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The Detection of Mutational Changes in Sorghum using RAPD

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Abstract

Sorghum is a multifunction plant due to its high economic value as a source of food, feed and industrial raw material of biofuel. Sorghum improvement can be done through mutation breeding and this research was conducted to evaluate the power of mutation breeding by observing the difference between mutant and its original counterpart. Varieties of sorghum Durra, Zhengzu and their mutants B-100 and Zh-30 were arranged in completely randomized design. DNA extraction was done using a modified CTAB method. After purification, quantification, and dilution, PCR was carried out for RAPD analysis. The result showed that Durra and Zhengzu varieties were significantly different in plant height, number of leaves and seed colour, however the mutant and its original counterpart cannot be differentiated morphologically. RAPD can be used to differentiate mutant and its original counterpart by observing the specific band pattern from each primer.

Keywords : Drought, mutation, molecular marker, sorghum

Introduction

Sorghum is fifth economic importance among the cereals crops worldwide (Zidenga, 2004). This crop is well adapted to a wide range of harsh environmental conditions and particularly adapted well to drought. The grain can be used for flour to make bread, porridge and for brewing beer. The large juicy sweet stems are utilized for chewing and making syrup, ethanol and sugar (Prakash *et al*., 2006). Sorghum grain and straw, taken after harvesting can be used to feed cattle or other livestock (Purseglove, 1972).

Mutation breeding is a supplementary approach for crop improvement and has played a productive role in sustainable agriculture. About 1790 different mutants have been listed in the database. Zh-30, for instance, was developed by the Centre for Application of Isotope and Radiation Technology ofIndonesia by using gamma radiation of Zhengzu variety which was originated from China. Zh-30 showed significant different from its original partner on its flour and starch quality (Santosa and Human, 2009). Different methods were available to investigate the effect of mutagens, with molecular markers allowed direct comparison of the effect at DNA level. RAPD (Randomly amplified Polymorphic DNA) analysis could be sued for the detection of DNA alteration after the influence of mutagenic agents (Selvi *et al*., 2007). The advantage of RAPD relies on its simplicity, rapidity, a small quantity of DNA and the ability to generate numerous polymorphisms (Kumar *et al*., 2009). RAPD is assay when the nucleotide sequence is not known (Akhare *et al*., 2008). The use of RAPD as molecular marker has been done to detect mutational changes in plants, because RAPD generated more number of polymorphic bands. Farooq *et al*. (1996) were able to distinguish different mutants of Basmati rice from their parents, therefore in this experiment RAPD also will be used to differentiate between mutant sorghum promising lines from their original parents.

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Materials and methods

Sorghum varieties Durra from ICRISAT-India, Zhengzu from China, their mutants B-100 and ZH-30 were provided by the Centre of Application Radiation Technology of Indonesia. The seeds of 4 accessions were planted in completely randomized design with 3 replications. Plant height and leaves number were collected to characterize the difference betwen mutant lines and their original parents morphologically. DNA was isolated from leaves of 3-4 weeks old plants and the extraction method used was a modified of CTAB (Cetyl trimethyl Ammonium Bromide) method. The leaves were cut for samples (85 mg) and only one replication was sampled for DNA extraction. The DNA was purified using Geneclean® III kit (MP Biomedicals). It was quantified using GeneQuant to find out the amount of isolated DNA. Dilution was done to obtain a final DNA concentration of 2.5 ng/µl. The PCR reaction was performed with a total volume of 10 µl for each sample using the PCR kit AmpliTaq®DNA Polymerase Stoffel Fragment (Applied Biosystem). Amplification was carried out using a PCR machine Perkin Elmer 9600. Initial heating was performed at 94°C for 3 seconds, denaturation at 95 °C for 1 minute, then followed by 45 cycles with each cycle at 94 °C for 30 seconds, annealing at 37 °C for 30 sec and elongation at 72 °C for 1 min 30 sec followed by final elongation at 72 °C for 7 minutes. The gel was made with 76 ml Psd H_2O, 1.4 g agarose (1.75%) and 4ml 20xTBE Buffer (made from 0.45M tris-HCl pH8, 0.45M boric acid, and 20mM EDTA). It was boiled and then added with 5µl ethidium bromide. The electrophoresis buffer was made from 5 l H_2O, 250 ml 20xTBE and 250µl ethidium bromide. Electrophoresis was carried out after adding 2 µl GL3 in each well containing amplified DNA from the PCR. As a marker, 10 µl of 100bp DNA ladder is used. Electrophoresis was carried out for 2.5 h at 120 Volt. Visualization was done using the Fotodyne Image Analyzer with a UV light. Scoring of visualized bands was done by scoring 1 for the presence of a band and 0 for no present of band for each individual plant. Analysis of Variance (ANOVA) was carried out using the SAS program (Anonym, 2002) for the morphological characteristics such as plant height and number of leaves. The scoring result was used to find out the amount and percentage of polymorphic locus using the computer program POPGENE 1.32.

