Phosphorus deficiency tolerance in rice: Development of molecular markers and a phenotyping system for *Pup1*, a major QTL for phosphorus deficiency tolerance

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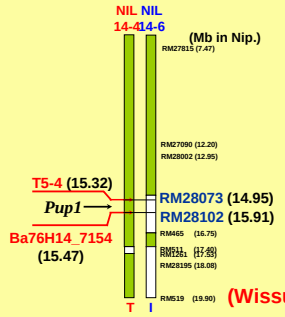
Phosphorus (P) deficiency and P-unavailability are major constraints to rice production, especially in rainfed ecosystems. A major QTL for P-deficiency tolerance, *Pup1* (Phosphorus Uptake 1) has recently been mapped in a Nipponbare x Kasalath population under upland conditions (Wissuwa et al., 2002, Theor. Appl. Gen.) and might be useful for breeders to develop rice varieties with improved performance under low P conditions. The *Pup1* locus was recently fine mapped and sequenced in the tolerant parent Kasalath, and we are now using the sequence information for development of molecular marker and functional assessment of *Pup1* (Wissuwa et al., unpublished; see poster Lu et al, this conference). In order to use *Pup1* for breeding it is of primary importance to develop a reliable phenotyping and genotyping system. As a first step, we have developed *Pup1* specific and *Pup1* flanking markers to monitor representation of *Pup1* in 81 diverse rice varieties and IRRI breeding lines. This analysis showed that *Pup1* is highly represented in upland varieties. For the development of a *Pup1* phenotyping system, we have performed first field screenings under P-deficiency conditions (upland and lowland), and have assessed a possible association of *Pup1* and drought tolerance in a greenhouse experiment with contrasting *Pup1* near isogenic lines (NILs). The latter experiment suggest that *Pup1* indeed has a beneficial effect on yield under drought. In order to identify the major determinant of tolerance we started an allelic survey of selected *Pup1* candidate genes. Interestingly, a putative protein kinase gene (Kas#43) is absent from the 93-11 and Nipponbare genome, but present in *O. rufipogon* and *O. glaberrima*. It is however, highly and specifically represented in most upland *indica* and *japonica* varieties tested. This suggests strong selection for this gene under unfavorable conditions suggesting a beneficial effect of this putative kinase. The *Pup1* candidate gene #18 codes for a dirigent-like gene thought to be involved in lignin and lignan biosynthesis. First allelic sequencing revealed an amino acid substitution that might associate with tolerance.

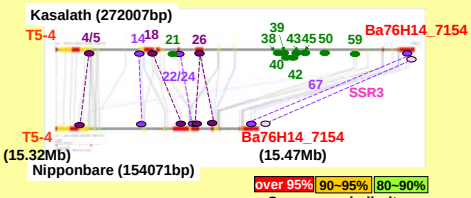
1. Development of *Pup1*-specific markers

Fine mapping of *Pup1* locus (red)
Flanking markers for MAB (blue)



(Wissuwa et al.)

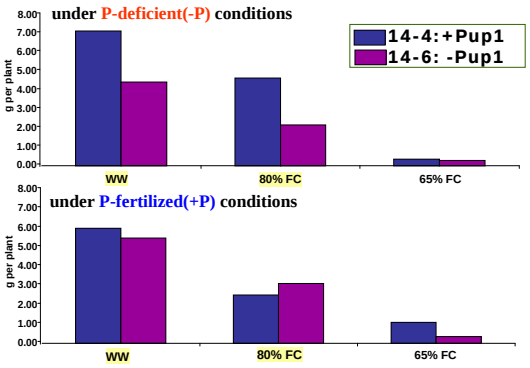
2. Development of allele-specific markers for *Pup1*



- Kasalath allele-specific dominant markers
- SSR markers developed
- Candidate gene-specific co-dominant markers
- Markers to be developed for candidate genes

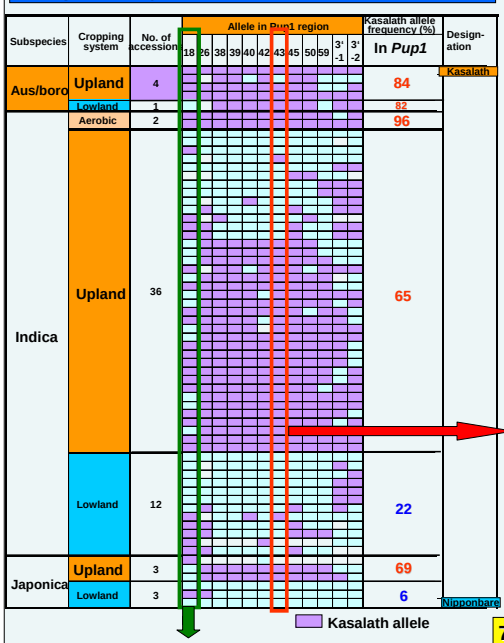
❖More information available in other poster (Lu et al.)

3. Average grain weight of contrasting Pup1 NILs



➢The higher root surface area per g root dry weight developed by NIL14-4
➢*Pup1* considerably improves P-nutrition under well watered and moderate water stress
Q) Under severe drought and +P condition : a higher P-uptake or to an P-unrelated, drought responsive mechanism conferred by *Pup1*?

4. *Pup1* survey in rice accession

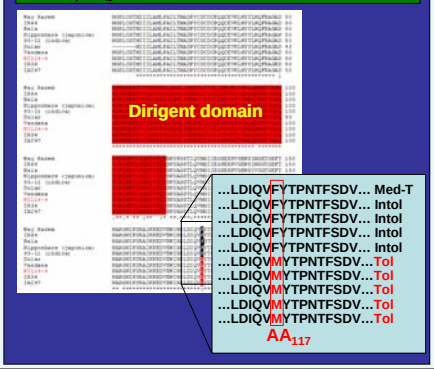


➢*Allele-specific PCR-based Pup1 markers* surveyed in 81 rice varieties and IRRI breeding lines of
O. sativa spp. *japonica*
spp. *indica*
spp. *aus*
➢Cultivated under upland and lowland conditions
➞*Pup1* is more widely distributed in upland varieties, suggesting beneficial effect of this QTL in P-deficiency- and drought-prone environments

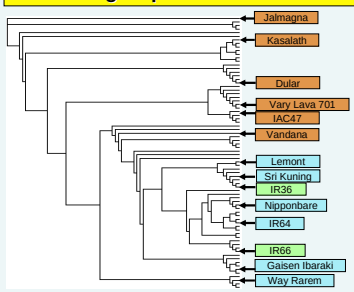
5. Gene43(Gene46n)

➢Putative protein kinase
➢Most similar to genes in *O.rufipogon* and *O.glaberrima*, while not present in Chr. 12 in Nipponbare and 93-11

6. Dirigent domain and allelic difference of Pup1 gene #18



7. Cladogram of 81 varieties and IRRI breeding lines, based on 12 *Pup1* specific PCR-based markers, showed 2 distinct groups



- Orange : Tentative P-tolerant varieties
- Blue : Tentative P-intolerant varieties
- Green : Lowland and tentatively P-tolerant varieties

The optimization of a *Pup1* phenotyping system and the analyses of gene/phenotype association is currently ongoing.