

Molecular breeding of *Pup1*-rice varieties and candidate gene characterization

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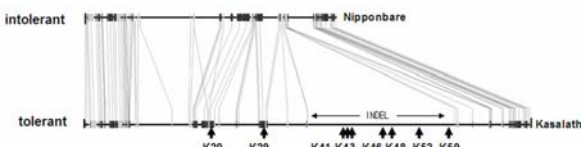
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The major QTL *Phosphate Uptake 1* (*Pup1*) confers tolerance of P deficiency by a yet unidentified mechanism. Sequencing of the *Pup1* genomic region in the tolerant donor parent Kasalath revealed a hot spot of transposon integration and unreliable gene-models. Six genes have now been selected for detailed analyses (fatty acid α -dioxigenase, unknown protein, dirigent, hypothetical protein, protein kinase, Zn-knuckle). Of particular interest is the protein-kinase gene *PupK46* which is not present in the Nipponbare reference genome and might be important for P sensing and signaling. Allelic sequencing revealed a tolerant-specific allele for the dirigent gene (*PupK20*). The putative function of *PupK20* was addressed by quantifying the lignin content in drought and P-stressed roots showing that lignification is a ubiquitous drought response. Root lignification was not observed in plants grown hydroponically with PEG-induced water stress.

Gene-based molecular markers were used to assess the *Pup1* haplotype in diverse rice varieties and breeding lines. This analysis showed that *Pup1* is conserved in upland rice but absent from most irrigated rice varieties suggesting that breeders have unknowingly selected for *Pup1*. The markers are now applied for the development of *Pup1*-introgression into three Indonesian upland (Dodokan, Situ Bagendit, Batur) and two modern irrigated (IR64, IR74) varieties.

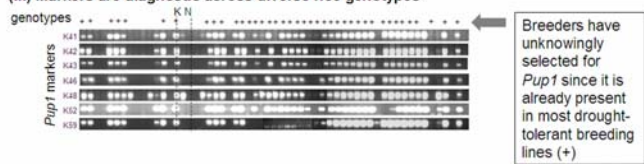
Pup1 BREEDING

(i) Genetic structure of the major QTL *Phosphate uptake 1* (*Pup1*)



(ii) Gene-based markers

(iii) Markers are diagnostic across diverse rice genotypes



(i) Comparative sequence alignment of the *Pup1* regions in Nipponbare (top, intolerant) and the *Pup1* donor Kasalath. Note the large INDEL region in Kasalath with several candidate genes.

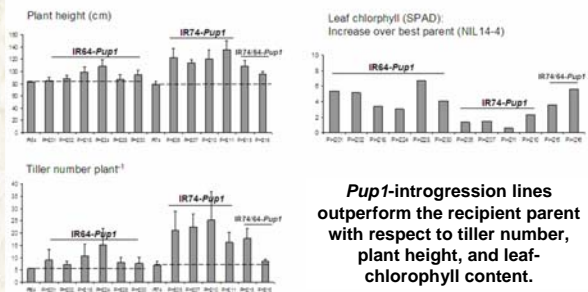
(ii) Position of markers targeting partially conserved genes (K20, K29) and Kasalath-specific genes (K41 to K59)

(i) Germplasm survey with Kasalath-specific, dominant markers



IR64 IR64-*Pup1* NIL14-4
BC₂F₁
(~6% NIL14-4 background remaining)

IR74 IR74-*Pup1* NIL14-4
BC₂F₁
(~12% NIL14-4 background remaining)

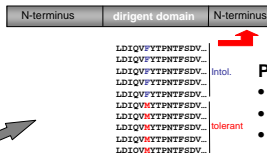


Pup1 CANDIDATE GENES

6 of 9 of 68: Current short list *Pup1* candidate genes

| Gene name | Annotation | Gene in Nipponbare | model validated? | Gene expressed? |
|-------------|----------------------|--|------------------|-----------------|
| OsPup1K01-1 | DOMON (DUF568) | absent | + | no |
| OsPup1K04-1 | fatty acid alpha DOX | highly conserved | + | yes |
| OsPup1K05-1 | CutC or part of DOX? | absent | still unclear | yes |
| OsPup1K20-1 | dirigent | conserved | + | yes |
| OsPup1K29-1 | hypothetical protein | corresponds to two genes in Nipponbare and 93-11 | + | yes |
| OsPupK46-1 | protein kinase | absent | + | yes |
| OsPup1K49-1 | Zn-knuckle | absent | + | yes |
| OsPup1K67-1 | aspartic proteinase | conserved | + | no |
| OsPup1K53-1 | Zn-finger protein? | absent | + | no |

Tolerant-specific allele of *PupK20*

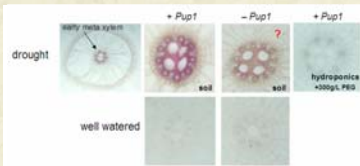


Putative function of dirigent genes:
• Lignin biosynthesis?
• Lignan biosynthesis?
• ROS scavenging?



Genes similar to *PupK46* are present in Nipponbare on Chr. 1, 5, and 8, but not in the *Pup1* region on Chr.12.

Drought-induced root lignification



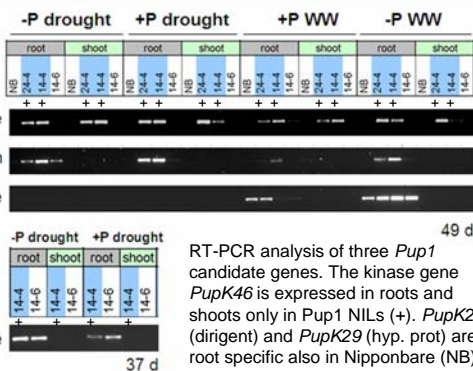
Fluoroglucinol staining of lignin in drought-stressed roots grown in soil or hydroponics with PEG. Staining is only observed in soil-grown, stressed roots.

Ongoing activities:

- Generation/ analysis of transgenic plants (RNAi and 35S)
- Promoter studies
- Field experiments with *Pup1* lines

In preparation:

- Yeast-2-Hybrid screen to identify target proteins of *PupK46*
- Cell wall analyses



RT-PCR analysis of three *Pup1* candidate genes. The kinase gene *PupK46* is expressed in roots and shoots only in *Pup1* NILs (+). *PupK20* (dirigent) and *PupK29* (hyp. prot) are root specific also in Nipponbare (NB).