

# Revitalizing Marginal Lands

Discovery of Genes  
for Tolerance of Saline  
and P-Deficient Soils to  
Enhance and Sustain  
Productivity

Abdelbagi M. Ismail  
IRRI, Philippines

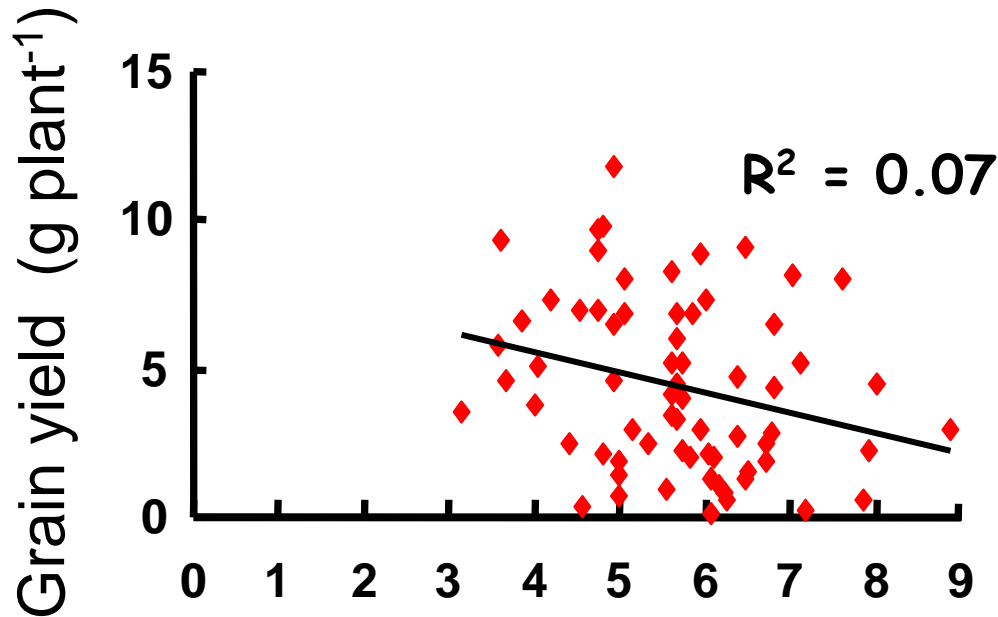
# Why Salinity and P-deficiency

- Both abiotic stresses are wide spread and are particularly important for rice (> 15 m ha are saline, > 50% of rice lands are P-deficient)
- Often coexist
- Both have substantial effect on productivity (0-2.5 t ha<sup>-1</sup>) and associated with poverty
- Amendments are expensive to poor farmers, yet solutions through germplasm are affordable
- Good understanding of the biology of tolerance to both stresses
- Major QTLs associated with tolerance identified with strong phenotypic effects ( $r^2 = 70-80\%$ )
- Major QTLs were fine-mapped to within < 1.0 cM, BACs spanning QTLs regions were identified

Rice is highly sensitive to salt stress particularly during seedling stage and reproduction



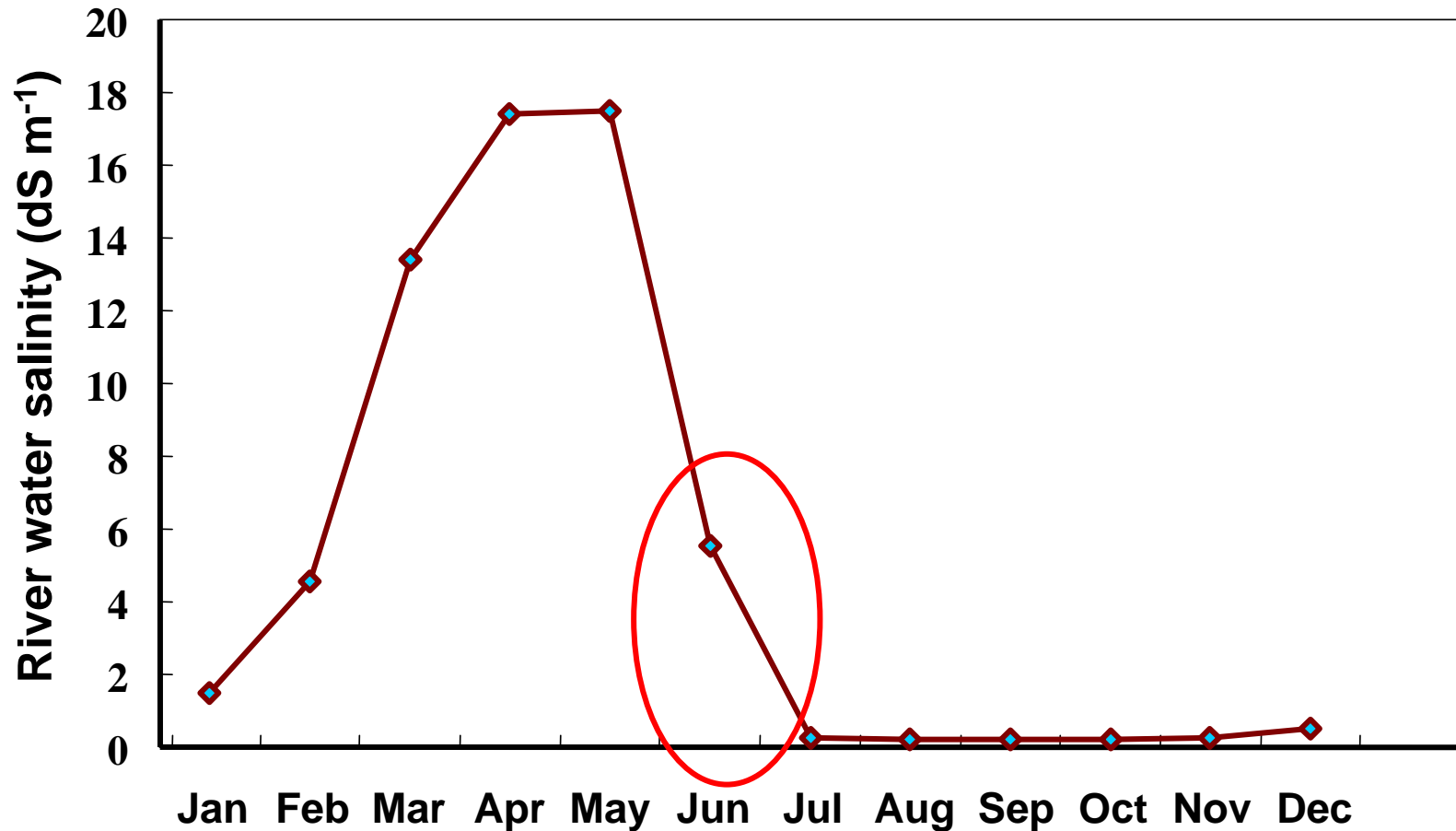
# 🌈 Tolerance at seedling stage does not ensure tolerance during reproduction



Tolerance scores at seedling stage

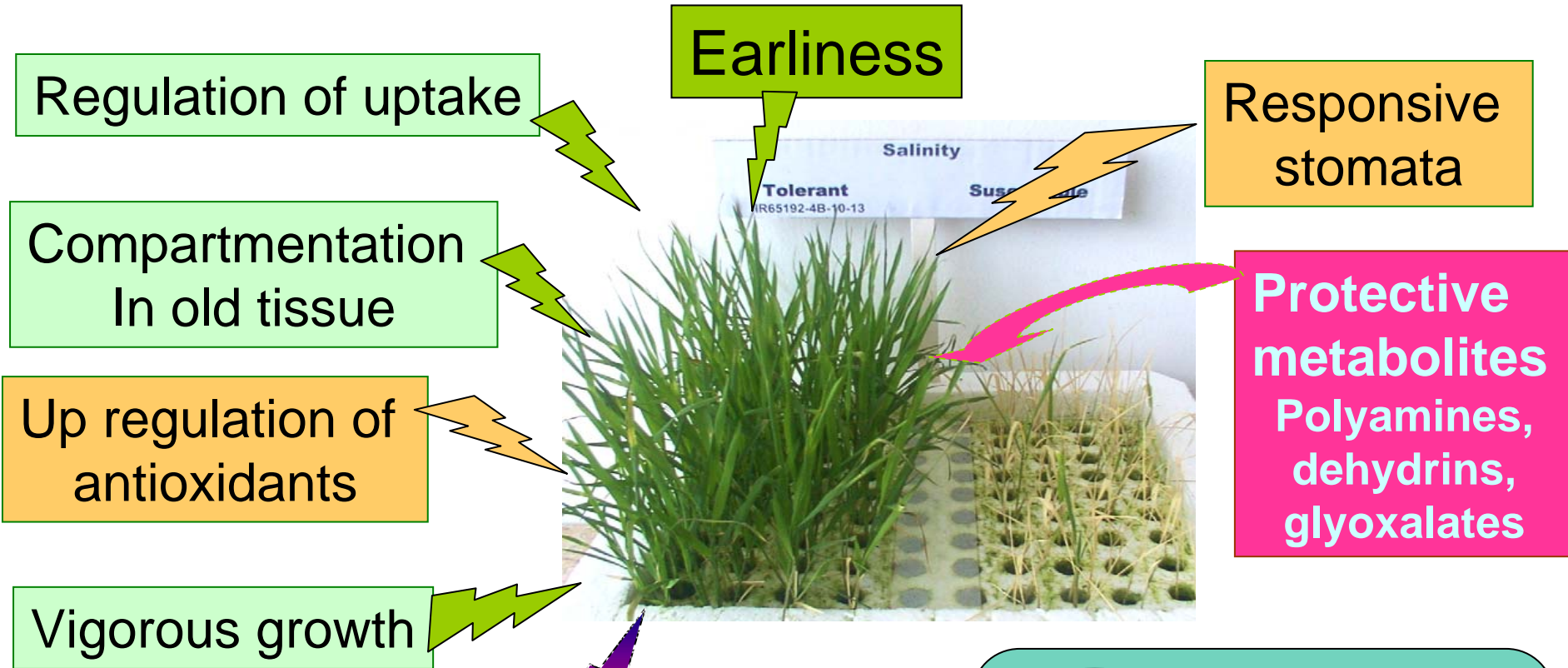
- 🌈 QTLs for salinity tolerance at seedling stage (Ch1) are different from those at reproductive stage (Ch 3,4,7,9)