Results and Discussion

Morphological charateristics

The Zhengzu plants (mutans and non-mutant) had a higher number of leaves than Durra plants even though Zhengzu were shorter in height (Table 1). This meant that higher plants does not always mean higher number of leaves because it only causes an increase in the length of internodes not the number of nodes per plant. Morphologically distinct accession plant could then be largely differentiated from each other at the genetic level (Damodaran *et al*., 2007). The plant height showed a significant difference between the two varieties but no difference between the non-mutant and mutant types. This meant that plant height was a specific trait for that variety and the mutation target did not change plant height but other yield component. Plant height might be genetically stable characteristics, it was therefore difficult to be altered through mutation. Santosa and Human (2009) mentioned that the main objective in developing Zh-30 for improving flour and starch quality, not other characteristics, therefore in this result, both evaluated characteristics were quite stable

Tabel 1. The plant height and number of leaves of the sorghum

Population	Plant height (cm)	Number of leaves
Durra	133.30 a	7.26 d
Zhengzu	101.00 b	9.70 a
B-100 (mutant of Durra)	128.90 a	7.88 c
Zh-30 (mutant of Zhengzu)	97.06 b	8.94 b

Remarks: means at the similar column followed by similar letter were not
signicantly different by Duncan Multiple Range Test at 5% significant level.

RAPD analysis

DNA markers might differ with respect to important features such as abundant in the genome, level of polymorphism detected, locus specificity, reproducibility, and technical requirement (Kumar *et al*., 2009). No marker was superior to all other for a wide range of application. The most appropriate DNA markers has depended on the specific application, the present level of polymorphism of polymorphism, the present of sufficient technical facilities and know-how, time constraint and financial limitation. RAPD generated more number of polymorphic bands (Data, 2010). Due to some specific RAPD bands could be identified at the rice mutant, it is reflected that RAPD markers might still be very useful for rapid and easy identification of most diverse genotypes (Asif *et al*., 2005). RAPD technique brought out greater genome varibility (Bhagwat *et al*., 1997), which was expressed through polymorphism. Out of 23 primers used for RAPD analysis which were some originated from Dahlberg *et al*. (2002), only 6 primers yielded good, scorable and polymorphic products i.e. A8, A13, A20, D3, B7 and T14 (Figure 1). Figure 1 showed the clear polymorphic bands among 4 accessions by using primer A20 and B7. Some monomorphic bands were observed using different primers.

In table 2, population Durra and Zhengzu have a higher percentage of polymorphism (78.12 – 79.17 %), and the mutant population B-100 and Zh-30 showed a lower percentage (23.96 – 59.38%). This level of polymorphism was comparable to the report by Akhare *et al*. (2008). A high level of polymorphism in original parents showed that there were still differences in the DNA sequence between individual plants. Those differences occurred because Durra and Zhengzu were naturally occurring populations. Even though sorghum was usually self pollinated there was enough possibility for cross pollination (Purseglove, 1972), or in other words because populations Durra and Zhengzu did not undergo selection thus therefore there were still variation in their genetic compositions.

Table 2. The percentage of polymorphic loci in each population using all 6 primers

Population	Number of polymorphic loci	Percentage of polymorphism (%)
Durra	75	78.12
Zhengzu	76	79.17
B-100	57	59.38
Zh-30	23	23.96

The level of polymorphism in the mutant populations (B-100 and Zh-30) were lower due to the selection activity carried out since the M2 generation. The characteristics of the mutant population tended to be similar i.e. low variance which can be seen as a low percentage of polymorphism. Table 3 showed that primer A8 produced a higher percentage of polymorphism in Zhengzu population and lowest in B-100. Population B-100 tended to be monomorphic which means that there is no difference in DNA sequence between individuals that could be amplified by primer A8. However using primer A13 the

