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Fingerprinting of Rice Genomes Using PCR with Arbitrary Primers

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Abstract

The arbitrary primed polymerase chain reaction (AP-PCR) has been used to detect the genetic alternations in the related species. Simple and reproducible fingerprints of complex genomes can be generated using single arbitrary chosen primers and the PCR. The technique was applied to the Oryza species and characterized the relationship among three cultivars of rice species based on the result of genomic DNA fingerprints. The results indicated that the polymorphism revealed in rice strains and the differences in the PCR product pattern could be represented for each strains. There were many variations in the PCR product pattern between cv. Dongjin (japonica type) and cv. Hyangdo (indica type), and our chosen AP-primers can be used as markers for strain identification and verification.

Key words : *Oryza sativa* cv. Dongjin, *Oryza sativa* cv. Hyangdo, arbitrary primed PCR (AP-PCR), fingerprinting, polymorphism

INTRODUCTION

Rice is an important plant which is the second largest crop after wheat and used as a staple food for 2/3 of world people since it supplies essential amino acids, proteins and enough calories. Therefore, it becomes an increasingly attractive plant for study to biologists. Rice is warm-season crop which is grown in mainly tropical or subtropical climate. Major species cultivating in the world is sativa. There is a wide variety of genetic diversity between wild type and cultivar because rice was originated from the wild variety of Asian area. In the ecogeographic race of *Oryza sativa* L., there are three races, indica, japonica, and javanica. Rice has 24 chromosomes, and its genome is 4.3 megabases, one tenth the size of human genome and only three times that of arabidopsis thaliana, a favorite plant for plant reseachers. Rice belongs to the grasses family (Poacea), which includes maize, sorghums, wheat, barley and oats (1).

Approximately 20,000 complementary DNAs (cDNAs) derived from a variety of tissues and cells cultured under different conditions were partially sequenced. One important finding from the analysis of cereal genomes in that, for much of the genome, the order of genes is well conserved among major cereals. This synteny extends to the nucleotide level of the chromosome suggesting that once genes with products of interest are isolated from rice, counterparts in wheat, maize, and other cereals can be easily identified (1-3).

Lately, foreign genes which invest resistance against various environmental stress are attempted to transformed into crop plants to make improvement of breed. In this case, the genetic transfer of the transformant to the descendent has to be confirmed. The methods to do that could be southern, Northern, and PCR analyses. Among the methods, PCR might be the adequate and fastest one. Not only validifying the genetic transfer of the transformants, but also generating primer sets from information of nucleotide sequence in some specific parts of DNA in molecular breeding have been developed ascribed the marker DNA (4-10).

DNA marker was used as a powerful tool in gene mapping, DNA fingerprinting and genotype detection. Genetic analysis using DNA marker can increase the efficiency of breeding with a decrease in time and labour than conventional breeding since it can make the diagnosis of genotype of cross-breeding be simple at laboratory level.

Especially when applying PCR to the protocol, a large number of ontogeny can be analyzed in a fast and simple

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way. Therefore, a variety of new PCR-based methods such as random amplified polymorphic DNAs (RAPD) (4,5), cleavable amplified polymorphic DNAs (CAPs), sequence target sites (STSs) (6,7), interrepeat amplification (IRA) (8), amplified fragments length polymorphism (AFLP) (9,10) were developed and used in the genetic analyses. As a consequence, a small part of sequence of DNA relevant to the beneficial phenotype is amplified with PCR, and the products are used in the detection of the genotype as in the CAPs. Otherwise; or as in the IRA, a highly repetitive sequences of the PCR products are used in the detection of genotype.

In the current study, a PCR-based DNA fingerprinting technique was used to investigate the polymorphism of rice cultivar, Dongjin (japonica type), Chilsung (japonica/indica derivative), Hyando (IR 841, indica type) The methods, called AP-PCR described by Welsh and McClelland (11), utilizes an amplification reaction with a single arbitrary primer. This approach has been applied for mapping DNA polymorphisms in various prokaryotic and eukaryotic system (11-14). Fingerprinting of rice genome was carried out using the AP-PCR method with the 12 different 12-mer arbitrary primers. Some intraspecies-specific genetic alteration and primer-specific pattern in the same rice genome could be readily detected by comparision of the AP-PCR fingerprints from the three cultivar of rice genome. With this primary exploration, DNA marker and genome pattern in rice of beneficial phenotype will be established in the future research.

