Tanzania Journal of Science 46(3): 828-839, 2020
ISSN 0856-1761, e-ISSN 2507-7961

Assessment of Genetic Diversity of the Wild Rice (*Oryza longistaminata*) Populations in Tanzania Using Microsatellite Markers

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Received 8 Jan 2020, Revised 11 Oct 2020, Accepted 13 Oct 2020, Published Oct 2020

Abstract

The genetic diversity of 132 samples of the perennial wild rice (*Oryza longistaminata*) populations from selected districts (Bagamoyo, Kibaha, Kilombero and Mbarali) in Tanzania was evaluated using twenty eight microsatellite markers. Genomic DNA was extracted using standard protocols. Polymerase Chain Reaction (PCR) was carried out in a total reaction volume of 10 µL, using Touchdown protocol. It was found that all 28 microsatellite markers generated polymorphic patterns, with average Polymorphic Information Content (PIC) value of 0.631. Moreover, a total of 243 alleles were detected by the 28 markers, with average of 8.68 alleles per marker. The percentages of genetic variations among and within populations were 10% and 90%, respectively, implying that there was more gene flow among populations than within populations. Furthermore, the *O. longistaminata* population from Kilombero had highest genetic diversity followed by population from Mbarali, while the lowest genetic diversity was recorded in the population from Kibaha. Generally the populations from Kilombero, Mbarali and Bagamoyo were found to have relatively high genetic diversity, hence have potential to be used in rice breeding. *In situ* conservation of this species is recommended, and it can be achieved by conserving all areas which harbour populations with high levels of genetic diversity.

Keywords: Genetic diversity, Microsatellites, SSR Markers, *Oryza longistaminata*, Wild rice.

Introduction

Oryza longistaminata is the perennial, rhizomatous wild rice species belonging to genus *Oryza*, family Gramineae (Poaceae), which is one of the largest and most widely distributed families of flowering plants. The genus *Oryza* consists of two cultivated species, namely *Oryza sativa* L. and *Oryza glaberrima* Steud. In addition to the two cultivated species, the genus also contains about twenty two (22) wild species (Ukoskit 2004) of which five are indigenous to Africa (Vaughan 1994). The five wild species which are indigenous to Africa are *O. longistaminata* A. Chev. et Roehr, *O. barthii* A. Chev., *O. brachyantha* A. Chev. et Roehr, *O. eichingeri* A. Peter, and *O. punctata* Kotschy ex Steud. The *O. longistaminata* is the most widely distributed wild rice species in Africa, and due to its extensive distribution in Africa it is sometimes referred to as African wild rice. The wide range of distribution of *O. longistaminata* is caused by its perennial growth habit, dual regeneration ability (through seeds and rhizomes) and tolerance to biotic and abiotic stresses (Melaku et al. 2013). Inventory studies and herbarium materials show that Tanzania is a

home to all the five wild rice species. *Oryza longistaminata*, which is the only cross-pollinated wild rice species in Tanzania, belongs to AA genome (Gichuhi 2017). Moreover, this species is regarded as the wild progenitor of the African cultivated rice called *O. glaberrima* (Kiambi et al. 2008).

Oryza longistaminata is one of the most important wild rice species in agriculture as it contains genes for bacterial blight resistance, high pollen production, long stigmas and drought tolerance (Khush et al. 1990, Kiambi et al. 2005). These genes may be introgressed into the cultivated rice through hybridization in order to improve the cultivated rice varieties or cultivars. In some countries, such as Ethiopia and Sudan, *O. longistaminata* is used as famine food and as fodder for livestock (Melaku et al. 2013). In Tanzania, *O. longistaminata* grows naturally in wetlands and swampy areas, such as ponds, rice fields, swamps, irrigation canals or riverbeds in various regions, including Dar es Salaam, Coast, Morogoro, Iringa, Mbeya, Kigoma, Mwanza and Shinyanga (Miezan and Second 1979, Katayama 1990, Mangosongo et al. 2019). However, fragmentation, loss and reduction of natural habitats of this species caused by human activities are among the main threats to the species genetic diversity. Habitat fragmentation and loss are believed to cause reduction or erosion of genetic diversity of *O. longistaminata* in different parts of the world (Kiambi et al. 2008), thus a need for conservation. Application of *in situ* and/or *ex situ* conservation strategies would help to ensure effective conservation of this species.

Possession of agronomically important genes makes the *O. longistaminata* an important reservoir of useful genes for tolerance to biotic and abiotic stresses that may be used in rice breeding programs (Song et al. 2005). Proper utilization of genetic resources from wild rice species in rice breeding may significantly improve cultivated rice cultivars and yield or productivity (Mangosongo et al. 2019). Rice, which is one of the most important cereal crops in the world, is the staple food to about a half of the global population (Song et al. 2003). Similarly, rice is one of the most important food crops in Tanzania, thus improved rice productivity may contribute to food security in the country. According to studies, effective conservation of genetic resources from wild rice species as well as selection and utilization of a wild rice genotype in rice breeding programs require knowledge or understanding on its genetic diversity (Govindaraj et al. 2015, Mangosongo et al. 2019). Moreover, Dashab et al. (2011) asserted that success in breeding programs depends on the amount of genetic variation in the population, thus lack of diversity limits success of any breeding program.

