ASSOCIATION OF *BETAINE ALDEHYDE DEHYDROGENASE 2.1* (*BADH2.1*) GENE ALLELE WITH AROMA IN POPULAR TRADITIONAL RICE VARIETIES IN TANZANIA

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ABSTRACT

Aromatic rice is highly cherished in many countries of the world and commands premium prices at all levels of the global rice trade. The presence of aroma in aromatic rice is controlled by *betaine aldehyde dehydrogenase 2.1 (BADH2.1)* gene allele which results from an eight base pair deletion and three single nucleotide polymorphisims (SNPs) in exon seven of *betaine aldehyde dehydrogenase 2 (BADH2)* gene. This mutation is responsible for the introduction of premature stop codon which produce a truncated protein, this results in loss of function of the enzyme *betaine aldehyde dehdrogenase 2* (*BADH2*) leading to accumulation of substrate (main aroma compound) 2-acetyl 1-pyrroline (2AP) in aromatic rice varieties. In this study, the association between *BADH2.1* aromatic allele and aroma in Tanzanian rice varieties was investigated. Leaf and grain aromatic tests for aroma evaluation and screening for *BADH2.1* gene allele using allele specific amplification (ASA) marker were conducted in 160 popular traditional rice landraces from different geographical regions of Tanzania. Of the 160 landraces genotyped and phenotyped; 95 varieties were classified as aromatic by the presence of aroma in both leaf and grain aromatic tests, most of these (91.6 %) carried *BADH2.1* gene allele. Evidence from sequencing of *BADH2/BADH2.1* alleles confirmed the association of *BADH2.1* gene allele with aroma in aromatic rice landraces as it was shown that all aromatic genotypes had eight base pair deletion and three SNPs in exon seven of *BADH2* locus. This suggests that *BADH2.1* gene allele is the main aroma allele in most of the Tanzanian aromatic rice varieties. Phylogenetic analysis of *BADH2/BADH2.1* nucleotide sequences, showed a large amount of genetic variability (39.41-100 % nucleotide sequence identity) among the varieties studied. These findings will contribute siginificantly in planning for effective rice breeding strategies especially in selection of appropriate parental materials for developing high yielding aromatic rice varieties in the country.

DECLARATION

I, William Erasto Moshi, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted to any other institution.

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LIST OF ABBREVIATIONS AND SYMBOLS

^{o}C	degrees Celicius
%	percentage
µg	microgram(s)
µl	microliter(s)
µM	micromole(s)
A	Absorbance
AfricaRice	Africa rice centre
2AP	2-acetyl-1-pyrroline
AFLP	amplified fragment length polymorphism
AfRGM	African rice gall midge
ASA	allele specific amplification
ARI	agricultural research institute
BADH2	betaine aldehyde dehydrogenase 2
Bp	base pair
BLAST	basic local alignment search tool
CARD	Coalition for Africa Rice Development
CAPS	cleaved amplified polymorphic sequences
CC	central corridor
cDNA	complimentary DNA
COSTECH	Tanzania Commission of Science and Technology
CTAB	Cetyl trimethyl ammonium bromide
DArT	diversity array technology
dH_2O	dionised water

DNA	deoxyribonucleic acid
dNTPs	dideoxynucleotide triphosphates
EAP	externa antsense primer
EAAPP	East Africa Agricultural Productivity Project
EDTA	ethylene diamine tetraacetic acid
ESA	east and southern Africa
ESP	external sense primer
ESTs	expressed sequence tags
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation Statistics
g	gram(s)
GC	gas chromatography
GCMS	gas chromatography/mass spectrometry
GRiSP	Global Rice Science Partnership
GT	gelatinisation temperature
IFAP	internal fragrant antisense primer
IMD	institute of molecular development
INSP	internal non-fragrant sense primer
IRRI	International Rice Research Institute
ISSRs	inter simple sequence repeats
KATRIN	Kilombero Agricultural Training and Research institute
KOH	potassium hydroxide
Kbp	kilo base pair
LB	Luria- Bertan
M	Ladder

MAFC	Ministry of Agriculture,Food Security and Cooperatives
MARI	Mikocheni Agricultural Research Institute
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
mM	millimole(s)
mRNA	messenger RNA
MS	mass spectrometry
N	negative control
NERICA	New Rice for Africa
ng	Nanogram(s)
NRDS	National Rice Development Strategy
PCR	polymerase chain reaction
Ppb	part per billion
QTL	quantitative trait loci
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RLDC	Rural Livelihood Development Company
RLDP	Rural Livelihood Development Program
RNA	ribonucleic acid
rpm	revolution per minute
RYMV	rice yellow mottle virus
SCARS	sequence characterized regions
SNP	single nucleotide polymorphism
SSIIA	starch synthase IIa

SSR	simple sequence repeat
STS	sequence tag sites
t	tonne(s)
TAP	Tanzania agricultural program
UK	United Kingdom
V	Voltage
WARDA	West Africa Rice Development Association

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Rice (*Oryza sativa* L.) is one of the most widely-grown crops in Tanzania, after maize. It is among the major sources of employment, income and food security for nearly half of the global population, especially in Asia, Africa and Latin America (FAO, 2004; MAFC 2009; Oko and Ugwu, 2011). Tanzania is the second largest rice producer and consumer after Madagascar in East, Central and Southern Africa region (FAO, 2001; AfricaRice, 2009; Matchmaker, 2010). However, its average yield remains low partly due to the fact that Tanzanian farmers are still growing their traditional rice varieties characterized by low yield potential (RLDC, 2009).

Grain quality in rice plays an important role in consumer acceptability. Juliano and Duff, (1991) concluded that grain quality is second after yield as the major breeding objective for rice improvement. The quality in rice is considered based on milling quality, grain size, shape, appearance, fragrance or aroma and other cooking characteristics (Dela Cruz and Khush, 2000).

Fragrance or aroma in rice is considered as a special trait with huge economic importance that determines the premium price in both local and international markets (Sakthivel *et al*., 2009; Yeap *et al.,* 2013). This is demonstrated by the retail price of jasmine aromatic rice produced in Thailand, which has a world market value that is two-fold higher than that of non-aromatic rice (Qiu and Zhang, 2003). The study by Matchmaker (2010) has confirmed that the demand for quality and branded aromatic rice, primarily produced in Tanzania, is increasing in urban centres of Tanzania.

Genetic analysis has revealed that a recessive gene (*BADH2.1*) on rice chromosome eight is responsible for rice aroma (Sood and Siddiq 1978; Lorieux *et al*., 1996; Jin *et al*., 2003). The eight base pair (bp) deletion and three single nucleotide polymorphisms (SNPs) in exon seven of *BADH2* have led to the introduction of premature stop codon to produce a truncated protein which result in loss of function of the enzyme *BADH2* leading to accumulation of substrate 2AP in aromatic varieties, while the functional *BADH2* gene codes for a mature protein which consumes the substrate in non-aromatic varieties (Bradbury *et al*., 2005b).

Breeding programs make use of a number of sensory based methods to distinguish aromatic and non-aromatic rice, one of which is to taste individual rice grains. Generally, a panel of analysts is required as the ability to detect rice aroma vary greatly between individuals. Chemical methods are also available which involve smelling leaf tissue or grains after these have been allowed to react with 0.1M potassium hydroxide (KOH) solution (Sood and Siddiq, 1978). An objective gas chromatography/mass spectrometry (GC/MS) method has been introduced which assay 2AP content directly (Chen *et al.*, 2006).

Molecular markers that are closely linked to aroma gene can be used for discriminating aromatic from non-aromatic rice varieties (Cordeiro *et al*., 2002; Jin *et al*., 2003). Moreover a perfect marker technique named allele specific amplification (ASA) was developed by Bradbury *et al*. (2005a) for aroma genotyping and discriminating aromatic from non-aromatic rice. This technique was found to be useful in identification and selection for fragrance trait enhancing the use of most appropriate parental materials for rice breeding programs.

Traditional varieties constitute a good source of unique genes for stress tolerance, grain quality and adaptability to stressful environment (Ogunbayo *et al*., 2007). Evaluation and characterization of locally adapted traditional varieties should form an important constituent of collection of efforts in improvement of rice production because of their enormous in built genetic diversity due to several generations of growing and selection by breeders and farmers.

The aim of this study was to determine presence or absence of *BADH2.1* allele in 160 popular traditional Tanzanian rice varieties. A 257 base pair (bp) DNA fragment spanning *BADH2.1* was amplified covering the seventh exon region and sequenced to determine if popular traditional rice varieties possess the eight base pair (bp) deletion mutation or not.

1.2 Problem statement and justification of the study

While the demand for aromatic rice has drastically shot up, commanding premium prices in both local and international markets, Tanzania is still growing local aromatic rice varieties descended from seeds originally imported by Arab traders before 1960. These varieties are characterized by long growth duration, susceptibility to diseases and relatively low yielding (MAFC, 2009). Therefore, determining the gene for aroma will enable rice breeders to correctly select and incorporate the aroma gene to highly yielding non-aromatic varieties, resulting in development of high yielding aromatic varieties leading to efficient and effective exploitation of both domestic and international rice markets.

Rice breeders in Tanzania have been using conventional methods in selection and breeding for aroma trait. These methods are based on tasting individual grains and using chemicals for instance smelling leaf tissue or grains after these have been allowed to

react with 0.1 M KOH. However evaluation of aroma using these methods is unreliable as the ability to detect aroma varies between individuals (Sood and Siddiq, 1978). For effective selection and breeding for rice aroma the present study combined conventional breeding methods and allele specific amplification (ASA) developed by Bradbury *et al*. (2005b); This technique is very convenient because it is less subjective, free of environmental effects on the phenotype, and young seedlings can be screened very early for the presence/absence of aroma. Also, the recessive alleles can be detected in the heterozygous state, eliminating the need for progeny testing to identify individuals possessing aroma after backcrossing with homozygous non-aromatic plants (Kiani, 2011; Shi *et al.,* 2008; Yi *et al*., 2009).

Molecular analysis of aroma gene has not been done in Tanzanian traditional aromatic rice varieties and information available on the genetic nature of this gene is lacking. It is expected that, after identifying and characterizing the gene for aroma, the information will be used for rice breeding improvement, whereby such alleles for aroma can be transferred to non-aromatic high yielding rice varieties. This will have a profound impact on food security, domestic and international rice markets and economic growth for both rice farmers and the nation in general.

1.3 Research objectives

1.3.1 Main objective

To determine the presence or absence of *BADH2.1* allele, in popular traditional rice varieties, in Tanzania.

1.3.2 Specific objectives

The specific objectives of this study included:

(i) To screen for *BADH2.1* gene allele in popular traditional Tanzanian rice varieties,

(ii) To characterize by sequencing *BADH2*/*BADH2.1* alleles detected in popular traditional Tanzanian rice varieties to verify presence or absence of eight base pair (bp) deletion associated with aroma, and

(iii) To determine the genetic diversity of *BADH2/BADH2.1* alleles in popular traditional Tanzanian rice varieties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Classification of rice

Rice is classified in the division *Magnoliophyta*, class *lilopsida*, and order *cyperales*. It belongs to the genus *Oryza* under the family *Graminae*. The genus *Oryza* has two cultivated species including *O. glaberrima* (African rice) and *O. sativa* (Asian rice), and 23 wild species (Vaughan *et al*., 2003). Based on morphological and grain characteristics, *O. sativa* varieties are separated into two main sub species: *indica* which is adapted to tropical and subtropical floating, lowland and irrigated agrosystems and *japonica* which is adapted to temperate and tropical upland ecosystems (Chang, 1984). These two subgroups differ in grain shape, apiculus hair length, leaf width and colour, and in their reaction to phenol (Kovach *et al*., 2007). The majority of rice varieties grown in Tanzania are *indica* types. Other groups have also been recognized but since the variation in morphological features among cultivars is continuous, it is often difficult to make an accurate classification. Isozyme and molecular data are generating new insights but are not entirely consistent with the morphological classifications (Glaszmann, 1987).

2.2 Botanical characteristics of rice

Rice plants are similar to other grasses and grains such as oats and wheat. The key difference is that they can grow in standing water or in very wet soil because they have efficient system of air passage from shoot to root. Rice, a monocot, is normally grown as an annual plant, although in tropical areas it can survive as a perennial and can produce a ratoon crop for up to 30 years (IRRI, 2009). The rice plant can grow to 1–1.8 m (3.3–5.9 feet) tall, occasionally more depending on the variety and soil fertility. It has long, slender leaves 50–100 cm (20–39 inches) long and 2–2.5 cm (0.79–0.98 inches)

broad. The small wind-pollinated flowers are produced in a branched arching to pendulous inflorescence 30–50 cm (12–20 inches) long. The edible seed is a grain (caryopsis) 5–12 mm (0.20–0.47 inches) long and 2–3 mm (0.079–0.118 inches) thick. Rice plants develop clusters of small wind-pollinated 'flowers' at the top of the plant called panicles. Once they are pollinated, the flowers develop rice grains. Although they are perennial (continue to grow from one season to the next), rice plants are treated as annuals whereas new seeds are usually planted each season for better yields (GRiSP, 2013).

2.3 Global rice production

World production of rice has risen steadily from about 200 million tones of paddy rice in 1960 to over 700 million tons in 2012. A total of 162.8 million hectares were cultivated in 2012 for rice production and the total production was about 738.1 million tones whereas the average world farm yield for rice was 4.5 tons per hectare (FAOSTAT, 2012). The world's largest rice producers by far are China and India. Although its area harvested is lower than India's, China's rice production is greater due to higher yields because nearly all of China's rice area is irrigated, whereas less than half of India's rice area is irrigated. After China and India, the next largest rice producers are Indonesia, Bangladesh, Vietnam, Thailand, and Myanmar (Table. 1).

In Africa, rice production has grown rapidly, but rice consumption has grown even faster, with the balance being met by increasing quantities of imports. Western Africa is the main producing sub region, accounting for more than 40 % of African production in 2006-10 (WARDA, 2007). In terms of individual countries, the leading producers of paddy (2006-10) are Egypt (6.1 million tons), Madagascar (4.1 million tons), and Nigeria (3.9 million tons) (GRiSP, 2013).

The production of rice in east and southern Africa (ESA) increased by 57 % from 1.19 million tons in 2000 to more than 1.87 million tons in 2010. During this period, the average yield increased by 17.5 % from 1.52 tons/ha to 1.78 tons/ha and the area of production increased by 37 % from 782 000 ha to 1.047 million ha. In Burundi, Kenya, Mozambique, Rwanda, Tanzania, and Uganda, total rice consumption in 2010 reached more than 3 million tons or 19 kg/person/year. Rice imports amounted to 1.2 million tons per year in paddy equivalent, or more than 40 % of all rice consumed in the region (FAOSTAT, 2012).

According to Wilfred and Consultant (2006), rice is the main staple food of the populations in Cape Verde, Comoros, Gambia, Guinea, Guinea–Bissau, Liberia, Madagascar, Egypt, Reunion, Senegal and Sierra Leone and is also an important food of the populations in Côte d'Ivoire, Mali, Mauritania, Niger, Nigeria, and Tanzania. In addition, rice has become an important food security factor in Angola, Benin, Burkina Faso, Chad, Ghana and Uganda.

Table 1: Top 20 rice producers in the world by 2012

Country	Production (Million metric tons)
China	204.3
India	152.6
Indonesia	69.0
Vietnam	43.7
Thailand	37.8
Bangladesh	33.9
Burma	33.0
Philippines	18.0
Brazil	11.5
Japan	10.7
Pakistan	9.4
Cambodia	9.3
United States	9.0
South Korea	6.4
Egypt	5.9
Nepal	5.1
Nigeria	4.8
Madagascar	4.0
Sri Lanka	3.8
Laos	3.5

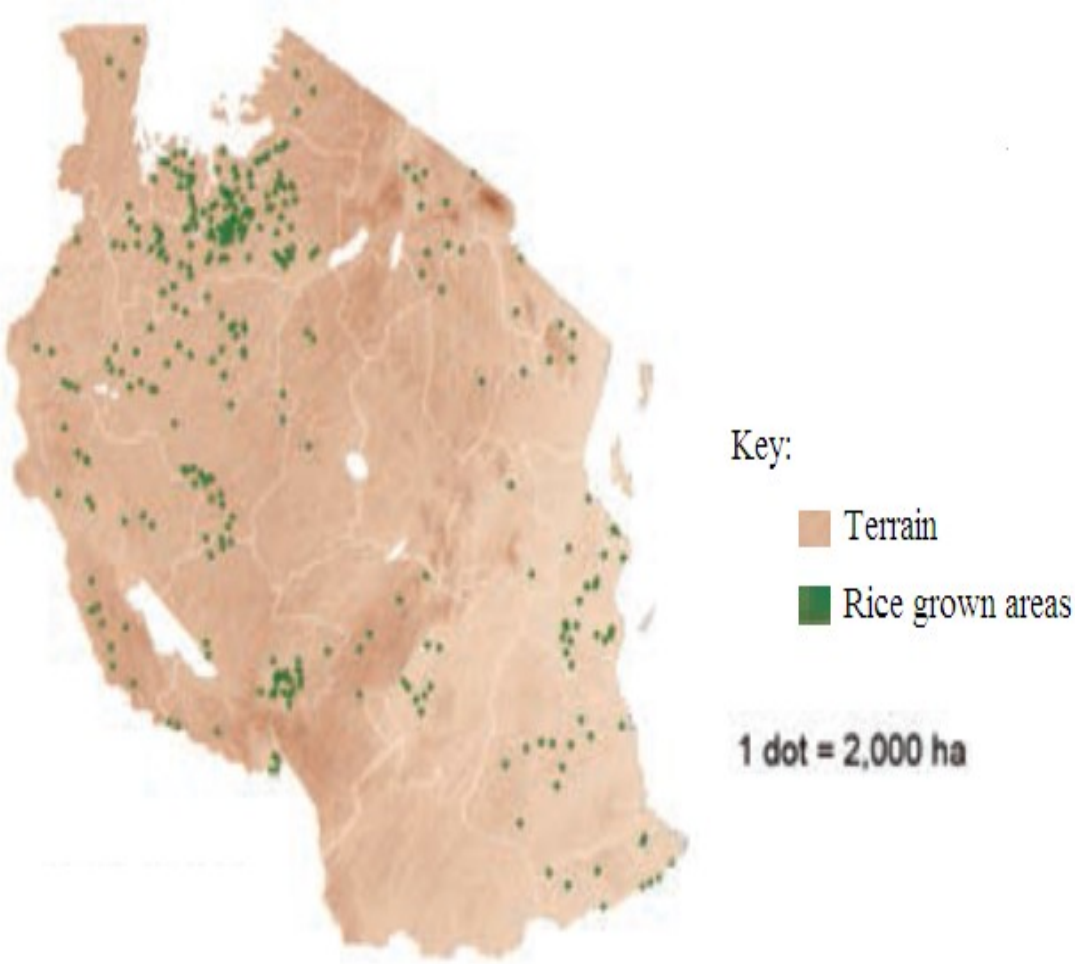
Source: FAOSTAT (2014).

