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

N. KISHIMOTO¹, F. FUJII¹, Y. SATO¹, K. TAKEUCHI¹, K. TOYOSHIMA¹, K. SHIMBO², Z. SHIMATANI², Y. NAGATA², A. HASHIMOTO², M. ISHIKAWA¹, J. YAZAKI¹, S. HONDA¹, K. SUZUKI³, K. KOJIMA³, K. YAMAMOTO¹, K. SAKATA¹, T. SASAKI¹ and S. KIKUCHI¹

- 1) National Institute of Agrobiological Sciences (NIAS), Kannondai, Tsukuba, Ibaraki, 305-8602 Japan
- 2) STAFF-Institute, Ippaizuka, Tsukuba, Ibaraki, 305-0854 Japan
- 3) Hitachi Software Engineering, Shinagawa, Tokyo, 140-0002 Japan

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). 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 micorarray 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

87 Research Notes

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	Shinniki	Sen-ichi	Surjamkhi	Dular				
Clone sup-regul	ated						BLASTN	BLASTX	
in 4x japonica	4x / 2x	4x / 2x	4x / 2x	4x / 2x	4x / 2x	Putative identification	E-value	E-value	chromosome
Up_Jp01	23 ↑	21 🕇	19.3 ↑	0.4 \$	0.7	Rice proteinase inhibitor		4e-17	3
Up_Jp02	29 ↑	24 ↑	3.6 ↑	1.6	0.8	Rice alcohol dehydrogenase 2	0.00		11
Up_Jp03	22 ↑	3.1 ↑	3.2 ↑	1.3	1.1	Rice alcohol dehydrogenase 1	4e-164		11
Up_Jp04	24 ↑	32 ↑	28 ↑	1.2	1.1	Rice alcohol dehydrogenase 1	1.0a - 109		11
Up_Jp05	25 ↑	3.5 ↑	3 ↑	1.3	1	Rice alcohol dehydrogenase 2	3e-174		11
up_dp06	2 1	27 ↑	4.9 ↑	4.3 🕇	1	Unknown protein			12
Clone s down -n	-								
in 4x japonica	2x / 4x	2x / 4x	2x / 4x	2x / 4x	2x / 4x	Putative identifi cation	E-value	E-value	chromosome
Down_Jp01	23 ↓	2 ↓	28 ↓	2.4 ↓	1	Rice chlorophyll a/b binding protein	0.00		3
Down_Jp02	21 ↓	21 ↓	26 ↓	26 1	1	Tobacco DNA binding protein ACBF		1e-21	4
Down_Jp03	21 ↓	21 ↓	23 ↓	24 1	1.3	Rice RuBisCO activas e small isoform	0.00		11
Down_ub04	23 ↓	21 ↓	26 ↓	2.5 ↓	0.9	Maize cysteine proteinase		1.0e -97	2
Down_Jp05	21 ↓	22 ↓	26 ↓	1.8	0.8	Rice Rieske iron -sulfur protein	0.00		7
Down_Jp06	32 ↓	24 ↓	25 ↓	1.2	1.3	Unknown protein			3
Table 1B:									
Table ID.	Sensho	Shinriki	Sen-ichi	Surjamkhi	Dular				
Clone up -regula		O I II I I I I	OCH ICH	Cujuliki	Date		BLASTN	BLASTX	
in 4x indica	4x / 2x	4x / 2x	4x / 2x	4x / 2x	4x/2x F	Putative identification	E-value	E-value	chromosome
Up_h01	0.7	3.6 🕇	1.2	21 †		Wheat hist one H2A	2e-49		1 or 3
Up.In02	1.4	1.7	0.7	22 ↑	24 ↑ F			2e-19	9
