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IDENTIFICATION OF M4 GAMMA IRRADIATED MAIZE MUTANT BASED ON RAPD MARKERS

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ABSTRACT

Gamma irradiation to induce mutation in plant has been used intensively since several decades ago. On maize, 275 Gy gamma irradiation has been known to increase genetic variability indicated by its morphological variation. Identification of genetic changes by molecular technique is important to detect mutation on DNA level of the plants. The objective of this research was to identify RAPD marker polymorphism on gamma irradiation mutants compared to their parents. The initial step of the research was to select random primers which could positively amplify the maize DNA. The result showed that among 60 random primers there were 15 primers that positively amplified the maize DNA. Amplification of both mutants and their parents by those 15 selected primers indicated that only 5 primers had polymorphism between mutants and their parents. Polymorphisms on mutant G1, G3 and G6 were detected at one locus, while on mutant G7, G8 and G9 were at two loci.

Keyword: maize, mutation, RAPD

INTRODUCTION

In Indonesia, the development of cultivars from induced mutation has been carried out in cooperation with PATIR BATAN. In 1972 rice varieties 'Atomita' has been released resulting from gamma irradiation (BATAN, 1996). Until recently, BATAN has produced thirteen lowland rice varieties, one upland rice variety, four soybean varieties, one mungbean variety, and one cotton variety (Sisworo *et al.*, 2008).

Physically induced mutations with gamma ray irradiation can be done to improve the genetic diversity of a base population (Micke and Donini, 1993). The successful attempt of irradiation to increase genetic variation of a population is largely determined by genotype radio sensitivity. Level of sensitivity of plant and crop highly varied between plants or genotypes (Banerji and Datta, 1992). Radio sensitivity can be measured based on LD50 which is the level of irradiation bringing about the death of 50% of the population. Some studies suggested that the optimum dose to produce mutant most commonly occurs in approximately LD50 (Ibrahim, 1999). In addition to the LD50, radio sensitivity is also observed from the growth inhibition or lethality, somatic mutations, chromosome fragments, and the number and size of chromosomes (Datta, 2001). In maize, a dose of 275 Gy irradiation increased the diversity of leaf number, length and width from 30% to 80%, while plant height increased by 250% to 1300% (Rustikawati *et al.*, 2008b).

Identification of induced mutation plants can be aided by morphology, enzymatic or molecular characterization. Morphological identification is the most inexpensive and the easiest to do, but errors of non-inherited epigenetic traits are relatively more frequent. Enzymatic identification on gamma ray induced carnation mutation which produced five mutants. The peroxidase enzyme (PER), the esterase enzyme (EST) and the acid phosphatase enzyme (ACP) showed a different banding pattern among the five carnation genotypes tested (Aisyah, 2006). The last few years, molecular markers have been widely used as tool in basic genetics studies and their application. Various size of DNA fragments produced by amplification can be easily separated by electrophoresis technique and the results can be observed as bands of DNA at

various size (Griffin and Griffin, 1994). DNA marker is more accurate because it is not influenced by environment and plant growth stage. Among the DNA markers, RAPD is the easiest to do, therefore, in this identification of gamma irradiation induced maize mutant is carried out using RAPD molecular markers.

MATERIALS AND METHODS

The study was conducted from September 2009 to August 2010. Plant materials used were six genotypes of maize and six S4 mutants induced by gamma irradiation at a dose of 275 Gy. The induced mutants were then selfed for 4 generations. The six mutants were G1-12-18a-1 (G1M), G3-15-17-4 (G3M), G6-6-19-19a (G6M), G7-15-9-3 (G7M), G8-4-8-6 (G8M), and G9-20-44-2 (G9M). DNA samples were isolated from leaves of 2-week-old plants. RAPD analysis was conducted at the Laboratory of Molecular and Cellular Department of Agronomy and Horticulture, Bogor Agricultural University.

DNA Extractions

DNA extractions were performed following a protocol based on DNA EXRACT KIT RED-AMP. DNA purification was done by the addition of the CIA and DNA precipitation using 95% alcohol. The quality and purity of DNA were determined by calculating the ratio of absorbance value of the prepared DNA at A_{260} to A_{280}. The value of 1.8 – 20 indicated good quality DNA (Herison *et al.*, 2003).