2.4 Rice production in Tanzania

In Tanzania, agriculture is the mainstay of the national economy, whereby rice is one of the major food and cash crops as acknowledged by AfricaRice (2009), for being the largest rice producer accounting for about 80 % of total production in Eastern Africa. However, there is an urgent need to enhance the yield as a key to promote food security from household to national and international levels (WARDA, 2007; Rugumamu, 2014).

Rice in Tanzania is grown in three major ecosystems; rain fed lowland and upland, and irrigated systems. About 71 % of the rice grown in Tanzania is produced under rain fed conditions, irrigated land presents 29 % of the total with most of it in small village level

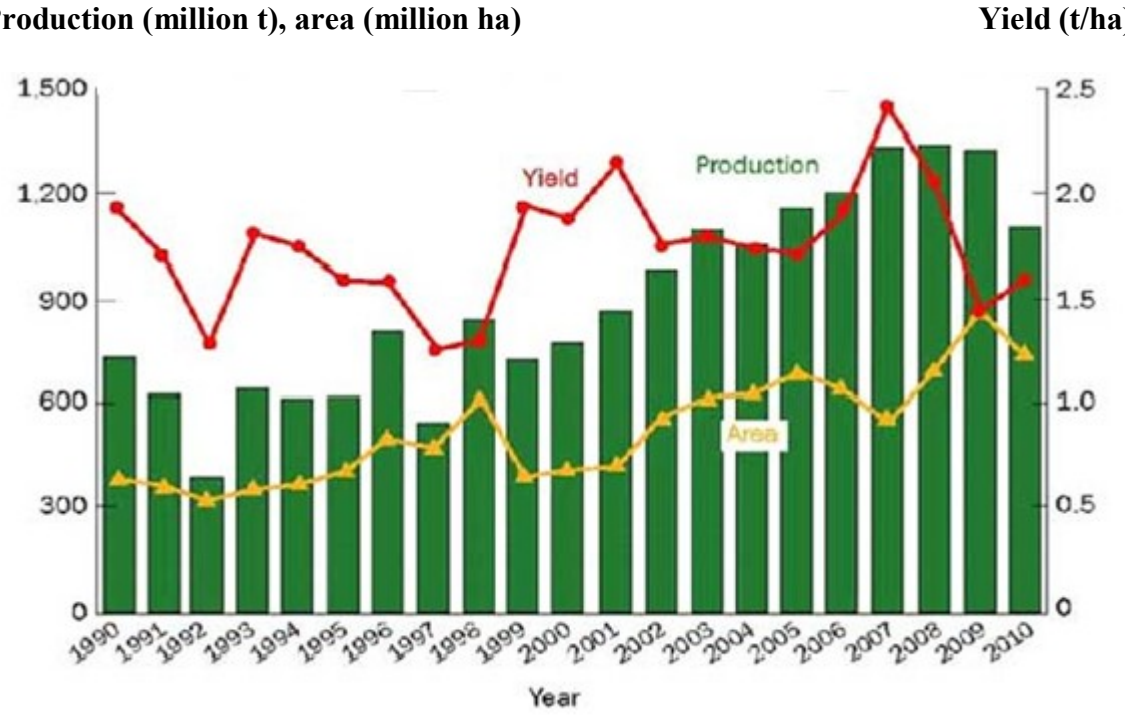
traditional irrigations. The average yield is very low, 1.6-1.8 tons per ha (MAFC, 2009). Rice is grown in swampy areas and river basins such as Rufiji, Ruvu in coast region, Kilombero, Wami, in Morogoro region, Pangani and Mombo in Tanga region (Fig. 1). Rice is a particularly important crop in Central Corridor (CC) which is comprised of Morogoro, Dodoma, Singida, Manyara, Shinyanga and Tabora regions) and 48 % of rice cultivated land in Tanzania is found in these regions (RLDC, 2009).



Source: GRiSP, 2013.

Figure 1: Rice growing areas in Tanzania. The leading regions in rice production are Shinyanga, Tabora, and Mwanza Mbeya, Rukwa and Morogoro. Others include Kilimanjaro, Arusha, Manyara, Iringa, Mara, Tanga and Kigoma.

The area under rice increased from about 0.39 million ha in 1995 to about 0.72 million ha in 2010 (Fig. 2). Under the CARD (Coalition for African Rice Development) initiative, Tanzania endorsed a national rice development strategy (NRDS) in 2009 that is fully in line with national policies and international commitments. The vision is to transform the existing subsistence-dominated rice subsector progressively into commercially and viable production systems (AfricaRice, 2014).



Source: GRiSP (2013)

Figure 2: Rice production Trend in Tanzania between 1990 and 2010. Production increased from about 0.62 million tons in 1995 to about 1.33 million tons of paddy rice in 2009 but dropped to 1.10 million tons in 2010. Average paddy yields across ecosystems have varied widely over the last 20 years (between 1.25 and 2.40 tons/ha) without a clear increasing or declining trend.

2.5 Rice varieties grown in Tanzania

Tanzania has traditionally grown local rice varieties descended from the seeds originally imported by Arab traders before 1960. These varieties are like *Supa*, *Behenge*, *Kula na bwana*, *Kalamata* and many others (Appendix 1) which are well adapted to the climate and the taste preference of the Tanzanians, but they are relatively low yielding, averaging 1–1.5 tons per hectare (RLDC, 2009). Improved varieties such as *IR64*, *SARO5 (TXD3O6), NERICA, IRO5N 221, IRO3A* and many others are also grown in Tanzania (KATRIN, 2012; GRiSP, 2013; IRRI, 2013).

2.6 Tanzanian rice market

Rice is usually sold to local agents and traders who transport and sell it into regional centers where the bigger millers operate. From there rice is hauled to large urban areas, primarily Dar es Salaam, which is the principal market in the country with 40 % of rice consumption. In Dar es Salaam, there is a network of brokers, wholesalers, middlemen, and retailers which ensure that the product gets to the final consumer. Overall, there is considerable cash transactions involved in this entire process, making rice an extremely good crop for stimulating economic activity (RLDC, 2009; Matchmaker, 2010).

Large portion of imported rice in the country is of poor quality. This is manly reflected in the price of imported rice, which is lower than domestic rice prices; For example, price data for 2010 show that high-quality domestic rice is sold at a premium of about 28 % over imported rice (USAID, 2010).

Tanzanian consumers have a preference for aromatic rice which is primarily produced in Tanzania and they pay relatively higher prices for local rice with aromatic qualities (Matchmaker, 2010).

2.7 Selection and breeding programs for rice improvement in Tanzania

A number of breeding programs for rice improvement have been carried out in Tanzania. Success has been recorded with the development of high-yielding rice varieties in by IRRI scientists based in Tanzania who worked in close collaboration with Tanzania's National Rice Research Program of the Agricultural Research Institute (ARI)-KATRIN to develop new varieties; *IR05N 221* (named *Komboka*, meaning be liberated) and *IR03A 262* (named *Tai*, meaning eagle) with yield potentials of 6.5–7 and 7.5 tons per hectare, respectively. These new varieties also possess grain quality that meets the needs and preferences of farmers and consumers in Tanzania that are essential for ensuring their adoption and marketability. *IRO5N 221* is strongly desired for its aroma, which is highly regarded by farmers and consumers, and therefore it is very marketable. *IRO3A 262*, on the other hand, is non-aromatic, yet has strong potential in parts of the country where aroma is less important (IRRI, 2013).

Breeding activities have been carried out to develop rice varieties suitable for different rice agro-ecosystems of Tanzania (Fig. 3). The main breeding approaches include; conventional breeding, mutational breeding and molecular markers assisted breeding. Conventional breeding has been implemented at (KATRIN-DAKAWA) while mutation breeding has been conducted at Sokoine University of Agriculture with assistance and collaboration with International Atomic Energy Agency. Molecular markers assisted breeding has been used in selection of breeding lines developed for rice yellow mottle virus (RYMV) resistance collaboratively implemented between ARI-KATRIN and ARI – Mikocheni and introduction of suitable varieties developed in international institutes such as IRRI and Africa Rice Centre has also been the sources of improved rice varieties in Tanzania (KATRIN, 2012).

Figure 3: Tanzania rice breeders inspect new rice varieties during field evaluation visit in Kyela, Tanzania

2.8 Opportunities for rice production in Tanzania

Tanzania has large land resources suitable for rice production (29 million ha) and abundant water resources (underground, rivers, and lake) for irrigation. There is also clear government political will to enhance rice production and productivity. There is currently a suitable policy environment, with tax exemption measures on the importation of agricultural machinery and subsidies provided to farmers on agricultural inputs such as fertilizer and seed. The government is providing an enabling environment for the private sector to participate more strongly in agricultural production, processing, and marketing (GRiSP, 2013).

The general objective of National Rice Development Strategy (NRDS) is to double rice production by 2018 vis-à-vis 2008, which would be achieved through better farmer access to improved varieties and improved crop management practices and postharvest technologies, strengthening seed systems for delivery of improved varieties to farmers and other end-users (public and private), strengthening the capacity of public and private institutions responsible for research, extension, and training in rice technology development and dissemination. Enhancing agro-processing and value addition, strengthening collaboration and linkages among national, regional, and international institutions involved in rice research and development (MAFC, 2009).

2.9 Challenges facing the rice sector in Tanzania

Challenges facing rice sector in Tanzania include insufficient use of improved seeds whereby most of rice farmers use their own seeds or through farmer to farmer seed exchange (RLDP, 2009; Matchmaker, 2010). Most of the rice grown depends on rainfall and many irrigation schemes need urgent rehabilitation. Upland systems are prone to drought, weed infestation (including *Striga*), and attacks by pests and diseases (*blast*). Rain-fed lowland systems suffer from floods during heavy rains but can also face drought. Weed infestation, pests (*African rice gall midge and stem borers)*, and diseases (*rice yellow mottle virus*, blast, *bacterial leaf blight*) cause low yields. Soil fertility is generally low. Rice competes with other crops such as maize, for land and labor. Inadequate postharvest technologies result in low-quality rice and low prices in the market. Farm operations are mostly (95 %) done manually. Farmers and processors do not have easy access to credit. The infrastructure for transportation, storage, and processing is often lacking or in need of rehabilitation (Balasubramanian *et al*., 2007; Rugumamu, 2014). Sustainable rice production would greatly depend on the establishment of appropriate policy to provide support to farmers especially in-terms of

input supply and output marketing. The development of lowland rice production with improved water supply and control would be essential.

2.10 Rice eating quality

The eating quality of rice is a complex trait involving many physicochemical properties, and thus it has been challenging to accurately evaluate eating quality for selection in rice breeding programs (Lestari *et al*., 2009). Some key physicochemical properties affecting the eating quality are amylose content, pasting properties, gel consistency, gelatinization temperature (GT) and protein content. Good eating quality is also associated with stickiness, sweet flavor, glossiness of the cooked rice, and palatability. Palatability, the trait directly related to rice eating quality, is determined by fragrance or aroma, appearance, taste, and texture (Ramesh *et al*., 2000). In addition to genetic determinants, such as genes involved in the synthesis of starch and protein, rice eating quality is also largely affected by environmental factors, cultural practices, and postharvest practices such as air temperature during ripening, the amount of fertilizer, irrigation management, grain-drying after harvest, and cooking method (Izumi *et al.,* 2007).

2.11 Chemistry of rice aroma

A total of 114 different volatile compounds have been identified to be associated with rice aroma (Yajima *et al*., 1979). One of these, 2-acetyl-1-pyrroline (2AP), is potent flavor component, with a lower odor threshold that gives both basmati and jasmine rices their distinctive fragrances (Buttery *et al*., 1983). 2AP is also present in non-aromatic rice varieties but at a concentration in the range of 10 to 100 times lower than that of aromatic rice (Buttery *et al*., 1983; Buttery *et al*., 1986; Widjaja *et al*., 1996; Wilkie and Wootton, 2004). The threshold concentration at which 2AP can be detected by the human nose is around 0.1 ppb when diluted in water (Buttery *et al*., 1983). Aromatic rice grain has 2AP

concentrations from about 3000 times this level and upwards, while non-fragrant rice has concentrations of 2AP of only about 30 times this threshold level of 2AP in water (Buttery *et al*., 1983; Buttery *et al*., 1986; Wilkie and Wootton, 2004). A wide range of 2AP concentrations have been observed in both aromatic and non-aromatic varieties in different studies.

2.11.1 Factors affecting concentration of 2AP in rice

The 2AP concentration may be due to the differences in rice varieties, differences in extraction procedure or quantification of 2AP, environmental influences on the level of aroma such as temperature and salt and drought stresses (Itani *et al*., 2004; Yoshihashi *et al*., 2004). Other factors include, harvest time or storage conditions of the rice (Bhattacharjee *et al*., 2002; Itani *et al*., 2004; Yoshihashi *et al*., 2004), whether the rice was milled or unmilled (Buttery *et al*., 1983; Philpot *et al*., 2005) and timing/level of nitrogenous fertilizer application to the growing plants (Wilkie and Wootton, 2004).

2.12 Genetic and molecular basis of aroma

The gene responsible for aroma in rice is *betaine aldehyde dehydrogenase* (*BADH2*) whereas an eight-base pair (bp) deletion and three single nucleotide polymorphisms in exon seven of this gene (Fig 4), named as *BADH2.1* gene allele, have led to the introduction of premature stop codon to produce a truncated protein which results in loss of function of the enzyme *BADH2* leading to accumulation of substrate 2AP in aromatic rice varieties, while the functional *BADH2* gene codes for a mature protein which consumes the substrate in non-aromatic varieties (Bradbury *et al*., 2005b).

In addition to eight bp deletion in exon seven, several variations including a seven bp insertion in exon eight, a seven bp deletion in exon two are also responsible for aroma development in rice (Shi *et al*., 2008; Sakthivel *et al*., 2011).

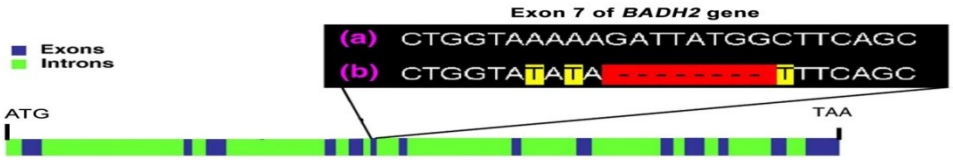


Figure 4: *BADH2* locus showing eight base pair deletions and three SNPs in exon seven of *BADH2* gene; (a) shows an intact exon seven of *BADH2* gene (b) shows eight base pair deletion and three SNPs whereas yellow colour indicates SNPs and red colour indicates eight base pair deletion.

2.13 Marker based evaluation of rice quality

DNA marker-based approaches have been developed for evaluation of rice quality. These methods offer the additional advantages of screening at early breeding generations as well as simplicity and accuracy. Markers based on the Polymerase Chain Reaction (PCR) have been tested for quality evaluation of rice varieties (Takeuchi *et al*., 2007).

Recently, sequence-tagged site (STS) primers developed from random amplified polymorphic DNA (RAPD) analysis were able to differentiate rice varieties according to their palatability (Ohstubo *et al*., 2003). Allele specific amplification (ASA) developed by Bradbury *et al*. (2005b) is very convenient because it is simply resolved in agarose and can be used to distinguish between aromatic and non-aromatic rice varieties and identify homozygous aromatic, homozygous non-aromatic and heterozygous individuals.

Several functional markers have also been developed to distinguish the physicochemical properties of rice, especially the effect of the waxy locus on pasting properties (Larkin *et al*., 2003), and those of amylase content and *starch synthase IIa* (SSIIa) on gelatinization temperature (Bao *et al*., 2006).

The advantages of applying markers in selection include managing target traits through genotyping, ensuring that the selection is independent of environmental factors and developmental stages, maintaining recessive alleles in backcrossing, and pyramiding multiple monogenic traits or several quantitative trait loci (QTL) for a single target trait (Xu *et al*., 2004).

The presence of various types of molecular markers and differences in their principles, methodologies and application, require careful consideration in choosing one or more of such methods. 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 populations and or species (Semagn *et al*., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample collection

Rice samples (representing both aromatic and non-aromatic rice landraces) from different geographical regions of Tanzania (Appendix 1) were previously collected from a genbank at KATRIN Agricultural Research Institute in Ifakara, Morogoro and grown in screen house at Mikocheni Agricultural Research Institute (MARI), Dar es Salaam, located at 39°14'02.85E to 6°45'25.16S and 22.3 metres above sea level.

3.2 Screen house establishment for experimental plants

A total of 160 rice varieties were raised in a screen house by planting 10 seeds of each variety in potted plastic containers containing clay loam soil. After 20 days, young leaves from seedlings were randomly sampled for DNA extraction.

3.3 Sensory evaluation of aroma in traditional Tanzanian rice varieties

Two sensory test methods were performed for aroma evaluation. For the first method, aroma was evaluated from 20 days old leaves following a modified protocol of Sood and Siddiq (1978). For the second method, aroma was evaluated by heating several rice seeds in water (Wanchana *et al*., 2005). Both sensory test methods were performed by five trained panels.

3.3.1 Leaf aromatic test

Approximately 0.3 g of leaf samples was taken from each variety and cut into tiny pieces (< 2mm) and put into glass Petri-plates. 15 ml of 1.9 % (1000 ml dH2O+ 1.7 g KOH) potassium hydroxide (KOH) was added to each of the Petri-plates containing the sample

and covered immediately. These Petri-plates were left under room temperature for overnight and then opened one by one for aroma test. The content in each Petri-plate was smelt and the results were recorded. This is a modified protocol of Sood and Siddiq (1978) where amount of leaf and concentration of KOH have been changed.

3.3.2 Grain aromatic test

One gram of each rice variety was placed in a test tube previously filled with 10 mls of water. The test tube was then covered with aluminum foil and cooked in boiling water for 15 minutes. After the samples were cool, the aluminum foil was opened and aroma was tested by smelling.

