

33. Prediction of organelle targeting of pentatricopeptide repeat protein and genes tagged with *Tos17*

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Pentatricopeptide repeat proteins (PPR) are nuclear-encoded proteins and are defined by the tandem array of PPR motif consisting of 35 amino acids. They have been reported to function in post-transcriptional RNA regulation processes such as editing, cleavage and splicing, and in translational regulations in mitochondria or chloroplasts. *PPR* genes form a huge gene family in higher plants. Bioinformatical analysis has revealed that there are 477 *PPR* genes in rice and 466 genes in *Arabidopsis thaliana* (Small and Peters, 2000 Andres et al., 2007 Saha et al., 2007 O'Toole et al., 2008). In *Arabidopsis*, it was predicted that half of PPRs are targeted to mitochondria, and a quarter may localize in plastids (Lurin et al., 2004). Prediction of organelle targeting has not yet been reported in rice PPR proteins.

To further examine importance of *PPR* genes for organelle biogenesis and their function, we searched *Tos17*-insertion lines (Hirochika, 2001) of the 477 *PPR* genes in rice. By searching a *Tos17*-flanking DNA sequence database (<http://tos.nias.affrc.go.jp/~miyao/pub/tos17/index.html.ja>), we identified 30 lines in which *Tos17* was inserted in a protein-coding region of 20 *PPR* genes (Table 1). The database of *Tos17* mutant panels has recorded some mutant phenotypes, such as low germination rate, lethal growth, yellow leaf color, and semi-dwarf. Similar phenotypes such as developmental defects (Falcon de Longevialle et al. 2007, 2008), reduced fertility (Lurin et al., 2004) and embryo-lethality (Gutierrez-Marcos et al., 2007) have been reported in *PPR* mutants of *Arabidopsis* and maize. Among 29 lines, we identified one homozygous *Tos17* inserted line NC0057. This line showed growth retardation. Genetic analysis indicated that this mutation was recessive. We also confirmed linkage between this phenotype and insertion of *Tos17*. This result lets us expect that at least some of the *PPR* genes in Table 1 are knocked out by the insertion of *Tos17*.

Then we predicted subcellular localization of the PPR proteins using four targeting predictors, WoLF PSORT (<http://wolfsort.org/>), MITOPROT (<http://ihg2.helmholtz-muenchen.de/ihg/mitoprot.html>), TargetP 1.1 Server (<http://www.cbs.dtu.dk/services/TargetP/>) and Predotar (<http://urgi.versailles.inra.fr/predotar/predotar.html>). Predicted localization is listed in Table 1. Eight proteins were predicted to be targeted to mitochondria, while six were to plastids, by at least three programs. Now we are experimentally analyzing subcellular localization of the PPR proteins encoded by the 20 genes in Table 1. Detail analyses of these *Tos17*-insertion lines will reveal essential functions of the *PPR* genes in mitochondria and plastids in rice.

References

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Score	Rice PPR genome browser ID (*1)	RAP locus ID (*2)	Chr. No and Position	Tosl7 line	Phenotypes recorded in Tosl7 mutant panel (*3)	Prabhar (*4)	TargetP (*5)	MITOPROT (*6)	WolfP/ORT (*7)
4	OsPPR_01g8530	Os01g913400	chr.1: 41,567,571_41,575,402	NC0559	ND	M	M	M	M
				NC0623	ND				
	OsPPR_04g7940	Os04g676200	chr.4: 34,915,279_34,917,033	NE1516	Albino, Smi dwarf,	M	M	M	M
	OsPPR_10g5420	Os10g497300	chr.10: 19,392,891_19,416,228	NG0791	Dwarf, Smi dwarf, Sterile, Low fertile	M	M	M	M
NG0806				ND					
3	OsPPR_01g5860	Os01g151900	chr.1: 2,804,586_2,807,152	NF9875	ND	M	M	M	P
	OsPPR_01g8120	Os01g176300	chr.1: 3,926,450_3,928,447	NG7065	Smi dwarf	M	M	M	P
	OsPPR_01g5840	Os01g880900	chr.1: 39,982,272_39,984,287	NE3006	Sterile	M	M	-	M
	OsPPR_02g8300	Os02g829800	chr.2: 36,561,685_36,563,274	NG6367	Low germination rate	M	M	M	P
	OsPPR_05g8190	Os05g455900	chr.5: 22,443,850_22,449,766	NG1405	Withering	Possibly M	M	M	P
2	OsPPR_03g2690	Os03g844000	chr.3: 36,324,824_36,330,690	NC6373	ND	M	P	M	P
	OsPPR_07g6390	Os07g548300	chr.7: 22,414,011_22,416,770	NG6033	Low fertile	P	M	M	P
1	OsPPR_01g8380	Os01g674700	chr.1: 29,481,170_29,487,353	NF6031	Narrow leaf	P	P	M	P
	OsPPR_01g1630	Os01g833000	chr.1: 37,414,591_37,416,792	NC2779	Smi dwarf, Low fertile, Small gran	p	P	M	P
	OsPPR_02g2950	Os02g121900	chr.2: 1,154,674_1,157,039	NC5121	Low fertile	none	-	M	Cy
	OsPPR_02g7050	Os02g167200	chr.2: 3,614,130_3,616,330	NC4052	Low fertile	P	P	M	P
	OsPPR_04g1350	Os04g602600	chr.4: 30,808,628_30,811,683	NC0057	Lethal, Yellow, Dwarf, Smi dwarf, Late heading	none	P	M	P
	OsPPR_05g0950	Os05g586900	chr.5: 29,320,221_29,321,645	NC0672	Lethal, Dwarf, Smi Dwarf, Low tillering, Late heading	Possibly P	P	M	P
	OsPPR_11g1860	Os11g109800	chr.11: 453,617_455,863	NG1026	Yellow, Sterile, Low fertile	Possibly P	P	M	P
NC2538				Low fertile					
NE5212				Low germination rate					
NE0172				Low germination rate					
ND0038				Deep green leaf, Dwarf, Long culm, Late heading, Shattering, Low fertile					
NG0557				Low fertile					
NG0344				Pale green leaf, Withering					
NG7176				Low germination rate					
0	OsPPR_01g2810	Os01g228400	chr.1: 7,091,012_7,094,295	NG2536	Low fertile	none	-	-	P
	OsPPR_05g5490	Os05g147600	chr.5: 2,713,413_2,715,042	ND4520	Sterile	none	-	-	P
	OsPPR_06g1040	Os06g613100	chr.6: 25,385,287_25,389,552	NF6025	Narrow leaf, Dwarf, Semi gran	none	-	-	Cy

*1 Rice PPR genome browser ID (Toole et al., 2008)
(<http://www.plantenergy.uwa.edu.au/cgi-bin/gro/wse/ricebrowser/>)

*2 RAP locus ID (<http://rap.dch.affrc.go.jp/>)

*3 <http://tos.nias.affrc.go.jp/~miyao/p/btos17/index.html>