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Fingerprinting and Assessing Relatedness of Selected Rice (*Oryza Sativa*) Genotypes in Kenya

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Abstract: *Rice is becoming an important food and cash crop in East Africa and is second to maize in terms of consumption. Genetic markers are very useful in managing germplasm and are greatly applied in biotechnology as breeding machinery in gene mapping and fingerprinting. Eighteen Rice accessions including landraces grown by the farmers and commercially released cultivars were used to assess the genetic diversity . Leaves were taken from two weeks old plants and the DNA extracted according to Mace.et.al., 2003. PCR was done and DNA fragments visualized by illumination device with UV light. . SSR bands were scored as present (1) or absent (0) for each DNA sample, and used to compute the measures of genetic distance for all pairs of individuals. Analysis of Molecular Variance. Results indicated that the five polymorphic markers used in this study showed a total of 11 alleles across the loci of the 18 rice genotype's studied. . The expected heterozygosity ranged from 0.191 in RM 261 to 0.5 in RM 22 with an average of 0.336. Genotypes TXD and B217 were identified by the 5markers*

Key words: *DNA, Fingerprinting, Microsatellites, Fingerprinting, Rice*

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I. Introduction

Rice is an annual grass being used as an important food crop all over the world and is believed to have originated from china (1). Rice is the grain with the third highest production globally after sugarcane and maize (2). . Wild and related genotypes are valuable resources to explore novel variations to widen the genetic background of cultivated rice ; (3,4,5).

Genetic markers can be used to managing germplasm in general and are widely used in biotechnology as a breeding tool (6,7). They are useful in gene mapping and finger printing commercial germplasm (8).Traits that serve as genetic markers are by definition polymorphic; the more polymorphic the trait, the greater its potential value to germplasm management (9). Polymerase chain reaction (PCR) is a procedure where particular portions of the sample DNA are amplified. It imitates the biological process of DNA replication, but confines it to specific DNA sequences of interest. This helps to enhance the discriminating power and the ability to recover information from very small (or degraded) starting samples. Inter-laboratory studies have demonstrated the importance of DNA quantitation on achieving reliable interpretation of typing and obtaining consistent results across laboratories (10). Rice microsatellites are useful for functions such as gene-tagging and marker-assisted selection (11,12).

2. Materials and Methods

Eighteen Rice accessions including forty four landraces grown by the farmers and four commercially released cultivars were used to assess the genetic diversity . Seeds for each accession were sown in trays containing soil and seedlings raised under standard glasshouse conditions at Kenya Bureau of Standards south C between , August and December, 2016. Leaves were taken from two weeks old individual plants, from each accession. DNA extraction was done using Cetyl Trimethyl Ammonium Bromide (CTAB) according to Mace (13)..Out of the 12 markers selected only 5 were polymorphic and were used in amplification of the 18 samples. The amplification was carried out using the profile developed by (14): DNA fragments

2.Data analysis

SSR bands were scored as present (1) or absent (0) for each DNA sample, and used to compute the measures of genetic distance for all pairs of individuals. In addition, genetic diversity was measured by the percentage of polymorphic bands (*PPB*), which was calculated by dividing the number of polymorphic bands at population, region or species levels, by the total number of bands surveyed. In order to describe population structure and variability among populations, the nonparametric Analysis of Molecular Variance (AMOVA) procedure was

used. Dendrogram was constructed showing the relationships between accessions The similarity matrix was converted into dendrogram using UPGMA with a FORTRAN program RAPDPLOT (15,16)

II. Results And Discussion

DNA fingerprints were obtained by coding the amplified fragments either 0 or 1.There were 50 polymorphic bands in total gotten from the 5 pairs of SSR primers. Some of the genotypes could be identified as unique using SSR primers. This shows that SSR primers can be used to clearly distinguish the rice genotypes Genotypes TXD and B217 were identified by 4 out of the 5 markers,

The present study found that SSRs can be used to fingerprint rice DNA and establish the level of relatedness within a population. SSR markers have an advantage over other previously used markers such as Snps due to their cost effectiveness. Successful differentiation of cotton has been achieved using SSR markers(16). Genetic diversity studies on Soybean has also been carried out using SSRs to identify soybean species(18), Past studies have found SSRs as useful in crops like cotton(17)

The phylogenetic tree obtained classified the rice genotypes under study into three major clusters I, II and III. In sub cluster Ia two genotypes Durado precise and Pishori were identical with a bootstrap value of 91%. In Cluster I Kenyan genotype B217 clustered with Tanzanian landraces Kahgo and Red afaa. Similar case of identical genotypes were observed in major cluster II. Mzungu and Ner1 genotypes were identical varieties with a bootstrap value of 64% while Supa saro and ITA 310 were also clustered as identical genotypes with a 53% bootstrap values. Generally, major cluster II had the lowest bootstrap value of 10% in the phylogenetic tree. Major cluster III consisted of two identical genotypes Ner11 and Ner10 in sub cluster IIIb with a bootstrap value of 62%. Tanzanian genotypes Saro 5 clustered with international genotypes as shown in sub cluster IIIa. The bootstrap value included in this phylogenetic tree showed the confidence limits of each clustering (Figure 1).