3.4 Molecular analysis of *BADH2 /BADH2.1* alleles in popular traditional Tanzanian rice varieties

3.4.1 Genomic DNA extraction

Genomic DNA was extracted from leaves of rice plants following Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (IMD, 2001) (Appendix 2). Deoxyribonucleic acid (DNA) was extracted from a total of 160 Tanzanian traditional rice varieties including both aromatic and non-aromatic rice landraces. Briefly, CTAB protocol aids the separation of polysaccharides from nucleic acids based on their differential solubilities in the presence of CTAB. In order to release the cellular constituents, the cell walls were broken by grinding the tissue in liquid nitrogen with a mortar and pestle. To release the DNA the cell membranes were disrupted by using a CTAB detergent. The time between thawing of the frozen, pulverized tissue and its exposure to the extraction buffer was minimized to avoid nucleolytic degradation of the DNA. Deoxy ribonucleic acid (DNA) was protected from the endogenous nucleases by addition of EDTA. Ethylene diamine tetraacetic acid (EDTA) is a chelating agent that binds magnesium ions, generally

considered a necessary cofactor for most nucleases. The buffer/tissue mixture was emulsified with either chloroform or phenol to denature and separate the proteins from the DNA. Deoxyribonucleic acid (DNA) was pelleted by centrifugation, the supernatant discarded and DNA recovered after air drying of the collection tube until all of the liquid evaporated. Shearing of DNA was minimized and DNA in solution was handled very gently.

3.4.2 Determination of yield and quality of the genomic DNA

Yield and quality of isolated DNA were determined using a spectrophotometer and agarose gel electrophoresis, respectively. The DNA concentration of each sample was determined by taking the reading directly using a Cecil CE 3021 spectrophotometer (Cecil Instruments, Cambridge, UK). The A260/A280 ratio was used to provide an estimate of DNA purity. The quality and purity of DNA was verified by running samples on a 0.8 % (w/v) agarose gel in one times Tris-acetate EDTA (TAE) (40 Mm Tris-HCl and one mM EDTA at pH 8.3 adjusted with acetic acid) buffer containing ethidium bromide (10 mg/ml) at 80 V for one hour. Visualization of the DNA bands was done using a Syngene Bioimaging System (Sony Corporation, Tokyo, Japan) fitted with analysis software. After determination of DNA concentration and quality, DNA was diluted to a working concentration of 100 ng/µl in double distilled water.

3.4.3 PCR amplification of *BADH2/BADH2.1* alleles

DNA amplification was done using a Gene Amp® PCR System 9700 Base Module (Applied Biosystems Inc, Carlsbad, CA). Primers targeting *BADH2/BADH2.1* alleles (Table 2) previously described by Bradbury *et al.* (2005b) were obtained from Bioneer Corporation (Daejeon, South Korea). Polymerase Chain Reaction (PCR) was performed in a total volume of 20 μl containing two μl of genomic DNA (100 ng/μl), two μl of ten

times reaction buffer, one μl of 10 mM dNTPs mix, 0.2 μl of taq DNA Polymerase, four primers all mixed at once, each contained one μl of 10 μM (external antisense primer-EAP, external sense primer-ESP, internal non-fragrant sense primer-INSP and internal fragrant antisense primer-IFAP). The primer sequences are shown in Table 2. Polymerase Chain Reaction (PCR) cycling consisted of an initial denaturation at 94 ^{0}C for five minutes followed by 35 cycles of 30 seconds at 94 ^{0}C, 45 seconds at 55 ^{0}C, one minute at 72 ^{0}C, concluded with final extension of seven minutes.

Polymerase chain reaction products were analyzed by gel electrophoresis using agarose (1.5 %) stained with ethidium bromide whereby 100 base pair (bp) ladder molecular weight standard (Bioneer Corporation, Daejeon, South Korea) was used to estimate PCR fragment size and visualization was done using a Syngene Bioimaging System (Sony Corporation, Tokyo, Japan) fitted with analysis software.

Table 2: Primers for analysis of aroma in rice

Primer name	**Primer sequence (5'→3')**
External Sense Primer (ESP)	TTGTTTGGAGCTTGCTGATG
Internal Fragrant Antisense Primer (IFAP)	CATAGGAGCAGCTGAAATATATACC
Internal Non-fragrant Sense Primer (INSP)	CTGGTAAAAAGATTATGGCTTCA
External Antisense Primer (EAP)	AGTGCTTTACAAAGTCCCGC

3.5 Sequencing of PCR products directly or after cloning

3.5.1 Purification of PCR products

PCR products *(BADH2/BADH2.1* fragments) were purified using GeneJET PCR Purification Kit (Thermal scientific, Massachusetts, USA), following manufactures' instructions (Appendix 3), Briefly a reaction mixture containing PCR products was

combined with the binding buffer and added to a purification column. A chaotropic agent in the binding buffer denatures proteins and promotes DNA binding to the silica membrane in the column. As an added convenience, the binding buffer contains a color indicator that allows for easy monitoring of the solution pH for optimal DNA binding. Impurities were removed with a simple wash step. Purified PCR products were then eluted from the column using an elution buffer. During purification process the flow through was discarded and the empty purification columns were repetitively centrifuged to completely remove any residual wash buffer that may inhibit subsequent reactions.

The purified PCR products were stored at -20 ^{o}C until sequencing.

3.5.2 Direct sequencing of PCR products

The purified PCR products (257 bp and 355 bp fragments) representing aromatic and non-aromatic genotypes were sequenced by dideoxynucleotide cycle sequencing using a 3730 x L genetic analyser (Applied Biosystems, Foster City, CA). Four primers (Table 2) targeting *BADH2/BADH2.1* sequences were used in sequencing. These primers were able to amplify the overlapping *BADH2/BADH2.1* fragments for all aromatic and non-aromatic varieties. Consensus sequences were obtained using the Sequence Scanner Software Version 2.0 (Applied Biosystems, Foster City, CA). Sequences were compared with published sequences using the basic alignment search tool (BLASTn) at the NCBI website. Multiple sequence alignment, nucleotide sequence identity calculation and phlogenetic analysis of the *BADH2/BADH2.1* alleles among Tanzanian aromatic and non-aromatic rice landraces were performed using Geneious computer software (Biomatters Ltd) package.

3.5.3 Indirect sequencing of PCR products

3.5.3.1 Preparation of competent cells

The Z-competent *E. coli* Transformation Kit from (Zymo Research Corporation, Orange County**,** USA) was used to make DH5α-T1®competent cells because cells prepared using this kit can be transformed without heat shock. Briefly, *E. coli* DH5α-T1®competent cells were grown in Luria-Bertani (LB) medium plates to log phase, then washed and the cells suspended in the provided buffers. The competent cells were then aliquoted and stored at -80 ^{o}C for transformations.

3.5.3.2 Ligation of PCR products into vectors

The amplified PCR products *(BADH2/BADH2.1* fragments) were cloned using sticky-end cloning Kit (Thermal-Scientific, Massachusetts, USA), according to manufacturer's instructions (Appendix 4). The ligation mixture was performed in a total volume of 20 µl containing ligation reaction (two µl) added to 18 µl blunting reaction. The blunting reaction contained, twice the reaction buffer six µl, three µl of purified PCR sticky-end DNA fragment (50-100 ng), water, nuclease-free to eight µl, and one µl DNA blunting enzyme. The blunt reaction was vortexed briefly and centrifuged at 13000 rpm for five second and the mixture was incubated at 70 °C for five minutes and then chilled on ice. The set up ligation reaction on ice contained one µl pJET1.2/blunt cloning vector (50 ng/µl) and one µl T4 DNA ligase. The ligation mixture was vortexed briefly and centrifuged at 13000 rpm for five second to collect drops and the ligation mixture incubated at room temperature (22 °C) for five minutes and the ligation mixture was used directly for transformation of bacterium.

3.5.3.3 Transformation of ligated plasmids

E. coli competent cells *DH5α-T1*® (Zymogen Research Corporation, Orange County, USA) were used for the transformation of ligated plasmids. Frozen (-80 ^{o}C) competent cells were placed on ice until thawed and gently mixed by flicking the tube. Fifty microlitres of competent cells were added to five µl of ligation mix in a 1.5 ml eppendorf tube, mixed gently by flicking the tube several times before returning the tube to ice for 10 min. The tube was heat shocked for 50 sec in a water bath at exactly 42 ^{o}C and then immediately placed on ice for two minutes. 400-450 µl Luria-Bertan (LB) medium containing 50 µg/ml ampicilin was added and incubated for 60 min at 37 ^{o}C with shaking at 150 rpm.

3.5.3.4 Culturing of transformed cells

After incubation of the transformed cell culture, 150 µl of it was spread on LB medium plates containing 50 µg/ml. One plate was spread with 50 µl of the competent cells as negative control. The plates were incubated for at least 18 hours at 37 ^{o}C to allow the development of white colonies. Single white colonies were picked from the plates and purified to isolate the plasmids DNA from bacterial cells tested by PCR for the insert.

3.5.3.5 Plasmid DNA purification

Plasmids DNA were isolated from bacterial cells using QIAGEN minipreps kit (QIAGEN, Hilden, Germany), according to manufacturer's instructions (Appendix 5). The extraction of recombinant plasmid DNA was done from the overnight cultures of *E. coli* competent cells DH5α-T1® grown in LB medium. The eluted DNA was tested by PCR (using primers as shown in Table 2) to confirm if we amplified the targeted *BADH2.1* gene allele.

3.5.3.6 Sequencing of cloned *BADH2/BADH2.1* alleles

The purified cloned PCR products (257 bp and 355 bp fragments) representing aromatic and non-aromatic genotypes were sequenced by dideoxynucleotide cycle sequencing using a 3730 x L genetic analyser (Applied Biosystems, Foster City, CA), at Ascefran LLC, North Carolina USA, using forward and reverse M13 primer. Consensus sequences were obtained using the Sequence Scanner Software Version 2.0 (Applied Biosystems, Foster City, CA). Sequences were compared with published sequences using the basic alignment search tool (BLASTn) at the NCBI website. Multiple sequence alignment of the *BADH2/BADH2.1* alleles among Tanzanian aromatic and non-aromatic rice landraces were performed using Geneious computer software (Biomatters Ltd) package. The aligned sequences were used to confirm if we amplified targeted *BADH2/BADH2.1* alleles.

CHAPTER FOUR

4.0 RESULTS

4.1 Leaf and grain aromatic tests

From both methods; grain and leaf aromatic tests, a total of 16 varieties were found to be non-aromatic in each method, while 31 varieties had mixed results that is, in grain aromatic test showed presence of aroma but the same variety showed absence of aroma in leaf aromatic test and vice versa. However, a total of 95 varieties showed presence of aroma in both methods. From the table of results (Appendix 6) 16 varieties were not tested using leaf aromatic tests, these varieties died in the screen house before leaves were produced.

4.2 Quality and quantity of rice genomic DNA

In the present study, DNA was extracted from rice leaves using CTAB protocol previously described by IMD (2001). The quality of DNA was investigated by agarose gel electrophoresis while quantity of DNA was examined by spectrophotometry (Table 3; Fig 5). Based on A 260/A 280 ratio (Table 3), the values were between 1.8 and 2.1 in all samples indicating that the genomic DNA was of high quality. High DNA concentrations between 800 and 3500 ng/µl were obtained (Table 3). The resulting DNA was diluted to 100 ng/µl for use during PCR amplification of *BADH2/BADH2.1* alleles.

Table 3: Deoxyribonucleic acid (DNA) quantity obtained from rice leaves using CTAB protocol previously described by IMD (2001) as determined by spectrophotometry

Variety	DNA conc (ng/µl)	A260	A280	A260/280
Lingwindimba	1809.2	0.784	0.428	1.83
Hali mgeni	1555.9	1.119	0.585	1.91
Zena	880.6	1.611	0.842	1.91
Shinyanga	1112.2	2.244	1.179	1.9
Kibata ngoto	983.6	1.673	0.877	1.91
Faya	1098.3	1.967	1.069	1.84
Kia la kondoo	2076	1.521	0.799	1.9
Machua	1253	1.059	0.583	1.80
Madevu	1882.8	1.656	0.86	1.93
Supa Buramba	1373.9	1.478	0.766	1.93
Kitenge	2104.5	2.09	1.09	1.92
Tambala	994.9	1.898	0.984	1.93
Kazole	1103.8	2.076	1.083	1.92
Kijicho	2108.1	2.162	1.119	2.10
Niwahi	1097.6	1.953	1.017	1.92
Kihogo red	1092.5	1.851	0.942	2.06
Purure	3483.6	1.672	0.86	1.95
Wahiwahi	874.6	1.491	0.772	1.93
Malemata	1096	1.92	0.988	1.94

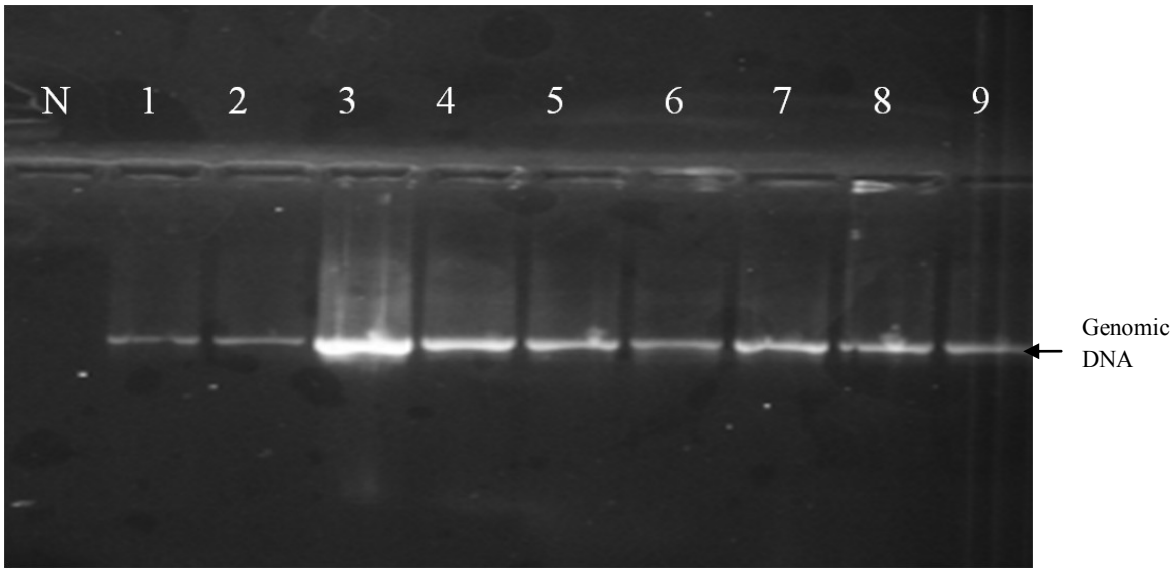


Figure 5: Agarose gel electrophoresis showing quality/integrity of DNA extracted from rice leaves. A single clear band of rice genomic DNA was observed in all samples (1 through 9) indicating good quality DNA. N indicates a negative control with no DNA added.

4.3 PCR amplfication of *BADH2/BADH2.1* alleles

PCR amplification of *BADH2/BADH2.1* alleles was carried out using allele specific amplification (ASA) as previously described by Bradbury *et al*. (2005b). Fragment sizes of 257 and 355 base pairs (bp) were amplified using external and internal primers (Table 2). External primers; External sense primers (ESP) and External antisense primers (EAP) paired to produce expected fragment of approximately 580 bp indicating positive control for all genotypes. Internal primers; Internal non-fragrant sense primer (INSP) and Internal fragrant antisense primer (IFAP) produced two bands of 355 and 257 bp when paired with external primers, EAP and ESP. Accordingly, ESP and IFAP primer pair amplified a 257 bp PCR products showing the marker for aromatic genotypes and INSP and EAP amplified a 355 bp band representing non-aromatic genotypes.

The appearance of both 257 and 355 bp bands indicate the presence of heterozygous non-aromatic genotypes. Each band produced by the primers was distinct and reproducible, (Fig. 6). Out of 160 landraces; 87 were homozygous aromatic (*BADH2.1/BADH2.1)*, 63 were homozygous non-aromatic *(BADH2/BADH2*) and 10 were heterozygous non-aromatic (*BADH2/BADH2.1)* (Appendix 7; Table 4).

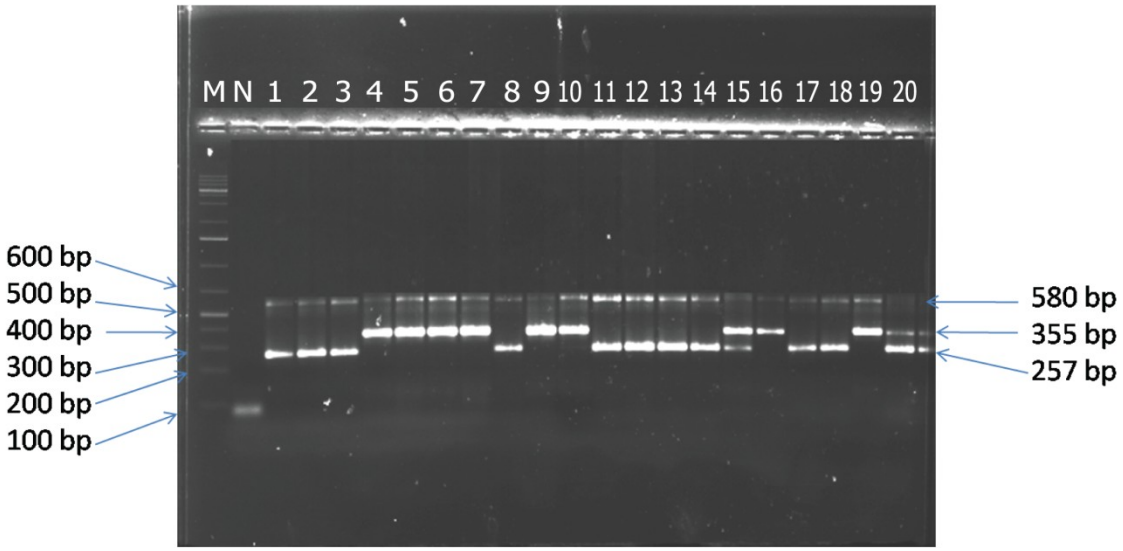


Figure 6: Agarose gel electrophoresis of PCR amplified *BADH2/BADH2.1* fragments from rice samples. A single band of 580 base pair was observed in all samples (1 through 20) indicating a positive control in all genotypes. Amplification of *BADH2* and *BADH2.1* alleles produced a distinct band of approximately 355 and 257 base pairs, respectively. The appearance of both 257 and 355 base pair (bp) bands indicate the presence of heterozygous non-aromatic genotypes. M indicates 100 bp DNA ladder, with band sizes given on the left hand side of the gel and N is a negative control (no DNA was added).

