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Loss-of-function and Gain-of-function Rice Mutants from Gamma-Ray Mutagenesis

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Gamma-ray irradiation is known to induce various mutations in plants caused by chromosome alterations. This study investigated disease responses of selected gamma-ray induced rice mutants generated from seven *Japonica*-type rice cultivars against three plant diseases. Among the tested 22 mutants, three gain-of-function mutants and six loss-of-function mutants against rice blast were obtained, as well as three loss-of-function mutants against bacterial leaf blight (BLB). Two of the loss-of-function mutants were susceptible to both rice blast and BLB. Gain-of-function mutation has not been frequently observed in rice plants, thus, the mutants can be used to identify loci of novel genes for the regulation of disease resistant response.

Keywords **:** Gain-of-function, gamma-ray, loss-of-function, rice mutants.

Mutagenesis is an important tool for the identification of plant genes and the understanding of plant gene function. Therefore, the plant functional genomic approach takes advantage of the massive generation of mutant pools. Chemical mutagenesis has been used to generate point mutations, and the mutants have been frequently used for genetic studies for subsequent map-based cloning of several plant genes (Bent et al., 1994). It has been known that chemical agents and radiation can cause various types of chromosomal DNA alteration in plants, such as deletion, point mutation, and inversion (Shirley et al., 1992). However, the process that occurs during chemical and physical mutagenesis and the cellular mechanism for the various types of mutation are not fully understood yet. Recently, a fast neutron deletion mutagenesis-based reverse genetic system has been developed in *Arabidopsis* (Li et al., 2001). Analysis of the fast neutron bombardment mutants revealed that there are several random deletions in each line.

In rice, a T-DNA insertional mutagenesis method has also been developed for functional genomic studies (Jeon et al., 2000; Jeong et al., 2002). T-DNA tagging is advantageous in identifying the chromosomal loci responsible for mutated phenotype. However, it may take long for the saturation mutagenesis of the whole rice genome, which is much larger than that of *Arabidopsis* (Sessions et al., 2002). Gamma-ray irradiation has been a general method in generating various mutations in plants, and the mutant plants have been often used for crop breeding program and classical plant genetic studies. Since rice draft genome sequences are available and the rice genome map is comprehensive (Chen et al., 2002; Goff et al., 2002; Wu et al., 2002; Yu et al., 2002), gamma-ray induced deletion mutants would be valuable as future functional genomic tools for rice plants.

Rice blast caused by *Magnaporthe grisea*, rice bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae*, and rice sheath blight (RSB) caused by *Corticium sasaki* are important rice diseases. It is expected that gamma-ray mutagenesis could cause many variations in the disease responses of rice plants. Therefore, this study investigated the alterations in disease responses of rice gamma-ray mutants using the above-mentioned three plant diseases. Gamma-ray irradiated mutants from seven *Japonica*-type rice cultivars were previously generated at the Korea Atomic Energy Research Institute. Mutants with alteration in quantitative traits were selected and several generations were created to fix the mutated phenotypes. In this study, seven wild type cultivars and their 22 selected mutants were used to evaluate the changes in the response to three plant diseases.

Rice cultivars (*Oryza sativa* L.) used in this study are all *Japonica*-type cultivars, such as Hwaseongbyeo, Chucheongbyeo, Seomjinbyeo, Suwon 345, Ilpumbyeo, Jakwangdo, Yongkeumbyeo, and Nagdongbyeo. Disease responses of these cultivars are variable according to diseases including rice blast and BLB, while all of them are susceptible to RSB. The gamma-ray irradiated and further selected mutants used in this study are listed in Table 1. The rice plants were routinely grown in a temperature-controlled glasshouse with natural lighting before evaluation for disease resistance. *M. grisea* (KJ-201), *X. oryzae* pv. *oryzae*

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Table 1. Rice cultivars and their selected mutants used in this study

No	Wild types	Mutants	No	Wild types	Mutants
1	Hwaseongbyeo		16	Suwon 345	Wonnong 9
2	Hwaseongbyeo	Wonpyungbyeo	17	Ilpumbyeo	
3	Chucheongbyeo		18	Ilpumbyeo	IPM-2503
4	Chucheongbyeo	Wonmibyeo	19	Ilpumbyeo	IPM-2506
5	Chucheongbyeo	Wonnong 6	20	Ilpumbyeo	IPM-3002
6	Chucheongbyeo	Wonnong 7-1	21	Ilpumbyeo	IPM-3008
7	Chucheongbyeo	Wonnong 7-2	22	Jakwangdo	
8	Chucheongbyeo	Wonnong 7-3	23	Jakwangdo	Wonnong 13
9	Chucheongbyeo	Wonnong 7-6	24	Jakwangdo	Wonnong 14 (R)
10	Seomjinbyeo		25	Jakwangdo	Wonnong 14 (W)
11	Seomjinbyeo	Wonnong 2	26	Yongkeumbyeo	
12	Seomjinbyeo	Wonkwangbyeo	27	Yongkeumbyeo	YKM-1009
13	Seomjinbyeo	Wonnong 4	28	Yongkeumbyeo	YKM-1501
14	Suwon 345		29	Yongkeumbyeo	YKM-2004
15	Suwon 345	Wonnong 8	30	Nagdongbyeo	