Genetic diversity is the amount of genetic variability among individuals of a variety or population of a species (Brown 1983). It is a measure of the magnitude of genetic variability within a population. Allele frequency, heterozygosity, allelic richness and percentage polymorphic loci are among the measures of within-locus genetic diversity. Genetic diversity results from many genetic differences between or among individuals and can be manifested in differences in DNA sequence, biochemical characteristics, physiological properties, or morphological characters (Rao and Hodgkin 2002). Genetic diversity or variation is fundamental to evolution as it provides basic materials on which adaptation and speciation depend (Amos and Harwood 1998). Variation in genes is necessary for organisms to adapt to ever changing environment. Therefore, populations with high levels of genetic diversity are regarded as healthy, thus conferring ability to respond to threats such as diseases, parasites and environmental changes. Conversely, low levels of genetic variability limits the species ability to respond to these threats in short and long terms. Genetic diversity may be gained through mutation or through gene flow from

neighbouring population and can be lost through genetic drift or through natural selection (Amos and Harwood 1998).

This study evaluated the genetic diversity of *O. longistaminata* populations in four districts of Tanzania using microsatellite markers. The microsatellite markers, which are also known as Simple Sequence Repeats (SSR) markers were preferred in this study due to their technical simplicity, relatively low cost, high power of genetic resolution, co-dominance nature and high polymorphism in plant species (Zhou et al. 2003, Semagn et al. 2006, Melaku et al. 2013). Human activities, including cultivation observed in the study area are likely to cause habitat fragmentation, hence affecting the genetic diversity of the species. Prior to this study, knowledge on the genetic diversity of *O. longistaminata* in the study area was limited and inadequate. This study aimed to provide knowledge on genetic diversity or variations among and within *O. longistaminata* populations in the four districts. Such knowledge is essential for development of proper conservation strategies and for selection of genotypes to be used in rice breeding programs to ensure improved rice quality and yield or productivity.

Materials and Methods

The study area

This study was conducted in four selected districts of Tanzania. The four districts were Bagmoyo, Kibaha, Kilombero and Mbarali (Figure 1). In these districts *O. longistaminata* was sampled in swampy areas, including in the rice fields.

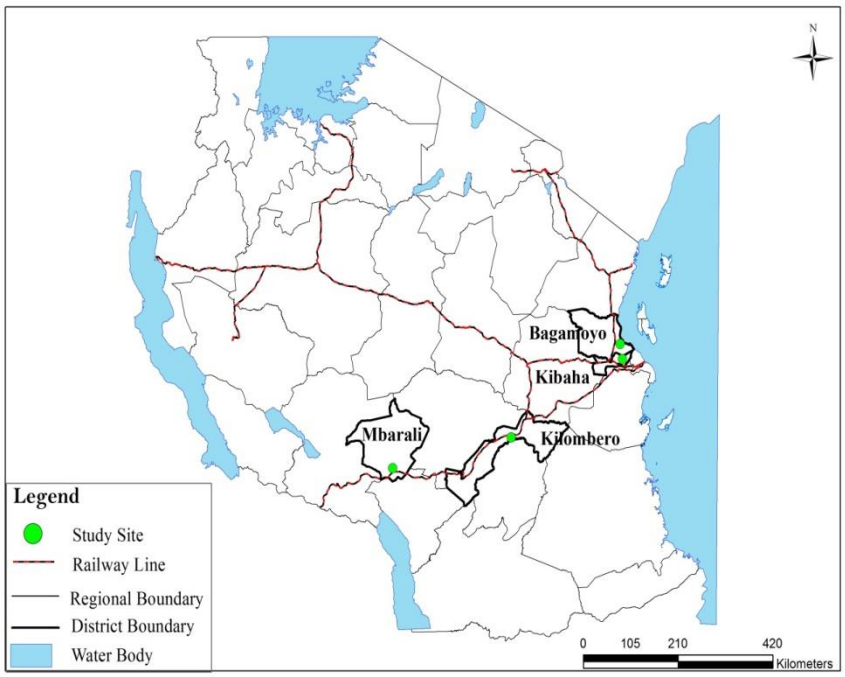

Figure 1: The map of Tanzania showing the location of study sites.

Leaf sampling and DNA extraction

Young leaf samples of *O. longistaminata* were collected from 132 randomly selected individual plants in the four study sites (districts). A total of 33 samples were collected from each site. Two to three newly

formed leaves were collected from each plant. Collected leaf samples were preserved in a zip locked plastic bag containing silica gel before DNA extraction. Genomic DNA (gDNA) was extracted using protocol adopted from Dellaporta et al. (1983), with minor modifications. 100 mg of leaf tissue was powdered by grinding in liquid nitrogen and incubated in extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 100 mM NaCl and 1.25% (w/v) SDS) containing 200 µL of 5 M potassium acetate at 65 °C for 30 minutes. The slurry was extracted with 0.8 µL of chloroform-isoamyl alcohol (24:1 v/v), and the emulsion was centrifuged at 5000 rpm at 4 °C for 15 minutes. Extracted DNA was precipitated from supernatant with 950 g/L alcohol and washed with 700 g/L alcohol three times. After drying, DNA was dissolved in Tris-EDTA, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA) containing 100 µL RNAase.

