



# Association studies and development of molecular markers for *Pup1*, a major QTL for phosphorus deficiency tolerance

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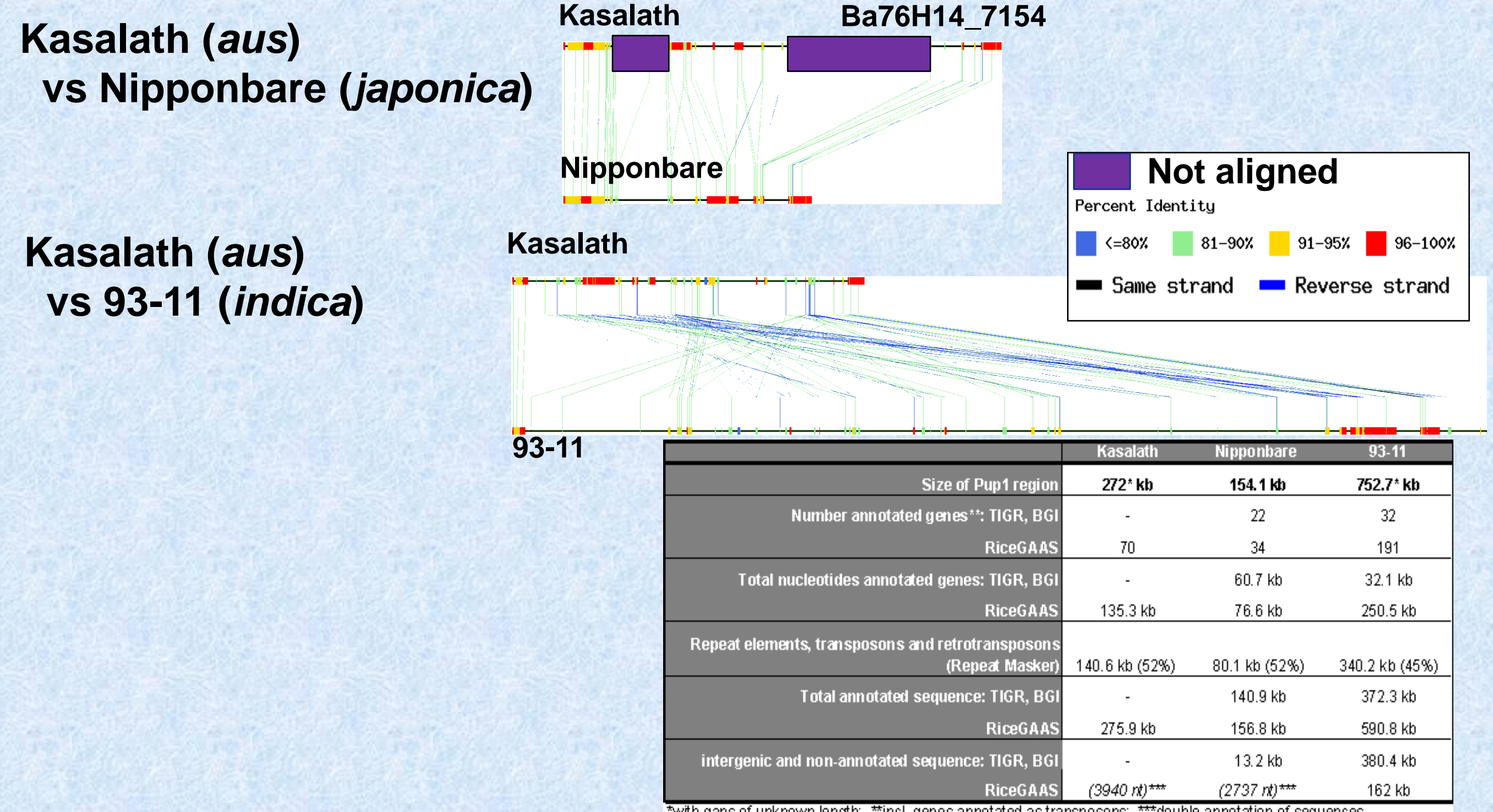
Phosphorus (P) deficiency and P unavailability are major constraints to rice production, especially in rainfed ecosystems. A major QTL for P-deficiency tolerance, *Pup1* (Phosphorus Uptake 1), has been mapped on chromosome 12 in a Nipponbare x Kasalath population under upland conditions (Wissuwa and Ae 2001). After fine mapping, we sequenced the *Pup1* region in tolerant donor Kasalath. Comparative sequence analyses revealed the unique structure of the Kasalath *Pup1* locus as compared with Nipponbare and 93-11 (Heuer et al. unpubl.; Ismail et al. 2007).