KXO85, and a strain of *C. sasaki* were used in this study to inoculate the rice plants as pathogens causing rice blast, BLB and RSB, respectively.

The *M. grisea* and *C. sasaki* fungal inoculum preparation and inoculation procedure followed that of Kim et al. (2001). Disease resistance to rice blast was scored by the absence of lesion and appearance of hypersensitive response spot on the sprayed leaves. Disease development was apparent by the lesion formation and leaf tissue collapse. Finally, the percentage of infected leaf area and infected sheath area were determined to estimate the responses of rice cultivars to rice blast and RSB, respectively. Bacterial inoculum for *X. oryzae* pv. *oryzae* KXO85 was prepared to have a cell density of approximately 10^8 cfu/ml, and inoculated on 4-week-old rice seedlings by the standard leaf clipping method (Kauffman et al., 1973). Disease incidence was scored by measuring the length of leaf blight lesion after maintaining the plants in an 80% relative humidity room for 5 days.

Screening the mutants for disease responses revealed that various alteration in the biotic stress response occurred in the gamma-ray rice mutants (Fig. 1). In the rice blast responses of the mutants, two of Chucheongbyeo mutants (Wonmibyeo and Wonnong 6) appeared to be resistant to the tested *M. grisea* strain, while the wild type Chucheongbyeo was highly susceptible to the disease. It is likely that Wonmibyeo is moderately resistant to rice blast (Fig. 1A). This gain-of-resistance in these mutants was limited in the rice blast response. Susceptibility of the wild type to RSB and BLB did not change in the rice blast resistant mutants (Fig. 1B). One of the Yongkeumbyeo mutants, YKM-1009, was also a gain-of-function type mutant to rice blast, while the wild type Yongkeumbyeo was susceptible to the rice blast. The mutant YKM-1009 had the same response to BLB and RSB with the wild type. There were six loss-of-function mutants against the tested *M. grisea* strain, while the wild types were resistant to the disease. These include the following, with their wild type in parentheses: Wonpyungbyeo (Hwaseongbyeo); Wonnong 2 and Wonnong 4 (Seomjinbyeo); and IPM-2506, IPM-3002, and IPM-3008 (Ilpumbyeo). Two of them (Wonnong 2 and Wonnong 4) were loss-of-function mutants to both rice blast and BLB. In the BLB responses of the mutants, there were three loss-of-function mutants including Wonnong 2 and Wonnong 4 as mentioned above. Interestingly, one of Seomninbyeo mutants, Wonkwangbyeo, was susceptible to BLB but resistant to rice blast, in contrast with the wild type Seomjinbyeo which was resistant to both diseases. Therefore, there might be a mutation in the locus specifically controlling the resistance to BLB but not to rice blast in the mutant Wonkwangbyeo. Among the tested mutants, no gain-of-function mutants were found against BLB and RSB. The gain-of-function mutants are interesting because those types are not frequently described in DNA insertion mutants. Furthermore, gain-of-function mutation is specific to rice blast but neither to BLB nor to RSB. It will be very interesting to define the loci responsible for the specific gain-of-function.

There have been some reports of the gain-of-function mutants generated from chemical or physical mutagenesis (Frye and Innes, 1998; Rate et al., 1999; Shirano et al., 2002; Vogel and Somerville, 2000). An *Arabidopsis* mutant, *edr1*, is a gamma-ray irradiated gain-of-function mutant resistant to *Erysiphe cichoracearum* causing powdery mildew. Expression of PR genes in the mutant was not constitutive, which was different from other previously