Polymerase chain reaction (PCR)

PCR was carried out in a programmable thermal cycler (Applied Biosystems 9700). The total reaction volume was 10 µL, containing 2.5 µL of genomic DNA, 5.82 µL of distilled water, 1.0 µL of 10x PCR buffer, 0.2 µL of dNTP, 0.2 µL of forward primer, 0.2 µL of reverse primer and 0.08 µL of Taq polymerase. Temperature cycles were programmed as follows: initial denaturation at 95 °C for 3 minutes followed by denaturation at 94 °C for 30 seconds, then annealing at 64 °C for 1 minute and then extension at 72 °C for 30 seconds (for 10 cycles). The content was then denatured at 94 °C for 30 seconds, annealing at 54 °C for 1 minute, extension at 72 °C for 30 seconds (for 25 cycles), and finally at 72 °C for 20 minutes for final extension. The content was then left to cool at 10 °C to infinite. The PCR products were electrophoresed in 1.8% agarose gels stained with gel red and observed under ultraviolet (UV) trans-illuminator. The PCR product was loaded into the DNA analyser (ABI 3730) for fragment analysis and the resulting fragments were scored on the Genemapper Version 1.41.

The microsatellite markers used and their sequences

A total of twenty eight microsatellite markers or primer pairs, dispersed across the rice genome were used to assess or determine the genetic diversity of the *O. longistaminata* populations from the four districts. The forward and reverse sequences of the microsatellite markers used in this study (Table 1) were downloaded from rice genome database, Rice Genome Microsatellite Markers (http//gramene.org/db/markers.html).

Data analysis

The genetic diversity of *O. longistaminata* samples was estimated on the basis of number of different alleles (Na), allele frequency, number of genotypes, observed heterozygosity (*Ho*) and expected heterozygosity (*He*) using POWER MARKER software package, Version 3.2 (Liu and Muse 2005) to evaluate variability at DNA levels. Other measures or indices of genetic diversity, including Shannon Information index, number of private alleles, and effective number of alleles were also determined for each of the four *O. longistaminata* populations. Polymorphism Information Content (PIC) for each locus was calculated according to Botstein et al. (1980). Analysis of Molecular Variance (AMOVA) was performed to assess the genetic variability within and among *O. longistaminata* populations using the GENALEX Version 6.3 software package (Peakall and Smouse 2006). Population assignment test (With Leave One Out Option) was performed to ascertain whether there was gene flow among populations or not.

Table 1: The list of twenty eight microsatellite markers, their sizes (range) and sequences

Microsatellite markers	Size (bp)	Forward sequence	Reverse sequence
RM297	95-131	TCTTTGGAGGCGAGCTGAG	CGAAGGGTACATCTGCTTAG
RM5427	120-132	CGCTGCTGTTGACACTTGAC	CCAAATACGAGCTCTCCACC
RM300	95-128	GCTTAAGGACTTCTGCGAACC	CAACAGCGATCCACATCATC
RM200	99-121	CGCTAGGGAATTTGGATTGA	CGATGAGCAGGTATCGATGAGAAG
RM569	149-179	GACATTCTCGCTTGCTCCTC	TGTCCCCTCTAAAACCCTCC
RM518	152-166	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
RM7200	142-174	TCGATGGTGACGATGATACG	ACACAACAAAGGGATGGTCC
RM3648	166-194	TACCCTTTCTTCCCCAAACC	ACCTCCTCCTCCACTTCTCC
RM19106	182-198	TTTCACTTGTGAGGGATGAGTCG	GCTCCCAGCATGTTACTCTTTGG
RM31	128-158	GATCACGATCCACTGGAGCT	AAGTCCATTACTCTCCTCCC
RM173	205-223	CCTACCTCGCGATCCCCCCCTC	CCATGAGGAGGAGGCGGCGATC
RM325A	204-222	GACGATGAATCAGGAGAACG	GGCATGCATCTGAGTAATGG
RM204	205-239	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG
RM11	116-130	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG
RM1377	134-174	ATTAGATACATCAGCGGGGG	GCTGCTGTACGATGTGATCC
RM5647	97-121	ACTCCGACTGCAGTTTTTGC	AACTTGGTCGTGGACAGTGC
RM5434	160-176	GTTGATTCTGCGCGAGTTTC	GAAACCGCCCACGCAAAC
RM284	142-148	ATCTCTGATACTCCATCCATCC	CCTGTACGTTGATCCGAAGC
RM231	160-184	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG
RM24545	127-161	ACAGCACAGCACCCGGAAGG	CGAGCAACAGGAAGGCGATAAGC
RM1125	149-195	GGGGCCAGAGTTTTCTTCAG	GTACGCGCAGAAAATGAGAG
RM330A	218-227	CAATGAAGTGGATCTCGGAG	CATCAATCAGCGAAGGTCC
RM332	143-169	GCGAAGGCGAAGGTGAAG	CATGAGTGATCTCACTCACCC
RM202	143-169	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA
RM5746	157-172	TCGCTACGTCGACTGATTTG	ATATCATCAGTCGGCAGCAG
RM7619	147-159	CTTGGTATGTATTGGCAGCG	GAGGCAATAGGAGGGGAGAG
RM19	199-214	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
RM1282	134-175	AAGCATGACAGCTGCAAGAC	GGGGATGAAGGGTAATTTCG

