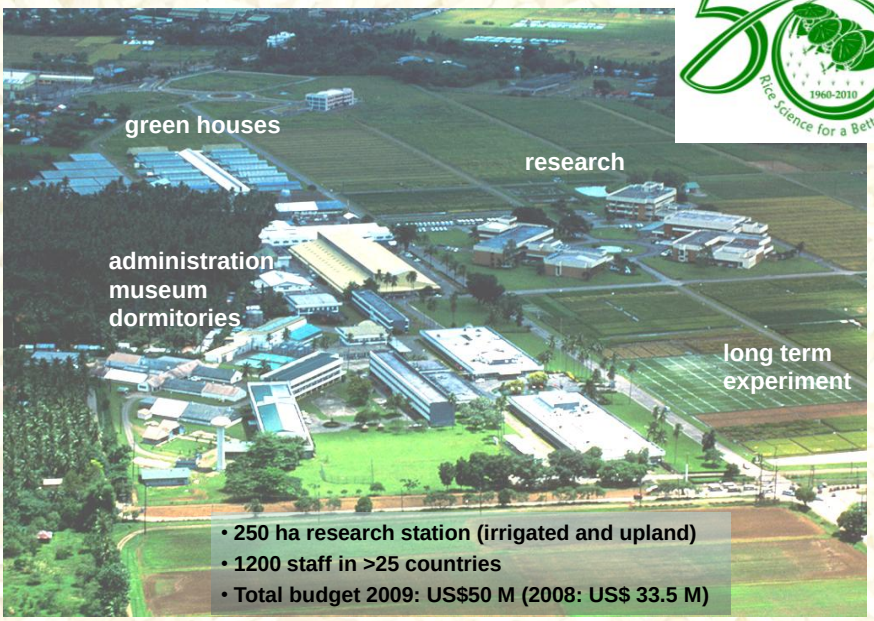
Marker-assisted breeding & molecular characterization of the major rice QTL *Phosphorus uptake 1* (*Pup1*)

Sigrid Heuer and Stephan Haefele
(IFA, Hanoi, November 10 2010)

Rice Science for a Better World

IRRI in Los Baños, Philippines

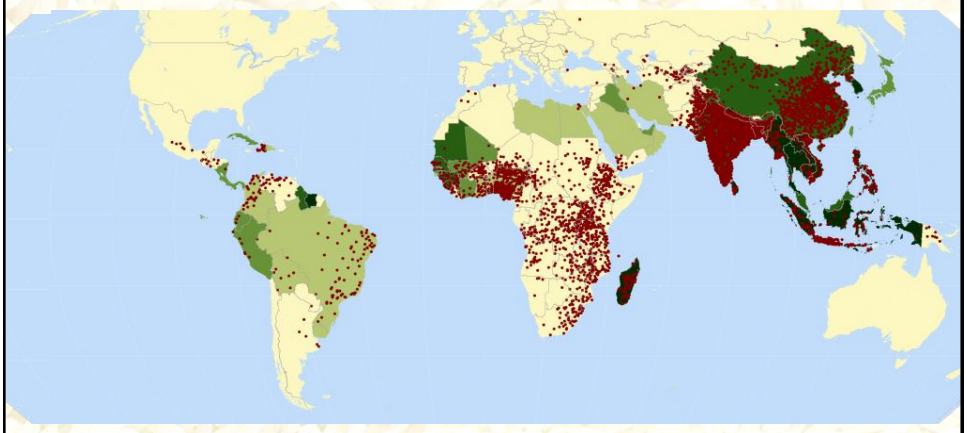


- 250 ha research station (irrigated and upland)
- 1200 staff in >25 countries
- Total budget 2009: US$50 M (2008: US$ 33.5 M)

IRRI's Mission:
To **reduce poverty and hunger**, **improve the health** of rice farmers and consumers, and ensure environmental sustainability **through collaborative research**, partnerships, and strengthening of NARES.

