

# Designer Rice: Improvement of Source to Sink in Rice for Higher Yields

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## Abstract

*The improvement of grain yield in rice is a major breeding objective that is primarily done by direct selection under field conditions for primary and secondary yield components. Understanding the basis of yield components in a 'holistic' systems biology approach integrating information from plant physiology, molecular genetics and plant breeding can enable the development of crop models for yield and crop improvement. Plant biomass and underlying biological processes of photosynthetic carbon metabolism are major components of grain yield that are quite drastically affected under environmental stress, which reprograms the plant's gene expression, physiology and development. We propose a systems biology analysis of perturbations that disturb grain yield, such as environmental drought stress, and an analysis of the coordinated reprogramming of a plant to resist or adapt to the stress will reveal the regulatory and functional mechanisms that underpin grain yield. This approach has been taken by identification of a regulator of photosynthetic carbon metabolism under stress that can increase grain yield and tolerance to environmental stresses in rice.*

**Keywords:** Grain yield, source, sink, rice, environmental stress

## Introduction

Grain yield is a function of plant biomass that can be converted to reserves in the sink tissue that can be harvested as grain. The basic process of living systems, photosynthesis, fixes carbon into carbohydrates and lipids through photosynthetic carbon metabolic processes. The evolution of plants went through a selection process under diverse environmental conditions, where plants matured within the environmental regime of temperature, water and day-length restrictions to produce the next generation of seed for survival. The plants naturally selected and adapted for an environment could survive under normal seasonal conditions indefinitely, unless challenged by random environmental stresses. Within the natural selection scheme is also built in an adaptability to resist environmental stresses, where the plant can respond to the stresses by physiological adaptation brought about by a cascade of molecular and physiological changes that manifest themselves to a new homeostasis that is more adapted and resistant to the environment.

The objective of human beings has been to domesticate plants for food and other needs, utilizing the basic photosynthetic carbon metabolic processes to capture

sunlight into carbohydrates, lipids and other building blocks to meet our nutritional requirements. The selection process of domestication has changed plants from being very adaptive to stresses and survival towards being a producer of our crop harvests under a more managed environment. One consequence is that our crop plants have not the capacity to survive and yield under unpredictable environmental conditions.

The proposed hypothesis is that crop plants are selected for high yield under optimal cultural conditions, at a cost of lacking the adaptability to adverse environmental stresses. The basic building blocks of grain yield, photosynthetic carbon metabolism and transfer of reserves from source to sink are thus not coordinated to respond to environmental stresses.

## Review of recent advances in research

Photosynthesis is acknowledged as the ultimate basis of yield, but yield improvement via improvement of photosynthetic efficiency has not yet been successful. Evidence that elevated CO<sub>2</sub> can increase leaf photosynthesis in crops by as much as 22.6% over the growing season suggests that increasing photosynthesis can increase yield (Zhu et al 2010). One of the primary

interests has been on engineering Rubisco to improve photosynthetic efficiency (Spreitzer et al 2002), however many more metabolic reactions in photosynthetic carbon metabolism and associated processes in sucrose synthesis and photorespiration have been shown to play an equivalent role. Analysis using a dynamic model of photosynthetic carbon metabolism (Zhu et al 2007) suggests that the partitioning of resources among enzymes of photosynthetic carbon metabolism in C3 crop leaves is not optimal for maximizing the light-saturated rate of photosynthesis, and under elevated CO<sub>2</sub> predicted for the future, this problem is amplified.

The selection of changes to the photosynthetic process intended to improve biomass production and crop yield must take into account a complex matrix of interacting elements. It is recognized that combining systems modeling with modern breeding and transgenic technologies holds promise to design new pathways, such as improved CO<sub>2</sub> fixation and photo-respiratory pathways (Kebeish et al 2007), or new genetic regulatory networks (Barrett et al 2006) to improve photosynthetic efficiency.

### **Innovative studies underway**

Our studies in rice towards genetic improvement of water use efficiency and resistance to abiotic stresses towards production of environmentally stable rice plants suggested that improvements in these production parameters contributed to improved yield. Since environmental stresses such as water deficiency or drought drastically affected a suite of metabolic processes involved in photosynthetic carbon metabolism, we reasoned that the analysis and understanding of the regulation of such coordinated down-regulation of essential metabolic processes would provide clues to identification of regulatory networks that could protect a plant under the stress. At the same time, the regulatory networks that protect a plant under stress maintaining yield and the associated coordinated regulatory pathways, could as well be a means to enhance yield under more favorable conditions.

