UTILIZATION OF ABIOTIC STRESS TOLERANCE GENES

Matthias Wissuwa

JIRCAS

1-1 Ohwashi, Tsukuba, Ibaraki 305-8686, Japan

ABSTRACT

Abiotic stresses such as drought, salinity, flooding or problem soils limit crop production worldwide. Resource-poor farmers are disproportionally affected because they typically lack the resources to alleviated effects of stress through improved water management or soil amendments. The development of cultivars with enhanced tolerance to abiotic stresses has therefore been advocated as a low-cost means to improve productivity in stressful environments (Ismail et al. 2007). In the past, efforts to develop such varieties have typically relied entirely on phenotypic selection in target environments but the tremendous success of marker assisted selection (MAS) for the *Sub1* locus enhancing submergence tolerance (Septiningsih et al. 2009) has clearly established MAS as the potential method of choice in breeding for abiotic stress tolerance.

Several other loci associated with tolerance of drought, P deficiency, salinity, or anaerobic germination have entered breeding programs by now. Among these is the *Pup1* locus enhancing tolerance to P deficiency, which had originally been mapped in 1998 in the Nipponbare x Kasalath mapping population. That it took more than a decade before *Pup1* was finally cloned and suitable markers developed for use in MAS was in large part due to the absence of *Pup1* from the rice reference genome based on Nipponbare, which is sensitive to P deficiency. The underlying gene was therefore only identified after sequencing the *Pup1* region in tolerant donor parent Kasalath (Chin et al. 2011). Similar difficulties were encountered for the *Sub1* locus: tolerance is conferred by a tolerance-specific allele, Sub1A-1, that is entirely absent from the reference genome. These examples highlight the need to have access to more detailed genomic information from the more exotic donors of key tolerance genes.

For MAS to fulfill its true potential it is crucial to shorten the period from QTL detection to gene identification and breeding application and to widen the genetic based beyond what is typically explored in a bi-parental cross. Genome wide association mapping (GWAM), based on representative sets of genebank accession that capture a very large part of the variation present in the rice gene pool, promises to accomplish this through the simultaneous identification of loci controlling key tolerance traits with an allele mining component (Zhao et al. 2011). In combination with low-cost high-throughput genotyping many limitations with respect to genotyping are presently being lifted. In the near future, the crucial task will be to design screening protocols that are equally high-throughput, while being specific enough to detect novel tolerance genes suitable for entering MAS breeding schemes. Currently such protocols are being developed and employed jointly between JIRCAS and IRRI to tag loci controlling traits enhancing phosphorus use efficiency and zinc uptake from zinc-fixing soils.

KEYWORDS

Problem soils, phosphorus efficiency, zinc deficiency, marker assisted selection, association mapping

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JIRCAS

Closing the yield gap:

Potential yield: 7-9 t/ha

Average yield: 4-5 t/ha
India: 2-4 t/ha
SS Africa: 2-4 t/ha

Improve tolerance to biotic and abiotic stresses

Main abiotic stresses:

- nutrient deficiency for P, N, Zn (K, S, B…)
- drought, heat, flooding
- salinity, sodicity (alkalinity)
- other soil problems (Al or Fe toxicity, acidity)

Breeding strategies:

- selection in target environments
- QTL mapping and marker assisted selection

The *Sub1* locus for submergence tolerance:

The model for a successful introgression of tolerance genes via MAS

Mapped as a major QTL in 1994/95

D. Mackill et al. IRRI

LETTERS

***Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice**

Kenong Xu[1], Xia Xu[1], Takeshi Fukao[2], Patrick Canlas[1], Reycel Maghirang-Rodriguez[3], Sigrid Heuer[3], Abdelbagi M. Ismail[4], Julia Bailey-Serres[2], Pamela C. Ronald[1] & David J. Mackill[3]

Sub1 was cloned in 2005/06

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Allele-specific markers

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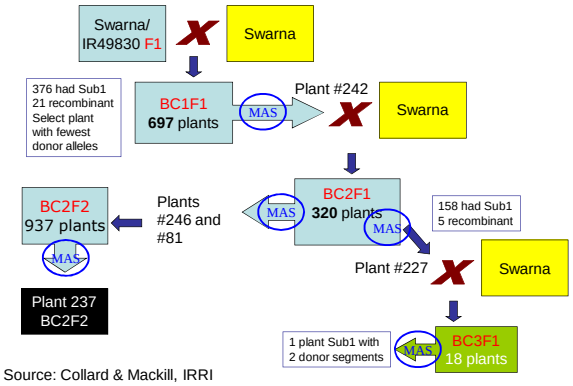
Marker assisted selection (MAS)

Selection for Swarna+*Sub1*



Source: Collard & Mackill, IRRI

Swarna-Sub1 was first variety released (India and the Philippines)

Septiningsih et al 2009, Annals of Botany; Singh S et al 2009, Field Crops Res

Sub1 is the model for stress tolerance genes utilized in plant breeding - but it is a rather special case

- almost qualitative trait (survival or not)
- simple screen, diagnostic at seedling stage
- rare gene, so almost all varieties can benefit

Most other traits/QTL are more complicated

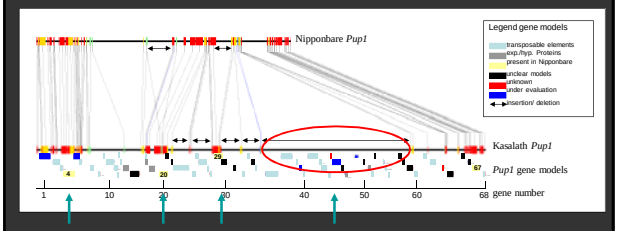
- truly quantitative (e.g. 30% effect vs. 70% background genes/noise)
- phenotyping difficult (field) – only detected as final yield
- presence/absence of gene not entirely clear based on phenotype

