

Exome resequencing reveals mutations in rice induced by heavy-ion beam with LETmax

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Heavy-ion beam irradiation induces mutations at a high rate without severely inhibiting growth at a relatively low dose. Heavy-ion beam with high linear energy transfer (LET) cause greater biological effects than low-LET radiation such as gamma rays and X-rays. LET can be controlled by adjusting the speed of the ions or choosing appropriate ion species. We found that the highest mutation rate is obtained at an LET of 30 keV/ μ m in *Arabidopsis thaliana*¹⁾. This high-efficiency LET (termed LETmax) mostly induced small deletions²⁾. In rice, the LETmax value was determined as 50-70 keV/ μ m. However, the mutations induced in rice when we irradiated heavy-ion beams with LETmax were unclear. We performed exome-resequencing analysis to reveal the nature of the mutations in rice induced by heavy-ion beams with LETmax in rice.

We selected seven rice (*Oryza sativa* L. cv. Nipponbare) mutants induced by LETmax irradiation to perform exome-resequencing analysis. Paired-end libraries were constructed using

SeqCap EZ developer library (Roche). Sequencing was performed using Hiseq 2000 (Illumina). Mutations were detected by using SAMtools and BEDTools software. We detected 16 deletions and 14 base substitutions among seven mutants (Table 1). Of the 16 deletions, 14 were small deletions (1-18 bp) and two were large deletions (739 bp and 102158 bp). Of the 16 deletions, 14 were located in coding regions. Two were located in the 5' untranslated region and intron. Of the 14 base substitutions, 9 induced alteration of the amino-acid sequence of each gene, such as missense mutation, nonsense mutation, and START gain mutation. Four base substitutions were located in 3' untranslated region. The one remain was a silent mutation. Of the 14 deletions located in coding regions, 1-18 bp deletions constituted 85.7% (12/14) of the total deletions. These findings indicate that heavy-ion beams with LETmax induce small deletions suitable for single-gene disruption in rice.

Table 1. Homozygous deletion and base substitution detected by Exome resequencing

Line	Ion / Dose / LET (Gy) (keV/ μ m)	Deletion		Base substitution	
		size (bp)	Remarks	change base	Remarks
3-14	C / 15 / 50	1*		A→G*	Missense mutation (K/R)
		1*			
5-12	C / 15 / 50	1*		A→G*	Missense mutation (Q/R)
		10	intron		
		18*			
4-13	C / 15 / 60	1*		A→T	3'UTR
		9*			
7-3B	Ne / 10 / 63	2+1*	Filler DNA	G→T*	START gain mutation
		3	5' UTR	T→G	Silent mutation
		739*	disruption of 2 genes	G→T*	Nonsense mutation
				C→T*	Missense mutation (G/S)
6-62	Ne / 15 / 63	1*		G→T*	Missense mutation (Q/K)
		1*		C→A*	Missense mutation (P/T)
		13*		A→T	3'UTR
		102158*	disruption of 7 genes		
Ne-1779	Ne / 15 / 63	14*		T→A*	Missense mutation (L/H)
				T→A*	Nonsense mutation
7-30	Ne / 15 / 70	12+1*	Filler DNA	C→T	3'UTR
				C→T	3'UTR

* Mutations that change the amino-acid sequences

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References

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