Table 4: Molecular screening for *BADH2*/*BADH2.1* alleles

Aroma Status	Number of Varieties	Percent
Homozygous aromatic (*BADH2.1/BADH2.1)*	87	54.36
Homozygous non-aromatic (*BADH2/BADH2)*	63	39.38
Heterozygous non-aromatic (*BADH2/BADH2.1)*	10	6. 26
Total	160	100.00

4.3.1 Comparison of sensory evaluation and molecular analysis for aroma

Based on sensory evaluation tests (Appendix 6) and genotyping results (Appendix 7; Fig.6), four homozygous aromatic varieties were found to be non-aromatic in both leaf and grain aromatic tests; moreover 12 homozygous non-aromatic and seven heterozygous non-aromatic landraces were found to be aromatic in both grain and leaf aromatic tests.

4.4 Cloning of *BADH2*/ *BADH2.1* PCR products

The pJET1.2/blunt vectors with the insert of *BADH2*/*BADH2.1* fragments were successfully transformed chemically to *DH5α-T1*® competent cells. The vector expressed a lethal restriction enzyme such that the vectors without inserts are not propagated and only recombinant clones appear on cultured plates. Blue/white screening was therefore, not required. The formation of white colonies on cultured plates indicated that *BADH2/BADH2.1* fragments were successfully ligated into pJET1.2/blunt vectors. The successful colonies were inoculated into LB media containing 50 µg/ml ampicilin in Falcon tubes overnight and the formation of cloudy colours indicates the growth of *DH5α-T1E.coli* cells. The overnight cultured plasmid DNA from Luria-Bertan (LB) media were used to confirm by PCR (Fig.7) expected inserts fragments of *BADH2*/*BADH2.1* allele with size of 257 and 355 bp by using primers (Table 2) previously described by Bradbury *et al*. (2005b). Amplification of *BADH2*/*BADH2.1* fragments produced distinct bands of approximately 257 and 355 bp wich confirms the

presence of *BADH2/BADH2.1* inserts after cloning of *BADH2*/*BADH2.1* PCR products (Fig.7).

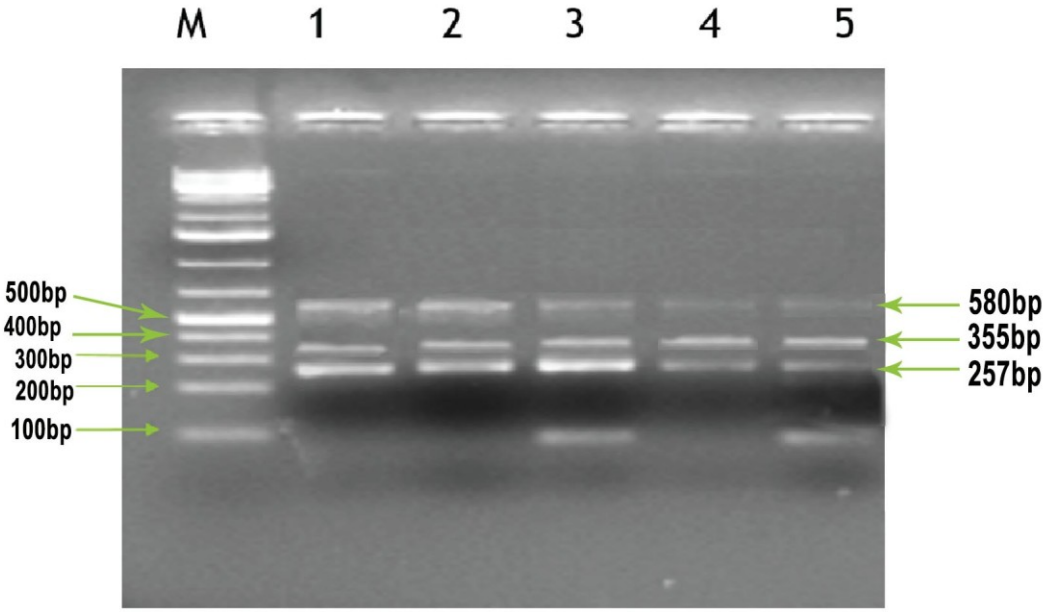


Figure 7: Agarose gel electrophoresis of PCR amplified *BADH2*/*BADH2.1* fragments from cloned *BADH2/BADH2.1* gene allele. A single band of 580 base pair was observed in all samples indicating a positive control for all genotypes. Amplification of *BADH2*/*BADH2.1* fragments produced distinct bands of approximately 257 and 355 bp respectively wich confirms the presence of *BADH2*/*BADH2.1* inserts after cloning of *BADH2*/*BADH2.1* PCR products.

4.5 Nucleotide Sequencing and sequence analysis of *BADH2/BADH2.1* alleles

Alignment of *BADH2/BADH2.1*nucleotide sequences revealed eight base pair (bp) deletion (5-'GATTATGG-3') and three SNPs in exon seven of all homozygous aromatic rice genotypes whereas homozygous non-aromatic rice genotypes were having an intact exon seven (Fig. 8). Bolded letters show SNPs.

Supa kijicho	GAGTTATGAAACTGGTAAAAAGATTATGGCTTCAGC
Niwahi	GAGTTATGAAACTGGTAAAAAGATTATGGCTTCAGC
Kivuli	GAGTTATGAAACTGGTAAAAAGATTATGGCTTCAGC
Pururе	GAGTTATGAAACTGGTAAAAAGATTATGGCTTCAGC
Kichwa cha nzi	GAGTTATGAAACTGGTAAAAAGATTATGGCTTCAGC
Kijicho	GAGTTATGAAACTGGTAAAAAGATTATGGCTTCAGC
Madevu	GAGTTATGAAACTGGTAAAAAGATTATGGCTTCAGC
Domo la fisi	GAGTTATGAAACTGGTAAAAAGATTATGGCTTCAGC
Kia la kondoo	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC
Mabula	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC
Karatasi	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC
Tarabinzuna	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC
Makaniki	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC
Tabora	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC
Kipeteli	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC
Ambali	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC
Bibi wa Unguja	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC
Wahiwahi	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC
Jobela	GAGTTATGAAACTGGTA**T**AT A - - - - - - - - **T**TTCAGC

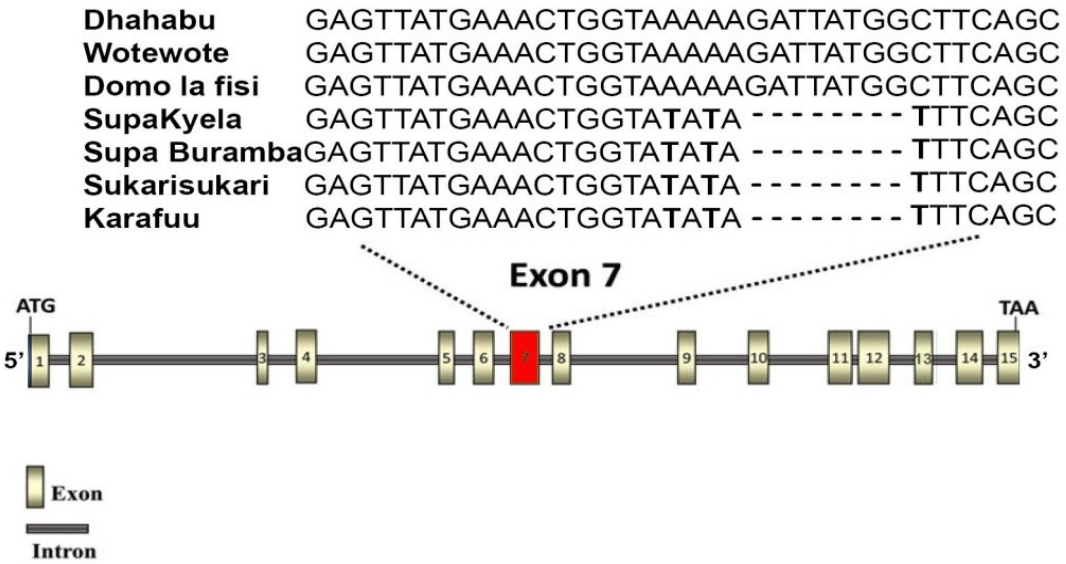


Figure 8: Structure of *BADH2* locus. The functional *BADH2* gene consists of 15 exons and 14 introns with its start codon (ATG) in exon one and stop codon (TAA) in exon 15. *BADH2.1* allele has eight-bp deletion (5'-GATTATGG-3'), this is demonstrated by four aromatic genotypes (*Supa Kyela, Supa Buramba, Sukarisukari* and *Karafuu*) and three single nucleotide polymorphisms (SNPs) in exon 7. The non-aromatic genotypes (*Dhahabu, wotewote* and *Domo la fisi*) have the functional *BADH2* alleles.

4.6 Genetic diversity of *BADH2/BADH2.1* gene alleles in popular traditional rice varieties in Tanzania

The genetic diversity of *BADH2/BADH2.1* alleles in Tanzanian rice varieties was investigated through sequencing and phylogenetic analysis of *BADH2/BADH2.1* nucleotide sequences. Results showed a large amount of genetic variability (39.41-100 % nucleotide sequence identity) among the varieties studied (Appendices 8 and 9).

Based on the percentage identity matrix calculation on *BADH2/BADH2.1* nucleotide sequences; Most genotypes displayed high nucleotide sequence identity (100 %) amongst each other (Appendices 8 and 9). Moreover, the following varieties displayed low nucleotide sequence identity amongst one another; *Supa India* and *Kipaku* (39.41 %), *Moshi wa taa* and *Supa India* (40 %), *Kipaku* and *Liwatawata* (41.55 %) and *Mzurinani* and *kipaku* (42.56 %) and others (Appendices 8 and 9).

The phylogenetic analysis (Appendix 8) based on sequence alignment of 160 landraces and published sequences from GenBank generated six main clusters irrespective of the collection regions. Following cluster analysis; it was observed that (in cluster IV) most of the aromatic landraces were clustered with published aromatic genotypes (Appendix 8).

Generally all clusters (Appendix 8) were commonly occupied with genotypes with higher/maximum nucleotide sequence identity and few diverged or distant related genotypes.

CHAPTER FIVE

5.0 DISCUSSION

Rice aroma, which is controlled by a recessive *BADH2.1* gene allele, is an attractive trait to consumers and plays a vital role in global rice trading. The *BADH2* locus encoding *BADH2* enzyme has been proven to be associated with rice aroma. The functuonal *BADH2* allele inhibits 2AP synthesis in non-aromatic rice varieties whereas *BADH2.1* gene allele with eight base pair deletion and three SNPs, lead to the introduction of premature stop codon in the coding sequence for *BADH2* enzyme resulting to a truncated protein which results in loss of function of the enzyme *BADH2* leading to accumulation of substrate 2AP in aromatic varieties (Bradbury *et al.,* 2005a; Vanavichit *et al.*, 2006); Shi *et al.*, 2008; Myint *et al.*, 2012; Yeap *et al.*, 2013).

In the present study, combination of sensory tests and molecular analysis for the detection of *BADH2.1* gene allele in Tanzanian rice landraces was used. In this context, sensory methods facilitated the identification of aromatic and non-aromatic landraces, whereas molecular analysis through the use of ASA confirmed the results by identifying the presence of *BADH2/BADH2.1* alleles. Apart from identification of specific alleles, ASA also aided in determination of zygosity (homozygous or heterozygous for *BADH2*/*BADH2.1* alleles) in Tanzanian rice landraces. In the light of this information rice breeders may decide not to cross heterozygous non-aromatic landraces as this may give rise to a mixture of aromatic and non-aromatic varieties; This is not possible by convetional/traditional breeding methods because it is not possible to identify heterozygous plants. In this case ASA marker is useful in the maintenance of grain aroma and quality rice seeds production (Bradbury *et al*., 2005a; Kiani, (2011); Ram *et al*., 2007; Yeap *et al*., 2013).

In molecular/genotypic analysis (Fig 6), all (160) genotypes produced a 580 base pair band indicating positive control for all genotypes, 87 genotypes showed bands of 257 bp indicating homozygous aromatic genotypes, 63 genotypes produced 355 base pair band indicating homozygous non-aromatic genotypes and 10 genotypes showed both 257 and 355 bp bands indicating heterozygous non-aromatic genotypes. Similar amplification pattern of *BADH2/BADH2.1* alleles was observed by Bradbury *et al*. (2005a); Bounphanousoy *et al*. (2008); Kiani, (2011) and Yeap *et al*. (2013).

Of the 160 landraces genotyped and phenotyped; 95 varieties (Appendices 6 and 7) were classified as aromatic by the presence of aroma in both leaf and grain aromatic tests. Most of these (91.6 %) carried the *BADH2.1* gene allele. This demonstrated that the majority of aromatic rice varieties from different geographical regions of Tanzania had eight base pair deletion and three single nucleotide polymorphisms (eight-bp deletion and three-SNPs) in exon seven of aroma gene.

Interestingly, the present study revealed that 19 (19.6 %) landraces (Section 4.3.1) which were previously identified as aromatic by both grain and leaf aromatic tests were found to be non-aromatic as a result of molecular analysis. Similar results were reported by Bouphanousoy *et al*. (2008) who reported similar findings between molecular and chemical analysis results in most of the rice varieties except for some contrasting results such as in local aromatic rice variety *Kai Noi Leuang* which produced aroma but was identified as homozygous non-aromatic by molecular marker analysis. Another study by Myint *et al*. (2012) reported similar findings except for contradicting results whereby two aromatic varieties *Pathein nyunt* and *Yangon saba* which were identified as aromatic in sensory tests but did not contain eight base pair deletion (*BADH2.1* gene allele).

Moreover a recent study by Yeap *et al*., 2013 reported similar findings except for aromatic rice variety *Kasturi* which produced aroma despite the fact that it was identified as homozygous non-aromatic by molecular marker analysis. It was suggested that these contradicting results may be caused by; different gene location or other less frequent mutations on the other regions of the *BADH2* gene might be responsible for the observed aroma or the presence of another major aromatic compound. Moreover, presence of minor genes or environmental factors and the fact that some rice varieties may carry minor Quantitative Trait Loci (QTLs) which influence aroma in rice may contribute to the stated findings (Bradbury *et al*., 2005a; Bouphanousoy *et al.,* 2008; Shi *et al*., 2008; Kovach *et al*., 2009; Yeap *et al*., 2013). Furthermore, four landraces (Section 4.3.1) which were identified as non-aromatic in sensory evaluation were found to be homozygous aromatic as a result of molecular analysis. This may be due to failure of *BADH2.1* gene allele expression as a result of epistasis or epigenetic effects. This suggests further investigation to yield concrete evidence to unveil this contradicting aspect.

Evidence from sequencing of *BADH2/BADH2.1* alleles confirmed the association of *BADH2.1* gene allele with aroma in aromatic rice landraces as it was shown that all aromatic genotypes had eight base pair deletion and three SNPs in exon seven of *BADH2* locus. This suggests that *BADH2.1* gene allele is the main aroma allele in most of the Tanzanian aromatic rice varieties (Fig. 8).

Traditional rice varieties, or landraces, have a higher level of genetic heterogeneity compared with modern varieties. This genetic variability is utmost important for the sustainability of small marginal farmers, because despite the low yield capacity, these varieties /landraces present high tolerant to abiotic and biotic stresses (Ram *et al*., 2007; Sarhadi *et al*., 2011).

The present study showed a wider/broad range of *BADH2/BADH2.1* nucleotide sequence identity (39.41-100 %) in popular Tanzanian rice landraces. Some aromatic genotypes showed higher genetic similarity with published aromatic genotypes (Appendix 8). Generally all clusters (Appendix 8) were commonly occupied with genotypes with higher/maximum nucleotide sequence identity and few diverged or distant related genotypes. Higher nucleotide sequence identity observed within aromatic and non-aromatic genotypes constitutes strong evidence that they are closely related to each other and they may be part of the same ancestral population or may have spread to different areas following germplasm exchange or importation of rice seeds (Bradbury *et al.,* 2005b; Fitzegerald *et al.,* 2008). However, the intra and inter genetic variation (between and within aromatic and non-aromatic genotypes) might be useful for breeders to improve aromatic rice landraces through selective breeding and crossbreeding programs (Ram *et al*., 2007; Yi *et al*., 2009).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The screening and sequencing of *BADH2/BADH2.1* alleles in popular Tanzanian rice landraces demonstrated that *BADH2.1* gene allele is associated with aroma in Tanzanian aromatic rice landraces. Despite the fact that this unique genotype (eight base pair deletion and three SNPs determines the aroma biosynthetic capability in most aromatic rice varieties; the present study has revealed that eight base pair deletion and three SNPs is not the only sequence variation that causes aroma and that there are other mutations that drive the accumulation of 2-acetyl-1- pyrroline (2AP). The findings of the present study indicated that, genotypic analysis or sensory tests alone could not represent the complete aromatic conditions. Marker-based identification and differentiation of rice landraces could be helpful to improve and preserve the integrity of the high quality aromatic rice varieties to benefit farmers, breeders and consumers.

6.2 Recommendations

i. Identification of multiple mutations for 2AP will enable rice breeding programs to actively select for multiple genetic sources of 2AP in different aromatic varieties.

ii. Integration of molecular analysis with sensory methods will facilitate rapid and reliable identification/screening of aromatic and non-aromatic rice varieties.

iii. Further work to determine the inhibitors of gene expression in those non-aromatic rice varieties which have the allele for aroma need to be done.