90% of the world's rice is produced and consumed in Asia. Over 70% of the world's poor are in Asia

IRRI

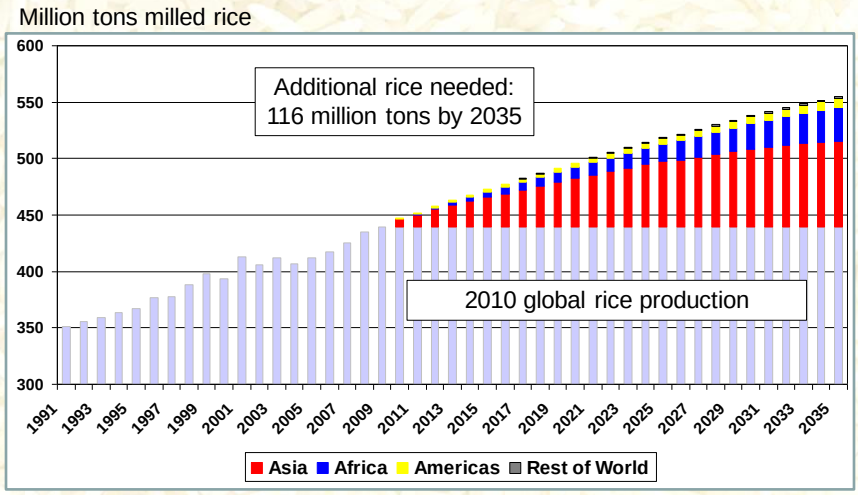


Rice Consumption
Annual consumption per capita
<25kg 25-50 50-75 75-100 >100kg

Poverty
Each dot represents 250,000 people living on less than $1.25 a day, 2005

A. Dobermann, IRRI 2010

Increasing rice production on less land, with less water, and less inputs



(i) Irrigated systems: ~80 Mha (50% area, **75%** production)

(ii) Rainfed systems: ~75 Mha (50% area, **25%** production)

A. Dobermann, IRRI 2010

Breeding activities and focus at IRRI: Overview

Intensive irrigated systems
- high yield (any new variety has to out-yield checks)
- hybrid rice (new consortium with private sector)
- C4 rice, golden rice, high iron rice
- disease resistance (blast, BB, tungro, sheath blight, brown spot...); rats

I. Enhancing yield

Rainfed drought-prone and stress environments (upland + lowland)
- submergence and drought
- salinity and acidity
- phosphorus and zinc deficiency
- heat and cold (AfricaRice)
- iron and aluminum toxicity
- all diseases + nematodes …

II. Reducing stress-related yield losses

Technologies/ agronomy
- variety testing in multi-location trials
- crop management and fertilizer trials
- alternate-wetting and drying technology (water saving)
- direct seeding (anaerobic germination)
- weed control (early vigor, water management)

III. Improved management and nutrient use

IRRI

Major crops: Share of global fertilizer use (2007)

Crop	Area (Mha)	Yield (t ha^{-1})	Total fertilizer use ($N + P_2O_5 + K_2O$) (% of total)	(Mt)	Total N use (% of total)	(Mt)	Total P_2O_5 use (% of total)	(Mt)	Total K_2O use (% of total)	(Mt)
Maize	158	5.0	15.3	25.8	16.8	16.9	12.4	4.9	14.2	4.1
Wheat	214	2.8	15.1	25.5	17.3	17.4	16.2	6.4	6.0	1.7
Rice	156	4.2	14.4	24.3	15.6	15.7	12.3	4.8	13.3	3.8
Other cereals	-	-	4.8	8.1	5.1	5.1	5.1	2.0	3.3	0.9
Sum			49.7	83.8	54.8	55.1	46.0	18.1	36.7	10.6

	nutrient-recovery rate (rice, 1st season):	nutrients not used in season (rice; Mt):
N	30-50%	11.0 - 7.85
P_2O_5	25%	3.6
K_2O	35-50%	2.47 – 1.9

at least 13.4 Mt of the applied 24,3 Mt fertilizer is not used
(rice in 1st season)

- **waste of resources (especially P*)**
- **waste of energy**
- **source of pollution (especially N)**
- **contribution to climate change**
- **high costs for (poor) farmers**

* **IFDC report**, SJ Van Kauwenbergh (2010):

- world rock P reserves: 340,000 – 460,000 mmt
- this will last for 300-400 years

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How can we improve nutrient uptake and use efficiency ?

