VARIATIONS IN COMPOSITION OF VOLATILE FLAVOR COMPOUNDS AND SELECTED AGRONOMIC TRAITS OF NERICA RICE VARIETIES GROWN UNDER DIFFERENT NITROGEN RATES AT DIFFERENT AGROECOLOGICAL ZONES IN LAKE VICTORIA BASIN

BETHER A. ODHIAMBO

A thesis submitted to the School of Graduate Studies, Maseno University in partial Fulfillment of requirements for the award of a Master of Science Degree in Chemistry.

MASENO UNIVERSITY

DECLARATIONS

Student

I declare that this work has not been previously submitted for a degree at Maseno University or any other university. The work reported herein was carried out by me and all sources of information have been acknowledged.

Bether A. Odhiambo

PG/MSc/00012/2012

Sign……Date……..

Supervisors

This thesis has been submitted for examination with our approval as the supervisors.

1. Prof. P. Okinda Owuor
Department of Chemistry
Maseno University

Sign……. Date…….

2. Prof John C. Onyango
Department of Botany
Maseno University

Sign…… Date…….

3. Dr. Julius O. Okora
Department of Agricultural Biosystems and Economics
University of Kabianga

Sign……. Date……

ACKNOWLEDGEMENT

All glory and honor returns to the Almighty God for granting me His grace, wisdom and knowledge to carry out this work to completion. My sincere appreciation goes to my supervisors Prof P. Okinda Owuor, Prof John C. Onyango and Dr. Julius O. Okora for their insightful direction, words of encouragement and availability during the times I needed their advice. From them, I obtained astute mentorship right from the conception of this work, its development and the final output in form of a thesis write-up. I greatly appreciate the efforts of Dr. Xavier Cheseto of ICIPE-NAIROBI for his unfailing support during the GC-MS analysis of the rice volatile organic compounds. Mr. Peter Olewe of Maseno University Botanic Garden; Mr. Sospeter Onyango of Kenya Agricultural and Livestock Research Organization (KALRO) in Kisii, Mrs. Celine Okoth and Mr. Lumumba Ayub of KALRO-Kibos ensured my success during the field work. Special thanks to Mrs. Winnie Kore, in-charge of rice program at KALRO-Kibos, for enabling me to access good quality rice seeds that ensured success in the field experiments. I also thank the field casuals at Kibos, Oyani and Maseno who assisted me in laying out and maintaining the field experiments. I am especially indebted to the Kenya Agricultural Productivity Programme (KAPAP), through KALRO-Kibos, and the Kenya Government through National Commission for Science and Technology Institute (NACOSTI) for funding my research. My sincere appreciation goes to my beloved husband, Dr. Benson Onyango and our children Joy, Grace and Glory for their consistent prayers and words of encouragement.

DEDICATION

This work is dedicated to my precious husband Dr. Benson Onyango and our three lovely daughters; Joy, Gracy and Glory.

ABSTRACT

Rice (*Oryza sativa* L.) is a staple food consumed worldwide. Some rice cultivars are preferred over others due to their distinctive aroma which contributes to consumer acceptability and increased economic importance. New Rice for Africa (NERICA) is an inter-specific high yielding upland rice variety, which is a promising crop for addressing food insecurity in Africa. NERICA 1, 4 and 10 were released to farmers in Kenya for cultivation in 2009. Although studies have shown variations in NERICA yield due to environmental factors such as soil and weather patterns, there is no information on their influence on volatile flavor compounds (VFCs) composition, hence aroma quality. Some NERICA varieties are said to be aromatic but the constituents contributing to the aroma have not been identified. Indeed, it is not documented if different varieties produce varying aroma complexes. Nitrogen (N) fertilizers are applied to increase crop yields, usually accompanied by changes in composition of plant secondary metabolites. Despite changes in physiological and chemical parameters of plants with environment and N-rates, it is not documented how NERICA varieties vary in growth parameters in Lake Victoria basin. The objective of this study was to investigate agronomic performance and composition of organic volatile compounds of cooked NERICA 1, 4 and 10 varieties and their variations due to location of production and nitrogen fertilizer rates. The experiments were set up at KALRO farms in Kibos, Oyani, and Maseno University Botanic garden in a split plot design with three NERICA varieties (sub plots) and four nitrogen fertilizer levels (20, 60, 100 and 140 KgN/ha as main plots) replicated three times. Field data obtained included plant height, number of tillers and leaf chlorophyll content. Grains were harvested at maturity, sun-dried for seven days, de-hulled and milled. The volatiles flavour compounds were extracted using Licken-Nickerson distillation method; quantified using Gas Chromatography with ethyl decanoate as an internal standard and identified by Gas Chromatography Mass Spectrometry (GC-MS). Agronomic performance was influenced by varietal differences and geographical location of production. Significant ($p \leq 0.05$) differences were observed in leaf chlorophyll content of the NERICA varieties at different location of studies, while tillering ability did not vary. Plant height numerically increased with increased N-rates with NERICA 4 cultivated in Kibos being significantly ($p \leq 0.05$) taller than those from Maseno and Oyani. This was partly due to favourable soil parameters at Kibos. A total of 110, 100, and 100 VFCs were detected in NERICA 1, 4 and 10, respectively. NERICA 1 was superior to NERICA 4 and 10 in terms of VFCs concentration. The classes of compounds detected were green leaf volatiles, terpenes and aromatic compounds. The main aroma compound 2-acetyl-1-pyrolline (2-AP) was detected in NERICA 1 samples from Oyani and Maseno at 60 KgN/ha and 100 KgN/ha, respectively. Kibos site at N-rate 140 KgN/ha was most suitable for growth parameters, while NERICA 4 had highest growth parameters at all sites. NERICA 1 is aromatic possibly due to the presence of 2AP, while NERICAs 4 and 10 lacked 2AP hence non-aromatic. Maximum VFCs occur at N-rates between 60 and 100 KgN/ha. The key aroma compound was detected at Maseno and Oyani sites. NERICA 4 is recommended for cultivation at Kibos site under N-rates 140 KgN/ha for maximum growth parameters while aromatic rice should be cultivated at Maseno and Oyani within N-rates 60 to 100 KgN/ha for increased quality of rice.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF SYMBOLS, ACRONYMS AND ABBREVIATIONS

µL	microliters
µm	micrometers
^{0}C	degrees celcius
^{0}C/Min	degrees celcius per minute
2 AP	2- acetyl-1-pyrolline
AAS	Atomic Absorption Spectroscopy
ANOVA	Analysis of Variance
AV	aroma values
$BaCl_{2-}$	Barium Chloride
BI	Biodiversity International
BRRI	Bangladesh Rice Research Institute
$CaCl_{2-}$	Calcium Chloride
CAN-	Calcium Ammonium Phosphate
COMESA	Common Market for Eastern and Southern Africa
DAP	Diammonium Phosphate
DAT	Days after transplanting
DMAPP	Dimethylallylpyrophosphate
EAC	East African Community
ERA	Economic Review of Agriculture
FID	Flame ionization detector
GC	Gas chromatography.
GC-MS	Gas Chromatography Mass Spectrometry.
GPP	Geranylpyrophosphate
ICIPE	International Center for Insect Physiology and Ecology
IPP	Isopentyl pyrophosphate
KALRO	Kenya Agricultural and Livestock Research Organization
Kg/ha	Kilogram per hactare
KgN/ha	Kilogram Nitrogen per hectare
KNIB	Kenya National Irrigation Board
LM	Lower midland
LM 2	Lower midland 2
LM 3	Lower midland 3
LPP	Linalylpyrophosphate
LSD	Least Significant Difference
m/z	Mass to charge ratio
MEP	Methyllerylthritol phosphate
Meq %	Milliequivalents percent
mg C ml^{-1}	Milligrams of carbon per millilitre
MG	Methyl glyoxal
mgN/l	Milligrams of nitrogen per litre
MVA	Mevalonic acid pathway
Na_2SO_4	Sodium sulphate
NACOSTI	National Commission for Science Technology and Innovation
$NaHCO_3$	Sodium hydrogen phosphate
NARL	National Agriculture Research Laboratory
NERICA	New Rice for Africa
NPP	Nerylpyrophosphate
NRTC	National Rice Technical Committee
ppm	parts per million
P5SC	Pyrolline-5-carboxylic acid synthetase
SDE	Simultaneous Distillation/Extraction
SFE	Supercritical Fluid Extraction
SPAD	Soil Plant Analysis Detector
SPE	Solid Phase Extraction
SPME	Solid Phase Micro Extraction
UM	Upper midland
USDA	United States Development Agency
VFCs	Volatile Flavor Compounds
VOCs	Volatile Organic Compounds
WARDA	West African Rice Development Association
α-	alpha
β-	beta
γ-	gamma
δ-	delta
Mo	Moybdenum

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Rice is a staple food consumed by many people worldwide (Bounphanousay *et al*., 2008). Rice belongs to the genus *Oryza* and comprises two cultivated species; *O. sativa* L. that originated from Asia and the African cultivated species *O. glaberrima* (Oikeh *et al*., 2008). It is estimated that by 2025, 10 billion people world-wide shall depend on rice as a main food and its demand shall reach about 880 million metric tons annually (Champagne, 2008). By August 2016, the annual world rice production was estimated at 481.08 million metric tons, a decrease from the previous year by 2.18% (USDA, 2016). The Economic Research Service of US, projects the global rice trade in 2017 at 40.5 million tons which is below the preceding year (USDA, 2016). Low rice production world-wide has led to high international and domestic prices, making the crop inaccessible to people in low income economies particularly in Sub-Saharan Africa. Indian consumers consider aroma, taste and grain length the most critical quality traits, while Asian consumers in the United States consider appearance, flavor and stickiness as the most important acceptance factors (Bergman *et al*., 2000; Suwansri *et al*., 2002). Flavor as a primary sensory quality attribute for consumers, is composed of odor (aroma) and taste. The latter is perceived by chemoreceptors within the taste buds on the surface of the tongue, while the former is assessed by chemoreceptors in the olfactory epithelium.

Africa has lagged behind in rice production with most of the rice consumed imported from Asia and other continents. Both O. *sativa* and *O. glaberrima* have been cultivated in Africa since the introduction of the former by the Portuguese in 1500AD (Porteres, 1950). However, the combined yield of the two species has been quite low due to utilization of unimproved technologies and over dependence on irrigated varieties by African farmers. As a result, close to 10 million tons of milled rice valued at over US$ 1.4 million is imported into Africa each year (Cho *et al*., 2014). Serious concerns have been raised on the quality of rice imports since Africa is considered a dumping ground for substandard rice (AfricaRice, 2015). The 2008–2009 global food-price crisis was a major incentive to increase domestic rice production in Africa through implementation of national rice development strategies (FAO, 2011). Consequently, rice production in Sub-Saharan Africa rose by 16–18% in 2008 and a further 4.5% in 2009 (FAO, 2011).

The increase in rice production in Africa was partly attributed to the entry into production of New Rice for Africa (NERICA). NERICA is an inter-specific hybrid of *O. sativa* and *O. glaberrima* developed by West African Rice Development Association (WARDA) in Cotonou, Benin (Somado *et al*., 2008). NERICA varieties are upland grown and have gained prominence in Africa due to their reputation in cooking quality, nutrition values and perceived high aroma (Kimani *et al*., 2010). The WARDA breeders combined the high yield potential and short growth cycle of *O. sativa* with the high tillering ability, tolerance to low water availability and resistance to pests and diseases of *O. glaberrima* to produce NERICA varieties (Mokuwa *et al*., 2013; Linares, 2010). Although the resultant NERICA varieties had better traits for survival and higher yield in the non-irrigated African soils than the parental lines (AfricaRice, 2010), the volatile flavor compounds that contribute to aroma in these NERICA varieties have not been identified.

Rice production in Kenya is below potential with the country producing only 20% of its national requirements (Economic Review of Agriculture, 2010). The annual consumption of rice in Kenya increased by 12% in 2015 to 560,000 metric tons from 500,000 metric tons in 2014 (USDA, 2016). This increase was attributed to population growth, higher incomes and changing dietary preferences of urban dwellers (Kamau *et al*., 2010). Despite the rapid increase in rice consumption, production has fluctuated between 150,000 and 155,000 metric tons per year during the same period (USDA, 2016). This deficit has often been met through imports from East African Community (EAC), Common Market for Eastern and Southern Africa (COMESA) and the world market, mostly from Asia. About 95% of Kenyan rice is grown under irrigation while the remaining is rain-fed (Kore *et al*., 2007). Irrigated ecosystems require heavy investments that are unaffordable to many small-scale rice farmers making the rain-fed ecosystems the viable option (Kore *et al*., 2007). The recent introduction of NERICA 1, 4 and 10 varieties in the Kenyan production systems provided a desirable alternative to mitigate the import deficit, while reducing the unsustainable irrigated rice area production. The Kenya National Irrigation Board (KNIB) and the County governments have promoted the expansion of NERICA varieties production in Kenya due to their numerous agronomic advantages (Gitonga, 2016). However, the optimal rates of nitrogen ferfilizer to maximize production and increase consumer acceptability of NERICA 1, 4 and 10 have not been developed.

The diverse agro-ecological sites of Lake Victoria Basin provide suitable growing conditions for the NERICAs (Olembo *et al*., 2010). NERICAs 1, 4 and 10 were tested and released to farmers

in Kenya for cultivation in 2009 by the Ministry of Agriculture, through the advice of National Rice Technical Committee (NRTC) (Onyango *et al*., 2010). The varieties are currently cultivated in several regions of Lake Victoria basin including Oyani in Migori County, Maseno and Kibos both in Kisumu County (Olembo *et al*., 2010). Although previous studies showed variations in yield due to environmental factors such as soil and weather patterns (Kore *et al*., 2007; Olembo *et al*., 2010), there is no information on the influence of these factors on the aroma profile of NERICA varieties and how the volatile flavor compounds affect the aroma. Developments of appropriate production protocols that influence aroma quality of the NERICAs may provide insightful information to rice breeders for varietal improvement so as to meet the rapidly changing consumer demands.

Growth parameters are important selection tools in assessment of the performance of any crop. In rice production, important growth parameters include plant height, number of tillers per hill and chlorophyll content. Chlorophyll is a major chloroplast component for photosynthesis and relative chlorophyll concentration has a positive relationship with photosynthetic rate (Anjum *et al.,* 2011). Assessment of chlorophyll content is an effective way of monitoring plant growth and estimating photosynthetic productivity (Chen *et al*., 2007). Similarly, higher tillering ability and greater plant height in rice reflect better plant establishment and strongly influences yield and other quality traits such as rice aroma (Chaunabasappa *et al*., 1998). Tillering, the production of lateral branches is an important agronomic trait that determines shoot architecture and grain production in rice (Hussein *et al*., 2014). Indeed, each tiller has the potential to produce a seed-bearing inflorescence and hence, increase yield. However, a balance between number and vigor of tillers is required, as unproductive tillers consume nutrients and reduce grain production (Hussein *et al*., 2014). Plant height is a selective morphological marker in rice breeding which modifies yield enhancing characters and finally shapes grain quality (WARDA, 2008). Rice plants under optimal nitrogen fertilizer rates tend to grow to maximum heights and produce more tillers which translate to higher yields and better-quality outputs (Li *et al*., 2003). There has been no study to evaluate variations in plant height, tillering ability and chlorophyll content of the released NERICA varieties due to different nitrogen rates in the different Lake Victoria regions.

Aroma is an important trait in rice and contributes to consumer acceptability and hence increases economic importance (Sakthivel *et al*., 2009). Progressive interest on scented rice production and flavour improvement has a direct influence in economic benefits due to

improved marketability. Superior rice flavor increases consumer satisfaction, overall acceptability and the probability of repeated purchase (Bergman *et al.,* 2000), hence selection for flavor in rice breeding programs is critical. Although the initial breeders of NERICA varieties aimed at producing lines that could withstand the harsh African growing conditions, aromatic rice varieties were also produced (Jones *et al*., 1997). Indeed, NERICA 1 was found to have favourable aroma giving it a premium market price because of superior quality of fragrance (Jones *et al*., 1997). The occurrence of fragrance in rice has been attributed to genetic factors, environmental conditions and pre-harvest and post-harvest treatment of the crop during cultivation. For instance, the genetic origin of fragrance in NERICA 1 grain was attributed to a mutation on *BADH2* gene which is responsible for fragrance in most modern rice varieties (Asante *et al*., 2010). WARDA breeders involved in the development of the NERICA varieties explained that several fragrant rice varieties were planted in a field near the breeding nursery during the development of NERICA 1 and this may have led to production of aromatic varieties (Jones *et al*., 1997). On the other hand, aroma synthesis was attributed to higher total soil nitrogen due to increased L-proline synthesis in some Asian rice varieties (Yang *et al*., 2012). It however remains to be determined if there are variations in the volatile compounds responsible for aroma in NERICAs 1, 4 and 10 cultivated in Lake Victoria basin.

As a quantitative trait, environmental factors affect the expression of rice aroma (Bounphanousay *et al*., 2008). Site characteristics such as biotic and abiotic components influence aroma quality in different rice varieties (Champagne, 2004). Each variety performed best, with respect to quality traits, in its own native area of cultivation (Ali *et al*., 1991). Some aromatic varieties express their aroma only in specific areas while others may show variations in aroma content depending on environmental condition (Champagne, 2004). The cropping systems of Lake Victoria basin have diverse agro-ecological zones and heterogeneous soils (Jaetzold *et al*., 2007). It has not been evaluated if variation in agro-ecological sites of production of NERICA varieties influences the concentration of volatile flavor compounds (VFCs) responsible for aroma.

1.2 Statement of the Problem

Despite introduction of NERICA varieties to overcome low and inadequate rice production, deficits in production still occur. This is possibly due to use of inappropriate agronomic inputs. For example, the optimal rates of nitrogen fertilizer application to increase production of NERICA 1, 4 and 10 have not been developed.

Plant growth parameters such as plant height, number of tillers per hill and chlorophyll content are important indicators of yield which are highly influenced by cultural practices such as nitrogen fertilization and prevailing environmental conditions. However, despite release of NERICA varieties into the cropping systems of Lake Victoria basin, no study has evaluated the effect of varying nitrogen fertilizer rates and sites of production on plant height, tillering ability and chlorophyll content of the rice varieties for increased rice production and quality.

Aroma is an important trait in rice which contributes to consumer acceptability and hence increases economic importance. The cropping systems of Lake Victoria basin with its diverse agro-ecological zones and heterogeneous soils may influence the composition of volatile flavor compounds in rice. NERICA 1 is classified as aromatic while NERICA 4, and 10 are non aromatic. The variations in the volatile flavor compounds of the NERICA varieties as influenced by varieties, agro-ecological sites of production and nitrogen fertilizer rates have not been quantified.

1.3 Objectives

1.3.1 Broad Objective

To assess variations in selected agronomic traits and composition and concentrations of volatile flavour compounds (VFCs) in selected NERICA varieties grown in Lake Victoria Basin and effect of nitrogen fertilizer on their levels.

1.3.2 Specific Objectives

1. To evaluate the effect of varieties, nitrogen fertilizer rates and sites of production on leaf chlorophyll, plant height and number of tillers of NERICA 1, 4 and 10.
2. To identify and compare the concentrations of volatile flavour compounds in NERICA 1, 4 and 10 varieties.
3. To evaluate the effect of nitrogen fertilizer rates on the composition and concentrations of volatile flavour compounds of NERICA 1, 4 and 10.
4. To determine the influence of agro-ecological zone of production on the composition and concentrations of volatile flavour compounds in the NERICA 1, 4 and 10.

1.4 Null Hypotheses, H_o

1. Leaf chlorophyll content, plant height and tiller number of NERICA 1, 4 and 10 do not vary with variety, nitrogen fertilizer rates and location of production.

2. There are no variations in the composition and concentration of volatile flavour compounds of NERICA 1, 4, and 10 varieties.
3. The composition and concentrations of volatile flavour compounds of NERICA 1, 4 and 10 do not vary with nitrogenous fertilizer rates.
4. The composition and concentrations of volatile flavour compounds of NERICA 1, 4 and 10 do not vary with location of production.

1.5 Justification

Selection criteria for breeding programmes in rice are currently unavailable. Understanding agronomic traits that can be linked to productivity shall lead to early of such programmes. The national and county governments currently spend a lot of revenue on importation of quality aromatic rice from Asia and other continents. Policy makers rely on qualitative scientific studies to increase investment in domestic rice production to save on revenue used to import aromatic rice. Development of agronomic practices that lead to high quality rice production shall improve demand and price of rice.

Aroma is an important quality parameter of rice. Determination and identification of rice with desirable aroma will lead to commercial exploitation of varieties that produce good aroma and enhance consumer preference and price of rice. Rice breeders' currently lack flavor quality assessment and progeny selection tool. Availability of scientific measure of aroma in NERICA is therefore necessary.

1.6 Significance of the study

Development of qualitative scientific studies is expected to increase investment in domestic rice thus increased production. This would save on revenue used to import aromatic rice from Asia and other continents. Development of agronomic practices that lead to high quality rice production shall improve demand and price of rice.

Determination and identification of rice with desirable aroma will lead to commercial exploitation of high quality aromatic varieties, which will enhance consumer preference hence price of rice. Creation of a scientific measure of aroma in NERICA varieties will enable rice breeders' use it as a tool for flavor quality assessment and progeny selections.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of NERICA varieties

The African cultivated rice, *Oryza glaberrima*, was domesticated by local farmers along the Niger River long before Asian cultivated rice, *Oryza sativa*, was introduced into Africa (Oikeh *et al*., 2008). *O. glaberrima* is well adapted to the harsh agro-ecological conditions characteristic of West Africa, and possesses tolerances to a wide range of biotic and abiotic stresses (Oikeh *et al*., 2008). However, *O. glaberrima* varieties are plagued by low yield potentials and some unfavorable traits for economic rice production, such as susceptibility to lodging and shattering. This prompted the West Africa Rice Development Association (WARDA) to develop inter-specific hybrids between *O. sativa* and *O. glaberrima*, in an effort to bring the beneficial tolerances of *O. glaberrima* into a high-yielding *O. sativa* genetic background (Jones *et al*., 1997).

Over 300 inter-specific inbred lines were developed by WARDA through crossing of *O. sativa* subspecies *japonica* variety WAB56-104 (recurrent parent), with the *O. glaberrima* variety CG14 (donor parent) (Jones *et al*., 1997). The resultant lines are collectively referred to as New Rice for Africa (NERICA) varieties. NERICA varieties are early maturing, high yielding, pest and disease resistant, acid soil tolerant and are able to withstand drought (Apaseku and Dowbe, 2013). The cultivars have acceptable organoleptic characteristics, including good taste and aroma which compare favorably with imported rice (WARDA, 2001). WARDA breeders set out to produce upland varieties that could exhibit stress tolerances under African environmental condition but other marketability features such as fragrance were produced (Oikeh *et al*., 2008). NERICA 1 is said to possess fragrance in the grain, a characteristic that both the WAB56-104 and CG14 parents lacked. Breeders involved in the development of NERICA explained that several fragrant varieties were planted in a field near the breeding nursery and this may have led to production of aromatic varieties (Jones *et al*., 1997). From its west-African origin, NERICA cultivation is now widespread in sub-Saharan Africa including East Africa where it is mostly cultivated in Uganda and to a lesser extent in Kenya. In Kenya, NERICA 1, 4 and 10 were released to farmers in 2009 by the Ministry of Agriculture, through the National Rice Technical Committee on Rice (MOA, 2010). The varieties are cultivated in several regions of Lake Victoria basin (Olembo *et al*., 2010). However, no scientific attention has been given to the quality attributes such as aroma of the released varieties.

2.2 Plant secondary metabolites

Plant secondary metabolites are naturally occurring organic compounds which are not directly involved in plant growth, development or reproduction (Purcaro, 2007). Volatile organic compounds can serve as attractants in plants pollinated by bees, beetles, butterflies, moths and bats (Dodson *et al.,* 1969; Galen & Kevan, 1983; Nilsson, 1983; Pellmyr, 1986; Williams, 1983; Williams and Whitten, 1983; Dudareva and Pichersky, 2000). Volatile compounds mediate many interactions between organisms, including plant response to pathogen infection (Shulaev *et al*., 1997), plant-parasitoid signaling in response to herbivory (Turlings *et al*., 1990) and plant-pollinator communication during flowering (Turlings *et al*., 1990). However, the volatiles emitted from the vegetative parts, especially those released after herbivory, protect plants by deterring herbivores or attracting the enemies of herbivores (Pichersky & Gershenzon, 2002). They have different pleasant odours and are therefore used in perfumery (Agarwaal, 1998; Charlwood and Charlwood, 1998). Other exploitable properties of monoterpenoids are anti-bacterial, anti-fungal and anti-cancer activities and in chemotherapy (Charlwood and Charlwood, 1998). Volatile organic compounds (VOCs) aid plants in functions such as tolerance to environmental stress, competition and species interactions. Biosynthesis of VOCs depends on the availability of carbon, nitrogen and sulfur as well as energy provided by primary metabolism. Majority of VOCs are synthesized through blends involving four classes; terpenoids, aromatics, fatty acid derivatives, and amino-acid derived products. The volatile compounds are synthesized from precursors whose hydrophilic functional groups have been removed or masked through reduction, methylation and acylation reactions. Their lipophilicity allows plant volatiles to traverse membranes readily and evaporate into the atmosphere.

