

# Solving the Drought Puzzle from the Genome to the Field

*As agriculture reaches into more marginal areas and the effects of climate change become more pronounced, the demand for drought-resistant cereal varieties will only increase. CIMMYT, with the support of the Rockefeller Foundation, works with Cornell University and Pioneer Hi-Bred to learn what really makes plants tolerate drought.*

Outside temperate ecologies, 15% of the world's maize crop, or 19 million tons, is lost every year to drought. (This loss is nearly equivalent to total annual maize production in Mexico, one of the developing world's largest maize producers.) Conventional breeding has been used to develop drought-tolerant cereals, but progress is often slow.

"Progress could be far more rapid if we understood more about key physiological and genetic aspects of the way plants respond to drought," says CIMMYT molecular geneticist and plant physiologist Jean-Marcel Ribaut. Ten years ago, Ribaut initiated research on drought tolerance at the flowering stage of development in maize, a critical period that makes the difference between crop failure and a sufficient harvest. Since then the effort to unravel the secrets of drought

tolerance has gained momentum. Thanks to a dedicated team of scientists and recent collaboration with Pioneer Hi-Bred International and Cornell University, a fuller understanding of how maize plants react to drought is emerging.

## ● Making a mosaic of the genome

Upon Ribaut's arrival at CIMMYT in 1993, he began developing segregating maize populations for drought. Segregating populations are a tool for learning about the genetic basis of a trait. A resistant or tolerant maize line is crossed with a susceptible line for a particular trait, creating what Ribaut calls "a mosaic of the genome." At this stage, by integrating phenotypic screening (an evaluation of a plant's physical characteristics, which indicate how the plant responds to the environment) with molecular analysis (an evaluation of a plant's genetic composition), scientists can begin to identify the genes and/or the genetic regions (quantitative trait loci, QTLs) that contribute to drought tolerance mechanisms.

In the early years, researchers identified QTLs related to yield components and secondary morphological traits of interest, such as flowering traits or senescence. This information is a powerful resource, but it has limitations. "It's great to characterize all those QTLs," Ribaut continues, "but we were really interested in what was going on *beneath* this level—in terms of physiological mechanisms and gene expression—to track the key pathways involved in drought response."

## ● A picture emerges

Plant physiologist Tim Setter of Cornell University had developed techniques to research exactly those aspects of maize drought response, and Ribaut began working with him to identify these pathways in the segregating populations. Setter was interested in CIMMYT's germplasm because it was well characterized at the morphological and genetic levels, and this information was complemented by a large QTL database. Since 2001, the collaboration has intensified. Setter has provided valuable data about levels of plant growth hormones, sugars, and the osmolyte proline in Ribaut's segregating material, generating about 20,000 measurements in 2002. Changes in the concentration of those components in target organs are indicative of metabolic activity, and they provide an understanding of why a given plant yields better than another when water is scarce.

"We already had the QTLs related to grain yield and traits of interest, and with Tim Setter's input, we identified QTLs for key physiological pathways related to drought response," Ribaut reflects. "Then the missing link was at the level of gene expression." The differential gene expression observed in plants that react differently under drought makes it possible to identify which particular genes, among the 40,000 present in the maize genome, play a role in regulating drought tolerance.

Ribaut's team wanted to pursue that missing link through functional genomics. Good fortune arrived in the form of Chris Zinselmeier and Jeff Habben, experts in maize functional genomics at Pioneer Hi-Bred International. Ribaut met them at a workshop on molecular approaches to drought tolerance funded by the

Rockefeller Foundation and held at CIMMYT in 1999. Soon the teams from Pioneer and CIMMYT entered a collaboration devoted to using microarrays (a genomics tool) to identify key genes with differential expression under water-limited conditions.

Ribaut's work received a big boost in 2001 when the Rockefeller Foundation funded a CIMMYT project devoted to innovative and integrated approaches to drought tolerance in maize, which was extended for an additional two years in 2003. "We've been incredibly fortunate, because all this new technology, the partners, and the support of the Foundation arrived right when we needed them," says Ribaut. "For the last decade we've been trying to put a picture of drought tolerance together like a puzzle." Ribaut gives great credit to lab companions Maria de la Luz Gutierrez and Mark Sawkins, as well as CIMMYT maize physiologist Marianne Bänziger, who played a critical role in selecting

germplasm with different responses under drought and evaluating segregating populations under drought in Zimbabwe. "Through a team effort, we're starting to see the bigger picture," he says.

### ● A wide view

The bigger picture brings three major components of understanding drought tolerance together—gene expression, metabolic pathways, and plant morphology—and reveals their interrelationships. For example, by combining information from functional genomics, data on sugar levels, and the QTL analyses, the important genomic regions involved in regulating glucose have been identified (see figure). "Through the collaborations we developed, we have those three knowledge components at our disposal. This puts us in a unique position to bridge the gap between changes in gene expression and plant phenotype," observes Ribaut.

Equipped with this knowledge, scientists have strong hopes of accelerating the development of drought-resistant maize in three ways: by creating a drought consensus map, which indicates the key genomic regions involved with drought tolerance and uses this information for marker-assisted selection; by identifying elite alleles at target genes, the presence of which would serve as predicting factors for plant breeders; and by using genetic engineering to alter specific genes or pathways.

"By engaging in a multidisciplinary approach with good collaborators, we've gained a much wider view of the problem," Ribaut concludes. "Best of all, there's more to come. The benefits of this research could extend to other cereals such as wheat, as some regulatory genes involved in drought tolerance might be common across genomes."

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