- Site specific nutrient management/ slow release fertilizers

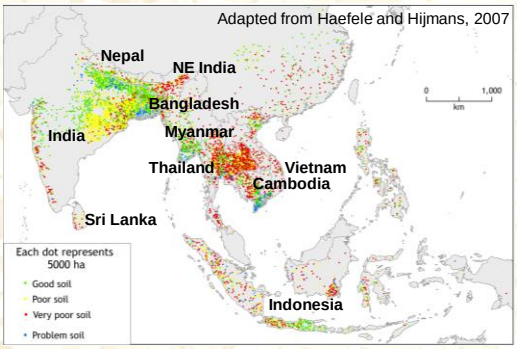
- Increased uptake efficiency of fertilizer (reduced net loss of fertilizer nutrients)
- More roots and/or different root architecture
- Better mobilization of “fixed” nutrients (P, K)
- High affinity transporters
- Increased internal efficiency
- Improve plant metabolism / photosynthesis
- Increase harvest index / new plant type
- Biological N fixation by exo- and/or endo-symbiosis

- Tolerant genotypes (reduce yield losses due to biotic and abiotic stresses)

- …..

Poor soils in poor countries

60% of rainfed rice in Asia is grown on problem soils



- **applied P fertilizer not plant available due to P-fixation in soil**
- **root growth impaired due to low pH, Al and Fe toxicity**
- **yield loss due to drought and submergence stress**
- **tolerant genotypes are needed to reduce production risk before farmers invest in fertilizer**

Nutrient uptake by roots : Phosphorus

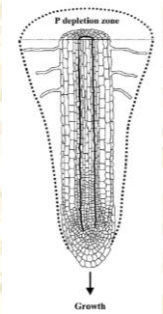
e.g., maize

Table 3. Percent of nutrients taken up by a corn crop normally supplied by root interception, mass flow and diffusion

Nutrient	Root interception	Mass flow	Diffusion
	% of uptake possible		
Nitrogen	<1	80	19
Phosphorous	2	5	93
Potassium	2	18	80
Calcium	150	375	0
Magnesium	33	600	0
Sulfur	5	300	0

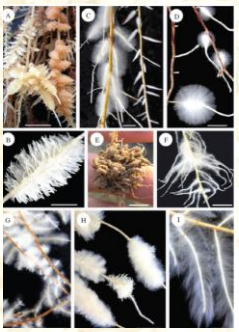
http://www.spectrumanalytic.com/support/library/ff/The_Nutrient_Uptake_Process.htm

Induction of root growth under low P



Pi

Cluster roots

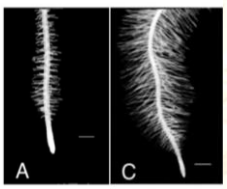


Lambers et al (2006)
Annals of Botany

A: Banksia prionotes
B: Hakea prostrata
C: Lepidosperma squamatum
D: Tetraria sp.
E: Aspalathus linearis
F: Aspalathus linearis in nutrient solution
G: Thamnochortus fracternus
H: Mastersiella digitata
I: Chondropetalum tectorum

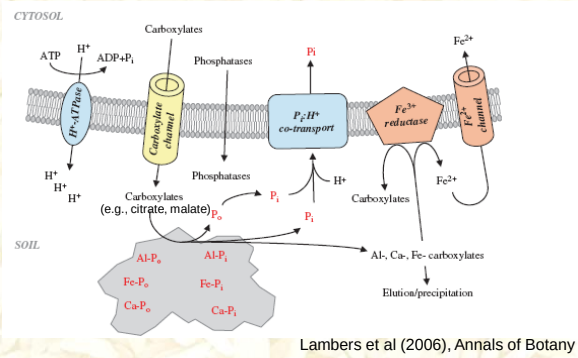
Root hair growth (*Arabidopsis*)

+P **–P**



adapted from Zhang et al (2003)
J Experimental Botany

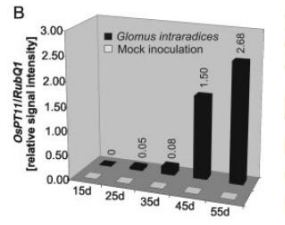
- **Mobilization of phosphorus by root exudates**