Primer Selection and Identification of RAPD Markers

A total number of 60 different primers from the Operon Tecknologies OPE, OPH and OPM, has been tested. Fifteen random selected primers were used for DNA amplification of maize mutant and the wild types. DNA band polymorphism of mutant and wild types was used to establish the occurrence of the mutant. Amplification was done with a DNA concentration of 10-25 ng. The PCR program included a pre-PCR (94°C, 5 seconds), 45 cycles of {denaturation (94°C, 5 seconds), annealling (TM-4°C, 30 seconds), elongation (72°C, 1 minute)}, and stop PCR (72°C, 10 minutes). Electrophoresis was performed on agarose gel (0.8 w/v) using TAE buffer, 100 volts voltage for 25 minutes. PCR products were visualized by ethidium bromide staining and UV transiluminator.

RESULTS AND DISCUSSION

RAPD Primer Selection

DNA template for primer selection was derived from line G1. There were 15 DNA primers out of the 60 random primers tested, which were suitable for maize. Sequences of each primer and the number of bands produced were listed in Table 1.

RAPD technique was usually chosen because of the requirement of very small amount of DNA, no information of plant DNA sequence required, no radioactive material used, easy implementation performed and no expensive expense spent. Random primer was usually plant species specific. Therefore, the primer selected in this study could also be used for amplification of DNA by other maize researchers so it could speed the time and save the cost of RAPD analysis. Primers which had more than 2 bands were likely to generate polymorphisms in analyzed plants. Based on the data above, the primers used to identify RAPD markers of the maize mutants were OPE-07, OPE-06, OPH7, OPH-19 and OPM-20. The primer of OPE-07 producing 4 bands was considered very good for polymorphism analysis.

Tabel 1. Primer bases for maize DNA amplification and the number of bands produced

No	Operon	Base	Number of bands
1	OPE-7	AGATGCAGCC	4
2	OPE-8	TCACCACGGT	1
3	OPE-10	CACCAGGTGA	1
4	OPE-17	CTACTGCCGT	1
5	OPH-1	GGTCGGAGAA	1
6	OPH-6	ACGCATCGCA	2
7	OPH-7	CTGCATCGTG	3
8	OPH-8	GAAACACCCC	2
9	OPH-9	TGTAGCTGGG	2
10	OPH-16	TCTCAGCTGG	1
11	OPH-19	CTGACCAGCC	3
12	OPM-1	GTTGGTGGCT	1
13	OPM-2	ACAACGCCTC	2
14	OPM-7	CCGTGACTCA	1
15	OPM-20	AGGTCTTGGG	3

RAPD Marker Identification

PCR template for identification of polymorphism was selected from the DNA of G1, G3, G6, G7, G9 either from the parents or the gamma-ray irradiated mutants. Each DNA was amplified by 5 selected primers. Visualization of PCR electrophoreses were done simultaneously with marker of 1 kb ladder. Markers were needed for quantification of DNA bands based on the length of the fragment. Figure 1 and Figure 2 presented some of the result of DNA amplification. Polymorphism banding pattern between the mutant and parentals was indicated by arrows.

The results obtained from RAPD analysis with the OPE-08 primer for the inbred line G1 presented a band of 1500 bp, whereas the G1M showed no band. The different band pattern indicated that both DNAs were also different. DNA band size differences resulting from the same primer was assumed that the diferences were from different loci. Based on the results of DNA amplification, all the gamma irradiation mutants were different in nucleotide sequences from their parents (Table 2).

Polymorphism on RAPD banding pattern was due to mutations in the DNA level of irradiated maize. Point mutations or changes in a DNA base was able to cause differences of the template, resulting in differences in RAPD banding pattern. Micke and Donini (1993) states that gamma irradiation could ionize atoms in the tissue by releasing electrons from the atoms. Ionization triggered molecule clustering along the ion orbits left due to irradiation. Such new grouping led to chemical changes bringing about to errors in organic bases pairing in DNA. Those resulted in gene mutations or the destruction or rearrangement of chromosomes.