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APPENDICES

Appendix 1: Popular traditional Tanzanian rice varieties

S/N	Variety	District	Region
1	Kanunga	Ulanga	Morogoro
2	1K	Ifakara	Morogoro
3	Liwatawata	Ulanga	Morogoro
4	2K	Ifakara	Morogoro
5	Chikweta	Ulanga	Morogoro
6	3K	Ifakara	Morogoro
7	Kalivumbura	Ulanga	Morogoro
8	4K	Ifakara	Morogoro
9	Africa	Ulanga	Morogoro
10	Lawama	Ulanga	Morogoro
11	Mzuri nani	Ulanga	Morogoro
12	Somba	Ulanga	Morogoro
13	Lingwelingweli	Ulanga	Morogoro
14	Taiwan	Ulanga	Morogoro
15	TOx	Ulanga	Morogoro
16	Tela	Ulanga	Morogoro
17	Mwanza	Ulanga	Morogoro
18	Kasegere	Ulanga	Morogoro
19	Dunduli	Ulanga	Morogoro
20	Nitalima wangu	Tunduru	Ruvuma
21	Zena	Tandahimba	Mtwara
22	Bora kupata	Nanyumbu	Mtwara
23	Ngwindimba	Mtwara vijijini	Mtwara
24	Lingwindimba	Tunduru	Ruvuma
25	Chiogo	Tunduru	Ruvuma
26	Chimbakata	Tandahimba	Mtwara
27	Sindano	Kilwa	Lindi
28	Ngoge	Kilwa	Lindi
29	Namahoki	Kilwa	Lindi
30	lichende	Tandahimba	Mtwara
31	Mpunga safi (ganda moja)	Mtwara vijijini	Mtwara
32	Jobela	Mtwara vijijini	Mtwara
33	Maganda manne	Nanyumbu	Mtwara
34	Wotewote	Kilwa	Lindi
35	Moto wa ngambo	Tunduru	Ruvuma
36	Wahiwahi	Mzega	Tabora
37	Nkunguru (supa kijivu)	Igunga	Tabora
38	Kihogo red	Igunga	Tabora
39	Malamata	Igunga	Tabora

S/N	Variety	District	Region
40	Malimata	Igunga	Tabora
41	Minoga mhuli (meno ya tembo)	Igunga	Tabora
42	Supa (Burundi)	Kibondo	Kigoma
43	Sindano	Kibondo	Kigoma
44	Tuliani	Igunga	Tabora
45	Nganyalo	Igunga	Tabora
46	Supa mbawambili	Igunga	Tabora
47	Moshi wa sigara	Igunga	Tabora
48	Bishori	Nzega	Tabora
49	Umano	Nzega	Tabora
50	Omano	Igunga	Tabora
51	Sembe	Sengerema	Mwanza
52	Sukarisukari	Bunda	Mara
53	Sengasenga	Bunda	Mara
54	Migiha (katumbo)	Sengerema	Mwanza
55	Kabangala	Shinyanga	Shinyanga
56	Umano	Shinyanga	Shinyanga
57	Kalamata	Kishapu	Shinyanga
58	Malamata	Sengerema	Mwanza
59	Gamti ndongo	Shinyanga	Shinyanga
60	Tela	Bunda	Mara
61	Mabeyenga	Shinyanga	Shinyanga
62	Mwanamwala	Shinyanga	Shinyanga
63	Malitela	Kishapu	Shinyanga
64	Supa Buramba/ Supa urambo	Musoma mjini	Mara
65	Mchina	Shinyanga	Shinyanga
66	Malamata/kalamata	Sengerema	Mwanza
67	Mwinula	Bunda	Mara
68	Supa India	Musoma mjini	Mara
69	Shinyanga	Bunda	Mara
70	Tondogoso	Bunda	Mara
71	Gamti Kubwa	Shinyanga	Shinyanga
72	Malombo-ga-mbiti	Sengerema	Mwanza
73	Moshi (madakawa)	Sengerema	Mwanza
74	Karafuu	Moro-vijijini	Morogoro
75	Kipakapaka	Moro-vijijini	Morogoro
76	Msesero	Moro-vijijini	Morogoro
77	Nyati	Moro-vijijini	Morogoro
78	Nyati-Matombo	Moro-vijijini	Morogoro
79	Mbawambili nyeupe	Moro-vijijini	Morogoro
80	Tambala	Moro-vijijini	Morogoro
81	Tule na bwana	Moro-vijijini	Morogoro
82	Faya	Moro-vijijini	Morogoro
83	Pawaga	Kilombero	Morogoro

S/N	Variety	District	Region
84	Mzuri kwao	Kilombero	Morogoro
85	Fara maria	Kilombero	Morogoro
86	Meri (Katashingo)	Kilombero	Morogoro
87	Meri matitu	Kilombero	Morogoro
88	Mla na bwana	Korogwe	Tanga
89	Kadomoli	Korogwe	Tanga
90	Supa ya mlimani	Korogwe	Tanga
91	Bora kupata	Korogwe	Tanga
92	Mropa	Korogwe	Tanga
93	Magugu	Korogwe	Tanga
94	Supa Zanzibar	Korogwe	Tanga
95	Moshi wa taa	Mvomero	Morogoro
96	Shingo ya mjakazi mjakazi	Mvomero	Morogoro
97	Supa shinyanga	Mvomero	Morogoro
98	Kizurizuri	Mvomero	Morogoro
99	Malemata	Sumbawanga VJ	Rukwa
100	Supa kyela	Sumbawanga VJ	Rukwa
101	Sindano	Sumbawanga VJ	Rukwa
102	Bhagalala	Kyela	Mbeya
103	Fwaja/faya	Kyela	Mbeya
104	Niwahi	Wete	Kas.Pemba
105	Kinuke	Wete	Kas.Pemba
106	Baramata	Wete	Kas.Pemba
107	Moshi wa sigara	Wete	Kas.Pemba
108	Kivuli	Wete	kas.Pemba
109	Ringa nyekundu	Micheweni	Kas.Pemba
110	Kibawa	Wete	Kas.Pemba
111	Kipaku	Micheweni	Kas.Pemba
112	Malikora	Wete	Kas.Pemba
113	Waya	Magharibi	Mjin magh
114	Kia la ngawa	Magharibi	Mjin magh
115	Tule na bwana	Magharibi	Mjin magh
116	Kitenge	Magharibi	Mjin magh
117	Dhahabu	magharibi	Mjin magh
118	Ulijuaje kama si umbea	Magharibi	Mjin magh
119	Kihogo	Magharibi	Mjin magh
120	Supa	Kas. B	Kaskazini
121	Lisutwa	Kas. B	Kaskazini
122	Kijicho	Kas. B	Kaskazini
123	Machiwa mekundu	Kas. B	Kaskazini
124	Supa kijicho	Kas. B	Kaskazini
125	Ringa nyekundu	Kas. B	Kaskazini
126	Purure	Kas. B	Kaskazini
127	Kazole	Kati	Kusini

S/N	Variety	District	Region
128	Kia la kondoo	Kati	Kusini
129	Mabula	Kati	Kusini
130	Karatasi	Kati	Kusini
131	Tarabizuna	Kati	Kusini
132	Makaniki	Kati	Kusini
133	Kijicho	Kati	Kusini
134	Madevu	Kati	Kusini
135	Waya kizimbani	Magharibi	Mjin magh
136	Ambali	Chakechake	Pemba
137	Kibata ngoto chekundu	Chakechake	Pemba
138	Sataya	Chakechake	Pemba
139	Kichwa cha nzi	Chakechake	Pemba
140	Chalao	Chakechake	Pemba
141	Bibi wa Unguja	Chakechake	Pemba
142	Shingo ya mjakazi	Chakechake	Pemba
143	Kikuba	Chakechake	Pemba
144	Machua	Chakechake	Pemba
145	Cheche hapwagwa	Chakechake	Pemba
146	Uchuki	Chakechake	Pemba
147	Kibata ngoto	Chakechake	Pemba
148	Msemwa	Chakechake	Pemba
149	TOX	Chakechake	Pemba
150	Kipeteli	Chakechake	Pemba
151	Mzuri hajipambi	Chakechake	Pemba
152	Hali huku mvunguni	Chakechake	Pemba
153	Domo la fisi	Chakechake	Pemba
154	Hali mgeni	Chakechake	Pemba
155	Tabora	Chakechake	Pemba
156	Sawasawa	Chakechake	Pemba
157	Ringa kijicho	Chakechake	Pemba
158	Mbawa mbili	Chakechake	Pemba
159	Mwanamatongo	Chakechake	Pemba
160	Nawa tule na bwana	Chakechake	Pemba

Appendix 2: CTAB-Nucleic acid extraction protocol

This method is based on the **CTAB-Nucleic acid extraction** procedures that make it possible to extract purified high molecular weight (> 50 kb) plant DNA. The basis for the separation of polysaccharides from nucleic acids is their differential solubilities in the presence of CTAB. Tissues as small as individual ovules and embryos, or small pieces of tissue from various parts of the same plant, can be used.

- In order to release the cellular constituents, the cell walls must be broken by grinding the tissue in liquid nitrogen with a mortar and pestle.
- To release the DNA into the Extraction Buffer, the cell membranes must be disrupted by using a detergent (CTAB; cetyltrimethyl ammonium bromide).
- The time between thawing of the frozen, pulverized tissue and its exposure to the Extraction Buffer should be minimized to avoid nucleolytic degradation of the DNA.
- To protect DNA from the endogenous nucleases, the detergents and also EDTA (ethylene diamine tetraacetic acid) are used. EDTA is a chelating agent that binds magnesium ions, generally considered a necessary cofactor for most nucleases.
- The buffer/tissue mixture is emulsified with either chloroform or phenol to denature and separate the proteins from the DNA.
- Shearing of the DNA should be minimized. DNA in solution should be handled very gently. Typically, 50-100 kb DNA can be obtained.

- **MATERIALS AND SOLUTIONS**

2xExtractionBuffer (100ml)

2% CTAB (w/v) ---------- 2 g

100 mM Tris-HCl (pH 8.0) ---------- 10 ml of 1 M Tris-HCl

20 mM EDTA (pH 8.0) ---------- 4 ml of 0.5 M EDTA

1.4 M NaCl ---------- 28 ml of 5 M NaCl

1% PVP (polyvinyl pyrrolidone; MW 40,000) ------- 1 g Add deionized H_2O to make a final volume of ------- 100 ml

- Warm up to 65^{o}C before use.

10% CTAB (Hexadecyl [or, Cetyl] trimethylammonium bromide) Solution (100 ml)

10% CTAB ---------- 10 g

0.7 M NaCl ---------- 14 ml of 5 M NaCl

Add deionized H_2O to make a final volume of ------- 100 ml

Precipitation Buffer (100 ml)

1% CTAB ---------- 1 g

50 mM Tris-HCl (pH 8.0) ---------- 5 ml of 1 M Tris-HCl

10 mM EDTA (pH 8.0) ---------- 2 ml of 0.5 M EDTA

Add deionized H_2O to make a final volume of ------- 100 ml

High-Salt TE Buffer (100 ml)

10 mM Tris-HCl (pH 8.0) ---------- 1 ml of 1 M Tris-HCl

1 mM EDTA (pH 8.0) ---------- 0.2 ml of 0.5 M EDTA

1 M NaCl ---------- 20 ml of 5 M NaCl

Add deionized H_2O to make a final volume of -------- 100 ml

RNase Stock Solution

1 mg/ml RNase A

100 U/ml RNase Tl

- The solution should be heated to boiling in a water bath for 30 min to destroy any DNases.
- It can be kept frozen until needed.

PROCEDURES

1. Grind 0.5 g tissue with the liquid nitrogen in a mortar and pestel (or in a microfuge tube).

2. Transfer the ground tissue to a microfuge tube.

3. Add 0.5 ml of 65^{o}C-warmed **2 X Extraction Buffer**

- If a low yield is expected add about 20 ug of yeast tRNA as a carrier to aid the precipitation in later steps.

4. Add one volume of chloroform/ isoamyl alcohol (24:1).

5. Mix gently to form an emulsion.

6. Centrifuge in a microfuge for 1 minutes at 12,000*g*.

7. Transfer the supernatant solution from the top (aqueous) phase to a new microfuge tube. Discard lower (chloroform) phase.

8. Add 1/10th volume of the **10% CTAB Solution** and mix.

9. Perform another chloroform/ isoamyl alcohol (24:1) extraction as in steps 4-7.

10. Add an equal volume of **Precipitation Buffer** and mix gently.

11. Centrifuge for 30 seconds at 7,500*g*. Discard the supernatant solution.

12. Rehydrate the pellet in **High-Salt TE buffer**.

13. Add two volumes of 100 % ethanol to the solution and mix gently.

14. Centrifuge for 5 minutes at 4000*g*. Discard the supernatant solution.

15. Add (up to the original volume) cold 80 % ethanol and centrifuge for 5 min. Discard the supernatant solution.

16. Remove residual ethanol on the wall of microfuge tube by Kimwipe.

Air dry until all of the liquid has evaporated. Do not over-dry.

17. Rehydrate in 100-500 ul of TE buffer.

18. To remove RNA contamination, incubate the nucleic acids with 1/10th volume of **RNase Stock Solution** at 37 ^{o}C for 1 hour.

NOTES

- The volume of **2 X Extraction Buffer** added should be approximately equal to the volume of the tissue. If drier tissues are being used (e.g., grass leaves or seeds), one to two volumes of **1 X Extraction Buffer** must also be added.
- High molecular weight DNA is not as soluble as lower molecular weight DNA or RNA.
- Frozen powdered tissue can be stored at -70 ^{o}C until needed. Alternatively, large amounts of tissue can be powdered in liquid nitrogen using a Waring blender with a stainless steel jar.
- When extracting from larger amounts of tissue, it is often easier to recover the DNA/CTAB precipitate with a hooked glass rod than to pellet it.
- DNA from grass leaves that appears undegraded when examined by agarose gel electrophoresis immediately after preparation or after storage at -20 ^{o}C may sometimes appear highly degraded after storage at 4 ^{o}C for several days. Residual nuclease is the apparent cause of this. It is therefore recommended that grass DNA
- Be stored at -70 ^{o}C.

Appendix 3: GeneJET PCR Purification protocol

Note:

- All purification steps should be carried out at **room temperature**.
- All centrifugations should be carried out in a table-top microcentrifuge at **>12 000g**
- (10 000-14 000 rpm, depending on the rotor type).

Step Procedure

1. Add a **1:1 volume** of **Binding Buffer** to completed PCR mixture (e.g. for every 100 µL of reaction mixture, add 100 µL of Binding Buffer). Mix thoroughly. Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.

2. For DNA ≤ 500 bp

Optional: if the DNA fragment is ≤500 bp, add a 1:2 volume of 100% isopropanol (e.g., 100 µL of isopropanol should be added to 100 µL of PCR mixture combined with 100 µL of Binding Buffer). Mix thoroughly.

Note: If PCR mixture contains primer-dimers, purification without isopropanol is recommended. However, the yield of the target DNA fragment will be lower.

3. Transfer up to 800 µL of the solution from step 1 (or optional step 2) to the GeneJET purification column. Centrifuge for 30-60 seconds. Discard the flow-through.

Note: If the total volume exceeds 800 µL, the solution can be added to the column in stages.

After the addition of 800 µL of solution, centrifuge the column for 30-60 s and discard flow through. Repeat until the entire solution has been added to the column membrane.

4. Add **700 µL** of **Wash Buffer** (diluted with the ethanol as described on p. 3) to the GeneJET purification column. Centrifuge for 30-60 s.

Discard the flow-through and place the purification column back into the collection tube.

5. Centrifuge the empty GeneJET purification column for an additional 1 min to completely remove any residual wash buffer.

Note: This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.

6. Transfer the GeneJET purification column to a clean 1.5 mL microcentrifuge tube. Add **50 µL** of **Elution Buffer** to the center of the GeneJET purification column membrane and centrifuge for 1 min.

Note:

- For low DNA amounts the elution volumes can be reduced to increase DNA concentration.
- An elution volume between 20-50 µL does not significantly reduce the DNA yield. However,
- Elution volumes less than 10 µL are not recommended.
- If DNA fragment is >10 kb, prewarm Elution Buffer to 65 °C before applying to column.
- If the elution volume is 10 µL and DNA amount is ≥5 µg, incubate column for 1 min at room temperature before centrifugation.

7. Discard the GeneJET purification column and store the purified DNA at -20 °C

Appendix 4: Sticky-End Cloning Protocol

- For cloning PCR products with 3'-dA overhangs generated by *Taq* DNA polymerase, DreamTaq™ DNA polymerase or enzyme mixtures containing *Taq* DNA polymerase.
- For cloning PCR products when DNA end structure of the generated PCR products is not specified by the supplier of the DNA polymerase.
- For cloning DNA fragments with 5'- or 3'-overhangs generated by restriction enzyme digestion. Gel-purify the DNA fragment prior to ligation and use in a 3:1 molar ratio with pJET1.2/blunt.

Note: The DNA Blunting Enzyme is a proprietary thermostable DNA polymerase with proofreading activity. It will remove 3'- overhangs and fill-in 5'-overhangs. Nucleotides for the blunting reaction are included in the reaction buffer.

1. Set up the blunting reaction **on ice**:

Component	**Volume**
2X Reaction Buffer	10 µl
Non-purified PCR product *or*	1 µl
purified PCR product/other sticky-end DNA fragment	0.15 pmol ends
Water, nuclease-free	to 17 µl
DNA Blunting Enzyme	1 µl
Total volume	18 µl

Vortex briefly and centrifuge for 3-5 s.

2. Incubate the mixture at 70 °C for 5 min. Chill on ice.

3. Set up the ligation reaction **on ice**. Add the following to the blunting reaction mixture:

Component	**Volume**
pJET1.2/blunt Cloning Vector (50 ng/µl)	1 µl (0.05 pmol ends)
T4 DNA Ligase	1 µl
Total volume	20 µl

Vortex briefly and centrifuge for 3-5 s to collect drops.

4. Incubate the ligation mixture at room temperature (22 °C) for 5 min.

Note: For PCR products >3 kb, ligation can be prolonged to 30 min. Ligation times longer than 30 min are not recommended and may decrease cloning efficiency.

5. Use the ligation mixture directly for transformation.

Note: Keep the ligation mixture at -20 °C if transformation is postponed. Thaw on ice and mix carefully before transformation.

Appendix 5: QIAprep® Spin Miniprep Kit

Notes before starting;

- Optional: Add LyseBlue reagent to Buffer P1 at a ratio of 1 to 1000.
- Add the provided RNase A solution to Buffer P1, mix, and store at 2–8°C.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 13 000 rpm (~17,900 x *g*) in a conventional table-top microcentrifuge.

1. Pellet 1–5 ml bacterial overnight culture by centrifugation at >8000 rpm (6800 x *g*) for 3. Min at room temperature (15–25 °C).
2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.
3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear. Do not allow the lysis reaction toproceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.
4. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. If using LyseBlue reagent, the solution will turn colorless.
5. Centrifuge for 10 min at 13 000 rpm (~17 900 x *g*) in a table-top microcentrifuge.
6. Apply the supernatant from step 5 to the QIAprep spin column by decanting or pipetting. Centrifuge for 30–60 s and discard the flow-through, or apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source.
7. Recommended: Wash the QIAprep spin column by adding 0.5 ml Buffer PB. Centrifuge for 30–60 s and discard the flow-through, or apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source.

Note: This step is only required when using *endA*+ strains or other bacteria strains with high nuclease activity or carbohydrate content.