The assessment of the genes present in the *Pup1* locus showed the absence of any known P-responsive gene (Heuer et al. unpubl.). Likewise, earlier physiological analyses revealed no evidence as to how *Pup1* confers tolerance for P deficiency (Wissuwa 2005). It is therefore likely that *Pup1* represents a novel P-deficiency tolerance mechanism. Candidate gene analyses are currently ongoing.

To breed P-deficiency-tolerant rice varieties, it is of primary importance to develop a reliable *Pup1* phenotyping system, as well as robust PCR-based foreground and flanking markers. This is also a prerequisite to the identification of putative recipient parents for *Pup1*. We recently made good progress in the development of a more efficient phenotyping system after discovering that *Pup1* physically overlaps with a major QTL for yield under drought. Indeed, the *Pup1* phenotype is more expressed under water-limited conditions.

PCR-based gene-specific and *Pup1* flanking markers were developed and are now being used for marker-assisted introgression of *Pup1* into Asian rice cultivars. These markers were also used for an allelic survey of 81 diverse rice accessions showing the presence of *Pup1* predominantly in upland varieties, suggesting that breeders have unknowingly selected for *Pup1* in varieties developed for unfavorable environments.

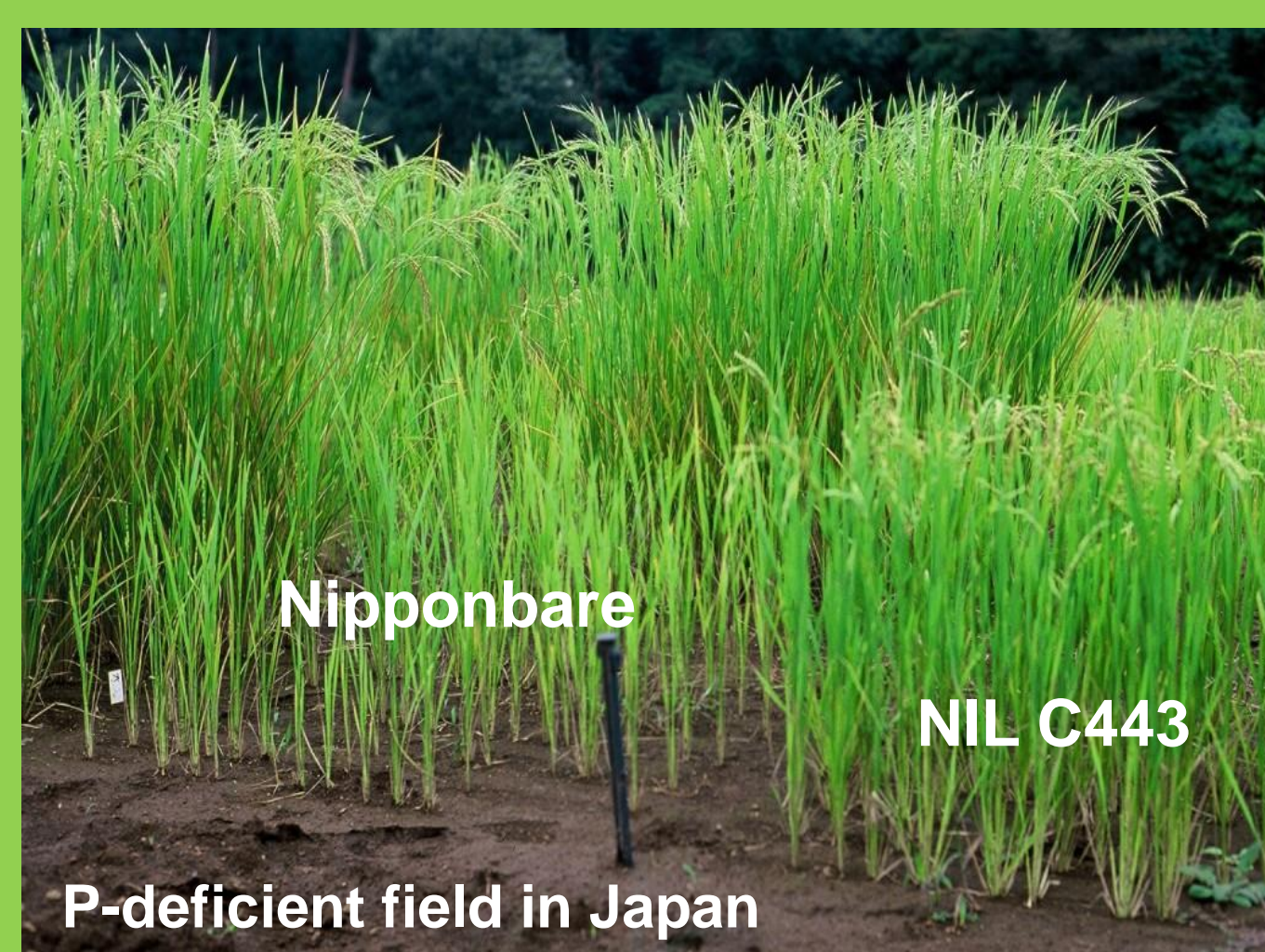
## Unique genomic structure of *Pup1* in Kasalath