# Salinity tolerance at seedling stage is important in coastal areas for good crop establishment

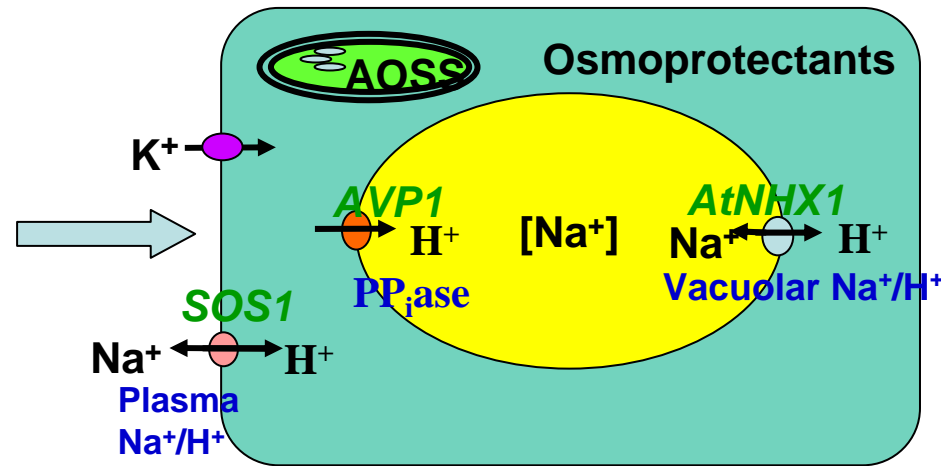


Typical salinity pattern in coastal saline areas  
(Mondal et al, 2005)

# Traits associated with tolerance during seedling stage in rice



**Compartmentation within tissue (tissue tolerance)**

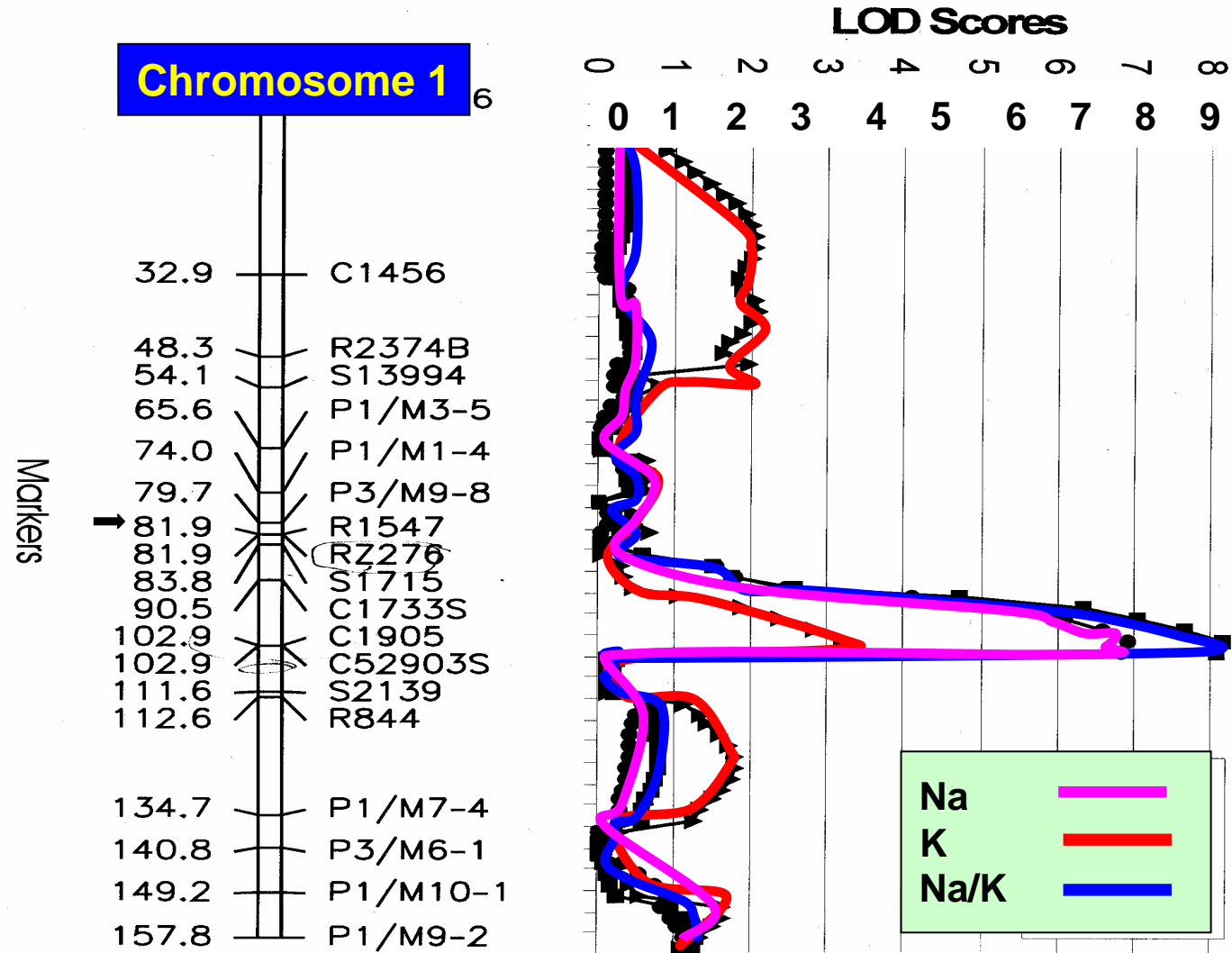


# Mapping of salt uptake in rice

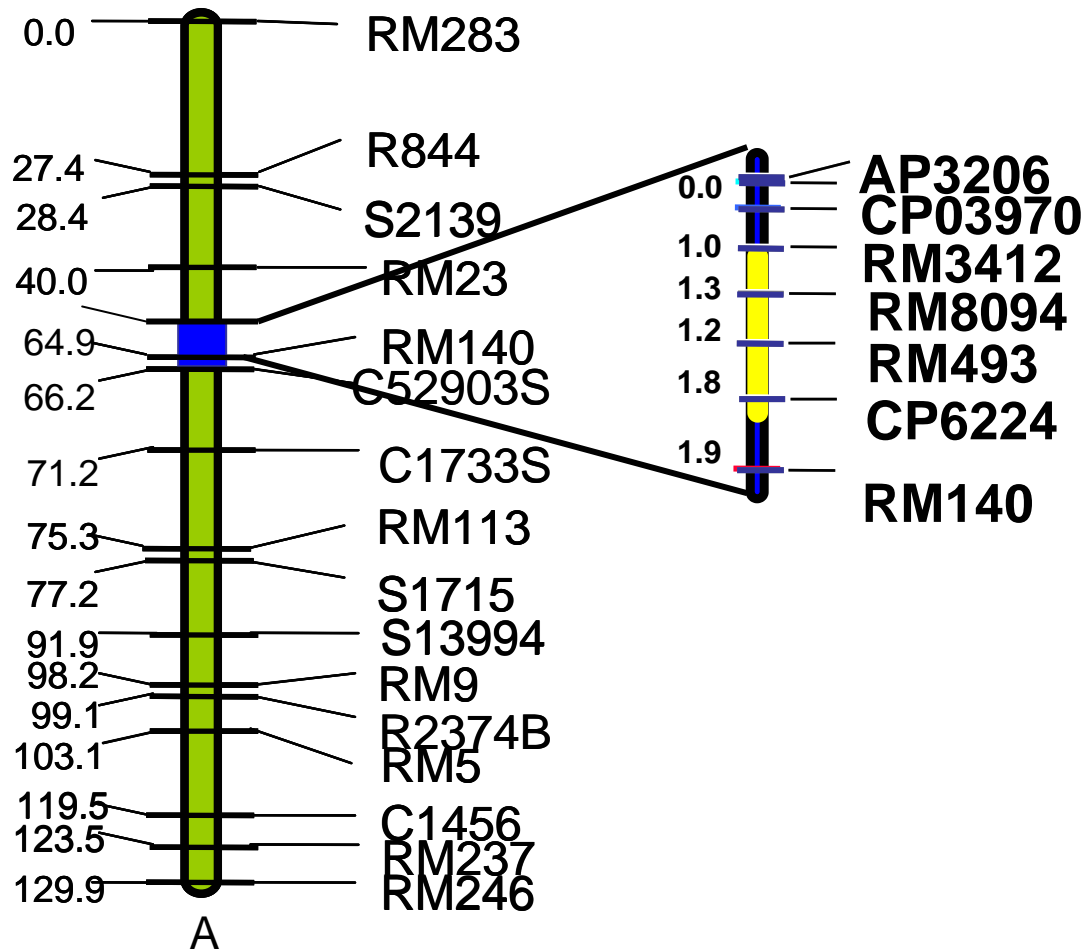
- Initial mapping achieved using  $F_8$  RIL population from a cross of Pokkali x IR29 using AFLPs
- A major QTL (*Salto1*) was mapped to within 15 cM on chromosome 1