Results

Microsatellites polymorphism and within-locus genetic diversity

All twenty eight microsatellite markers generated polymorphic patterns. Considering the populations separately, the percentage of polymorphic loci in the *O. longistaminata* populations from Bagamoyo, Kibaha, Kilombero and Mbarali were 93%, 96%, 93% and 100% respectively. The loci which did not show polymorphism in Bagamoyo population were RM284 and RM569 while in Kilombero population were RM19 and RM284 and in Kibaha population was RM19. The polymorphic information content (PIC) of the twenty eight microsatellite markers varied from 0.051 (RM284) to 0.907 (RM1125) with an average of 0.631. The observed heterozygosity of the markers ranged from 0.023 (RM284) to 0.826 (RM1125) with the average of 0.363. A total of 243 alleles were detected by the twenty eight microsatellite markers. The number of alleles per locus varied from 3 (RM284 and RM5746) to 22 (RM1125) with an average of 8.68 alleles per locus. The allele frequency for the markers varied from 0.205 (RM1125) to 0.973 (RM 284) with the average of 0.443. The expected heterozygosity (gene diversity) detected by the markers ranged from 0.052 (RM284) to 0.913 (RM1125) with an average of 0.443. The number of *O. longistaminata* genotypes ranged from 4 (RM284) to 44 (RM1125). The summary of the number of alleles generated by each primer, number of genotypes, heterozygosity and the PIC values is presented in Table 2.

Table 2: Summary results on the genetic diversity indices of *Oryza longistaminata* samples from the study area as revealed by 28 microsatellite markers

Marker	Allele frequency	Genotype number	Allele number	Expected heterozygosity	Observed heterozygosity	PIC
RM11	0.314	17.000	8.000	0.771	0.470	0.736
RM19	0.955	6.000	5.000	0.088	0.061	0.087
RM31	0.288	24.000	13.000	0.825	0.348	0.803
RM173	0.318	24.000	10.000	0.817	0.432	0.795
RM200	0.292	27.000	10.000	0.822	0.682	0.800
RM202	0.367	23.000	9.000	0.763	0.386	0.730
RM204	0.352	21.000	10.000	0.754	0.576	0.719
RM231	0.489	18.000	8.000	0.661	0.455	0.611
RM284	0.973	4.000	3.000	0.052	0.023	0.051
RM297	0.208	34.000	14.000	0.867	0.667	0.853
RM300	0.511	11.000	7.000	0.545	0.621	0.444
RM325A	0.489	15.000	7.000	0.677	0.591	0.634
RM330A	0.580	8.000	4.000	0.592	0.106	0.542
RM332	0.587	13.000	8.000	0.587	0.508	0.538
RM518	0.390	11.000	7.000	0.687	0.098	0.632
RM569	0.917	6.000	5.000	0.158	0.045	0.154
RM1125	0.205	44.000	22.000	0.913	0.826	0.907
RM1282	0.383	7.000	5.000	0.713	0.061	0.663
RM1377	0.239	43.000	14.000	0.888	0.606	0.880
RM3648	0.227	29.000	11.000	0.852	0.477	0.835
RM5427	0.428	10.000	5.000	0.676	0.045	0.619
RM5434	0.277	20.000	9.000	0.821	0.417	0.800
RM5647	0.231	21.000	9.000	0.827	0.462	0.805
RM5746	0.879	4.000	3.000	0.216	0.061	0.197
RM7200	0.311	26.000	11.000	0.831	0.288	0.813
RM7619	0.780	9.000	6.000	0.375	0.129	0.355
RM19106	0.223	26.000	9.000	0.827	0.598	0.804
RM24545	0.182	18.000	11.000	0.879	0.129	0.867
Average	**0.443**	**18.536**	**8.68**	**0.660**	**0.363**	**0.631**

Comparison of genetic diversity of the four *Oryza longistaminata* populations

When the four *O. longistaminata* populations were compared based on various measures or indices of genetic diversity, the results showed that the population from Kilombero was the most diverse followed by a population from Mbarali, while population from Kibaha was the least diverse. With exception of the number of private alleles, all other indices of genetic diversity assessed were highest in the population from Kilombero and lowest in the population from Kibaha. On the other hand, the number of private alleles was highest in the population from Mbarali and lowest in the population from Bagamoyo. A summary of the results on comparison of the four populations based on various measures of diversity is presented in Table 3.

