Relationship between gene expression level and LET immediately after heavy-ion beam irradiation in rice

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In plant mutagenesis by heavy-ion beam irradiation, linear energy transfer (LET) is one of the most important factors that determines the mutation rate. The most effective linear energy transfer (LETmax) has been defined and reported for *Arabidopsis thaliana*[1] and rice.[2] In wet seeds of rice, the mutation rate was low at low LET (22.5 keV/μm) and high at LETmax (50−70 keV/μm).[2] The relationship between the mutation rate and LET curved sigmoidally, however, the reason is still unknown. In this study, we tried to reveal the relationship between the gene expression level and LET after heavy-ion beam irradiation and to show whether the change of gene expression corresponds to that of the mutation rate.

Wet seeds of rice (*Oryza sativa* L. cv. Nipponbare) were imbibed for three days and used as samples. Heavy-ion beam irradiation (C-ions for LETs of 22.5 and 50 keV/μm and Ne-ions for LETs of 63 and 80 keV/μm), RNA extraction, and gene expression analysis were conducted as previously described.[3] All the analyses in this study were conducted on the samples sampled two hours after irradiation.

Out of 45,221 probe sets, 81 probe sets showed significantly ($p < 0.01$) higher expression levels when irradiated at all LETs (22.5, 50, 63, and 80 keV/μm) than when not irradiated (control). These probe sets contained the homologs of *Rad51*, *Rad21/Rec8*, and *Artemis*, which are the genes involved in DNA double-strand break repair. It was shown that the gene expression analysis successfully reflected the immediate response to the heavy-ion beam irradiation.

It was found that 1575 probe sets showed significantly ($p < 0.05$) higher expression levels when irradiated at 63 or 80 keV/μm of LET than when not irradiated. On each probe set, the relationship between the expression level and LET was fitted to both the linear and sigmoid functions (Fig. 1).

Moreover, 826 probe sets were significantly ($R^2 \geq 0.8$) fitted to either function. Among these 826 probe sets, the relationships between the expression level and LET of 594 probe sets (72%) were fitted better to linear function, whereas those of the remaining 232 probe sets (28%) were fitted better to sigmoid function [Table 1]. In these 232 probe sets, the relationships between the expression level and LET of only three probe sets (0.4%) were coincident with the relationship between the mutation rate and LET.

This is the first observation of the relationship between the gene expression level and LET immediately after heavy-ion beam irradiation. On almost all the researched genes, the relationship between the gene expression level and LET was not coincident with that between the mutation rate and LET. The three exceptional genes can be used as the indicators of LETmax.
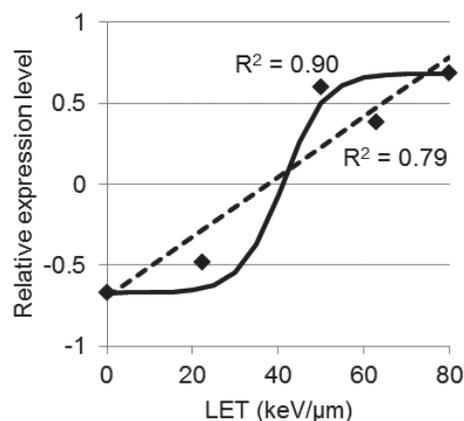

Fig. 1. Example of curve fitting of the relationship between expression level and LET. Squares indicate the measured values of a typical probe set. Line and dashed line indicate sigmoidal and linear regression curves, respectively.

Table 1. Classification of probe sets based on the nature of relationship between their expression level and LET.

Classification	Probe set (number)			
	80 keV/μm > ctrl.*	63 keV/μm > ctrl.**	Subtotal	(%)
Fitted to linear function	559	35	594	72
Fitted to sigmoid function	197	35	232	28
LETmax > 22.5 keV/μm†	(2)	(1)	(3)	
Sum total	756	70	826	

*Expression level when irradiated at 80 keV/μm was significantly higher than that of control.
**Expression level was not significantly higher than control when irradiated at 80 keV/μm but significantly higher when irradiated at 63 keV/μm.
†Expression levels when irradiated at 50 and 63 keV/μm were significantly higher than that when irradiated at 22.5 keV/μm.

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