Terpenoids are derived from condensation of a C_5 precursor isopentenyl pyrophosphate and its allylic isomer, dimethylallyl pyrophosphate (Baldwin, 2016). In plants, two independent, compartmentally separated pathways – the mevalonic acid (MVA) and methylerythritol phosphate (MEP) – are responsible for the formation of these C_5-isoprene building units (McGarvey and Croteau, 1995). The MVA pathway gives rise to sesquiterpenes (C_{15}), while the MEP pathway provides precursors to volatile hemiterpenes (C_5), monoterpenes (C_{10}) and diterpenes (C_{20}) (Dudareva *et al*., 2005). Aromatic VOCs comprise phenylpropanoid and benzenoid compounds (Knudsen *et al*., 2006), which originate from phenylalanine through chain-shortening of trans-cinnamic acid and incorporation of structures from lignin biosynthesis. Plant phenolics are a heterogeneous group of aromatic VOCs soluble in organic

solvents although a few are large, insoluble polymers. Fatty acids derived VOCs such as 1-hexanal, cis-3-hexenol, nonanal and methyl jasmonate arise from C_{18} unsaturated linoleic or linolenic fatty acids (Song *et al*., 2005). Numerous VOCs are derived from amino acids such as alanine, valine, leucine, isoleucine, and methionine, or intermediates in their biosynthesis (Knudsen *et al*., 2006). The biosynthesis of amino acid derived volatiles in plants is believed to proceed in a similar way to that found in bacteria or yeast (Dickinson *et al*., 2000; Beck *et al*., 2002; Tavaria *et al*., 2002). The amino acids undergo an initial deamination or transamination catalyzed by aminotransferases, leading to the formation of the corresponding a keto-acid. These α-keto-acids can be further subjected to decarboxylation, reductions, oxidations and/or esterifications, forming aldehydes, acids, alcohols and esters (Reineccius, 2006).

2.1.1 Composition of volatile flavor compounds in aromatic rice

2.2.1.1 Role of nutrients on aroma production in rice

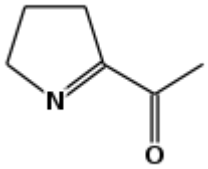
The primary chemical components in rice grains are lipids, starch and proteins, which vary with production environment, soil chemical factors and the rice cultivar (Tok, 2007). For example, the lipid content ranges from 1 % to 4 %, protein from 6 % to 8 % and starch from 60 % to 80 % between different cultivars (Kennedy and Burlingame, 2003). Rice lipids are generally classified as starch lipids which are associated with the starch granules and non-starch lipids which compose other cellular components. Non-starch lipids are primarily located near the surface (bran); and as a consequence, are significantly reduced during milling. The free fatty acids in rice are mainly palmitic, stearic, oleic and linoleic acid (Zhou *et al*., 2002). Volatile compounds formation from lipids results from lipolysis (breakdown of lipids to produce fatty acids), lipid oxidation and decomposition (Zhou *et al*., 2002). Lipase produces free fatty acids that may undergo oxidation during lipolysis. In addition to enzymatic action, heat during cooking also results in lipolysis. Hydroperoxides formed with lipid oxidation readily decompose yielding a variety of products with varying molecular weights and odor thresholds. Decomposition products include aldehydes, ketones, alcohols, furanones, acids, lactones and hydrocarbons, many of which impact flavor (Zhou *et al*., 2002).

The protein content of brown rice is between 6.6 to 7.3 % (Tok, 2007). Much of rice protein is present in protein bodies that are stable even during heating. The primary protein in rice is glutenin, which is insoluble in water. Albumin and globulin also present, are water soluble, and are prevalent in the bran (Zhou *et al*., 2002). Volatile sulfur compounds (e.g., hydrogen sulfide, dimethyl sulfide) concentration decreases during protein oxidation during cooking (Zhou *et al*,

2002). Carbonyl compounds formed by lipid oxidation react with the sulfuhydryl groups in cysteine or methionine decreasing the formation of volatile sulfur compounds.

2.2.1.2 Volatile flavor chemistry in rice

A large number of compounds contribute to the aroma and flavor of rice. Previous studies on VFCs revealed over 200 volatile compounds in rice, but only a few contributed to the characteristic rice aroma (Yajima *et al*., 1978; Buttery *et al*., 1988; Widjaja *et al*., 1996; Jezussek *et al*., 2002). Later studies reported more than 200 volatile compounds in rice, although only a few were identified as influencing the aroma and flavor of cooked rice (Champagne, 2008), but determining which volatile compounds are responsible for the perceived aroma in rice is a difficult task (Jezussek *et al*., 2002). With the exception of 2-acetyl-1-pyrroline (2AP) (**1**), no one single compound can be said to contribute a characteristic aroma (Jezussek *et al*., 2002). Several compounds are thought to work collectively to influence aroma quality. Perceived aroma is not strictly additive but may result from interactions of several volatile compounds (Buttery *et al*., 1988).



(1) C_6H_9NO

The Maillard reaction is the primary route for aroma formation in rice and it's enhanced by heat treatment prior to analysis (Tok, 2007). In studying rice aroma, all samples are parboiled in distilled water which hastens the reaction for formation of important volatiles such as 2AP (Fig. 10). Volatile compounds are produced when during heating the carbonyl group of a reducing sugar and an amino group of an amino acid or protein react forming N-substituted glycosylamine or fructosylamine (Reineccius, 2006). Subsequent fragmentation and release of the amino group results in deoxyosones, which can react in countless ways to produce Maillard reaction products. Such compounds include furans, nitrogen-containing heterocyclic compounds, oxygen-containing heterocyclic compounds and sulfur-containing heterocyclic compounds (Van Moerkercke, 2012). Resultant compounds including ornithine, proline and glutamate can be converted to a common metabolite β- pyrroline-5-carboxylic acid, via three distinct enzymes: ornithine amino-trasferase, proline dehydrogenase and β- pyrroline-5-carboxylic acid synthetase. On this basis, a biosynthetic pathway to 2AP was proposed (Costello and Henschke, 2002; Hofmann and Schieberle, 1998; Huang *et al*., 2008; Schieberle, 1990;

Yoshihashi *et al.*, 2002). The glutamic acid is converted by the bi-functional enzyme 1-Pyrroline-5-Carboxylate Synthetase (P5CS) to glutamic-gamma-semialdehyde, which cyclizes spontaneously to P5CS. The latter undergoes decarboxylation from glucose with the intermediacy of fructose-1-6, diphosphate, to give 2AP. Alternatively, P5CS may react directly with methyl glyoxal (MG) to give final product, P5CS can also be obtained from proline by proline dehydrogenase (Huang *et al.*, 2007).

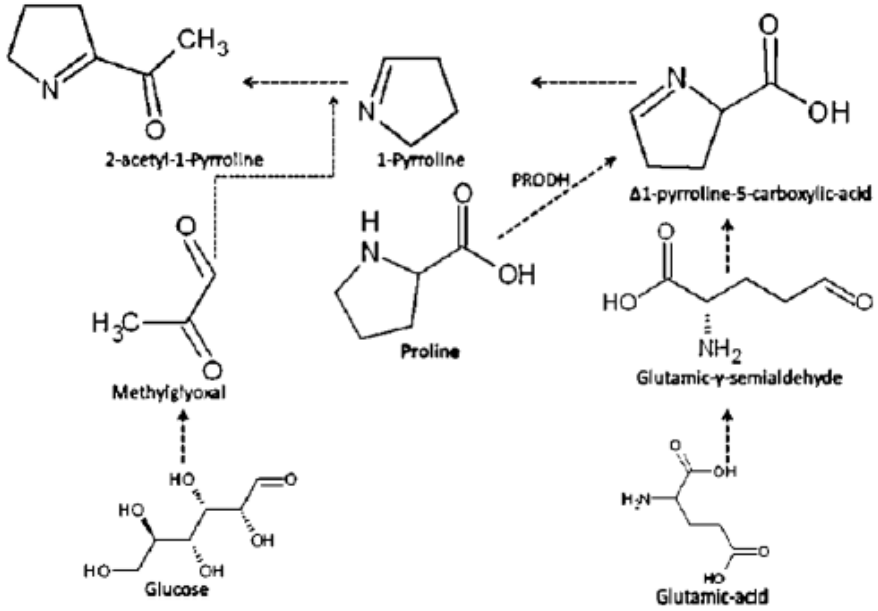


Fig 1. The biochemical pathway of 2-acetyl-1-pyrroline synthesis

Several studies (Buttery *et al*., 1983; Jezussek *et al*., 2002; Lam and Proctor, 2003) have used methodical approaches to determining which volatile compounds are important contributors to the aroma and flavor of rice. The variations in 2AP concentrations of 10 rice varieties are shown in Table 1 (Buttery *et al.*, 1983). The range of concentration was from 6 ppb to 90 ppb in the milled rice. The brown rice had concentrations of 2-acetyl-1-pyrroline from 100 ppb to 200 ppb. The variations demonstrated that the aroma quality changed with varieties. The presence of 2AP and variations in other VFCs due to cultivar differences in the NERICAs cultivated in Lake Victoria basin is still unknown.

Table 1. Concentration of 2-acetyl-1-pyrroline (ppm) in cooked rice varieties

Variety	Brown	Milled
Malagkit Sungsong	0.09	0.2
IR841-76-1	0.07	0.2
Khao Dawk Mali 105	0.07	0.2
Milagross	0.07	0.17
Basmati	0.06	NR
Seratus Malam	0.06	NR
Azucena	0.04	0.16
Hieri	0.04	0.1
Texas Long Grain	<0.008	NR
Calrose	<0.006	NR

KEY: ppm = parts (weight) of compound per million.NR = Not Recorded: (Buttery *et al*., 1983)

Rice volatiles have been intensively studied as important aspects of consumer acceptance as shown in Table 2 (Yajima *et al.,* 1979; Tsuzuki *et al.,* 1981; Maga, 1984; Tsugita, 1985; Widjaja *et al*., 1996; Lam and Proctor, 2003; Wongpornchai *et al*., 2005; Champagne, 2008). Although much progress has been made in identification of volatile flavor compounds and determination of aroma values (AV) in rice flavor, it is still unclear if environmental factors of production would cause variation in levels and composition of volatile flavor compounds. It was previously assumed that volatiles with low odor threshold would be more probable contributors to the overall aroma or flavor of rice. For instance, the aldehydes (E) 2-nonenal (threshold [T] = 0.08 ppb) and (E, E) - 2, 4-decadienal (T = 0.07 ppb) had the lowest odor threshold, and were considered to likely contribute to the aroma (Buttery *et al*., 1988). Other aldehydes with relatively low thresholds but likely to contribute to aroma were (E)-2-decenal (T = 0.4 ppb), octanal (T = 0.7 ppb), nonanal (T = 1 ppb), and decanal (T = 2 ppb) (Buttery *et al*., 1988). It was concluded, based on their aroma value, hexanal (grassy flavor) and 2-pentylfuran contributed more to flavor change in milled rice early in storage rather than later (Lam and Proctor, 2003). 2-nonenal (rancid flavor) and octanal (fatty flavor) contributed more to the overall flavor of milled rice during long-term storage (Lam and Proctor, 2003). The variations of these volatile compounds in NERICA varieties have not been studied.

Table 2. VFCs previously identified in rice.

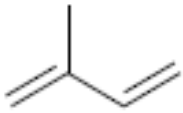
Compound	Formula	Reference	Compound	Formula	Reference
Benzaldehyde	C_7H_6O	Tsuzuki *et al* 1975	Tridec-2-enal	$C_{13}H_{24}O$	Yajima *et al* 1979
Octanal	$C_8H_{16}O$	Yajima *et al* 1979	Hexanal	$C_6H_{12}0$	Tsuzuki *et al* 1981
3-Methyl butanal	$C_5H_{10}O$	Yajima *et al* 1979	Heptanal	$C_7H_{14}O$	Widjaja *et al*., 1996
E-2-Heptenal	$C_7H_{12}O$	Yajima *et al* 1979	Furfural	$C_5H_4O_2$	Widjaja *et al*., 1996
5 methyl furfural	$C_6H_6O_2$	Tsuzuki *et al* 1981	3-Furaldehyde	$C_5H_4O_2$	Tsuzuki *et al* 1975b
Undecanal	$C_{11}H_{22}O$	Yajima *et al* 1979	β-Cyclocitral	$C_{10}H_{16}O$	Tsugita, 1985
Z-2-Nonenal	$C_9H_{16}O$	Buttery *et al*., 1988	Acetaldehyde	C_2H_4O	Tsuzuki *et al* 1975b
E, E-2,4-Nonadienal	$C_9H_{14}O$	Buttery *et al*., 1988	Pentanal	$C_5H_{10}O$	Buttery *et al*., 1988
2-Furaldehyde	$C_5H_4O_2$	Tsugita, 1985	Nonanal	$C_9H_{18}O$	Tsugita, 1985
3-Cyclohexene-1-carboxaldehyde	$C_7H_{10}O$	Tsugita, 1985	β-ionone	$C_{13}H_{20}O$	
6-Dodecanone	$C_{12}H_{24}O$		Alpha ionone	$C_{13}H_{20}O$	Jezussek *et al*., 2002
2-Decanone	$C_{12}H_{24}O$	Buttery *et al*., 1988	Cryptone	$C_9H_{14}O$	Buttery *et al*., 1988
3-Octen-2-one	$C_8H_{14}O$	Yajima *et al* 1979	Acetophenone	C_8H_8O	Jezussek *et al*., 2002
Geranyl acetone	$C_{13}H_{23}O$	Widjaja *et al*., 1996	2,3-Octanedione	$C_8H_{14}O_2$	Widjaja *et al*., 1996
2,5-Octanedione	$C_8H_{14}O_2$	Widjaja *et al*., 1996	2-hexanone	$C_6H_{12}O$	Maga, 1984
2-Nonanone	$C_9H_{18}O$	Yajima *et al* 1979	2-Pentadecanone-6,10,14-trimethyl	$C_{18}H_{36}O$	Buttery *et al* 1982
6-Methyl-5-hepten-2-one	$C_8H_{14}O$	Buttery *et al* 1983b	2-Heptadecanone	$C_{17}H_{34}O$	Buttery *et al* 1982
2 –Heptanone	$C_7H_{14}O$	Yajima *et al* 1979	2- Acetyl-1-pyrroline	$C_6H_{10}ON$	Buttery *et al*., 1983
Methyl pyrazine	$C_5H_6N_2$	Buttery *et al* 1982	Pyrrolidine	$C_6H_{10}ON$	Tsugita, 1985
Pyridine	C_5H_5N	Buttery *et al* 1982	Cyclopentane, Ethyl	C_7H_{14}	Buttery *et al*., 1988
Tetradecane	$C_{14}H_{30}$	Maga, 1984	Cyclopentane	C_5H_{10}	Maga, 1984
1-Decene	$C_{10}H_{20}$	Tsugita, 1985	Octane	C_8H_{18}	Widjaja *et al*., 1996
1-Heptene	C_7H_{14}	Tsugita, 1985	Dodecane,2,6,10 ,trimethyl	$C_{15}H_{32}$	Buttery *et al*., 1988
Heptane,2-methyl	C_8H_{18}	Widjaja *et al*., 1996	Cyclopentane, butyl	C_9H_{18}	Widjaja *et al*., 1996
Nonadecane	$C_{19}H_{40}$	Tsuzuki *et al* 1981	Tridecane	$C_{13}H_{28}$	Widjaja *et al*., 1996
Benzene acetaldehyde	$C_{10}H_{12}O_3$	Yajima *et al* 1979			

Table 2. Contd...VFCs previously identified in rice.

Compound	Formula	Reference	Compound	Formula	Reference
Octadecane	$C_{18}H_{38}$	Tsuzuki *et al* 1981	Dodecane	$C_{12}H_{26}$	Tsugita, 1985
Eicosane	$C_{20}H_{42}$	Tsugita, 1985	Heptadecane	$C_{17}H_{36}$	Tsugita, 1985
p-Cymene	$C_{10}H_{14}$	Tsuzuki *et al* 1981	Pentane,2,3,4-trimethyl	C_8H_{18}	Jezussek *et al*., 2002
Limonene	$C_{10}H_{16}$	Tsuzuki *et al* 1981	Methyl octadecanoate	$C_{19}H_{38}O_2$	Yajima *et al* 1979
2,4-Dimethylfuran	C_6H_6O	Widjaja *et al*., 1996	Naphthalene	$C_{10}H_8$	Tsuzuki *et al* 1981
2,5-Dimethylfuran	C_6H_8O	Tsugita, 1985	o-Cymene	$C_{10}H_{14}$	Tsuzuki *et al* 1981
2-Heptylfuran	$C_{11}H_{18}O$	Yajima *et al* 1979			Tsugita, 1985
1,2-Benzothiazole	C_7H_5NS	Tsuzuki *et al* 1981	Naphthalene,2,3,6-trimethyl	$C_{13}H_{14}$	Jezussek *et al*., 2002
Dimethyl disulfide	$C_2H_6S_2$	Tsuzuki *et al* 1978	Styrene	C_8H_8	Widjaja *et al*., 1996
Decanoic acid	$C_{10}H_{20}O_2$	Buttery *et al* 1982	Vinylfuran	C_6H_6O	Jezussek *et al*., 2002
Hexadecanoic acid	$C_{16}H_{32}O_2$	Tsugita, 1985	2- Pentylfuran	$C_9H_{14}O$	Yajima *et al* 1979
Carvacrol	$C_{10}H_{13}O$	Tsugita, 1985	2-Acetyl furan	$C_6H_6O_2$	Yajima *et al* 1979
Methyl chavicol	$C_{10}H_{12}O$	Yajima *et al* 1979	Dimethyl trisulfide	$C_3H_6S_3$	Tsuzuki *et al* 1978
Dodecan-1-ol	$C_{12}H_{26}O$	Yajima *et al* 1979	Thymol	$C_{10}H_{14}$	Buttery *et al*., 1988
Hexanoic acid ethyl ester	$C_8H_{16}O_2$	Tsugita, 1985	Octadecanioic acid	$C_{18}H_{36}$	Tsugita, 1985
Linalool	$C_{10}H_{18}O$	Yajima *et al* 1979	Methyl tetradecanoate	$C_{15}H_{30}O_2$	Yajima *et al* 1979
Isobutyl 2-methylbutanoate	$C_8H_{16}O_2$	Jezussek *et al*., 2002	Octylformate	$C_9H_{18}O_2$	Buttery *et al* 1982
Methyl propanoate	$C_4H_8O_2$	Buttery *et al* 1982	Nonanol	$C_9H_{20}O$	Yajima *et al* 1979
Dec-1-en-3-ol	$C_{10}H_{20}O$	Buttery *et al*., 1988	Decan-1-ol	$C_{10}H_{22}O$	Yajima *et al* 1979
Methyl linoleate	$C_{19}H_{34}O_2$	Yajima *et al* 1979	Terpinen-4-ol	$C_{10}H_{18}O$	Buttery *et al* 1982
Cedrol –epi	$C_{15}H_{26}O$	Buttery *et al* 1982			

2.2.1.3 Terpenoid biosynthesis

Monoterpenes, diterpenoids, triterpenoids and sesquiterpenoids are the most important constituents of essential oils (Solomons, 1997). They are products of secondary metabolism synthesized in most cellular organelles and stored in specialized secretory structures. Apart from the rare hemiterpenoids (containing a single C_5 skeleton), there exist monoterpenes. Isoprene **(2)** is a classic example of hemiterpenoids (Agarwaal, 1998).



2)

Monoterpenes consist of two isoprene (C_5 carbon) units, formed by head-to-tail condensation to produce a C_{10} branched chain or ring. The metabolic pathway of terpenes starts with the condensation acetyl coenzyme A (**3**) from acetic acid and malonyl coenzyme A (**4**) from malonic acid, (Fig 2). They condense to form (**5**), which decarboxylates to form aceto acetyl coenzyme A (**6**). Another molecule of malonyl coenzyme combines with aceto acetyl coenzyme to form (**7**). The latter then decarboxylates to form hydroxyl methyl glutarate (**8**), on addition of NADPH, mevalonic acid (**9**) arises. The isoprene unit, normally in the form of isopentyl pyrophosphate (IPP) (**10**) readily converted to dimethylallylpyrophosphate (DMAPP) (**11**) as shown in the figure below.

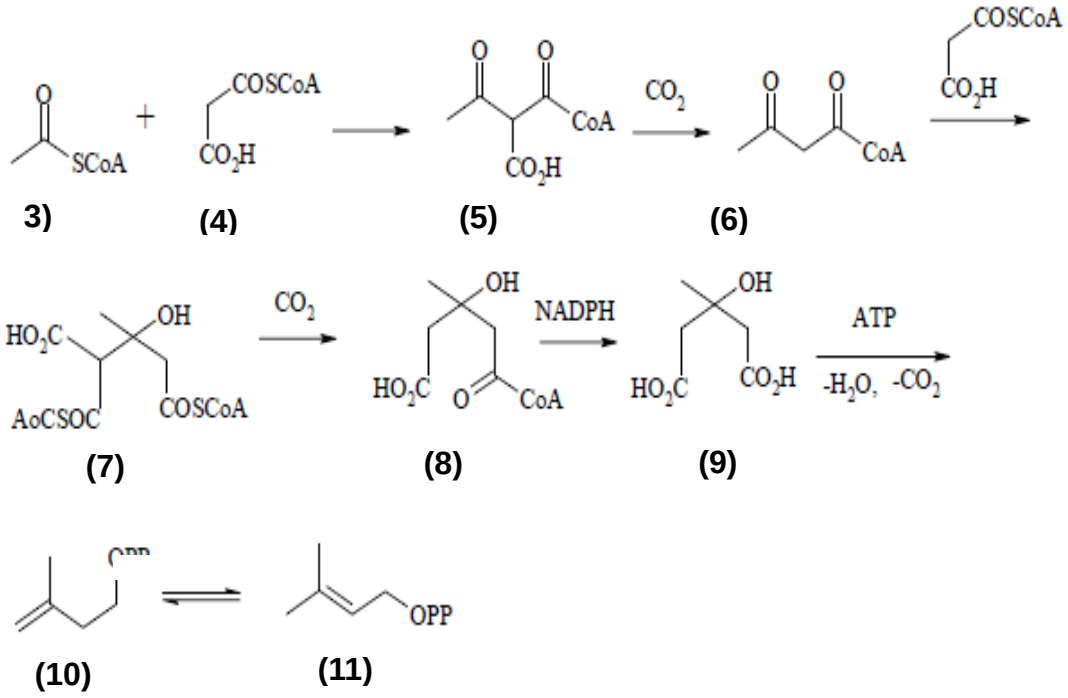


Fig 2: Biosynthesis of IPP from acetyl coenzyme A (Mann, 1978)

The intermediate compound IPP (**10**) is readily converted to monoterpenes by condensation with DMAPP (**11**) to give nerylpyrophosphate (NPP) (**12**) and geranylpyrohosphate (GPP) (**13**) (Fig 3). The cyclic, bicyclic and acyclic species are derived from NPP (**12**) and GPP (**13**) (Fig 3). When the phosphate group is eliminated, linalyl (**14**) and neryl (**15**) cations are formed. NPP, LPP and GPP are in isomerism. They form menthyl (α-terpenyl) cation which also forms

various cations. Neryl cation (**16**) gives the cyclic classes. Rationalization of many monoterpenoid skeletons is made possible through hydride shifts, internal additions and rearrangements

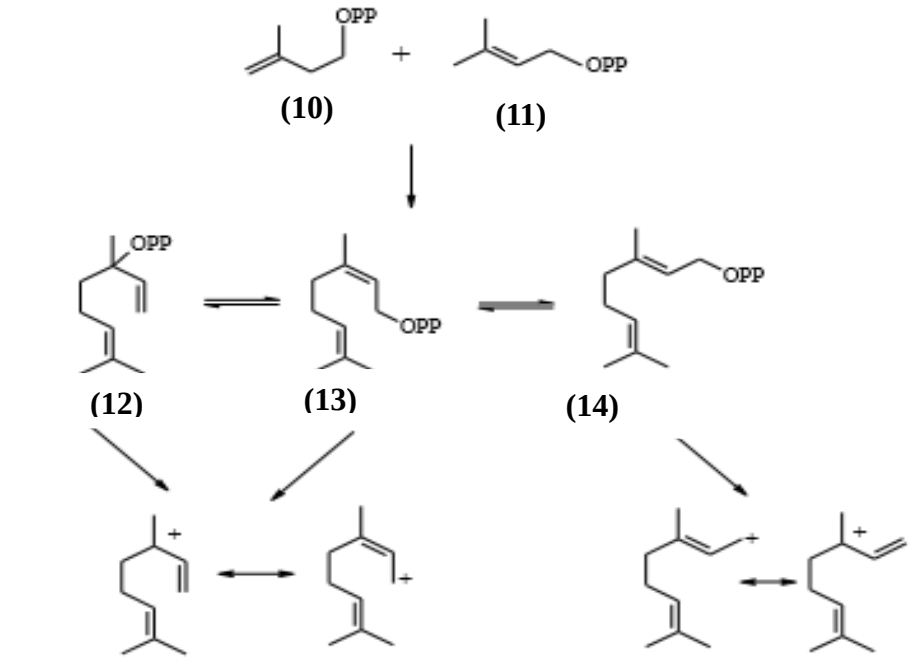


Fig 3: Formation of neryl pyrophosphate (NPP) and geranyl pyrophosphate (GPP) (McCaskill & Croteau, 1997; Fraga, 1998)

