35. Neighboring nucleotide bias around MNU-induced mutations in rice.

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N-methyl-*N*-nitrosourea (MNU) is one of alkylating agents and has been used as a chemical mutagen to generate mutant populations for genetic studies in rice (Kurata et al., 2005). Since MNU is thought to be able to induce mutations in every gene, MNU-induced mutants are expected to contribute functional genomics in rice (Suzuki et al., 2007). Alkylating agents are known to add alkyl group to guanine residue, yielding O^6-alkylguanine that induces frequently G to A or C to T transition mutations on replication. The mutated G residue by MNU frequently follows a purine residue (Shioyama et al., 2000). Therefore, mutagenesis with MNU in rice would show biases depending on local sequence compositions, although MNU-induced mutations are thought to disperse evenly in all genomic regions. In this study, the sequence compositional biases in MNU-mutagenesis were deduced from sequences around MNU-induced mutations in *Oryza sativa*.

To date, 54 mutations have been obtained for a total of 9.9 kb genomic regions for six genes in our rice TILLING project using MNU-mutagenized population of japonica rice Taichung 65. Of these mutations, 51 were transitions of G to A or C to T, two were A to G transitions, and the other was A to T transversion. Expected frequencies for each of four nucleotides at adjacent positions from 51 mutated G residues were estimated by a formula, (number of that nucleotide at a particular position from mutated G available in the reference fragments) x 51 ÷ (number of all available G), and then ratios of the observed frequencies to the expected frequencies were calculated (Table 1). The results revealed local biases due to neighboring nucleotides around the mutated G residues in MNU-mutagenesis. In the -1 nucleotide from the mutated G, strong purine bias was detected ($P < 10^{-6}$), while no significant difference was detected in the +1. Approximately 88% of G/C to A/T substitutions were detected in the middle nucleotide of 5'-purine-G-N-3' sequences. This bias was the same as reported previously in other organisms (Richardson and Richardson, 1990; Shioyama et al., 2000). Interestingly, weaker but still significant biases were detected at +2 and +3 positions ($P < 0.05$), but no biases were at -2 and -3. At +2, purines were more frequent than pyrimidines, and at +3, G residues frequently appeared and pyrimidines less frequently.

These results suggested that the MNU-induced mutations might preferentially occur at the second residues of 5'-purine-G-N-purine-(G or not-pyrimidine)-3' quintuplets, supporting a hypothesis for EMS-mutagenesis in *Arabidopsis* that the neighboring bases would influence the ability to repair chemically induced lesions (Greene et al., 2003). However, the neighboring nucleotide biases in MNU-mutagenesis were different from those in *Arabidopsis* EMS-mutagenesis, in which the mutated Gs were at the middle of 5'-(C or not-G)-purine-G-purine-(G or not-C)-3' sequences. Although further studies are necessary to understand mechanisms of alkylation adducts removal and mismatch repair in rice, it is suggested that sequences favoured by the machinery of damage and mismatch repair might be different between rice and *Arabidopsis*, or that molecules involved in the alkylation damage repair might be different between for methylguanine and for ethylguanine.

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Table 1 Ratios of observed/expected frequencies on either side of the mutated G.

	-4	-3	-2	-1	0	+1	+2	+3	+4
A	1.4	1.3	1.4	1.7		1.0	1.6	1.1	0.8
C	0.7	0.9	0.8	0.3		1.2	0.6	0.5	1.4
G	1.1	1.0	1.2	1.9		0.9	1.4	1.5	0.8
T	0.8	0.8	0.6	0.2		0.8	0.4	0.5	1.0
P	0.33	0.79	0.19	$< 10^{-6}$		0.68	0.01	0.01	0.36

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