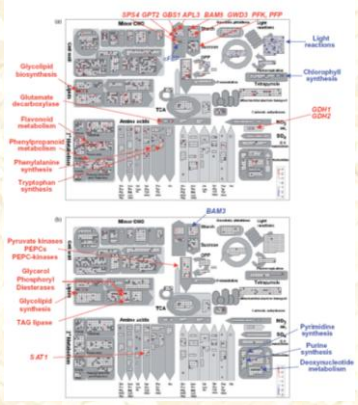
Lambers et al (2006), Annals of Botany

- **Induction of high-affinity P transporters**

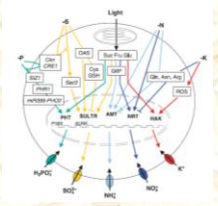
- **P-uptake in interaction with Mycorrhiza, e.g., OsPT11**

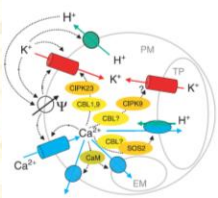


Molecular studies reveal insight in the regulation of nutrient uptake

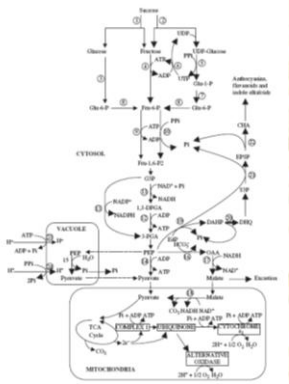


Morcuende et al (2007), *Plant Cell Environment*





Amtmann and Blatt (2009) *New Phytologist*



Hammond et al (2004) *Annals of Botany*

Many pathways/genes with complex regulation -
manipulate to enhance P uptake ?

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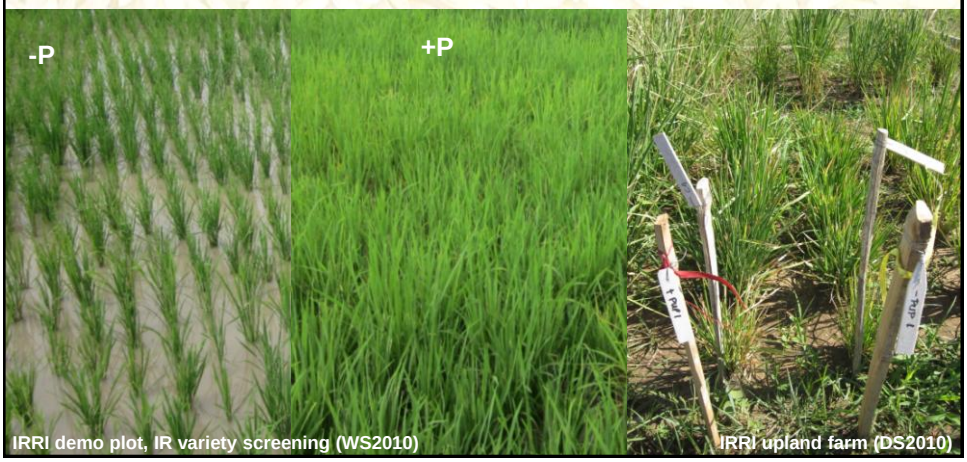
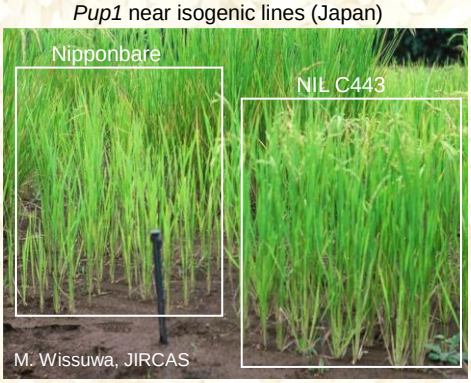
The major QTL Phosphorus uptake 1 (*Pup1*): A different story