# *Saltol* constitute 3 overlapping traits



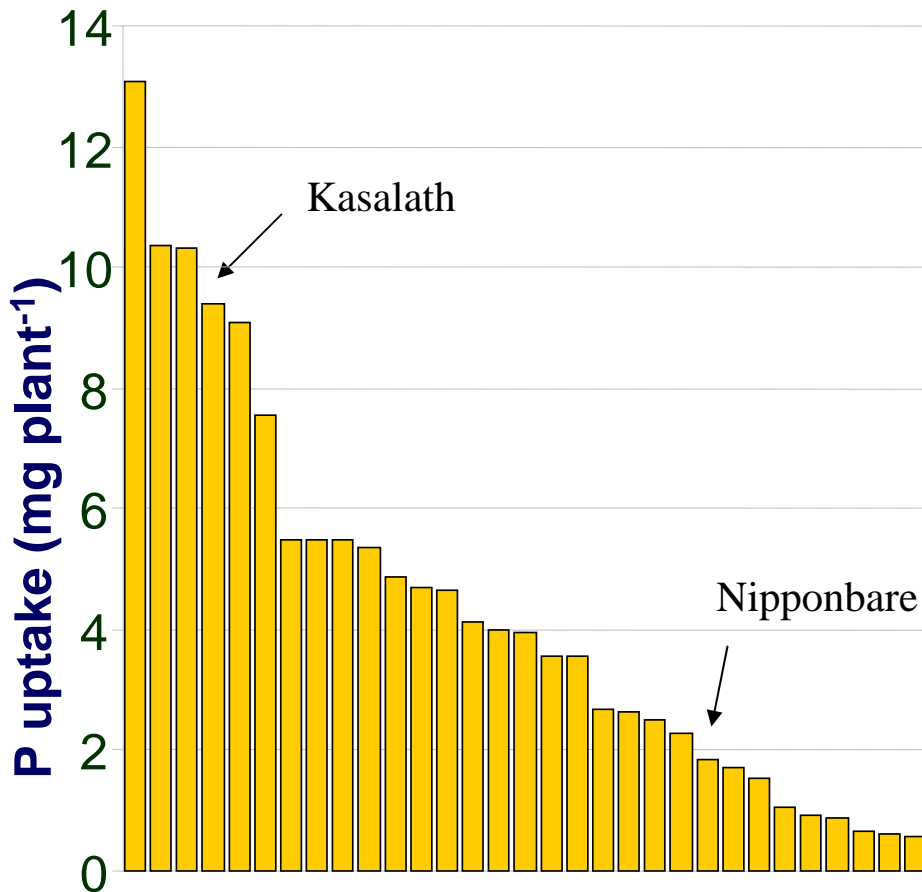
# Fine-mapping of *Saltol* locus using NILs



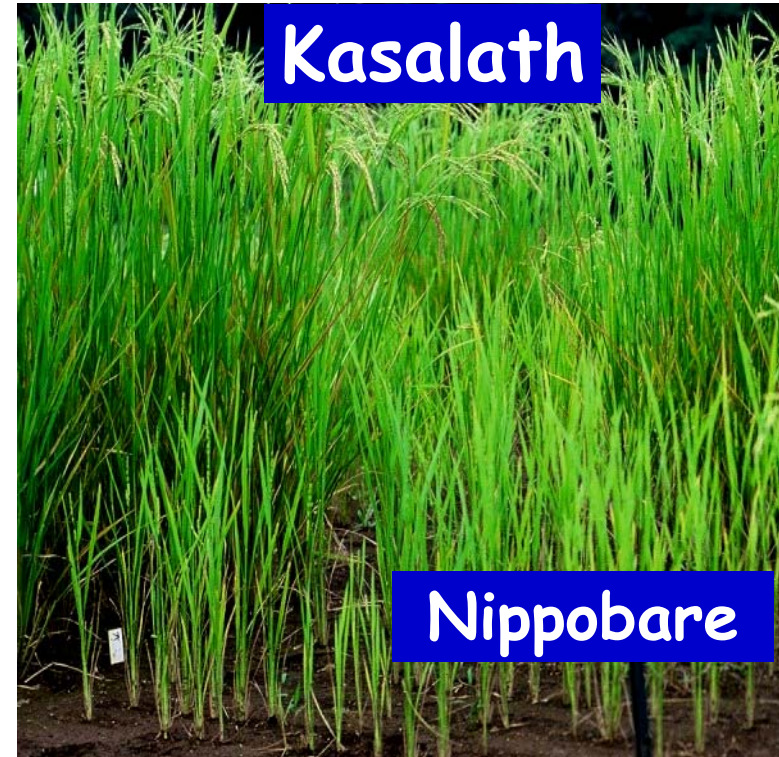
**Mapping of tolerance to  
to P-deficiency**

# Genotypic variation for P uptake and QTL mapping

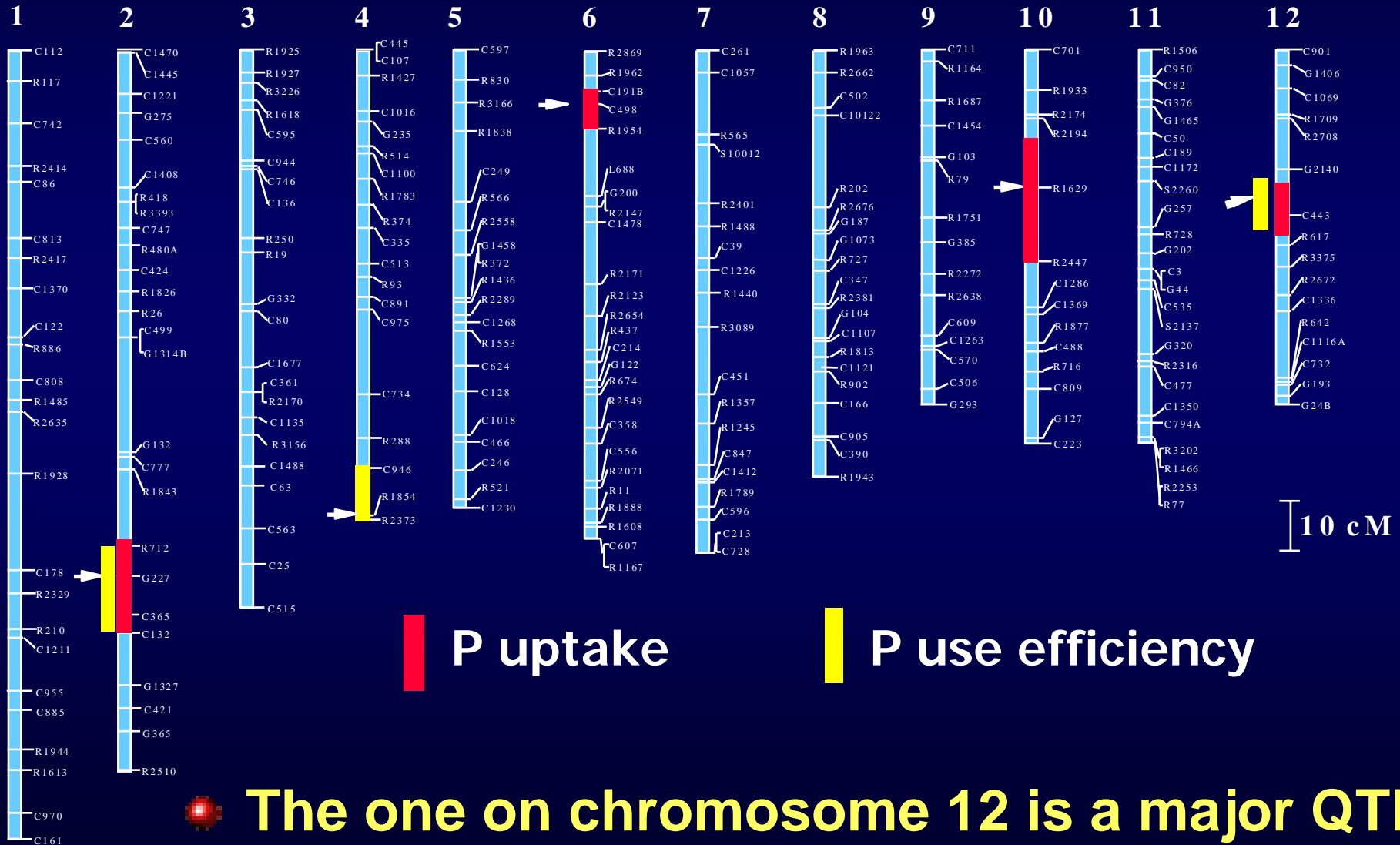
## P uptake of 30 cultivars



- 20-fold difference in P uptake with traditional varieties being superior
- Mapping population developed from Nipponbare x Kasalath and used in QTL mapping



