GENETIC DIVERSITY OF SOME RICE (*Oryza sativa* L.) LANDRACES GROWN IN TANZANIA USING SIMPLE SEQUENCE REPEAT (SSR)

BY

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ABSTRACT

Simple sequence repeat (SSR) microsatellite markers were used to analyze genetic variation of 70 samples of Rice (*Oryza sativa* L.) Landraces grown in Tanzania. The objective of the study was to assess the extent of genetic variation in traditional rice varieties and establish their genetic relationships for developing breeding strategies. Ten (10) SSR markers were found to be polymorphic with a minimum of 1 and a maximum of 6 alleles. Variability in the number of alleles was obtained with an average of 4.9 alleles per locus. The genetic analyses were carried out using the NTSYs pc (Version 2.02) Software and a cluster diagram constructed base on genetic distances detected between landraces by the UPGMA method. Results showed a large amount of variability within *Oryza sativa* species. The overall pattern of variation as well as the degree of relatedness among accessions is shown in the dendograms generated from similarities or genetic distance matrices. Rice landraces Lifumba, Tunduru and Dunduli ya mlimani had wider genetic base with distant coefficient range of 0.66-0.98. It is advocated to include them in the National Rice Improvement Programme in Tanzania. Some landraces had narrow genetic base range of 0.76-0.98 distant coefficient. Rice landraces Katumahi Simzito, Tuliani and Afaa Kikangaga, were found to be genetically distant at 0.98 coefficient and can be used for intended breeding purposes. The reaction of landraces to RYMV infection was evaluated using three strains of the virus. The tested strains were S4 ex Kyela, S6 ex Mang'ula, and S6 ex Dakawa. Most of landraces were susceptible to RYMV. However, only a few landraces expressed high levels of resistance to all three strains, characterized by lack of symptoms until harvest. These landraces were Afaa Mzinga,

Lingwelingweli, Mbega, Jambo twende, Kalibumbula, Msonga, Mzinga, Lunyuki, and Tosa can be used for breeding purposes.

DECLARATION

I, Bakari Mndolwa Mohamed, do hereby declare to the Senate of SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO (SUA), that from the best of my knowledge this dissertation is my own original work, and has never been submitted to any other university.

SIGNATURE OF STUDENT…….DATE……

SIGNATURE OF SUPERVISOR…… DATE……..

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DEDICATION

To my beloved wife Elizabeth, my children Mndolwa, Tamaly, Said, Neema, Kusaka, Ibrahim Chamdoma and Daudi. Special dedication goes to Mkufu, Mama Zaina, Albertina and beloved mother Chabezigwa. You are so blessed.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree centigrade
µl	Micro liter
AFLP	Amplified Fragment Length Polymorphism
Bp	base pair
D	Dead
DNA	Deoxyribonucleic acid
dNTP	deoxyribonucleic triphosphate
EDTA	ethylenediamine tetraacetic acid
EtOH	Ethanol
FAO	Food and Agriculture Organization
HCl	hydrochloric acid
IPGRI	International Plant Genetic Resource Institute
JC	Jaccard's coeffient
kb	kilobase
Kcl	potassium chloride
M	marker
mA	milli Ampere
mg	Milligram
MgCl	magnesium chloride
ml	millilitre
Min	Minute
m–M	Milli molar
NaCl	sodium chloride

NB	Note ben
n–g	nanogram
NTSYs	numerical taxonomy and multivariate analysis system
PCA	Principle component analysis
PCR	polymerase chain reaction
pH	Hydrogen ion concetration
RAPD	random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic Acid
RM	Rice maker
Rpm	Revolutions per minute
RYMV	Rice yellow mottle virus
s	seconds
S4	Ex- Kyela
S6	Ex- Dakawa
S6m	Ex- Mang′ula
SDS	Sodium dodecyl sulfate
SSR	simple sequence repeats
Tag	*themus aquaticus*
TBE	tris-borate EDTA
TE	tris- EDTA
Tris	tris(hydroxymethyl)methylamine
UPGMA	unweighted pair group method with arithmetic
UVL	Ultraviolet light
v	Scored mark

V	Volt
v/v	volume by volume

CHAPTER ONE

1.0 INTRODUCTION

1.1 Origin and Distribution

Rice belongs to the genus *Oryza* under the family *Graminae*. The genus *Oryza* comprises several species distributed throughout tropical and subtropical regions. *Oryza* has two cultivated and 22 wild species. The cultivated species are *Oryza* sativa and *Oryza glaberrima*. *Oryza sativa* is grown all over the world while *Oryza glaberrima* has been cultivated in West Africa for the last 3500 years. *Oryza graberrima* is an upland crop but is being replaced by *O. sativa* which matures earlier and also gives high yields (IRRI, 1988; FAO, 1994).

1.2 Botanical and Genetic Characteristics

Oryza sativa varieties have been separated into Indica, Japonica and Bulu (Javanica) types based on their places of origin. The majority of rice varieties grown in Tanzania are Indica types which is tropical rice. Rice is grown under many different conditions and production systems, but submerged in water is the most common method used worldwide. Rice is the only cereal crop that can grow for long periods of time in standing water because it has an efficient system of air passage from shoot to root.

Rice can be grown in mixed and multiple cropping patterns with crops like wheat and soybeans (Swaminathan, 1984). Traditional rice varieties require about 150 days of growth to reach the mature grain stage whereas the modern, high yielding and early maturing varieties can be harvested in 90 days after sowing (Heinrichs, 1994).

1.3. Importance of Rice

Rice is a stable diet of over half of the world's population. In 2 000 598 million metric tones were produced from 153 hectares at an average yield of 3895 kg/ha (FAO, 2001). Rice is unique among the important grain plants because it is the only cereal that is almost always cooked and eaten as whole grain (Jonson, 1985). The consumption grain per capital varies from 186 kg/year in Burma to 4 kg/year in USA (IRRI, 1988). In Tanzania rice has many uses: the grain being used as both human food and as cash crop, while stalks are used as animal feed and for thatching, husks as litter in poultry and as mulch.

1.4 Rice Production

Rice is a popular food almost everywhere in Africa, south of Sahara (FAO, 1994) and the demand for rice in Sub-Sahara Africa is becoming higher due to a general dietary shift from conventional foods (Houston, 1972). Major rice producing areas of Africa are concentrated in the Eastern and Western parts of the continent. Tanzania is the second largest producer and consumer after Madagascar in East, Central and Southern Africa (ECSA) region (Chinganga, 1985; FAO, 2001). Tanzania operates an agrarian economy and rice is considered second to maize both in production (Fig.1) and consumption (Ministry of Agriculture, 1999). Rice in Tanzania is grown in all regions of the country but at varied levels of importance. It is mainly grown by peasant farmers under varied ecological conditions. Kanyeka *et al.* (1995) reported that rice production in lowland and upland ecosystem is 80% and 20% respectively, with lowland production consisting of 74% rainfed and 6% irrigated. It is grown in swampy areas and river basins such as the Rufiji, Ruvu in Coast region, Kilombero, and Wami, in Morogoro region Pangani and Mombo in Tanga region. It is also

cultivated in Shinyanga, Mwanza and Kigoma region (Monyo and Kanyeka, 1978). The demand for rice as a staple food in Africa especially in Tanzania is increasing where self sufficiency in rice production is declining as demand increases. Therefore there is a need to improve the production of rice in Africa in order to cope with the high demand (Ogunbayo *et al.* 2005).The swelling high imbalance between rice production and consumption has compelled this country to import rice despite its vast potentials for rice production.

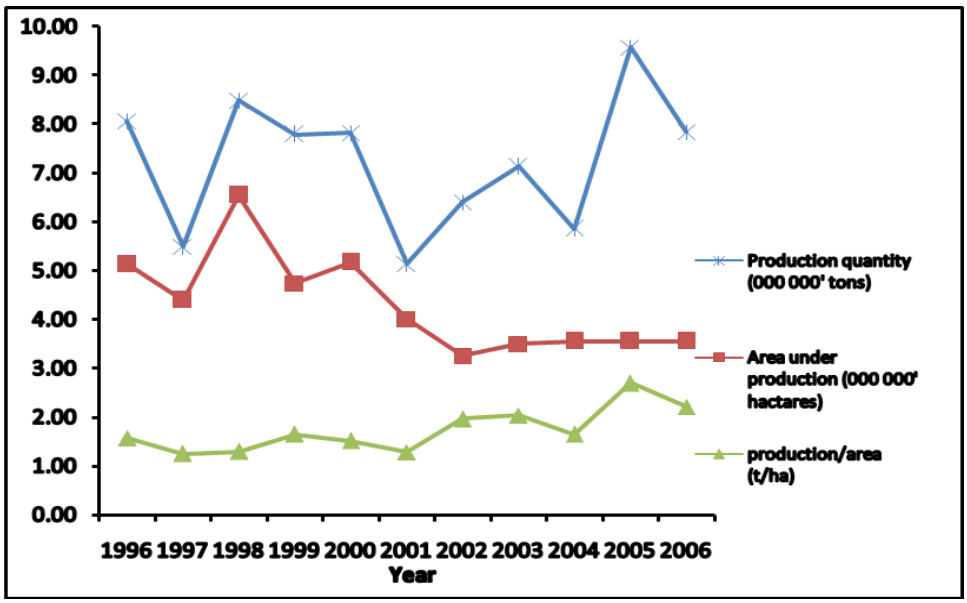


Figure 1: Rice production trend in Tanzania (1996 to 2006) Source: FAO (2006).

In order to improve rice production, equal emphasis is required on cultural practices and management as well as suitability of rice varieties. Evaluation and characterization of landraces should form an important constituent of collection efforts because of their enormous in-built genetic diversity due to several generations of growing and selection by breeders and farmers. The landraces constitute a good source of unique genes for stress tolerance, quality of grain and adaptability to stressful environments (Ogunbayo *et al.* 2007).

1.5 Occurrence and Importance of Rice Yellow Mottle Virus (RYMV).

RYMV belongs to the *Sobemovirus* group and it is the main virus affecting the rice crop in lowland ecosystems. This virus is restricted to the African continent and causes severe yield losses in farmers' fields Abo *et al.* (1998). RYMV is transmitted by several species of beetles and is not seed-borne Banwo *et al*. (2001). The disease is characterized by mottling and yellowing of the rice leaves depending on the genotype. Although diseased plants usually survive, they are severely stunted and produce fewer tillers. Flowering is delayed, with incomplete emergence of panicles and unfilled and discolored grains Bakker, (1974). The disease severity depends on genotype, time of infection, presence of host plants, presence of the chrysomelid beetle vectors and cultural practices (Bakker,1970). Under natural conditions, yield losses up to 90% have been reported Kanyeka *et al*. (1996). One of the first reports on incidence of RYMV in mainland Tanzania was in Mkindo irrigation scheme Kanyeka *et al*. (1996). Although it is thought to have been noted much earlier. Recent surveys conducted in Tanzania (Ali, 1999). Show that RYMV is rapidly spreading between and within the major rice growing areas of Tanzania including Mbeya, Mwanza and Shinyanga. RYMV is known to be naturally very destructive and therefore threatening rice production in Tanzania and the African continent at large.

