

## Phylogenic-analysis of wild rice using AFLP and nutrients analysis

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AFLP 및 양분 분석을 이용한 야생벼의 계통학적 연구

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### Objective

To illuminate a genetic relationship between nutritional- and genomic distance in wild rices, 14 accessions of 4 *Oryza* species were analyzed using 7 selective AFLP primers comparing nutrient contents in grain.

### Materials and Methods

#### 1. Plant materials

*O. sativa* accession : 5-species

*O. officinalis* accession : 7-species

*O. ridleyi* accession : 1-species

*O. brachyantha* : 1-species

#### 2. AFLP and nutrient analysis

Leaf samples for DNA extraction were obtained from 15-old-day rice seedling. According to Vos et al. (1995), selective amplification was carried out in 25  $\mu$ l reaction volumes using 5  $\mu$ l template(Table 1). PCR products were resolved on 5% polyacrylamide gel. Each AFLP fragment/marker was treated as a unit character and scored as binary code(1/0=+/-). The 1/0 matrix was used to calculate dissimilarity coefficients following Nei and Li(1979). The resulting distance matrices were used to construct an unweighted pair-group method with arithmetic means(UPGMA) phenogram using software package NTSYS-PC 2.11. These phylogenic distance matrix was compared to dissimilarity matrix on a basis of nutrient contents in the grains, resulting

### RESULTS

AFLP analysis revealed total 219 fragments. In total, 211 fragments were identifiable(96.3 %). Genetic distances exhibit 4 well-distinguishable groups corresponding to AA, BB, BBCC, CCDD. Species of *Sativa* complex, *O. barthi* and *O. glaberrima* were well-differentiated from other accessions, *O. glumaepatula* and *O. Nivara*. On a basis of nutrient contents, *O. barthi* and *O. glaberrima* were also showed most different to *O. nivara*.

Table 1. The sequence of adapters and primers

	Name	Sequence
Ligation	Mse-adapter I	5'-GACGATGAGTCCTGAG-3' TACTCAGGACTCAT
	Pst-adapter I	5'-CTCGTAGACTGCGTACATGCA-3' CATCTGACGCATGT
Pre-amplification	M00	5'-GATGAGTCCTGAGTAA-3'
	P00	5'-AGACTGCGTACATGCAG-3'
Selective-amplification	M primer	M40(MseI+AGC), M46(MseI+ATT), M38(MseI+ACT), M43(MseI+ATA)
	P primer	P33(PstI+AAG), P63(PstI+GAA), P34(Pst I+AAT)

Table 2. No. of fragments and polymorphism according to AFLP primer combinations

Primer combination	No. of total fragment	No. of polymorphic fragment	polymorphism(%)
P33/M40	52	48	92.3
P63/M46	36	33	91.7
P34/M38	55	54	98.2
P63/M43	76	76	100.0
Total	219	211	96.3
Mean	54.8	52.7	