8. Wash the QIAprep spin column by adding 0.75 ml Buffer PE. Centrifuge for 30–60 s and discard the flow-through, or apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source. Transfer the QIAprep spin column to the collection tube.
9. Centrifuge for 1 min to remove residual wash buffer.
10. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA,add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

Appendix 6: Results for grain and leaf aromatic tests

Results For Grain and Leaf Aromatic Test in Tanzanian Rice Landraces Conducted at Mikocheni Agricultural Researchi Institute (MARI)

Lab No.	Variety	District of collection	Region	Result of grain aromatic test	Result of leaf aromatic test
1	Kanunga	Ulanga	Morogoro	Presence of aroma	Presence of aroma
2	1K	Ifakara	Morogoro	Absence of aroma	Absence of aroma
3	Liwatawata	Ulanga	Morogoro	Absence of aroma	Absence of aroma
4	2K	Ifakara	Morogoro	Absence of aroma	Absence of aroma
5	Chikweta	Ulanga	Morogoro	Absence of aroma	Absence of aroma
6	3K	Ifakara	Morogoro	Presence of aroma	Presence of aroma
7	Kalivumbura	Ulanga	Morogoro	Absence of aroma	Absence of aroma
8	4K	Ifakara	Morogoro	Presence of aroma	Presence of aroma
9	Africa	Ulanga	Morogoro	Absence of aroma	Absence of aroma
10	Lawama	Ulanga	Morogoro	Absence of aroma	Presence of aroma
11	Mzuri nani	Ulanga	Morogoro	Absence of aroma	Presence of aroma
12	Somba	Ulanga	Morogoro	Absence of aroma	Presence of aroma
13	Lingwelingweli	Ulanga	Morogoro	Absence of aroma	Presence of aroma
14	Tela	Ulanga	Morogoro	Absence of aroma	Presence of aroma
15	Mwanza	Ulanga	Morogoro	Absence of aroma	Presence of aroma
16	Kasegere	Ulanga	Morogoro	Presence of aroma	Presence of aroma
17	Dunduli	Ulanga	Morogoro	Absence of aroma	
18	Nitalima wangu	Tunduru	Ruvuma	Absence of aroma	
19	Zena	Tandahimba	Mtwara	Presence of aroma	Absence of aroma
20	Bora kupata	Nanyumbu	Mtwara	Presence of aroma	Presence of aroma
21	Ngwindimba	Mtwara vijijini	Mtwara	Presence of aroma	Presence ofaroma
22	Lingwindimba	Tunduru	Ruvuma	Presence of aroma	Presence of aroma
23	Chimbakata	Tandahimba	Mtwara	Absence of aroma	Presence of aroma
24	Sindano	Kilwa	Lindi	Presence of aroma	Presence of aroma

Lab No.	Variety	District of collection	Region	Result of grain aromatic test	Result of leaf aromatic test
25	Chiogo	Tunduru	Ruvuma	Presence of aroma	Absence of aroma
26	Ngoge	Kilwa	Lindi	Absence of aroma	Absence of aroma
27	Namahoki	Kilwa	Lindi	Presence of aroma	Presence of aroma
28	Lichende	Tandahimba	Mtwara	Presence of aroma	Presence of aroma
29	Mpunga safi (ganda moja)	Mtwara vijijini	Mtwara	Absence of aroma	Presence of aroma
30	Jobela	Mtwara vijijini	Mtwara	Presence of aroma	Presence of aroma
31	Maganda manne	Nanyumbu	Mtwara	Presence of aroma	
32	Wotewote	Kilwa	Lindi	Presence of aroma	Presence of aroma
33	Moto wa ngambo	Tunduru	Ruvuma	Presence of aroma	Presence of aroma
34	Wahiwahi	Nzega	Tabora	Presence of aroma	Presence of aroma
35	Nkunguru (supa kijivu)	Igunga	Tabora	Presence of aroma	Presence of aroma
36	Kihogo red	Igunga	Tabora	Presence of aroma	Presence of aroma
37	Malamata	Igunga	Tabora	Absence of aroma	Presence of aroma
38	Malimata	Igunga	Tabora	Presence of aroma	Presence of aroma
39	Minoga mhuli (meno ya tembo)	Igunga	Tabora	Presence of aroma	Presence of aroma
40	Mchina	Igunga	Tabora	Presence of aroma	
41	Bungala	Kibondo	Kigoma	Presence of aroma	Presence of aroma
42	Supa (Burundi)	Kibondo	Kigoma	Presence of aroma	Presence of aroma
43	Sindano	Kibondo	Kigoma	Absence of aroma	Absence of aroma
44	Tuliani	Igunga	Tabora	Presence of aroma	
45	Nganyalo	Igunga	Tabora	Presence of aroma	Presence of aroma
46	Supa mbawambili	Igunga	Tabora	Absence of aroma	Presence of aroma
47	Moshi wa sigara	Igunga	Tabora	Presence of aroma	
48	Bishori	Nzega	Tabora	Absence of aroma	Absence of aroma
49	Umano	Nzega	Tabora	Presence of aroma	Presence of aroma
50	Omano	Igunga	Tabora	Presence of aroma	

51	Sembe	Sengerema	Mwanza	Presence of aroma	Presence of aroma

Lab No.	Variety	District of collection	Region	Result of grain aromatic test	Result of leaf aromatic test
52	Sukarisukari	Bunda	Mara	Presence of aroma	Presence of aroma
53	Sengasenga	Bunda	Mara	Absence of aroma	Presence of aroma
54	Migiha (katumbo	Sengerema	Mwanza	Absence of aroma	Absence of aroma
55	Kabangala	Shinyanga	Shinyanga	Absence of aroma	Presence of aroma
56	Umano	Shinyanga	Shinyanga	Presence of aroma	Presence of aroma
57	Kalamata	Kishapu	Shinyanga	Presence of aroma	Presence of aroma
58	Gamti ndongo	Shinyanga	Shinyanga	Presence of aroma	Presence of aroma
59	Tela	Bunda	Mara	Presence of aroma	Presence of aroma
60	Mabeyenga	Shinyanga	Shinyanga	Presence of aroma	Presence of aroma
61	Mwanamwala	Shinyanga	Shinyanga	Presence of aroma	Absence of aroma
62	Malitela	Kishapu	Shinyanga	Presence of aroma	Presence of aroma
63	Supa Buramba/ Supa urambo	Msoma vijijini	Mara	Presence of aroma	Absence of aroma
64	Mchina	Shinyanga	Shinyanga	Presence of aroma	Presence of aroma
65	Malamata/kalamata	Sengerema	Mwanza	Presence of aroma	Presence of aroma
66	Supa Buramba/ Supa urambo	Bunda	Mara	Presence of aroma	Presence of aroma
67	Supa India	Msoma vijijini	Mara	Presence of aroma	
68	Shinyanga	Bunda	Mara	Presence of aroma	Presence of aroma
69	Tondogoso	Bunda	Mara	Presence of aroma	Presence of aroma
70	Gamti Kubwa	Shinyanga	Shinyanga	Presence of aroma	Presence of aroma
71	Malombo-ga-mbiti	Sengerema	Mwanza	Presence of aroma	Presence of aroma
72	Moshi (madakawa)	Sengerema	Mwanza	Presence of aroma	Presence of aroma
73	Karafuu	Moro-vijijini	Morogoro	Presence of aroma	Absence of aroma
74	Kipakapaka	Moro-vijijini	Morogoro	Absence of aroma	Absence of aroma
75	Msesero	Moro-vijijin	Morogoro	Presence of aroma	
76	Waya	Moro-vijijini	Morogoro	Absence of aroma	Presence of aroma
77	Nyati	Moro-vijijini	Morogoro	Presence of aroma	Presence of aroma

Lab No.	Variety	District of collection	Region	Result of grain aromatic test	Result of leaf aromatic test
78	Nyati (Matombo)	Moro-vijijini	Morogoro	Absence of aroma	Absence of aroma
79	Mbawambili nyeupe	Moro-vijijini	Morogoro	Presence of aroma	Presence of aroma
80	Tambala	Moro-vijijini	Morogoro	Presence of aroma	Absence of aroma
81	Tule na bwana	Moro-vijijini	Morogoro	Presence of aroma	Presence of aroma
82	Faya	Moro-vijijini	Morogoro	Presence of aroma	
83	Pawaga	Kilombero	Morogoro	Presence of aroma	Presence of aroma
84	Mzuri kwao	Kilombero	Morogoro	Presence of aroma	Presence of aroma
85	Fara maria	Kilombero	Morogoro	Presence of aroma	Presence of aroma
86	Meri matitu	Kilombero	Morogoro	Presence of aroma	Presence of aroma
87	Mla na bwana	Korogwe	Tanga	Presence of aroma	Presence of aroma
88	Kadomoli	Korogwe	Tanga	Presence of aroma	Presence of aroma
89	Supa ya mlimani	Korogwe	Tanga	Presence of aroma	Presence of aroma
90	Bora kupata	Korogwe	Tanga	Absence of aroma	Presence of aroma
91	Mropa	Korogwe	Tanga	Presence of aroma	Presence of aroma
92	Magugu	Korogwe	Tanga	Presence of aroma	
93	Supa Zanzibar	Korogwe	Tanga	Presence of aroma	Presence of aroma
94	Moshi wa taa	Mvomero	Morogoro	Presence of aroma	Presence of aroma
95	Shingo ya mwali	Mvomero	Morogoro	Presence of aroma	Presence of aroma
96	Supa shinyanga	Mvomero	Morogoro	Presence of aroma	Presence of aroma
97	Kizurizuri	Mvomero	Morogoro	Presence of aroma	Presence of aroma
98	Malemata	Sumbawanga VJ	Rukwa	Presence of aroma	Presence of aroma
99	Supa kyela	Sumbawanga VJ	Rukwa	Presence of aroma	Presence of aroma
100	Sindano	Sumbawanga VJ	Rukwa	Absence of aroma	
101	Malemata	Sumbawanga VJ	Rukwa	Presence of aroma	Presence of aroma
102	Bhagalala	Kyela	Mbeya	Presence of aroma	Presence of aroma
103	Fwaja/faya	Kyela	Mbeya	Presence of aroma	Presence of aroma
104	Kinuke	Wete	Kas.Pemba	Presence of aroma	

105	Niwahi	Wete	Kas.Pemba	Presence of aroma	Presence of aroma

Lab No.	Variety	District of collection	Region	Result of grain aromatic test	Result of leaf aromatic test
106	Malikora	Wete	Kas.Pemba	Presence of aroma	Presence of aroma
107	Baramata	Wete	Kas.Pemba	Presence of aroma	Presence of aroma
108	Moshi wa sigara	Wete	Kas.Pemba	Presence of aroma	Presence of aroma
109	Kivuli	Wete	Kas.Pemba	Absence of aroma	Presence of aroma
110	Rangi nyekundu	Micheweni	Kas.Pemba	Presence of aroma	Presence of aroma
111	Kibawa	Wete	Kas.Pemba	Presence of aroma	
112	Kipaku	Micheweni	Kas.Pemba	Presence of aroma	Presence of aroma
113	Waya	Magharibi	Mjin magh	Presence of aroma	Presence of aroma
114	Kia la ngawa	Magharibi	Mjin magh	Presence of aroma	Presence of aroma
115	Tule na bwana	Magharibi	Mjin magh	Presence of aroma	Presence of aroma
116	Kitenge	Magharibi	Mjin magh	Absence of aroma	Absence of aroma
117	Dhahabu	Magharibi	Mjin magh	Presence of aroma	Presence of aroma
118	Ulijuaje kama si umbea	Magharibi	Mjin magh	Presence of aroma	Presence of aroma
119	Kihogo	Magharibi	Mjin magh	Absence of aroma	Presence of aroma
120	Supa	Kas. B	Kaskazini	Presence of aroma	Presence of aroma
121	Lisutwa	Kas. B	Kaskazini	Presence of aroma	Presence of aroma
122	Kijicho	Kas. B	Kaskazini	Presence of aroma	Presence of aroma
123	Machiwa mekundu	Kas. B	Kaskazini	Presence of aroma	Presence of aroma
124	Supa kijicho	Kas. B	Kaskazini	Presence of aroma	Absence of aroma
125	Rangi nyekundu	Kas. B	Kaskazini	Presence of aroma	Presence of aroma
126	Pururе	Kas. B	Kaskazini	Presence of aroma	Presence of aroma
127	Kia la ngawa	Kati	Kusini	Presence of aroma	Presence of aroma
128	Kazole	Kati	Kusini	Presence of aroma	Presence of aroma
129	Kia la kondoo	Kati	Kusini	Presence of aroma	Presence of aroma
130	Mabula	Kati	Kusini	Presence of aroma	Presence of aroma
131	Karatasi	Kati	Kusini	Presence of aroma	Presence of aroma
132	Tarabizuna	Kati	Kusini	Presence of aroma	Presence of aroma
133	Makaniki	Kati	Kusini	Presence of aroma	Presence of aroma

Lab No.	Variety	District of collection	Region	Result of grain aromatic test	Result of leaf aromatic test
134	Kijicho	Kati	Kusini	Presence of aroma	Presence of aroma
135	Madevu	Kati	Kusini	Presence of aroma	Absence of aroma
136	Waya kizimbani	Magharibi	Mjin magh	Presence of aroma	
137	Ambali	Chakechake	Pemba	Presence of aroma	Presence of aroma
138	Kibata ngoto chekundu	Chakechake	Pemba	Absence of aroma	Presence of aroma
139	Kichwa cha nzi	Chakechake	Pemba	Presence of aroma	Presence of aroma
140	Chalao	Chakechake	Pemba	Absence of aroma	Absence of aroma
141	Bibi wa Unguja	Chakechake	Pemba	Presence of aroma	Presence of aroma
142	Madevu	Chakechake	Pemba	Presence of aroma	Absence of aroma
143	Shingo ya mjakazi	Chakechake	Pemba	Absence of aroma	Absence of aroma
144	Kikuba	Chakechake	Pemba	Presence of aroma	Absence of aroma
145	Machua	Chakechake	Pemba	Presence of aroma	Presence of aroma
146	Cheche hapwagwa	Chakechake	Pemba	Presence of aroma	
147	Uchuki	Chakechake	Pemba	Presence of aroma	Absence of aroma
148	Msemwa	Chakechake	Pemba	Absence of aroma	Presence of aroma
149	TOX	Chakechake	Pemba	Presence of aroma	Absence of aroma
150	Sataya	Chakechake	Pemba	Presence of aroma	Presence of aroma
151	Kipeteli	Chakechake	Pemba	Presence of aroma	Presence of aroma
152	Mzuri hajipambi	Chakechake	Pemba	Presence of aroma	Presence of aroma
153	Hali huku mvunguni	Chakechake	Pemba	Presence of aroma	Presence of aroma
154	Domo la fisi	Chakechake	Pemba	Absence of aroma	Presence of aroma
155	Hali mgeni	Chakechake	Pemba	Presence of aroma	Absence of aroma
156	Tabora	Chakechake	Pemba	Presence of aroma	Presence of aroma
157	Sawasawa	Chakechake	Pemba	Absence of aroma	Presence of aroma
158	Ringa kijicho	Chakechake	Pemba	Absence of aroma	Absence of aroma
159	Mwanamatongo	Chakechake	Pemba	Presence of aroma	Presence of aroma
160	Nawa tule na bwana Chakechake	Chakechake	Pemba	Precence of aroma	Precence of aroma

Appendix 7: Aroma status for popular traditional rice varieties in Tanzania based on molecular analysis

S/N	Variety	District	Region	Ar	Nar	Ht
1	Kanunga	Ulanga	Morogoro	-	+	-
2	1K	Ifakara	Morogoro	-	+	-
3	Liwatawata	Ulanga	Morogoro	-	+	-
4	2K	Ifakara	Morogoro	-	+	-
5	Chikweta	Ulanga	Morogoro	-	+	-
6	3K	Ifakara	Morogoro	+	-	-
7	Kalivumbura	Ulanga	Morogoro	-	+	-
8	4K	Ifakara	Morogoro	+	-	-
9	Africa	Ulanga	Morogoro	+	-	-
10	Lawama	Ulanga	Morogoro	-	+	-
11	Mzuri nani	Ulanga	Morogoro	-	+	-
12	Somba	Ulanga	Morogoro	-	+	-
13	Lingwelingweli	Ulanga	Morogoro	-	-	+
14	Taiwan	Ulanga	Morogoro	+	-	-
15	TOx	Ulanga	Morogoro	+	-	-
16	Tela	Ulanga	Morogoro	+	-	-
17	Mwanza	Ulanga	Morogoro	+	-	-
18	Kasegere	Ulanga	Morogoro	+	-	-
19	Dunduli	Ulanga	Morogoro	-	+	-
20	Nitalima wangu	Tunduru	Ruvuma	+	-	-
21	Zena	Tandahimba	Mtwara	-	+	-
22	Bora kupata	Nanyumbu	Mtwara	-	+	-
23	Ngwindimba	Mtwara vijijini	Mtwara	-	+	-
24	Lingwindimba	Tunduru	Ruvuma	+	-	-
25	Chiogo	Tunduru	Ruvuma	+	-	-
26	Chimbakata	Tandahimba	Mtwara	-	+	-
27	Sindano	Kilwa	Lindi	-	-	+
28	Ngoge	Kilwa	Lindi	-	+	-
29	Namahoki	Kilwa	Lindi	+	-	-
30	lichende	Tandahimba	Mtwara	-	+	-
31	Mpunga safi (ganda moja)	Mtwara vijijini	Mtwara	-	+	-
32	Jobela	Mtwara vijijini	Mtwara	+	-	-
33	Maganda manne	Nanyumbu	Mtwara	+	-	-
34	Wotewote	Kilwa	Lindi	+	-	-
35	Moto wa ngambo	Tunduru	Ruvuma	+	-	-
36	Wahiwahi	Mzega	Tabora	+	-	-
37	Nkunguru (supa kijivu)	Igunga	Tabora	+	-	-
38	Kihogo red	Igunga	Tabora	+	-	-
39	Malamata	Igunga	Tabora	+	-	-