Despite the big losses caused, no forces has been applied to control the disease, Today, cultural practices and prophylactic methods help reduce the negative impact of RYMV in rice production. However, these methods have to be combined with

resistance breeding to be more efficient. Partial and high resistance gene, used alone or in pyramiding approaches, are of particular interest in breeding new varieties.

1.6 Problem Identification and Justification

Utilization of rice genetic resources is limited to only the preferred genotypes which leads to a threat of disappearance of other landraces, and varieties and promotes existence of a narrow genetic diversity Caldo *et al*. (1996). The narrow genetic diversity makes the crop vulnerable to losses from biotic and abiotic environmental stresses such as insect pests and diseases, drought, water logging, and salinity. Wide genetic diversity is a pre-requisite to any successful plant breeding for yield and resistance to biotic and abiotic factors Ogunbayo *et al.* (2007). In Tanzania, there are various landraces cultivated locally but most of them are being replaced by improved varieties, thus land area planted to landraces is decreasing rapidly Kihupi and Pillai (1989). Therefore, the reduction in genetic variability due to adoption of new rice varieties calls for a need to collect landraces for *ex situ* conservation and characterize them for future rice breeding programs. Both morphological and molecular characterizations are needed because the evaluation of phenotypic diversity usually reveals important traits of interest to plant breeders. We need to relate phenotypic expression with molecular characteristics to see if the traits are genetically determined.

More over phenotypic characterization alone may not be efficient in discriminating genotypes because of environmental influences on phenotypes. In Tanzania, a number of landraces grown in different rice growing areas are collected at Katrin

research station in Ifakara district. Such landraces differ from improved varieties in adaptation to soil types, sowing, ripening periods and yielding stability in areas where seasons are unpredictable. Also they constitute a good source of unique genes for stress tolerance and are genetically dynamic Guei and Traore (2001). The effects of narrowness in crop genetic diversity are reported by several plant breeders around the globe. For instance, Louwaars and Marrewijk (1994) reported that there was a danger resulting from planting single crop with limited genetic base in a large area. During 1840's for instance, Ireland experienced a great famine due to an epidemic of potato blight (*Phytophthora infestans*) which lead to people's starvation and migration to America.

Secondly, the risk of narrow genetic base in maize was reported by Louwaars and Marrewijk (1994), when 15% of the harvest was lost due to the outbreak of southern corn leaf blight (*Drechslera maydiys*). This was due to the adaptation of the pathogen to cytoplasmic factors which was incorporated in 90% of the USA-maize varieties at the time. It is important to note that in Tanzania phenotypic expression of rice genotypes in terms of traits of interest such as resistance to biotic and abiotic factors, quality, stature, yield components, has been done and well documented. However molecular characterization of the genotypes to complement the phenotypic expression has not been done (Reuben personal communication, 2009).

My hypothesis is that traditional rice varieties locally cultivated by farmers that were either not used, or poorly used as parents in the Tanzanian rice breeding program represent alternative genetic pools to the improved varieties. In the present investigations, microsatellite markers were used to determine genetic diversity within

a sample of 70 traditional varieties, representing a significant portion of the rice cultivated in Tanzanian. This study was aimed at assessing the extent of genetic variation in the traditional rice varieties and establishing their genetic relationships. Results will complement breeding activities, and help to identify potential rice genotypes with resistance to (RYMV).

1.7 Objectives

1.7. 1. General objective

To establish the level of genetic diversity and relationships of some selected rice landraces cultivated in rice growing areas of Tanzania.

1.7.2 Specific objectives

(i) To determine genetic differences and similarities among rice landraces using SSR molecular marker technique.

(ii) To determine genetic relationships among the selected rice landraces grown in major rice growing areas of Tanzania.

(iii) To identify potential rice genotypes with resistance to rice yellow mottle virus and recommend them for use as source of breeding materials for rice improvement programs.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Molecular Markers for Genetic Studies

Genetic diversity is a crucial level of concern in the maintenance of biological diversity for three main reasons: (i) short-term viability of individuals and populations - it enables organisms to respond to environmental change and shape the ecosystems in which they live (i.e., it provides insurance against future adverse conditions); (ii) direct use of genetic resources - it is the raw material that farmers and plant breeders use to improve quality and productivity); and (iii) evolutionary potential of populations and species - species' potential for evolutionary change is dependent on the existence of genetic diversity. Diverse types of data sets can be used by researchers to estimate genetic diversity parameters: pedigree data, morpho-agronomic data, biochemical data obtained by analysis of isozymes and storage proteins, and DNA-based markers (molecular markers) data. The ability to generate molecular data using a wide range of molecular markers (Semagn *et al*. 2006a) and the variety of statistical programs for data analyses are expanding at an ever-increasing pace. This has vastly expanded the accuracy and types of information for the analysis of genetic diversity and structure in any species.

The presence of various types of molecular markers, and differences in their principles, methodologies, and applications require careful consideration in choosing one or more of such methods. Semagn *et al*. (2006) provided detailed reviews for restriction fragment length polymorphism (RFLP), random amplified polymorphic

DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeats (ISSRs), sequence characterized regions (SCARs), sequence tag sites (STSs), cleaved amplified polymorphic sequences (CAPS), microsatellites or simple sequence repeats (SSRs), expressed sequence tags (ESTs), single nucleotide polymorphisms (SNPs), and diversity arrays technology (DArT). The desirable properties of molecular markers are high polymorphism, codominant inheritance, frequent occurrence and even distribution throughout the genome, selectively neutral behavior, easy access, easy and fast assay, low cost and high throughput, high reproducibility, and transferability between laboratories, populations and/or species. No molecular markers are available yet that fulfill all requirements needed by scientists. Nevertheless, according to the kind of study to be undertaken, one can choose among the variety of molecular marker systems Semagn *et al*. (2006) using a number of factors: (a) Marker system availability; (b) Simplicity of the technique and time availability; (c) Anticipated level of polymorphism in the population; (d) Quantity and quality of DNA available; (e) transferability between laboratories, populations, pedigrees and species; (f) the size and structure of the population to be studied; (g) availability of adequate skills and equipment ; (h) cost per data point and availability of sufficient funding; and (i) marker inheritance (dominant versus codominant) and the type of genetic information sought in the population (Staub and Serquen, 1996; Karp *et al.* 1997; Wolfe and Liston, 1998; Mackay, 2001; Rungis *et al.* 2005). At present, SSRs and SNPs are the markers of choice for several applications.

2.2. SSR Markers

Simple sequence repeats (SSRs) markers are short tandem repeats usually consisting of 1-6 base pairs (bp′s) of nucleotides, found in all eukaryotic genomes. They were first referred to as microsatellites by Litt and Lutty (1989) and later as simple sequence repeats (SSRs) by Jacob *et al*. (1991). These sequences usually form a single locus (microsatellites) and can vary between individuals in repeat lengths i.e. the number of copies of the basic repeat, the basic repeat can be repeated 10, 12, 13 or more times.

The SSR loci are randomly dispersed in the genome and suitable for wide comparisons Morgante and Oliveri (1983). These loci are highly variable on account of number of repeat units found for each locus in any given population Morgante and Oliveri, (1983). The high level of heterozygosity, codominant and PCR-based nature of these repeats loci have made SSR the molecular marker of choice for genetic mapping and diversity studies Gupta *et al*. (1996). Therefore it is a powerful ideal tool for genotype assignment, marker assisted breeding, genetic mapping, and diversity assessment Gupta and Varshney, (2000). Furthermore, SSR polymorphism can be detected easily and robustly by polymerase chain reaction (PCR) and DNA sequence.

According to Callow and Newburry (1997), an SSR is 1 to 6 nucleotides in length, which shows a high degree of polymorphism. Specific microsatellites can be isolated using hybridized probes followed by their sequencing. Like any DNA fragment, SSRs are detected by specific dyes or by radiolabelling using gel electrophoresis. The advantage of using SSRs as molecular markers is the extent of polymorphism

shown, which enables the detection of differences at multiple loci between strains coupled with chemical and morphological data, they can identify the plant species or strains of interest Tao *et al.* (1993). Further more assays involving SSRs are more robust than random amplified polymorphic DNA (RAPDs), making them up to seven times more efficient. The latest research suggests that SSRs can be involved in new methods of detection of alterations of specific sequences in the DNA.

In many rice growing countries, the landraces carrying a vast amount of genetic diversity were distributed in remote villages. The number of landraces began to decline since 1968's when high-yielding varieties were introduced. Most of the old landraces are now available in certain gene banks only, not in the hands of farmers. In Tanzania, collection of rice landraces was initiated in 1970′s over the whole country, and more than 400 accessions were collected. They are now preserved by the Katrin Agriculture Institute Ifakara. It is essential to rationalize conservation and use of genetic resources to guide in the establishment of strategies that ensure the maintenance of genetic variability, essential in plant breeding.

Although the use of molecular markers to study the genetic diversity and relationships among the different cultivars has been previously reported (Davierwala *et al.* 2000; Porreca *et al*. 2001; Neeraja *et al.* 2002; Saker *et al*. 2005), the information on the genetic relationships among Tanzanian accessions is limited.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Plant Materials

Rice landraces seeds (Table 1) were collected from gene bank at KATRIN Agricultural Research Institute in Ifakara, Morogoro, grown in a screenhouse at Agricultural Research Institute Mikocheni (ARI- Mikocheni). The research institute is located at 39º 14' 02. 85¨E to 6º 45' 25.16¨S and 22.3 meters above sea level. Equipments, reagents and tools for DNA extraction were available in the laboratory at Mikocheni.