## *Pup1* enhances P uptake in P-deficient soil

*Pup1* QTL (chr.12):  
 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 <sup>-1</sup> )	
	+P	-P
Nipponbare	13.7	1.8
NIL-C443	13.9	3.2
Kasalath	10.9	3.2



(Wissuwa and Ae 2001)

## Development of *Pup1* NILs

Nipponbare / Kasalath  
 (Intolerant) (Tolerant)

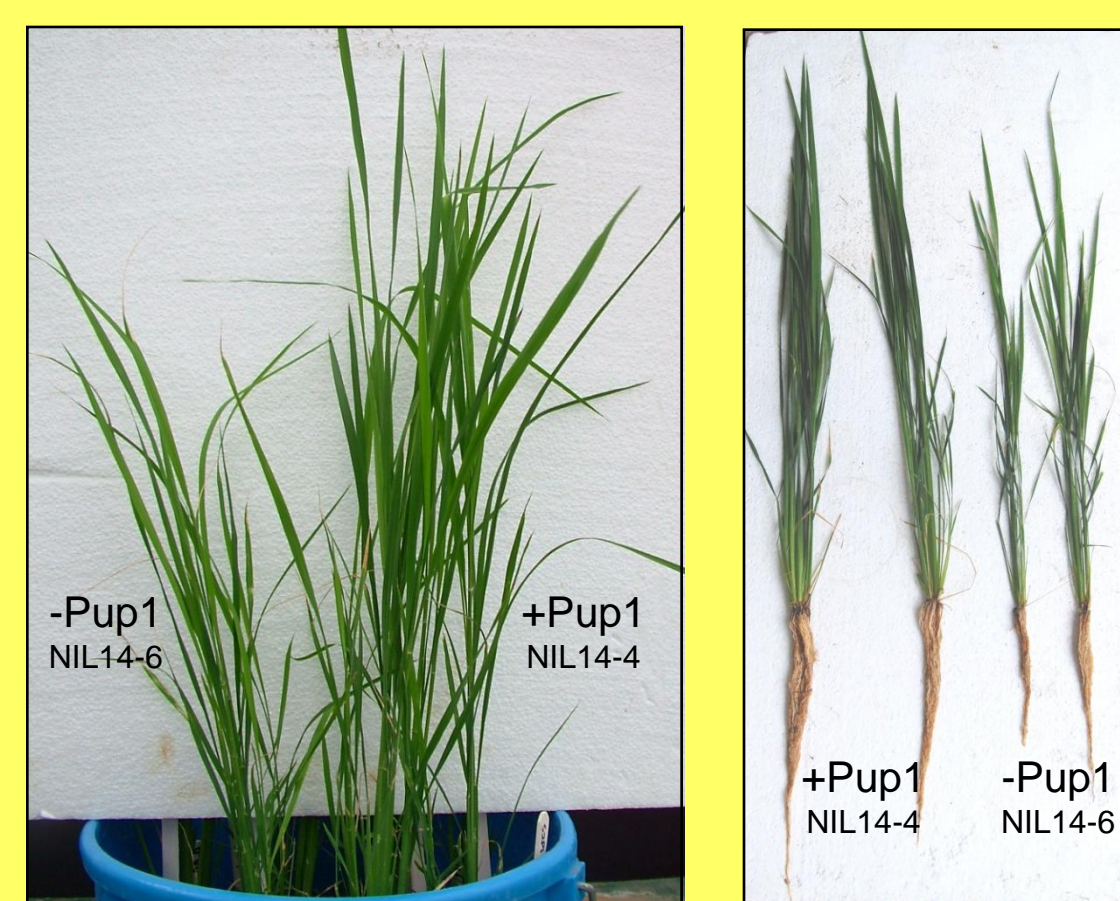
QTL analysis (G X P association)  
 for P uptake

NIL-C443 selected as tolerant line

Backcrossed with Nipponbare to remove  
 additional introgression outside of *Pup1*

