A TILLING Rice Population Induced by Gamma-ray Irradiation and its Genetic Diversity

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Abstract : TILLING (Targeting Induced Local Lesions IN Genomes) is broadly regarded as an excellent methodology for reverse genetics applications. Approximately 15,000 M₃ TILLING lines have been developed via the application of gamma-ray irradiation to rice seeds (cv. Donganbyeo), followed by subsequent selections. In an effort to evaluate the genetic diversity of the TILLING population, we have employed the AFLP multiple dominant marker technique. A total of 96 (0.64%) TILLING lines as well as Donganbyeo were selected randomly and their genetic diversity was assessed based on AFLP marker polymorphisms using 5 primer combinations. An average of 100.4 loci in a range of 97 to 106 was detected using these primer combinations, yielding a total of 158 (31.4%) polymorphic loci between Donganbyeo and each of the 96 lines. A broad range of similarity from 80% to 96% with an average of 89.4% between Donganbyeo and each of the 96 lines was also observed, reflecting the genetic diversity of the TILLING population. Approximately 28 polymorphic loci have been cloned and their sequences were BLAST-searched against rice whole genome sequences, resulting in 20 matches to each of the gene bodies including exon, intron, 1 kb upstream and 1 kb downstream regions. Six polymorphic loci evidenced changes in the coding regions of genes as compared to the rice pseudomolecules, 4 loci of which exhibited missense mutations and 2 loci of which exhibited silent mutations. Therefore, the results of our study show that the TILLING rice population should prove to be a useful genetic material pool for functional genomics as well as mutation breeding applications.

Key words : Functional genomics, Gamma ray irradiation, Genetic diversity, Rice, TILLING

INTRODUCTION

Rice (Oryza sativa L) is one of the most important plants on earth. Not only is it one of the world’s most popular edible crops, but it is also a popular model plant for molecular biology and genomic research into monocots, owing to its small genome size (370 Mb), ease of transformation, and prodigious molecular and genetic information. Additionally, completed rice genome sequences might be extended and applied to the sequences of other grasses via comparative analysis, thus further expanding our knowledge of functional genomics in the plant kingdom (Delsney 2003, Devos 2005, International Rice Genome Sequencing Project 2005)

TILLING (Targeting Induced Local Lesions IN Genomes) is an excellent reverse genetic technique, which allows for functional identification of mutations in specific genes in functional genomics research (Till et al. 2003). McCallum et al. (2000) was the first to report a methodology for the target screening of mutations induced by treatment with ethyl methanesulfonate (EMS), and has combined the ability of denaturing high-performance liquid chromatography to detect base pair changes in the genome of Arabidopsis. TILLING populations have also been developed in other plant organisms such as maize (Till et al. 2004), wheat (Dong et al. 2009), rice (Till et al. 2007), soybean (Cooper et al. 2008), and tomato (Minoia et al. 2010). With regard specifically to the rice genome, the UCD Genome Center (University of California at Davis) has launched a large-scale rice TILLING project to develop useful functional genomics resources by treatment with chemical mutagens...

As of August 2009, more than 3000 mutant varieties have been developed (http://mvgs.iaea.org). Approximately two-thirds of those were developed via the direct use of induced mutants generated via the application of different mutagenic influences such as ionizing radiations or chemicals (http://mvgs.iaea.org). These mutagens could induce a variety of useful alterations in the genomes of crop plants, many of which constitute potential improvements. Gamma rays are an extremely high-energy form of electromagnetic radiation, and are frequently utilized as a source of induced mutagenesis for crop plant breeding. For example, Kim et al. (2004a, 2004b) have used gamma-ray irradiation techniques to develop some useful rice mutants with improved agronomic traits such as high lysine and high essential amino acids. However, there have been relatively few reports thus far regarding the induction of TILLING populations by gamma-ray irradiation.

We have developed approximately 15,000 M₃ TILLING lines after gamma-ray irradiation was applied to Donganbyeo at two doses—200 Gy and 300 Gy. The seeds were irradiated with gamma rays using a gamma phytotron at the Korea Atomic Energy Research Institute, Jeongeub, Korea. To assess the genetic diversity of the TILLING population, we randomly selected 96 TILLING lines, in addition to Donganbyeo, which was employed as a control.

**Fingerprinting assay**

Total genomic DNA from each of 96 lines, including Donganbyeo was extracted via a modified CTAB method (Saghai-Maroof et al. 1984). The quality and quantity of the extracted DNA were assessed via 1.5% agarose gel electrophoresis and spectrophotometric measurements (Eppendorf, Biophotometer plus), respectively. AFLP fingerprinting was conducted as described previously by Vos et al. (1995) with some modifications, as described below. Genomic DNA (250 ng/μl) was digested with 5 U each of EcoRI and MseI (New England Biolabs, USA), and 10x AFLP DL Buffer [100 mM Tris-HCl (pH7.5), 100 mM magnesium acetate, 500 mM potassium acetate, 50 mM dithiothreitol, and 100 ng/μl of bovine serum albumin] for 1 h at 37°C. Each of both cohesive digested genomic DNA sites were ligated with EcoRI and MseI adapters, respectively, and a ligation reaction buffer including 10 mM ATP, 1 U T4 ligase (Promega, USA), and 10 x AFLP DL Buffer at 37°C for 3 hrs. Pre-amplification was conducted using template DNA (1/10 diluted ligated DNA) with a primer pair (complementary to the EcoRI and MseI adapters, with one selective nucleotide adenine and cytosine, respectively). The cycling profile is as follows: 2 min at 72°C for pre-heating, 20 cycles at 94°C for 30 sec, 65°C for 30 sec, 72°C for 60 sec, and 2 min at 72°C, and a 15 min final extension at 60°C. The diluted (50-fold) pre-amplified products were subsequently employed as templates for selective amplification. Five EcoRI/MseI primer combinations were utilized for selective amplification. The oligonucleotide sequences used for AFLP analysis and the primers employed herein are listed in Table 1. The PCR program was conducted as follows: 12 cycles with annealing temperatures decreasing for 0.7°C per cycle, beginning with 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s and ending with 23 cycles at 94°C for 30 sec, 56°C for 60 sec, 72°C at 60 sec.

**MATERIALS AND METHODS**

**Plant materials**

Approximately 15,000 M₃ TILLING lines were developed after gamma-ray irradiation was applied to Donganbyeo at two doses—200 Gy and 300 Gy. The seeds were irradiated with gamma rays using a gamma phytotron at the Korea Atomic Energy Research Institute, Jeongeub, Korea. To assess the genetic diversity of the TILLING population, we randomly selected 96 TILLING lines, in addition to Donganbyeo, which was employed as a control.