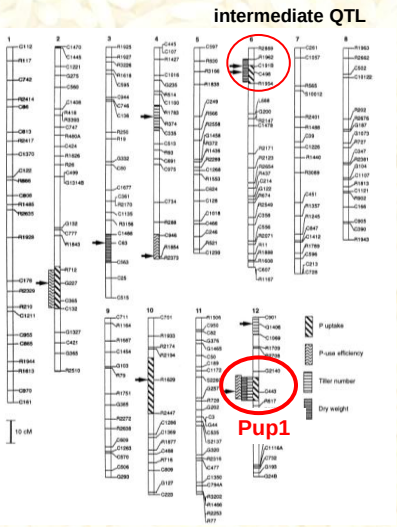


Pup1 mapping

IRRI

Pup1 near isogenic lines (Japan)

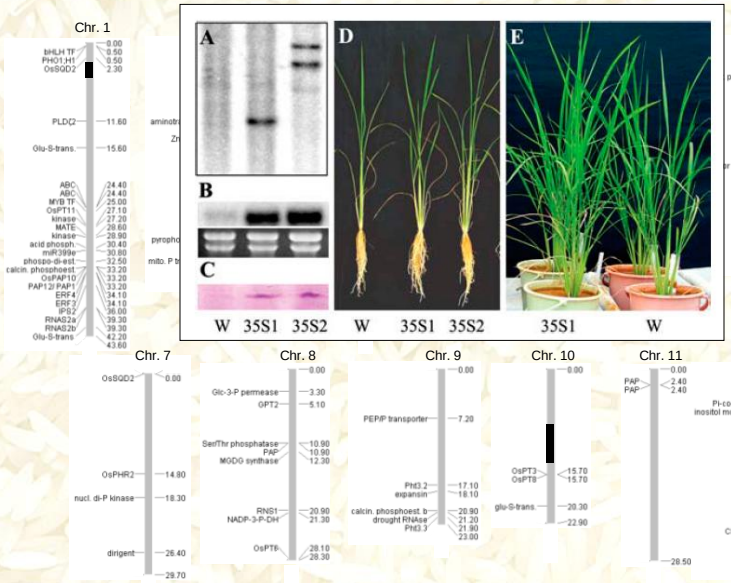




P-uptake: LOD 10.7 (28%)
dry weight: LOD 10.5 (27%)
tiller number: LOD 7.9 (21%)
(P-use efficiency: LOD 6.6 (19%) NB allele)

P uptake (mg root weight^{-1})	+P	-P
Nipponbare	13.7	1.8
NIL-C443	13.9	3.2
Kasalath	10.9	3.2

P-responsive genes in rice: No candidate gene in *Pup1* region

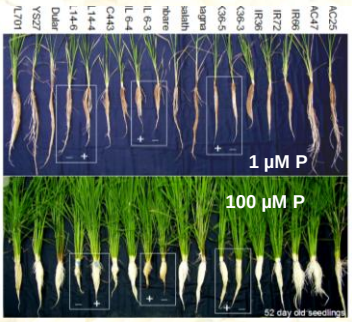


Heuer et al (2009), *Plant Biotech J*

How is *Pup1* functioning?

- no effect of *Pup1* on root growth in hydroponics

- root length? No
- longer root hairs? Yes in two Pup1 NILs but not in NIL6-4
- organic acid exudation? **No**
- interaction with mycorrhiza? No difference in autoclaved soil



- Microarray studies of contrasting *Pup1* materials

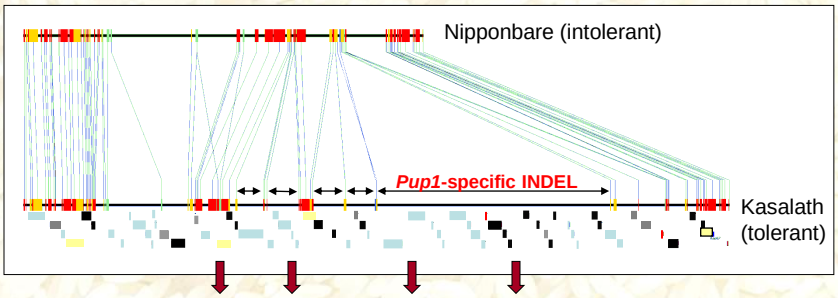
(i) high affinity P transporters are up-regulated in tolerant and intolerant lines

(ii) main differences are related to cell walls

Novel genes or P-uptake mechanisms?