The biosynthesis of sesquiterpenes is shown in Fig 4. Sesquiterpenes are C_{15} hydrocarbons which arise from the cyclisation of 2*E*, 6*E*-farnesyl pyrophosphate (FPP) (17**)** with nerolidyl pyrophosphate (NPP) (**18**) and 2*Z*, 6*E*-FPP (**19**) which are in isomerism. Subsequent rearrangements results in farnesyl (**20**) and nerolydyl cation (**21**) (Bouwmeester *et al*., 1999; Mckaskill & Croteau, 1997). Cyclization followed by hydride shifts results in numerous sesquiterpene skeletons: farnesane (**22**), bisabolane (**23**), cadinane (**24**), humulane (**25**), caryophyllane (**26**), eudesmane (**27**), eremophillane (**28**), elemane (**29**), germacrane (**30**), bicyclogermacrane (**31**), carotaene (**32**), aromandrane (**33**) and guainane (**34**)

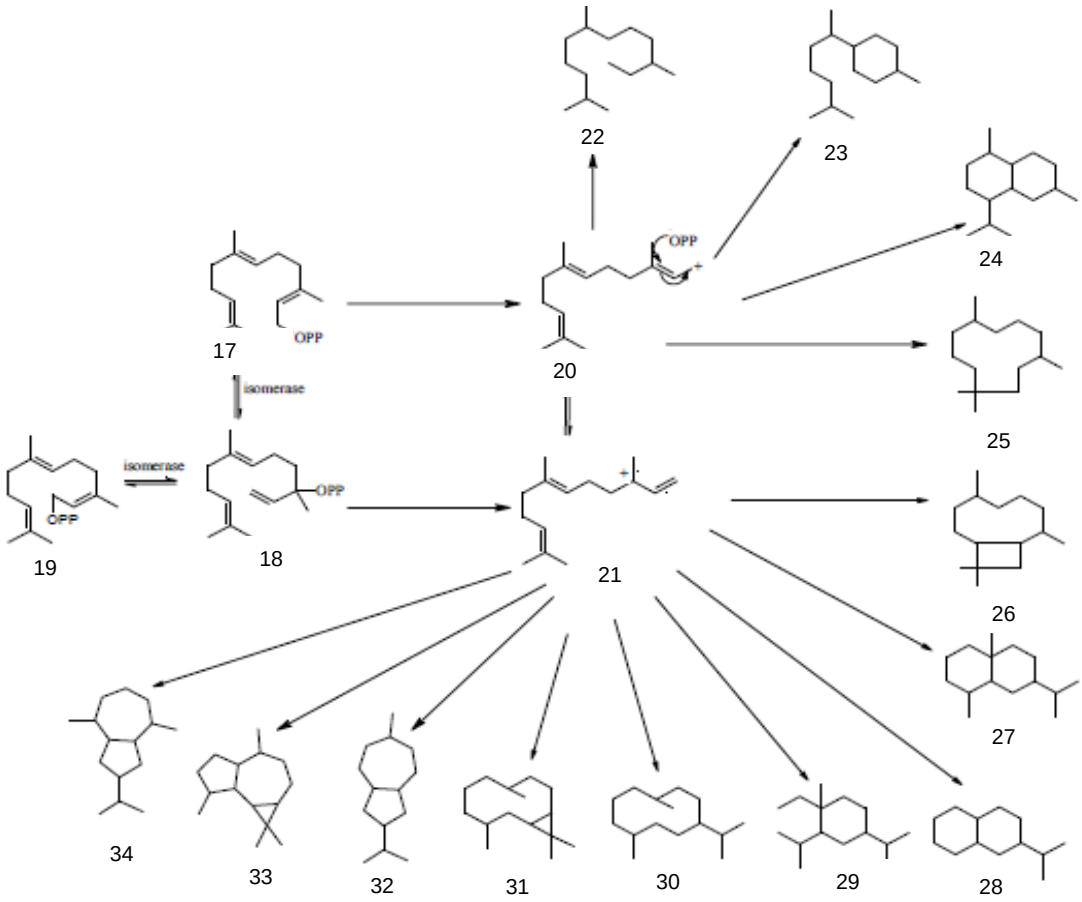


Fig 4. Biosynthesis of sesquiterpenes and homoterpenes (McCaskill & Croteau, 1997; Fraga, 1998).

Other important compounds previously implicated with the fragrance trait in rice included alkanals, alk-2-enals, alka-2, 4-dienals, 2-pentylfuran and 2-phenylethanol (Widjaja *et al*., 1996). Recently, while reporting the volatile chemistry of black rice (aromatic specialty rice popular in Asia), guaiacol, indoles, and p-xylene, in addition to 2AP, were reonsible for its unique flavour (Yang *et al*., 2008). Sensory panels observed that rice with lower protein content had higher levels of desirable sweet aroma/taste and lower levels of undesirable flavor attributes (Park *et al*., 2001; Champagne, 2008). The volatile flavor compounds responsible for the desirable sweet aroma in aromatic NERICA varieties have not been quantified.

2.3 Effects of ecological factors on plant secondary metabolites

Similar plant species growing under different environmental conditions show significant differences in the production and accumulation of secondary metabolites (Turtola *et al*., 2005). The plant-environment interaction induces plant-stresses mediated by synthesis of secondary metabolites as adaptive responses. Ecological factors known to influence plant secondary metabolites production include seasonal weather variations, edaphic factors of the site and storage (Champagne *et al*., 2008). Under induced stress conditions of drought, the total amount of terpenes and resin acids increased, simultaneously with a decrease in the growth of *Pinus sylvestris* and *Picea abies* seedlings (Turtola *et al*., 2005). Higher artemisin secretion (Brown, 2010) and flavonoid biosynthesis activation (Jaakola *et al*., 2004) have been observed, with increased temperatures. Conversely, more anthocyanin was produced with lower temperatures (Lin-Wang et al., 2011). In *Pinus elliotii*, monoterpene emission rates, particularly those of α - and β -pinene, myrcene, limonene and β -phellandrene, increased exponentially with an increase of temperature, between 20°C and 46°C. In black tea, the volatile compounds varied with location of production (Mahanta *et al*., 1988; Owuor *et al*., 1988; Robinson and Owuor 1992), cultivars (Owuor *et al*., 1990). Similarly, the overhead volatile compounds of tea varied with cultivars (Odak *et al*., 2016). Indeed, the unsaturated fatty acids in tea that produce green leaf volatiles also varied with region of production (Okal *et al.*, 2012; Owuor *et al*., 2011) and the precursors to non-volatile flavor compounds (Kwach *et al*., 2016) varied with location of production. However, the variations in the volatile flavor compounds in NERICA varieties due to agro ecological conditions within the Lake Victoria basin have not been documented.

2.3 Quality of aromatic rice as dependent on ecological sites

Prevailing environmental conditions of the cultivation site and crop management practices highly affect rice yield and aroma (Champagne *et al*., 2008). Site characteristics such as biotic and abiotic components influence aroma quality in different rice varieties. Reportedly, each variety performs best, with respect to quality traits, in its own native area of cultivation (Ali *et al*., 1991). Although some varieties can be cultivated widely without any effect on yield, the quality of resultant grains, especially aroma vary with geographical area of production (Champagne, 2004). Some aromatic varieties express their aroma only in specific areas while others may show variations in aroma content depending on environmental conditions (Champagne, 2004).

Day/night temperatures of 25/15 ºC during ripening result in better aroma of basmati rice (Bhattacharjee *et al*., 2002), while early transplanting diminishes the aroma (Ali *et al*., 1991). 2-AP concentration in Khao Dawk Mali 105 variety differed due to production location ranging from 87 to 532 ppb (Yoshihashi *et al*., 2004). The 2-AP concentrations in Hieri, Miyakaori and Sari Queen (Japanese aromatic cultivars) were higher in brown rice harvested early and ripened at a low temperature (Itani *et al*., 2004). The basmati and jasmine types had stronger aromas when harvested at the beginning of winter (Lorieux *et al*., 1996). High altitude and low soil moisture also increased 2-AP concentration in aromatic rice (Nakamura, 1998), the latter through an increased proline concentration - a substrate in 2-AP biosynthesis (Yang and Kao, 1999). For superior quality, cultivation at a cool temperature and high altitude, application of low levels of nitrogen, early harvesting, and drying at a low air temperature are recommended (Itani *et al*., 2004). Environmental factors such as cool weather during flowering and grain development; fertile soil, direct sowing, production on lighter soils and upland conditions, low soil moisture during grain filling, and manual deshelling were also observed to affect rice aroma (Singh *et al*., 1997). Soil factors affect rice quality traits presumably through the interaction of volatile nutrients with aroma related volatile compounds (Singh *et al*., 1997). The type and composition of the soil as one of the determinant factors in secondary metabolites composition and that of volatiles in particular has been reported in other plants (Sampaio *et al*., 2016). The growth of many plant species is severely depressed in poorly drained soils, greatly reducing crop and essential oil yields and affecting volatiles composition (Pang *et al*., 2007). Supplementation of soil with important nutrients increased volatile flavor compounds concentration although the separate addition of the same nutrients gave different results in the

yield and composition of the same oils (Hornok, *et al*., 2004). In farmers' perception, lighter soils and upland conditions favor the formation of aroma (Singh *et al*., 1997). Evidently, environmental characteristics of site of cultivation have a great bearing on the production of volatile compounds in plants and rice in particular. NERICA varieties have been introduced into Lake Victoria Basin and appropriate planting sites need to be determined that will maximize aroma to increase market appeal. Currently, it is not known how the aromatic qualities of NERICA varieties vary when grown in different agro-ecologies.

2.4 The influence of nitrogen and nitrogen regimes on rice aroma quality

Soil chemical composition especially the level of inorganic ions such as nitrogen during field production influence the eventual aroma of cooked rice (Rohilla *et al*., 2000). That NERICA varieties adapt well to difficult production environments (Oikeh *et al*., 2008) and low levels of farming inputs (Apaseku and Dowbe, 2013) cannot be gainsaid. High rates of nitrogen fertilizers application reduce the quality of rice including aromatic flavor (Itani *et al*., 2004). To obtain higher concentrations of 2-AP in aromatic rice, it is recommended that the crop be grown in a cool climate with relatively low levels of nitrogen fertilization and harvested earlier than ordinary cultivars (Itani *et al*., 2004). Aroma, softness, whiteness, stickiness and glossiness of cooked milled rice of Khao Dawk Mali 105 variety were deteriorated with increasing dosages of applied nitrogen (Suwanarit *et al*., 1996). Thus, soils low in nitrogen generally produced higher quality aromatic rice grains (Suwanarit *et al.,* 1996). Late nitrogen fertilizer application especially during flowering, affected the nutritional quality of paddy rice which has a bearing on the aroma of cooked rice (Perez *et al*., 1996). At the same time, split nitrogen applications were recommended for obtaining high paddy rice grain quality (Perez *et al.,* 1996). Although higher yield of NERICA varieties were reported in Western Kenya due to variation in nitrogen levels (Atera *et al*., 2007), it remains to be determined if such variations have a significant effect on volatile flavor compounds. However, the influence of nitrogen fertilization on the concentration of the concentration of volatile flavor compounds in NERICA varieties.

Nutritional composition of rice is highly affected by fertilization and cultural practices. For instance, amylose and protein contents of rice cultivars vary with nitrogen fertilizer application which in turn may influence the aroma and flavor of the cooked rice (Juliano *et al.,* 1965). Low protein content was recorded in rice samples of the same cultivar which were more flavored than those with higher protein content (Juliano *et al.,* 1965). This observation was corroborated by two descriptive sensory panels (Park *et al* 2001; Champagne *et al.,* 2004), that rice with

lower protein content had higher levels of desirable sweet aroma/taste and lower levels of undesirable flavor attributes. In 17 diverse cultivars grown over two crop years in one location, hay-like and sweet aromatic flavors were correlated positively and negatively respectively, with protein content (Champagne *et al*., 2004). Protein content of short grain cultivars milled to different degrees (8- 14%) correlated highly and positively with hay-like puffed corn, raw rice, and wet cardboard, and negatively with sweet taste (Park *et al*., 2001).

2.5 Effect of nitrogen on other growth parameters

The efficiency of fertilizer use in upland rice production systems is a factor of plant height, tiller number and dry matter content (Kore *et al*., 2007). Increase in yield components with increased nitrogen levels was observed in Malian upland rice varieties at different growth stages (30 days after transplanting (DAT), 45 DAT and at 60 DAT) (Haque *et al*., 2004). At maturity, the tallest plant, the highest number of tillers and the highest dry matter content were found where higher amount of nitrogen was applied (Haque *et al.,* 2004). However, excess nitrogen application made the crop succulent and enhanced lodging during grain filling stage (Sidhue *et al.,* 2004).

The number and length of panicles and the percentage of spikelet sterility increased with the increase of nitrogen levels (BRRI, 2002). Maximum grain number per panicle was also observed with application of 25 KgN/ha and beyond that rate, there was a decline due to lodging of the crop during grain filling (Kumar *et al.,* 2003). The highest number of grains per panicle was observed up to 60 KgN/ha (Kumar *et al.,* 2003). In a separate study, higher doses of nitrogen made the crop tall and increased vegetative growth that enhanced crop lodging during grain filling stage resulting into higher spikelet sterility (Moriwaki, 1999). Excess nitrogen application reduced carbohydrate content resulting into abnormal development of pollen grains (Sharma and Singh, 1999). However, no significant difference was found in 1000-grain weight due to the application of nitrogen (Shivay and Singh, 2003). The individual grain weight is usually a stable varietal character and the management practice has less effect on its variation (Yoshida, 1981). In a separate study vigorous crop growth for nitrogen treatments resulted in higher straw yields of fine rice (Salam *et al.,* 2004). The straw yield increased significantly with the nitrogen rates up to 75 KgN/ha. However, there was no significant difference in straw yield between 75 and 100 KgN/ha, but 100 KgN/ha showed higher straw yield than other levels of nitrogen. Influence of nitrogen rates on growth parameters of the NERICA varieties has not been studied. It is also not documented if the responses to nitrogen rates of these growth parameters vary with location of production.

2.6 Analysis of aroma by gas chromatography - olfactometry

One key objective of flavor research is to identify the potent aroma components of a food. The methods involved in characterization of key aroma compounds include isolation and separation using different techniques. These are followed by sensory evaluation as to qualify and quantify the derived data (Reineccius, 2000).

Isolation techniques used in flavor analysis include steam distillation-solvent extraction in which the volatile profile obtained is influenced by volatility of the aroma compounds during the concentration of the solvent extract (Reineccius 2006) and solubility during solvent extraction of the distillate. Headspace trapping is the most prefered of these methods (Knudsen *et al*., 1993; Dobson, 1991; Hills and Schutzmann, 1990) since it generates floral volatile profiles devoid of wound related compounds and extraction artifacts (Bergström *et al*., 1980; Mookherjee *et al*., 1990; Dobson, 1991). In dynamic headspace technique, scent compounds are concentrated in a small glass or plastic chamber and are swept by a flow of filtered air over a cartridge packed with adsorbent particles (Dobson, 1991; Bicchi & Joulain, 1990; Williams, 1983). In static headspace method, the adsorbent satchets hang on the floral parts without passing air. In the purge and trap method, the compounds with the highest vapor pressure are preferentially removed and trapped on Tenax depending on their polarity. Solid phase micro extraction (SPME) (Sides *et al*., 2000), is a fast technique (Flamini *et al*., 2003) that does not use a solvent, and is based on the adsorption of chemical compounds onto an extracting phase like polydimethylsiloxane (PDMS) on a fused silica fiber (Shang *et al*., 2001, Fernando & Grun, 2001). The adsorbed volatiles are thermally desorbed from the fiber in the injector port of GC. Solvent extraction, especially simultaneous distillation extraction (SDE) using the Likens-Nickerson's method (Sides *et al*., 2000), is a classical method that employs reduced pressure in order to reduce boiling point of flavor components and minimize thermally induced artifacts. Classical steam distillation allows the investigation of higher boiling point compounds, which may be relevant for the odor characteristics determination of the total concentration of the volatiles (Scheirer, 1984). It is practical for use even in laboratories with low technological endowment (Marsili, 2007). The SDE has the advantage that it is easy to use and several injections into the gas chromatography column may be made from one isolate (Sides *et al*., 2000). The procedure involves boiling the sample in water at atmospheric pressure making it suitable only for pre-boiled foods (Marsili, 2007). A major disadvantage of SDE is the need to remove some of the solvents from the isolate in order to produce a sample sufficiently

concentrated for analysis, inevitably resulting in losses of some volatile compounds. In this study, Lickens and Nickerson's apparatus, using a modified simultaneous distillation and extraction (SDE) method (Gu *et al*., 2009) was used in the extraction process to allow total extraction of the compounds, even those with high boiling points. This method closely mimicks how aroma is perceived during the rice cooking and preparation The resultant concentrate was then subjected to analysis by a gas chromatography-mass spectrometry (GC-MS) for identification of peaks, because key aroma compounds are usually volatiles or semi-volatiles (Ameenah, 1994; Flamini *et al*., 2002; Fernando & Grun, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study sites

Field trials were conducted on three NERICA varieties (1, 4 and 10) at three sites; KALRO-Kibos Research fields and Maseno University Botanic Garden in Kisumu County and KALRO-Oyani Research sub-station in Oyani County within the Lake Victoria Basin. KALRO-Kibos is located at an altitude of 1173 m above the sea level, longitudes of 34^{o} 24" 13' E and latitudes of 00^{o} 03" 01'S, within the lower midland ecological zone (LM 3). The site has heavy black clay soils (vertisols), fairly typical of the Kano plains (Jaetzold *et al.*, 2009). Mean annual rainfall data during the last 25 years is 1323 mm and temperature ranges between 16-31^{o}C (Jaetzold *et al*, 2009). Maseno University Botanical Garden lies within the upper midland agro-ecological zone (UM 1), at an altitude of 1500 m above sea level within longitudes 34^{o}25'47' E and latitudes $00^{o}1^{'}$N 0'12$^{'}$S. The area receives a bimodal mean annual rainfall of 1750 mm and a mean annual temperature of 28.7^{o}C (Mwai, 2001). The soils at the site are classified as acrisol/dystric nitosols, with pH ranging between 4.6 and 5.4 (Sikuku *et al*., 2010). These soils are reddish brown and well drained, deep-reddish brown and slightly friable clay (Mwai, 2001). KALRO – Oyani is located within the lower midland ecological zone (LM 2), at an altitude of 1138 m above the sea level, longitudes of 34^{o} 28" 00'E and latitudes of 01^{0} 04" 00'S (Jaetzold *et al.*, 2009). The soils at the site are classified as andosols. Such soils are moderately deep, firm, dark grey, brown, firm, clay soils in valley bottoms (Jaetzold *et al.*, 2009).

3.2 Field experiments

3.2.1 Soil sampling

Soil sampling was done at the selected experimental sites. At each site, four replicate samples were collected within an area of 10 m^2 by randomly sampling four corners within the experimental site. One kilogram of soil was collected from a depth of 5 - 20 cm using a shovel. To avoid cross contamination, the shovel was sprayed in between sampling with 5% sodium hypochlorite solution in a wash bottle, rinsed with water three times and dried using a sterile cloth. The soils were placed in 1 Kg brown paper bags and put in bucket containers away from sunlight for preparation for chemical analysis.

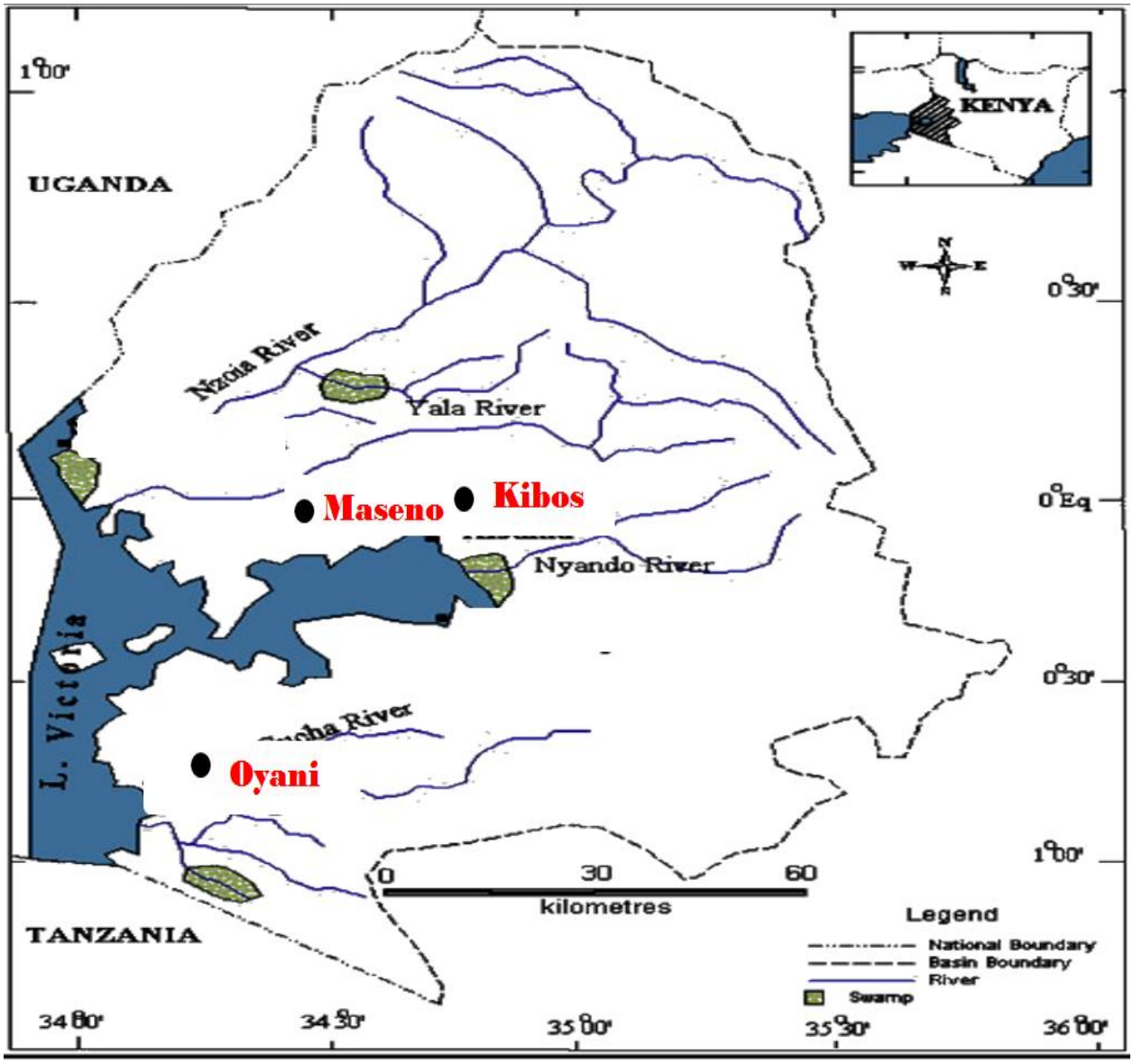


Fig 5. The experimental sites in Lake Victoria basin.

3.2.2 Chemical analysis of soil samples

The soil samples were air dried, crushed using a wooden roller and passed through a 0.5 cm mesh to remove any plant or grass fragment. Four hundred grams of the prepared soil samples in quadruplicates were placed in brown paper bags, tightly sealed and submitted to KALRO's National Agricultural Research Laboratories in Nairobi for soil chemical analysis.

3.2.2.1 Determination of soil pH

Soil pH was determined using the $CaCl_2$ method (Thomas, 1996). To a soil sample weighing 10 g, 25 ml of 0.01M $CaCl_2$ solution was added. The mixture was shaken on a shaker for 30 minutes. Prior to taking the pH reading from a pH meter, the electrode was thoroughly rinsed to

free it of buffer solution. The suspension was stirred up and the electrode immersed into the suspension making sure it did not touch the base of the beaker. When the pH reading was stable, the displayed value was recorded.