MATERIALS AND METHODS

Plant materials and growth condition

The seeds of *Oriza sativa* cv. Dongjin, Hyangdo, Chilsung were soaked in 1% sodium hypochlorite for 15 min, washed with sterilized distilled deionized water three times. The sterilized seeds were immersed in sterilized water and grown in a growth chamber at 28°C for three days until germination. The sprouts were planted in several flower pots containing vermilculate and cultivated with sufficient watering in a growth chamber at 28°C for 1 week.

Isolation of rice genomic DNA

Total DNA was extracted from 7-day-old rice seedlings by the methods of Cenis (15) with RNase treatment and phenol extraction. The leaves of three rice cultivar were frozen with liquid nitrogen separately and ground to a powder. The 3ml of extraction buffer (200 mM Tris HCl pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) were added to powder and grinded. After that 1.5 ml of 3 M sodium acetate, pH 5.2 were added, and tubes are placed at -20°C for about 10 minutes. Tubes are then centrifuged in a microfuge and the supernatant transferred to another tube. Then, an equal volume of isopropanol is added, and after at least 5 min at room temperature, the precipitated DNA is pelleted by centrifugation in a microfuge. After a wash with 70% ethanol, the pellet is vacuum dried for some minutes and resuspended in a 50 μl of TE.

Arbitrary primed polymerase chain reaction

Primers for PCR amplification of segment of rice genomic DNA were supplied by Dr. Ahn, K. S. (Samsung Biomedical Research center, Seoul, Korea). The primers consisted of 12 short sequence of 12-mer (Table 1). PCR reaction mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 8.2), 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM each 4 dNTPs, 500 nM of each primer, 100 ng of genomic DNA, and polymerase (Bioneer Co., Seoul, Korea) in 25 μl. Amplification consisted of 30 cycles of 1 min at 94°C (denaturation), 2 min at 42°C (annealing) and 1 min at 72°C (elongation). The reaction products (8 μl) were loaded on 2% agarose gels and electrophoresed at 100 V.

RESULTS AND DISCUSSION

AP-PCR is widely used technique in various field of

Table 1. Primer sets used in the arbitrary primed polymerase chain reactiom (AP-PCR)

Primer number	Nucleotide sequence	GC contant (%)
Primer 1	5'-GCAACTGATGCC-3'	54
Primer 2	5'-CGCAACTGATGC-3'	54
Primer 3	5'-CCGCAACTGATG-3'	54
Primer 4	5'-GCCGCAACTGAT-3'	54
Primer 5	5'-TGCCGCAACTGA-3'	54
Primer 6	5'-ATGCCGCAACTG-3'	54
Primer 7	5'-GATGCCGCAACT-3'	54
Primer 8	5'-TGATGCCGCAAC-3'	54
Primer 9	5'-CTGATGCCGCAA-3'	54
Primer 10	5'-ACTGATGCCGCA-3'	54
Primer 11	5'-AACTGATGCCGC-3'	54
Primer 12	5'-CAACTGATGCCG-3'	54

biology to identify and classify intraspecies and interspecies genotypic relationships because of its simplicity and products amplified with arbitrary primers differences in quality or in quantity of genomes among different species can be easily illustrated. Actually the PCR is a technique for *in vitro* amplification of specific segments of DNA from complex mixtures such as the DNA of a whole eukaryotic genome(16,17). In this procedure, though the sequence of *in vitro*-amplified DNA short, the specific character of the sequence of arbitrary primers could represent total cell genome (4,11,12). In general, the AP-PCR involved two cycles of low stringency amplifications followed by PCR at higher stringency. However, in our study, the AP-PCR was carried out fixed PCR condition.

To investigate the genetic polymorphism of the three rice cultivars, e. g., Dongjin (japonica type), Chilsung (japonica/indica derivate), Hyangdo (indica type), genomic DNAs of the plants were isolated and subjected to AP-PCR. Various temperatures in the amplification reaction were tested to find out the best condition for the amplification reaction to get band pattern in the gels. In the PCR, the annealing temperature was appeared to be best at the range of 42°C to 50°C. The annealing temperature of 42°C was appeared to be adequate temperature (Fig. 1). The results of AP-PCR in rice DNAs were showed in Figs. 2 and 3, each using the 12mer arbitrary primers. More than 10 DNA fragments of sizes ranging from < 250 to ~3000 base pairs were reproducibly amplified with each of the single arbitrary primers. The genomic DNA fingerprints were different, depending on the primer. For a given primer, differences in the band pattern were also apparent. These results showed the ability of the selected primer to detect of rice genomic polymorphism.

