Journal of Tropical Crop Science Vol. 4 No. 1, February 2017
www.j-tropical-crops.com

Biodiversity Assessment of Foxtail Millet (*Setaria italica* L.) Genotypes Based on RAPD Marker

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Abstract

Foxtail millet (*Setaria italica* L.) is an important crop in areas where harsh environmental condition limit crop productivity, including in high salinity and drought prone areas. In Indonesia millet is cultivated in certain areas, however, superior varieties are less developed in the country. The objective of this study was to analyze the genetic diversity among foxtail genotypes using RAPD markers. Genomic DNA of ten foxtail millet genotypes was amplified using 26 random primers through RAPD analysis. Of these primers, 22 produced reproducible amplicons and were polymorphic among the 10 foxtail millet genotypes. The number of polymorphic markers for each primer varied from 1 (primer E15) to 14 (primer M17). The amplified product size ranged from 120 to 2500 base pairs (bp). A dendrogram constructed based on the UPGMA clustering method put all genotypes in 5 distinct groups at 0.64 coefficient level. Diverse genotypes identified in this study can be used as potential parents in an efficient crop improvement program.

Keywords: genetic diversity, molecular marker, underutilized crop

Introduction

Foxtail millet (*Setaria italica* L. Beauv.), together with other types of millets, is ranked the sixth most important cereal in the world and is cultivated mainly in semiarid and tropical regions in Asian and African countries (Kajuna, 2001). Millet has a relatively short growing season and can survive under dry and warm temperature regions. It can adapt to a range of soils including heavy clays provided the crop is not subjected to prolonged waterlogging. The grain of foxtail millets is an important carbohydrate source since it was reported to have a low glycemic index (Jali et al., 2012), high in dietary fiber and protein (Amadou et al., 2013), contains antioxidants (Suma and Urooj, 2012), and also has a therapeutic potential in reducing colon cancer effects (Shan et al., 2015). Foxtail millet is an underutilized crop in Indonesia, as only limited area partly used this species as carbohydrate source. However, the short life cycle of foxtail millet (Doust et al., 2009) and its comparable tolerant level to drought (Kafi et al., 2009) and salinity (Ardie et al., 2015) has made this plant an important crop in marginal areas where drought and salinity stresses occur.

There is wide genetic diversity in foxtail millet (Wang et al., 2012, Lin et al., 2012), and characterizing these resources is a prerequisite for the genetic improvement and development of new cultivars. Assessing millet's genetic diversity can provide genetic resources which is crucial for conservation and to ensure agricultural sustainability and food security.

The relative genetic diversity of a species' population can be determined using morphological and molecular markers. Different to phenotypic traits which are affected by environmental conditions, molecular markers which are based on DNA sequence polymorphism, are independent of environmental conditions. In the last decade, molecular marker techniques, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP), have been used routinely to assess genetic variations at DNA level (Agarwal et al., 2008). Among these techniques, RAPD has been shown to be an effective method for detecting polymorphism in foxtail millet (Schontz and Rether, 1999). RAPD is based on polymerase chain reaction (PCR) which is convenient, economical and sensitive compared to other techniques (Williams et al., 1990). Thus, the objective of this research was to evaluate the genetic diversity of 10 foxtail millet genotypes in Indonesia using RAPD-marker.

Materials and Methods

A total of ten (10) foxtail millet genotypes, collection of Indonesian Cereals Research Institute (ICERI), were used in this study. Twenty seeds of each of the 10 entries were germinated on filter paper in closed petri dishes for 7 days in 15 mL deionized water. Genomic DNA was extracted from the 7-day-old seedlings using GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich) with slight modification. Twenty six 10-mer oligonucleotides with arbitrary sequence were used in RAPD analysis, however only 22 primers produced reproducible amplicons (Table 1). The PCR reaction mixture consisted of 5 mL genomic DNA, 1.5 mM $MgCl_2$, 0.08 mM dNTP, 1x reaction buffer, 2-3 units of Taq DNA polymerase (KAPA2GTM Fast PCR Kit), and 50 pmol/ mL primer (Operon Tech., USA) in a final volume of 20 mL. The amplification conditions were 94°C for 5 minutes to pre-denature, followed by 45 cycles of 5 sec at 94°C, 30 seconds at annealing temperature, 1 minute at 72°C, with a final extension at 72°C for 10 minutes. Samples were then kept at 4°C. Amplified DNA fragments were analyzed by gel electrophoresis in a 1.5% agarose gel electrophoresis in 1x TAE buffer (Tris borate EDTA) for 45 minutes at 90 V. The gel were then stained with ethidium bromide and photographed by UV transilluminator (EagleyeTM, Stratagene).