3.2.2.2 Determination of soil organic carbon

A modified Walkley-Black dry combustion method was used to determine the percentage of organic carbon in the soil samples (Anderson and Ingram, 1983). To prepare a working standard, about 15 g of sucrose was dried at 105^{o}C for 2 hrs and 11.87 g of it dissolved in water. The solution was made up to 100 ml in a volumetric flask, to make a 50 mg C ml^{-1} solution. Twenty-five mililitres of the solution were pipetted into labeled 100 ml volumetric flasks, and made to the mark with deionized water. Two milliliters of each of these working standards were pipetted into labeled 150 ml conical flask and dried at 105^{o}C in an oven. One gram of air-dried soil sample was weighed into 150 ml flask to which 10 ml of 1N $K_2Cr_2O_7$ was added and the contents were gently swirled until the sample was completely wet. This was followed by the addition of 20 ml of concentrated H_2SO_4 with further swirling to ensure through mixing of the solution in a fume cupboard. After cooling the solution, 50 ml of 0.4 % $BaCl_2$ was added. The solution was left to stand over-night and the absorbance of the samples and the standards were read at 600 nm on UV visible spectrophotometer (Spectronic-20-Bausch and Lamb).

3.2.2.3 Determination of exchangeable cations

Total exchangeable cations were determined following extraction using 1 M acidified ammonium acetate (Lindsay and Norvell, 1978). Air-dried soil samples weighing 10 g were put in 150 ml plastic containers to which 40 ml of I M ammonium acetate were added. The containers were tightly closed and put on a reciprocating shaker (Stuart Flask model) for 1 hr. The solution was then filtered through a Whatman number 125 filter paper into 250 ml flasks. The remaining soil was washed using 1 M ammonium acetate making the volume in the collecting flask to about 90 ml. Fresh 1 M ammonium acetate was used to raise the mark to 100 ml. After leaching the bases, potassium (K) and sodium (Na) emission were read on a flame spectrophotometer with the filter set on either K or Na while magnesium (Mg), manganese (Mn) and calcium (Ca) absorption were read on an atomic absorption spectrophotometer (AAS) model SpectrAA50 using the respective lamps (Thomas, 1982) and the peaks evaluated against the Inductive Coupled Plasma (ICP) standards (Jones, 2001).

3.2.2.4 Determination of available phosphorus

Soil available phosphorus (P) was measured using the Olsen method (Olsen *et al*., 1954). An air-dried soil sample weighing 2.5 g was placed into 250 ml polythene shaking bottle followed by addition of Olsen's extracting solution (0.5 M $NaHCO_3$ at pH 8.5). The mixture was put on a shaker for 30 minutes and the suspension filtered through Whatman No. 42. Five mililiters of 0.8 M boric acid and 10 ml of ascorbic acid reagent were added and allowed to stand for 1 hr. The P content was determined colorimetrically from a phosphorus molybdate complex formed by addition of acidified ammonium molybdate (Okalebo *et al*., 2002).

3.2.2.5 Determination of total nitrogen

Total soil N was determined using the semi-Kjeldhal method (Anderson and Ingram, 1983). An air-dried soil sample weighing 0.5 g was digested using a digestion solution made of Kjetab (1.72 g). An acid resistant 5 ml pippete was used to add 3.5 ml of concentrated sulphuric acid. The mixture was placed on a pre-heat alluminium digester at 360 ^oC for 2 hrs. Three reagent blanks were included to each batch. The mixture was allowed to cool and 25 ml of distilled water added. A further 75 ml of distilled water was added and allowed to settle. Standards were prepared by oven drying 7 g of ammonium sulphate [$(NH_4)_2SO_4$] at 105^oC for 2 hrs. About 4.714 g of dry ammonium sulphate was dissolved in 1000 ml of deionized water (1000 mg N/litre) and 50 ml of the solution was pipetted into 500 ml volumetric flask. Two and a half mililitres of the digested blank was added to each flask before marking it, to the mark with distilled water. Total nitrogen was determined on a Flow Injection Analyzer (Jones, 2001).

3.2.3 Planting site preparation

At each site, a research field of 9.5 m by 32 m was selected. Field preparation was done through manual groundbreaking after clearing and burning the debris, followed by one plough and one harrowing using disc plough. The plot areas were leveled using a rake and left for 2 weeks and prepared to a fine tilth.

3.2.4 NERICA seed treatment and planting procedure

Seeds of NERICA 1, NERICA 4 and NERICA 10 were obtained from KARLO - Kibos station. The seeds were treated with Apron star 42 WS (thiamethoxam + difenoconazole + metalaxyl–m) at the rate of 1 sachet 10g/Kg of seed 2-3 days before planting. Planting consisted of opening up of the spots to dibble in the filled NERICA seeds of each variety at the rate of 50 Kg/ha, at a spacing of 25 cm × 15 cm.

3.2.5 Field experimental design and layout

Field experiments at each of the three sites (Plates 1-3) were arranged in a split plot with nitrogen fertilizer levels as the main plots and the NERICA varieties as sub plots (Fig. 6). Experimental sub plots measured 2.1 m by 2.5 m, separated by 0.5 m wide footpaths, while 1 m wide footpaths between the treatments were used. Replication was done three times at each site. Four inorganic nitrogen fertilizer regimes (20 KgN/ha, 60KgN/ha, 100 KgN/ha and 140 KgN/ha) were broadcast in form of Calcium Ammonium Nitrate (CAN) in two split doses at the rate of half a dose at 21 days (tiller initiation stage) and the next half at 42 days (maximum tillering stage) post emergence.

3.3 Collection of agronomic data

3.3.1 Chlorophyll content

A flag leaf was selected per hill and used to determine the chlorophyll content of the NERICA varieties due to N-fertilization. Measurement was taken at weeks 6 8, 10 and 12 post-emergence (Sikuku *et al*., 2007). A Minolta SPAD-502DL (Konica Minolta Optics Inc. Korea) hand-held meter was used for non-destructive data collection. Leaf transmittance was taken in the wavelength of 600 – 700 nm. The flag leaf was gently pressed using the SPAD meter until a consistent reading was observed. This was done on 12 plants in each plot and average values recorded in arbitrary SPAD units.

3.3.2 Plant height

Four hills in each plot were randomly picked and marked for evaluation of plant height. A 1-meter ruler was vertically held from the soil level to the tip of the tallest plant in the hill and the height obtained in centimeters. Measurement was taken at weeks 2, 4, 6, 8, 10 and 12 post-emergence (Sikuku *et al*., 2007). Results obtained were averaged and recorded.

3.3.3 Number of tillers

In each plot, four hills were randomly identified and marked at the onset of the experiment and used to determine tiller numbers fortnightly from 28 days post emergence. Number of tillers were counted on each hill and the four values averaged, and represented in whole numbers.

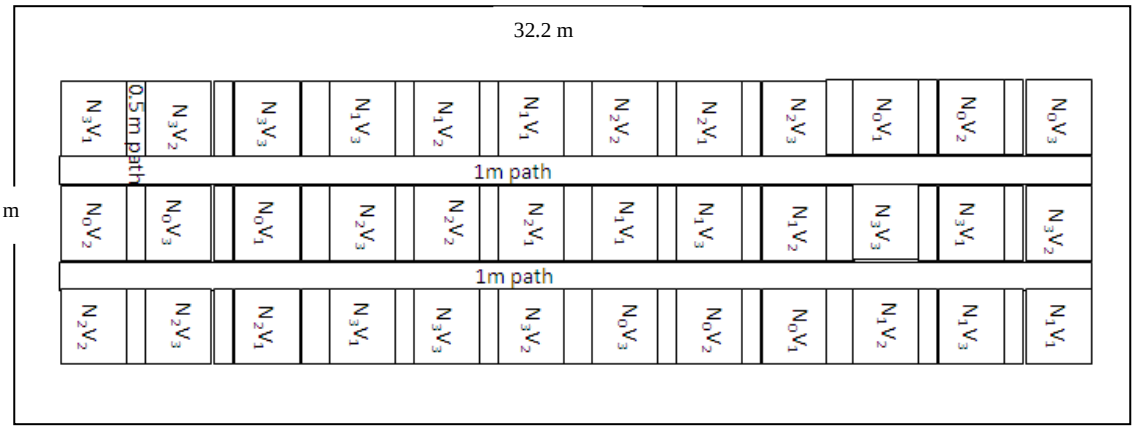


Fig 6. Field layout of the trials on NERICA 1, 4 and 10 at varying nitrogen fertilizer rates

KEY: N_0 – 20 KgN/ha; N_1 – 60 KgN/ha; N_2 - 100 KgN/ha; N_3-140 KgN/ha
V_1 – NERICA 1, V_2 – NERICA 4, V_3 – NERICA 10

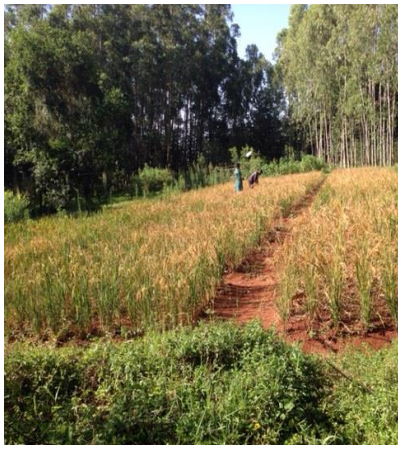


Plate 1. Research field at Maseno University, Kisumu County.

Plate 2. Research field at KALRO Kibos, Kisumu County.

Plate 3. Research field at KALRO Oyani, Migori County.

Plate 4. NERICA 1 grains

Plate 5. NERICA 4 grains

Plate 6. NERICA 10 grains

3.4 Sampling of rice grains for volatile flavor compound analysis

Sampling of rice grains (Plates 4-6) for volatile flavor analysis was done when the crop was mature and completely browned. Sampling was done manually using a sickle by cutting the panicle and placing the harvested crop in an upright position to dry before threshing. The grain samples were sun dried on a concrete floor for 6 hours a day for 7 days with 3 turnings per day to reduce the moisture content to 14% for quality storage awaiting analysis. This was followed by threshing by hand and winnowing using traditional baskets to separate chaff from the grains. The samples were bulked by putting together each variety under specific nitrogen treatment from each of the three replications. Bulking was done to obtain a manageable number of samples for analysis. Each bulked sample was individually finely ground using mortar and pestle and weighed using an electric balance into 50 g portions, stored in zip-lock bags and placed in a well labeled air-tight plastic container in a refrigerator at 5 °C until further testing.

3.5 Extraction and analysis of volatile flavor compounds

3.5.1 Extraction of volatile compounds

A modified Likens-Nickerson apparatus was used for extraction through the simultaneous steam distillation and solvent extraction method (Terashi and Kint, 1993). A sample of 50 g was crushed and weighed on an electronic balance, then poured into a 500 mL round bottomed flask. Five milliliters of silicon antifoam/antibump (Sigma Aldrich) were mixed with 6 litres of distilled water and boiled for 2 hours to obtain a volatile free mixture. Two hundred milliliters of the water-antifoam mixture were transferred into a 2.0000 quick fit FR500/4S 500 ml round bottomed flask containing 50 g of the sample, 2.5 ml of n-Hexane (99% Sigma Aldrich) and 125 µL of ethyl decanoate (99% Sigma Aldrich), as an internal standard. The mixture was bolied for 1 hour in a modified Lickens and Nickerson apparatus. From time to time, the organic volatiles

from the mixture was condensed and accumulated in the U-tube of the apparatus, from where they were tapped into a clean, dry, 2 ml glass vial. The organic layer was dried with anhydrous sodium sulphate (Na_2SO_4) to remove any traces of water, transferred into a clean 2 ml glass vial and the sample kept refrigerated at -80 ^{0}C for further analysis.

3.6 Volatile analysis and identification

Both GC and GC-MS were used for analysis. The GC was performed on Shimadzu model GC-2010 equipped with a flame ionization detector. A 50 m silica gel capillary column (film thickness 0.2µm and 0.25mm inner diameter) was used. Oven temperature was programmed from 35 ^{0}C to 250 ^{0}C, followed by 5 min hold at 230 ^{0}C. The flow rate of the carrier gas nitrogen was 3.0 ml/min and detector gases 40.0 ml/min (hydrogen) and 400.0 ml/min (air) respectively. The detector temperature was set at 230^{0}C. A sample volume of 1µl was injected in a split-less mode. Analysis on GC-MS was conducted using an Agilent 7890A Series Gas Chromatograph coupled to a Mass Specrtometer Agilent 5973 quadruple detector). The gas chromatographic conditions were as follow; helium was used as a carrier gas at a flow rate of 1.2 ml/min, inlet temperature of 270^{0}C, transfer line temperature of 280^{0}C and column (250µm film thickness and 0.25mm internal diameter). Oven temperature was programmed from 35^{o}C for 5 minutes, then 10^{o}C /minute to 280 ^{o}C for 10.5 minutes. Parameters for electron impact sample ionization were as follow: mass selective detector maintained at an ion source temperature of 250^{0}C and quadruple temperature of 180^{0}C; electron energy, 70 eV; source temperature of 250^{0}C. Fragment ions were analyzed at 40-550 m/z mass range in the full scan. The compounds were quantified as ratio of the peak area of each compound to that of ethyl decanoate (internal standard) (Baruah *et al*., 1986). Identification of the compounds was done by comparing the fragmentation patterns with mass spectral data in mass spectral library (NIST/EPA/NIH., 2008) and literature. Confirmation of compounds was done by injecting samples with VOCs authentic stanadards which led to the enhancement of peaks being analyzed at a particular time. The authentic standard was then analysed alone. Formation of peaks with the same retention time and fragmentation pattern confirmed the identity of the compound being analysed.

3.7 Data analysis

Data from the field experiments and laboratory analysis were organized into a matrix and subjected to two and three-way analysis of variance (ANOVA) using MSTAT-C and where significant, means were separated using Least Significant Difference at [$LSD_{5\%}$] and percentage

coefficient of variation calculated. The percentage variation of each compound was obtained as a percentage of the quotient of standard deviation and mean.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Chemical analysis of soil samples

The chemical properties of soil samples from the three study sites are shown in Table 3. Kibos site had a near neutral pH of 6.51 which differed with the acidic pH of Maseno site (3.76) and the moderately acidic pH of Oyani site (5.07). Soil pH influences the uptake of inorganic nitrogen by most plants including rice. Optimum soils for NERICA varieties are within pH ranges of 5.5 – 6.5 (Oikeh *et al*., 2008). Soils from Kibos with an average pH of 6.5 were in the optimum range while Maseno and Oyani had acidic and slightly acidic soils, respectively. Thus, Kibos soils have a potential for increased yield of NERICA varieties.

The soil nitrogen values ranged between 0.17 - 0.21 % for all sites and were deficient in nitrogen for optimal NERICA production (Nwilene *et al*., 2008). Less than 1.0 % of nitrogen may not support proper plant growth and such soils need amendments (Nwilene *et al*., 2008). For rice production, soil nitrogen content of 1% per kilogram of nitrogen is considered optimal. In Lake Victoria basin, there is a high variability in soil nitrogen (Jaetzold *et al*., 2007), although most soils have inherently low nitrogen (Ojiem *et al*., 2001). The NERICA varieties may require additional nitrogen fertilization to optimize production under rain fed systems (Ronoubigouwa, 2008). Low input farmers who have adopted NERICA cultivation in Lake Victoria basin need to apply nitrogenous to fertilizer to remedy the deficiency.

Available phosphorus levels varied with soils from Kibos having a high value of 289.00 ppm compared to Maseno and Oyani, which ranged from 8 to 20 ppm. These low levels of available phosphorus were attributed to fixation due to the acidic nature of the soils (Bambara and Ndakidemi, 2010). Levels below 25 ppm phosphorus per kilogram of soil are inadequate for NERICA production (Oikeh *et al*., 2013) since it serves the plant in root development, tillering, early flowering and ripening (IRRI, 2002). Maseno and Oyani soils were therefore suitable in terms of phosphorus levels for NERICA production. Potassium is required in large amounts of more than 0.1% of the plant's dry weight for root growth and plant vigour (IRRI, 2002). Potassium levels of 0.21 – 0.78 meq % for Maseno and Oyani, and 1.11 meq % for Kibos were therefore inadequate. Microelement composition of the soils varied as follows; Ca (4.90 meq % for Kibos and a range of 1.40 – 3.00 meq% for Maseno and Oyani); Mg (5.17 meq % for Kibos and a range of 2.87 – 0.93 meq % for Maseno and Oyani); Mn (0.45 meq % for Kibos and a

range of 0.81 – 0.67 meq% for Maseno and Oyani); Cu (1.14 meq % for Kibos and a range of 2.03 – 3.90 meq % for Oyani and Maseno); Fe (17.20 meq % for Kibos and a range of 21.30 – 23.00 meq % for Maseno and Oyani); Zn (3.72 meq % for Kibos and a range of 2.40 – 5.42 meq % for Maseno and Oyani) and Na (0.32 meq % for Kibos and 0.08 – 0.18 meq % for Maseno and Oyani). All microelement levels were below optimum for rice production (IRRI, 2007) except the levels of iron. Deficiency of microelements in the soil hinder root growth and development, cause leaf chlorosis, irregularity in soil pH, increase iron toxicity, and increase convertion of nitrate to nitrites (IRRI, 2011). This has a great bearing on rice growth and crop productivity. The major cause of low microelement is continual removal through subsequent cropping without fertilization or organic matter cycling (Okalebo *et al*., 2002). Yet, microelements influence the intake of important plant growth enhancing compounds such as nitrates and phosphates (IRRI, 2011). At Oyani and Maseno, the levels of calcium, potassium, and magnesium in the soils were below the recommended rates for optimum growth in the NERICA (IRRI, 2011). Therefore, the levels of copper and iron were higher in Oyani and Maseno than in Kibos soils. Negative effect of heavy metals on plant metabolism include stunted growth, long duration of growth and impaired flower and seed setting (Tisdale *et al*., 1993). The high levels of heavy metal in Maseno and Oyani could cause slow growth, poor tillering and low chlorophyll content in these sites, while Kibos soils showed potential for better tillering and chlorophyll content, indicating that the soils had potential for high yield.

The pH values, percentage nitrogen and available phosphorus at Kibos were within recommended ranges (IRRI, 2011) for optimal growth and production of NERICA varieties. However, Maseno and Oyani soils may require soil ammendments through liming (Ojiem *et al*., 2001) and fertilization (Oikeh *et al*., 2013) to optimize production of the NERICA varieties.

Table 3. Variation in soil chemical status of the three sites.

Site	pH	N (%)	Org. C (%)	Av. P (ppm)	K (meq)	Ca (meq)	Mg (meq)	Mn (meq)	Cu (ppm)	Fe (ppm)	Zn (ppm)	Na (meq)
Kibos	6.51	0.21	2.08	289.0	1.11	4.90	5.17	0.45	1.14	17.20	3.72	0.32
Maseno	3.76	0.16	1.57	8.00	0.21	1.40	0.93	0.81	3.90	23.00	5.42	0.08
Oyani	5.07	0.21	2.04	20.00	0.78	3.00	2.87	0.67	2.03	21.30	2.40	0.18

NOTE: Values show averages of seven sampling points per site. Av – available.

4.2 Agronomic performance of NERICA varieties

4.2.1 Plant height

4.2.1.1 Effect of varieties on plant height

Variations in plant height of NERICA 1, 4 and 10 varieties are shown in Fig. 7 and Table 4. An average height of 44.0 cm was observed in NERICA 4 plants in week 6, which was significantly ($p \leq 0.05$) taller than 42.90 cm and 38.69 cm for NERICA 1 and 10 respectively. A similar trend was observed in Week 12, in which NERICA 4 produced significantly ($p \leq 0.05$) taller plants (82.58 cm) compared to 78.53 cm and 75.7 cm obtained from NERICA 1 and 10 respectively. NERICA 4 performed better than NERICA 1 and 10 making it a preferable variety in the Lake Victoria basin. NERICA 4 grows faster and shows weed competitiveness, an indicator of a potential high yielding variety. Varietal responses of NERICA to different growing conditions were previously attributed to inherent genetic traits and prevailing environmental conditions (Kore *et al*., 2007). In particular, varietal plant height enhancement in rice has been attributed to genetic improvements through classical breeding (Yu *et al*., 2012). In this study, NERICA 4 performed better than NERICA 1 and 10 in terms of plant height, an important yield enhancing trait (WARDA 2008). The variety had taller plants in weeks 6 and 12, which may be attributed to its inherently longer inter-nodal lengths (Ashrafruzzamann *et al*., 2009; Krishnan *et al*., 2011). A study conducted in Alupe within Lake Victoria basin showed that amongst the upland cultivars recently introduced into the cropping systems, NERICA 4 registered the highest panicle length at all stages of growth (Sikuku *et al*., 2016). Indeed, the maximum height of 82.58 cm achieved by NERICA 4 in Week 12 in the current study is within the IRRI index of intermediate group of rice plants (BI-IRRI-WARDA, 2007), and compared favorably with previous work on rain-fed upland NERICA varieties (Fukuta *et al*., 2012; Anyang *et al*., 2015). Plant height is a selective morphological marker in rice breeding which modifies yield enhancing characters and finally shapes grain quality (WARDA 2008). For instance, lush growth of rice plants with great heights result in better inflorescence setting, eventually producing larger grains with higher proximate nutrient concentration (Sarkar *et al*., 2014). Taller rice varieties have a competitive advantage over weeds which results in higher grain biomass (Anyang *et al*., 2015). Compared to other rain-fed upland varieties, NERICA 4 have erect leaves that allow good light penetration deep into the canopy (Fischer *et al*., 2001), a higher panicle production and greater ability to partition photosynthates for plant development (Oikeh *et al*., 2008). NERICA 4 had taller plants, although this was significantly different in Weeks 6 and 12. Thus NERICA 4 may have a better growth potential in Lake Victoria basin soils.

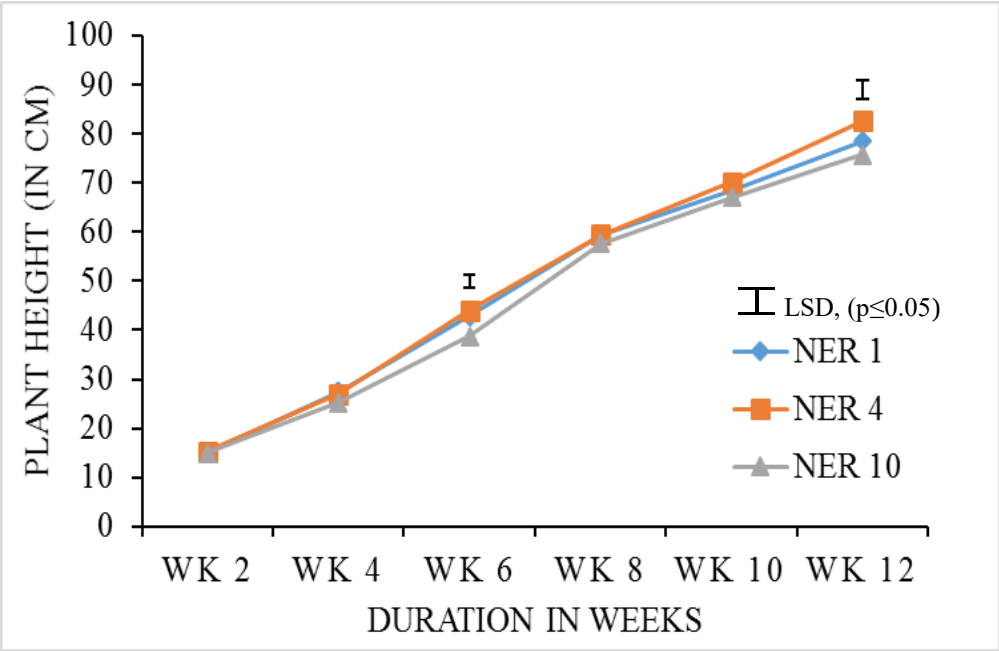


Fig 7. Effect of varieties on plant height (cm)

4.2.1.2 Variation in plant height with nitrogen fertilizer rates

Although nitrogen fertilizers are key inputs for increased rice yields (Alam *et al*., 2009; Dastan *et al*., 2012), variation in nitrogen levels did not cause a significant difference in plant height (Fig 8) except in Week 12. A rate of 140 KgN/ha was significantly higher in week 12, indicating a late response of the NERICA varieties. In Marakwet District in Kenya, NERICA varieties showed no significant difference in plant height even upto 56 DAS, although a significant difference was observed at maturity (Kinyumu *et al*., 2009). The results were however contrary to previous studies where aromatic paddy rice varieties showed significant increases in plant height with increase in nitrogen rate (Awan *et al*., 2011; Ghanbari-Malidarreh *et al*., 2009; Rahman *et al*., 2007). Among the factors of rice production, fertilizers play an important role and higher productivity requires increased nutrient inputs (Linquist *et al*., 2001). The amount of nitrogen required and its management varies depending on variety, soil conditions, cultural practices, crop rotations, and other factors (Linquist *et al*., 2001). Plant height modifies yield enhancing characters and finally shapes grain quality (WARDA 2008). Thus, nitrogen being a primary nutrient in plant metabolism, positively influences plant growth at later stages of development. Evidently, high levels of fertilizer (140 KgN/ha) at later stages of growth is suitable for enhanced plant height in the NERICA varieties.