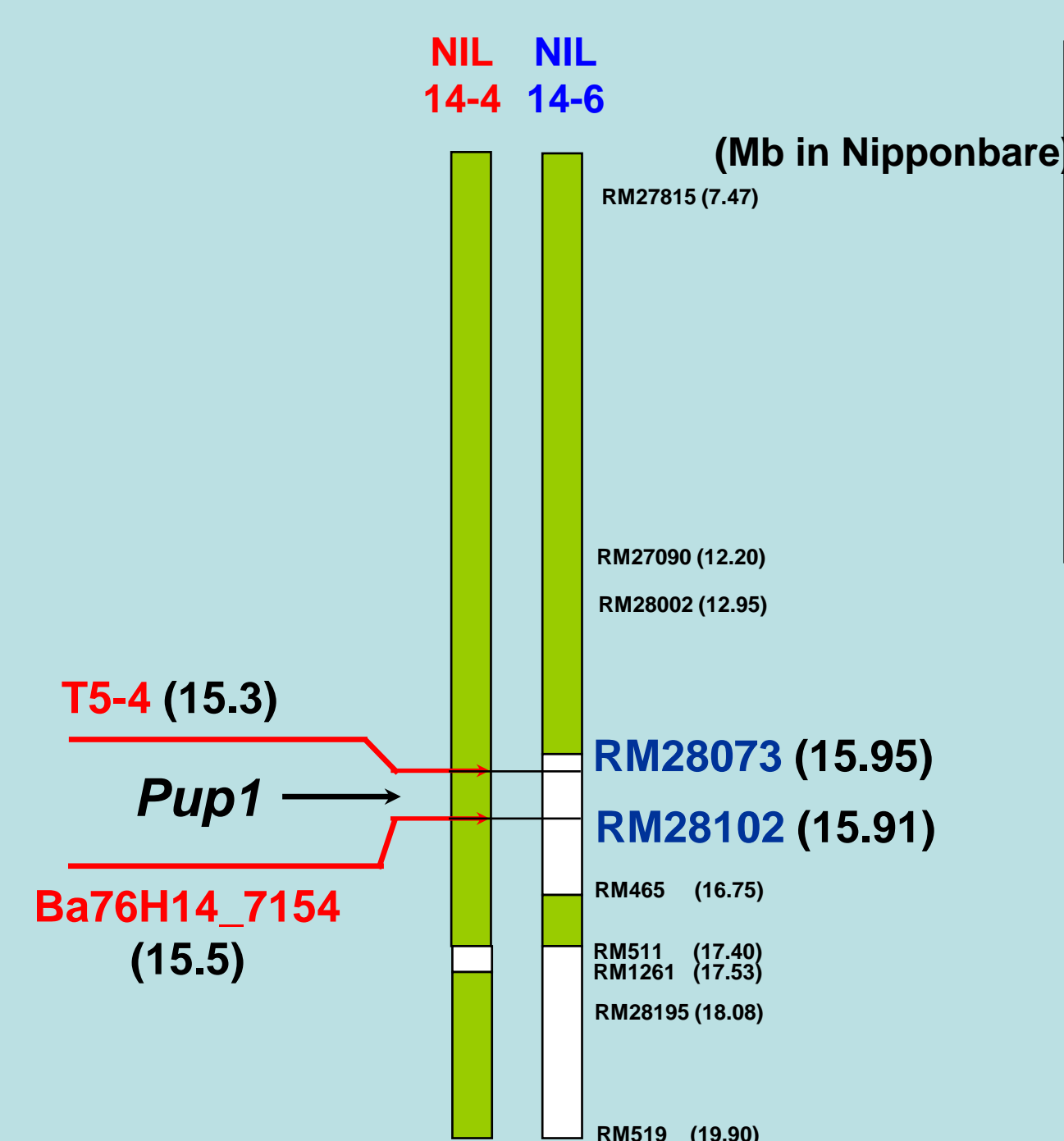
NIL14-4

Repeated backcross and selection to  
 eliminate additional introgressions that  
 might have undesired effects



*Pup1* phenotype in P-deficient soil. *Pup1* near-isogenic lines (NILs) 14-4 (+*Pup1*) and 14-6 (-*Pup1*) were grown under long-day conditions in P-deficient soil from Pangil (Philippines). NIL14-4 showed a clear growth advantage under these conditions.