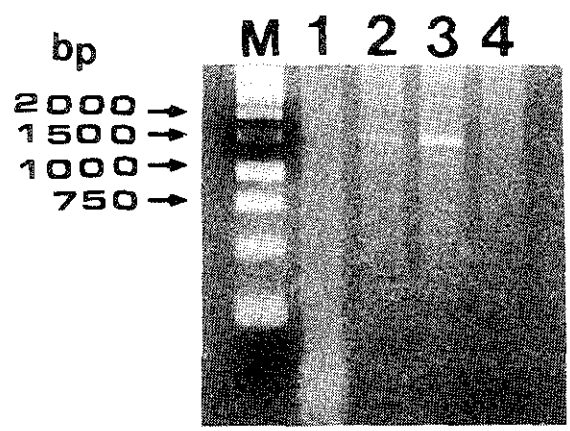

Fig. 1. Effects of annealing temperature on AP-PCR product from cv. Dongjun genomic DNA and primer 5′-GCAACTGATGCC-3′.
Lane M: 1kb DNA ladder (promega, Madison, USA)
Lane 1: 37°C, Lane 2: 42°C, Lane 3: 50°C, Lane 4: 55°C

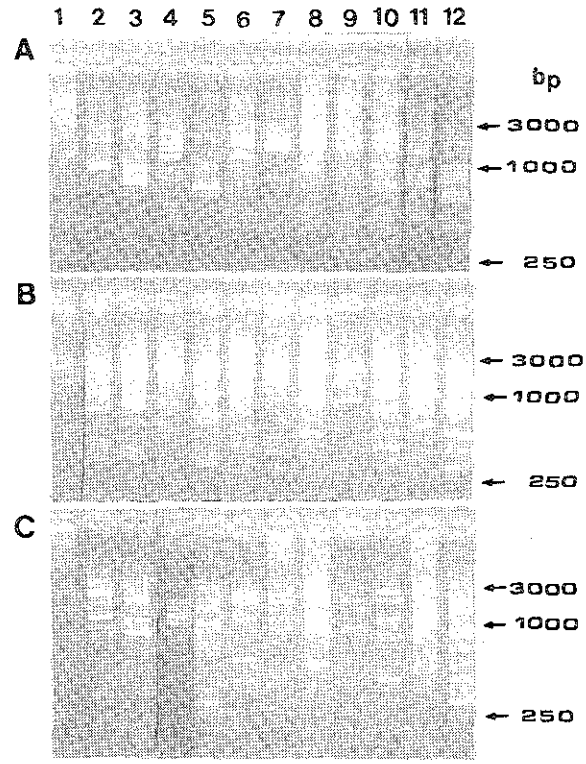

Fig. 2. Amplification product of rice DNA from AP-PCR with twelve primer sets on a 2% agarose gel (Annealing temperature: 42°C).
(A) *Oriza sativa* cv. Dongjin, (B) *Oriza sativa* cv. Chilsung, (C) *Oriza sativa* cv. Hyungdo.
Lane 1: primer 1, Lane 2: primer 2, Lane 3: primer 3, Lane 4: primer 4, Lane 5: primer 5, Lane 6: primer 6, Lane 7: primer 7, Lane 8: primer 8, Lane 9: primer 9, Lane 10: primer 10, Lane 11: primer 11, Lane 12: primer 12.

A set of 12 arbitrary primer was used to find out the polymorphism depending on the primer of the 3 cultivars of rice. With Dongjin which is a japonica type, in the amplification of genomic DNA with the 12 different 12-mer primers, the primer 3 (5′-CCGCAACTGATG-3′), the primer 4 (5′-GCCGCAACTGAT-3′), the primer 10 (5′-ACTGATGCCGCA-3′), the primer 11 (5′-AACGA TGCCGC-3′), and the primer 12 (5′-CAACTGATGC CG-3′) showed superior polymorphism as depicted in the gel (Fig. 2). In comparison to that, with Chilsung, the primer 1 (5′-GCAACTGATGCC-3′) showed the least polymorphism in the gel (Fig. 2). However, in the case of Hyangdo, an indica type, all the 12 primers showed a