The genetic variation within and among the populations

Analysis of Molecular Variance (AMOVA) results showed that the populations had much more genetic variations within populations than among populations, as indicated in Figure 2.

Table 3: Comparison of *O. longistaminata* populations based on various indices of genetic diversity

Parameters	Study site (District)			
	Bagamoyo	Kibaha	Kilombero	Mbarali
Number of different alleles	5.250 ± 0.539	4.821 ± 0.353	6.321 ± 0.632	6.214 ± 0.576
Number of effective allelles	3.210 ± 0.294	2.928 ± 0.260	3.745 ± 0.386	3.559 ± 0.404
Shannon's information index	1.179 ± 0.106	1.111 ± 0.098	1.313 ± 0.128	1.268 ± 0.120
Number of private alleles	0.107 ± 0.079	0.464 ± 0.131	0.750 ± 0.175	1.107 ± 0.220
Expected heterozygosity	0.596 ± 0.044	0.561 ± 0.046	0.609 ± 0.053	0.599 ± 0.049

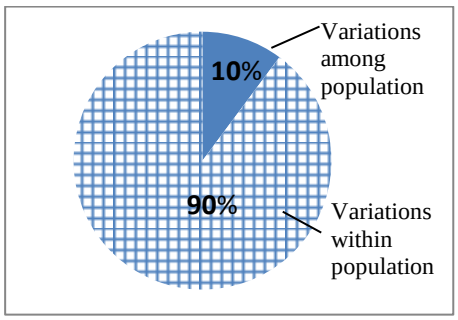

Figure 2: Partitioning of genetic variations within and among populations.

Population assignment test results

Population assignment test or analysis results showed that the percentages of number of individuals sampled from Bagamoyo, Kibaha, Kilombero and Mbarali that were assigned to self-population were 76%, 97%, 82% and 91%, respectively, while the percentages of individuals assigned to other populations were 24%, 3%, 18% and 9% respectively, as shown in Table 4.

Table 4: The results on population assignment to 'Self' or 'Other' populations

Population/ Study site	Individuals assigned to self-population		Individuals assigned to other-populations	
	Number	Percent (%)	Number	Percent (%)
Bagamoyo	25	76	8	24
Kibaha	32	97	1	3
Kilombero	27	82	6	18
Mbarali	30	91	3	9

Discussion

Microsatellites polymorphism and genetic diversity

The twenty eight microsatellite markers used in this study generated polymorphic patterns, whereby the degree of polymorphism varied among the loci. The degree of polymorphism of a marker, which reflects its informativeness was measured as Polymorphic Information Content (PIC) (Chesnokov and Artemyeva 2015). In this study, the PIC of the markers ranged from 0.051 (RM284) to 0.907 (RM1125) with an average of 0.631. The implication of these results is that most of the microsatellite markers used in this study had high power to detect genetic variations among and within *O. longistaminata* populations. Based on their PIC values, the best markers were RM1125, RM31, RM200, RM297, RM1377, RM3648, RM5434, RM5647, RM7200, RM19106 and RM24545. According to Dashab et al. (2011) a marker is regarded as highly polymorphic when it has the PIC value of 0.5 or above. The microsatellite markers used in this study demonstrated high ability in detecting genetic variations in this species, thus are suitable for studies on genetic variations within and among *O. longistaminata* populations. This finding is similar to what was reported by Chesnokov and Artemyeva (2015) that SSR markers are better markers for studies on genetic diversity of plant populations compared to other markers. This is because

Simple Sequence Repeats, which are widely distributed across all eukaryotic genomes, have high sensitivity to detect alleles compared to other molecular markers (Kuroda et al. 2007). High level of polymorphism associated with SSR markers may be due to the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Powell et al. 1996, Zhou et al. 2003). Many recent studies on genetic diversity revealed that microsatellites possess hypervariability and higher resolving power among various molecular markers (Guadagnuolo et al. 2001, Zhou et al. 2003), resulting into high polymorphism.

Based on the allele frequency, number of different alleles and number of genotypes, results of the present study showed that the study area harbours a relatively high genetic diversity of *O. longistaminata*. The allele frequency, which refers to the number of times an allele occurs in a population or populations seemed to be inversely related to polymorphism (PIC) of the markers, such that more polymorphic markers generated relatively lower allele frequency than less polymorphic (monomorphic markers), which generated higher allele frequencies. This is similar to what was reported in the study by Sun et al. (2001) that more polymorphic markers can detect a large number of different alleles each with low frequency of occurrence, while less polymorphic (monomorphic) markers detect few alleles each with high frequency of occurrence. High polymorphism in plant populations, as reflected by low allele frequency is an indicator of existence of high genetic variations in the populations concerned (Sun et al. 2001). Most of the markers used in the present study exhibited high polymorphism, resulting in relatively low allele frequencies (average allele frequency was 0.443), and implying availability of high genetic diversity. Based on the number of different alleles, a total of 243 alleles were detected by the twenty eight microsatellite markers among the 132 *O. longistaminata* samples, hence the average number of alleles per marker was 8.68. The number of alleles reflects the abundance of genes in a population (Sun et al. 2001), thus a large number of alleles implies high abundance of genes and therefore high genetic diversity. The large number of different alleles detected in this study also implies availability of high genetic diversity in the studied *O. longistaminata* populations. The average number of alleles per marker recorded in this study is also slightly high compared to that reported by Mwangi (2011) in his study on the genetic diversity of *O. longistaminata* populations from Kenya in which the average number of alleles per marker was 8.0.