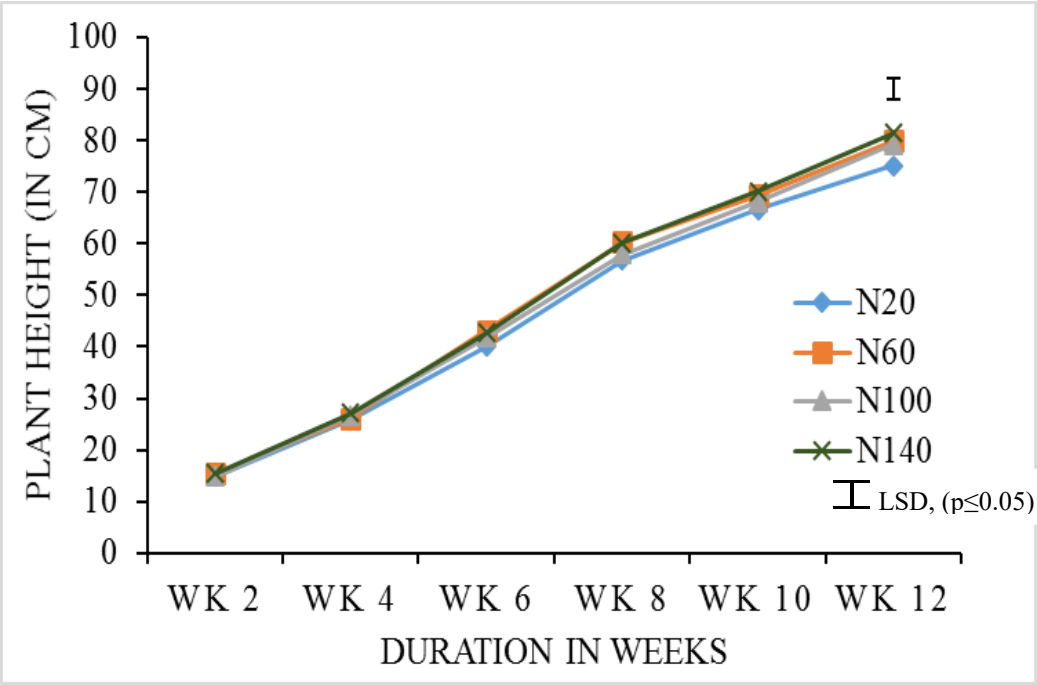


Fig 8. Effect of N-rates on plant height (cm)

4.2.1.3 Effect of sites on plant height

Plant height varied ($p \leq 0.05$) with site of production throughout the growing season (Table 4, Fig. 9). Kibos site had significantly ($p \leq 0.05$) taller plants while the shortest plants were obtained from Maseno. Agro-ecological factors which contribute immensely to rice plant height include soil chemical status, soil moisture and prevailing environmental conditions (Pascual *et al*., 2016). Results of chemical analysis of soil samples from the three sites (Table 3) revealed variation in macro-elements such as nitrogen, phosphorus and potassium. The highest plant height of NERICA varieties at Kibos site could be attributable to available phosphorus level, pH, calcium and nitrogen levels which were within the recommended rates for NERICA cultivation (IRRI, 2011). Inherently high soil phosphorus at Kibos significantly influenced overall height of the NERICA plants. Secondly, since most nutrients produce the best effects under a balanced nutrition, the optimal levels of P and N as exhibited in the Kibos soils may have resulted in better plant growth as observed in taller plants. Lastly, at Oyani and Maseno study sites, the levels of Ca, K, and Mg in the soils were below the recommended rates for optimum growth (IRRI, 2011). Soil acidity is a main hindrance to availability of inorganic nutrients such as nitrogen, calcium, magnesium and molybdenum. These nutrients influence the plant growth (Bambara and Ndakidemi, 2010), which may have negatively influenced plant height at Maseno and Oyani. Rainfall regimes and environmental temperature positively influence overall plant growth (Pascual *et al*., 2016). NERICA varieties, cultivated under rain-

fed conditions require even distribution of rainfall (IRRI, 2011). These conditions may not have been optimum during the field trials in this study (Appendix 3a-f). Kibos site experienced longer duration of rainfall days (118) with a total rainfall of 1211.9 mm which may have led to taller plants, relative to Maseno and Oyani. Kibos site is therefore potential for increased rice production.

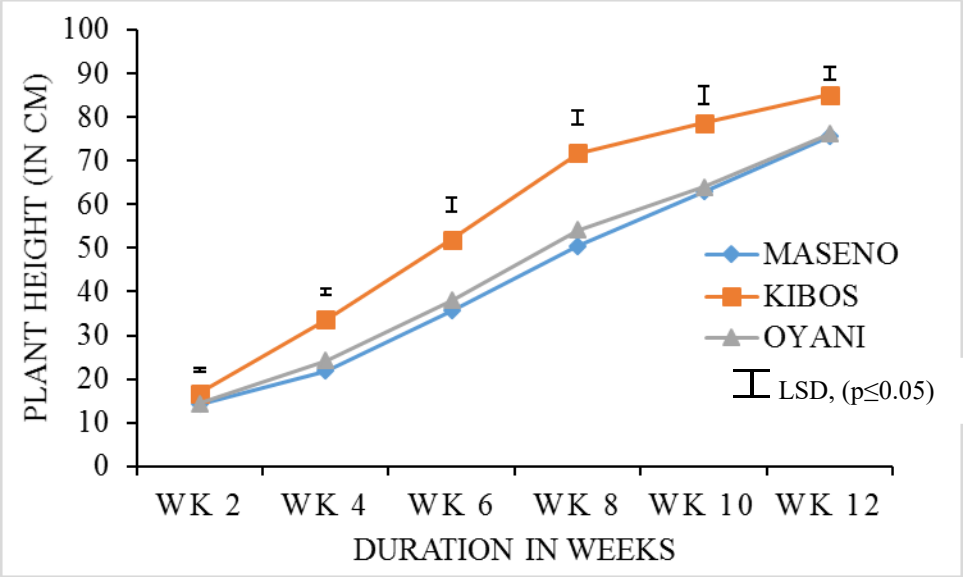


Fig 9. Effect of sites on plant height (cm)

Table 4. Effect of site, N-rate and NERICA varieties on plant height (in cm) during Weeks 2 - 12

WEEK	SITE	VAR (NERICA)	N-RATES 20	60	100	140	VAR MEAN	SITE MEAN	C.V %
WEEK 2	MASENO	1	14.19	14.11	13.33	14.52	14.04		
		4	14.43	13.21	14.13	15.41	14.29	14.20	8.41
		10	14.04	13.75	13.88	15.42	14.27		
		MEAN N	14.22	13.69	13.78	15.11			
		$LSD_{p\leq0.05}$			NS		NS		
	KIBOS	1	13.58	17.50	17.00	17.90	16.49		
		4	16.77	19.56	15.83	16.03	17.05	16.73	15.01
		10	17.12	17.48	15.52	16.48	16.65		
		MEAN N	15.83	18.18	16.12	16.80			
		$LSD_{p\leq0.05}$			NS		NS		
	OYANI	1	14.58	14.90	15.15	15.34	14.99		
		4	14.18	13.92	15.19	14.14	14.35	14.44	7.21
		10	14.18	14.08	13.75	13.87	13.97		
		MEAN N	14.31	14.30	14.70	14.45			
		$LSD_{p\leq0.05}$			NS		NS		
	ALL SITES	1	14.12	15.50	15.16	15.92	15.17		
		4	15.13	15.56	15.05	15.19	15.23	15.12	12.06
		10	15.11	15.10	14.38	15.25	14.96		
		MEAN N	14.79	15.39	14.86	15.45			
		$LSD_{p\leq0.05}$			NS		NS	0.88	
WEEK 4	MASENO	1	23.11	23.24	21.31	22.57	22.56		
		4	24.60	21.67	20.36	24.63	22.81	21.82	10.03
		10	19.57	20.09	19.50	21.20	20.09		
		MEAN N	22.43	21.67	20.39	22.80			
		$LSD_{p\leq0.05}$			NS		1.85		
	KIBOS	1	32.33	32.91	36.71	34.79	34.18		
		4	34.44	31.33	36.79	35.23	34.45	33.58	9.32
		10	32.81	33.00	29.54	33.12	32.12		
		MEAN N	33.19	32.41	34.34	34.38			
		$LSD_{p\leq0.05}$			NS		NS		
	OYANI	1	23.60	25.63	27.17	26.28	25.67		
		4	21.21	23.53	26.24	23.98	23.74	24.27	10.07
		10	22.91	24.50	23.48	22.72	23.40		
		MEAN N	22.57	24.55	25.63	24.33			
		$LSD_{p\leq0.05}$			NS		NS		
	ALL SITES	1	26.34	27.26	28.39	27.88	27.47		
		4	26.75	25.51	27.80	27.95	27.00		11.3
		10	25.10	25.86	24.17	25.68	25.20		
		MEAN N	26.06	26.21	26.79	27.17			
		$LSD_{p\leq0.05}$			NS		NS	1.46	

WEEK	SITE	VAR (NERICA)	N-RATES 20	60	100	140	VAR MEAN	SITE MEAN	C.V %
WEEK 6	MASENO	1	36.40	41.05	35.45	33.68	36.64		
		4	39.93	35.90	32.92	40.60	37.34	35.64	12.67
		10	31.43	34.98	31.18	34.20	32.95		
		MEAN N	35.92	37.31	33.18	36.16			
		$LSD_{p\leq0.05}$			NS		NS		
	KIBOS	1	51.64	49.10	52.23	54.78	51.94		
		4	53.05	56.42	58.82	54.12	55.60	51.99	8.81
		10	45.63	52.14	46.32	49.61	48.42		
		MEAN N	50.10	52.55	52.46	52.83			
		$LSD_{p\leq0.05}$			NS		3.81		
	OYANI	1	34.04	40.98	42.43	42.99	40.11		
		4	34.75	39.71	41.04	40.71	39.05	37.95	11.02
		10	31.61	38.17	35.81	33.16	34.68		
		MEAN N	33.46	39.62	39.76	38.95			
		$LSD_{p\leq0.05}$			4.09		3.54		
	ALL SITES	1	40.69	43.71	43.37	43.82	42.90		
		4	42.58	44.01	44.26	45.14	44.00	41.86	13.11
		10	36.22	41.76	37.77	38.99	38.69		
		MEAN N	40.10	43.16	41.80	42.65			
		$LSD_{p\leq0.05}$			NS		2.67	3.23	
WEEK 8	MASENO	1	50.11	55.97	51.91	49.71	51.92		
		4	53.49	50.31	44.83	54.66	50.82	50.51	11.68
		10	47.47	52.67	45.68	49.29	48.78		
		MEAN N	50.36	52.98	47.47	51.22			
		$LSD_{p\leq0.05}$			NS		NS		
	KIBOS	1	72.94	65.54	74.08	74.31	71.72		
		4	70.33	71.87	75.26	71.66	72.28	71.68	9.18
		10	67.61	76.83	66.76	72.96	71.04		
		MEAN N	70.29	71.41	72.03	72.97			
		$LSD_{p\leq0.05}$			NS		NS		
	OYANI	1	48.22	55.06	54.41	58.28	53.99		
		4	49.43	57.74	55.92	57.50	55.15	54.13	8.27
		10	50.97	56.26	53.25	52.57	53.26		
		MEAN N	49.54	56.35	54.52	56.12			
		$LSD_{p\leq0.05}$			4.37		4.37		
	ALL SITES	1	56.12	58.86	60.13	60.77	59.21		
		4	57.75	59.97	58.67	61.27	59.42		9.58
		10	55.35	61.92	55.23	58.27	57.69		
		MEAN N							
		$LSD_{p\leq0.05}$			NS		NS	3.28	

NOTE: NS – Not significant; S – Site; N – Nitrogen; VAR – Variety

Table 4. Contd….

WEEK	SITE	VAR (NERICA)	N-RATES 20	60	100	140	VAR MEAN	SITE MEAN	C.V%
WEEK 10	MASENO	1	66.18	65.55	65.13	59.13	63.99		11.93
		4	67.62	64.21	54.43	68.50	63.69	62.99	
		10	62.03	64.43	58.29	60.37	61.28		
		MEAN N	65.27	64.73	59.28	62.67			
		$LSD_{p\leq0.05}$		NS			NS		
	KIBOS	1	78.45	74.32	79.43	80.46	78.16		
		4	77.36	80.72	84.36	80.33	80.69	78.70	8.44
		10	75.81	80.28	73.10	79.73	77.23		
		MEAN N	77.21	78.44	78.96	80.17			
		$LSD_{p\leq0.05}$		NS			NS		
	OYANI	1	54.67	65.38	65.05	68.48	63.39		
		4	59.57	67.08	67.83	69.96	66.11	64.03	6.66
		10	58.31	62.21	65.44	64.36	62.58		
		MEAN N	57.52	64.89	66.10	67.60			
		$LSD_{p\leq0.05}$		3.60			NS		
	ALL SITES	1	66.43	68.41	69.87	69.36	68.52		
		4	68.18	70.67	68.87	72.93	70.16	68.57	9.94
		10	65.38	68.97	65.61	68.15	67.03		
		MEAN N	66.67	69.35	68.12	70.15			
		$LSD_{p\leq0.05}$		NS			NS	3.96	
WEEK 12	MASENO	1	78.17	78.19	79.36	69.91	76.41		12.59
		4	78.43	75.71	69.26	80.08	75.87	75.50	
		10	70.66	77.46	70.89	77.86	74.22		
		MEAN N	75.26	78.40	80.85	79.60			
		$LSD_{p\leq0.05}$		NS			NS		
	KIBOS	1	84.85	80.68	86.20	86.46	84.55		
		4	83.62	92.38	96.33	87.81	90.04	85.13	6.85
		10	79.31	82.62	78.46	82.81	80.80		
		MEAN N	78.22	84.49	82.30	85.30			
		$LSD_{p\leq0.05}$		NS			4.93		
	OYANI	1	62.77	76.32	76.99	82.41	74.62		
		4	72.62	85.37	81.32	88.01	81.83	76.18	5.29
		10	65.75	71.50	74.05	77.04	72.08		
		MEAN N	71.90	77.19	74.46	79.23			
		$LSD_{p\leq0.05}$		3.93			3.41		
	ALL SITES	1	75.75	77.12	73.17	75.95	78.52		
		4	82.59	85.23	87.00	85.69	82.58	78.93	9.36
		10	67.05	77.73	77.45	82.49	75.70		
		MEAN N	75.13	80.02	79.20	81.38			
		$LSD_{p\leq0.05}$		4.27			3.70	3.70	

NOTE: NS – Not significant; S – Site; N – Nitrogen-rate; VAR – Variety

4.2.2 Leaf chlorophyll content

4.2.2.1 Effect of varieties on leaf chlorophyll content

Inter-varietal comparisons of leaf chlorophyll contents on NERICA 1, 4 and 10 showed significant ($p \leq 0.05$) differences in weeks 6 and 8. (Fig. 10 and Table 5). NERICA 4 leaves had the highest chlorophyll content throughout the study season. Similar results had been recorded by (Sikuku *et al.*, 2016). Plant leaf chlorophyll content is responsible for photosynthetic efficiency (Gietson *et al.*, 2016) and varietal differences have been detected in several plant species (Dey *et al.*, 2016) including rice (Bekere *et al.*, 2014). Most varietal differences were attributed to the genetic capacity of the plant to metabolize soil nitrogen, which is an important nutrient in the biosynthesis of chlorophyll (Gietson *et al.*, 2016). Although significant ($p \leq 0.05$) differences in chlorophyll content were observed, a genetic characterization of 131 quantitative trait loci of NERICA varieties reported a close genetic constitution between them (Fukuta *et al.*, 2012). However, NERICA 4 has erect leaves that allow good light penetration deep into the canopy (Fischer *et al.*, 2001). NERICA 4 leaves had higher leaf chlorophyll content, an indication of a greater potential for growth and increased production of grains, and is therefore more suitable for cultivation in Lake Victoria basin.

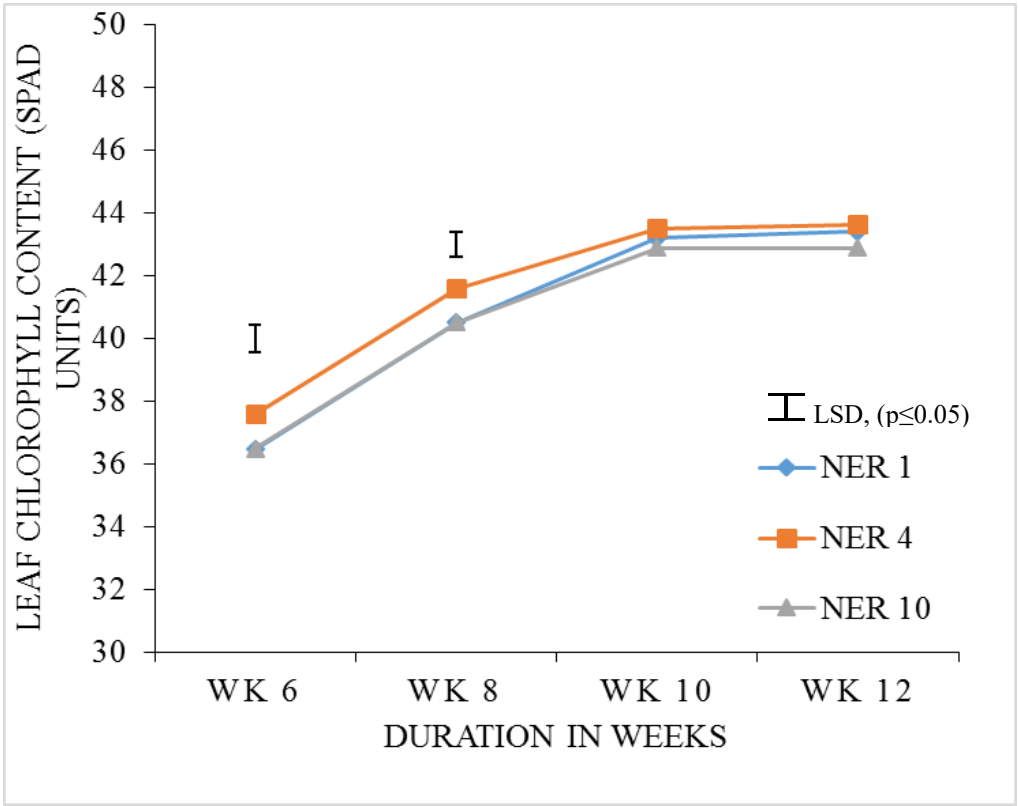


Fig 10. Variations in leaf chlorophyll content with varieties

4.2.2.2 Effect of nitrogen rates on leaf chlorophyll content

Leaf chlorophyll content significantly ($p \leq 0.05$) varied with nitrogen rates between weeks 6 and 12 (Fig. 11 and Table 5). In week 6 and 12, 140 KgN/ha resulted in the highest leaf chlorophyll content, while in weeks 8 and 10, the highest values were observed at nitrogen rates of 140 KgN/ha and 60 KgN/ha repectively. Thus, the higher nitrogen rate of 140 KgN/ha positively influenced leaf chlorophyll content similar to previous research (Sikuku *et al*., 2016). Metabolic nitrogen is required for chlorophyll synthesis and its deficiency leads to loss of green color in the leaves, decrease leaf area and intensity of photosynthesis (Anjum *et al.*, 2011). Thus, assessment of chlorophyll content is an effective way of monitoring plant growth and estimating photosynthetic productivity (Chen *et al*., 2007). Indeed, higher leaf chlorophyll intensity due to high nitrogen fertilization is a reflection of the NERICA plant nitrogen status, and its overall yield potential (Sikuku *et al*., 2016). In contrast, excessive use of fertilization has been associated with nitrogen losses through ammonia volatilization and leaching (Peng *et al*., 2010). Recommendations for nitrogen-use efficiency in rice cropping systems include adjustment of nitrogen rates, based on leaf chlorophyll readings (Hu *et al*., 2010). Thus, the highest nitrogen fertilizer rates tested (140 KgN/ha) had the greatest leaf chlorophyll content and is preferable for the NERICA varieties for growth and weed competetiveness.

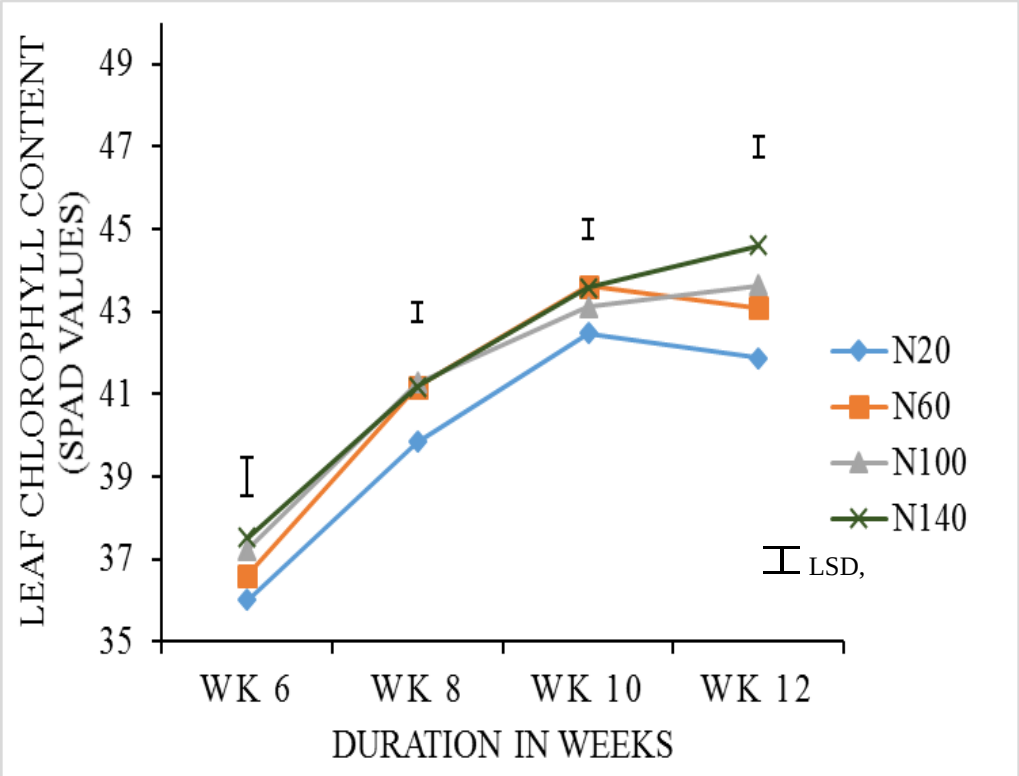


Fig 11. Effect of N-rates on leaf chlorophyll content (SPAD units)

4.2.2.3 Effect of sites on leaf chlorophyll content

Chlorophyll content of the NERICA varieties varied at the three sites (Table 5, Fig. 12). The highest ($p \leq 0.05$) chlorophyll contents were recorded at Kibos site throughout the study period. The major site factors that influence enhanced chlorophyll content include soil type, topography and environmental conditions. Kibos soils had the highest amounts of phosphorus, magnesium and nitrogen (Table 3) which are key metabolites in chlorophyll synthesis, and their higher levels at Kibos soils may have culminated in the observed higher leaf chlorophyll content. Maseno and Oyani sites experienced lower environmental temperatures (Appendix 1b and 1c) which was not favourable for high leaf chlorophyll development (Akparobi, 2009). Low growing temperatures for rice plants results in chlorosis, retarded growth and reduced yield (Akparobi, 2009). Though Oyani site had better rainfall distribution (Appendix 1c), the soil moisture was low due to high temperatures experienced throughout the growing season (Appendix 1d). Further, Oyani soils have low water retention capacity (Jaetzold, 2009) which may have negatively influenced plant metabolic processes such as chlorophyll synthesis. Maseno site received the least amount of rainfall (1120.56 mm within 117 days) throughout the growing season (Appendix 1f), which may have contributed to the low SPAD values detected. Kibos site has a potential for production of high yielding NERICA varieties.