Up_in02 Up_in03	1.4	1.7 7.4 †	0.7 4.8 †	22 ↑ 9.1 ↑		tice leucine −rich repeat protein Veize gibberellin −responsive gene 1a	1-	2e−19 1e−28	9 2
					23 ↑ 1	Rice leucine -rich repeat protein	2e-101		
UpJh03	1	7.4 🕇	4.8 🕇	9.1 ↑	23 ↑ 1	ice leucine -rich repeat protein Maize gibbereilin -responsive gene 1a			2
UpJh03	0.6	7.4 🕇	4.8 🕇	9.1 ↑	23 ↑ 1	ice leucine -rich repeat protein Maize gibbereilin -responsive gene 1a			2
Up_ln03 Up_ln04	0.6	7.4 🕇	4.8 🕇	9.1 ↑	23 ↑ I 21 ↑ \	ice leucine -rich repeat protein Maize gibbereilin -responsive gene 1a	2e-101	1e-28	2
Up_in03 Up_in04 Clone downreg	1 0.6 gulated	7.4 ↑ 3.4 ↑	4.8 † 1.9	9.1 ↑ 2.8 ↑	23 ↑ F 21 ↑ V 2x/4x F	Roe Isucine — rich repeat protein Maize gibberellin — responsive gene 1a Wheat histone H2A	26-101 BLASTN	1e-28 BLASTX	2 3
Up_In03 Up_In04 Clone down -reg in 4x indica	1 0.6 uuleted 2x / 4x	7.4 ↑ 3.4 ↑	4.8 † 1.9 2x / 4x	9.1 ↑ 2.6 ↑ 2x/4x	23 ↑ ↑ 21 ↑ ↑ 2x/4x F 28 ↓ F	Rice (sucine - rich repeat protein Maize gibberellin - responsive gene 1a Mheat histone H2A Putative identification	26-101 BLASTN	1e-28 BLASTX E-value	2 3 chromosome
Up_in03 Up_in04 Clone down = reg in 4x indica Down_in01	1 0.6 x./sted 2x / 4x 1.3	7.4 ↑ 3.4 ↑ 2x / 4x 1.4	4.8 † 1.9 2x / 4x 0.1 †	9.1 ↑ 2.6 ↑ 2.x / 4x 3.8 ↓	23 ↑ ↑ 21 ↑ ↑ 2x/4x F 28 ↓ F	Noe leucine — rich repeat protein Maize gibberellin — responsive gene 1a Wheat histone H2A Putative identification Noe serpin	26-101 BLASTN	1e-28 BLASTX E-value 1e-12	2 3 chromosome 1 12
Up_in03 Up_in04 Clone down = reg in 4x indica Down_in01	1 0.6 x./sted 2x / 4x 1.3	7.4 ↑ 3.4 ↑ 2x / 4x 1.4	4.8 † 1.9 2x / 4x 0.1 †	9.1 ↑ 2.6 ↑ 2.x / 4x 3.8 ↓	23 ↑ ↑ 21 ↑ \ 2x/4x	Noe leucine —rich repeat protein Maize gibberellin —responsive gene 1a Wheat histone H2A Putative identification Noe serpin Rice pentatricopeptide (PPR)	26-101 BLASTN	1e-28 BLASTX E-value 1e-12 2e-07	2 3 chromosome 1 12
Up.h03 Up.h04 Clone down —reg in 4x indica Down.h01 Down.h02	1 0.6 2x / 4x 1.3 1.2	7.4 ↑ 3.4 ↑ 2x / 4x 1.4	4.8 † 1.9	9.1 ↑ 2.6 ↑ 2x / 4x 3.8 ↓ 2.7 ↓	23 ↑ ↑ 21 ↑ \ 2x/4x	Noe leucine —rich repeat protein Maize gibberellin —responsive gene 1a Wheat histone H2A Putative identification Noe serpin Rice pentatricopeptide (PPR) repeat —containing protein	26-101 BLASTN	BLASTX E-value 1e-12 2e-07 (Identities	2 3 chromosome 1 12 946)