Table 1: List of rice landraces evaluated and their origins

Code	Rice landraces	Place originated
1	Afaa Mwanza	Mwanza
2	Serena	Tabora
3	Mbawa mbili nyekundu	Kilombero
4	Suba nburi	Kilombero
5	Kivuri	Zanzibar
6	Afaa mzinga	Kilombero
7	Mpaka wa bibi	Zanzibar
8	Gamti	Tunduru
9	Sindano	Tunduru
10	Pishori brown	India/ Kilombero
11	Kihogo red	Kilombero
12	Lingwelingweli	Mahenge
13	Tondogoso	Shinyanga
14	Mbega	Kilombero
15	Wahiwahi	Shinyanga/Mwanza
16	Kisegese	Kilombero
17	Kialangawa	Zanzibar
18	Chambena	Kilombero
19	Rangi mbili nyekundu no2	Kilombero
20	Supa	Mwanza
21	Gigante	Kilombero/Katrin
22	Zambia	Zambia/ Kilombero
23	Sotea	Zanzibar
24	Faya mafuta	Tabora
25	Nondo	Tabora
26	Tunduru	Tunduru
27	Nylon	Shinyanga
28	Lifumba	Msumbiji
29	Supa kijivu	Kilosa
30	Mbawa mbili nyeupe	Kilombero
31	Jambo twende	Kilombero
32	Mwarabu	Morogoro Rural
33	Sifara	Zanzibar
34	Kaling´anaula	Kilombero
35	Supa surungai	Dodoma
36	Sindano kubwa	Zanzibar
37	Mwanza	Mwanza
38	Kalibumbula	Mahenge
39	Msonga	Kilombero
40	Ringa	Zanzibar
41	Dunduli ya mlimani	Kilombero
42	Mzinga	Kilombero
43	Katumbo	Shinyanga
44	Katumahi	Tabora
45	Kalamata	Mwanza
46	Simzito	Zanzibar
47	Rangi mbili	Kilombero
48	Lunyuki	Zanzibar
49	Ngadija	Shinyanga
50	Sukari	Tabora
51	Kalundi	Tabora
52	Uchuki	Zanzibar
53	Mzungu	Kilombero
54	Tuliani	Tuliani Morogoro
55	Magongo ya wayungu	Shinyanga
56	Afaa kikangaga	Kilombero
57	Kagiha	Tabora
58	Tosa	Mahenge
59	Moshi	Tanga
60	Limota	Limota
61	Mbawa mbili rangi mbili	Tunduru
62	Faya(chikuyu manyoni)	Manyoni
63	Mabawa ya njiwa	Tunduru
64	Shingo ya mwali	Kyela
65	Afaa	Mwanza
66	Loya	Tabora
67	Usiniguse	Kilombero
68	Mkiwa wa nyumbu	Kitere Mtwara
69	Chamota	Kilombero
70	Salama	Kilombero

3.2 Methods

3.2.1 Screenhouse Establishment of Experimental Plants

A single factor (landrace) experiment was established in the screen house using Complete Randomized Design (CRD). Seventy landraces of rice were replicated three times where by for each variety, ten seeds were planted in a potted container containing clay loam soil, and placed in a screen house. After 20-days, young leaves were sampled from seedlings (Fig. 2) for DNA extraction in the laboratory.

Figure 2: Twenty days old seedlings ready for DNA extraction

3 .2 .2 Assessment of Genetic Variation of Rice Landraces

3 .2 .2.1 Sample Preparations

Young leaves from each landrace from individual plants were harvested in a screen-house at ARI- Mikocheni (Fig. 3). Seventy percent of ethanol was used as disinfectant to wash hands before harvesting. Motor and pestle were cleaned using 70% ethanol before a new set of leaves was extracted.

Figure 3: Sample collection from rice seedlings for DNA extraction

3.2.2.2 DNA Extraction Procedure.

Extraction of genomic DNA was done using a protocol developed by Dellaporta *et al.* (1983). However, some modifications to fit the prevailing conditions due to hardness of rice leaves was done using liquid nitrogen. One 1gram freshly harvested leaves were ground in liquid nitrogen with a mortar and pestle. The powder was transferred into 50ml Falcon tube containg 16 ml of extraction buffer (3% (w/v) CTAB, 1.4 M NaCl, 1% (w/v) PvP, 20 mM EDTA PH 8.0,dH_2O and 0.2 (w/v) Mercaptoethanol in water bath at 65 ºC, and 1ml of 20% SDS were added. The tubes were shaken vigorously until the tissue became dispersed in the buffer then continued to mix for another 2mins. Thereafter tubes were transferred to 65ºC water bath and mixed gently by inverting 5-6 times, every 5min, for 15min. The tubes were removed from 65ºC water bath and allowed to cool at room temperature for approximately 10min. Five ml of ice-cold 5M potassium acetate was added to each tube and mixed gently by inverting five times.

Thereafter, tubes were incubated at -20ºC for 20min, and then centrifuged at 3000 rpm in a table top centrifuge for 20min. The supernatant was filtered through two layers of Mira cloth into a new 50ml falcon tube, then one volume of ice-cold iso-proponol (approximately 18ml) added and mixed by inverting gently 10 times. The tubes were incubated at -20ºC for 2hr, and centrifuged at 3000 rpm for 20min. After centrifugation, the supernatant was poured off from tubes into the beaker. The last drops of iso-propanol from tubes were removed by placing inverted tubes on paper towels leaving the pellets sticking to the bottom of the tubes.

Subsequent to removal of iso-propanol, The pellet was allowed to dry by leaving it on paper towels for 1h. Then 300 µl of 10m–M tris HCI/1m–M EDTA, containing 10mg/ml RNAse, was added in tubes, stored at 4ºC overnight to dissolve pellet. The solution was transferred into an eppendorf tube and 2µl loaded on a 3% agarose gel to check quality. The stock solution of DNA was stored at -20ºC and when required for use was transferred and stored at 4ºC.

3.2.2.3 Deoxyribonucleic (DNA) quantification

According to Herzberg *et al*. (2004) 20n–g of DNA concentration was required for amplification. DNA quantification was done with the aim of getting the right quantity of 20n–g that was used in PCR amplification. The following standards were used to compare with DNA sample: 150n–g, 100ng, 50n–g. The DNA sample of each of seventy landraces was diluted with tris- ethlymethlytetra acetate (TE) buffer in different concentrations viz. 1:50, 1:25 and 1:10 dilution. Both the standards (lambda) and DNA sample were electrophoresed on one gel tank; the band intensity of the DNA sample was compared with the standard bands and the dilution of 1:20 which is equivalent to 20n–g of DNA was used throughout the experiment.

3.2.2.4 DNA amplification.

Ten SSRs markers were tested at Mikocheni laboratory in Dar-Es-Salaam using PCR machine to amplify the isolated DNA and their composition as established by Mba *et al*. (2000). For PCR amplification, 20µl reaction mixture containing 10.5µl distilled water, 2µl of DNA, 1µl of each primer (forward and reverse), 0.5µl of concentration taq polymerase (Promega Madison W1 USA), 2.5µl of 10xPCR buffer (Promega

Madison W1 USA), 1.5µl of $MgCl_2$ and 1µl of dNTP (deoxyribonucleoside triphosphate) were applied. Amplifications were performed in DNA amplification thermocycler (GeneAmp® PCR SYSTEM 9700, version 3.08) apparatus.

The PCR thermocycle was programmed to execute the following conditions: 94ºC for 5 minutes; 94ºC for 1 minute (denaturation); 61ºC for 1minute (annealing); 72ºC for 2 minutes (extension temperature) for 35 cycles and a final extension of 72ºC for 5 minutes were done automatically. Source of primers is Invitrogen Ex Uk 890 G02, each primer has a different annealing temperature calculated based on base composition. The SSR marker screened with the corresponding annealing temperature were Primer RM. 307 forward and reverse (57ºC); Primer RM. 338 forward and reverse (59ºC); Primer SSR no. 431 forward and reverse (55ºC); Primer RM. 452 forward and reverse (57ºC); Primer RM. 237 forward and reverse (64ºC); Primer RM. 433 forward and reverse (55ºC); Primer RM. 552 forward and reverse (53ºC); Primer RM. 161 forward and reverse (67ºC); Primer RM. 124 forward and reverse (69ºC) and Primer RM. 510 forward and reverse (57ºC) (App. 3).

To minimize possibility of cross contamination in the amplification reactions a master reaction mixture was routinely prepared and negative two controls were used. One control consisted of reaction mixture excluding DNA template and the second control consisted of reaction mixture and milliq (distilled water) excluding DNA template. The procedure was performed several times and only reproducible products were taken into account for further data analysis. The amplification products were separated in 3% agarose gels in 1x TBE buffer and detected by staining with

ethidium bromide 0.1µl for one and half hours at 55volts in electrophoresis machine (Sambrook *et al*. 1989).

3.2.2.5 Genetic diversity data analysis.

Genetic diversity data of rice landraces were obtained by scoring polymorphic bands (80) for the 70 rice landraces as present (1) and absent (0). Missing values e.g. resulting from failed PCR reaction or faint (2) (uncertain bands)) were not considered. Coded data of genetic diversity were computed and arranged in similarity matrix on the bases of Jaccard´s coefficient (*Ibid*). The coefficient was calculated as:

$$JCij = a / (a + b + c)$$

Whereby:

a- is the number of fragments present in both i and j

b- is the number of fragments presents in i and absent in j

c- the number of fragments present in j and absent in i.

The genetic diversity was computed from the binary data for all pair wise combinations of landraces. The genetic similarity among landraces based on the observed SSR marker data were determined using NTSYS 2.1 statistical software (Rohlf, 1993) where by cluster analysis based on similarity matrices using the Un-weighted Pair Group Method with Arithmetic mean (UPGMA) to obtain a dendrogram was done.

3.2.3 Mechanical inoculation of RYMV in rice landraces.

To identify potential rice genotypes with resistance/tolerant to rice diseases, 70 rice landraces were planted and screened for RYMV. A total of 420 seeds were collected (six seeds from each of the 70 rice landraces from KATRIN, Ifakara in Morogoro) then planted in 10 liter bucket containing clay loam soil. The seedlings were maintained in the screen house throughout the growing period under natural light and temperature ranging from 24º to 30ºC. NPK fertilizer was applied to enhance growth.

Leaves from naturally infected plants collected from KATRIN, Ifakara in Morogoro designated as strain Kyela (S_4), Mang`ula (S_6) and Dakawa (S_6) were macerated and blended in a sterile blender containing sterile cold 0.1M Phosphate buffer (pH 7.4) so as to give inoculums suspension. The inoculums were used to inoculate healthy rice landraces plants kept in the screen house for 19 days after sowing (Figure 4). Young expanded leaves of rice landraces were dusted with carborundum powder (600 grit) and inoculated by using a cotton swab containing inoculum. Excess inoculum was washed off with tap water after inoculation. Inoculation process was done in the screenhouse at temperature ranging between 24º-30ºC and rice landraces were observed for RYMV development after the ten days from inoculation.

Inoculated rice landraces were scored three times for RYMV at an interval of 7 days (Table 3) and then analyzed using a standard evaluation system for rice (IRRI, 1999). In this standard; 1 = Resistant, 3 = moderately resistant, 5 = moderately susceptible, 7 = susceptible and 9 = highly susceptible.

Figure 4: The inoculation process in the screen house.