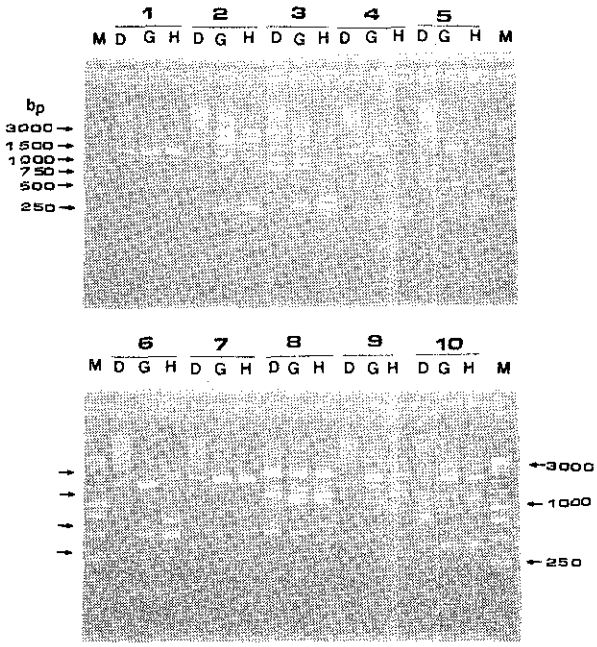

Fig. 3. Amplification product of rice DNA from AP-PCR with twelve primer sets on a 2% agarose gel (Annealing temperature: 50°C).
D: *Oriza sativa* cv. Dongjin, G: *Oriza sativa* cv. Chilsung, H: *Oriza sativa* cv. Hyungdo.
1: primer 1, 2: primer 2, 3: primer 3, 4: primer 4, 5: primer 5, 6: primer 6, 7: primer 7, 8: primer 8, 9: primer 9, 10: primer 10.

similar degree of polymorphism in the band pattern (Fig. 2). Among them, only the two primers, e. g., the primer 1 (5′-GCAACTGATGCC-3′) and the primer 7 (5′-GATGCGCAACT-3′) showed a comparatively less degree of polymorphism in the gel (Fig. 2). Collectively, the best primer sets to reveal the interspecific polymorphism among the three cultivars of Dongjin, Chilsung, Hyangdo was appeared to be the primer 3 (5′-CCGCAACTGATG-3′), the primer 4 (5′-GCCGCAACTGAT-3′), the primer 5 (5′-TGCCGCAACTGA-3′), the primer 11 (5′-AACTGATGCCGC-3′), and the primer 12 (5′-CAACTGATGCCG-3′).

In the present study, to find out which primer set was adequate to show polymorphism of differentially expressed pattern of genes on the gels, fingerprints of complex genomes were generated using 12 different 12mer arbitrary chosen primers and PCR (13,14). In the fingerprints, the polymorphism of differentially expressed bands on the gels depends on the combination of primers with three different strains of rice, e. g., Dongjin, Chilsung, and Hyangdo were compared. The primers selected were very similar in sequences. The primers were designed in each way that the first base at 3′ end of the primer 1 was removed to the end of the other side (5′ end) to make the primer 2. Sequentially the new base at 3′ end was removed to the 5′end to make the next primer so that twelve 12mer primers were made (Table 1). As mentioned above, the 12mer primers were appeared to be adequate to pick up polymorphism of complex genomes.

With the strain, Dongjin which is a japonica type, it was resulted that primer 3 (5′-CCGCAACTGATG-3′), the primer 4 (5′-GCCGCAACTGAT-3′), the primer 10 (5′-ACTGATGCCGCA-3′), the primer 11 (5′-AACTGATGCCGC-3′), the primer 12 (5′-CAACTGATGCCG-3′) were prominent to show polymorphism of band patterns of differentially amplified genomic DNA than other primer did (Fig. 2A). Otherwise the primer 1 (5′-GCAACTGATGCC-3′) with Chilsung, showed the least polymorphism of the amplified PCR products than other primers did (Fig. 2B). In the case of Hyangdo which is an Indica type, all the 12 primer sets showed almost similar level of polymorphism in the band pattern (Fig. 2). However, among the primers, the primer 1 (5′-GCAACTGATGCC-3′) and the primer 7 (5′-GATGCCGCAACT-3′) showed relatively less degree of in the amplified products (Fig. 2C).

From the results, it can be said that with Dongjin, the primer 3, 4, 10, 11, and 12 are adequate primers in PCR amplification and with chilsung, all the primers except the primer 1 appeared to be adequate in PCR. With Hyangdo, the primers except two primers e. g., the primer 1 and 7 are adequate in PCR. The knowledge in the selectivity adequacy of the primers in PCR will be adopted to rice gene mapping, RAPD, RFLP (Restriction fragment length polymorphism), DDRT-PCR (Differential Display of Reverse transcription Polymerase Chain Reaction) and gene targeting etc. Addition to that the primers would be used in direct gene cloning and direct sequencing.

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