# Four QTLs for P uptake detected on chromosomes 2, 6, 10, 12



The one on chromosome 12 is a major QTL

# NIL with the major QTL developed: NIL-Pup1

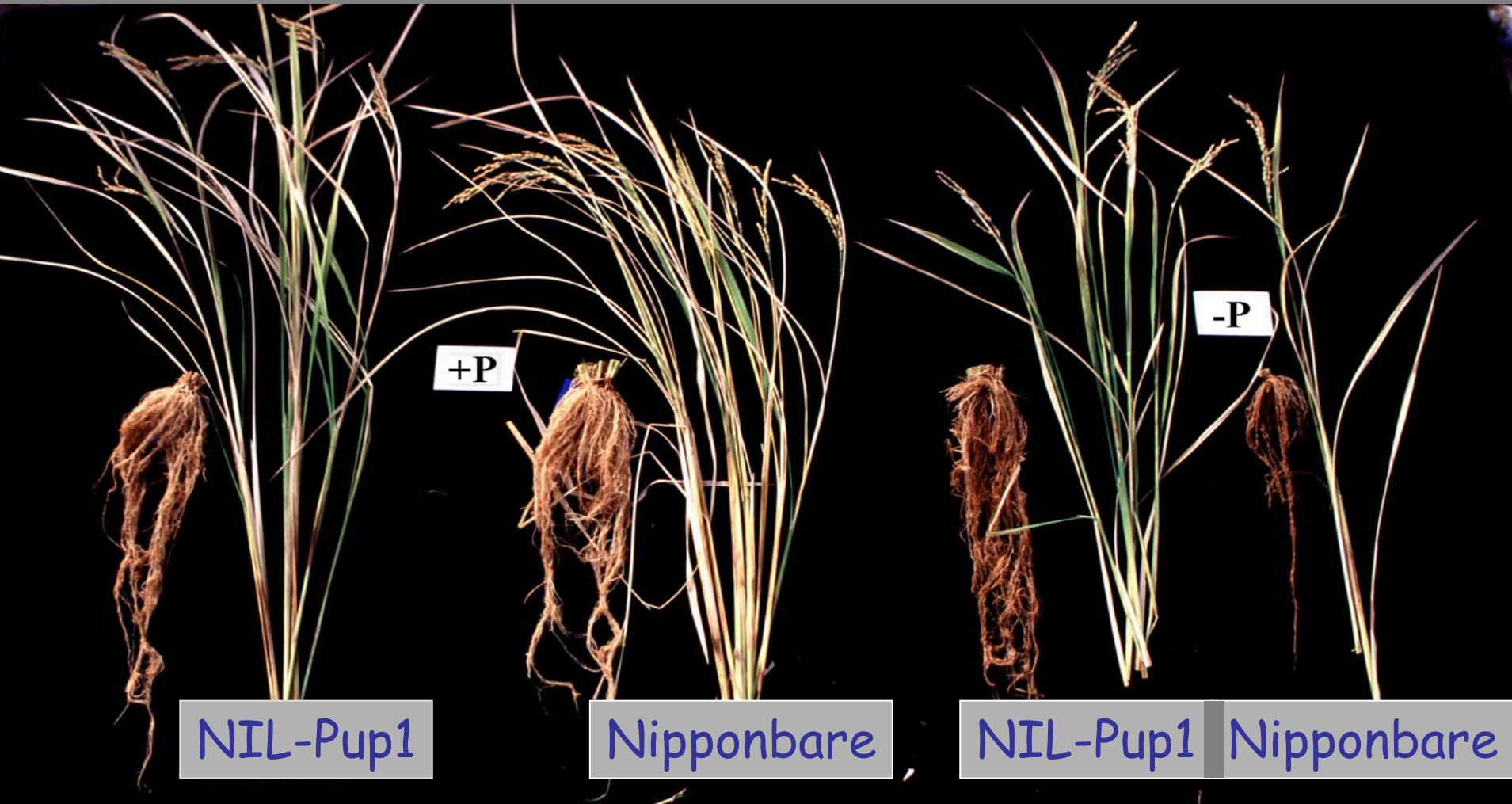
**Kasalath**



**Nippobare**

**NIL-Pup1**

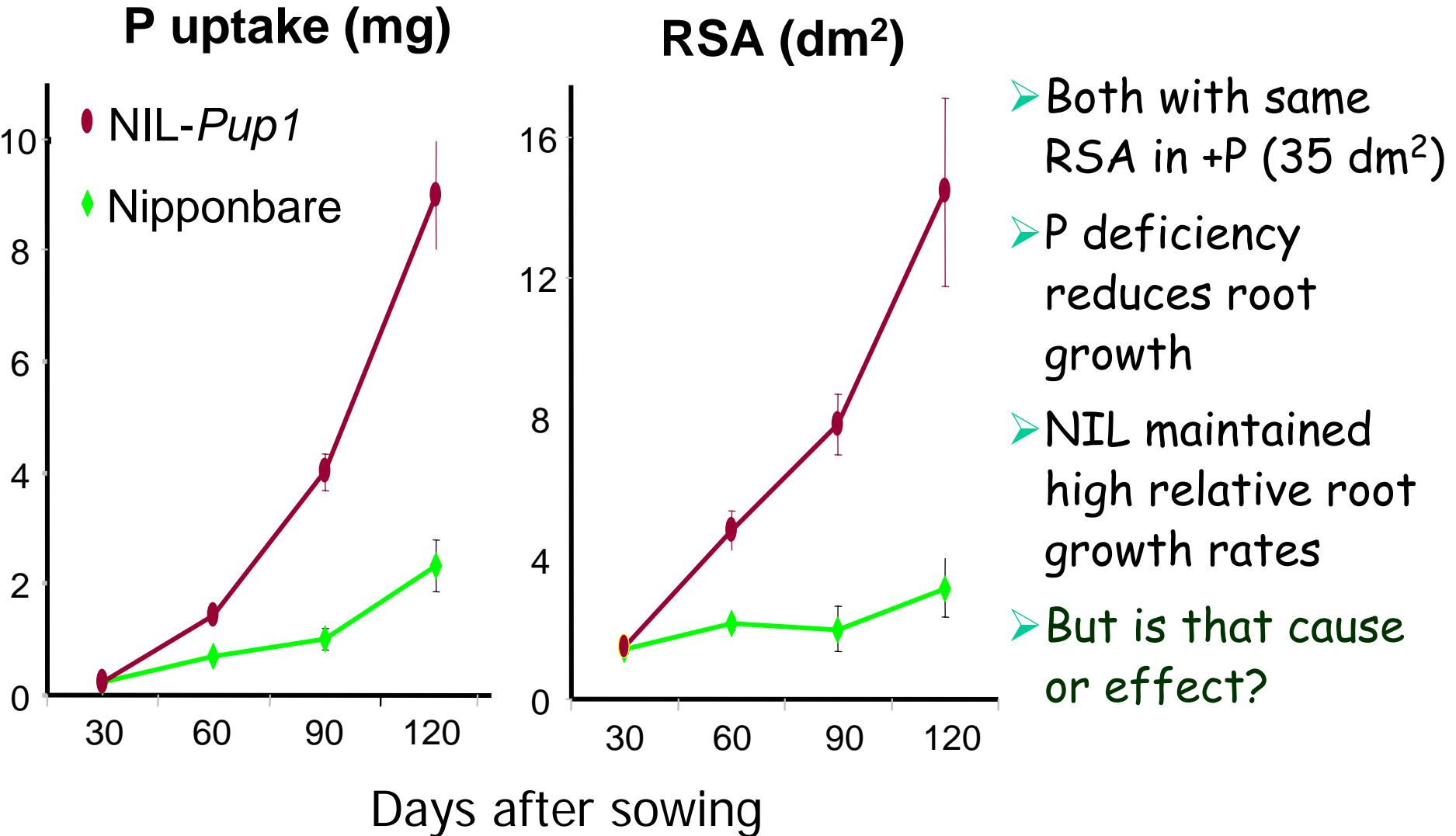
# Near Isogenic Line for Pup1 Locus



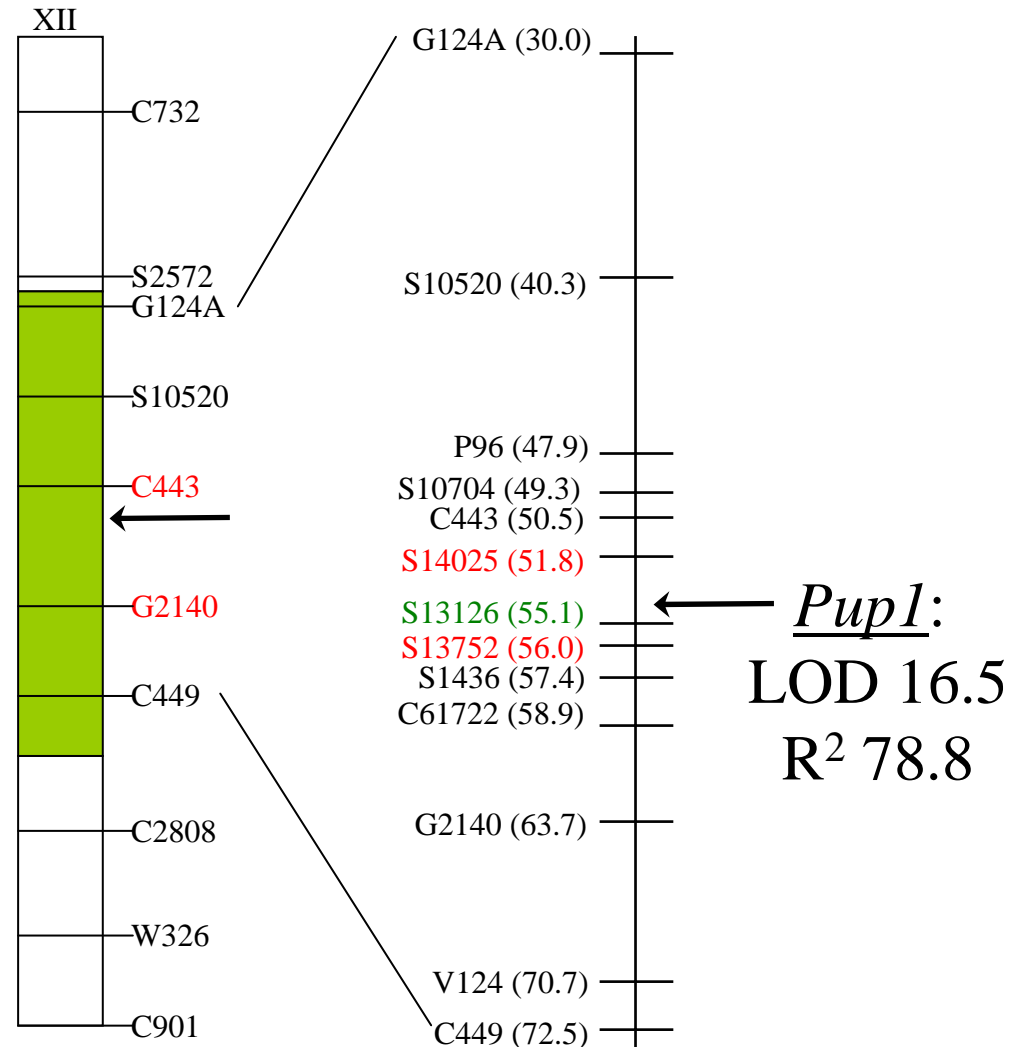
Q 1: How does Pup1 confer tolerance? -> physiology

Q 2: Can we identify the gene? -> fine mapping, genetics

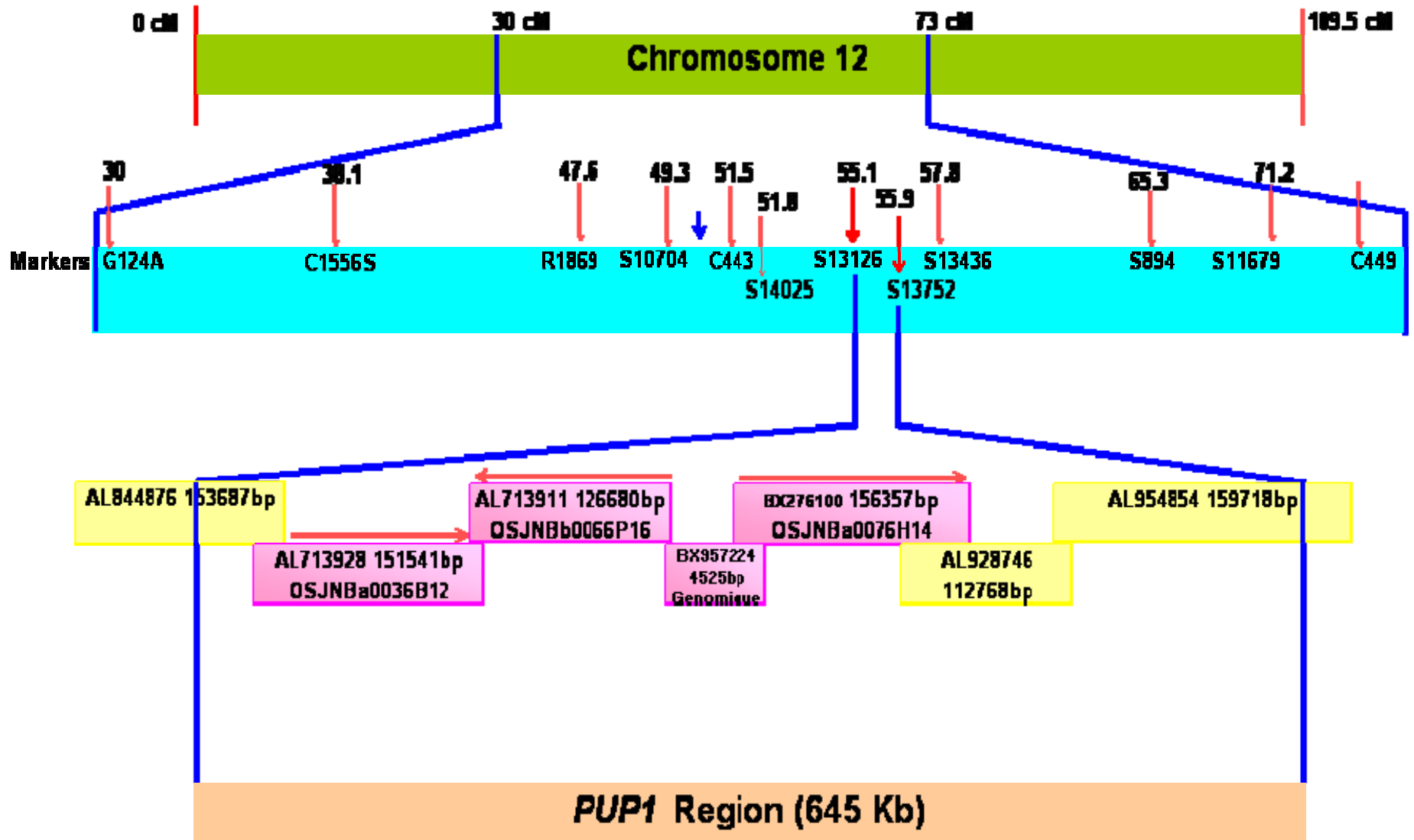
# *Pup1* is associated with high relative root growth rates



# Screened 3000 lines for additional recombinants – (M. Yano, NIAS, Japan)



# Fine mapping of *Pup1* locus



# OBJECTIVES

**To identify genes associated with salinity and P-deficiency tolerance, with emphasis on those associated with *Pup1* and *Saltol* loci and to develop a marker system to incorporate them into varieties popular in target areas**

# Specific Objectives

- Further precision mapping of *Saltol* & *Pup1* loci
- Develop/validate markers for both QTLs & a MAB system for incorporating them into popular varieties
- Attempt to isolate genes involved at the two loci
- Validate roles of isolated genes through different approaches (expression, proteomic, complementation etc)

# Specific Objectives (cont.)

- Functional confirmation and assessment of positive and any potential negative impacts using NILs
- Comparative studies of genes/pathways identified in other crops for effectiveness in rice
- Capacity building of NARES partners in MAB to incorporate the two QTLs into popular local varieties