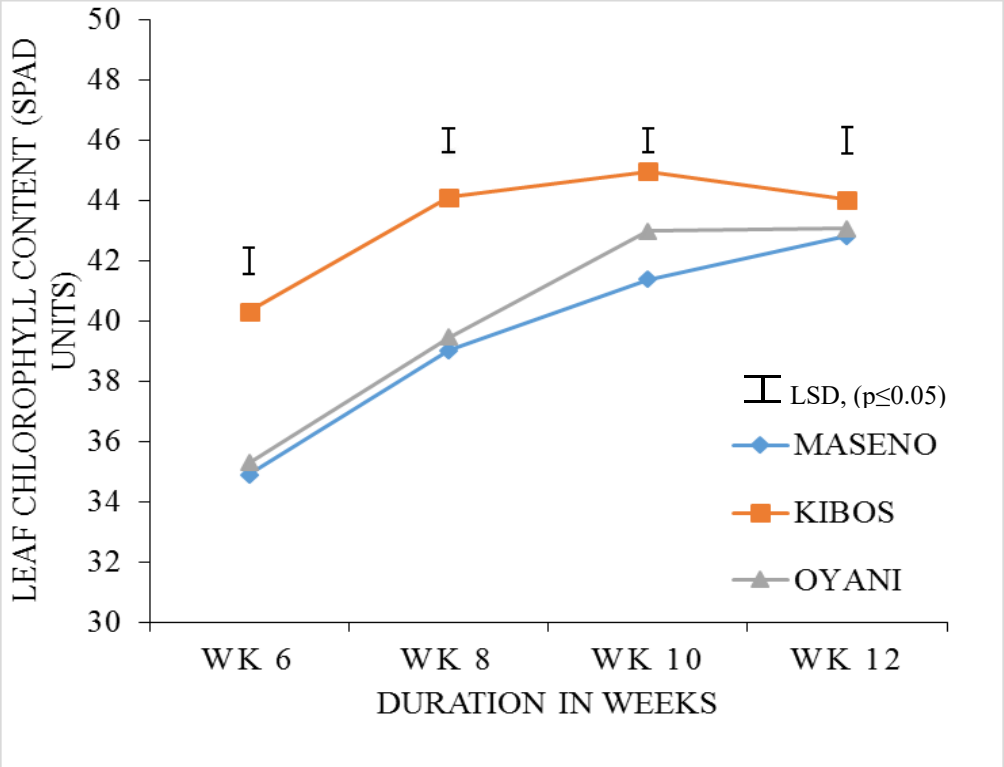


Fig 12. Effect of site on leaf chlorophyll content

Table 5. Influence of site, N-rate and NERICA varieties on leaf chlorophyll content

WEEK	SITE	VAR (NERICA)	N-RATES 20	60	100	140	VAR MEAN	SITE MEAN	C.V %
WEEK 6	MASENO	1	34.57	33.70	35.19	33.93	34.34		
		4	35.56	34.61	35.68	37.20	35.76	34.89	4.72
		10	34.35	34.46	34.44	34.98	34.56		
		MEAN N	34.83	34.25	35.10	35.37			
		$LSD_{p\leq0.05}$			NS		NS		
	KIBOS	1	39.77	38.88	39.80	40.98	39.85		
		4	40.01	39.27	41.80	41.64	40.68	40.32	3.72
		10	40.90	40.31	40.45	40.10	40.44		
		MEAN N	40.22	39.48	40.68	40.90			
		$LSD_{p\leq0.05}$			NS		NS		
	OYANI	1	32.33	35.28	36.94	36.02	35.14		
		4	34.32	37.15	36.10	37.59	36.29	35.29	5.0
		10	32.24	35.80	34.56	35.15	34.44		
		MEAN N	32.96	36.08	35.87	36.25			
		$LSD_{p\leq0.05}$			1.72		NS		
	ALL SITES	1	35.55	35.95	37.31	36.98	36.45		
		4	36.63	37.01	37.86	38.81	37.58		4.8
		10	35.83	36.86	36.48	36.74	36.48		
		MEAN N	36.00	36.61	37.22	37.51			
		$LSD_{p\leq0.05}$			0.99		0.86	0.86	
		S×N			1.72				
WEEK 8	MASENO	1	38.92	39.53	39.23	38.58	39.06		
		4	39.44	40.03	39.01	38.97	39.36	39.03	5.13
		10	37.85	39.19	39.25	38.41	38.68		
		MEAN N	38.74	39.58	39.16	38.65			
		$LSD_{p\leq0.05}$			NS		NS		
	KIBOS	1	42.00	44.39	44.11	44.70	43.80		
		4	43.52	44.99	45.34	44.31	44.54	44.11	2.44
		10	42.41	43.82	45.06	44.75	44.01		
		MEAN N	42.64	44.40	44.83	44.59			
		$LSD_{p\leq0.05}$			0.91		NS		
	OYANI	1	37.47	37.58	40.07	39.64	38.69		
		4	38.31	41.70	41.42	41.96	40.84	39.46	3.86
		10	38.68	39.43	38.03	39.27	38.85		
		MEAN N	38.15	39.57	39.84	40.29			
		$LSD_{p\leq0.05}$			1.49		1.29		
	ALL SITES	1	39.46	40.50	41.13	40.97	40.51		
		4	40.42	42.24	41.92	41.75	41.58		4.05
		10	39.65	40.81	40.78	40.81	40.51		
		MEAN N	39.84	41.18	41.28	41.17			
		$LSD_{p\leq0.05}$			0.93		0.80	0.80	

WEEK	SITE	VAR (NERICA)	N-RATES 20	60	100	140	VAR MEAN	SITE MEAN	C.V %
WEEK 10	MASENO	1	41.66	41.61	41.07	41.76	41.66		
		4	41.54	42.55	41.29	41.31	41.54	41.39	3.88
		10	41.25	41.10	40.34	41.04	41.25		
		MEAN N	41.48	41.75	40.90	41.37	41.48		
		$LSD_{p\leq0.05}$			NS		NS		
	KIBOS	1	42.65	45.48	45.88	46.15	42.65		
		4	45.01	45.95	44.80	46.02	45.01	44.97	2.64
		10	44.20	44.66	46.27	45.65	44.20		
		MEAN N	43.95	45.36	45.65	45.94	43.95		
		$LSD_{p\leq0.05}$			1.16		NS		
	OYANI	1	42.18	43.18	42.88	43.82	43.01		
		4	41.88	44.19	43.75	43.87	43.42	42.98	4.12
		10	41.89	43.88	41.80	42.51	42.52		
		MEAN N	41.99	43.75	42.81	43.40			
		$LSD_{p\leq0.05}$			NS		NS		
	ALL SITES	1	42.16	43.42	43.27	43.91	43.19		
		4	42.81	44.23	43.28	43.73	43.51	43.19	3.71
		10	42.45	43.21	42.80	43.06	42.88		
		MEAN N	42.47	43.62	43.12	43.57			
		$LSD_{p\leq0.05}$			0.90		NS	0.77	
WEEK 12	MASENO	1	42.36	42.17	43.56	44.07	43.04		
		4	43.80	42.52	43.12	42.79	43.06	42.82	11.93
		10	43.40	40.91	42.33	42.82	42.36		
		MEAN N	43.18	41.87	43.00	43.23			
		$LSD_{p\leq0.05}$			NS		NS		
	KIBOS	1	41.56	45.18	44.49	45.13	44.09		
		4	43.12	44.26	43.74	44.92	44.01	44.02	8.44
		10	44.69	41.76	45.07	44.39	43.98		
		MEAN N	43.12	43.73	44.43	44.81			
		$LSD_{p\leq0.05}$			NS		NS		
	OYANI	1	39.95	43.86	42.83	45.69	43.08		
		4	39.87	45.09	44.45	45.99	43.85	43.07	6.66
		10	38.20	42.19	43.06	45.72	42.29		
		MEAN N	39.34	43.71	43.45	45.80			
		$LSD_{p\leq0.05}$			1.80		NS		
	ALL SITES	1	41.29	43.74	43.63	44.96	43.40		
		4	42.26	43.96	43.77	44.57	43.64		9.94
		10	42.10	41.62	43.48	44.31	42.88		
		MEAN N	41.88	43.10	43.63	44.61			
		$LSD_{p\leq0.05}$			1.02		NS	0.88	
		S×N			1.77				

NOTE: NS – Not significant; S – Site; N – Nitrogen VAR – Variety

4.2.3 Tiller number

4.2.3.1 Effect of varieties on tiller number

The number of tillers did not significantly ($p>0.05$) vary with varieties (Fig. 13 and Table 6). In the three varieties, the number of tillers increased progressively with time. The absence of significant ($p>0.05$) varietal differences in the tillering of the NERICA varieties may be due to similarity in their genetic make-up (Jones *et al*., 2007). Previous studies on tillering in wheat, rice, barley and rye have demonstrated that it is a continual organogenesis process which is highly dependent upon the plant genetic networks (Hussein *et al*., 2014; Domagalska and Leyser, 2011; Wang and Li, 2008). Thus, the NERICA varieties responded similarly in terms of tiller production, which may be attributed to their close genetic make-up (Jones *at al*., 2007). The three varieties have similar tillering ability and therefore have similar a poteintial for high yields.

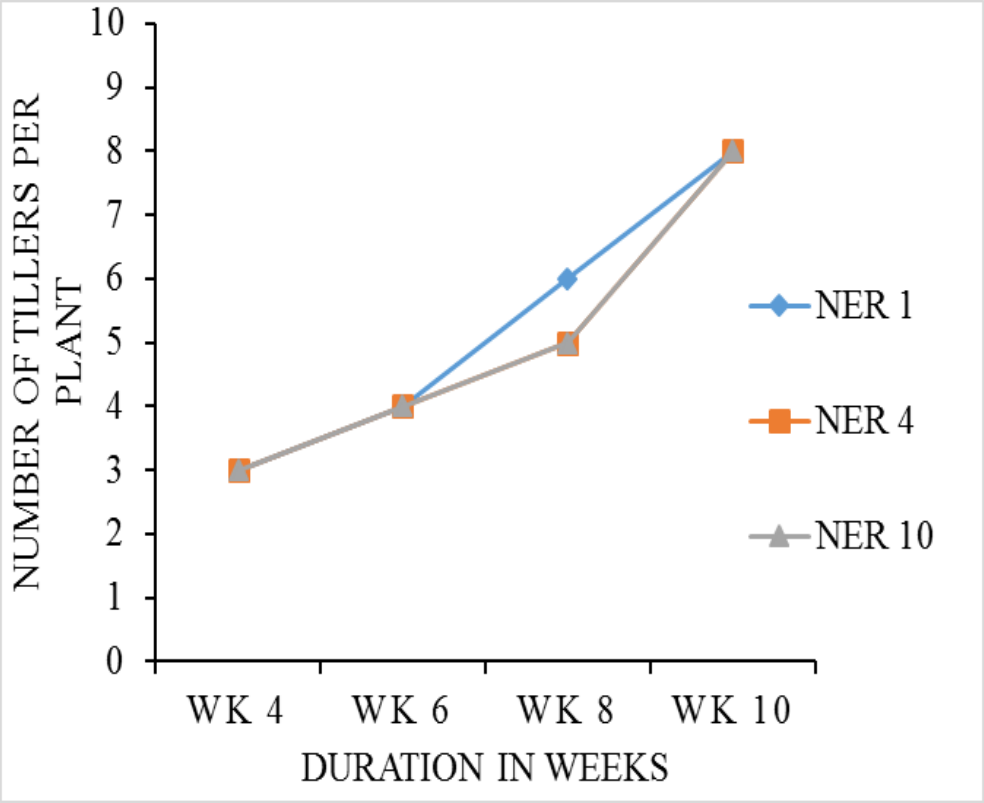


Fig 13. Varietal variations in number of tillers per hill

4.2.3.2 Effect of nitrogen fertilizer rates on tiller number

Nitrogen rates significantly ($p \leq 0.05$) influenced the number of tillers per hill in weeks 6, 8 and 10 at specific sites (Table 6, Fig. 14). In week 6, application of 100 KgN/ha had significantly ($p \leq 0.05$) higher tiller numbers that all other nitrogen rates applied. In week 8, significantly ($p \leq 0.05$) the highest number of tillers were counted at 60 KgN/ha. Thus, 100 KgN/ha was suitable for maximum tiller number in the NERICA varieties at earlier stages of plant growth, while 60 KgN/ha resulted in more tillers at later stages of growth. Application of nitrogen fertilizer was done in two split doses during week 4 and week 6. Significant variations in tiller number was observed two weeks after each dose, due to availability of nitrates to the plants from inorganic nitrogen applied (Mattson *et al*., 2009). These findings are similar to (Fairhurst *et al.* 2007) in which application of nitrogen nutrients resulted in increased number of tillers and consequently higher grain yield. Tillering in rice is highly influenced by environmental factors of growth including agronomic inputs (Kebrom *et al*., 2013). Maximization of tillering through application of appropriate rates of nitrogen fertilizer is a major target of agronomic programs to enhance crop yield. In this study, 100 KgN/ha increased tiller numbers in Week 6, while 60 KgN/ha favored tiller formation at later stages of growth. Thus, application of 100 KgN/ha in week 4 and 60 KgN/ha in week 6 is suitable for higher tiller number of the NERICA varieties in Lake Victoia basin.100KgN/ha causes higherr tillering in the early stages. Such tillers have vigour and grow to bear seeds, while late tillering is disadvantageous because such tiller die before beraing any seeds. 100 KgN may be suitable for production of NERICA varieties.

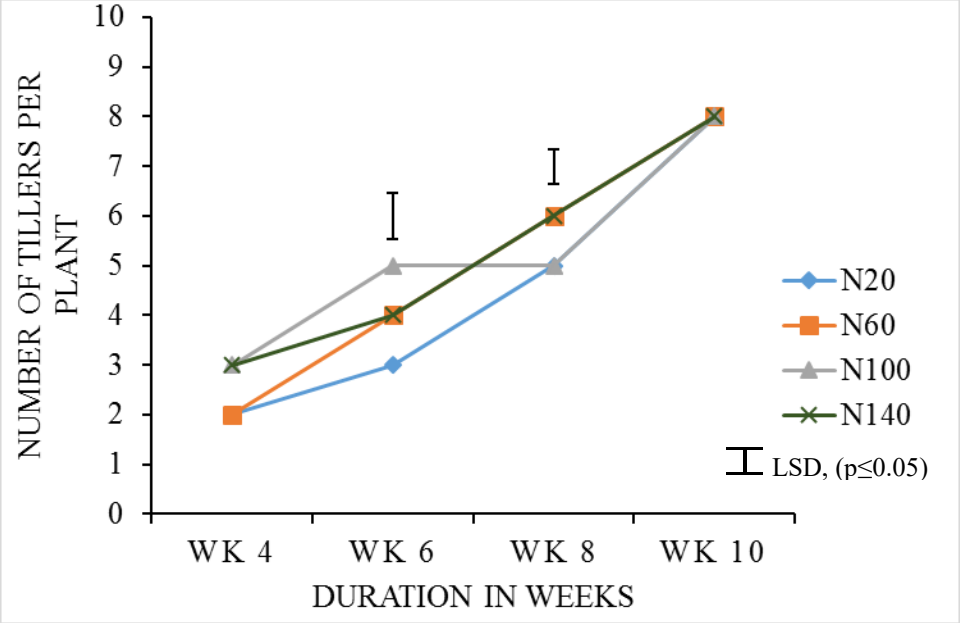


Fig 14. Effect of N-rates on number of tillers per plant

4.2.3.3 Effect of sites on tiller number

There was a significant ($p \leq 0.05$) difference in tiller number of the NERICA varieties at the three study sites throughout the growing season (Table 6, Fig. 15). Kibos site had the highest tiller number per hill throughout the growing season. High tillering ability of the NERICA varieties observed in Kibos site was interpreted as a good yielding attribute (Sikuku *et al*., 2006). However, previous work (Espino, 2014) reported that a good rice stand should produce plants with one to three tillers, unlike poor stands whose plants may produce up to 12 tillers. When many tillers per plant are produced, panicle maturity will be uneven, compromising grain quality at harvest (Singh *et al*., 1997). When stands are very dense, tillers may not even develop or may die before they can produce a panicle due to shading (Espino, 2014). As previously reported (Table 3), Kibos site had better soils and received sufficient rainfall (Appendix 1a) for growth and development of the NERICA varieties, although more tiller numbers may not have a direct relationship to higher yields. Thus, productivity of NERICA varieties in terms of tiller number was higher at Kibos site, in comparison to Maseno and Oyani, indicating that Kibos was a more suitable site for NERICA cultivation.

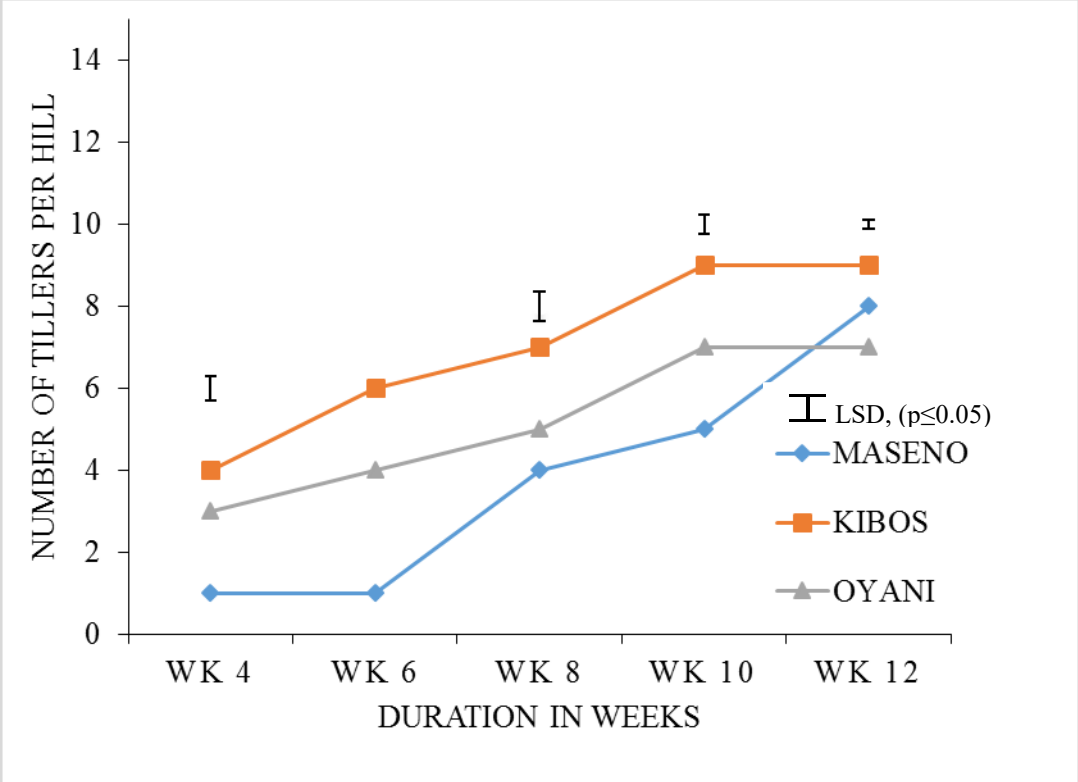


Fig 15. Influence of sites on number of tillers per hill

Table 6. Effect of site, N-rate and NERICA varieties on number of tillers per hill

WEEK	SITE	VAR (NERICA)	N-RATES 20	60	100	140	VAR MEAN	SITE MEAN	C.V%
WEEK 4	MASENO	1	0	1	1	1	1		
		4	1	0	0	1	0	1	39.70
		10	0	1	0	0	0		
		MEAN N	0	1	0	1			
		LSD$_{p\leq0.05}$		NS			NS		
	KIBOS	1	5	2	4	5	4		
		4	5	4	4	5	5	4	25.72
		10	5	4	3	5	4		
		MEAN N	5	3	4	5			
		LSD$_{p\leq0.05}$		NS			NS		
		V×N		1.77					
	OYANI	1	3	3	4	2	3		
		4	1	2	4	3	2	3	38.61
		10	2	2	3	2	2		
		MEAN N	2	2	3	2			
		LSD$_{p\leq0.05}$		0.96			NS		
	ALL SITES	1	3	2	3	3	3		
		4	2	2	3	3	2		39.75
		10	2	2	2	2	2		
		MEAN N	2	2	3	3			
		LSD$_{p\leq0.05}$		NS			NS	0.59	
		S×N		0.79					
WEEK 6	MASENO	**1**	1	2	2	1	2		
		4	2	1	2	1	1	1	64.8
		10	1	2	1	2	1		
		MEAN N	1	2	2	1			
		LSD$_{p\leq0.05}$		NS			NS		
	KIBOS	**1**	7	5	6	8	7		
		4	7	7	6	4	6	6	16.5
		10	6	7	5	8	7		
		MEAN N	6	6	6	7			
		LSD$_{p\leq0.05}$		0.66			NS		
	OYANI	**1**	3	5	6	4	4		
		4	2	4	5	5	4	4	33.4
		10	3	3	4	3	3		
		MEAN N	3	4	5	4			
		LSD$_{p\leq0.05}$		0.94			NS		
	ALL SITES	**1**	3	4	5	5	4		
		4	4	4	4	3	4		22.88
		10	3	4	3	4	4		
		MEAN N	3	4	4	4			
		LSD$_{p\leq0.05}$		NS			NS	0.46	
		S×N		0.93					

WEEK	SITE	VAR (NERICA)	N-RATES 20	60	100	140	VAR MEAN	SITE MEAN	C.V%
WEEK 8	MASENO	1	4	5	4	4	4		
		4	4	4	4	4	4	4	25.17
		10	5	6	3	4	4		
		MEAN N	4	5	4	4			
		LSD$_{p\leq0.05}$		NS			NS		
	KIBOS	1	7	8	7	9	8		
		4	7	7	8	6	7	7	16.14
		10	7	8	6	8	7		
		MEAN N	7	8	7	8			
		LSD$_{p\leq0.05}$		NS			NS		
	OYANI	1	3	5	7	6	5		
		4	3	6	5	6	5	5	28.87
		10	4	5	5	4	4		
		MEAN N	3	5	6	5			
		LSD$_{p\leq0.05}$		1.36			NS		
	ALL SITES	1	5	6	6	6	6		
		4	5	6	6	5	5		22.88
		10	5	6	5	5	5		
		MEAN N	5	6	5	6			
		LSD$_{p\leq0.05}$		0.70			NS	0.61	
		S×N		1.21					
WEEK 10	MASENO	1	8	9	7	8	8		
		4	8	8	8	7	8	5	9.53
		10	9	10	8	7	9		
		MEAN N	5	6	5	5			
		LSD$_{p\leq0.05}$		0.76			NS		
	KIBOS	1	9	9	8	10	9		
		4	7	8	8	7	8	9	8.52
		10	8	9	7	9	8		
		MEAN N	8	9	8	8			
		LSD$_{p\leq0.05}$		0.74			NS		
	OYANI	1	6	7	7	8	7		
		4	6	7	7	8	7	7	15.69
		10	6	7	6	7	6		
		MEAN N	6	7	7	7			
		LSD$_{p\leq0.05}$		NS			NS		
	ALL SITES	1	7	8	7	9	8		
		4	8	8	8	8	8		12.59
		10	8	8	8	8	8		
		MEAN N	8	8	8	8			
		LSD$_{p\leq0.05}$		NS			NS	0.48	

NOTE: NS – Not significant; S – Site; N – Nitrogen-rate; VAR – Variety

4.3 Volatile flavor compounds detected on NERICA 1, 4 and 10

4.3.1 Identities of volatile flavor compounds

Chemicals present in the endosperm of rice grains determine the overall aroma, and rice plants with very similar genetics, such as the NERICA varieties would be expected to have very similar volatile profiles. Volatile compounds in rice are secondary metabolites under genetic control and are responsible for aroma production (Chen *et al*., 2008; Vanavichit *et al*., 2006; Srivong *et al*., 2005). A total of 112 volatile compounds were identified collectively in NERICA 1, 4 and 10 cultivars (Table 7). The compounds were classified as green leaf volatiles, aromatics and terpenoids. More than 200 volatile compounds have been identified in rice although only a few were observed to contribute to the characteristic aroma (Yajima *et al*., 1978; Buttery *et al*., 1988; Widjaja *et al*., 1996; Jezussek *et al*., 2002). NERICA varieties are currently classified as non-aromatic rice (IRRI, 2011). However, the findings of this study indicate that they have a variety of compounds that could contribute to aroma.

