Variation at the *Pup1* locus within the genus *Oryza* predates domestication

The deficiency of phosphorus (P) in soil is a worldwide problem, and though there are many approaches to tackle this problem, the development of rice cultivars with enhanced P efficiency would represent a sustainable strategy to improve the livelihood of resource-poor farmers. Recently, the *Pup1* locus (Fig. 1), a major QTL for tolerance to P deficiency, was successfully narrowed down to a single-candidate gene, the protein kinase: P starvation tolerance (*OsPSTOL1*). The aim of this study was to search for novel *OsPSTOL1* alleles and to survey *Pup1* locus variation in Asian (*O. sativa*)- and African (*O. glaberrima*)-cultivated rice and their wild progenitors. This information would help in designing a suitable strategy for marker-assisted introgression of *Pup1/PSTOL1* into rice megavarieties.

A novel *OsPSTOL1* allele was detected in *O. glaberrima*. This allele has 35 base-pair changes (when aligned to Kasalath allele), but none of the functional domains were affected and it is expressed. Allele-specific markers were then developed for single PCR and/or duplex PCR system, which produce a band pattern clearly distinguishable on agarose gels (Fig. 2), and are therefore suitable for most marker laboratories throughout the world. Using these markers to survey allelic distribution of *PSTOL1* across the genus *Oryza* showed that the novel allele is common in accessions belonging to *O. glaberrima* and its ancestor *O. bartii*, but is not restricted to African rice as *O. sativa*, *O. rufipogon*, and *O. nivara* accessions do carry the *glaberrima* allele at low frequency (Fig. 1).

Using additional allele-specific markers across the entire *Pup1* locus revealed two main patterns in the Africa rice (*O. glaberrima* and *O. barthii*): the more typical 'Africa pattern' characterized by the novel *PSTOL1* allele and partial presence of the *Pup1*-specific INDEL region, but general absence of 90 kb of a region upstream of *PSTOL1* (pattern G, Fig. 1); and the less common pattern (K) with Kasalath alleles across most of *Pup1*. Within *O. sativa,* the Kasalath (K) and Nipponbare (N) patterns could be distinguished as described earlier, but in addition a mixed pattern (m) with partial presence of Kasalath, *O. glaberrima*, and novel alleles was detected. These three patterns were already present in *O. rufipogon* and *O. nivara*, the wild ancestors of *O. sativa*. Results suggested that *Pup1* locus variation was already a common feature within wild ancestors of cultivated Asian and African rice. Thus, divergence at *Pup1* appears to predate domestication of rice.

Since the function of other genes within the *Pup1* locus remains unclear, it would be desirable to transfer the entire *Pup1* region from Kasalath into recipient varieties during marker-assisted selection. Thus, we propose using two foreground markers (K46-K and K20-K) in breeding programs aimed at introgressing *Pup1*.

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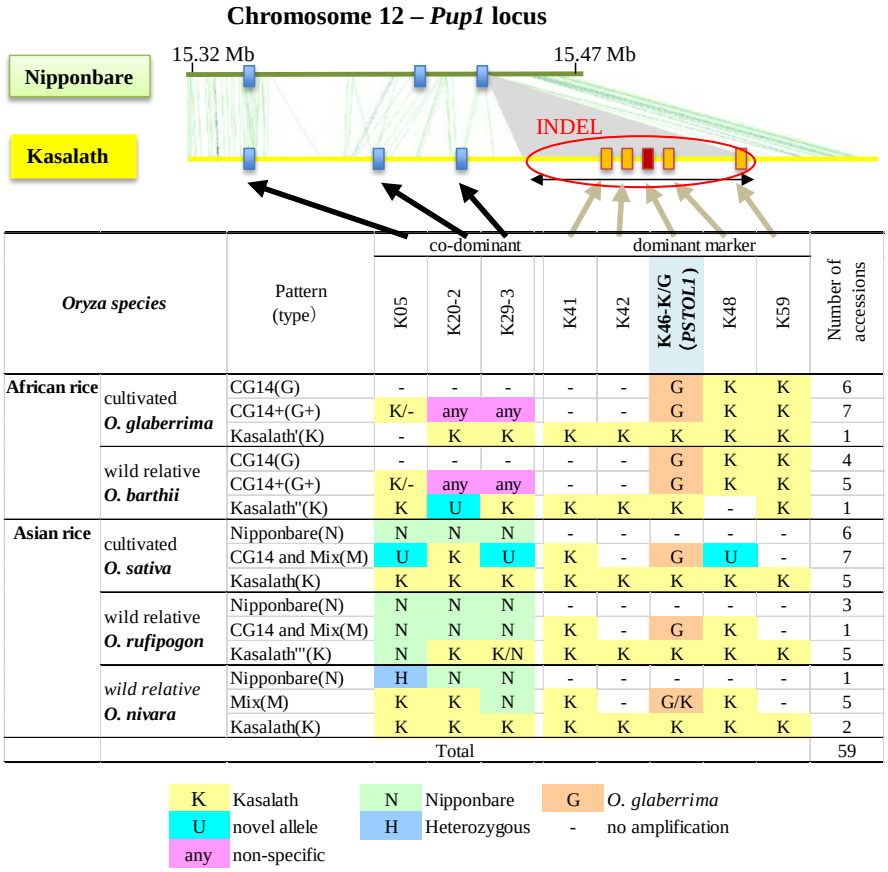