On the basis of expected and observed heterozygosity, the study revealed that *O. longistaminata* populations in the study area are under deficiency of heterozygotes. Expected heterozygosity refers to the heterozygosity expected if the population is in Hardy-Weinberg equilibrium (Pagnotta 2018), while observed heterozygosity is the proportion of heterozygous individuals in the populations (Sun et al. 2001). Rao and Hodgkin (2002) pointed out that heterozygote deficiency in a plant population may be caused by various factors, including unrecognized genetic structure within populations, inbreeding, which may result from consanguineous mating, and presence of null (non-amplifying) alleles. Null alleles may be caused by divergence in the sequences flanking the microsatellites as well as undetectable or unquantified DNA temperate, thus causing heterozygotes to be wrongly scored as homozygotes (Smulders et al. 1997). However, unrecognized genetic structure is unlikely to be the cause of heterozygosity deficit revealed in this study due to hyper-variability and higher resolution power of the microsatellite markers used (Zhou et al. 2003). From the results, mating among genetically related individuals (inbreeding) is most likely to be the main

cause of heterozygotes deficiency within *O. longistaminata* populations in the study area.

When the four populations were compared, the results revealed that there were variations in the extent of genetic diversity among populations. Generally, the *O. longistaminata* population from Kilombero was the most diverse followed by the population from Mbarali, whereas the population from Kibaha was the least diverse. Studies show that differences in levels of genetic variations between or among populations may be caused by a number of factors, including mutation rate, breeding system, migration and dispersal mechanisms (Hamrick and Godt 1989, Forrest et al. 2011), as well as biotic and abiotic selection intensities which are determined by location, climate and soil characteristics (Nevo et al. 1979, Kark et al. 1999, Parsons et al. 1999, Kiambi et al. 2005). A relatively low genetic diversity recorded in *O. longistaminata* population from Kibaha, which unlike other three populations investigated, was small in size may be due to its small size of the population. According to Rao and Hodgkin (2002), the smaller the population size, the lower is its genetic diversity. Moreover, the amounts of genetic diversity found in geographically or ecologically marginal areas of a crop or species may be very much reduced due to small population size and reduced gene flow (Yen 1991). However, such populations may possess unique genes which confer resistance to stresses or diseases. This is because when a population grows in ecologically or geographically isolated area, various selection pressures and genetic drift at different habitats result in local adaptations where population-specific allelic genes evolve and eventually fixed in a limited area (Hamrick and Godt 1989).

The genetic diversity within and among populations

The study further revealed that the genetic variation within *O. longistaminata* populations was higher than variation among the populations. The percentages of genetic variations within and among *O. longistaminata* populations were 90% and 10%, respectively. Studies show that partitioning of genetic variations within and among populations is related to the breeding system of the species under consideration (Rao and Hodgkin 2002, Nybom 2004). This is because breeding system affects allele distribution in plant populations. Generally, in-breeding species are characterized by high levels of genetic variation among populations and low genetic variation within population, whereas out-breeding (or out-crossing) species are characterized by low genetic variation among populations and high genetic variation within population (Kiambi et al. 2005). Existence of higher genetic variation within populations than among populations observed in the present study seems to be similar to what have been found in several other studies on various out-crossing species which demonstrate higher genetic variation within populations than among populations (Hamrick and Godt 1989, Kiambi et al. 2005, Mwangi 2011, Melaku et al. 2013). Out-crossing characteristic is genetically advantageous to plants, as it enables plant populations to maintain high levels of genetic diversity (Perera et al. 1998). Another factor which can also be used to account for low genetic variation among populations is the rate of gene flow among populations, whereby high rate of gene flow among populations may lead to low genetic differentiation among populations (Ukoskit 2004). On the other hand, a limited gene flow through cross pollination may be responsible for high levels of genetic differentiation. Therefore, low genetic variation among *O. longistaminata* populations revealed in the study area may be associated with occurrence of gene flow among the populations.