The green leaf volatiles reported were (*E, E*)-2, 4-decadienal, nonanal, hexanal, *E*-2-nonenal, octanal, decanal, (*E, E*)-2, 4-nonadienal, E-2-undecanal, 2E-tridecen-1-al, furfural, decadienal <2E 4Z>, 3-methyl butanal and undecanal. Amongst the green leaf volatiles detected, (*E, E*)-2, 4-decadienal, nonanal, hexanal, *E*-2-nonenal, octanal, decanal, (*E, E*)-2, 4-nonadienal and E-2-undecanal have been identified as primary compounds responsible for variations in the aroma of irrigated aromatic rice varieties (Mahattanatawee and Rouseff, 2010; Maraval *et al*., 2008; Yang *et al*., 2008). These compounds were observed in NERICA 1, 4 and 10 and confirms the likelihood that NERICA varieties are aromatic. Indeed, the three NERICA varieties had similar green leaf volatile profiles most of which were observed in previous studies. For instance, octanal, (E)-2-nonenal, nonanal, heptanal, hexanal, decanal and (E)-2-octenal were major contributors in the aroma of seeds of Basmati-370, Ambemohar-157 (non-basmati scented), and IR-64 (non-scented) rice cultivars (Hinge *et al*., 2016), although heptanal content was significantly higher in the scented rice cultivars than non-scented one (Hinge *et al*., 2016). High concentrations of hexanal in food products give a pungent taste, while low concentration evokes a wide range of pleasant aroma (Jezussek *et al*., 2002). Aldehydes are contributors of rice aroma because of their low odor threshold value (Yang *et al*., 2008). In a sensory study of two Basmati varieties, the aldehydes (E) 2-nonenal and (*E, E*)-2, 4-decadienal had the lowest odor threshold but were still major contributors of aroma (Buttery *et al*., 1988). Other aldehydes with relatively low thresholds that contribute to rice aroma were (E)-2-decenal, octanal, nonanal, and decanal (Buttery *et al*., 1988). Aliphatic

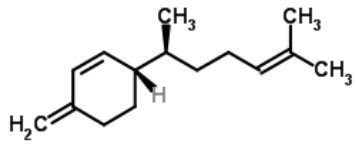
aldehydes are associated with lipid breakdown products and can be linked with flavors like grassy, fatty, and soapy (Lam and Proctor 2003, Grosch, 1982). Octyl formate, methyl octadecanoate, isobutyl butanoate, methyl tetradecanoate, methyl hexadecanoate, phthalic acid isobutyl octyl ester, dibutyl phtalate, hexanoic acid isobutyl ester and methyl linoleate were among the esters detected in the NERICA varieties (Table 8). Esters have nutritional and medicinal benefits and are abundant in brans of grain such as rice (Jariwalla, 2001; Escribano *et al*., 2004). Ethyl hexanoate, ethyl heptanoate, ethyl octanoate, and ethyl laurate were detected in the leaves of Basmati-370, Ambemohar-157 (non-basmati scented), and IR-64 (non-scented) rice cultivars while ester acetic acid, 1,7,7-trimethyl-bicyclo(2,2,1)hept-2-yl, ester were reported in the leaves of Ambemohar-157 (non-basmati scented) cultivar, but not in grains (Hinge *et al*., 2016). Amongst the esters detected are medicinal and flavor active compounds that have previously been detected in other food compounds (Yang *et al*., 2008a; Zeng *et al.,* 2008). Esters reported in NERICA varieties were octyl formate, methyl octadecanoate, isobutyl butanoate, methyl tetradecanoate, methyl hexadecanoate while the alkanols detected were 1-octen-3-ol, 3-methylpentanol and nonanol. In a separate study, 1-pentanol, 1-heptanol, 1-octen-3-ol, and 3-octen-2-one were reported in black rice (Yang *et al*., 2008), although 1-pentanol, 1-octen-3-ol and 3-octen-2-one did not appear to be significant contributors to the distinctive different aroma (Yang *et al*., 2008). The hydrocarbons reported in NERICA varieties were dodecane, 2,6,10-trimethyl, nonadecane, tridecane, dodecane, octadecane, eicosane, trisocane, tetratrioctane, hexadecane, cyclopentane nonyl, cyclopentane undecyl, 3-methyl tridecane, heptadecane, cyclopentane, methyl cyclopentane, octane, pentane,2,3,4-trimethyl, cyclopentane butyl, 2-methylhexane, hept-1-ene and tetradecane. Most hydrocarbons are byproducts of lipid oxidation and have little effects on the aroma attributes of food (Bryant and McClung, 2011). The presence of similar compounds including octadecane, hexadecane, and tridecane suggests that their presence in the NERICA varieties does not influence aroma. For instance, tridecane, dodecane, octadecane, eicosane, hexadecane, cyclopentane have previously been reported in non-fragrant rice (Widjaja *et al*., 1996; Bryant and McClung, 2011). Hydrocarbons are mostly released due to incomplete burning of food during cooking (Food Safety Authority of Ireland, 2016) and are not principally involved in conferring a pleasant aroma. The furans 2,4-dimethylfuran, 2,5-dimethylfuran, vinylfuran, 2-pentylfuran, 2-heptylfuran, benzofuran,2-methyl, furan,2-(2furanylmethyl)-5-methyl, 1H-pyrrole,1-(2-furanylmethy), furan, tetrahydro-2,5-dimethyl, furan,2,3-dihydro-4-methyl were also detected. These have been reported as odor active compounds in aromatic rice (Widjaja *et al*., 1996; Yang *et al*., 2008). The extraction process employed in this study involved heating of the NERICA samples which may have resulted in the high concentration

of aromatic furans including Pyrazine-2-methyl-6-ethyl, 2,4-dimethylfuran, 2,5-dimethylfuran, vinylfuran, 2-pentylfuran, 2-heptylfuran. Cooking duration affects the concentration of furans in foods and may eventually result in toxicity (Kim *et al*., 2009). Aromatic furans are found in cooked or thermally processed foods (Becalski and Seaman, 2005) and are the major compounds formed during the Maillard reaction (Becalski *et al*. 2005). Furan and its derivatives in foods contribute to their flavoring properties (Maga, 1979). Pentylfuran<2> was detected in high concentrations in BA-370 as one of the major contributors of aroma in basmati flavor (Hinge *et al*., 2016), in California long-grain rice (Buttery *et al*., 1988) and brown rice cultivars (Jessuzek *et al*., 2002).

Volatile aromatic compounds detected in NERICA 1, 4 and 10 varieties were 1-cyclohexene carboxaldehyde, benzaldehyde, benzene acetaldehyde, o-cymene, naphthalene-2,3,6-trimethyl, p-cymene, naphthalene. The aromatic nitrogen containing compound pyrazine-2-ethyl-6-methyl was reported in NERICA varieties. Nitrogen containing compounds detected were methyl pyrazine, 2-acetyl-1-pyrolline and pyridine, many of which are derived from rice bran (Buttery *et al*., 1983). Analysis of volatile flavor compounds in this study was done using rice grains with intact brans which may have contributed to the nitrogen containing compounds detected. A diverse range of nitrogen-containing heterocyclic compounds including pyrazine, methoxypyrazine, pyrrole, pyridine, pyrroline, pyrrolidine, pyrrolizine and piperine are formed in the Maillard reaction (Reineccius, 2006). In rice varieties with high protein content in the grain, the biosynthesis of nitrogen based volatiles is usually high since the protein serves a nitrogen source (Park *et al*., 1999).

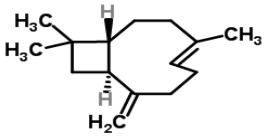
Terpenoids were the second most abundant group of compounds detected. Some terpenoids reported in this study were sesquiphellandrene (37), β-caryophellene (38), α-cedrene (39), α-isocomene (40), sabinene (41), γ-gurjurene (42), α-cubebene (43), longifolene (44), β-phellandrene (45), β-acoradiene (46), α, -bisabolene (47), β- biabolene (48), copaene (49), gamma-humulene (50), α-muurolene (51), α-funebrene (52) were also reported in NERICA varieties. Rice plants damaged by *C. suppressalis* for 24 hours increase release of terpenes including limonene, copaene, β-caryophyllene, α-bergamotene, germacrene D, δ-selinene, and α-cedrene (Zhou *et al*., 2011; Liu *et al*., 2015). Infestation of rice by chewing herbivores, such as *C. suppressalis* induces the release of a blend of volatiles that increase the efficiency of natural enemies (Lou *et al*., 2013). Terpenoids, the most common group of secondary metabolites, can directly affect insect performance or indirectly attract natural enemies of the attacking herbivore (Stam *et al*., 2015; Dicke *et al*., 2010; Hagenbucher *et al*., 2013; Liu *et al*., 2015).

(37)



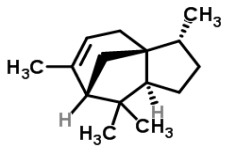
$C_{15}H_{24}$

(38)



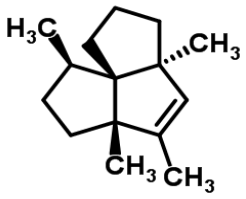
$C_{15}H_{24}$

(39)



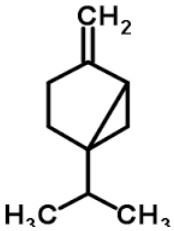
$C_{15}H_{24}$

(40)



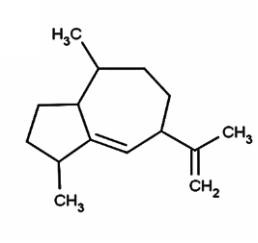
$C_{15}H_{24}$

(41)



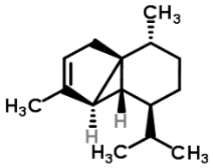
$C_{10}H_{16}$

(42)



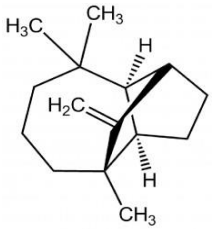
$C_{15}H_{24}$

(43)



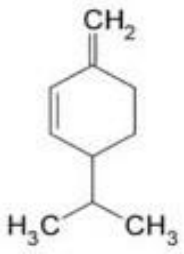
$C_{15}H_{24}$

(44)



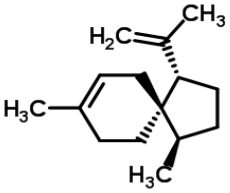
$C_{15}H_{24}$

(45)



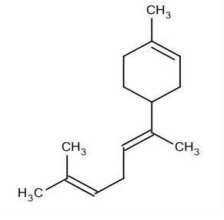
$C_{10}H_{16}$

(46)



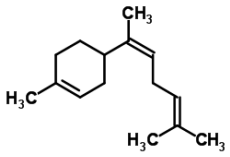
$C_{15}H_{24}$

(47)



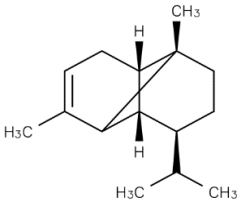
$C_{15}H_{24}$

(48)



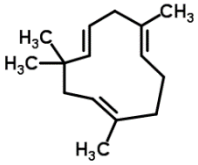
$C_{15}H_{24}$(

(49)



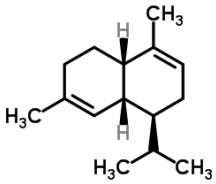
$C_{15}H_{24}$

(50)



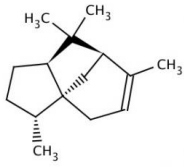
$C_{15}H_{24}$

(51)



$C_{15}H_{24}$

(52)



$C_{15}H_{24}$(

The terpenoids detected in NERICA varieties were mainly isomeric bicyclic and tricyclic sesquiterpenes and monoterpenes. Terpenoids detected in NERICA 1, 4 and 10 including linalool, cedrol-epi, and terpinen-4-ol were already reported in other rice varieties (Buttery *et al*., 1983). Linalool, for instance elicits attractant response to red spider mites (Odak *et al.,* 2016) and is a male pheromone attractant to bee *Colettes cunucularius* (Borg-Karlson *et al*., 2003) Among plant secondary metabolites**,** terpenoids are the most diverse class of natural compounds known to play a

role in food flavour (Caputi and Aprea, 2011). Terpenoids offer a wide variety of pleasant scents from flowery to fruity, to woody scents (Caputi and Aprea, 2011). Cedrol, α-cedrene, (*E*)-β-caryophyllene, linalool, (*E*)-β-ocimene, (*Z*)-β-ocimene have been reported in different tea cultivars (Odak, 2016). Detection of a large group of terpenoids in the NERICA cultivars reveal the potential of NERICA varieties for production of highly aromatic rice, which warrants further integrated breeding and chemical analysis. Aromatics, monoterpenes and sesquitepenes defend plants against pests (Khan *et al*., 2008; Erbilgin *et al*., 2007; Jaenson *et al*., 2006; Zhang *et al*., 2015)

Sulphur containing compounds detected included dimethyl disulphide, 1, 2-benzothiazole and dimethyl trisulphide, imparting an off-flavor scent. Dimethyl disulfide is the principal volatile flavour component of processed tomatoes (Fischer and Scott, 1997). The sulfur group of any compound has a high flavour impact because it binds strongly to the olfactory receptors (Fischer and Scott, 1997). Volatile sulfur compounds are not major contributors to the aroma for several reasons. Carbonyl compounds formed by lipid oxidation can react with sulfhydryl groups on cysteine or methionine decreasing the formation of volatile sulfur compounds (Zhou, 2002). Likewise, protein oxidation reduces the level of volatile sulfur compounds (e.g., hydrogen sulfide, methylmercaptan, dimethyl sulfide, dimethyl disulfide and sulfur dioxide) formed during cooking (Zhou, 2002).

Table 7. Table ofidentities and retention times of VFCs detected in NERICA varieties at Kibos, Maseno and Oyani study sites.

No.	COMPOUND	RT	No.	COMPOUND	RT	No.	COMPOUND	RT
1	3-methyl pentanol	3.1336	41	Nonanone<2>	12.827	81	α-Bisabolene	18.8175
2	3-Methyl-butanal	3.2799	42	Linalool	12.9557	82	β-Bisabolene	18.8170
3	3-Methylhexane	3.4671	43	Benzofuran,2-methyl	13.131	83	δ-Cidanene	18.9753
4	Cyclopentane,1,3-dimethyl	3.5782	44	6-Dodecanone	13.4881	84	Naphthalene,1,6,7-trimethyl	19.2563
5	2,2,7,7-Tetramethyloctane	3.6130	45	Decene<1>	14.096	85	Amorphrene delta	19.2971
6	2,2,3,5-Tetramethyl heptane	3.6192	46	Nonanol	14.0965	86	Tridecane, 7-hexyl	19.3380
7	Cyclopentane,1,2-dimethyl	3.6543	47	Terpinen-4-ol	14.2603	87	Tetratrioctane	19.3907
8	Furan,2,5-dimethyl	3.9820	48	Furan,2-(2furany lmethyl)-5-methyl	14.278	88	Hexadecene-1	19.6188
9	Furan, tetrahydro-2,5-dimethyl	4.0462	49	1H-Pyrrole,1-(2-furanylmethyl	14.3246	89	γ-Muurolene	19.7417
10	Vinylfuran	4.3446	50	Naphthalene	14.3773	90	Hexadecane	19.8322
11	Cyclopentane, ethyl	4.5080	51	2-n-Heptylfuran	14.4475	91	Cedrol	20.0867
12	1H-Pyrrole,1-methyl	4.7658	52	Dodecane	14.5411	92	Cyclopentane, nonyl	20.3911
13	Dimethyl disulphide	4.795	53	Methyl chavicol	14.5703	93	Cyclopentane, undecyl	20.5020
14	Pyridine	5.292	54	2E,4E-Nonadienal	14.7809	94	Tetradecane, 3-methyl	20.5022
15	Heptane,2-methyl	5.4561	55	β Cyclocytral	14.939	95	Hexadecanol	20.5548
16	Heptane,3-methyl	5.6840	56	Carvacrol, methyl ether	15.2372	96	Heptadecane	20.8883
17	Heptane,4-methyl	5.6841	57	1,2-Benzothiazole	15.3192	97	Methyl tetradecanoate	21.2042
18	Isobutyl 2-methylbutanoate	5.6842	58	Decanol	15.5999	98	Decanoic acid	21.5259
19	1-Pentene	5.5731	59	1-Cyclohexene Carboxaldehyde	15.7462	99	Octadecane	21.9764
20	2-hexanone	6.2224	60	Decadienal<2E,4Z)	15.9392	100	2-Pentadecanone -6,10,14-trimethyl	22.2513
21	3-hexanol	6.3745	61	Tridecane	15.9919	101	2-heptadecanone	23.1056
22	Hexanal	6.4682	62	Carvacrol	16.0387	102	Nonadecane	23.0177
23	Pyrazine,methyl	7.0708	63	Thymol	16.044	103	Methyl hexadecanoate	23.3102
24	Cyclotrisiloxane, hexamethyl	7.2112	64	Undecanal	16.1147	104	Phthalic acid, isobutyloctyl ester	23.5148
25	Furfural	7.3574	65	E,E-2,4-decadienal	16.261	105	Hexadecanoic acid	23.5149
26	2- Acetyl-1-pyrroline	9.5452	66	Furan,2,3-dihydro-4-methyl	16.6471	106	Dibutyl phthalate	23.6963
27	Benzaldehyde	10.364	67	2E –Undecanal	16.8986	107	Hexadecanioc acid ethyl ester	23.7782
28	2,3-Octanedione	10.873	68	Tridecen-1-al<2E>	16.899	108	Eicosane	24.0063
29	6-methyl-5-hepten-2-one	10.926	69	Dodecane,2,6,10, trimethyl	17.045	109	Methyloctadecanoate	25.0008
30	2- pentylfuran	11.008	70	α-Copaene	17.173	110	Octadecanoic acid	25.3752
31	Dimethyl trisulphide	11.100	71	α Cubebene	17.1736	111	Methyl linoleate	25.5566
32	Pyrazine,2-ethyl-6-methyl	11.1131	72	Tetradecane	17.3549	112	Trisocane	26.4926
33	p-Cymene	11.6336	73	Longifoline	17.624			
34	o-Cymene	11.6337	74	b-Caryophyllene	17.7878			
35	Limonene	11.7150	75	α-Cedrene	17.706			
36	β-Phellandrene	11.7155	76	Geranyl acetone	18.069			
37	Octen-2-one<3E>	11.8083	77	Nonadecane	18.1564			
38	Benzene acetaldehyde	11.9027	78	γ-gurjurene	18.4720			
39	Acetophenone	12.4060	79	α-Humulene	18.2266			
40	Octylformate	12.4468	80	β-ionone	18.5600			

4.3.1 Composition and concentration of volatile compounds in NERICA varieties

A total of 112 volatile compounds were detected in the NERICA varieties as shown on Table 8. The number of volatile compounds varied between the varieties with 110 compounds detected in NERICA 1, and 100 in NERICA 4 and 10. NERICA 1 had more volatile compounds than NERICA 4 and 10 and was therefore likely to be more aromatic, since highly scented rice varieties are superior to non-scented varieties in terms of the number and concentration of volatiles accumulated (Hinge *et al*., 2016; Dorward *et al*., 2008; WARDA, 2008). The major contributor of flavor in rice, 2-acetyl-1-pyrroline (2AP) was detected in NERICA 1, with an average concentration of 0.002 at all sites but was absent in NERICA 4 and 10. Only fragrant rice cultivars possess the genetic potential to accumulate 2AP (Bradbury *et al*., 2005), and its detection in NERICA 1 samples elucidates this work. Indeed, NERICA 1 grains had a favourable aroma giving it a premium market price because of its superior quality of fragrance (Jones *et al*., 1997). The genetic origin of fragrance in NERICA 1 grain was attributed to a mutation on *BADH2* gene which is responsible for fragrance in most modern aromatic rice varieties (Asante *et al*., 2010). Thus, NERICA 1 varieties cultivated in Lake Victoria basin had unique aroma inducing compounds compared to NERICA 4 and 10.

Other major compounds that influence rice aroma and hence consumer acceptability detected in this study include 2-pyrrolidone, pyridine, 2-methoxyphenol, 1H-indole, p-xylene, and 1-octen-3-ol (Hinge *et al*., 2016). Pyridine and 2-octen-3-ol were found in NERICA 1, 4 and 10 varieties, although in lower concentrations in NERICA 1. Variations in individual volatile flavor compound concentrations has a significant effect on its aroma profile since some compounds evoke a wide range of pleasant aroma even in very low concentrations (Jezussek *et al*, 2002). Thus, although low concentrations of pyridine and 2-octen-3-ol were observed NERICA 1 relative to NERICA 4 and 10, it was previously classified as having a favourable aroma (Jones *et al*., 1997). Thirteen odor active compounds were detected in six rice varieties including 2AP, hexanal, (E)-2-nonenal, octanal, heptanal, nonanal, (E)-2-octenal, (E,E)-2,4-nonadienal, 2-heptanone, (E,E)-2,4-decadienal, decanal, and 2-methoxyphenol (Ajayarasiri and Chaiseri, 2008). All these compounds except 2-methoxyphenol, 1H-indole and 2-pyrrolidone were detected in NERICA 1 in this study, further confirming its high aroma quality. Hexanal, (E,E)-2,4-nonadienal, E,E)-2,4-nonadienal were detected in all the three varieties, with the lowest concentrations in NERICA 1. In particular, hexanal has elicited much scientific interest since its odor-effect varies with concentration. High concentrations of hexanal in Malagkit Sungsong, Texas Long Grain, Calrose, IR841-76-1, Khao

Dawk Mali, 105, Milagross, Basmati, Seratus Malam, Azucena, and Hieri rice varieties give a pungent taste, while low concentration evoke a wide range of pleasant aroma (Jezussek *et al*., 2002).

Amongst the aromatic compounds, acetophenone was solely detected in NERICA 1, further confirming the superior quality of the variety in terms of volatile flavor compounds concentration. Acetophenone was first reported in cooked rice (Maga *et al*., 1984) and was also identified in two *O. sativa* cultivars (Sj1 and Si1) where it exhibited a 'floral' or 'almond' odor (Cho *et al*., 2014), while in peanut, it was described as 'fruity' and 'sweet' (Schirack *et al*., 2006). Acetophenone obtained from other plants has been associated with acute insecticidal activities (Mohsen *et al*., 1995). Tea cultivar TRIT 201/50 produced the highest amounts of acetophenone and had the least red crevice mite infestations (Odak *et al*., 2016).Thus, the concentration of volatile compounds and variation due to varieties has a significant influences on the aroma of rice, as was observed in NERICA 1, 4 and 10.

Terpenoids were the second most abundant group of compounds detected in this study, and were evenly distributed amongst the three NERICA varieties. Sesquiterpenes detected included β-sesquiphellandrene**,** (-)-β-caryophyllene, (−)-α-cedrene, α-isocomene, sabinene, γ-gurjurene, α-cubebene, longifoline, β-phellandrene, β-acoradiene, β-bisabolene, α-bisabolene, α-copaene, γ-humulene, α-muurolene, α-funebrene and δ-cadinene. The high number of terpenoids detected in the NERICA varieties could possibly explain their disease and pest resistance (Apaseku and Dowbe, 2013). This is because sesquiterpenes are known to defend plants against pest attack by attracting their natural enemies such as insects or birds, eliciting a biological control mechanism (Metcalf and Kogon, 1987; Eller *et al*., 2014). Many plants contain mixtures of volatile monoterpenes that lend a characteristic odor to their foliage and grains (Eller *et al*., 2014). Evidently, varietal differences in NERICA 1, 4 and 10 influenced the concentrations of volatile flavor compounds responsible for aroma. NERICA 1 had the highest number of compounds, confirming its superior quality compared to NERICA 4 and 10. Further, the major contributor of flavor in rice, 2-acetyl-1-pyrroline (2AP) was solely detected in NERICA 1 samples. Although significant aroma compounds such as hexanal were detected in low concentrations in NERICA 1, previous odor threshold studies revealed more favourable aroma profiles at such low concentrations (Jezussek *et al*, 2002).

Table 8. Table of influence of NERICA 1, 4 and 10 on concentration of volatile compounds.