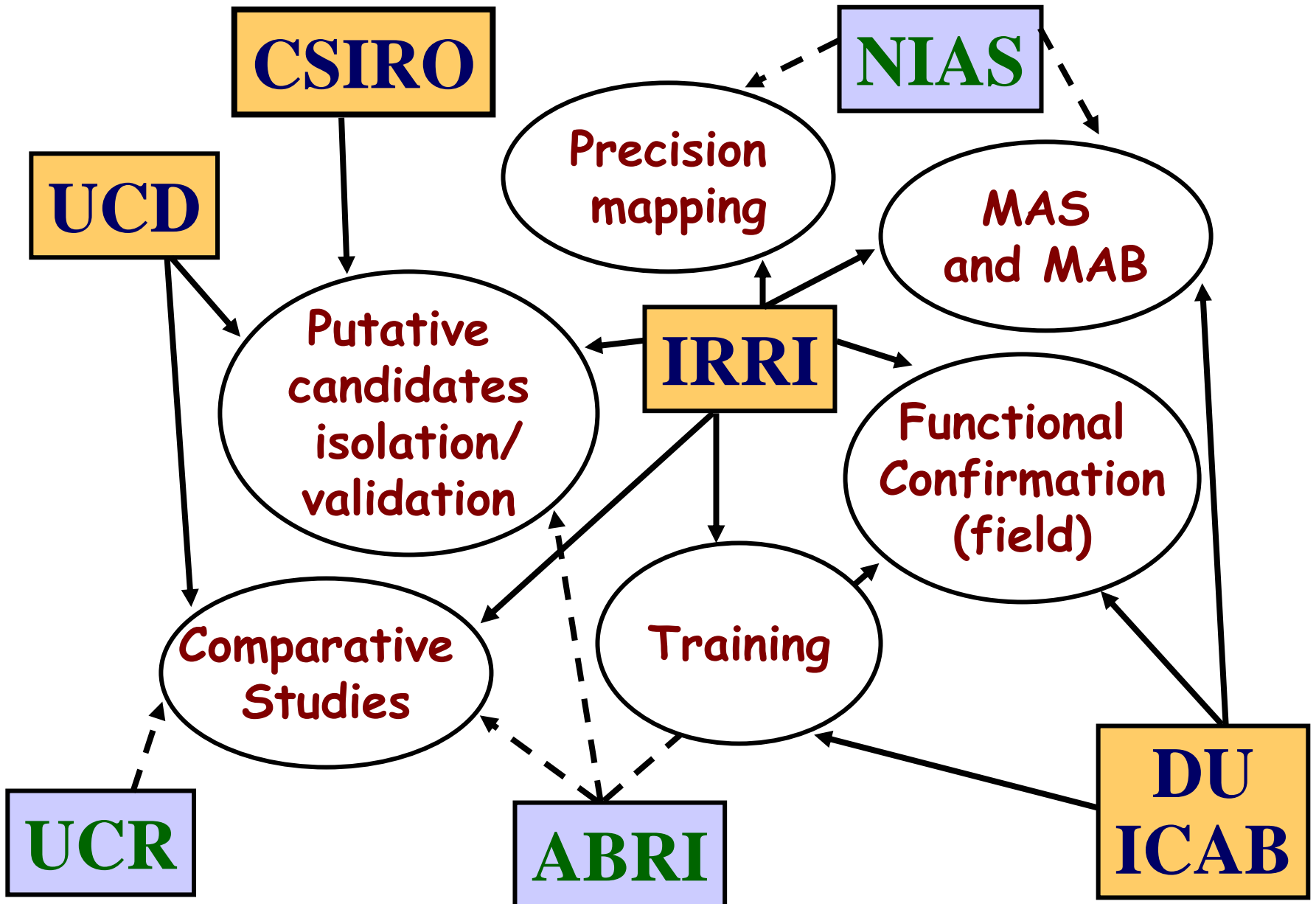
# OUTPUTS

- **Precision mapping of *Pup1* and *Saltol* to a small physical region and list of putative genes annotated in the two loci**
- **New markers developed and validated for the two QTLs**
- **MAB system developed and implemented at NARES to introduce them into locally preferred varieties**

# OUTPUTS (cont.)

- Candidate genes at the two loci identified based on converging evidences
- Target genes validated through functional verification in transformants
- Better understanding of mechanisms involved
- Enhanced capacity of NARES through degree and non-degree training

# Partners and Roles



*"Saltol"*

Further progress

# Gene discovery in *Saltol* region



Growth in the presence/absence of NaCl

Expression analyses (MA, RNA blot analysis, RT-PCR, proteomics)

Identification of candidate genes in the QTL region (*Salto*)

Overexpression in IR29

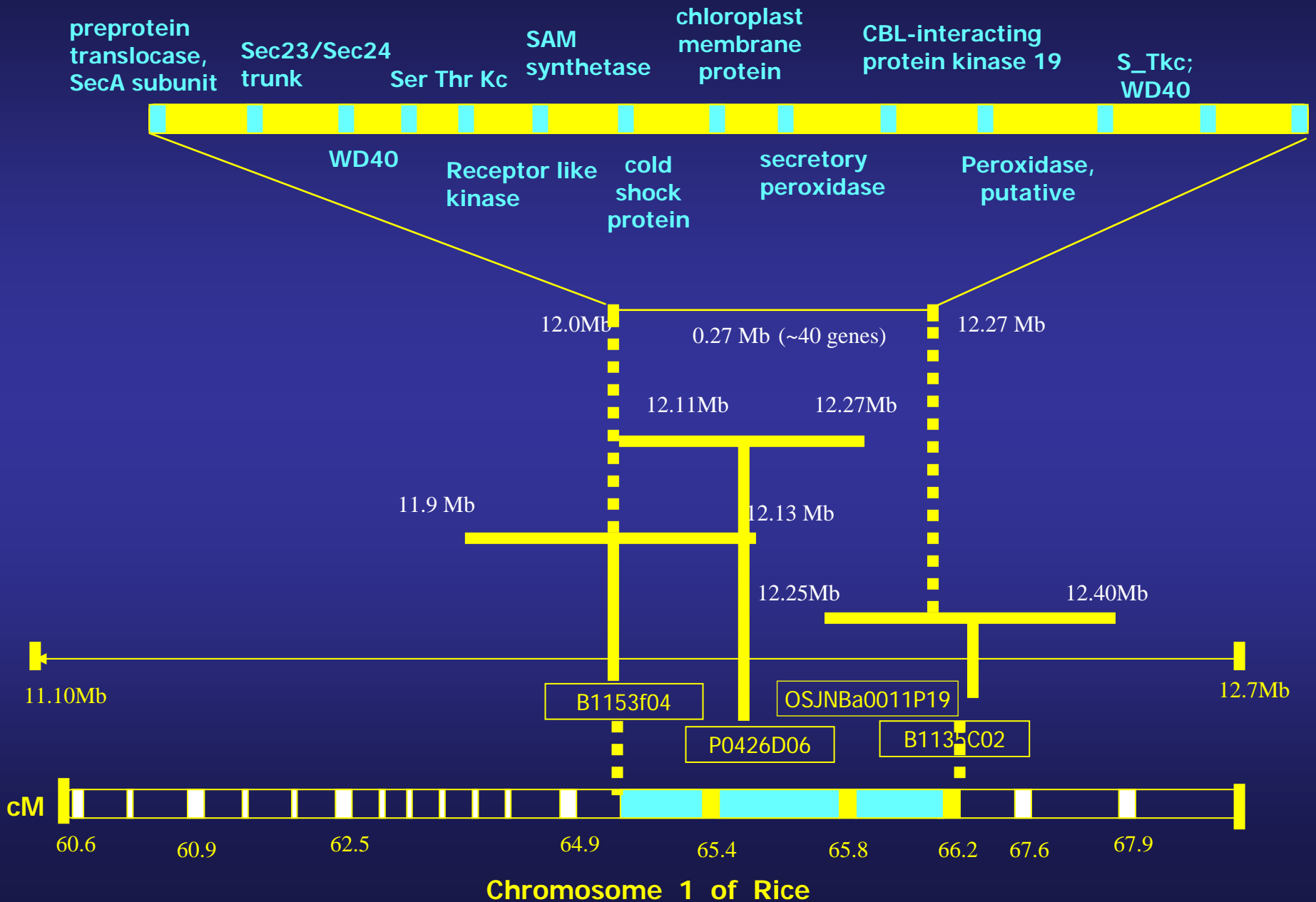
KO or silencing in Pokkali

Phenotypical analyses

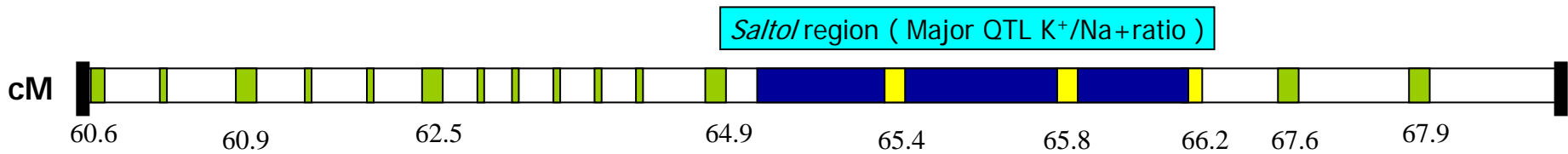
Phenotypical analyses

Gene-specific markers

# Physical map of *Saltol* Region



# Rice Chromosome 1



- CP12 domain, putative
- Stress-inducible membrane pore protein
- Zinc finger, C3HC4 type (RING finger), putative
- Universal stress protein family
- Cation-chloride co-transporter
- Receptor like protein kinase
- Myb-like DNA-binding domain, putative
- Peroxidase, putative
- Cell wall protein type (Extensin,Hydroxyproline rich, glycine rich)
- Cation transporter
- Phospholipase D. Active site motif, putative
- Protein kinase domain, putative
- Dual specificity phosphatase, catalytic domain, putative
- Pectinemethylesterase/invertase inhibitor
- Pectinesterase

- preprotein translocase, SecA subunit
- Sec23/Sec24 trunk domain, putative
- Ser Thr Kc
- Protein kinase domain
- S-adenosylmethionine synthetase
- chloroplast membrane protein
- Cold shock protein
- secretory peroxidase
- CBL-interacting protein kinase 19
- Peroxidase, putative
- Cell wall protein type (Extensin, Hydroxyproline rich, glycine rich)

- phospholipid/glycerol acyltransferase –like
- Mitochondrial carrier protein, putative
- GDSL-like Lipase/Acylhydrolase, putative
- organic cation transporter
- major facilitator superfamily protein
- Cell wall protein type (Extensin,Hydroxyproline rich, glycine rich)



- ◆ Microarray data of Pokkali, FL 478 and IR29 from Korea (Dr. Jukon Kim) is available.
- ◆ RNA blot analysis is on-going to confirm the genes which are differentially expressed in the array data.