CHAPTER FOUR

4.0 RESULTS

4.1 Determination of genetic differences and similarities among rice landraces.

4.1.1 Deoxyribonucleic acid (DNA) quantification.

The following standards were used to compare with DNA sample: 150n–g, 100n–g, 50n–g as a result of quantification. The results were observed 150n–g is very brighter sample no.4, 6, 7, 8. 100n–g is. sample no. 1, 5, 11,12,13,14,16 and 50n–g samples no. 2,3,9,10,15,17,18,19 compared in DNA.

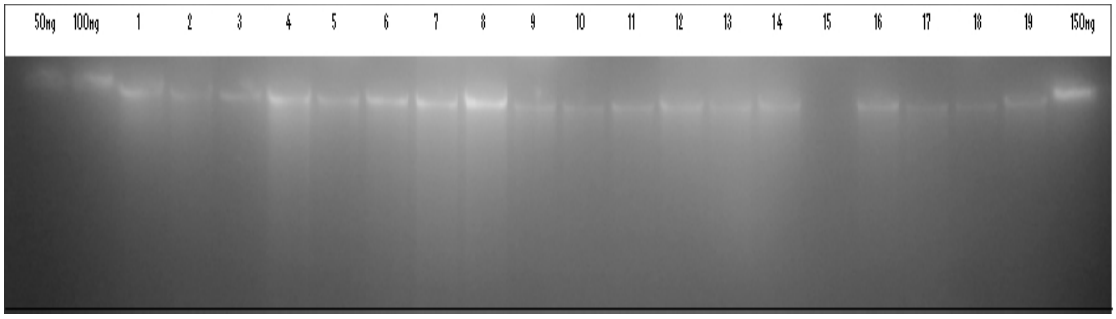


Plate 1: DNA quantification of rice landraces from sample 1-19 in 3% agarose gel.

4.1.2 Genetic diversity evaluation of rice landraces.

The amplification products were separated in 3% agarose gels in 1x TBE buffer and detected by staining with ethidium bromide 0.1µl after electrophoresis, run for one and half hours at 55volts (Sambrook *et al.* 1989). Plates 2 and 3 show a sample DNA band amplified from the landraces using SSR marker no. RM 431.

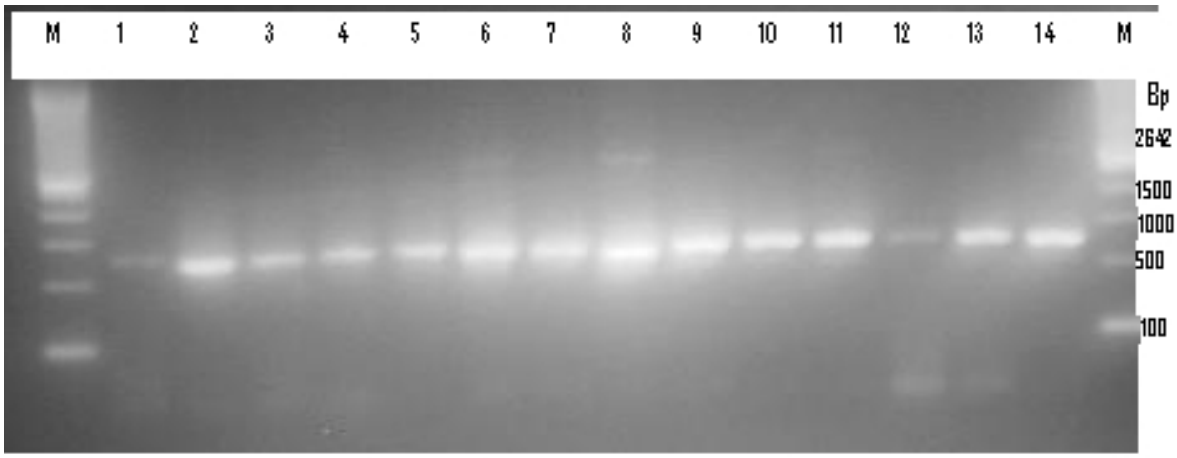


Plate 2: Amplification of DNA from landraces no. 1-14 using RM 431.

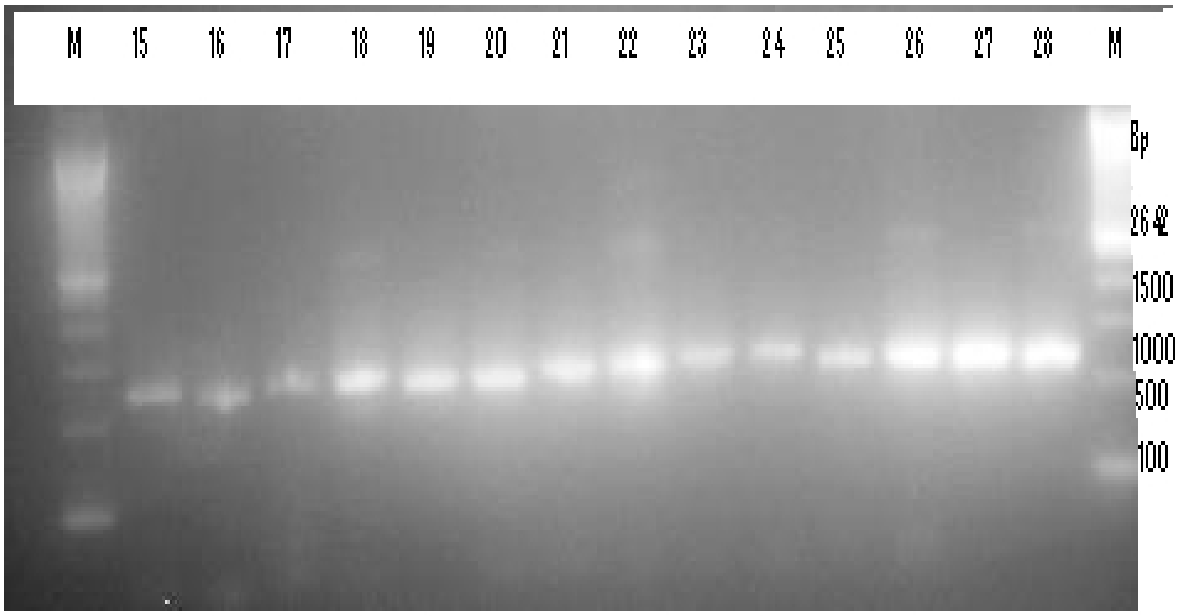


Plate 3: Amplification of DNA from landraces no. 15-28 using RM 431.

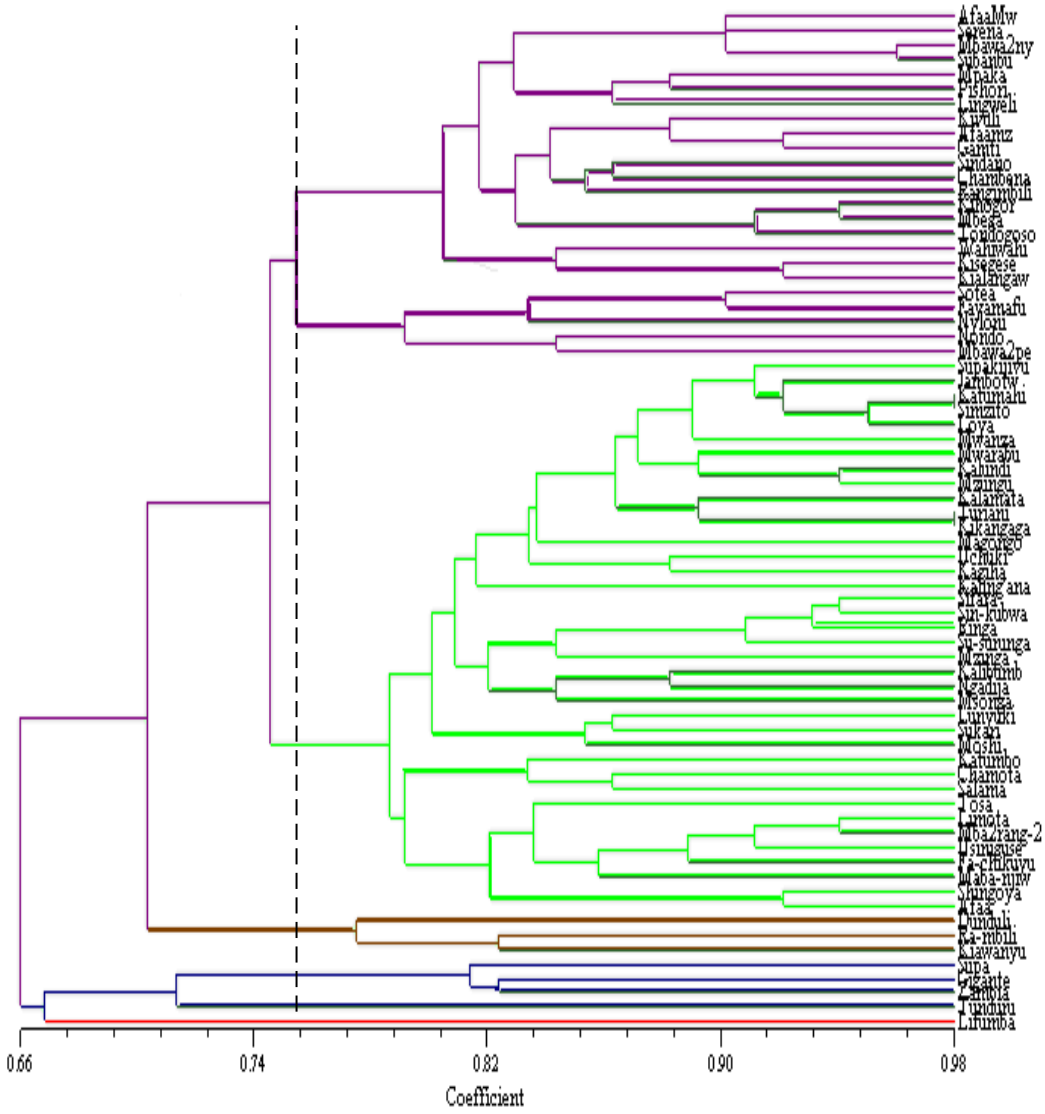
Further results from the PCR products show 10 informative markers which detected polymorphism among 70 landraces. These markers revealed a total of 80 distinct bands with a mean of 8 bands (Table 2, App. 4 and 5). Forty nine out of 80 were polymorphic among the rice landraces used in this study. The number of loci per primer ranged from 1-6 includes SSR markers RM 124, RM 161, RM 338, 433, RM 431 and RM 552. The minimum number of loci detected polymorphic were RM 124 that range from (1 – 3) polymorphic loci and RM 433 had the highest range (1-6).The highest polymorphic loci include number RM 452, RM 237, RM 510, RM 307 with RM 237 having the highest polymorphic bands ranged from (2-6) (Table 2).

