Figure 1. *EvoNet v1: Extreme survivor species in Chile (Red) and paired “sister” species (black).* Preliminary phylogenetic analysis of 32 pairs of “extreme survivors” to available sister species identified 1,157 genes that provide recurrent (>3 independent origins) support for the appearance of low-N/Drought adaptations. Our *EvoNet* analysis captured 19 independent origins of low-N adaptation.

References

1. Lee E et. al., A functional phylogenomics view of the seed plants. PLoS Genet 7(12):e1002411.
2. Delaux et. Al., Comparative phylogenomics uncovers the impact of symbiotic associations on host genome evolution. PloS Genet 10(7):e1004487.

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analysis pipeline [1] identified 1,157 genes that provide recurrent support (> 3 independent origins) for the divergence of low-N adapted Chilean species from their available sister species. This set of genes is enriched in the processes Nitrogen compound metabolism and Photosynthesis (GO term analysis FDR <0.01). Crucially, we identified 19 transcription factors that provide recurring support for low-N adaptation and are functionally validating them through transgenic lines in the model monocot *Brachypodium*.

Aim 3. Combine phylogenomics (protein sequence) and gene networks (gene expression) to identify genes and network modules associated with adaptations to marginal, low-N soils (NYU, Chile).

Progress: We exploit a comparative analysis of gene regulatory networks by developing a new module *PhyloExpress* that extends the *BigPlant* phylogenomic pipeline to include gene expression data.

Aim 4. Functionally validate top-ranked candidate master genes for low-N adaptation in Arabidopsis and Brachypodium (NYU, Chile, U Wisconsin).

Progress: We (Wisconsin) are transforming Brachypodium with the most promising candidate from our *EvoNet v1* analysis (Fig 1).