The RAPD bands were scored as "1" for the presence or "0" for absence of a particular DNA fragment of a similar length. To assess the reproducibility of the profiles the same template DNA was amplified in 3 different amplification reactions using the same primer. Only reproducible and clear amplification bands were scored for the construction of the data matrix. The data were entered into NTSYS-pc, anumerical taxonomy and multivariate analysis system program (Rohlf, 1998). The 0/1 matrix was used to calculate the similarity in the matrices using 'Simqual' which is a subprogram of the NTSYS-pc software. Dendogram was built based on the unweighted pair group method with the Bootstrap value of 1,000.

Results and Discussion

In this study, DNA analysis was applied to identify the diversity among 10 foxtail millet genotypes. Reproducibility is of a major concern during

Table 1. List of RAPD primers along with their sequences, band sizes, the number of monomorphic, polymorphic and total bands, and the polymorphism ratio of all tested foxtail millet genotypes.

Primer	Sequence (5'-3')	Annealing temperature (°C)	Band size (bp) min-max	Polymorphic bands	Monomorphic bands	Total bands	Polymorphism ratio (%)
E1	CCCAAGGTCC	37.3	350-2250	6	5	11	54.5
E2	GGTGCGGGAA	44.9	500-1750	6	3	9	66.7
E3	CCAGATGCAC	29.2	580-2100	2	4	6	33.3
E4	GTGACATGCC	28.4	300-1800	6	10	16	37.5
E7	AGATGCAGCC	33.6	350-1800	3	3	6	50.0
E9	CTTCACCCGA	34.9	450-2000	4	3	7	57.1
E10	CACCAGGTGA	29.1	280-3500	7	3	10	70.0
E15	ACGCACAACC	34.8	400-1800	1	9	10	10.0
E18	GGACTGCAGA	28.3	200-1800	11	4	15	73.3
E19	ACGGCGTATG	36.2	350-2500	9	6	15	60.0
H1	GGTCGGAGAA	33.2	300-2250	12	1	13	92.3
H2	TCGGACGTGA	36.4	400-1800	4	6	10	40.0
H5	AGTCGTCCCC	36.2	600-2250	7	9	16	43.8
H13	GACGCCACAC	34.6	580-2250	5	3	8	62.5
H16	TCTCAGCTGG	29.1	120-1580	9	4	13	69.2
H19	GTGACCAGCC	33.6	200-1500	6	4	10	60.0
M1	GTTGGTGGCT	33.2	250-2250	4	8	12	33.3
M6	CTGGGCAACT	33.8	420-1800	3	2	5	60.0
M16	GTAACCAGCC	28.4	190-1600	11	8	19	57.9
M17	TCAGTCCGGG	39.5	180-2500	14	6	20	70.0
M20	AGGTCTTGGG	31.8	280-2500	7	10	17	41.2
M24	GGCGGTTGTC	39.2	280-1280	7	9	16	43.8

amplification of RAPD markers (Jones et al., 1998). Twenty six 10-mer oligonucleotides with arbitrary sequence were used in RAPD analysis, however only 22 primers produced reproducible amplicons (Table 1). The four primers were M18 (5'-CACCATCCGT-3'), H12 (5'-ACGCGCATGT-3'), H15 (5'-AATGGCGCAG-3'), and H18 (5'-GAATCGGCCA-3').

The size and the number of bands produced were strictly dependent upon the nucleotide sequence of the primer used for the template DNA (Table 1). The amplified product size ranged from 120 to 2500 bp and the total number of bands produced ranged from 5 to 20 with an average of 12 bands per primer. The 22 primers produced 264 amplification products, of which 119 were monomorphic and 145 were polymorphic. The number of polymorphic markers for each primer varied from 1 (primer E15) to 14 (primer M17). The primers M16, E18, H1 and M17 produced greater number (11-14) of polymorphic bands, compared to E15, E3, M6, and E7 which produced only 1-3 polymorphic bands. Different primers showed variation in their ability to detect polymorphism which ranged from 10 to 92.3%. Primer H1 revealed 92.3% polymorphism whereas E15 showed 10% polymorphism. The RAPD profiles produced by primers H1 and E15 are shown in Figure 1.

All the 22 primers were effective in bringing out differences among the 10 foxtail millet genotypes. To better understand the genetic relationships among