The locus Pup1 (P uptake 1) confers tolerance to P deficiency

QTL mapped in 1998 Wissuwa et al. 1998; 2001; 2002; 2005)
Conceptual proof that MAS for Pup1 works in 2001
Precision mapping in 2005 and sequencing of Pup1 in Kasalath in 2007

Kasalath sequence at the Pup1 locus:

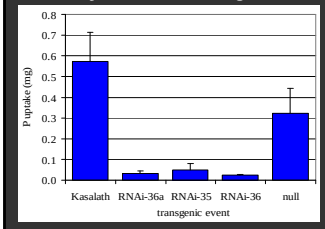
- 120 kb larger than in Nipponbare (100 kb INDEL region)
- 30+ more predicted genes than in Nipponbare
- none of these have any known relation to P uptake or metabolism



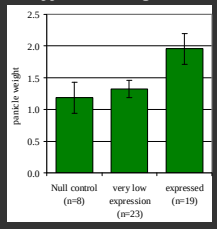
Several candidate genes based on expression patterns

Confirming the Pup1 candidate gene using transgenics

Knockout lines without expression of Pup1 in a Kasalath background



Lines over-expressing Pup1 in a Nipponbare background

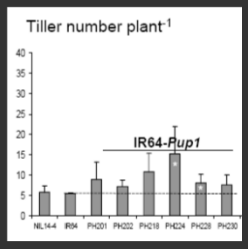


Pup1 increases P uptake and grain yield by up to 60 %

Possibly confirm in double-transgenic (Pup1 may depend on 2 genes)
Screen gene bank for novel Pup1 alleles
Promote MAS based on marker diagnostic of 'strong' Pup1 allele

Breeding application:

MABc of Pup1 into IR64 background (IRRI)

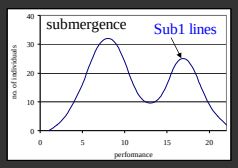


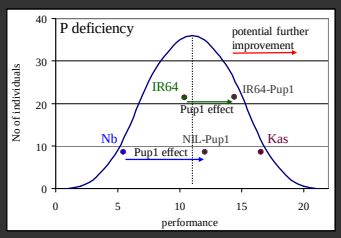
IR64 **IR64-*Pup1*** NIL14-4

Chin & Heuer, IRRI

First yield trials in the field in 2012
Similar activity at early stage with AfricaRice

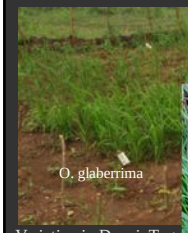
QTL effects :





Unless a trait is controlled by one major locus, the story ain't over yet

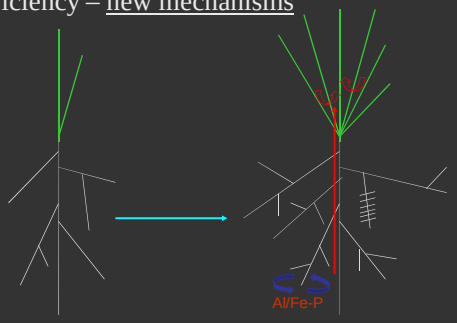
Search for additional sources of tolerance to P deficiency – new germplasm



Variation in Danyi, Togo

QTL mapping in NERICA-based populations ongoing at JIRCAS

Search for additional sources of tolerance to P deficiency – new mechanisms



1. enhanced root growth, root hairs (Pup1)
2. Root exudation – P solubilization
3. P translocation – remobilization (PUE)

Search for mechanism-specific genes

Design mechanism-specific screening experiments

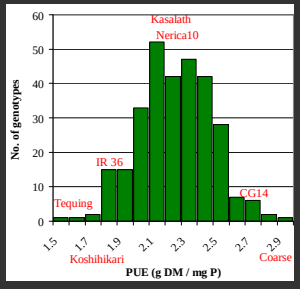
Evaluate a representative sub-sample of the rice gene pool

Genome-wide association mapping

>> Identification of novel alleles present in the rice gene pool

Internal P utilization efficiency (PUE):

Assessing the variation present in the rice gene pool

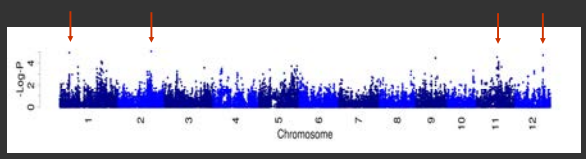


PUE = biomass / mg P (0.8 mg P)

- most modern varieties are inefficient
- highest PUE among the 'aus' group (but not all)
- followed by traditional 'indicas'

350 genotypes x 2 P levels x 3 replicates: 2100 units – a major logistical headache!

Genome-wide association mapping



44k SNP chip (McCouch group)

Detected several novel loci for PUE

From here to application in breeding is still a long way, but possibly much shorter than from traditional QTL mapping

Conclusion & Outlook

1. At present the bottleneck in molecular breeding for abiotic stress tolerance is the limited number of precisely mapped, high impact genes (Sub1, Pup1, saltol, anaerob germ.,...)
2. High-throughput pipelines in MAS should allow us to combine several QTL/genes synergistically controlling a trait → marker assisted pyramiding as a target
3. The 'genomics' advances have outpaced our phenotyping ability (dimension but also conceptually)
4. With more emphasis on phenotyping, new genomics tools hold huge potential to identify novel tolerance alleles and to accelerate their utilization in plant breeding