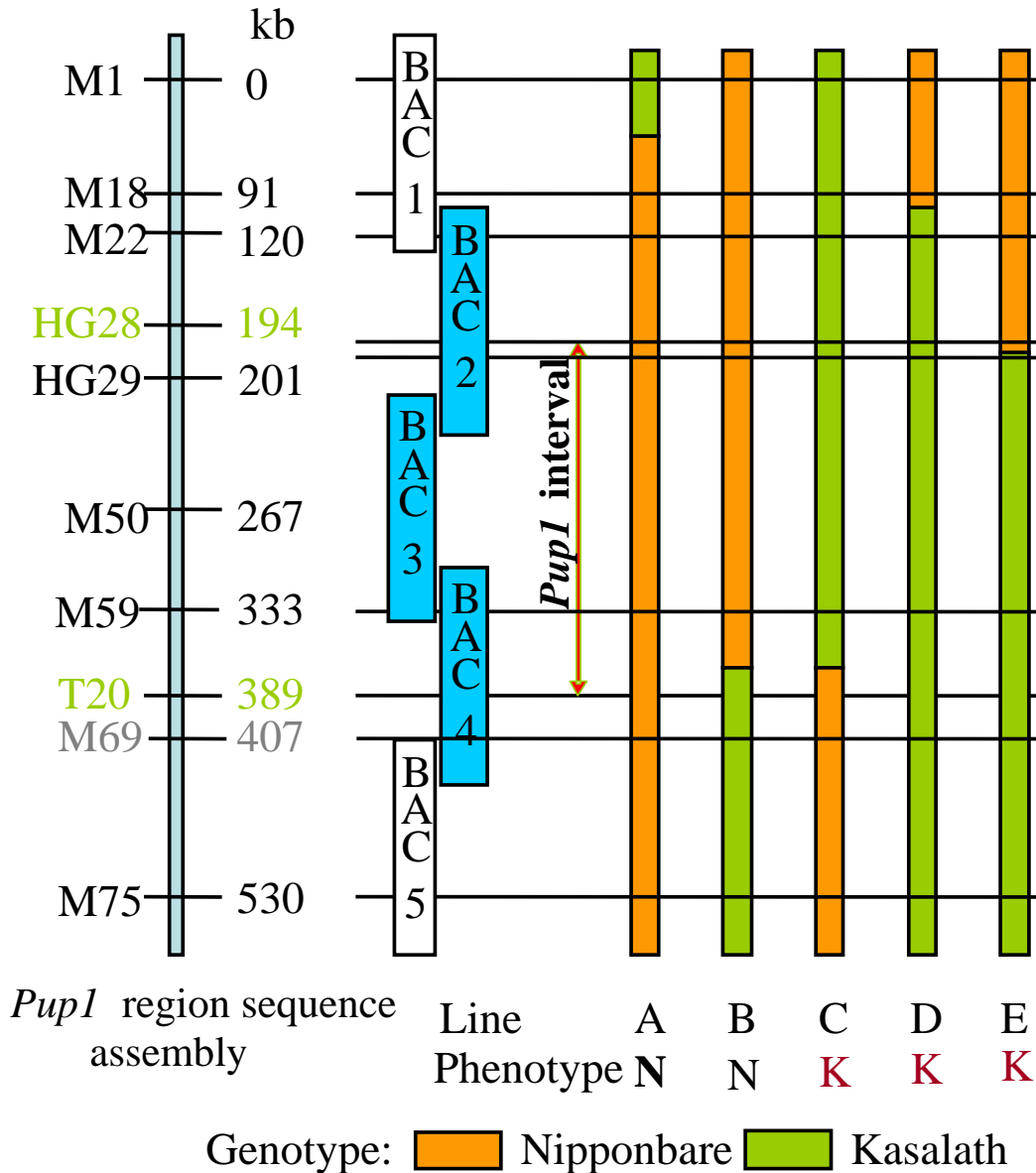
# List of genes that are located in the region of QTL and up-regulated by high salinity in rice

Gene name	Insertion lines	Clone ID full length cDNA	Rice 60k chip data under high salinity (fold-induction)			References
			0.5 h	2 h	6 h	
Pectinesterase	1B-23740, 1B-23741 CG408589	Ak105998	1.1	3.3	4.9	
Ser/thr kinase		AK065231		2.3	2.7	Guo <i>et al.</i> , 2001
Phospholipase D	1515	AK120868		3.5	2.6	Kacperska, 2004 Zhu, 2002
SecA/protein transport factor	CL520490 CL520492	AK070488	3.1	1.5		
Peroxidase		AK099187		2.6	3.05	Pastori and Foyer, 2002 Sottosanto <i>et al.</i> , 2004
Alkaline Invertase		AK120720	4.0	2.2	4.2	
Unknown cDNA		AK099887	0.37	1.6	2.4	

*"Pup1"*

Further progress

# High resolution mapping of Pup1



- Using new markers, *Pup1* is now mapped to a 195 kb region on chromosome 12.
- Transition from map-based to sequence based approach

# Functional annotation of *Pup1* region

- Annotation of 46 hypothetical genes using combined evidence from 3 programs (RiceGASS, TIGR, Fgenesh):
  - 3 putative genes with similarity to known genes
  - 13 putative genes with some similarity to known genes
  - 19 hypothetical proteins
  - 11 transposable elements (mostly retrotransposons)
- None obviously related to P metabolism or P uptake
- *Pup1* most likely a novel gene

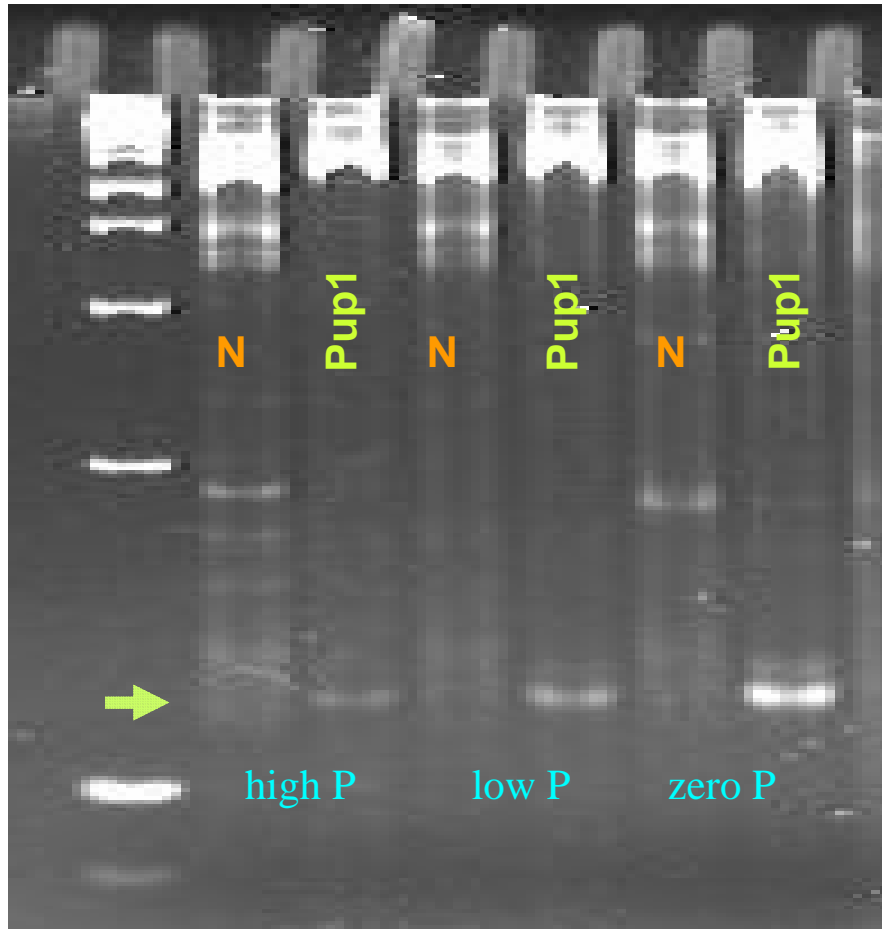
# Next: identify candidate genes among 46 putative genes