Table 2: RM sequences consisting of polymorphic genetic variation of rice landraces

Primer RM	Primer Sequences Forward	Primer Sequence Reverse	Total No of Loci (bands)	Polymorphic Loci (bands)	
				Lowest	Highest

452	CTGATCGAGAGCGTTAAG GG	GGGATCAAACCACGTTTC TG	8	2	5
124	ATCGTCTGCGTTGCTGCTGCT G	CATGGATCACCGAGCTCCCCC C	8	1	3
161	TGCAGATGAGAAGCGGCGCCT C	TGTGTCATCAGACGGCGCTCC G	8	1	4
237	CAAATCCCGACTGCTGTC C	TGGGAAGAGAGCACTACA GC	8	2	6
510	AACCGGATTAGTTTCTCG CC	TGAGGACGACGAGCAGAT TC	8	2	4
338	CACAGGAGCAGGAGAAGA GC	GGCAAACCGATCACTCAG CT	8	1	4
307	CTGCTATGCATGAACTGC TC	GTACTACCGACCTACCGTTCA C	8	2	4
433	TGCGCTGAACTAAACACA GC	AGACAAACCTGGCCATTC AC	8	1	6
431	TCCTGCGAACTGAAGAGT TG	AGAGCAAAACCCTGGTTC AC	8	1	4
552	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGT CC	8	1	4
Total			80	14	49
Mean			8	1.4	4.9

Results from the dendrogram generated based on UPGMA from similarities or a genetic distance matrix has shown an overall pattern of variation as well as the degree of relatedness among accession of rice landraces. The five clusters (Fig. 5) of the SSR dendrogram together with their internal groups have demonstrated the polymorphic nature of the 70 rice landraces used in the current study. The basic shape enclosed the genotypes in each cluster, showing four clusters and one out group.



KEY

First cluster
Second cluster
Third cluster
Fourth cluster
Five cluster

Figure 5: A Dendrogram showing the genetic similarity of 70 rice landraces based UPGMA using NTSYS

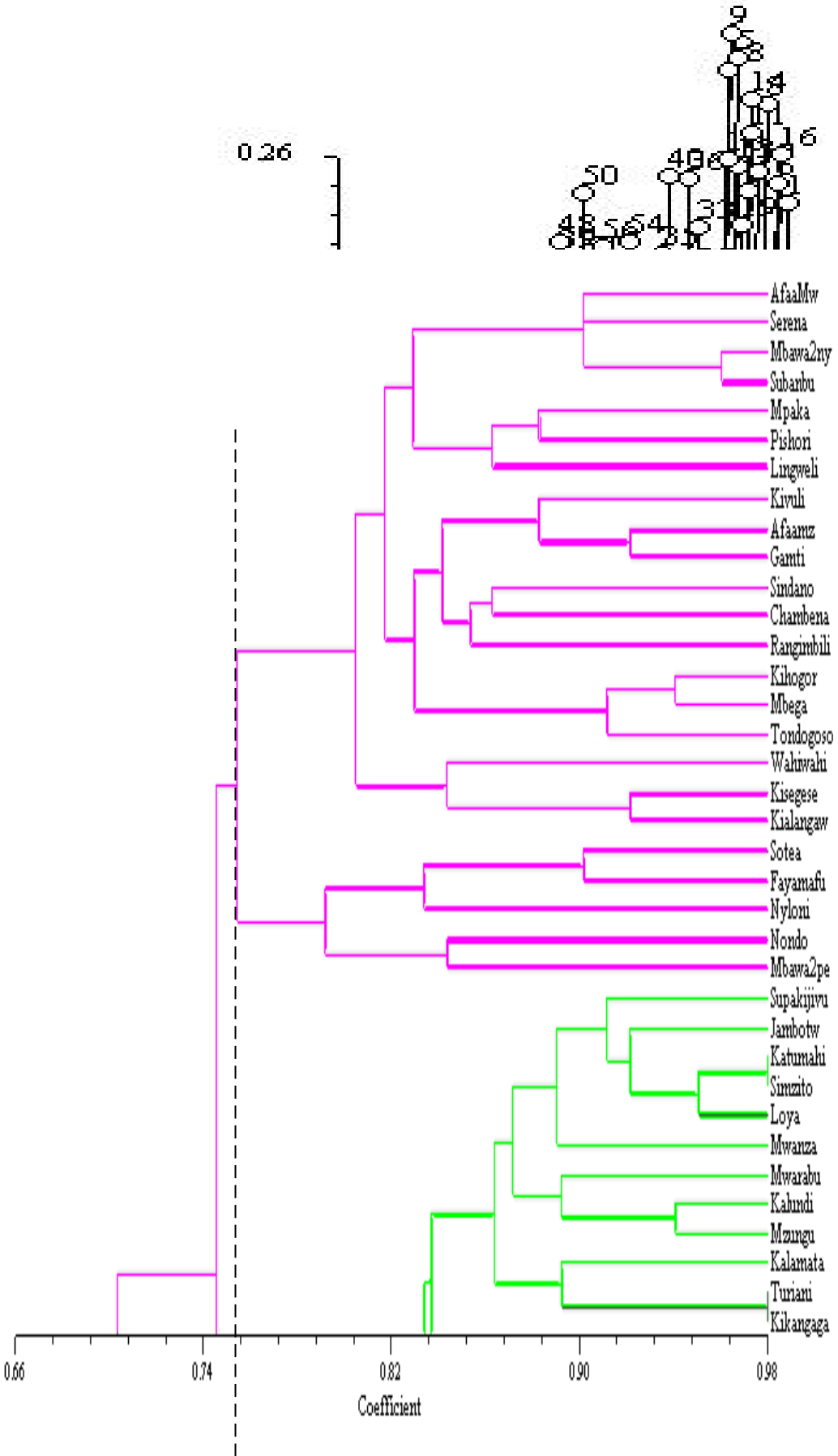
A total of 49 clear and scorable DNA fragments were detected among the 70 rice landraces using the 10 SSR primers. The similarity matrix generated by the 49 SSR loci based on the NTSYS analysis ranged from 0.66 to 0.98. Dendrogram obtained

using Un weighted Pair Group Method with Arithmetic (UPGMA) analysis method in NTSYS software package revealed five cluster. Distinct DNA cluster ranged at 0.76-0.98 similarity coefficient (Fig. 5). The dendrogram results revealed five clusters wherein the first cluster had 24 rice landraces which were Afaa Mwanza, Serena, Mbawa mbili nyekundu, Suba nburi, Mpaka wa bibi, Pishori brown, Lingwelingweli, Kivuri, Afaa mzinga, Gamti, Sindano, Chambena, Rangi mbili nyekundu no.2, Kihogo red, Mbega, Tondogoso, Wahiwahi, Kisegese, Kialangawa, Sotea, Faya mafuta, Nylon, Nondo, and Mbawa mbili nyeupe . (Fig.7).

The second cluster consisted of 38 rice landraces which were Supa kijivu, Jambo twende, Katumahi, Simzito, Loya, Mwanza, Mwarabu, Kalundi, Mzungu, Kalamata, Tuliani, Afaa kikangaga, Magongo ya wayuni, Uchuki, Kagiha, Kaling′naula, Sifara, Sindano kubwa, Ringa, Supa surungai, Mzinga, Kalibumbula, Ngadija, Msonga, Lunyuki, Sukari, Moshi, Katumbo, Chamota, Salama, Tosa, Limota, Mbawa mbili rangi mbili, Usiniguse, Faya (chikuyu manyoni), Mabawa ya njiwa, Shingo ya mwali, and Afaa.on (Fig. 7 and 8.) The third cluster had 3 rice landraces with Dunduli ya mlimani, Rangi mbili, and Mkiya wa nyumbu.(Fig. 8). The fourth cluster had 4 rice landraces of Supa, Gigante, Zambia and Tunduru. (Fig 8). Lifumba was the only one landrace observed in the fifth cluste or out-group. (Fig. 8).

The microssatellite analysis indicated that, rice landraces were genetically related based on their similarity coefficients. The results obtained in the dendrogram were also supported by the Principle Component Analysis as shown in (Fig. 6). Although PCA is based on a different algorithm using Eigenvector (Appendix 2), the grouping of rice landraces was similar to that of dendrogram as indicated in (Fig. 5). The Principle Component Analysis (PCA) performed on the bases of the similarity matrix

of 70 landraces which revealed five major clusters confirms the results of cluster analysis using as UPGMA as shown in (Fig. 7 and 8).



based

the 70

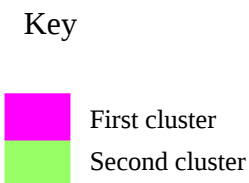


Figure 7: Dendrogram of genetic analysis computed from a similarity matrix of genetic distance based on (Rolf. 1993). Rice landraces no. 1-35

