

## 25. Preliminary screening of rice genes whose expression levels alter by autotetraploidy using cDNA microarray

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There have been several reports showing gene dosage effects on polyploid plants by mainly based on isozyme analyses. However, there have been few studies on gene dosage effect using the microarray technique. As the first step to investigate gene regulation in autopolyploidy, we have carried out cDNA microarray analysis to compare gene expression levels in tetraploid rice with those in diploid rice. To perform transcription profiling with microarray, we used our second generation microarrays with unique 8,987 EST clones of rice ([http://cdna01.dna.affrc.go.jp/RMOS/array9000g\\_en.html](http://cdna01.dna.affrc.go.jp/RMOS/array9000g_en.html)). We isolated total RNAs from young leaves of seedlings (with 3.5-5.5 leaves) of five diploid lines and five tetraploid lines derived from five different rice cultivars consisting of three japonica lines ('Shinriki', 'Sensho' and Sen-ichi) and two indica lines ('Surjamukhi' and 'Dular'). The seed materials were distributed by NIAS GeneBank. These plants were grown in a green house (26-36 degrees C) under natural light condition for 29 days after sowing in bulk for each rice line (from the late May to June 2002). For expression profiling by microarray, we used 20 microgram of total RNA from each material per slide of microarray. Methods on our microarray analysis and data analysis were as described by Yazaki et al. (2003).

Differential expression data obtained with microarray between diploid and autotetraploid were compared among japonica lines or among indica lines. In this series of experiments, we used the two following criteria to identify genes whose transcription was increased or decreased as shown by comparisons between diploid and autotetraploids with the same genetic background (the same cultivar background): a significant difference of the expression level of a given gene was [1] 1.5-fold or more, or [2] 2-fold or more, in three japonica diploid and autotetraploid sets or two indica diploid and autotetraploid sets.

In the criterion [1], we detected 88 clones showing change of transcriptional level (up-regulated: 19 clones; down-regulated: 69 clones) in the three japonica sets, and 131 clones (up-regulated: 67 clones; down-regulated: 84 clones) in the two indica sets. There was only one clone overlapped in both japonica Table 1. Microarray clones showing change of transcriptional level between diploids and autotetraploid in

the criterion [2] where a significant difference of the expression level of a given gene was 2-fold or more in the three japonica diploid and autotetraploid sets or in the two indica diploid and autotetraploid sets. The first three cultivars ('Sensho', 'Shinriki' and 'Sen-ichi') are japonica; the other two cultivars ('Surjamkhi' and 'Dular') are indica. "4X / 2X" represents ratios of expression level in autotetraploid to that in diploid. "2X / 4X" represents ratios of expression level in diploid to that in autotetraploid. Upward- and downward-arrows in the table indicate up-regulation and down-regulation, respectively. Table 1A: Clones showing up- or down-regulation common in all the three japonica autotetraploids. Table 1B: Clones showing up- or down-regulation common in all the two indica autotetraploids.

Table 1A:

	Sensho	Shinriki	Sen-ichi	Surjamkhi	Dular		BLASTN	BLASTX	
Clones up-regulated							E-value	E-value	chromosome
in 4x japonica	4x / 2x	4x / 2x	4x / 2x	4x / 2x	4x / 2x	Putative identification			
Up <sub>Jp01</sub>	23 ↑	21 ↑	193 ↑	0.4 ↓	0.7	Rice proteinase inhibitor		4e-17	3
Up <sub>Jp02</sub>	29 ↑	24 ↑	36 ↑	1.6	0.8	Rice alcohol dehydrogenase 2	0.00		11
Up <sub>Jp03</sub>	22 ↑	31 ↑	32 ↑	1.3	1.1	Rice alcohol dehydrogenase 1	4e-164		11
Up <sub>Jp04</sub>	24 ↑	32 ↑	28 ↑	1.2	1.1	Rice alcohol dehydrogenase 1	1.0e-109		11
Up <sub>Jp05</sub>	25 ↑	35 ↑	3 ↑	1.3	1	Rice alcohol dehydrogenase 2	3e-174		11
Up <sub>Jp06</sub>	2 ↑	27 ↑	49 ↑	4.3 ↑	1	Unknown protein			12
Clones down-regulated							E-value	E-value	chromosome
in 4x japonica	2x / 4x	2x / 4x	2x / 4x	2x / 4x	2x / 4x	Putative identification			
Down <sub>Jp01</sub>	23 ↓	2 ↓	28 ↓	2.4 ↓	1	Rice chlorophyll a/b binding protein	0.00		3
Down <sub>Jp02</sub>	21 ↓	21 ↓	26 ↓	2.6 ↓	1	Tobacco DNA binding protein ACP		1e-21	4
Down <sub>Jp03</sub>	21 ↓	21 ↓	23 ↓	2.4 ↓	1.3	Rice RuBisCO activase small isoform	0.00		11
Down <sub>Jp04</sub>	23 ↓	21 ↓	26 ↓	2.5 ↓	0.9	Maize cysteine proteinase		1.0e-97	2
Down <sub>Jp05</sub>	21 ↓	22 ↓	26 ↓	1.8	0.8	Rice Rieske iron-sulfur protein	0.00		7
Down <sub>Jp06</sub>	32 ↓	24 ↓	25 ↓	1.2	1.3	Unknown protein			3