Genetic relationship and gene flow among populations

Results of the present study based on population assignment tests (individual-based

assignment tests), which assign individuals probabilistically to candidate populations by their multi-locus genotype (Berry et al. 2004), revealed that the percentage number of individuals from the four populations, Bagamoyo, Kibaha, Kilombero and Mbarali that were assigned to self-population were 76%, 97%, 82% and 91%, respectively. The implication of these results is that most of the individuals sampled from each of the districts had higher genetic relationship or similarity to each other than to individuals from other districts. On the other hand, population assignment tests which are widely believed to hold the potential in estimating contemporary rates of gene flow and dispersal (Waser and Strobeck 1998) assigned some of the individuals from each of the four districts to other districts (i.e. to districts other than where they have been sampled). According to the results, the percentage number of individuals from Bagamoyo, Kibaha, Kilombero and Mbarali that were assigned to other populations were 24%, 3%, 18% and 9% respectively, implying these individuals showed genetic similarity to individuals from other districts or populations. The observed genetic similarity among individuals from different districts or populations implies occurrence of gene flow among populations. Gene flow between two or more populations can be achieved through pollen or through seed dispersal by wind, water or animals. However, due to long geographic distance separating the four districts, the gene flow among *O. longistaminata* populations in the study area reported in the present study is most likely to occur through seed dispersal or transfer. This is because the geographic distance between the populations (or districts) is too long, making the gene flow through pollen unlikely. According to Hamrick and Godt (1989), gene flow between or among populations of a species is important for maintenance of high genetic variability within populations. Therefore, high levels of genetic diversity in *O. longistaminata* populations revealed by this study may be associated by occurrence of gene flow among populations. For *in situ* conservation of genetic resources of this species, the conservation priorities should be given to areas which experience high levels of threats to erosion of the genetic diversity of the species or areas harbouring populations with rare alleles and to populations with high levels of genetic diversity.

Conclusion

In the light of the findings of this study, it can be concluded that most of the microsatellite markers used in this study were highly informative and suitable for studies on genetic diversity of *O. longistaminata* populations. Among the markers used in the present study, RM1125 and RM1377 were the most informative, while RM 284 and RM569 were the least informative. Moreover, the four *O. longistaminata* populations in the study area contain a considerable amount of genetic diversity, in which genetic variation was higher within populations than among populations. The four *O. longistaminata* populations in the study area are connected by gene flow which is probably accomplished by various agents of seed dispersal or transfer, including wind, water and animals. The *O. longistaminata* populations from Kilombero, Mbarali and Bagamoyo had relatively high genetic diversity, thus are potentially suitable for utilization in rice breeding. The *in situ* conservation of the four *O. longistaminata* populations is recommended for protection of genetic resources from this species.

References

Amos W and Harwood J 1998 Factors affecting levels of genetic diversity in natural populations. *Phil. Trans. R. Soc. Lond. B.* 353: 177-186.

Berry O, Tocher MD, Sarre SD 2004 Can assignment tests measure dispersal? *Mol. Ecol.* 13: 551-561.

Botstein D, White RL, Skolnick M and Davis RW 1980 Construction of a genetic

linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-331.

Brown WL 1983 Genetic diversity and genetic vulnerability - an appraisal. *Econ. Bot.* 37(1): 4-12.

Chesnokov YV and Artemyeva AM 2015 Evaluation of the measure of polymorphism information of genetic diversity. *Agric. Biol.* 50(5): 571-578.

Dashab GR, Aslaminejad A, Nassiri M, Esmailizadeh AK, Saghi DA 2011 Analysis of genetic diversity and structure of Baluchi sheep by microsatellite markers. *Trop. and Subtrop. Agroecosyst.* 14: 1047-1054.

Dellaporta SL, Wood J and Hicks JB 1983 A plant DNA minipreparation Version II. *Plant Mol. Biol. Rep.* 1(4): 19-21.

Forrest CN, Ottewell KM, Whelan RJ and Ayre BJ 2011 Tests for inbreeding and outbreeding depression and estimation of population differentiation in bird-pollinated shrub, *Gravillea mucronulata*. *Ann. Bot.* 108: 185-195.

Gichuhi EW 2017 *Genetic analysis for high productivity derived from Oryza longistaminata under low-input conditions in rice.* PhD dissertation, Okayama University.

Govindaraj M, Vetriventhan M, Srinivasan M 2015 Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet. Res. Int*. 2015: 1-14.

Guadagnuolo R, Bianchi DS, and Felber F 2001 Specific genetic markers for wheat, spelt and four wild relatives: comparison of isozymes, RAPDs and wheat microsatellites. *Genome* 44: 610-621.

Hamrick JL and Godt MJW 1989 Allozyme diversity in plants species. In: Brown AHD, Clegg MT, Kalher AL and Weir BS (eds) Plant Population Genetics, Breeding and Genetic Resources. Sinauer, Sunderland, Massachusetts. pp 42-63.

Kark S, Alkon PU, Safriel UN and Randi E 1999 Conservation priorities for chukar partridge in Israel based on genetic diversity across an ecological gradient. *Conserv. Biol.* 13(3): 542-552.

Katayama TC 1990 Wild rice of Tanzania collected in 1984 and 1988. *Occas. Pap.* 18: 181-192.

Kiambi DK, Ford-Lloyd BV, Jackson MT, Guarino L, Maxted N and Newbury HJ 2005 Collection of wild rice (*Oryza* L.) in east and southern Africa in response to genetic erosion. *Plant Genet. Resour. Newslett.* 142: 10-20.