Tabel 2. RAPD polymorphism band pattern of gamma irradiation mutants and their respective parents DNA

Primer	G1	G1M	G3	G3 M	G6	G6M	G7	G7M	G8	G8M	G9	G9M
$OPE07_{500}$									+	-		
$OPE08_{450}$											-	+
$OPE08_{600}$									+	-	-	+
$OPE08_{1500}$	+	-										
$OPE08_{1600}$							+	-				
$OPE08_{2000}$							+	-			+	-
$OPH07_{600}$					+	-						
$OPM20_{1100}$			+	-								

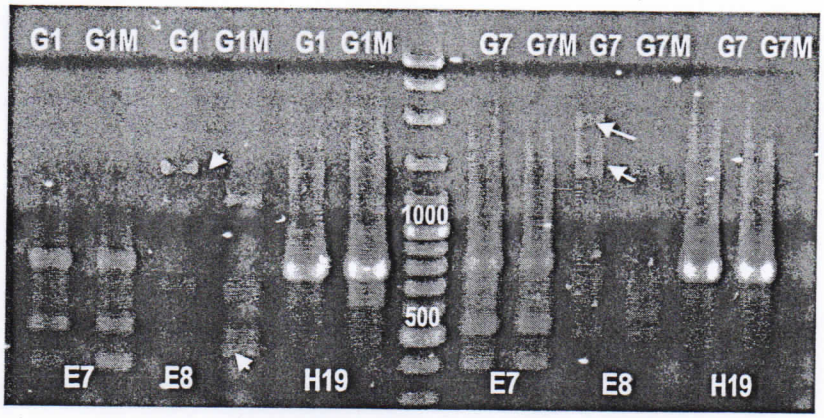

Figure 1. Product amplification of maize mutants and their parents of G1 and G7 with OPE-07, OPE-08 and OPH-19 primer

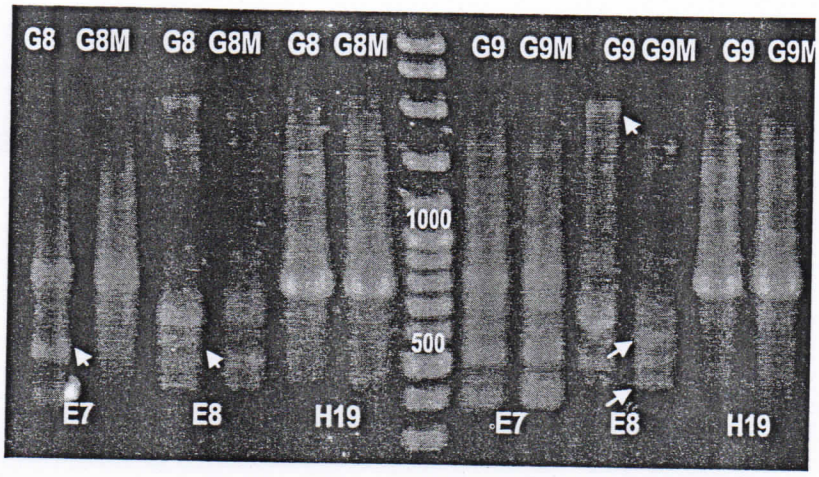

Figure 2. Product amplification of maize mutants and their parents of G8 and G9 with OPE 07,OPE-08 and OPH-19 primer

Consistency of RAPD bands as molecular markers of plants has been demonstrated by Herison *et al.* (2010) in the banding pattern identification BC2 plants chili with recurrent elders. The same primer used for the identification of BC3 plants produce an identical banding pattern. RAPD markers have also been used for the analysis of gene linkage to CMV resistance in hot pepper (Rustikawati *et al.*, 2008a).

CONCLUSIONS AND SUGGESTIONS

All gamma irradiation maize mutants produced polymorphism DNA banding pattern from their parents. Based on the generated RAPD banding pattern, polymorphism in the mutant G1, G3 and G6 occurred at one locus, while the G7, G8 and G9 occurred at two loci. Thus, the 275 Gy of gamma irradiation was consider successfully induced mutations in the DNA of maize.

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