➡ **Sequencing of the *Pup1* region in tolerant donor (Kasalath)**

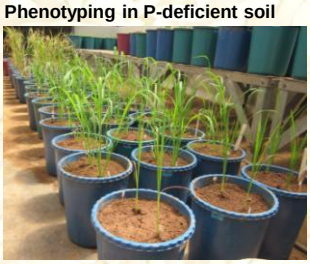
Sequence comparison of the *Pup1* genomic region



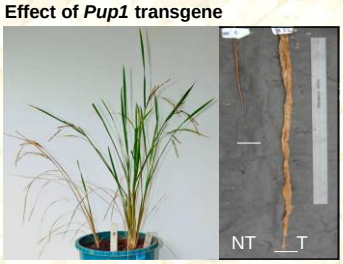
1. Identification of candidate genes

2. gene validation by transgenic approaches

Phenotyping in P-deficient soil



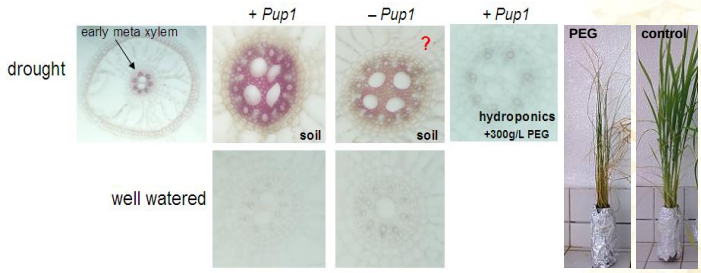
Effect of *Pup1* transgene



***Pup1* is a novel, regulatory gene located in the INDEL region**

Other *Pup1* candidate genes:

Lignification of roots under drought - role of *Pup1* dirigent gene?



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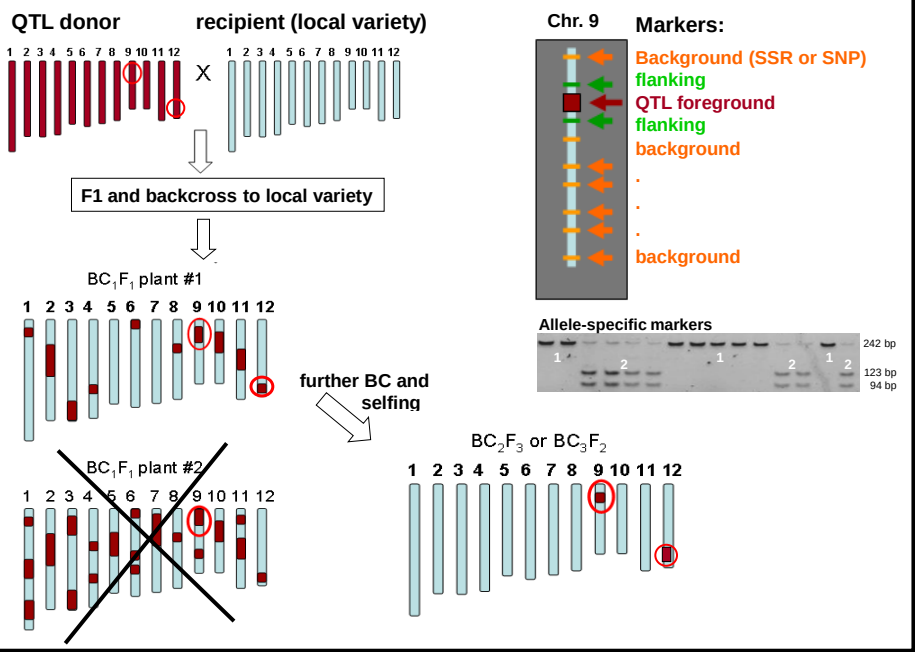
Marker-assisted backcrossing:
Targeted introgression of high-value genes/QTLs into widely grown and well adapted mega varieties

Marker assisted backcrossing (MABC)