- Expression analysis using RT-PCR
- Microarray analysis
- Map-based cloning (one recombinant at 150:50 kb)
- Bioinformatics (function, sequence analysis)
- Sequencing of Kasalath BACs (partial sequence available, larger & with more genes)
- Sequence comparison between sets of genotypes
- Proteomics analysis
- Converging evidence for strongest candidate(s)
- Further validation through transformation

# Expression analysis of hypothetical genes using RT-PCR

- ◆ Nipponbare and NIL-Pup1 grown in nutrient solution at different levels of P contents and in P-deficient soil
- ◆ Sample RNA from roots and shoots after 36 and 42 days
- ◆ RT-PCR on most hypothetical genes, ignore transposons and very small genes (<100 bp)

# Candidate gene # 1: not predicted by TIGR and latest GAAS

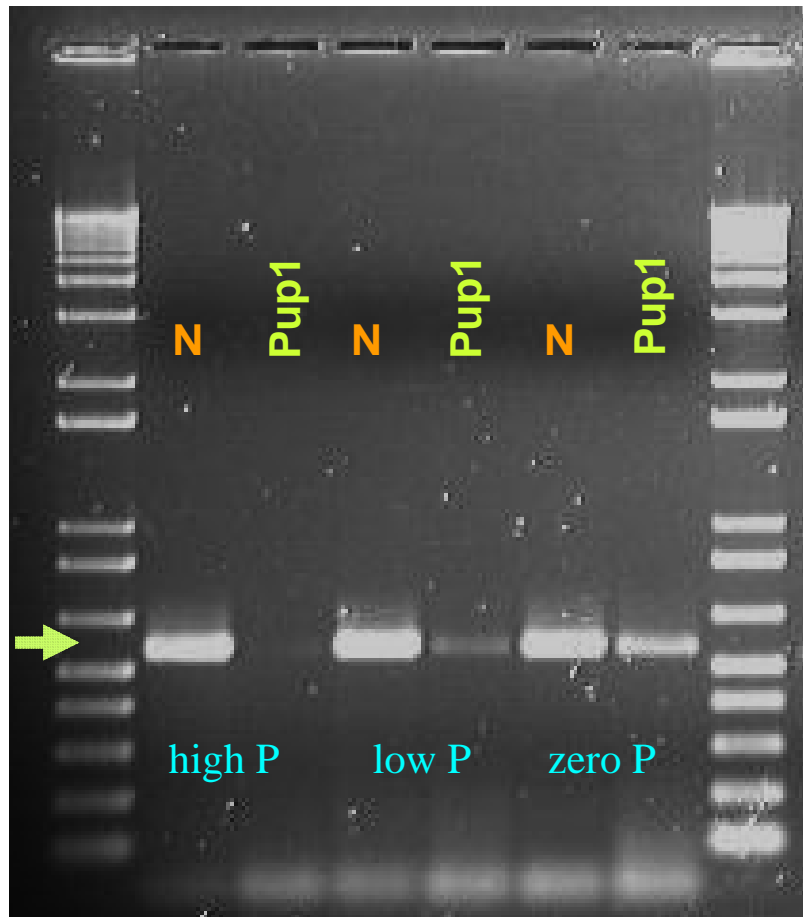


## Hypothetical gene 66P16\_28

- Predicted size of gene: 159bp; of amplified product: 110bp
- Not expressed in Nipponbare but induced by low P supply in NIL-*Pup1*.
- A soluble protein, possibly found in the nucleus.

# Candidate gene #2

## Hypothetical gene #25

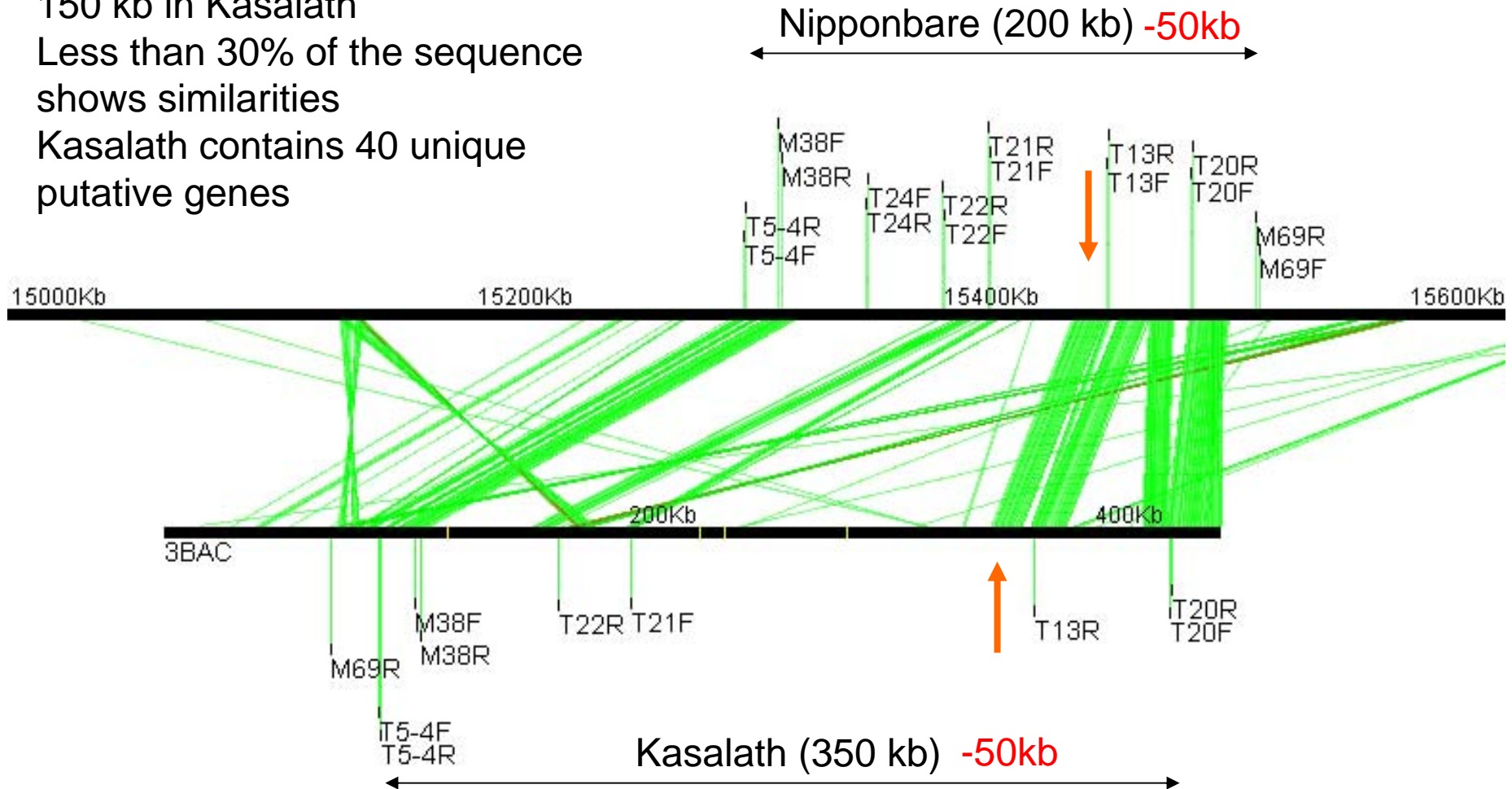


- ✦ Predicted size of gene: 591bp;
- ✦ of amplified product: 550bp
- ✦ Constitutively expressed in Nipponbare but induced by low P supply in NIL-*Pup1*.
- ✦ A membrane protein of one transmembrane helix.
- ✦ Well conserved in indica (BGI, Kasalath)

# Sequence comparison at Pup1 interval between Nipponbare and Kasalath

The Pup1 interval has an additional 150 kb in Kasalath  
Less than 30% of the sequence shows similarities  
Kasalath contains 40 unique putative genes

Latest fine-mapping:



# Data overkill !!!

- Once the step is made from mapping to sequence analysis, the flow of data is overwhelming
- At some point the need arose to find a platform that would make the maximum amount of data available in a highly ordered fashion
- Particularly important for collaborative projects for exchange of information
- **Gbrowse** at link <http://i4ws1243a/cgi-bin/gbrowse/pup1c>
- Next problem: find a common platform for data/information exchange: **Pup1WIKI** at [http://cropwiki.irri.org/pup1/index.php/Main\\_Page](http://cropwiki.irri.org/pup1/index.php/Main_Page)

# Project team

**Glenn Gregorio**  
**Ellen Tumimbang**  
**Zeba Seraj (Bangladesh)**  
**Ed Blumwald (UCD)**  
**Tim Close (UCR)**  
**Richard Bruskiwich**  
**RK Singh**

**Mathias Wissuwa**  
**Kristy Anne Gatdula**  
**M. Yano (NIAS)**  
**Manny Delhaize (CSIRO)**  
**Mae Bustaman (Indonesia)**  
**Hosseini Salekdeh (ABRII)**  
**Ken McNally**

**Sigrid Heuer**  
**Mike Thomson**  
**Xiaochun Lu**