The basic biological processes in rice, including photosynthetic carbon metabolism and other mechanisms such as stress response pathways are affected by drought stress, for which we developed methods to study interactions of networks of genes. A Rice Environment Coexpression Network RECoN was made, integrating gene expression data from 129 samples (datasets) of 45 gene expression experiments of rice challenged by some environmental challenge (Ambavaram et al 2011). Coexpression networks provide information on networks of genes that are

expressed similarly and thus likely to be involved in the same biological process. RECoN was constructed to have 34,792 rice genes with 18.5 million edges, and this stringently selected for 1,744 dense clusters of 28,421 closely associated genes, that could provide information on biological processes such as metabolic pathways working together. With RECoN we can query any sets of genes, for example in carbohydrate metabolism, and get a network of closely interacting genes that are most probably associated with a query gene set.

We next developed methods to identify genes associated to photosynthetic carbon metabolism (e.g. photosynthesis, carbohydrate biosynthesis) that were potential transcriptional regulators (e.g. transcription factors, TFs), whose actions were also perturbed/associated to environmental stress. A 'regulatory association network' was developed in rice by aggregating genome-wide expression profiles of all rice genes under various developmental stages in the form of a normalized gene expression matrix, with expression values derived from the experiment of development of rice reproductive process (Jain et al 2007). Rice transcription factors curated from multiple public databases were used to calculate correlations between gene-TF pairs and generate a gene-TF co-expression network. By setting an appropriate threshold (few false positives, less indirect interactions), significant correlations between TFs and gene clusters could be obtained which were available for experimental validation. The gene clusters were annotated with functional annotations e.g. GO biological process categories.

Hence, gene-level correlation scores were summarized into more robust process-level association scores to the TF, generating a TF-process association network in rice. To identify TFs regulating stress processes we selected TFs that were significantly up- or down-regulated under the perturbation of drought stress (compared to well-watered) from our gene expression results and identified TFs strongly associated with biological processes of interest, e.g. photosynthetic metabolism.

### **Prospects of reaching the breeding goal**

A TF strongly positively associated to photosynthetic carbon metabolism under normal well-watered conditions and negatively associated under drought stress was identified. Overexpression of the TF was able to maintain photosynthetic carbon metabolism under drought stress and under normal conditions. The phenotype observed was drought resistant by maintenance in expression of genes involved in essential biological processes including photosynthetic carbon metabolism, and conferring yield advantage

under reproductive stage drought stress over wild-type to an extent of 15-40%. Interestingly, expression of the TF coordinately expressed genes in photosynthetic carbon metabolism at an enhanced level. This resulted in enhanced photosynthesis, sugars, starch, biomass with more shoot and root growth and finally higher yield to an extent of 30% increase in some genotypes.

The regulatory network of downstream TFs and target genes in photosynthetic carbon metabolism are all part of the regulatory network model involved in enhanced yield. The results also provide us systematic data on genes and yield parameters enhanced that directly functionally contribute to increased grain yield. This provides us with functional gene markers to be used to search for natural favorable alleles to be used in molecular breeding. The enhancement in yield parameters by overexpression in a variety of elite cultivars provides us independent networks of genes that work under diverse (*japonica* and *indica*) backgrounds, and the specific alleles of downstream genes involved.

#### REFERENCES

- Ambavaram MM, Krishnan A, Trijatmiko KR and Pereira A.** 2011. Coordinated activation of cellulose and repression of lignin biosynthesis pathways in rice. *Plant Physiol* 155: 916-931.
- Barrett CL, Kim TY, KimHU, Palsson BØ and Lee SY.** 2006. Systems biology as a foundation for genome scale synthetic biology. *Curr Opin Biotechnol* 17:488-492.
- Jain M, Nijhawan A, Arora R, Agarwal P, Ray A, Sharma P, Kapoor S, Tyagi AK and Khurana JP.** 2007. F-box proteins in rice. Genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. *Plant Physiol* 143: 1467-1483.
- Karaba A, Dixit S, Greco R, Aharoni A, Trijatmiko KR, Marsch-Martinez N, Krishnan A, Nataraja K, Udayakumar M and Pereira A.** 2007. Improvement of water use efficiency in rice by expression of *HARDY* an *Arabidopsis* drought and salt tolerance gene. *Proc Natl Acad Sci USA* 104:15270-5.
- Kebeish R, Niessen M, Thiruveedhi K, Bari R, Hirsch H-J, Rosenkranz R, Stähler N, Schönfeld B, Kreuzaler F and Peterhänsel C.** 2007. Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*. *Nat Biotechnol* 25:593-599.
- Spreitzer RJ and Salvucci ME.** 2002. RUBISCO: Structure, regulatory interactions, and possibilities for a better enzyme. *Annu Rev Plant Biol* 53:449-475.
- Zhu XG, de Sturler E and Long SP.** 2007. Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. *Plant Physiol* 145:513-26.
- Zhu XG, Long SP and Ort DR.** 2010. Improving photosynthetic efficiency for greater yield. *Annu Rev Plant Biol* 61:235-61.
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