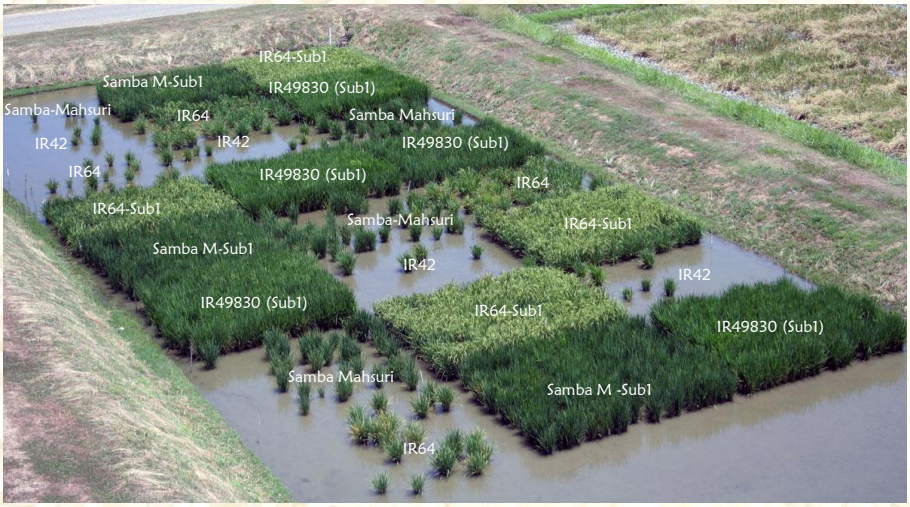
IRRI



First MABC product at IRRI: *Sub1* mega varieties

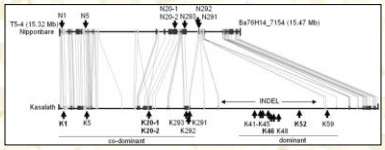
IRRI

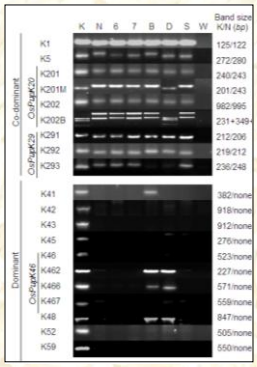
Recovery of Sub1 varieties after 17 days complete submergence



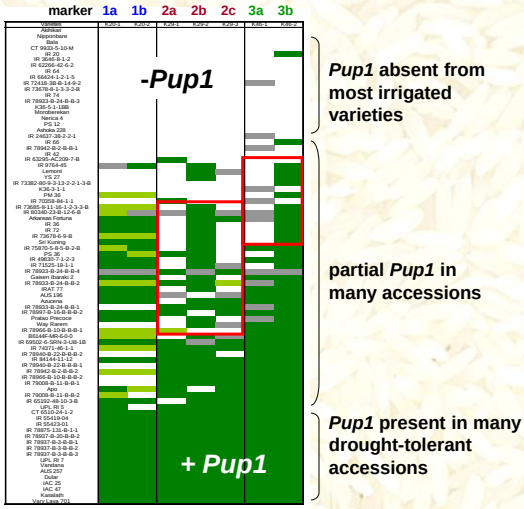
Pup1 gene-based markers

IRRI





Pup1 survey



***Pup1* absent from most irrigated varieties**

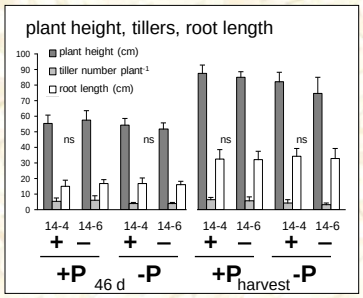
partial *Pup1* in many accessions

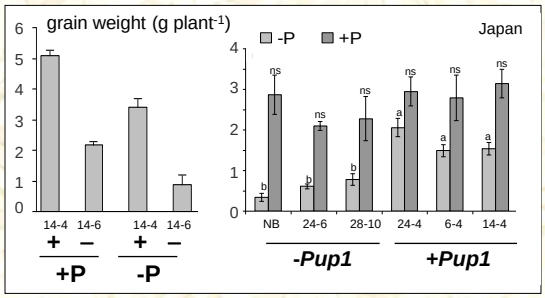
***Pup1* present in many drought-tolerant accessions**