Up.h03 Up.h04 Clone down —reg in 4x indica Down.h01 Down.h02	1 0.6 2x / 4x 1.3 1.2	7.4 ↑ 3.4 ↑ 2x / 4x 1.4	4.8 † 1.9	9.1 ↑ 2.6 ↑ 2x / 4x 3.8 ↓ 2.7 ↓	23 ↑ ↑ 1 21 ↑ ↑ 2x / 4x	Noe leucine —rich repeat protein Maize gibberellin —responsive gene 1a Wheat histone H2A Putative identification Noe serpin Rice pentatricopeptide (PPR) repeat —containing protein	26-101 BLASTN	BLASTX E-veius 1e-12 2e-07 (identities 3e-04	2 3 chromosome 1 12 946)
Up_h03 Up_h04 Clone down -reg in 4x indica Down_h01 Down_h02 Down_h03	1 0.6 nuinted 2x / 4x 1.3 1.2	7.4 ↑ 3.4 ↑ 2x / 4x 1.4 1	48 † 1.9 2x / 4x 0.1 † 1.3	9.1 ↑ 2.6 ↑ 2x/4x 3.8 ↓ 2.7 ↓ 2 ↓	23 ↑ ↑ ↑ 21 ↑ ↑ 2x/4x	Noe leucine —rich repeat protein Malze gibberellin —responsive gene 1a Minest historie HZA Putative identification Noe serpin Rice pentatrioopeptide (PPR) repeat —containing protein Noe cytochrome P450	26-101 BLASTN	BLASTX E-value 1e-12 2e-07 (Identities and a constitution of the c	2 3 chromosome 1 12 94%)
Up_in03 Up_in04 Clone downreg in 4x indox Down_in01 Down_in02 Down_in03 Down_in04	1 0.6 2x / 4x 1.3 1.2 0.8	7.4 ↑ 3.4 ↑ 2x / 4x 1.4 1 1.6	48 † 1.9 2x / 4x 0.1 † 1.3 1.2	9.1 ↑ 2.6 ↑ 2.7 4x 3.8 ↓ 2.7 ↓ 2 ↓ 4.6 ↓	23 ↑ ↑ 1 21 ↑ 1 2x/4x	Noe leucine —rich repeat protein Malze gibberellin —responsive gene 1a Minest historie HZA Putative identification Noe serpin Rice pentatrioopeptide (PPR) repeat —containing protein Noe cytochrome P450	26-101 BLASTN	BLASTX E-value 1e-12 2e-07 (Identities and a constitution of the c	2 3 chromosome 1 12 94%)
Up.jn03 Up.jn04 Clone downreg in 4x indica Down.jn01 Down.jn02 Down.jn03 Down.jn04 Down.jn06	1 0.6	7.4 ↑ 3.4 ↑ 2x / 4x 1.4 1 1.6 0.7 1.4	2x / 4x 0.1 1 1.3 1.2 0.2 1 0.4 1	2x / 4x 38 \$\dag{1}\$ 27 \$\dag{1}\$ 48 \$\dag{1}\$ 21 \$\dag{1}\$	2x / 4x 1 2x / 4x 1 2x 4 1 2x	Noe leucine —rich repeat protein Malze gibberellin —responsive gene 1a Minest histone HZA Putative identification Noe serpin Rice serpin repeat —containing protein Noe optochrome P450 Noe mannose —binding lectin	26-101 BLASTN	BLASTX E-value 1e-12 2e-07 (Identities and a constitution of the c	2 3 chromosome 1 12 94%)