Table 1B:

	Sensho	Shinriki	Sen-ichi	Surjamkhi	Dular		BLASTN	BLASTX	
Clones up-regulated							E-value	E-value	chromosome
in 4x indica	4x / 2x	4x / 2x	4x / 2x	4x / 2x	4x / 2x	Putative identification			
Up <sub>Ji01</sub>	0.7	3.6 ↑	1.2	2.1 ↑	2.9 ↑	Wheat histone H2A	2e-49		1 or 3
Up <sub>Ji02</sub>	1.4	1.7	0.7	2.2 ↑	2.4 ↑	Rice leucine-rich repeat protein		2e-19	9
Up <sub>Ji03</sub>	1	7.4 ↑	4.8 ↑	9.1 ↑	2.3 ↑	Maize gibberellin-responsive gene 1a		1e-28	2
Up <sub>Ji04</sub>	0.6	3.4 ↑	1.9	2.6 ↑	2.1 ↑	Wheat histone H2A	2e-101		3
Clones down-regulated							E-value	E-value	chromosome
in 4x indica	2x / 4x	2x / 4x	2x / 4x	2x / 4x	2x / 4x	Putative identification			
Down <sub>Ji01</sub>	1.3	1.4	0.1 ↑	3.8 ↓	2.8 ↓	Rice serpin		1e-12	1
Down <sub>Ji02</sub>	1.2	1	1.3	2.7 ↓	2.8 ↓	Rice pentatricopeptide (PPR) repeat-containing protein		2e-07	12
Down <sub>Ji03</sub>	0.8	1.6	1.2	2 ↓	2.8 ↓	Rice cytochrome P450		3e-04	1
Down <sub>Ji04</sub>	0.7	0.7	0.2 ↑	4.6 ↓	2.7 ↓	Rice mannose-binding lectin		3e-32	1
Down <sub>Ji05</sub>	0.3 ↑	1.4	0.4 ↑	2.1 ↓	2.2 ↓	Unknown protein			
Down <sub>Ji06</sub>	1.6	2.5 ↓	0.9	3 ↓	2.6 ↓	Unknown protein			
Down <sub>Ji07</sub>	1.4	1.1	1	2.4 ↓	3.9 ↓	Unknown protein			
Down <sub>Ji08</sub>	1.7	0.5 ↑	2.8 ↓	2.6 ↓	2.3 ↓	Barley Mglucanase subunit XANTH A-F		8e-28	3
Down <sub>Ji09</sub>	—	2.3 ↓	0.1 ↑	2 ↓	2.8 ↓	Rice class III peroxidase 40		3e-23	3
Down <sub>Ji10</sub>	—	3.4 ↓	—	2.3 ↓	2.1 ↓	Rice tyrosine-specific protein phosphatase		9e-37	6
Down <sub>Ji11</sub>	—	3.3 ↓	1.4	2.3 ↓	3 ↓	Unknown protein			4
Down <sub>Ji12</sub>	—	1.6	0.9	2.3 ↓	2.2 ↓	Rice potassium transporter KUP1		9e-15	4
Down <sub>Ji13</sub>	—	3.5 ↓	2.1 ↓	2.2 ↓	2.5 ↓	Rice alpha/beta fold family-like hydrolase		4e-41	11

and indica sets in the criterion [1]. A BLAST search showed that this down-regulated clone coded homeodomain protein and was located on chromosome 1.

In the criterion [2], we detected 12 clones (0.13% of the microarray clones) showing change of transcriptional level (up-regulated: 6 clones; down-regulated: 6 clones) in the japonica sets, and 23 clones (0.26% of the microarray clones) showing change of transcriptional level (up-regulated: 4 clones; down-regulated: 19 clones) in the indica sets. There is no overlapped clone in the criterion [2]. Table 1 shows the clones detected in the criterion [2], with their putative functions and results of BLAST search. There was no trend in gene function and chromosomal location (Table 1). It is interesting that genes showing expression change in japonica autotetraploids differ from those in indica autopolyploids. It remains to be solved whether this is due to difference of gene dosage response between japonica and indica or due to limited number of lines used in this study.

Getting together, the results obtained in this study suggest that almost none of the genes tested in this study exhibit a gene dosage effect caused by autotetraploidy and that very small number of genes (less than 0.3 % of genes) in rice genome could be involved in the gene dosage.

## References

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