Results also showed that there were molecular. diversity in the genotypes tested.. When grouped into different populations it was noted that there was less diversity within populations and high diversity betweem populations. This could have been caused. In comparative research, genetic diversity studies in soybean exploited SSRs as useful tools in identifying varying species (18) In another study on Jute plants, SSRs were also found to be very effective in molecular DNA fingerprinting (18,

Gene diversity is also referred to as expected heterozygosity. Rice genotypes studies showed relative heterozygosity. The value obtained was higher than an average of 0.358 that was obtained from a study on 300 rice accessions as reported by Chen (11). This value was also lower than 0.4181 obtained in diversity studies on selected Kenyan and Tanzanian rice based on gel consistency and alkali digestion (19). The low gene diversity value obtained is attributed to presence of a common gene pool amongst the rice varieties studied. Observed heterozygosity was lower than the expected heterozygosity this is attributed to the high inbreeding coefficient. This is evident by the inbreeding coefficient of 1 attained in this study. Similar conclusion have been reported by Musyoki et al. (20). This is attributed to genetic improvement of rice using a common gene pool hence lowering the gene diversity.

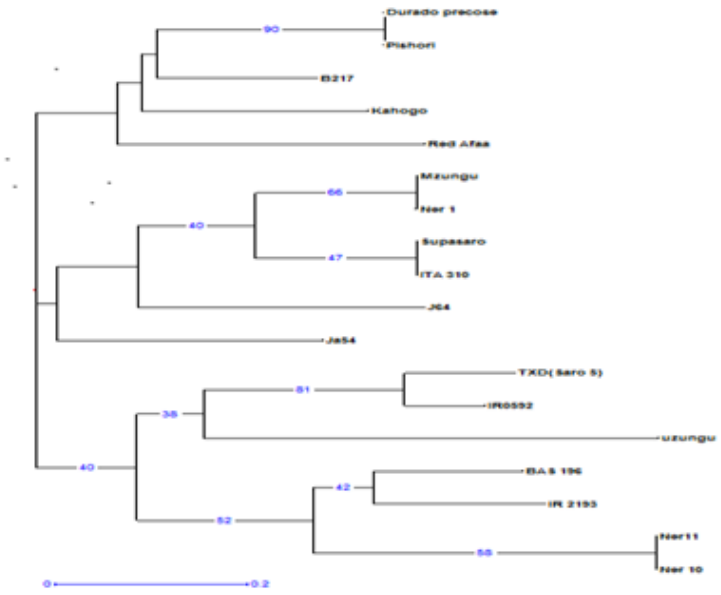

Table 1. Fingerprinting and scoring of genes using SSR markers

Genotype	Region	Marker 1			Marker 2		Marker 3			Marker 4		Marker 5	TOTAL
		100	150	200	100	150	100	150	250	100	150	100	
Uzungu	TZ	0	0	0	0	0	0	1	0	0	0	0	1
Ner 1	KE	1	0	0	1	0	1	0	0	0	0	0	3
IR0592	KE	1	0	0	1	0	0	1	0	0	0	1	4
TXD(Saro 5)	TZ	1	0	0	1	0	0	1	0	1	0	1	5
Pishori	KE	0	0	0	0	1	1	0	0	0	0	1	3
Durado precise	KE	0	0	0	0	1	1	0	0	0	0	1	3
Red Afaa	TZ	0	0	1	0	1	1	0	0	0	1	0	4
Ja54	INT	0	0	1	1	0	1	0	0	0	0	1	2
B217	KE	1	0	0	0	1	1	0	0	0	1	1	5
J64	INT	0	0	0	0	0	1	0	0	0	0	0	1
Kahogo	TZ	0	0	0	0	0	1	0	0	0	1	1	3
IR 2193	INT	0	0	0	0	0	0	0	1	0	0	1	2
BAS 196	KE	1	0	0	0	0	0	0	1	0	0	1	3
Ner 10	KE	0	0	0	0	0	0	0	1	0	0	0	1
Ner11	KE	0	0	0	0	0	0	0	1	0	0	0	1
ITA 310	INT	0	0	0	1	0	1	0	0	0	0	0	2
Supasaro	TZ	0	0	0	1	0	1	0	0	0	0	0	2
Mzungu	TZ	1	0	0	1	0	1	0	0	0	0	0	3
TOTAL		6	0	2	7	4	11	3	4	1	3	9	50

III. Conclusion

There were differences among the genotypes studied as identified by the DNA fingerprints obtained. SSR markers are therefore useful for genetic identification in rice genotypes. The phylogenetic tree generated revealed genetic relatedness based on the five microsatellite markers. The divergence observed by clustering of the rice genotypes into three major clusters is indicative of genetic divergence. The long branches of the Ner 10, Ner 11 and Uzungu justifies the high dissimilarity index observed in the dissimilarity matrix. The long branched observed signifies that these genotypes have undergone much evolutionary change compared to other genotypes under study. This study also revealed that most traded seeds may be genetic replicates even though the names are different. Fingerprints are therefore necessary in ensuring purity and quality assurance by rice traders and farmers.

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