Up_in03 Up_in04 Clone downreg in 4x indica Down_in01 Down_in02 Down_in03 Down_in04 Down_in05 Down_in06	1 06 2x/4x 1.3 1.2 08 0.7 0.3 1 1.6	74 ↑ 34 ↑ 2x / 4x 14 1 16 07 14 25 ↓	2x / 4x 01 1 13 12 02 1 04 1 09	2x / 4x 3.6 \(\preceq 2 \) 2x / 4x 4.6 \(\preceq 2 \) 21 \(\preceq 4.6 \) \(\preceq 2.1 \) \(\preceq 3.1 \) \(\pre	2x /4x 1 2x /4x 1 2x 1 2	No leucine —rich repeat protein Malze gibberellin —responsive gene 1a Minest histone HZA Putative identification Noe serpin Rice pertatricopeptide (PPR) repeat —containing protein Noe optochrome P450 Noe mannose —binding lectin Jirknown protein	26-101 BLASTN	BLASTX E-value 1e-12 2e-07 (Identities and a constraints)	2 3 chromosome 1 12 94%)
Up_in03 Up_in04 Clone downreg in 4x indica Down_in01 Down_in02 Down_in03 Down_in04 Down_in05 Down_in06 Down_in06 Down_in07	1 06 2x/4x 1.3 1.2 08 0.7 0.3 1 1.6 1.4	7.4 ↑ 3.4 ↑ 2x / 4x 1.4 1.6 0.7 1.4 2.5 ↓ 1.1 0.5 ↑ 2.3 ↓	2x / 4x 01 1 13 12 02 1 04 1 09 1 28 1 01 1	2x / 4x 38 \$\frac{1}{2}\$ 27 \$\frac{1}{4}\$ 45 \$\frac{1}{2}\$ 21 \$\frac{1}{4}\$ 22 \$\frac{1}{4}\$ 22 \$\frac{1}{4}\$ 22 \$\frac{1}{4}\$ 24 \$\frac{1}{4}\$ 25 \$\frac{1}{4}	2x / 4x 1 2x / 4x 1 2x 1 1 2	No leucine —rich repeat protein Malze gibberellin —responsive gene 1a Minest histone HZA Putative identification No serpin Rice serpin repeat —containing protein Nos entrome P450 No mannose —binding lectin Jirknown protein	26-101 BLASTN	BLASTX E-value 1e-12 2e-07 (Identities: 3e-04 (Identities: 3e-32	2 3 chromosome 1 12 9460 1
Up_in03 Up_in04 Clone downreg in 4x indica Down_in01 Down_in02 Down_in03 Down_in04 Down_in06 Down_in06 Down_in07 Down_in08 Down_in08 Down_in08 Down_in08 Down_in08	1 06 2x/4x 1.3 1.2 08 0.7 0.3 1 1.6 1.4	7.4 ↑ 3.4 ↑ 2x / 4x 1.4 1.6 0.7 1.4 2.5 ↓ 1.1 0.5 ↑ 2.3 ↓ 3.4 ↓	2x / 4x 01 1 13 12 02 1 04 1 09 1 28 1 01 1	2x/4x 38 ↓ 27 ↓ 2 ↓ 46 ↓ 21 ↓ 3 ↓ 24 ↓ 26 ↓ 2 ↓ 23 ↓	2x / 4x 1 28 1 1 28 1 1 22 1 1 22 1 1 23 1 1 23 1 1 24 1 1 1 1 1 1 1 1 1	No leucine —rich repeat protein Melze gibberellin —responsive gene 1a Minest histone HZA Autative identification Noe serpin Rice serpin Rice pentatricopeptide (PPR) repeat —containing protein Rice cytochrome P450 Nice mannose —binding lectin Anknown protein Harlown protein Barley Mg —chelatase subunit XANTH —A—F Rice class III peroxidase 40 Rice tyrosine —specific protein phosphatase	26-101 BLASTN	BLASTX E-value 1e-12 2e-07 (identities 3e-04 (identities 3e-32	2 3 chromosome 1 12 9460 1 1 9460