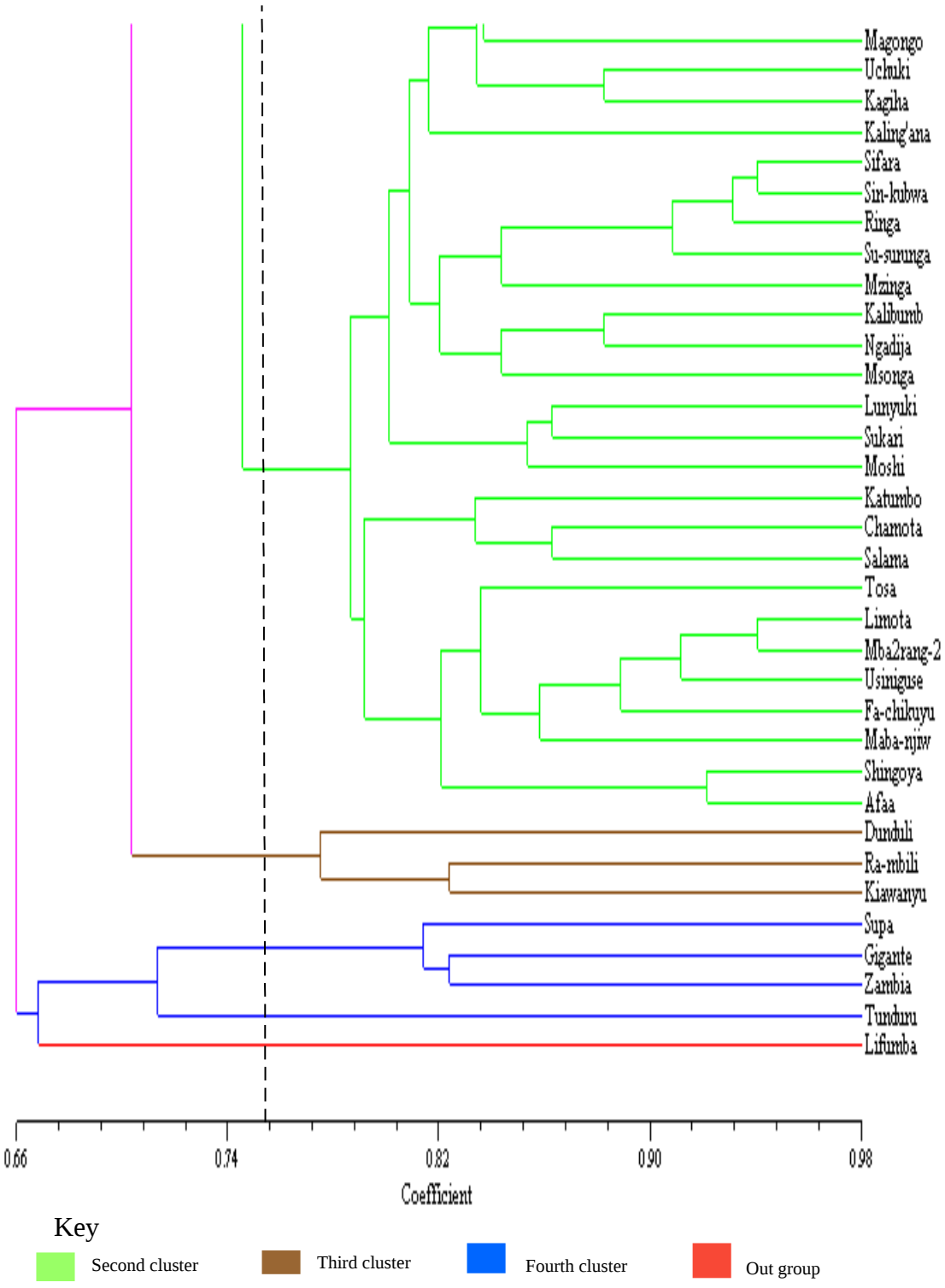


Figure 8: Dendrogram of genetic analysis computed from a similarity matrix of genetic distance based on (Rolf. 1993). Rice landraces no. 36-70

4.2 Screening of Rice Genotypes for Resistance to RYMV.

4.2.1. Scoring for RYMV disease

Table 3: RYMV average ratings for 70 Rice landraces following inoculation with various strains (S4- Kyela. S6 - Mang'ula, S6-Dakawa), done on 7days, 14 days, and 28days after inoculation.

Code	Rice land racers	S4 Kyela			S6 Mang'ula			S6 Dakawa		
		14/4	21/4	28/4	14/4	21/4	28/4	14/4	21/4	28/4
1	Afaa Mwanza		+	+ +		+	+		+	+ +
2	Selena			+ +	+	+ +	+ + +	+	+ +	+ + +
3	Mbawa mbili nyekundu no 1	+	+ +	+ + +		+ +	+ + +		+	+ +
4	Supa nburi					+	+ +			
5	Kivuri			+ +	+	+ +	+ + +	D	D	D
6	Afaa mzinga									
7	Mpaka wa bibi		+	+ +	+	+	+ +	+	+ +	+ + +
8	Gamti		+ +	+ + +	+	+ +	+ + +	+	+ +	+ + +
9	Sindano	+	+	+ + +	+	+	+ +	+	+ +	+ + +
10	Pishori brown			+ +	+	+ +	+ + +		+ +	+ + +
11	Kihogo red		+	+ + +	+	+ +	+ + +	+	+ +	+ +
12	Lingwelingweli									
13	Tondogoso	+	+ +	+ + +	+	+ +	+ + +		+	+ + +
14	Mbega									
15	Wahiwahi	+	+	+ + +	+	+ +	+ + +		+	+ + +
16	Kisegese	+	+ +	+ +	+	+ +	+ + +		+	+ + +
17	Kialangawa		+	+ +	+	+ +	+ + +		+ +	+ +
18	Chambena	+	+ +	+ + +	+	+ +	+ + +	D	D	D
19	Rangi mbili nyekundu no2			+ +	+	+	+ +	+	+ +	+ + +
20	Supa		+	+ +		+ +	+ + +	+	+ +	+ +
21	Gigante					+	+ +	+	+ +	+ +
22	Zambia		+	+ +	+	+	+ +	+	+ +	+ +
23	Sotea	+	+ +	+ + +	+	+ +	+ + +		+	+ + +
24	Faya mafuta		+	+ +	+	+	+ +		+	+ + +
25	Nondo		+	+ +		+	+ +	+	D	D
26	Tunduru	+	+ +	+ + +	+	+	+ +		+	+ +
27	Nylon		+ +	+ + +	+	+ +	+ + +		+	+ + +
28	Lifumba	+	+ +	+ + +	+	+ +	+ + +		+	+ +
29	Supa kijivu		+		+	+ +	+ + +		+	+ +
30	Mbawa mbili nyeupe		+	+ +	+	+	+ +	+	+ +	+ + +
31	Jambo twende									
32	Mwarabu		+	+ +	+	+ +	+ + +	+	+ +	+ + +
33	Sifara					+	+ +	+	+ +	+ + +
34	Kaling'anaula				+	+	+ +		+ +	+ + +
35	Supa surungai		+	+ +	+	+ +	+ + +	+	+	+ +
36	Sindano kubwa		+	+ +	+	+	+ +	+	+	+
37	Mwanza		+	+ +	+	+ +	+ + +	+	+ +	+ +
38	Kalibumbula									
39	Msonga									
40	Ringa	+	+ +	+ + +		+	+ + +	+	+ +	+ + +
41	Dunduli ya mlimani	+	+ + +		+	+	+ +	+	+ +	+ + +
42	Mzinga									
43	Katumbo	+	+ +	+ + +	+	+	+ +	+	+	+ +
44	Katumahi				+	+ +	+ + +		+	+ +
45	Kalamata		+	+ +	+	+	+ +	+	+ +	+ + +
46	Simzito		+	+ +	+	+	+ +	+	+ +	+ + +
47	Rangi mbili		+	+ +		+	+ +	+	+ +	+ + +
48	Lunyuki									
49	Ngadija		+	+ +	+	+	+ +	+	+ +	+ + +
50	Sukari	+	+ +	+ + +	+	+ +	+ + +	+	+ +	+ + +
51	Kalundi	+	+	+ +	+	+ +	+ + +		+	+ +
52	Uchuki				+	+	+			
53	Mzungu	+	+ +	+ + +	+	+	+	+	+ +	+ + +
54	Tuliani	+	+ +	+ + +					+	+ +
55	Magongo ya wayungi	+	+ +	+ + +	+	+ +	+ + +	+	+ +	+ + +
56	Afaa kikangaga	+	+ +	+ + +	+	+ +	+ +	+	+ +	+ + +
57	Kagiha	+	+ +	+ + +	+	+	+ +	+	+	+ +
58	Tosa									
59	Moshi		+	+ +		+	+	+	+	
60	Limota		+	+	+	+ +	+ + +		+	+ +
61	Mbawa mbili rangi mbili	+	+ +	+ + +	+	+ +	+ + +	D	D	D
62	Faya(chikuyu manyoni)	+	+ +	+ + +	+	+ +	+ + +	+	+ +	+ + +
63	Mabawa ya njiwa		+	+ +	+	+ +	+ + +		+	
64	Shingo ya mwali	+	+ +	+ + +	+	+ +	+ + +	+	+ +	+ + +
65	Afaa	+	+ +	+ + +	+	+ +	+ + +	+	+ +	+ + +
66	Loya	+	+ +	+ + +	+	+	+		+	+ +
67	Usiniguse	+	+ +	+ + +	+	+ +	+ + +		+	+ +
68	Mkiwa wa nyumbu	+	+ +	+ + +		+	+ +	+	+ +	+ + +
69	Chamota	+	+ +	+ + +	+	+ +	+ + +	+	+ +	+ + +
70	Salama									+

KEY

\+ Weak molting. ++ Extensive molting plus yellowing. ++ + generalizing necrosis plus stunting. D. Dead

4.2.2. Symptoms of the disease on leaves

RYMV disease Symptoms analyzed were shown changes from the 7th day after inoculation but the extent of distribution were high from the 10th day (Fig. 9) to the 20th day (Fig. 10).

Figure 9: RYMV effects on rice landraces ten days after inoculation.

Figure 10: RYMV effects on rice landraces twenty days after inoculation.

Analyzed data for RYMV collected on the 7th, 14th, and 28th days showed landraces to display a wide range of resistance of accessions against the RYMV.

4.2.3. Resistance of rice landraces on RYMV disease.

Results on scored rice landraces for RYMV disease in a standard evaluation system for rice, indicated that nine rice landraces (out of seventy) were resistant to all three strains viz. S4 (from Kyela), S6 (from Mang`ula), S6 (from Dakawa) Afaa Mzinga (6) example (Fig. 11), Lingwelingweli, Mbega, Jambo twende, Kalibumbula, Msonga ,Mzinga, Lunyuki and Tosa. The reaction of RYMV disease ranged from some landraces being resistant while others were highly susceptible to an extent of causing death of plants. There was also a continuum of response to various strains between the moderately resistant and highly susceptible ones. Some landraces which were resistant to one or two other strains, for example Suba nburi (4), which was resistant to S4 Kyela and S6 Dakawa was moderately susceptible to S6 Mang'ula (Table 4).

Figure 11: Afaa mzinga reaction to RYMV.

Some of the landraces including Suba nburi and Uchuki were resistant to S4 Kyela and S6 Dakawa but susceptible to S6 Mang′ula, and Salama resistant to S4 Kyela and S6 Mang′ula but susceptible to strain S6 Dakawa. Landraces Gigante, Sifara and Kaling`anaula were resistant to strain S4 Kyela. Tuliani and Limota were resistant to strain S6 Mang′ula (Table 4).

Moderately resistance to rice landraces in a specific strains of RYMV were shown at Afaa mwanza S6 Mang′ula, Sindano kubwa S6 Dakawa, Uchuki S4, Mzungu S6 Mang′ula, Limota S4 Kyela, Loya S6 Mang′ula ,Usiniguse S6 Mang′ula and Salama S6 Dakawa. The Landraces that showed moderate resistance to two strains of RYMV included Supa Kijivu against S4 Kyela and S6 Mang′ula, Moshi, and Mabawa ya njiwa against S6 Mang′ula and S6 Dakawa (Table 4).