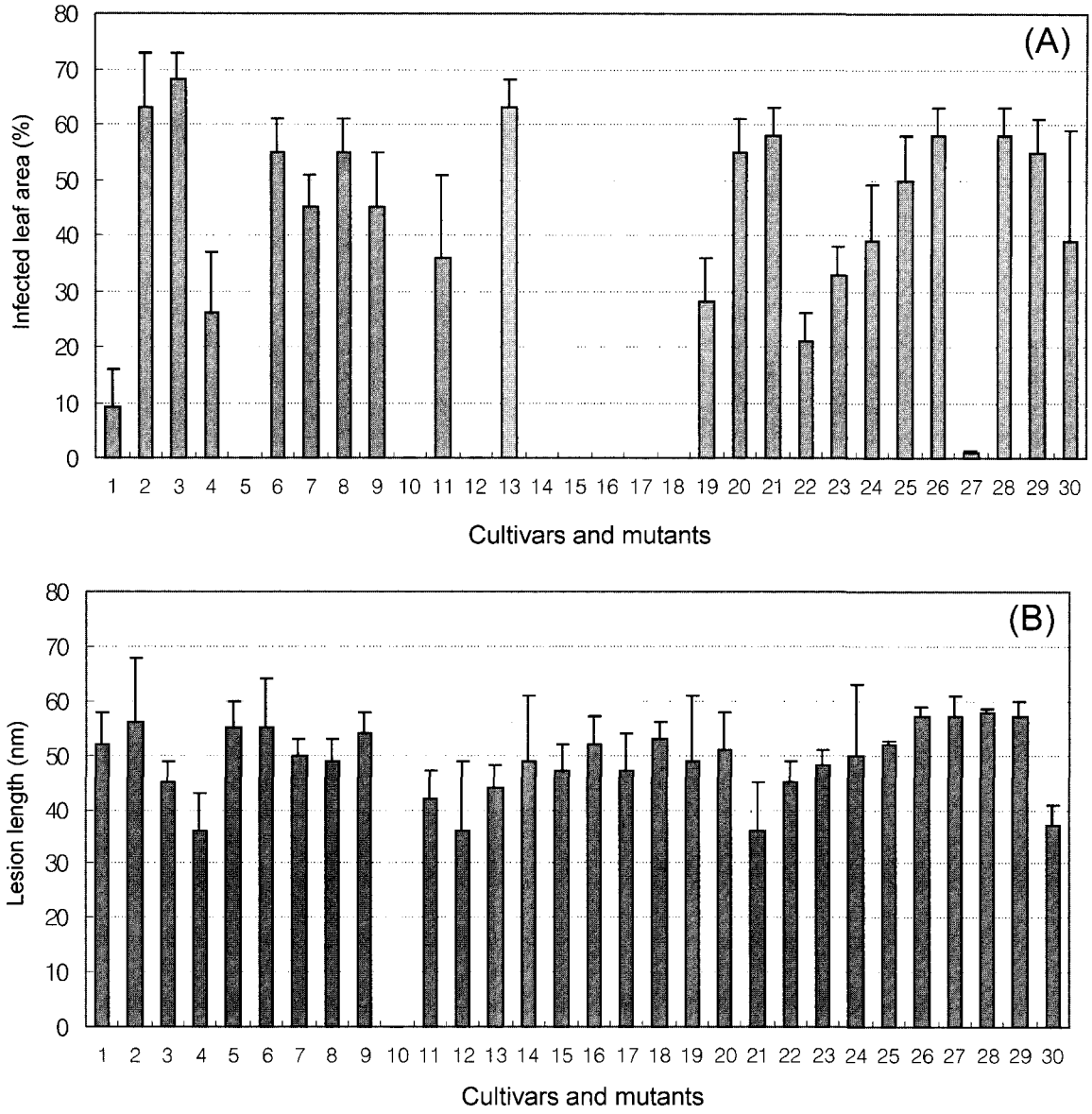

Fig. 1. Rice plant responses of rice cultivars and their mutants against rice blast (**A**) and bacterial leaf blight (**B**). Standard deviations from three replications are indicated above each bar. Rice plants used in this study are listed in Table 1.

described gain-of-function mutants (Frye and Innes, 1998). Expression of PR genes in the gain-of-function mutants in this study has not been analyzed yet. Characterization of defense-related gene expression and identification of the mutated loci in these gain-of-function mutants would provide valuable information on rice disease resistance gene expression.

This study described rice mutants generated from gamma-ray irradiation with altered disease responses, and suggested that gamma-ray mutants could be important materials for rice genetic study to identify novel genetic loci involved in disease resistance signaling. Gamma-ray mutagenesis would also be a good alternative for functional genomic study in rice to supplement the T-DNA tagging mutagenesis. Mapping the mutated loci in the mutants could facilitate the isolation of chromosomal gene responsible for mutant phenotype, since rice gene mapping is highly comprehensive and rice genome draft sequence is publicly available now.

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