

Genetic diversity of candidate genes for root development in rice

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Water stress is responsible for significant losses in rice production. A deep and thick root system was clearly shown to be correlated with a better yield under water stress situations in **rice**. A large number of candidate genes co-localizing with QTLs for root morphology have been detected. Nevertheless, the most valuable genes and alleles to improve **drought tolerance** still remain to be identified.

Our goal is to survey rice **genetic resources** to identify genes and alleles contributing to a deeper, thicker and faster root development using a combination of approaches such as meta QTL analysis, **population genetics**, **association mapping** and **transcriptomics**.

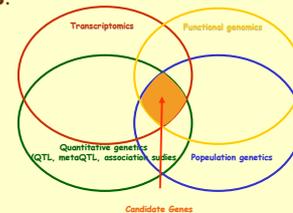


Figure 1: Approaches leading to the identification of candidate genes

Adapted from Leung, 2005

Method

We sequenced segments of 8 candidate genes and surveyed their allelic and haplotypic diversity in a panel of 32 rice accessions representative of *O. sativa* 4 isozymic groups. The level of genetic variation at the nucleotide level was estimated as nucleotide polymorphism (θ) and nucleotide diversity (π). θ and π were calculated for coding regions, for non coding regions and for the whole gene. The neutrality of mutation was tested employing Tajima's D test for the whole set of accessions as well as for the two main varietal groups.

Material

32 rice accessions belonging to 4 different isozymic groups (indica (10), japonica (temperate (8) and tropical (8), aus boro (3), sadri basmati (3) 19 different geographic origins (Australia, Brazil, China, France, Guinea, India, Indonesia, Iran, Italy, Ivory coast, Laos, Madagascar, Pakistan Philippines, Russia, Senegal, Spain, Thailand, USA). 8 genes known to contribute to root development in rice and *Arabidopsis* or co-segregating with meta-QTLs for root development in rice (Courtois *et al*, 2009): *CRL1/ARL1*, 4 transcription factors belonging to the MADS box family (*OsMADS23*, *25*, *27* and *61*), 2 Auxin Efflux Carriers, and the early auxin-responsive gene of auxin/indoleacetic acid component 8 (*IAA8*).

Six out of the 8 genes had sequences of good quality for the 32 accessions. Among these 6 genes, *IAA8* exhibited the highest diversity ($\pi=5.52 \cdot 10^{-3}$). Tajima's test of neutrality of mutations in *IAA8* revealed a significant departure from neutral expectations. D was significant for japonica varieties for *IAA8*. This value is negative indicating an excess of rare alleles. The tests were not significant for the other genes.

Table 1: Diversity rate for *MADS23*, *MADS25*, *MADS27*, *MADS61*, *AEC6* and *IAA8*

	Taille exon	Taille intron	π codant ($\times 10^{-3}$)	π non-codant ($\times 10^{-3}$)	π total ($\times 10^{-3}$)	θ_w codant ($\times 10^{-3}$)	θ_w non-codant ($\times 10^{-3}$)	θ_w total ($\times 10^{-3}$)	Tajima D	Tajima indicas	Tajima japonicas
<i>MADS 23</i>	337	494	0.00	0.64	0.38	0.00	0.99	0.59	-0.69 ns	1.44 ns	-1.16 ns
<i>MADS 25</i>	414	1199	1.56	4.92	4.06	1.81	3.13	2.79	1.54 ns	0.65 ns	-0.85 ns
<i>MADS 27</i>	658	777	2.54	0.61	1.60	2.02	0.71	1.38	0.47 ns	-0.80 ns	-1.25 ns
<i>MADS 61</i>	63	718	0.00	0.43	0.40	0.00	1.05	0.97	-1.37 ns	-1.40 ns	0.00 ns
<i>AEC 6</i>	102	666	0.60	4.94	4.37	2.44	3.00	2.82	1.50 ns	-1.40 ns	-1.76 ns
<i>IAA 8</i>	469	403	1.33	5.52	3.68	0.54	3.01	1.97	2.49 *	0.36 ns	-2.02 *

The gene *IAA8* shows 4 haplotypes. Haplotype 1 is the most frequent (56%) whereas haplotype 4 is the scarcest (3%). Indica and japonica could be discriminated based on their haplotypes. The number of haplotypes was higher among indicas than among japonicas.

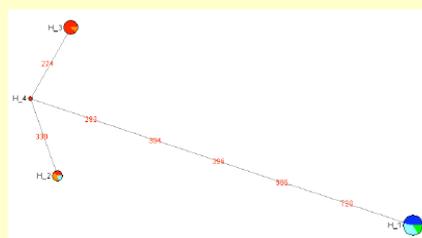


Figure 2: Haplotype network for *IAA8*

The circle size is related to the haplotype frequency. Red:Indica, Dark blue: Temperate japonica, light blue: Tropical japonica, Green: sadri basmati, Orange : aus boro. Red figures indicate the polymorphic site position and the number of mutations separating the haplotypes.

The correlation between the function of *IAA8* and the selection evidences allows considering *IAA8* as a good candidate gene for root development in tropical japonica. We could distinguish isozymic groups based on the haplotypic organization. This bipolarity was reported with neutral markers such as isozymes, RFLP, SSR (Glaszman, 1987; Garris *et al.*, 2005) but it is not always the case.

The polymorphism of *IAA8* and its promoter will be assessed in an extended population of 200 tropical japonica accessions and correlated to phenotypic results on root development through association mapping. The level and tissue localization of the gene expression will be investigated

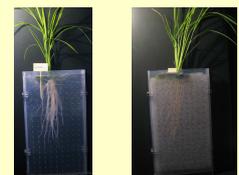


Figure 3: Phenotyping of rice varieties for root development

Glaszman JC (1987) Isozymes and classification of Asian rice varieties. Theor Appl Genet 74: 21-30

Garris A, Tai T, Coburn J, Kresovich S, McCouch S (2007) Genetic structure and diversity in *Oryza sativa* L. Genet 169: 1631-1638

Courtois B, Ahmadi N, Khowaja F, Price A, Rami JF, Frouin J, Hamelin C, Ruiz M (2009) Rice root genetic architecture : meta-analysis from a QTL database improves resolution to a few candidate gene level. Rice 2 (2): 115-118