The Landraces that showed moderate susceptibility to specific strains included Serena against S4 Kyela, Mbawa mbili nyekundu S6 Dakawa, Supa nburi S6 Mang ′ula, Kivuri S4 Kyela, Sindano S6 Mang′ula, Pishori brown S4 Kyela, Kihogo red S6

Dakawa, Lifumba S6 Dakawa, Supa kijivu S6 Dakawa ,Mwarabu S4 Kyela , Sifara S6 Mang′ula, Kaling`anaula S6 Mang′ula, Dunduli ya mlimani S6 Mang′ula, Katumahi S6 Dakawa, Ngadija S6 Mang′ula,Tuliani S6 Dakawa, Afaa kikangaga S6 Mang′ula, Moshi S4 Kyela, Limota S6 Dakawa, Mabawa ya njiwa S4 Kyela Loya S6 Dakawa, Usiniguse S6 Dakawa, Mkia wa nyumbu S6. Mang′ula Moderately susceptible to two strains were displayed for Afaa Mwanza S4 Kyela and S6 Dakawa, Mpaka wa bibi S4 Kyela and S6 Mang′ula, Kialangawa S4 Kyela and S6 Dakawa, Rangi mbili nyekundu no.2 S4 Kyela and S5 Mang′ula, Supa S4 Kyela and S6 Dakawa, Gigante S6 Mang′ula and S6 Dakawa, Faya mafuta S4 Kyela and S6 Mang′ula, Nondo S4 Kyela and S6 Mang′ula, Tunduru S6 Mang′ula and S6 Dakawa, Mbawa mbili nyeupe S4 Kyela and S6 Dakawa, Supa surungai S4 Kyela and S6 Dakawa, Sindano kubwa S4 Kyela and S6 Mang′ula, Mwanza S4 Kyela and S6 Dakawa, Katumbo S6Mang′ula and S6 Dakawa, Kalamata S4 Kyela and S6 Dakawa, Simzito S4 and S6 Dakawa, Rangi mbili S4 Kyela and S6 Dakawa, Kalundi S4 and S6 Dakawa and Kagiha S6 Mang′ula and S6 Dakawa. Only Zambia was moderately susceptible to all strains viz. S4 Kyela, S6 Mang′ula and S6 Dakawa (Table 4).

Landraces which were susceptible to one strain included Lifumba S4 Kyela, Sifara S6 Dakawa, Supa surungai S6 Mang′ula, Ringa S4 Kyela, Mzungu S6 Dakawa, Tuliani S4 Kyela, and Loya S4 Kyela while landraces which were found susceptible to two strains included Pishori brown S4 Kyela and S6 Mang′ula, Chambena S4 Kyela and S6 Mang′ula, Dunduli ya mlimani S4 Kyela and S6 Dakawa, Ngadija S4 Kyela and S6 Dakawa, Mbawa mbili rangi mbili S4 Kyela and S6 Mang′ula (Table 4).

Landraces which were highly susceptible to one strain included Mpaka wa bibi S6 Dakawa, Kialangawa S6 Mang′ula, Rangi mbili nyekundu na.2 S6 Dakawa, Faya mafuta S6 Dakawa, Tunduru S4 Kyela, Lifumba S6 Mang′ula, Mbawa mbili nyeupe S6 Dakawa, Kaling`anaula S6 Dakawa, Mwanza S6 Mang′ula, Katumbo S4 Kyela, Katumahi S6 Mang′ula, Mzungu S4 Kyela, Kagiha S4 Kyela, Limota S6 Mang′ula and Usiniguse S4 Kyela (Fig. 15). Landraces which were found to be highly susceptible to two strains included Serena S6 Mang′ula and S6 Dakawa, Mbawa mbili nyekundu S4 Kyela and S6 Mang′ula, Kivuli S6 Mang′ula and S6 Dakawa, Sindano S4 Kyela and S6 Dakawa, Kihogo red S4 Kyela and S6 Dakawa, Mwarabu S6 Mang′ula and S6 Dakawa, Ringa S6 Mang′ula and S6 Dakawa, Afaa kikangaga S4 Kyela and S6 Dakawa, Mkia wa nyumbu S4 Kyela and S6 Dakawa, and Chamota S4 Kyela and S6 Dakawa (Table 4).

Figure 12: Reaction of susceptible S6 ex Dakawa to the disease RYMV.

Highly susceptible landraces to all strains viz. S4 Kyela, S6 Mang′ula, and S6 Dakawa included Gamti, Tondogoso, Wahiwahi, Kisegese, Sotea, Nylon, Sukari, Magongo ya wayuni, Faya, Shingo ya mwali and Afaa. It was also interesting to note that most of those landraces were highly susceptible to strain S6 Dakawa to an extent that some landraces caused death of the plants. Landraces into which strain S6 Dakawa caused death to plants included Kivuri , Chambena , Nondo , and Mbwa mbili rangi mbili (Table 4).

Code	**Rice Landraces**	**1= Resistant**			**3=Moderately Resistant**			**5=Moderately Susceptible**			**7=Susceptible**			**9=Highly Susceptible**		
		S4	S6m	S6	S4	S6m	S6	S4	S6m	S6	S4	S6m	S6	S4	S6m	S6
1	Afaa Mwanza					V		V		V						
2	Serena							V							V	V
3	Mbawa mbili nyekundu									V				V	V	
4	Suba nburi	V		V					V							
5	Kivuri							V							V	V
6	Afaa mzinga	V	V	V												
7	Mpaka wa bibi							V	V							V
8	Gamti													V	V	V
9	Sindano								V					V		V
10	Pishori brown							V				V	V			
11	Kihogo red									V				V		V
12	Lingwelingweli	V	V	V												
13	Tondogoso													V	V	V
14	Mbega	V	V	V												
15	Wahiwahi													V	V	V
16	Kisegese													V	V	V
17	Kialangawa							V		V					V	
18	Chambena										V	V				D
19	Rangi mbili nyekundu no2							V	V							V
20	Supa							V		V						V
21	Gigante	V							V	V						
22	Zambia							V	V	V						
23	Sotea													V	V	V
24	Faya mafuta							V	V							V
25	Nondo							V	V							D
26	Tunduru								V	V				V		
27	Nylon													V	V	V
28	Lifumba									V	V				V	
29	Supa kijivu				V	V				V						
30	Mbawa mbili nyeupe							V	V							V
31	Jambo twende	V	V	V												
32	Mwarabu							V							V	V
33	Sifara	V							V				V			
34	Kaling'anaula	V							V							V
35	Supa surungai							V		V		V				
36	Sindano kubwa					V		V	V							
37	Mwanza							V		V					V	
38	Kalibumbula	V	V	V												
39	Msonga	V	V	V												
40	Ringa										V				V	V
41	Dunduli ya mlimani								V		V		V			
42	Mzinga	V	V	V												
43	Katumbo								V	V				V		
44	Katumahi	V								V					V	
45	Kalamata							V	V							V
46	Simzito							V	V							V

47	Rangi mbili							V	V							V
48	Lunyuki	V	V	V												
49	Ngadija								V		V	V				
50	Sukari													V	V	V
51	Kalundi							V		V						V
52	Uchuki	V		V		V										
53	Mzungu					V							V	V		
54	Tuliani		V							V	V					
55	Magongo ya wayuni													V	V	V
56	Afaa kikangaga								V					V		V
57	Kagiha								V	V				V		
58	Tosa	V	V	V												
59	Moshi					V	V	V								
60	Limota				V				V						V	
61	Mbawa mbili rangi mbili										V	V				D
62	Faya(chikuyu manyoni)													V	V	V
63	Mabawa ya njiwa					V	V	V								
64	Shingo ya mwali													V	V	V
65	Afaa													V	V	V
66	Loya						V			V	V					
67	Usiniguse						V			V				V		
68	Mkiwa wa nyumbu								V					V		V
69	Chamota		V											V		V
70	Salama	V	V				V									

Table 4: Evalution of rice landraces for resistance to RYMV strains

CHAPTER FIVE

5.0 DISCUSSION

The assessment of genetic diversity is an essential component in germplasm characterization and conservation. The results derived from analyses of genetic diversity of rice landraces at the DNA level could be used for designing effective breeding programs aiming to broadening the genetic bases of rice landraces.

In this study 70 rice landraces were evaluated with 10 SSR markers. The genotypes studied represent rice landraces cultivated by farmers in Tanzania. All 10 SSRs were polymorphic across the 70 genotypes. A total of 49 alleles were detected with an average number of alleles of 4.9 per locus (range 1 to 6 per locus). This value is quite low compared with those reported for the worldwide collection (range 2 -11, mean = 6.3) and other large scale studies (range = 3-17, mean = 7.4) (Olufowote *et*

al. 1997; Yu *et al*. 2003). Most of rice landraces grouped at range of 0.76-0.98 using Jaccard's coefficient of similarity thus supporting the reports of narrow genetic variation in Latin American cultivars (Cuevas-Perez *et al.* 1992; Guimaraes *et al*. 1995; Fuenters *et al*. 1999). Similar narrow genetic variation values have been reported for Japanese (Hashimoto *et al.* 2004). And Korean (Song *et al.* 2002) cultivars for which a narrow genetic base has been documented by means of SSR markers.

Result obtained from the genetic diversity study of rice landraces show narrow genetic base as reported by Latin American commercial rice landraces, mainly based on pedigree and/or biochemical analyses (Cuevas-Perez *et al.* 1992; Guimaraes *et al*. 1995; Rangel *et al*. 1996; Fuenters *et al*. 1999; Anguirre *et al*. 2005). To our knowledge this work represents the first study of genetic diversity and molecular characterization of Tanzanian rice landraces germplasm using SSRs markers. The study is supported by few published reports of DNA fingerprinting of Latin American commercial rice cultivars (Fuentes *et al*. 1999; Aguirre *et al*. 2005; Giarrocco *et al*. 2007).

SSR′s were chosen for the analysis of genetic diversity of rice landraces because several works have shown that these markers are very powerful for differentiating individual germplasm accessions, particularly when they are closely related (Bligh *et al.* 1999; Xu *et al*. 2004; Jeung *et al.* 2005). Additionally, SSRs show a series of advantages when compared with other DNA-based markers, such as abundance in the genome, high level of polymorphism, repeatability, co-dominance and cost-effectiveness Ni *et al*. (2002).

The markers which had the highest number of alleles suggested that they could distinguish closely related rice landraces. These include Primer no. RM 307, Primer no. RM 338, Primer no. RM 431, Primer no. RM 452, Primer no. RM 237, Primer no. RM 433, Primer no RM. 552, Primer no. RM 161, Primer no. RM 124 and Primer no. RM 510.

The genetic characterization using the UPGMA procedure revealed four cluster and one out-group where all samples had a range of 0.66- 0.98 Jaccard's coefficient of similarity. Rice landraces with the respective Jaccard's coefficient similarity viz Tunduru (0.61), Dunduli ya mlimani (0.63), Lifumba (0.66), Zambia (0.67), Supa (0.67) and Gigante (0.73), in pair wise similarity had a genetic distance ranging between 0.66-0.76 while the rest rice landraces ranged from 0.76-0.98. Further more rice landraces Katumahi, Simzito, Tuliani, and Afaa Kikangaga had equal genetic distance level of 0.98 coefficient (Fig. 5).These four landraces were given different names by farmers may be due to traditional naming and usage of crops. However results obtained by the use of molecular techniques (SSR) were regarded more reliable than those obtained from phenotypic expression of resistance to RYMV. In crop improvement it is important for breeder to know this to avoid duplication of accessions in breeding lines, thus lowering handling costs and improving genetic gain.