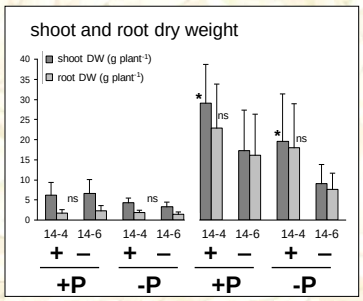
Pup1 phenotyping

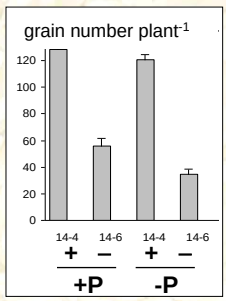
Phenotyping in P-deficient soil, aerobic/drought

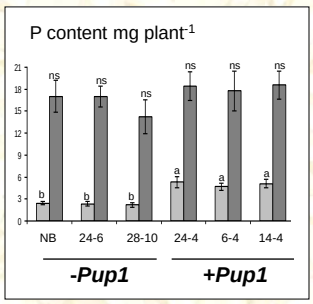
IRRI











Chin et al (2010), *Theor Appl Genet*

IRRI upland farm experiments (drought/ ww but high residual P)

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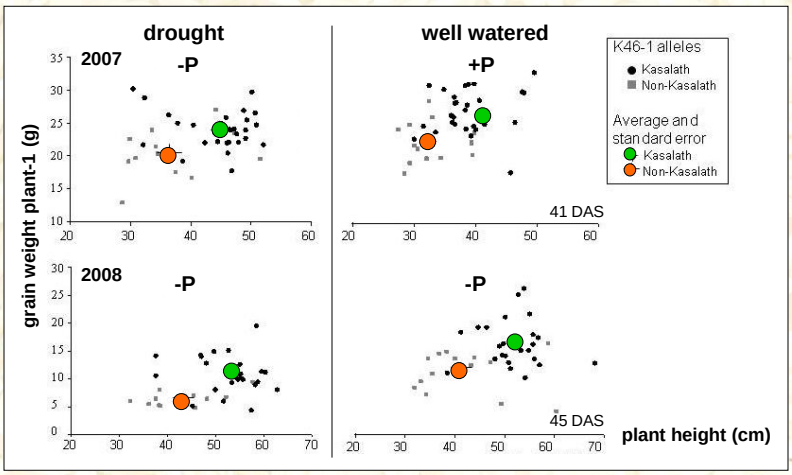

Pup1 in mega varieties

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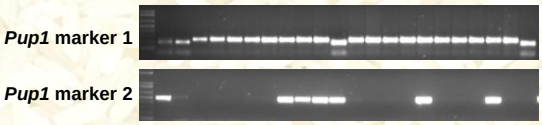
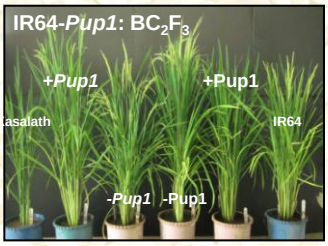
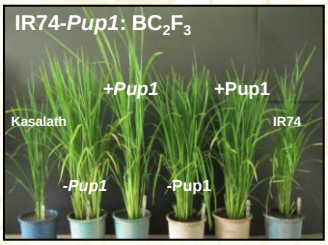
BC_2F_3 genotyping







IRRI

Summary and outlook:

- ***Pup1*** **does not function via known P-uptake mechanisms**

- ***Pup1*** **major gene is a novel regulatory gene with large effect on root growth; other** ***Pup1*** **genes are under validation (cell wall related)**

- **mapping of QTLs + MABC into mega varieties is a straight forward and successful breeding approach --- candidate gene approach is limited to known mechanisms/genes**

- **important:**
  (i) Good QTL donor (IRRI's strongest QTLs come from <u>un-adapted</u> germplasm)
  (ii) QTLs <u>must</u> have effect in different environments and genetic backgrounds

- ***Pup1*** **is most likely best suitable for aerobic, drought-prone environments**

- **For intensive systems we need to**
  (i) clearly define targeted mechanisms (higher and/or faster nutrient uptake? Higher internal nutrient-use efficiency?...)
  (ii) develop well defined screening protocols to identify donor varieties for this trait