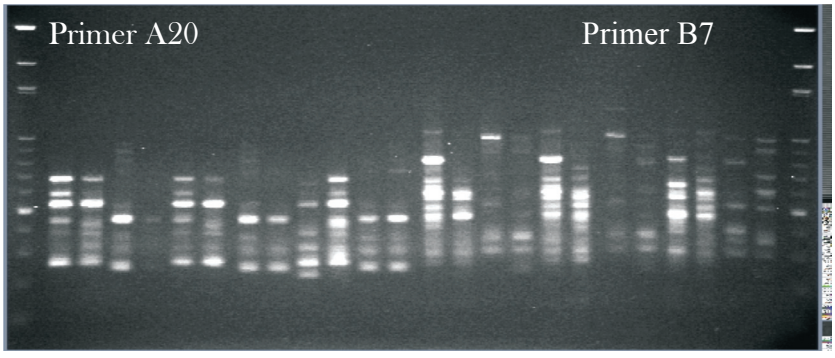

Figure 1. Polymorphic bands produced by primers A20 and B7.

Table 3. The percentage of polymorphic loci in each population for each primer

Primer	Primer sequence (5′-3′)	Popu-lation	Number of amplified bands	Number of poly-morphic loci	Poly-morphism (%)
A8	GTG ACG TAG G	Durra	16	9	56.25
		Zhengzu	16	13	81.25
		B-100	16	0	0
		Zh-30	16	5	31.25
A13	CAG CAC CCA C	Durra	16	13	81.25
		Zhengzu	16	16	100
		B-100	16	15	93.75
		Zh-30	16	9	56.25
A20	GTT GCG ATC C	Durra	16	10	62.50
		Zhengzu	16	10	62.50
		B-100	16	10	62.50
		Zh-30	16	8	50.00
D3	GTC GCC GTC A	Durra	16	14	87.50
		Zhengzu	16	12	75.00
		B-100	16	6	37.50
		Zh-30	16	0	0
B7	GGT GAC GCA G	Durra	16	14	87.50
		Zhengzu	16	12	75.00
		B-100	16	13	81.25
		Zh-30	16	0	0
T14	AAT GCC GCA G	Durra	16	15	93.75
		Zhengzu	16	13	81.25
		B-100	16	13	81.25
		Zh-30	16	1	6.25

percentage of polymorphism in B-100 was high (93.75%) which show that there might be a difference in a certain characteristic that cannot be seen directly.

Different methods were actually available to investigate the effect of mutagenesis; however molecular markers seemed more powerful in this experiment because they allow direct comparison the effect at DNA level. RAPD could be used for the detection of DNA alteration after the influence of mutagenic agent. Irradiation made the increasing level of DNA break formation which leads to formation of new variation that could be detected by changes in RAPD profiles (Selvi *et al.*, 2007). The appearance of new bands could be explained as a result of DNA structural changes.

Using primer A8, a specific band was found in the Durra population. Mutant Durra could be identified using primer A13 with the presence of bands at 480, 450 and 420 bp, while mutant Zhengzu had a specific band at 380 bp (Table 4). Using primer A20, a band at 650 bp showed for the mutant Durra population while primer D3 showed a specific band at 520 bp for mutant Zhengzu. Primer B7 showed a specific band at 650 bp for mutant Zhengzu and 420 bp for mutant Durra which can be used to differentiate individuals in the F3. Primer T14 showed a band at 420 bp which occured only in Zhengzu population.

Table 4. Specific markers from each primer for mutant population

Primer	**Size (bp)**	**Population showing the specific band**
A8	480	Durra, B-100
	700	Durra, B-100
A13	480	B-100
	450	B-100
	420	B-100
	380	Zh-30
A20	650	B-100
D3	520	Zh-30
B7	650	Zh-30
	420	B-100
T14	420	Zh-30

From all the primers used, primer A13 was potential to be used as a marker to detect mutation in the sorghum. Primer A13 showed specific bands for the mutant populations B-100 and Zh-30 and not found on the original populations of Durra and Zhengzu. This might be due to specific changes in the DNA which occur during the induced mutation.

In this experiment, evident was found that molecular markers in this case RAPD offer several advantages over the morphological one as they provide data that can be analyzed objectively (Farooq & Azam, 2002).

It is concluded that sorghum varieties Durra and Zhengzu are different in the number of leaves, height and grain color but there were no difference between the mutant and original plants and RAPD can be used to distinguish the mutants from its parents.

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