Potential rice genotypes with resistance to RYMV are indicated in Table 4, For immunity to the disease, rice landraces study in a screen house indicated considerable variation in resistance among landraces , when tested against S4-Kyela, S6- Mang'ula and S6-Dakawa strains and some of the 70 landraces were found to be immune (Table 4). The behavior of the tested landraces in relation to the disease was not homogeneous. On basis of the symptoms developed among the landraces, some

showed resistance to RYMV, some had moderate reaction, while the majority was highly susceptible. For breeder genetic homoestasis in which pathogens don’t mutate to new races as some levels of infection or survival of pathogen are allowed, especial for qualitative resistance. However commercial requirements of uniformity my not favor mixture in crop production.

Landraces Afaa mzinga, Lingwelingwe, Mbega, Jambo twende, Kalivumbula Msonga, Mzinga, Lunyuki, and Tosa, showed resistance to three strains S4 Kyela, S6 Mang'ula, and S6 Dakawa, thus can be used for breeding purposes and included in National gene bank. The fact that some landraces showed resistance to all three strains they show promise that they might be used as sources of genes of resistance to RYMV and to solve the problem of some RYMV strains that break resistance Kanote *et al*. (1997). Therefore, additional source of resistance with different genetic determinants should also be sought to increase the stability and durability of the resistance. Indeed, RYMV is characterized by a variability which is able to break the varietals resistance in time and space Konate *et al*. (2006). Landraces which have shown resistance to RYMV strains are of particular interest for breeding as they have been reported to provide resistance to some other diseases, for example rice blast Awoderu *et al*. (1987).

Strain S6 from Dakawa (Morogoro), was very aggressive on landraces and sometimes leading to death of plants for example, with landraces Kivuri, Chambena, Nondo and Mbawa mbili rangi mbili. Response to the virus shows a large variability depending on the genotype, but also on screening conditions, severity of inoculation and resistance evaluation method N`guessan *et al*. (2001).

RYMV disease affects the vegetative and floral parts of infected plants. An importance occurs in plants that exhibit strong mosaic or yellow discoloration together with stunting. In the present study, only the foliar symptom was used in evaluation. Considering the development of foliar symptoms alone, one would confuse a resistant landrace with a tolerant landrace and vice-versa. A good selection of resistant rice landraces in relation to the RYMV can not be limited to the evaluation of the resistance of cultivars on the basis of foliar reaction, further evaluation of yield growth and number of tillering parameter are recommended.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6. 1 Conclusions

Results indicate that there was no significant difference among landraces as coefficient of similarity was concentrated at a range of 0.76- 0.98 genetic distances for most varieties and few of them being at 0.66- 0.76 genetic distance levels. The accessions were grouped under five cluster similarity levels (Fig.5).

The first cluster had 24 landraces as shown in Fig. 7.While the second cluster consisted of 38 landraces (Fig. 7 and 8).

The third cluster included 3 landraces viz. Dunduli ya mlimani, Rangi mbili, and Mkiya wa nyumbu (Fig. 8). The fourth cluster included 4 rice landraces viz. Supa, Gigante, Zambia and Tunduru, while the five clusters consisted of landrace Lifumba, which was found to be the most distant landrace with genetic distance 0.66 coefficient. (Fig. 8). Rice landraces Katumahi, Simzito, Tuliani and Afaa Kikangaga, were found to be the most genetically similar with genetic distance 0.98 coefficient thus indicating that genetically these landraces do not differ in genetic distance significantly, hence acquired different names because of the movement of seed materials, usage and traditional names.

Landraces Afaa Mzinga, Lingwelingwe, Mbega, Jambo twende, Kalibumbula, Msonga, Mzinga, Lunyuki and Tosa, were resistant to all three strains of RYMV

studied. Some of the landraces including Suba nburi S4 and S6, Uchuki S4 Kyela and S6 Mang'ula, and Salama S4 Kyela and S6 Mang'ula were resistant to two out of the three strains. Landraces Gigante, Sifara and Kaling`anaula were all resistant to strain S4 Kyela, while Tuliani, was resistant to strain S6 Mang'ula.

Highly susceptible landraces to all strains included Mbawa mbili nyekundu, Gamti, Tondogoso, Wahiwahi, Kisegese, Sotea, Nylon, Sukari, Magongo ya wayuni, Faya, Shingo ya mwali and Afaa. This is a preliminary step in identifying resistant rice landraces which are potential sources of genes for resistance against RYMV.

6.2 Recommendations

Simple sequence repeat (SSR) markers should be used whenever possible in identifying rice landraces.

Landraces Afaa mzinga, Lingwelingwe, Mbega, Jambo twende, Kalivumbula Msonga, Mzinga, Lunyuki, and Tosa, showed resistance to three strains S4, S6 Mang'ula, and S6 Dakawa, thus can be used for breeding purposes and included in National Gene Bank.

Rice landraces Lifumba, Tunduru and Dunduli ya mlimani had wider genetic base ranging from 0.66-0.98 distant coefficient. It advocated to include them in a National Rice improvement programmer in Tanzania. While some landraces had narrow genetic base ranging from 0.76-0.98 distant coefficient, rice landraces Katumahi, Simzito, Tuliani and Afaa Kikangaga, were found to be genetically distant at 0.98 coefficients and can be used for intended breeding purposes.

Because of the biological diversity of RYMV isolates, it is necessary to test the landraces which have proved to be resistant to the three isolates through tests in the field in various localities and also assess the impact of RYMV on yield components as related to the severity of symptoms.

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APPENDICES

Appendix 1: Genetic relationship of 70 Rice landraces based on Jaccard's coefficient similarity.

Appendix 2: Eigenvector different algorithm showing in a table.

code	Eigenvalue	Percent	Cumulative
1	53.25440425	76.0777	76.0777
2	2.97857663	4.2551	80.3328
3	1.44438448	2.0634	82.3962
4	1.22155480	1.7451	84.1413
5	1.15755277	1.6536	85.7950
6	0.98794330	1.4113	87.2063
7	0.84079402	1.2011	88.4074
8	0.79589614	1.1370	89.5444
9	0.69520461	0.9931	90.5376
10	0.56604580	0.8086	91.3462
11	0.53096897	0.7585	92.1048
12	0.48555574	0.6937	92.7984
13	0.47407166	0.6772	93.4756
14	0.44848980	0.6407	94.1163
15	0.41371814	0.5910	94.7074
16	0.37218986	0.5317	95.2391
17	0.32568691	0.4653	95.7043
18	0.31019159	0.4431	96.1475
19	0.29692904	0.4242	96.5717
20	0.23944636	0.3421	96.9137
21	0.22646155	0.3235	97.2372
22	0.21606011	0.3087	97.5459
23	0.18193665	0.2599	97.8058
24	0.16135508	0.2305	98.0363
25	0.14969390	0.2138	98.2502
26	0.13636862	0.1948	98.4450
27	0.12456243	0.1779	98.6229
28	0.11143372	0.1592	98.7821
29	0.10152268	0.1450	98.9271
30	0.09660238	0.1380	99.0651
31	0.09066596	0.1295	99.1947
32	0.07858798	0.1123	99.3069
33	0.06490877	0.0927	99.3997
34	0.05854205	0.0836	99.4833
35	0.05340058	0.0763	99.5596
36	0.04525293	0.0646	99.6242
37	0.04357095	0.0622	99.6865
38	0.03937609	0.0563	99.7427
39	0.03261697	0.0466	99.7893
40	0.03163090	0.0452	99.8345
41	0.02896631	0.0414	99.8759
42	0.02604674	0.0372	99.9131
43	0.01959388	0.0280	99.9411
44	0.01452544	0.0208	99.9618
45	0.01191238	0.0170	99.9789
46	0.00762883	0.0109	99.9898
47	0.00528774	0.0076	99.9973
48	0.00188350	0.0027	> 100%
49	0.00000000	0.0000	> 100%
50	0.00000000	0.0000	> 100%
51	0.00000000	0.0000	> 100%
52	0.00000000	0.0000	> 100%
53	0.00000000	0.0000	> 100%
54	0.00000000	0.0000	> 100%
55	0.00000000	0.0000	> 100%
56	0.00000000	0.0000	> 100%
57	0.00000000	0.0000	> 100%
58	0.00000000	0.0000	> 100%
59	0.00000000	0.0000	> 100%
60	0.00000000	0.0000	> 100%
61	0.00000000	0.0000	> 100%
62	0.00000000	0.0000	> 100%
63	0.00000000	0.0000	> 100%
64	0.00000000	0.0000	> 100%
65	0.00000000	0.0000	> 100%
66	0.00000000	0.0000	> 100%
67	0.00000000	0.0000	> 100%
68	0.00000000	0.0000	> 100%
69	0.00000000	0.0000	> 100%
70	0.00000000	0.0000	> 100%
Sum of eigenvalues =	70.000000		

Appendix 3: Difference primers with annealing temperatures used

Primer no.	**Frequencies Forward**	**Frequencies Reverse**	**Annealing Temperature**
: RM 237	CAA ATC CCG ACT GCT GTC C	TGG GAA GAG AGC ACT ACA GC	64ºc
RM 431	TCC TGC GAA CTG AAG AGT TG	AGA GCA AAA CCC TGG TTC AC	63ºc
RM 452	CTG ATC GAG AGC GTT AAG GG	GGG ATC AAA CCA CGT TTC TG	65ºc
RM 338	CAC AGG AGC AGG AGA AGA GC	GGC AAA CCG ATC ACT CAG CT	59ºc
RM 307	CTG CTA TGC ATG AAC TGC TC	GTA CTA CCG ACC TAC CGT TCA C	57ºc
RM 124	ATC GTC TFC GTT GCT GCT GCT G	CAT GGA TCA CCG AGC TCC CCC C	69ºc
RM 161	TGC AGA TGA GAA GCG GCG CCT C	TGT GTC ATC AGA CGG CGC TCC G	67ºc
RM 510	AAC CGG ATT AGT TTC TCG CC	TGA GGA CGA CGA GCA GAT TC	57ºc
RM 552	CGC AGT TGT GGA TTT CAG TG	TGC TCA ACG TTT GAC TGT CC	57ºc
RM 433F	TGC GCT GAA CTA AAC ACA GC	AGA CAA ACC TGG CCA TTC AC	55ºc

Appendix 4: Sample 1-35 SSR PCR products show 10 informative markers which detected polymorphism among 70 landraces

Appendix 5: Sample 36-70 SSR PCR products show 10 informative markers which detected polymorphism among 70 landraces.