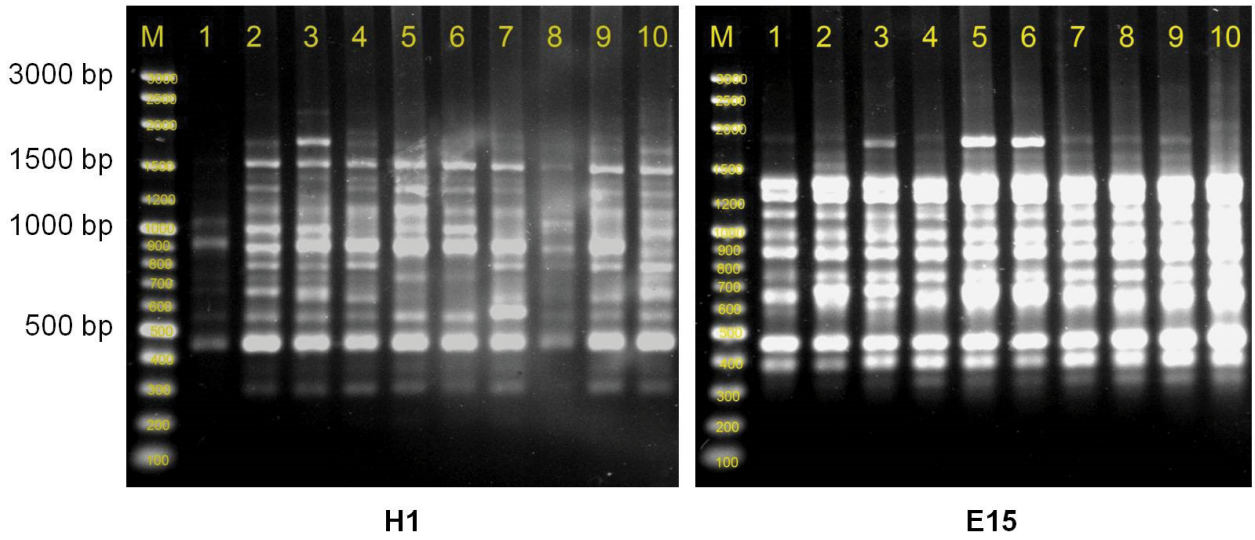

Figure 1. RAPD profiles of 10 foxtail millet genotypes amplified by primer H1 and E15

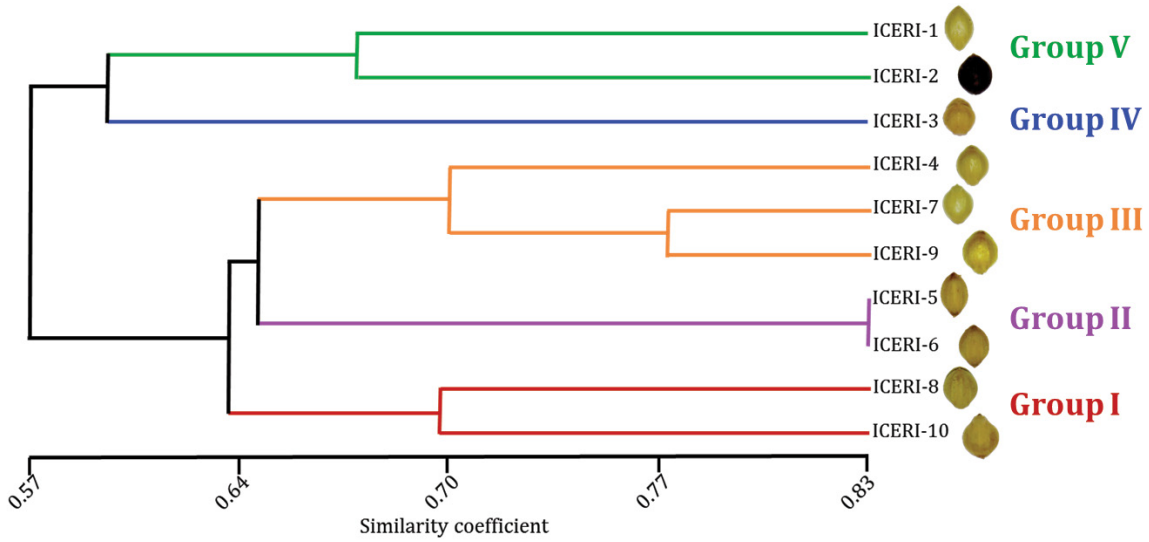

Figure 2. UPGMA dendogram showing the relationship among 10 foxtail millet genotypes

the 10 genotypes, the foxtail millet genotypes were clustered into different defined groups based on their genetic distances (Figure 2).

A dendrogram construction was based on the UPGMA clustering method putting all genotypes in five distinct groups at 0.64 coefficient level. The first group consisted of ICERI-8 and ICERI-10, while the second group consisted of ICERI-5 and ICERI-6. The third group consisted of three genotypes (ICERI-4, 7, and 9), while the fourth group consisted of only one genotypes (ICERI-3). ICERI-3 showed great divergence from the rest of the genotypes and was not included in any of the other groups. The last group consisted of two genotypes (ICERI-1 and ICERI-2). Our previous study showed that ICERI-5 and ICERI-6 were identified as potentially salt-tolerant genotypes based on their germination and seedling growth under salinity (Ardie et al., 2015). It is interesting to note that these two genotypes were grouped together in the Group II.

Conclusion

The result of this study has confirmed that there is a great biodiversity in the 10 Indonesian foxtail millet genotypes evaluated using the 22 RAPD primers. These diverse genotypes can be useful for selective breeding of specific traits and in enhancing the genetic base for the future crop improvement programs.

Acknowledgement

This project was funded by the Ministry of Research, Technology and Higher Education, Republic of Indonesia.

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