Oryza species		Pattern (type)	co-dominant			dominant marker					Number of accessions
			K05	K20-2	K29-3	K41	K42	**K46-K/G (*PSTOL1*)**	K48	K59	
African rice	cultivated ***O. glaberrima***	CG14(G)	-	-	-	-	-	G	K	K	6
		CG14+(G+)	K/-	any	any	-	-	G	K	K	7
		Kasalath'(K)	-	K	K	K	K	K	K	K	1
	wild relative ***O. barthii***	CG14(G)	-	-	-	-	-	G	K	K	4
		CG14+(G+)	K/-	any	any	-	-	G	K	K	5
		Kasalath''(K)	K	U	K	K	K	K	-	K	1
Asian rice	cultivated ***O. sativa***	Nipponbare(N)	N	N	N	-	-	-	-	-	6
		CG14 and Mix(M)	U	K	U	K	-	G	U	-	7
		Kasalath(K)	K	K	K	K	K	K	K	K	5
	wild relative ***O. rufipogon***	Nipponbare(N)	N	N	N	-	-	-	-	-	3
		CG14 and Mix(M)	N	N	N	K	-	G	K	-	1
		Kasalath'''(K)	N	K	K/N	K	K	K	K	K	5
	wild relative ***O. nivara***	Nipponbare(N)	H	N	N	-	-	-	-	-	1
		Mix(M)	K	K	N	K	-	G/K	K	-	5
		Kasalath(K)	K	K	K	K	K	K	K	K	2
			Total								59

K	Kasalath	N	Nipponbare	G	*O. glaberrima*
U	novel allele	H	Heterozygous	-	no amplification
any	non-specific				

Fig. 1. Characterization of the *Pup1* locus in Nipponbare and Kasalath, and cultivated and wild rice genotypes. A main difference is the absence of a 90 kb INDEL region in Nipponbare containing *OsPSTOL1* and 20 other Kasalath-specific genes.

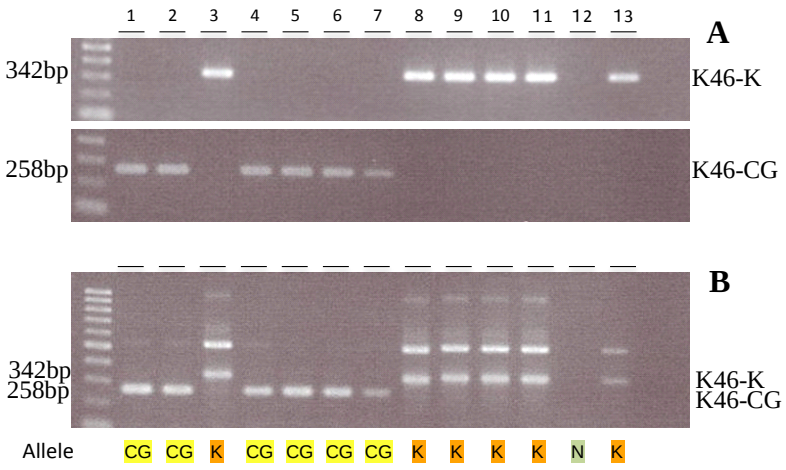

Fig. 2. Amplification of *OsPSTOL1* alleles using allele-specific markers for Kasalath and CG14 in single PCR (A), and duplex PCR system to detect both alleles in one reaction (B).
(1: CG14, 2: IRAT, 3: NERICA16, 4: WAB56-50, 5: NERICA1, 6: NERICA10, 7: WAB181-18, 8: IDSA, 9: IR12979, 10: WAB56-104, 11: IAC165, 12: Nipponbare, 13: Kasalath)