

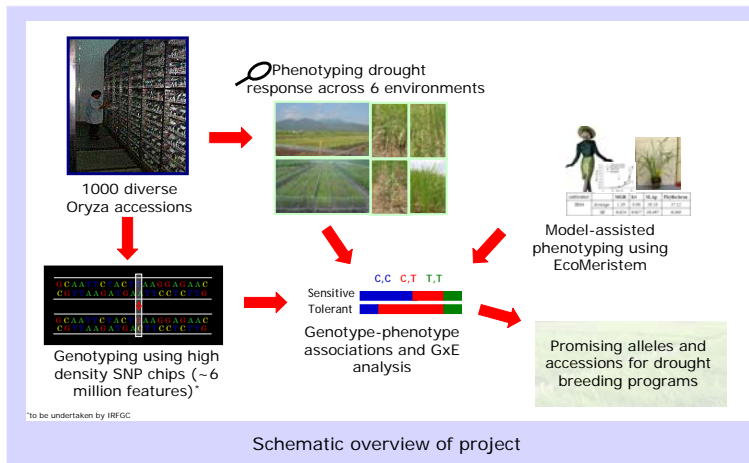
Deciphering the **CODE**: identifying alleles linked to performance in drought-prone environments through genotype-phenotype associations

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If translated into text, the rice genome would take up the equivalent space of 130 copies of Tolstoy's epic *War and Peace*. Unraveling this code and assigning value to key genes or regions is an immense task but advances in technology are helping pave the way. Association studies offer great potential to identify key alleles by linking phenotypes to single polymorphisms within key genes or genomic regions. The identification of alleles linked to performance under drought stress will help improve yields in drought-prone environments.

We used association analysis to look at variation within eight drought-responsive candidate genes and phenotypic response to drought stress, linking field performance to molecular variations within genes. Advances in genome-wide SNP arrays will allow this approach to be applied to thousands of genes. Currently 20 varieties have been genotyped using this technology with a further 2,000 to be genotyped. Here we report on a new project to phenotype a collection of 1000 of these accessions for drought response, and apply association analyses to identify key regions responsible for reproductive success, and ultimately yield stability under drought.



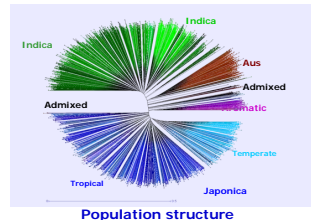
Validation of key drought-responsive genes

A mini-core of 1,536 *Oryza* accessions were chosen to represent the genetic diversity within *O. sativa* L. The mini-core was phenotyped for performance under drought stress and miss-matches (SNPs or indels) within key drought-responsive candidate genes identified.

Population structure was determined by 48 SSR markers using Instruct (GCP Project 2005-01g). Within indica and japonica, four and two subgroups were identified, respectively.

Candidate genes	
Gene	Bioprocess
14-3-3	Membrane associated signal cascade
ADF	Actin-depolymerizing factor
BZIP	Transcriptional control
DREB2B	Dehydration-responsive element binding protein
ERF3	ABA responsive element binding factor
MAPK	Protein kinase domain containing protein
Sucrose synthase	Sucrose synthase
TPP	Trehalose-6-phosphatase

Eight candidate genes were selected using evidence from functional annotation, expression analyses, co-localization with QTL and shifts in allele frequencies under selection. EcoTILLING was used to identify polymorphisms within candidate genes.

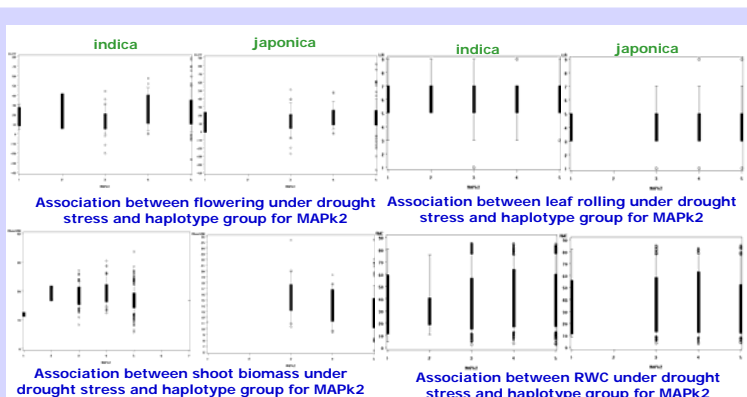


Field drought screening

Accessions were screened in the field under upland conditions over 3 years (2004-2006). Drought was imposed at the vegetative stage. Secondary traits associated with performance were measured.

Association between molecular variation in candidate genes and field performance under water-deficits

Large phenotypic variation was observed for all traits within each isozyme group. Significant associations were observed between haplotype groups of genes and performance under drought, although this varied depending on trait and level of variation within a gene.



Upscaling

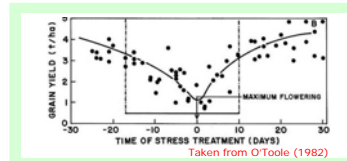
Recent advances in genome-wide SNP arrays allow this approach to be upscaled to thousands of candidate genes/regions. Under OryzaSNP, the International Rice Functional Genomics Consortium (IRFGC) in collaboration with Perlegen Sciences genotyped twenty *Oryza* accessions for more than 400,000 SNPs (McNally et al. 2006). A similar approach will now be applied to ~2,000 rice accessions.

OryzaSNP accessions



Phenotyping for performance under water-limited conditions

To exploit this genetic information a large-scale phenomics initiative has just started. Phenotyping will be conducted under reproductive stage drought, the most sensitive stage to drought, providing a valuable knowledge base for breeders.



Partner	Ecosystem	Indica	Japonica	Aus
IRRI	Upland		x	
	Lowland	x		x
CIRAD	Greenhouse		x	
	Upland	x		
TNAU	Upland	x		
	Lowland	x		x
CRRI	Upland	x	x	
BAU	Lowland	x		x
WARDA	Upland		x	
BIOTEC	Lowland	x		x

Environments and isozyme groups to be phenotyped by partners

Key isozyme groups will be targeted and accessions are being screened for phenology in each environment. By carefully targeting reproductive stage processes under drought stress across a range of environments, a large, robust, phenotypic dataset will be generated for genome-wide association analysis.

Future Perspectives

This project will build on previous research within the GCP to generate large phenotypic data sets on the response of rice to water deficits at the reproductive stage. This information can be used to dissect performance across environments and compare observed polymorphisms with trait variability leading to the identification and validation of specific genes and mining of favourable alleles.

This work is part of the Generation Challenge Program Commissioned Funded Project G4008.05 "Connecting genotypes to performance under water-limited environments through phenotype associations."