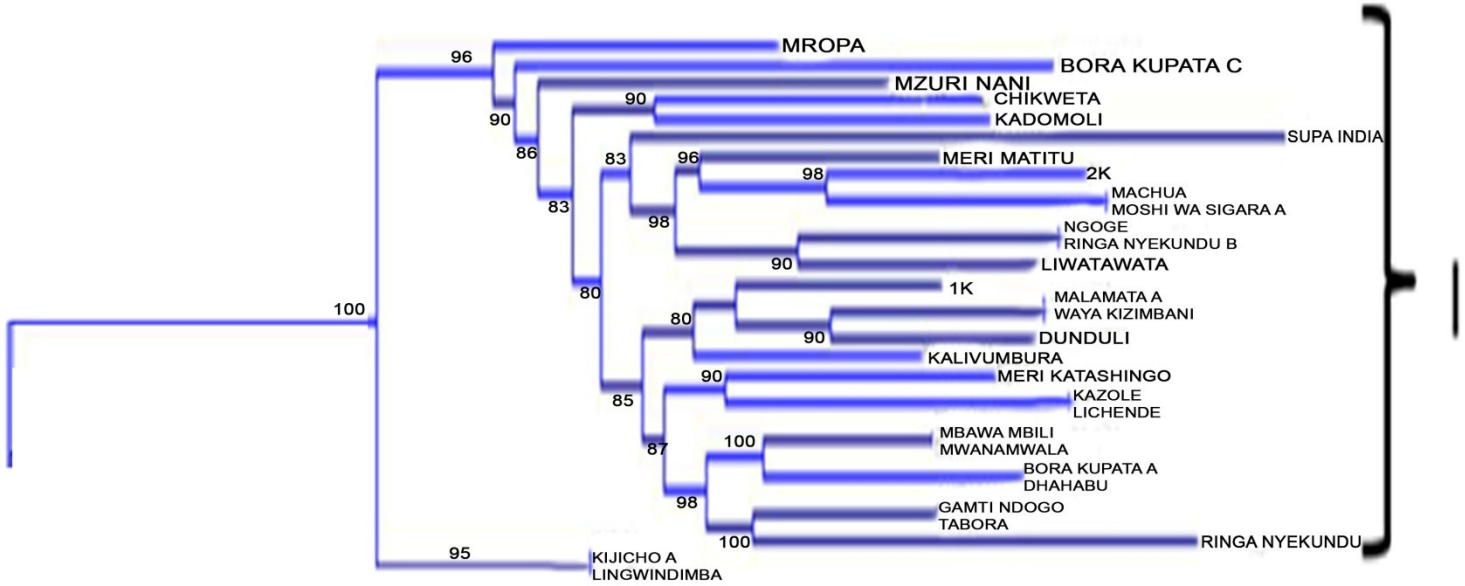
S/N	Variety	District	Region	Ar	Nar	Ht
40	Malimata	Igunga	Tabora	+	-	-
41	Minoga mhuli (meno ya tembo)	Igunga	Tabora	-	+	-
42	Supa (Burundi)	Kibondo	Kigoma	-	-	+
43	Sindano	Kibondo	Kigoma	+	-	-
44	Tuliani	Igunga	Tabora	+	-	-
45	Nganyalo	Igunga	Tabora	+	-	-
46	Supa mbawambili	Igunga	Tabora	+	-	-
47	Moshi wa sigara	Igunga	Tabora	-	+	-
48	Bishori	Nzega	Tabora	-	+	-
49	Umano	Nzega	Tabora	+	-	-
50	Omano	Igunga	Tabora	-	+	-
51	Sembe	Sengerema	Mwanza	+	-	-
52	Sukarisukari	Bunda	Mara	+	-	-
53	Sengasenga	Bunda	Mara	+	-	-
54	Migiha (katumbo)	Sengerema	Mwanza	+	-	-
55	Kabangala	Shinyanga	Shinyanga	-	+	-
56	Umano	Shinyanga	Shinyanga	-	+	-
57	Kalamata	Kishapu	Shinyanga	+	-	-
58	Malamata	Sengerema	Mwanza	+	-	-
59	Gamti ndongo	Shinyanga	Shinyanga	+	-	-
60	Tela	Bunda	Mara	+	-	-
61	Mabeyenga	Shinyanga	Shinyanga	+	-	-
62	Mwanamwala	Shinyanga	Shinyanga	-	+	-
63	Malitela	Kishapu	Shinyanga	+	-	-
64	Supa Buramba/ Supa urambo	Musoma mjini	Mara	+	-	-
65	Mchina	Shinyanga	Shinyanga	+	-	-
66	Malamata/kalamata	Sengerema	Mwanza	+	-	-
67	Mwinula	Bunda	Mara	-	+	-
68	Supa India	Musoma mjini	Mara	-	+	-
69	Shinyanga	Bunda	Mara	+	-	-
70	Tondogoso	Bunda	Mara	+	-	-
71	Gamti Kubwa	Shinyanga	Shinyanga	+	-	-
72	Malombo-ga-mbiti	Sengerema	Mwanza	+	-	-
73	Moshi (madakawa)	Sengerema	Mwanza	+	-	-
74	Karafuu	Moro-vijijini	Morogoro	+	-	-
75	Kipakapaka	Moro-vijijini	Morogoro	-	+	-
76	Msesero	Moro-vijijini	Morogoro	+	-	-
77	Nyati	Moro-vijijini	Morogoro	+	-	-
78	Nyati-Matombo	Moro-vijijini	Morogoro	-	+	-
79	Mbawambili nyeupe	Moro-vijijini	Morogoro	+	-	-
80	Tambala	Moro-vijijini	Morogoro	+	-	-
81	Tule na bwana	Moro-vijijini	Morogoro	+	-	-

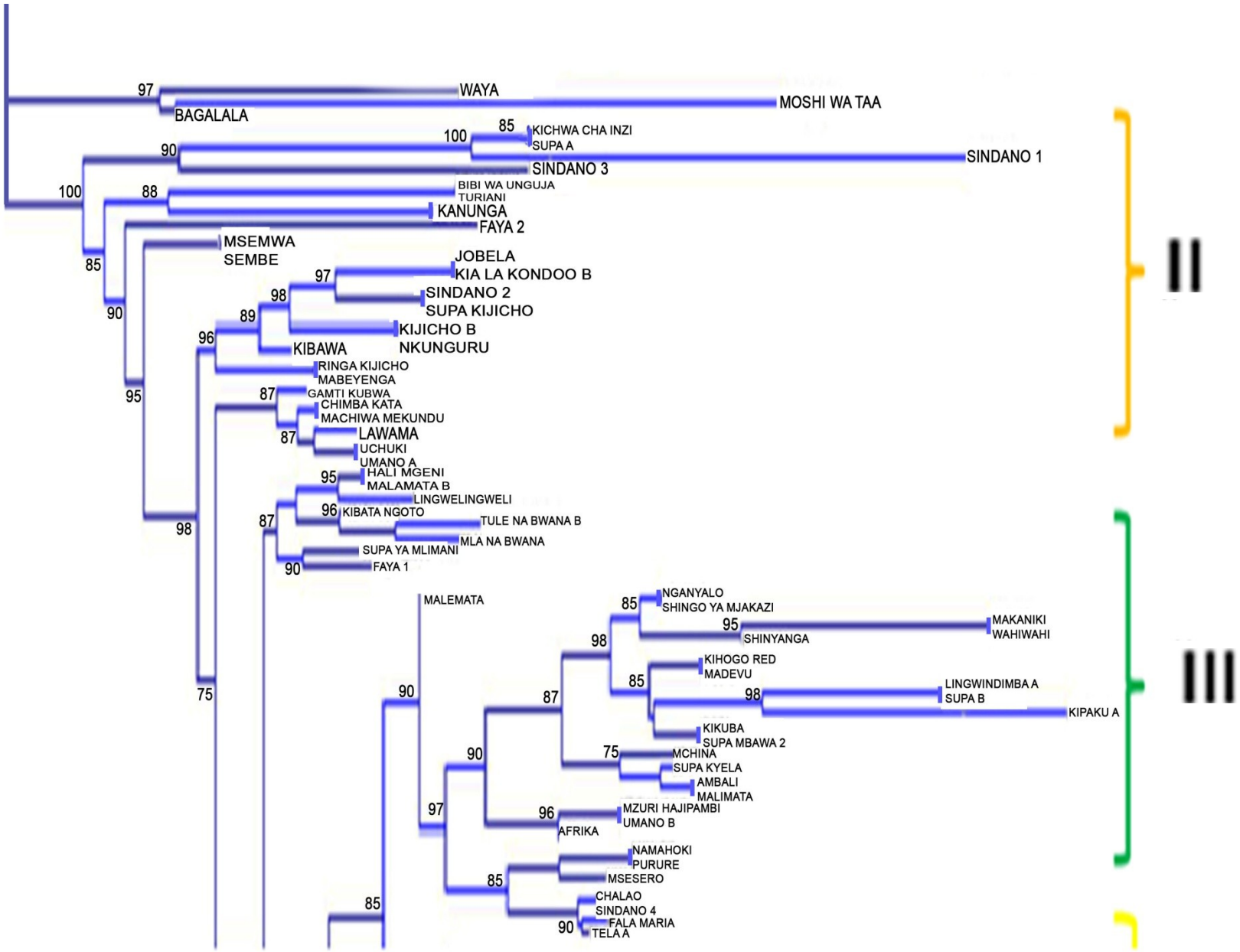
S/N	Variety	District	Region	Ar	Nar	Ht
82	Faya	Moro-vijijini	Morogoro	-	-	+
83	Pawaga	Kilombero	Morogoro	+	-	-
84	Mzuri kwao	Kilombero	Morogoro	-	+	-
85	Fara maria	Kilombero	Morogoro	+	-	-
86	Meri (Katashingo)	Kilombero	Morogoro	-	+	-
87	Meri matitu	Kilombero	Morogoro	-	+	-
88	Mla na bwana	Korogwe	Tanga	+	-	-
89	Kadomoli	Korogwe	Tanga	+	-	-
90	Supa ya mlimani	Korogwe	Tanga	+	-	-
91	Bora kupata	Korogwe	Tanga	-	+	-
92	Mropa	Korogwe	Tanga	-	+	-
93	Magugu	Korogwe	Tanga	+	-	-
94	Supa Zanzibar	Korogwe	Tanga	+	-	-
95	Moshi wa taa	Mvomero	Morogoro	+	-	-
96	Shingo ya Mjakazi	Mvomero	Morogoro	+	-	-
97	Supa shinyanga	Mvomero	Morogoro	+	-	-
98	Kizurizuri	Mvomero	Morogoro	-	+	-
99	Malemata	Sumbawanga VJ	Rukwa	-	+	-
100	Supa kyela	Sumbawanga VJ	Rukwa	+	-	-
101	Sindano	Sumbawanga VJ	Rukwa	+	-	-
102	Bhagalala	Kyela	Mbeya	-	+	-
103	Fwaja/faya	Kyela	Mbeya	+	-	-
104	Niwahi	Wete	Kas.Pemba	-	+	-
105	Kinuke	Wete	Kas.Pemba	+	-	-
106	Baramata	Wete	Kas.Pemba	+	-	-
107	Moshi wa sigara	Wete	Kas.Pemba	+	-	-
108	Kivuli	Wete	kas.Pemba	-	+	-
109	Ringa nyekundu	Micheweni	Kas.Pemba	+	-	-
110	Kibawa	Wete	Kas.Pemba	+	-	-
111	Kipaku	Micheweni	Kas.Pemba	-	+	-
112	Malikora	Wete	Kas.Pemba	+	-	-
113	Waya	Magharibi	Mjin magh	-	-	+
114	Kia la ngawa	Magharibi	Mjin magh	+	-	-
115	Tule na bwana	Magharibi	Mjin magh	+	-	-
116	Kitenge	Magharibi	Mjin magh	-	-	+
117	Dhahabu	magharibi	Mjin magh	-	+	-
118	Ulijuaje kama si umbea	Magharibi	Mjin magh	+	-	-
119	Kihogo	Magharibi	Mjin magh	+	-	-
120	Supa	Kas. B	Kaskazini	-	-	+
121	Lisutwa	Kas. B	Kaskazini	+	-	-
122	Kijicho	Kas. B	Kaskazini	-	+	-
123	Machiwa mekundu	Kas. B	Kaskazini	-	-	+
124	Supa kijicho	Kas. B	Kaskazini	-	+	-

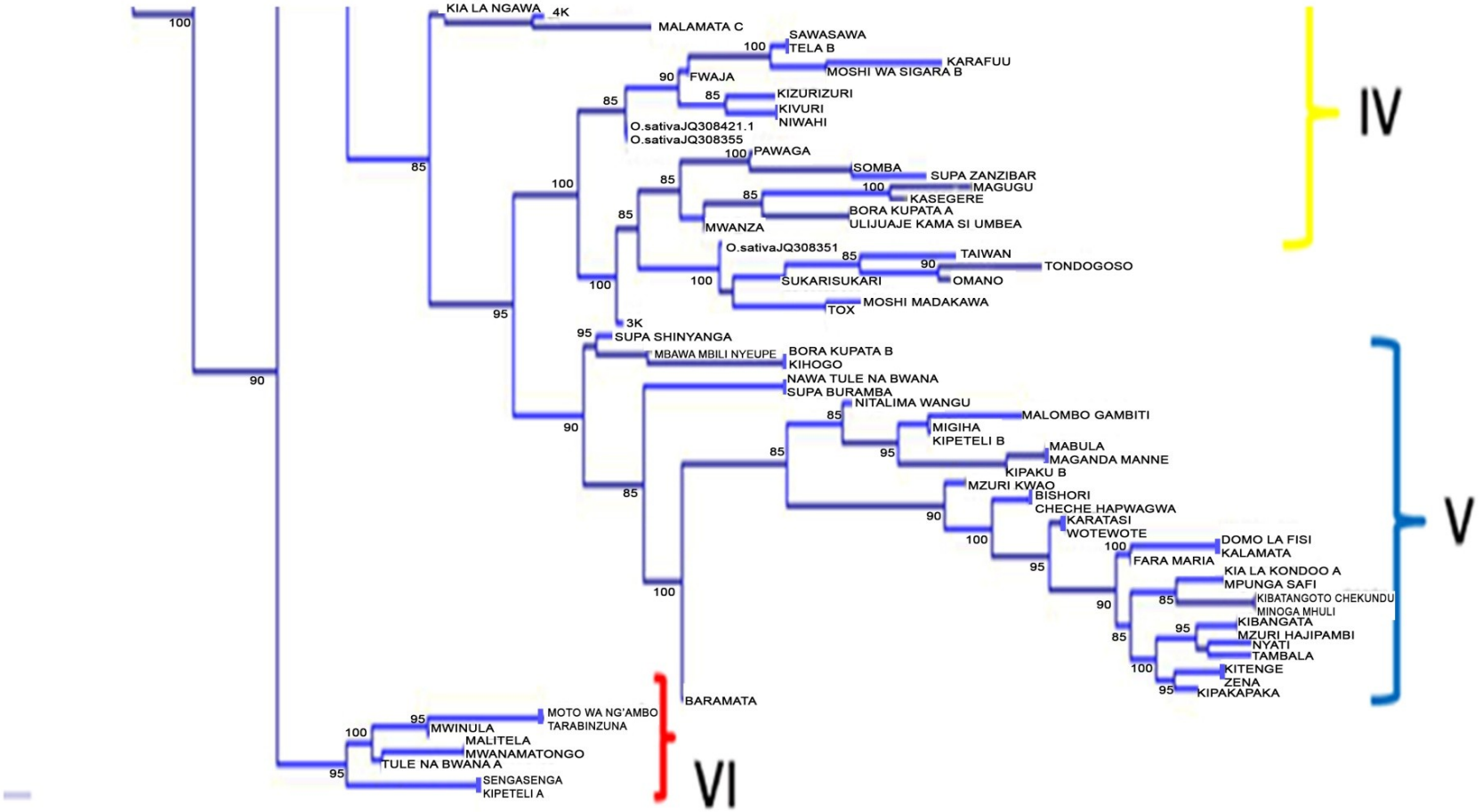
S/N	Variety	District	Region	Ar	Nar	Ht
125	Ringa nyekundu	Kas. B	Kaskazini	-	-	+
126	Purure	Kas. B	Kaskazini	-	+	-
127	Kazole	Kati	Kusini	-	+	-
128	Kia la kondoo	Kati	Kusini	+	-	-
129	Mabula	Kati	Kusini	+	-	-
130	Karatasi	Kati	Kusini	+	-	-
131	Tarabizuna	Kati	Kusini	+	-	-
132	Makaniki	Kati	Kusini	+	-	-
133	Kijicho	Kati	Kusini	-	+	-
134	Madevu	Kati	Kusini	-	+	-
135	Waya kizimbani	Magharibi	Mjin magh	-	+	-
136	Ambali	Chakechake	Pemba	+	-	-
137	Kibata ngoto chekundu	Chakechake	Pemba	-	+	-
138	Sataya	Chakechake	Pemba	+	-	-
139	Kichwa cha nzi	Chakechake	Pemba	-	+	-
140	Chalao	Chakechake	Pemba	-	+	-
141	Bibi wa Unguja	Chakechake	Pemba	+	-	-
142	Shingo ya mjakazi	Chakechake	Pemba	-	+	-
143	Kikuba	Chakechake	Pemba	-	-	+
144	Machua	Chakechake	Pemba	-	+	-
145	Cheche hapwagwa	Chakechake	Pemba	-	+	-
146	Uchuki	Chakechake	Pemba	-	+	-
147	Kibata ngoto	Chakechake	Pemba	-	+	-
148	Msemwa	Chakechake	Pemba	-	+	-
149	TOX	Chakechake	Pemba	-	+	-
150	Kipeteli	Chakechake	Pemba	+	-	-
151	Mzuri hajipambi	Chakechake	Pemba	-	+	-
152	Hali huku mvunguni	Chakechake	Pemba	-	+	-
153	Domo la fisi	Chakechake	Pemba	-	+	-
154	Hali mgeni	Chakechake	Pemba	-	+	-
155	Tabora	Chakechake	Pemba	+	-	-
156	Sawasawa	Chakechake	Pemba	-	+	-
157	Ringa kijicho	Chakechake	Pemba	-	+	-
158	Mbawa mbili	Chakechake	Pemba	+	-	-
159	Mwanamatongo	Chakechake	Pemba	+	-	-
160	Nawa tule na bwana	Chakechake	Pemba	+	-	-

Key:Ar-Aromatic,Nar-Non-Aromatic,Ht-Heterozygous

Appendix 8: The phylogenetic tree (1000 Boostrap replications) showing diversity of *BADH2/BADH2.1* alleles in Tanzania rice landraces