## *Pup1* fine mapping and markers for MAB

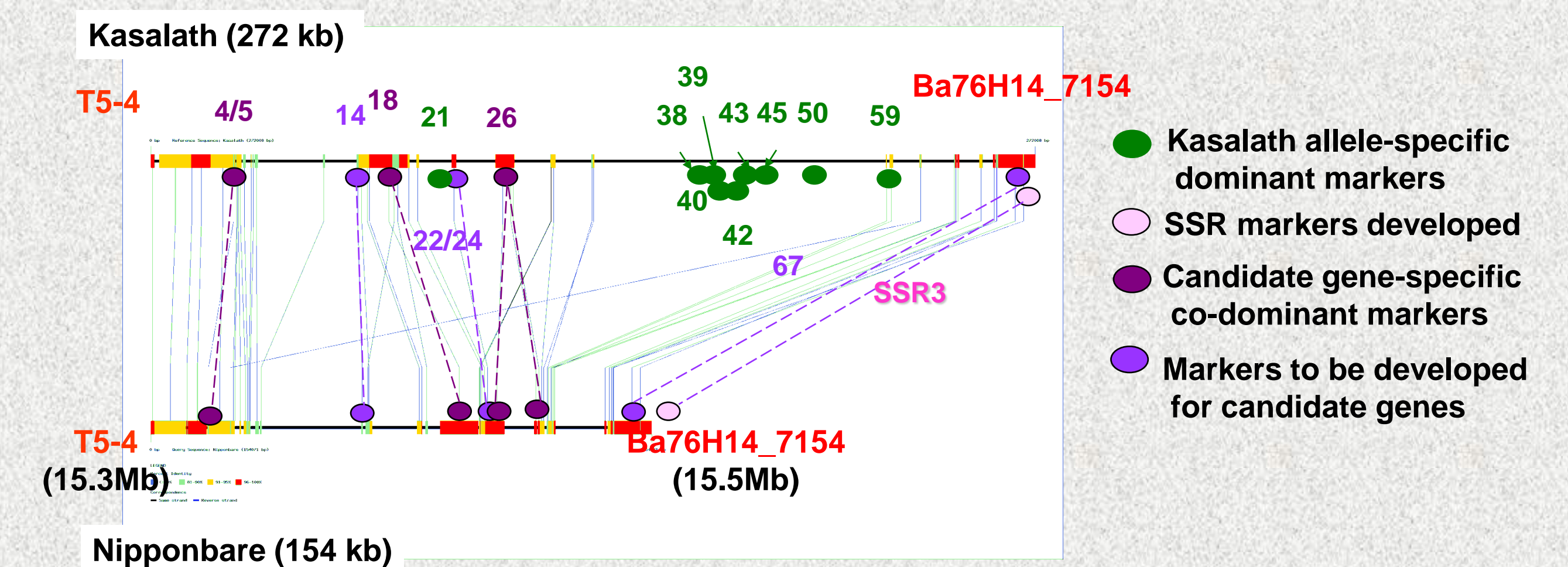


MAB STRATEGY

- Foreground selection by *Pup1* gene-specific markers
- Recombinant selection by tightly linked PCR markers
- Background selection by polymorphic markers for all chromosomes (population-specific)

- Fine mapping markers for the *Pup1* locus (red)
- Tightly linked recombinant markers (blue)

## Development of gene-specific markers in *Pup1*



## *Pup1* survey in rice accessions

Subspecies	Major cropping system	Accession no.	Allele in <i>Pup1</i> region													Kasalath allele frequency in <i>Pup1</i> (%)	Designation
			18	26	38	39	40	42	43	45	50	59	3'-1	3'-2			
Aus/boro	Upland	4	[Grid]													84	Kasalath
	Lowland	1	[Grid]													82	
	Aerobic	2	[Grid]													96	
Indica	Upland	36	[Grid]													65	Nipponbare
	Lowland	12	[Grid]													22	
Japonica	Upland	3	[Grid]													69	
	Lowland	3	[Grid]													6	

Legend: [Grid] indicates presence of Kasalath allele

*Pup1* allele-specific PCR-based markers surveyed in

- > 81 varieties and IRRI breeding lines
- > *O. sativa* ssp. *japonica* and *indica*
- > upland and lowland cultivars

*Pup1* is more widely distributed in upland varieties, suggesting beneficial effect of this QTL in P-deficient and drought-prone environments

## References

- Wissuwa M and Ae N 2001 Plant Breeding 120: 43-48.
- Wissuwa M 2005 Plant Soil 269: 57-68.
- Wissuwa M, Wegner J, Ae N, Yano M 2002 Theor Appl Genet 105: 890-897.
- Ismail A, Heuer S, Thomson MJ, Wissuwa M 2007 Plant Mol Biol DOI 10.1007/s11103-007-9215-2