Up_in03 Up_in04 Clone downreg in 4x indica Down_in01 Down_in02 Down_in03 Down_in04 Down_in06 Down_in06 Down_in07 Down_in08 Down_in09 Down_in09 Down_in10 Down_in10	1 06 2x/4x 1.3 1.2 08 0.7 0.3 1 1.6 1.4	7.4 ↑ 3.4 ↑ 2x / 4x 1.4 1 1.6 0.7 1.4 2.5 ↓ 1.1 0.5 ↑ 2.3 ↓ 3.4 ↓ 3.3 ↓	2x / 4x 01 1 13 12 02 1 04 1 09 1 28 1 01 1 — 14	2x / 4x 38 ↓ 27 ↓ 2 ↓ 46 ↓ 21 ↓ 3 ↓ 24 ↓ 26 ↓ 2 ↓ 23 ↓ 23 ↓	2x / 4x 1 28 1 1 28 1 1 22 1 1 22 1 1 23 1 1 23 1 1 23 1 1 23 1 1 23 1 1 23 1 1 3 1 1 3 1 1 3 1 1	No leucine —rich repeat protein Meize gibberellin —responsive gene 1a Minest histone HZA Autative identification No serpin Rice serpin Rice pentatrioopeptide (PPR) repeat —containing protein Rice cytochrome P450 Rice mannose —binding lectin Anknown protein Barley Mg —chelatase subunit XANTH —A—F Rice class III percoidase 40 Rice tyrosine —specific protein phosphatase	26-101 BLASTN	BLASTX E-value 1e-12 2e-07 (identities 3e-04 (identities 3e-32 8e-28 3e-23 9e-37	2 3 chromosome 1 12 9460 1 3 3 3 6 4
Up_in03 Up_in04 Clone downreg in 4x indica Down_in01 Down_in02 Down_in03 Down_in04 Down_in06 Down_in06 Down_in07 Down_in08 Down_in08 Down_in08 Down_in08 Down_in08	1 06 2x/4x 1.3 1.2 08 0.7 0.3 1 1.6 1.4	7.4 ↑ 3.4 ↑ 2x / 4x 1.4 1.6 0.7 1.4 2.5 ↓ 1.1 0.5 ↑ 2.3 ↓ 3.4 ↓	2x / 4x 01 1 13 12 02 1 04 1 09 1 28 1 01 1	2x/4x 38 ↓ 27 ↓ 2 ↓ 46 ↓ 21 ↓ 3 ↓ 24 ↓ 26 ↓ 2 ↓ 23 ↓	2x / 4x 1 28 1 1 28 1 1 22 1 1 23 1 1 23 1 1 24 1 1 1 1 1 1 1 1 1	No leucine —rich repeat protein Melze gibberellin —responsive gene 1a Minest histone HZA Autative identification Noe serpin Rice serpin Rice pentatricopeptide (PPR) repeat —containing protein Rice cytochrome P450 Nice mannose —binding lectin Anknown protein Harlown protein Barley Mg —chelatase subunit XANTH —A—F Rice class III peroxidase 40 Rice tyrosine —specific protein phosphatase	26-101 BLASTN	BLASTX E-value 1e-12 2e-07 (identities 3e-04 (identities 3e-32	2 3 chromosome 1 12 9460 1 3 3 3

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 autoploids. 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

Yazaki, J., N. Kishimoto, Y. Nagata, M. Ishikawa, F. Fujii, A. Hashimoto, K. Shimbo, Z. Shimatani, K. Kojima, K. Suzuki, M. Yamamoto, S. Honda, A. Endo, Y. Yoshida, Y. Sato, K. Takeuchi, K. Toyoshima, C. Miyamoto, J. Wu, T. Sasaki, K. Sakata, K. Yamamoto, K. Iba, T. Oda, Y. Otomo, K. Murakami, K. Matsubara, J. Kawai, P. Carninci, Y. Hayashizaki and S. Kikuchi, 2003. Genomics approach to abscisic acid- and gibberellin-responsive genes in rice. DNA Res. 10: 249-261.