Kiambi DK, Newbury HJ, Maxted N and Ford-Lloyd 2008 Molecular genetic variation in the African wild rice *Oryza longistaminata* A. Chev. et Roehr. and its association with environmental variables. *Afr. J. Biotechnol.* 7(10): 1446-1460.

Kuroda Y, Sato YI, Bounphanousay C, Kono Y and Tanaka K 2007 Genetic structure of three *Oryza* AA genome species (*O. rufipogon, O. nivara* and *O. sativa*) as assessed by SSR analysis on the Vientiane Plain of Laos. *Conserv. Genet*. 8: 149-158.

Khush GS 1990 Report of meeting to discuss chromosome numbering system in rice. *Rice Genet. Newslett.* 7: 12-15.

Liu K and Muse SV 2005 PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21(9): 2128-2129.

Mangosongo HM, Lyaruu HV and Mneney EE 2019 Agro-morphological characterization of the wild rice (*Oryza longistaminata*) populations from selected areas of Tanzania. *Tanz. J. Sci.* 45(1): 9-17.

Melaku G, Haileselassie T, Feyissa T and Kiboi S 2013 Genetic diversity of the African wild rice (*Oryza longistaminata* Chev. et. Roehr) from Ethiopia as revealed by SSR markers. *Genet. Resour. Crop Ev.* 60: 1047-1056.

Miezan K and Second G 1979 Prospection of the traditional varieties and wild species of rice in Tanzania. *Mission Report*.

Mwangi JN 2011 *Genetic structure analysis of wild rice populations (Oryza longistaminata) from Kwale district of Kenya using microsatellite markers.* MSc. Thesis, University of Nairobi.

Nevo E, Zohary D, Brown AH and Haber H 1979 Genetic diversity and environmental associations of wild barley *Hordeum spontaneum* in Israel. *Evolution* 33(3): 815-833.

Nybom H 2004 Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Mol. Ecol.* 13: 1143-1155.

Pagnotta MA 2018 Comparison among methods and statistical software packages to analyze germplasm genetic diversity by means of codominant markers. *Multidiscip. Sci. J.* 1: 197-215.

Parsons BJ, Newbury HJ, Jackson MT, Ford-Lloyd BV 1999 The genetic structure and conservation of *aus, aman* and *boro* rices from Bangladesh. *Genet. Resour. Crop Ev*. 46: 587-598.

Peakall R and Smouse PE 2006 GENALEX 6: Genetic analysis in excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* 6(1): 288-295.

Perera L, Russell JR, Provan J, McNicol JW and Powell W 1998 Evaluating genetic relationships between indigenous coconut (*Cocos nucifera* L.) accessions from Sri Lanka by means of AFLP profiling. *Theor. Appl. Genet.* 96: 545-550.

Powell W, Machray GC and Provan J 1996 Polymorphism revealed by short sequence repeats. *Trends Plant Sci.* 1: 215-222.

Rao VR and Hodgkin T 2002 Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell Tissue Organ Cult.* 68: 1-19.

Semagn K, Bjørnstad A and Ndjiondjop MN 2006 An overview of molecular marker methods for plants. *Afr. J. Biotechnol.* 5: 2540-2568.

Smulders MJM, Bredemeijer G Rus-Kortekass W, Arens P and Vosman B 1997 Use of short microsatellites from database sequences to generate polymorphism among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor. Appl. Genet. 97: 264-272.

Song ZP, Lu BR, Zhung YG, Chen JK 2003 Gene flow from cultivated rice to the wild species *Oryza rufipogon* under experimental field conditions. *New Phytol*. 157: 657-665.

Song ZP, Li B, Chen JK and Lu BR 2005 Genetic diversity and conservation of common wild rice (*Oryza rufipogon*) in China. *Plant Species Biol*. 20: 83-92.

Sun CQ, Wang XK, Li ZC, Yoshimura A. and Iwata N 2001 Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. *Theor. Appl. Genet.* 102: 157-162.

Ukoskit K 2004 Genetic diversity of Wild Rice (*Oryza* spp.) in the Northern region of Thailand. *Thammasat Int. J. Tech.* 9: 11-20.

Vaughan DA 1994 The wild relatives of rice: a genetic resources handbook. International Rice Research Institute, Los Banos, Philippines.

Waser PM and Strobeck C 1998 Genetic signatures of interpopulation dispersal. *Trends Ecol. Evol.* 13: 43-44.

Yen DE 1991 Domestication: lessons from New Guinea. In: Pawley AK (ed) Man and a half: Essays in Pacific anthropology and ethnobiology in honour of Ralph Bulmer, Auckland. *Polyn. Soc.* 558-569.

Zhou HF, Xie ZW and Ge S 2003 Microsatellite analysis of genetic diversity and population genetic structure of a wild rice (*Oryza rufipogon* Griff.) in China. *Theor. Appl. Genet.* 107: 332-339.