WAYA
MOSHI WA TAA
BAGALALA
KICHWA CHA INZI
SUPA A
SINDANO 1
SINDANO 3
BIBI WA UNGUJA
TURIANI
KANUNGA
FAYA 2
MSEMWA
SEMBE
JOBELA
KIA LA KONDOO B
SINDANO 2
SUPA KIJICHO
KIJICHO B
NKUNGURU
KIBAWA
RINGA KIJICHO
MABEYENGA
GAMTI KUBWA
CHIMBA KATA
MACHIWA MEKUNDU
LAWAMA
UCHUKI
UMANO A
HALI MGENI
MALAMATA B
LINGWELINGWELI
KIBATA NGOTO
TULE NA BWANA B
MLA NA BWANA
SUPA YA MLIMANI
FAYA 1
MALEMATA
NGANYALO
SHINGO YA MJAKAZI
MAKANIKI
WAHIWAHI
SHINYANGA
KIHOGO RED
MADEVU
LINGWINDIMBA A
SUPA B
KIPAKU A
KIKUBA
SUPA MBAWA 2
MCHINA
SUPA KYELA
AMBALI
MALIMATA
MZURI HAJIPAMBI
UMANO B
AFRIKA
NAMAHOKI
PURURE
MSESERO
CHALAO
SINDANO 4
FALA MARIA
TELA A
II
III



Phylogenetic tree showing diversity of *BADH2/BADH2*.1 alleles in Tanzanian rice landraces. The numbers above nodes indicate Boostrap support based on 1000 replicates constructed using Geneious computer software package. The following GenBank accession numbers were involved; *Oryza sativa BADH*2.1-JQ308421.1, *O. sativa BADH*2.1-JQ308355 and *O. sativa BADH*2.1-JQ308351

Appendix 9: Percent similarity(in the lower triangle) in the nucleotide sequences of *BADH2/BADH2.1* alleles in Tanzanian rice landraces

	CHALAO	SINDANO 2	TELA A	FARA MARIA	MZURI AJIPAMBI B	UMANO B	AFRICA	4K	LINGA KIJICHO	MABEYENGA	MAGUGU	MWANZA	3K	BORA KUPATA A
CHALAO	1													
SINDANO 2	100	1												
TELA A	98.172	98.172	1											
FARA MARIA	97.417	97.417	97.253	1										
MZURI AJIPAMBI B	77.915	77.915	96.364	95.064	1									
UMANO B	77.915	77.915	96.364	95.064	100	1								
AFRICA	97.441	97.441	96.364	95.803	97.814	97.814	1							
4K	95.978	95.978	96.175	94.343	97.628	97.628	96.903	1						
RINGA KIJICHO	90.744	90.744	90.217	90.381	90.942	90.942	91.501	90.399	1					
MABEYENGA	90.744	90.744	90.217	90.381	90.942	90.942	91.501	90.399	100	1				
MAGUGU	71.124	71.124	95.109	94.891	73.46	73.46	96.539	95.628	92.559	92.559	1			
MWANZA	95.255	95.255	95.082	94.171	96.357	96.357	96.357	95.628	93.625	93.625	97.436	1		
3K	96.133	96.133	95.238	95.948	95.78	95.78	96.507	95.78	93.59	93.59	97.053	97.431	1	
BORA KUPATA A	82.718	82.718	95.29	94.891	82.916	82.916	96.903	95.811	92.21	92.21	86.431	97.08	97.053	1
ULIJUAJE KAMA SIUMBEYA	82.718	82.718	95.29	94.891	82.916	82.916	96.903	95.811	92.21	92.21	86.431	97.08	97.053	100
SOMBA	94.891	94.891	94.545	93.26	96.727	96.727	96.175	96	91.47	91.47	96.533	96.715	95.956	96.715
SUPA ZANZIBAR	73.394	73.394	95.281	93.978	71.429	71.429	96.364	95.636	91.486	91.486	73.227	96.715	96.324	85.862
PAWAGA	95.073	95.073	94.364	93.625	96.545	96.545	96.182	95.636	91.848	91.848	95.628	97.445	97.059	96.539
SUKARISUKARI	96.533	96.533	96	94.9	95.818	95.818	96.539	95.636	92.74	92.74	96.715	97.445	97.794	97.08
TAIWAN	76.933	76.933	95.463	95.073	75.83	75.83	96	95.091	92.029	92.029	78.457	96.168	97.243	84.978
JQ308421.1	99.203	99.203	99.402	98.606	98.805	98.805	99.004	98.805	94.632	94.632	99.202	99.002	99.401	99.202

	CHALAO	SINDANO 2	TELA A	FARA MARIA	MZURI AJIPAMBI B	UMANO B	AFRICA	4K	LINGA KIJICHO	MABEYENGA	MAGUGU	MWANZA	3K	BORA KUPATA A
TONDOGOSO	70.588	70.588	95.644	94.708	69.585	69.585	96.168	95.628	91.818	91.818	71.709	95.612	96.685	86.411
SUPA SHINYANGA	95.788	95.788	95.612	94.689	95.612	95.612	95.788	95.978	91.788	91.788	95.963	96.33	97.967	96.33
MBAWAMBILI NYEUPE	95.78	95.78	95.612	94.505	96.709	96.709	96.344	96.161	92.532	92.532	96.154	97.436	97.426	96.703
AMBALI	74.805	74.805	95.273	94.891	74.615	74.615	95.803	95.438	91.257	91.257	71.204	96.703	96.869	83.013
MALIMATA	74.805	74.805	95.273	94.891	74.862	74.862	95.803	95.438	91.257	91.257	71.204	96.703	96.869	83.013
SUPA KYELA	95.985	95.985	96.168	95.238	94.545	94.545	95.064	94.698	90.693	90.693	95.978	95.413	96.31	94.718
KIHOGO RED	77.315	77.315	95.652	95.073	78.085	78.085	96.357	95.091	92.015	92.015	74.19	96.533	96.875	83.776
MADEVU	77.315	77.315	95.652	95.073	78.085	78.085	96.357	95.091	92.015	92.015	74.19	96.533	96.875	83.776
KIKUBA	93.078	93.078	92.21	92.883	92.029	92.029	93.625	92.545	90.563	90.563	93.636	93.978	94.485	92.727
SUPA MBAWAMBILI	93.078	93.078	92.21	92.883	92.029	92.029	93.625	92.545	90.563	90.563	93.636	93.978	94.485	92.727
NGANYALO	95.455	95.455	94.746	94.9	95.29	95.29	96	95.455	92.754	92.754	96.37	96.35	96.875	96.37
SHINGO YA MJAKAZI	95.455	95.455	94.746	94.9	95.29	95.29	96	95.455	92.754	92.754	96.37	96.35	96.875	96.37
BORA KUPATA B	74.443	74.443	95.446	95.055	72.515	72.515	95.803	95.446	91.636	91.636	74.356	95.978	96.317	83.309
KIHOGO	74.443	74.443	95.446	95.055	72.515	72.515	95.803	95.446	91.636	91.636	74.356	95.978	96.317	83.309
KASEGERE	96.182	96.182	95.636	95.073	96.545	96.545	97.445	96.715	92.35	92.35	97.086	96.886	96.869	96.539
LINGWINDIMBA A	81.818	81.818	81.851	80.874	80.616	80.616	81.341	80.581	76.311	76.311	80.832	80.692	80.952	80.832
SUPA B	81.818	81.818	81.851	80.874	80.616	80.616	81.341	80.581	76.311	76.311	80.832	80.692	80.952	80.832
KIPAKU A	74.597	74.597	74.91	74.82	73.975	73.975	74.91	73.477	70.483	70.483	74.51	74.64	73.779	73.797
MAKANIKI	83.424	83.424	82.909	82.664	83.485	83.485	83.485	82.727	79.529	79.529	82.971	82.664	82.385	83.152
WAHIWAHI	83.424	83.424	82.909	82.664	83.485	83.485	83.485	82.727	79.529	79.529	82.971	82.664	82.385	83.152

	CHALAO	SINDANO 2	TELA A	FARA MARIA	MZURI AJIPAMBI B	UMANO B	AFRICA	4K	LINGA KIJICHO	MABEYENGA	MAGUGU	MWANZA	3K	BORA KUPATA A
SHINYANGA	74.099	74.099	95.455	94.343	76.404	76.404	96	95.636	91.107	91.107	73.146	95.438	95.78	83.505
KIBATANGOTO	96.52	96.52	96.52	95.604	96.527	96.527	95.985	95.803	90.893	90.893	95.978	96.154	96.133	96.161
MALAMATA C	71.094	71.094	95.985	94.689	76.29	76.29	96.168	96.533	91.257	91.257	73.288	95.971	96.869	84.047
NAWA TULE NA BWANA	72.615	72.615	96.161	95.413	72.197	72.197	96.154	96.52	91.408	91.408	71.766	96.324	97.227	83.704
SUPA BURAMBA	72.615	72.615	96.161	95.413	72.197	72.197	96.154	96.52	91.408	91.408	71.766	96.324	97.227	83.704
BARAMATA	96.147	96.147	96.154	95.963	96.33	96.33	96.514	97.064	91.956	91.956	97.243	96.875	97.963	97.059
JQ308355	99.203	99.203	99.402	98.606	98.805	98.805	99.004	98.805	94.632	94.632	99.202	99.002	99.401	99.202
KIA LA NGAWA	95.795	95.795	96.161	94.689	97.075	97.075	96.527	96.892	91.606	91.606	95.795	96.881	96.679	96.161
MLA NA BWANA	73.341	73.341	94.9	93.601	74.828	74.828	95.62	95.795	90.71	90.71	73.227	96.154	95.212	83.013
KIPETELI A	74.468	74.468	96.175	94.698	73.549	73.549	96.35	96.715	91.803	91.803	70.483	96.52	97.053	82.718
SENGASENGA	74.468	74.468	96.175	94.698	73.549	73.549	96.35	96.715	91.803	91.803	70.483	96.52	97.053	82.718
MALEMATA	96.709	96.709	96.892	95.413	96.715	96.715	96.709	96.161	91.241	91.241	96.168	96.147	96.679	95.985
TULE NA BWANA A	95.1	95.1	94.928	94.364	96.377	96.377	96.727	95.818	91.123	91.123	95.471	95.993	95.971	94.928
MOTO WA NGAMBO	74.19	74.19	95.993	94.881	74.55	74.55	96.168	96.892	91.075	91.075	74.43	95.788	96.869	83.013
TARABINZUMA	74.19	74.19	95.993	94.881	74.55	74.55	96.168	96.892	91.075	91.075	74.43	95.788	96.869	83.013
MWINULA	95.118	95.118	95.628	94.516	96.383	96.383	96.715	96.709	90.893	90.893	95.118	96.337	96.317	94.575
FWAJA	94.526	94.526	94.526	93.978	94.891	94.891	94.891	95.255	91.621	91.621	95.43	96.344	97.059	95.612
KIZURIZURI	85.782	85.782	92.986	92.599	84.069	84.069	93.333	93.514	90.288	90.288	84.992	94.937	95.636	86.19
KIVULI	93.49	93.49	93.321	92.96	93.682	93.682	93.514	93.863	90.647	90.647	94.404	95.118	95.841	94.224

Variety	CHALAO	SINDANO 2	TELAA	FARAMARIA	MZURI AJIPAMBI B	UMANO B	AFRICA	4K	LINGA KIJICHO	MABEYENGA	MAGUGU	MWANZA	3K	BORA KUPATA A	ULIJUAJE KAMA SIUMBEYA	SOMBA	SUPA ZANZIBAR	PAWAGA	SUKARI SUKARI	TAIWAN	JQ308421.1	TONDOGOSO
1K	55.69	55.69	61.053	60.247	56.127	56.127	61.268	61.444	60.387	60.387	55.606	60.954	62.1	55.775	55.775	61.239	56.383	61.376	61.728	56.839	62.235	55.387
KALIVUMBURA	54.449	54.449	59.79	59.331	54.737	54.737	59.72	59.123	59.019	59.019	54.887	59.507	60.461	55.038	55.038	60.494	55.204	60.351	60.457	55.287	61.036	54.449
MALAMATA A	52.648	52.648	56.894	57.118	52.64	52.64	56.643	57.443	55.769	55.769	52.489	56.942	58.23	51.735	51.735	57.394	52.194	57.793	57.895	52.95	58.621	52.042
WAYA KIZIMBANI	52.648	52.648	56.894	57.118	52.64	52.64	56.643	57.443	55.769	55.769	52.489	56.942	58.23	51.735	51.735	57.394	52.194	57.793	57.895	52.95	58.621	52.042
DUNDULI	53.707	53.707	59.266	59.155	54.299	54.299	58.494	59.474	58.319	58.319	54.6	59.507	60.638	53.846	53.846	60.317	54.614	60.175	60.105	55.455	60.845	54.917
MER MATITU	56.146	56.146	60.702	59.965	55.825	55.825	60.633	61.796	60.105	60.105	54.697	60.601	61.032	54.173	54.173	60.708	55.083	61.092	61.023	55.539	61.464	54.628
MACHUWA	53.729	53.729	59.683	58.761	54.863	54.863	58.73	59.894	58.554	58.554	54.407	59.397	59.821	54.033	54.033	59.858	54.573	59.894	59.292	54.046	60.348	53.963
MOSHI WASIGARA A	53.729	53.729	59.683	58.761	54.863	54.863	58.73	59.894	58.554	58.554	54.407	59.397	59.821	54.033	54.033	59.858	54.573	59.894	59.292	54.046	60.348	53.963
GAMTI NDOGO	55.083	55.083	60.915	60.177	55.235	55.235	60.071	60.424	60.424	60.424	55.015	60.993	61.786	55.015	55.015	61.279	55.945	61.77	61.239	55.945	62.355	55.623
TABORA	55.083	55.083	60.915	60.177	55.235	55.235	60.071	60.424	60.424	60.424	55.015	60.993	61.786	55.015	55.015	61.279	55.945	61.77	61.239	55.945	62.355	55.623
MBAWAMBILI	54.614	54.614	60.281	59.259	55.371	55.371	60.141	60.141	59.683	59.683	55.909	60.531	61.032	55.606	55.606	61.348	55.775	60.424	60.247	55.319	61.079	55
MWANAMWALA	54.614	54.614	60.281	59.259	55.371	55.371	60.141	60.141	59.683	59.683	55.909	60.531	61.032	55.606	55.606	61.348	55.775	60.424	60.247	55.319	61.079	55
MROPA	52.807	52.807	57.774	57.904	51.897	51.897	57.092	57.624	58.333	58.333	53.343	58.007	58.961	53.191	53.191	58.289	54.116	58.259	58.259	53.495	59.188	52.888
NJOGE	52.352	52.352	57.469	56.89	53.858	53.858	57.645	58.099	58.172	58.172	53.858	58.127	59.075	53.253	53.253	59.292	53.718	58.803	58.201	53.253	58.574	54.021
RINGA NYEKUNDU B	52.352	52.352	57.469	56.89	53.858	53.858	57.645	58.099	58.172	58.172	53.858	58.127	59.075	53.253	53.253	59.292	53.718	58.803	58.201	53.253	58.574	54.021
MERI KATASHINGO	53.485	53.485	58.07	57.218	53.776	53.776	58.07	57.996	57.093	57.093	53.625	58.377	58.865	53.707	53.707	59.187	54.173	59.051	59.155	54.091	59.423	54.091
2K	51.662	51.662	56.415	55.83	51.964	51.964	56.614	56.966	56.085	56.085	52.648	56.814	57.398	52.194	52.194	57.447	52.959	57.42	57.244	52.576	56.95	51.891
RINGA NYEKUNDU	51.672	51.672	56.743	56.239	52.432	52.432	56.415	56.766	55.965	55.965	52.816	57.143	57.447	52.359	52.359	57.597	53.74	57.923	57.218	53.13	57.663	53.577
BORA KUPATA	54.325	54.325	60.494	59.646	54.091	54.091	59.541	60.071	59.083	59.083	54.78	59.929	60.606	53.869	53.869	60.036	54.49	60	60	54.49	60.501	54.021
DHAHABU	54.325	54.325	60.494	59.646	54.091	54.091	59.541	60.071	59.083	59.083	54.78	59.929	60.606	53.869	53.869	60.036	54.49	60	60	54.49	60.501	54.021
MZURI NANI	53.354	53.354	58.082	58.214	54.268	54.268	58.824	58.824	58.467	58.467	54.504	59.213	59.82	54.351	54.351	59.677	54.824	59.643	59.464	54.656	59.806	54.809
CHIKWETA	51.982	51.982	57.244	56.128	52.664	52.664	57.522	57.447	57.345	57.345	53.272	57.473	57.706	51.903	51.903	57.576	52.824	58.333	58.259	53.13	57.67	51.756
LIWATAWATA	52.187	52.187	58.042	57.118	52.108	52.108	57.268	57.719	57.618	57.618	53.614	57.57	58.688	53.163	53.163	58.275	52.64	58.421	58.172	53.916	58.349	53.625

Variety	CHALAO	SINDANO 2	TELAA	FARAMARIA	MZURIAJIPAMBI B	UMANO B	AFRICA	4K	LINGA KIJICHO	MABEYENGA	MAGUGU	MWANZA	3K	BORAKUPATA A	ULIJUAJE KAMASIUMBEYA	SOMBA	SUPAZANZIBAR	PAWAGA	SUKARISUKARI	TAIWAN	JQ308421.1	TONDOGOSO
SUPA INDIA	43.485	43.485	47.359	46.903	42.965	42.965	47.09	46.29	46.561	46.561	43.57	46.809	46.607	43.57	43.57	46.195	44.175	45.76	46.903	44.175	46.228	43.247
KADOMOLI	55.136	55.136	60.175	59.788	57.164	57.164	60.808	61.444	59.051	59.051	55.958	61.661	61.388	55.204	55.204	62.124	56.278	61.62	60.494	55.589	61.657	55.219
BORA KUPATA C	50.076	50.076	53.616	52.566	49.696	49.696	53.18	53.628	53.263	53.263	50.608	53.819	54.107	49.544	49.544	54.093	49.848	53.982	54.433	49.39	55.34	49.695
KIJICHO A	58.182	58.182	64.499	64.488	59.455	59.455	64.374	64.903	64.374	64.374	59.848	64.248	65.419	58.788	58.788	65.371	59.697	65.548	65.018	59.181	67.375	58.877
LINGWINDIMBA B	58.182	58.182	64.499	64.488	59.455	59.455	64.374	64.903	64.374	64.374	59.848	64.248	65.419	58.788	58.788	65.371	59.697	65.548	65.018	59.181	67.375	58.877
KAZOLE	54.299	54.299	59.019	58.803	55.053	55.053	59.051	58.875	60.105	60.105	54.683	59.259	60.036	54.079	54.079	60.141	55.219	59.683	59.331	54.242	60.077	54.683
LICHENDE	54.299	54.299	59.019	58.803	55.053	55.053	59.051	58.875	60.105	60.105	54.683	59.259	60.036	54.079	54.079	60.141	55.219	59.683	59.331	54.242	60.077	54.683