COMPOUND	VARIETIES			MEAN	SD	%VAR	COMPOUND	VARIETIES			MEAN	SD	%VAR
	NER 1	NER 4	NER 10					NER 1	NER 4	NER 10			
E,E-2,4-decadienal	0.0790	0.4250	0.0860	0.197	0.198	100.661	Cyclotrisiloxane, hexamethyl	0.0080	0.0170	0.0130	0.013	0.005	39.370
Geranyl acetone	0.0610	0.3380	0.2040	0.201	0.138	68.657	Furfural	0.0450	0.0230	0.0230	0.030	0.012	39.604
β-ionone	0.0360	0.0470	0.0850	0.056	0.026	46.429	2,3-Octanedione	0.0020	0.0110	0.0070	0.007	0.004	59.702
2-hexanone	0.1150	0.1070	0.0450	0.089	0.038	42.697	Pyrazine,2-ethyl-6-methyl	0.0020	0.0000	0.0010	0.001	0.001	100.000
2- pentylfuran	0.0500	0.2320	0.1100	0.131	0.093	71.155	p-Cymene	0.0150	0.0010	0.0110	0.009	0.007	77.778
Limonene	0.0010	0.0130	0.0010	0.005	0.007	140.000	o-Cymene	0.0050	0.0470	0.0200	0.024	0.021	87.500
6-methyl-5-hepten-2-one	0.0180	0.0160	0.0240	0.019	0.004	20.725	β-Phellandrene	0.0140	0.0140	0.0010	0.010	0.007	72.165
2- Acetyl-1-pyrroline	0.0020	0.0000	0.0000	0.001	0.001	142.857	Octen-2-one<3E>	0.0080	0.0220	0.0300	0.020	0.011	55.000
Benzaldehyde	0.0760	0.0190	0.0270	0.041	0.031	76.167	Benzene acetaldehyde	0.0240	0.0240	0.0340	0.027	0.006	21.978
Acetophenone	0.0130	0.0000	0.0000	0.013	0.007	53.846	Octylformate	0.0060	0.0020	0.0060	0.005	0.002	42.553
3-methyl pentanol	0.8270	1.2060	0.5280	0.854	0.340	39.827	Nonanone<2>	0.0200	0.0140	0.0380	0.024	0.013	54.167
Dimethyl sulphide	0.0006	0.0009	0.0003	0.001	0.000	50.000	Linalool	0.0160	0.0160	0.0070	0.013	0.005	38.462
Cyclopentane, ethyl	0.0020	0.0070	0.0060	0.005	0.003	60.000	Benzofuran,2-methyl	0.0010	0.0000	0.0040	0.002	0.002	117.647
1H-Pyrrole,1-methyl	0.0020	0.0010	0.0010	0.001	0.001	76.923							
3-Methyl-butanal	0.0110	0.0040	0.0100	0.025	0.004	16.000	Nonanol	0.0040	0.0010	0.0350	0.013	0.019	142.857
3-Methylhexane	0.0150	0.0060	0.0200	0.041	0.007	17.073	Terpinen-4-ol	0.0220	0.0250	0.0110	0.019	0.007	36.269
Cyclopentane,1,3-dimethyl	0.0020	0.0010	0.0570	0.020	0.032	160.000	Decene<1>	0.0020	0.0080	0.0000	0.003	0.004	121.212
2,2,7,7-Tetramethyloctane	0.0020	0.0060	0.0010	0.003	0.003	100.000	Furan,2-(2furanylmethyl)-5-methyl	0.0030	0.0010	0.0060	0.003	0.003	90.909
2,2,3,5-Tetramethyl heptane	0.0020	0.0010	0.0110	0.005	0.006	127.660	1H-Pyrrole,1-(2-furanylmethyl	0.0010	0.0010	0.0010	0.001	0.000	0.000
Cyclopentane,1,2-dimethyl	0.0090	0.0050	0.0000	0.005	0.004	85.106	Naphthalene	0.0160	0.0150	0.0080	0.013	0.004	30.769
Furan,2,5-dimethyl	0.0050	0.0090	0.0090	0.008	0.002	25.974	2-n-Heptylfuran	0.0010	0.0000	0.0050	0.002	0.003	150.000
Furan, tetrahydro-2,5-dimethyl	0.0110	0.0160	0.0130	0.013	0.003	22.556	Dodecane	0.0140	0.0130	0.0250	0.017	0.007	40.462
Vinylfuran	0.0000	0.0010	0.0020	0.001	0.001	100.000	Methyl chavicol	0.0170	0.0130	0.0120	0.014	0.002	14.286
Heptane,2-methyl	0.0050	0.0770	0.0050	0.029	0.041	141.379	Nonadienal<2E,4E>	0.0420	0.1290	0.0510	0.074	0.048	64.865
Heptane,3-methyl	0.0040	0.3280	0.0050	0.112	0.187	166.518	Cyclocytral<beta>	0.0230	0.0200	0.0320	0.025	0.006	24.000
Heptane,4-methyl	0.0010	0.0070	0.0120	0.007	0.006	89.552	1,2-Benzothiazole	0.0480	0.2310	0.0490	0.109	0.106	96.981
Isobutyl 2-methylbutanoate	0.0050	0.0040	0.0030	0.004	0.001	25.000	Carvacrol, methyl ether	0.0470	0.0660	0.0820	0.065	0.018	27.692
1-Pentene	0.0010	0.0000	0.0050	0.002	0.003	150.000	Decanol	0.0060	0.0020	0.0050	0.004	0.002	46.512
Pyridine	0.0004	0.0001	0.0027	0.001	0.001	127.273	1Cyclohexene-carboxaldehyde	0.0260	0.0020	0.0220	0.017	0.013	77.844
3-hexanol	0.1030	0.0020	0.1530	0.086	0.077	89.535	Decadienal<2E,4Z)	0.0360	0.0690	0.0310	0.045	0.021	46.358
Hexanal	0.4140	0.0160	0.4840	0.305	0.253	83.033	Tridecane	0.0420	0.0310	0.0480	0.040	0.009	22.333
Pyrazine,methyl	0.0040	0.0030	0.0070	0.005	0.002	42.553	Carvacrol	0.0130	0.0740	0.0120	0.033	0.036	109.091

Table 8. Contd.....

COMPOUND	VARIETIES			MEAN	SD	%VAR
	NER 1	NER 1	100			
Thymol	0.0320	0.0030	0.0050	0.0133	0.02	120.30
Undecanal	0.0030	0.0450	0.0010	0.0163	0.03	153.37
Furan,2,3-dihydro-4-methyl	0.0010	0.0010	0.0010	0.0010	0.00	0.00
Undecanal<2E>	0.0130	0.0080	0.0770	0.0327	0.04	116.21
Tridecen-1-al<2E>	0.0010	0.0010	0.0000	0.0007	0.00	142.86
Dodecane,2,6,10, trimethyl	0.0630	0.0000	0.0000	0.0210	0.04	171.43
α-Copaene	0.0340	0.0330	0.0470	0.0380	0.01	21.05
Cubebene<alpha>	0.0010	0.0000	0.0350	0.0120	0.02	166.67
Tetradecane	0.2140	0.2070	0.2510	0.2240	0.02	10.71
Longifoline	0.0620	0.1160	0.0630	0.0803	0.03	38.61
b-Caryophyllene	0.0520	0.0490	0.0440	0.0483	0.00	8.28
α-Cedrene	0.0010	0.0010	0.0000	0.0007	0.00	142.86
Nonadecane	0.1010	0.0010	0.0110	0.0377	0.06	145.89
α-Humulene	0.0780	0.0510	0.0900	0.0730	0.02	27.40
Dodecanol	0.0350	0.0120	0.6580	0.2350	0.37	155.74
γ-Muurolene	0.0020	0.0010	0.0000	0.0010	0.00	100.00
α-Bisabolene	0.0020	0.0000	0.0010	0.0010	0.00	100.00
β-Bisabolene	0.0010	0.0010	0.0020	0.0013	0.00	76.92
δ-Cidanene	0.0900	0.1410	0.0370	0.0890	0.05	58.43
Naphthalene,1,6,7-trimethyl	0.0020	0.0000	0.0010	0.0010	0.00	100.00
Amorphrene delta	0.0020	0.0010	0.0420	0.0150	0.02	153.33
Tridecane, 7-hexyl	0.0010	0.0020	0.0000	0.0010	0.00	100.00
Tetratrioctane	0.0020	0.0010	0.0000	0.0010	0.00	100.00
Hexadecene-1	0.1050	0.0640	0.0560	0.0750	0.03	34.67
Hexadecane	0.1120	0.0480	0.0220	0.1820	0.05	25.27
Cedrol	0.0400	0.0010	0.0010	0.0140	0.02	157.14
Cyclopentane, nonyl	0.0010	0.0010	0.0030	0.0017	0.00	58.82
Cyclopentane, undecyl	0.0360	0.0340	0.0280	0.0327	0.00	12.23
Tetradecane, 3-methyl	0.0010	0.0010	0.0200	0.0073	0.01	150.68
Hexadecanol	0.0100	0.0020	0.0020	0.0047	0.00	85.11
Heptadecane	0.0510	0.0050	0.0030	0.0197	0.03	137.06
Methyl tetradecanoate	0.0400	0.0000	0.0530	0.0310	0.03	90.32
Decanoic acid	0.0110	0.0050	0.0000	0.0053	0.01	113.21
Octadecane	0.0440	0.0810	0.0540	0.0600	0.02	31.67
Dimethyl trsulphide	0.0005	0.0014	0.0002	0.0007	0.00	85.71
2-Pentadecanone,6,10,14-trimethyl	0.4060	0.3480	0.5750	0.4430	0.12	26.64
2-heptadecanone	0.0590	0.0630	0.0960	0.0727	0.02	27.51
Nonadecane	0.0250	0.0030	0.0490	0.0257	0.02	89.49
Methyl hexadecanoate	0.2180	0.1570	0.8050	0.3933	0.36	91.02
Phthalic acid,isobutyloctyl ester	0.0080	0.0050	0.0120	0.0083	0.00	48.19
Hexadecanoic acid	0.6160	0.5740	0.3180	0.5027	0.16	32.03
Dibutyl phthalate	0.2460	0.1870	0.0400	0.1577	0.11	67.22
Hexadecanioc acid ethyl ester	0.0160	0.1250	0.3160	0.1523	0.15	99.80
Eicosane	0.0110	0.1100	0.0050	0.0420	0.06	140.48
Methyloctadecanoate	0.0080	0.0490	0.3280	0.1283	0.17	135.62
Octadecanoic acid	0.0030	0.0640	0.0000	0.0223	0.04	161.44
Methyl linoleate	0.1450	0.0520	0.1420	0.1130	0.05	46.02
Tricosane	0.0750	0.0110	0.0010	0.0290	0.04	137.93

4.5 Influence of nitrogen fertilizer rates on volatile flavor compounds

The identities and concentrations of volatile flavor compounds detected in the NERICA varieties varied with nitrogen fertilizer application rates (Table 9). A total of 101 and 104 volatile compounds accumulated at 20 KgN/ha and 60 KgN/ha respectively, with percentage variations of upto 190%. The results showed that increasing soil nitrogen through fertilization led to increase in concentration of the volatile flavor compounds with maximum levels achieved at between 60 and 100 KgN/ha. Beyond 100 KgN/ha, there was a general decrease in concentration of volatile flavor compounds with a few exceptions. It is plausible to argue that nitrogen fertilizer rates of between 60 and 100 KgN/ha provide suitable conditions for biosynthesis of aroma active volatile compounds, but above this rate causes their deteroriation. This agrees with (Suwanarit *et al*., 1996) that the aroma of cooked milled rice of KhaoDawk Mali 105 deteriorated with increased dosages of applied nitrogen of above 100 KgN/ha. High rates of nitrogen fertilizers application reduce the quality of rice including aromatic flavor (Itani *et al*., 2004).

Several nitrogen containing heterocyclic compounds responsible for aroma were detected at fertilizer rates of between 60 KgN/ha to 100 KgN/ha. For instance, the main aroma compound 2AP was detected at nitrogen rates of 60 KgN/ha and 100 KgN/ha with concentrations of 0.0005 and 0.0008 respectively. A study on the aroma chemistry of West African rice cultivars and their inter-specific hybrids failed to detect 2AP amongst the 41 volatiles identified across representative cultivars (Cho *et al*., 2014). Thus, the detection of 2AP in this study could be attributed to the supply of inorganic nitrogen which promotes synthesis of amino acid and increases protein nitrogen; which is responsible for the nitrogen containing aroma compounds (Berendse, 2003). Indeed, to obtain higher concentrations of 2-AP in aromatic rice, it is recommended that the crop be grown with relatively low levels of nitrogen fertilization and harvested earlier than ordinary cultivars (Itani *et al*., 2004). Nitrogen promotes biosynthesis of 2AP by increased electron transport rate and leaf photosynthesis which provides ATP requirements and carbon substrate availability for proline synthesis (Ormeno and Fernandez, 2012). Other nitrogen containing compounds detected in this study included pyrazine 2-ethyl-6-methyl, methyl pyrazine, and pyridine all of which impart a pleasantly sweet aroma to rice (Bryant and McClung, 2011; Yoshihashi, 2002). The relationship between nitrogen availability and accumulation of proline and pyrazine in rice was positive (Andersen *et al*., 1995), and both are bio-indicators of nitrogen intake by plants (Sánchez *et al*., 2002). The findings of this study indicate that nitrogen rates of 60 – 100 KgN/ha is optimum for cultivation of aromatic NERICA varieties. Production of aromatic NERICA varieties is favoured by low nitrogen levels between 60 KgN/ha and 100 KgN/ha.

Table 9. Table of Effect of nitrogen rates on the concentration of volatile flavor compounds

COMPOUND	N-RATES				MEAN	SD	%VAR	COMPOUND	N-RATES				MEAN	SD	%VAR
	20	60	100	140					20	60	100	140			
E,E-2,4-decadienal	0.0992	0.0989	0.5228	0.0694	0.217	0.198	109.84	Pyrazine,2-ethyl-6-methyl	0.0011	0.0000	0.0024	0.0016	0.001	0.001	77.27
Geranyl acetone	0.0951	0.0565	0.4388	0.0538	0.186	0.161	115.53	p-Cymene	0.0237	0.0088	0.0000	0.002	0.011	0.009	124.80
β-ionone	0.0829	0.0792	0.0005	0.0614	0.038	0.056	68.16	o-Cymene	0.0043	0.0307	0.041	0.0197	0.016	0.024	65.73
2-hexanone	0.0027	0.1955	0.1522	0.0055	0.100	0.089	111.90	β-Phellandrene	0.0182	0.0006	0.0011	0.0186	0.010	0.010	105.58
2- pentylfuran	0.0447	0.0779	0.2906	0.0494	0.118	0.116	101.60	Octen-2-one<3E>	0.0079	0.0325	0.0103	0.0286	0.013	0.020	63.25
Limonene	0.0015	0.0008	0.0002	0.0176	0.008	0.005	168.00	Benzene acetaldehyde	0.0323	0.0646	0.0084	0.0038	0.028	0.027	101.99
6-methyl-5-hepten-2-one	0.0192	0.03	0.0076	0.0192	0.009	0.019	48.15	Octylformate	0.0000	0.0068	0.0012	0.0106	0.005	0.005	105.35
2- Acetyl-1-pyrroline	0.0000	0.0005	0.0008	0.0000	0.000	0.000	131.59	Nonanone<2>	0.0374	0.0409	0.0014	0.0168	0.019	0.024	76.80
Benzaldehyde	0.0236	0.0345	0.0826	0.0224	0.028	0.041	69.63	Linalool	0.0299	0.0004	0.0004	0.0213	0.015	0.013	115.13
Acetophenone	0.0002	0.0011	0.0161	0.0007	0.008	0.005	171.68	Benzofuran,2-methyl	0.0008	0.0008	0.0012	0.0044	0.002	0.002	96.86
3-methylpentanol	1.1184	0.8026	0.51	0.9832	0.263	0.854	30.82	6-Dodecanone	0.0002	0.0002	0	0.0002	0.000	0.001	16.67
cyclopentane, ethyl	0.0031	0.0111	0.003	0.0024	0.004	0.005	84.59	Nonanol	0.0156	0.0359	0.0038	0.0015	0.016	0.014	110.77
1H-Pyrrole,1-methyl	0.0008	0.0006	0.0012	0.0014	0.000	0.001	36.51	Terpinen-4-ol	0.0435	0.0003	0.0008	0.0323	0.022	0.019	114.81
3-Methyl-butanal	0.0035	0.0102	0.0102	0.0074	0.003	0.008	40.66	Decene<1>	0.0006	0.0000	0.0018	0.0117	0.006	0.004	157.18
3-Methylhexane	0.005	0.0255	0.0153	0.0097	0.009	0.014	63.45	Furan,2-(2furanylmethyl)-5-methyl	0.0009	0.0038	0.0033	0.0061	0.002	0.004	60.94
Cyclopentane,1,3-dimethyl	0.0021	0.0021	0.0001	0.0772	0.038	0.020	185.76	1H-Pyrrole,1-(2-furanylmethyl	0.0007	0.0011	0.001	0.0006	0.000	0.001	26.45
2,2,7,7-Tetramethyloctane	0.0065	0.0021	0.001	0.0018	0.002	0.003	85.42	Naphthalene	0.0291	0.0014	0.0017	0.0196	0.014	0.013	105.57
2,2,3,5-Tetramethyl heptane	0.0005	0.0161	0.0011	0.0011	0.008	0.005	161.81	2-n-Heptylfuran	0.0015	0.0023	0.0027	0.0007	0.001	0.002	49.27
Ctclopentane,1,2-dimethyl	0.0048	0.0036	0.0006	0.0011	0.002	0.003	80.22	Dodecane	0.0251	0.024	0.002	0.0184	0.011	0.017	61.27
Furan,2,5-dimethyl	0.0048	0.0173	0.0064	0.0017	0.007	0.008	89.30	Methyl chavicol	0.0264	0.0004	0.0044	0.0181	0.012	0.012	98.06
Furan,tetrahydro-2,5-dimethyl	0.0144	0.011	0.009	0.0081	0.003	0.011	26.35	Nonadienal<2E,4E>	0.071	0.052	0.1017	0.072	0.021	0.074	27.67
Vinylfuran	0.001	0.006	0.0009	0.0023	0.002	0.010	23.40	Cyclocytral<beta>	0.0372	0.037	0.0000	0.026	0.018	0.025	69.72
Heptane,2-methyl	0.0044	0.0057	0.0963	0.0094	0.045	0.029	155.00	1,2-Benzothiazole	0.0723	0.0522	0.278	0.0355	0.113	0.110	103.50
Heptane,3-methyl	0.002	0.0066	0.4346	0.0067	0.215	0.113	190.90	Carvacrol, methyl ether	0.0843	0.0806	0.0098	0.0841	0.037	0.065	56.63
Heptane,4-methyl	0.0006	0.0512	0.0083	0.0016	0.024	0.015	156.45	Decanol	0.0016	0.0025	0.0052	0.0072	0.003	0.004	62.39
Isobutyl 2-methylbutanoate	0.0043	0.0041	0.0046	0.0031	0.001	0.004	16.25	1-Cyclohexene-carboxaldehyde	0.0636	0.0021	0.0007	0.0000	0.031	0.017	188.83
1-Pentene	0.0074	0.0004	0.0000	0.0004	0.004	0.002	170.08	Decadienal<2E,4Z)	0.0318	0.0555	0.061	0.0326	0.015	0.045	33.65
3-hexanol	0.0014	0.2027	1.2203	0.0402	0.576	0.366	157.31	Tridecane	0.0529	0.0528	0.0168	0.0391	0.017	0.040	42.12
Hexanal	0.5523	0.5662	0.0337	0.0668	0.294	0.305	96.53	Carvacrol	0.0176	0.0136	0.0704	0.0299	0.026	0.033	78.91
Pyrazine,methyl	0.0008	0.0094	0.0037	0.0053	0.004	0.005	74.75	Thymol	0.0313	0.0048	0.0053	0.0092	0.013	0.013	99.12
Cyclotrisiloxane, hexamethyl	0.0043	0.0142	0.0141	0.0183	0.006	0.013	46.83	Undecanal	0.0021	0.0027	0.0016	0.0586	0.028	0.016	173.23
Furfural	0.0292	0.0223	0.0325	0.0369	0.006	0.030	20.38	Furan,2,3-dihydro-4-methyl	0.0007	0.002	0.0000	0.0011	0.001	0.001	83.47
2,3-Octanedione	0.001	0.0187	0.0026	0.0044	0.008	0.007	121.43	Undecanal<2E>	0.0371	0.0753	0.0116	0.0063	0.031	0.033	96.62

Table 9. Contd...

COMPOUND	N-RATES				MEAN	SD	%VAR
	20	60	100	140			
Tridecen-1-al<2E>	0.0000	0.0012	0.0007	0.0000	0.001	0.001	117.05
Dodecane,2,6,10,trimethyl	0.0034	0.027	0.0003	0.0538	0.025	0.021	117.69
α-Copaene	0.0819	0.0213	0.0058	0.0415	0.033	0.038	87.60
Cubebene<alpha>	0.0008	0.0437	0.0018	0.0026	0.021	0.012	172.10
Tetradecane	0.3494	0.1682	0.142	0.2363	0.093	0.224	41.33
Longifoline	0.0773	0.0814	0.1559	0.0064	0.061	0.080	76.05
b-Caryophyllene	0.1121	0.0049	0.0121	0.0615	0.050	0.048	104.38
α-Cedrene	0.0016	0.0000	0.0081	0.0007	0.004	0.003	143.26
Nonadecane	0.0152	0.1352	0.0000	0.0000	0.065	0.038	174.10
α-Humulene	0.114	0.0862	0.0213	0.0692	0.039	0.073	53.52
Dodecanol	0.0127	0.8728	0.0359	0.0189	0.425	0.235	180.89
γ-Muurolene	0.0000	0.0007	0.0000	0.002	0.001	0.001	134.71
α-Bisabolene	0.0021	0.0004	0.0015	0.0000	0.001	0.001	96.95
β-Bisabolene	0.0000	0.0019	0.0012	0.0023	0.001	0.001	72.02
δ-Cidanene	0.067	0.1042	0.1107	0.0752	0.021	0.089	23.98
Naphthalene,1,6,7-trimethyl	0.0011	0.0006	0.0031	0.0000	0.001	0.001	112.01
Amorphrene delta	0.0515	0.0000	0.0056	0.0024	0.025	0.015	164.59
Tridecane, 7-hexyl	0.0019	0.0007	0.0000	0.0000	0.001	0.001	128.04
Tetratrioctane	0.001	0.0011	0.0007	0.0000	0.000	0.001	70.95
Hexadecene-1	0.1378	0.0575	0.0255	0.0787	0.047	0.075	63.16
Hexadecane	0.0159	0.0412	0.0125	0.0359	0.014	0.026	54.13
Cedrol	0.0432	0.0012	0.0069	0.0044	0.020	0.014	141.41
Cyclopentane, nonyl	0.0000	0.0015	0.0005	0.0047	0.002	0.002	124.17
Cyclopentane, undecyl	0.0778	0.0057	0.0048	0.0431	0.035	0.033	106.01
Hexadecanol	0.0027	0.0051	0.0102	0.0000	0.004	0.005	96.30
Heptadecane	0.0627	0.0547	0.0166	0.0039	0.029	0.035	83.00
Methyl tetradecanoate	0.0042	0.0703	0.0047	0.0000	0.034	0.020	170.37
Decanoic acid	0.0000	0.0102	0.0003	0.0106	0.006	0.005	111.72
Octadecane	0.0671	0.052	0.0261	0.0938	0.028	0.060	47.36
2-Pentadecanone,6,10,14-trimethyl	0.3671	0.7951	0.129	1.7431	0.338	0.430	78.42
2-heptadecanone	0.1434	0.0628	0.0031	0.0807	0.058	0.073	79.43
Nonadecane	0.0312	0.0663	0.0024	0.0015	0.031	0.025	120.42
Methyl hexadecanoate	0.2574	1.849	0.0912	0.1433	0.845	0.585	144.46
Phthalic acid,isobutyloctyl ester	0.006	0.0067	0.0124	0.0064	0.003	0.008	38.36
Hexadecanoic acid	0.5311	0.7494	0.2758	0.4529	0.196	0.502	39.09
Dibutyl phthalate	0.1056	0.1492	0.2769	0.097	0.083	0.157	52.81
Hexadecanioc acid ethyl ester	0.0614	0.3735	0.0164	0.1581	0.159	0.152	104.23
Eicosane	0.0068	0.0075	0.0101	0.0135	0.003	0.010	31.96
Methyloctadecanoate	0.0079	0.4342	0.0064	0.0684	0.205	0.129	158.95
Octadecanoic acid	0.028	0.0258	0.0000	0.0346	0.015	0.021	73.43
Methyl linoleate	0.1677	0.1599	0.064	0.0598	0.059	0.113	52.21
Tricosane	0.0882	0.0078	0.0085	0.0114	0.040	0.030	132.15

4.6 Influence of sites on volatile flavor compounds

The effect of sites on the identities and concentration of volatile flavor compounds is presented on Table 10. Inter-site variations of upto 165% were observed in the concentration of volatile compounds. Maseno site had a total of 104 compounds against Kibos and Oyani with 101 and 100 compounds respectively. Variations in the volatile compounds in the three sites may be attributed to the climatic conditions and inherent soil factors (Appendix 2). Rice aroma is influenced by environmental factors of the location of production and the cultural conditions subjected on the stand crop (Goufo *et al*., 2010). Factors of the site which majorly influence crop yield and associated qualities include inherent soil chemical factors and weather conditions (Hashemi *et al*., 2013), cultural activities, biotic and abiotic stresses and the topography of the area (Goufo *et al*., 2010).

The key aroma compound 2AP was detected in samples from Oyani and Maseno with concentrations of 0.001 and 0.0005 respectively. High amounts of rainfall at Kibos site during grain filling stage (Appendix 1a) could have led to high soil moisture. The heavy black vertisol soils of Kibos have high water retention capacity (Jaetzold *et al*., 2009), and could have led to maximum absorption and transport of the available nutrients including nitrogen fertilizers. High nitrogen level increases protein production (Ibrahim *et al*., 2011) which increases the use of phenylalanine for protein synthesis. Phenylalanine is also used in the biosynthesis of plant secondary metabolites (Margna 1989). This competition could have negatively affected accumulation of secondary metabolites such as 2AP at Kibos. This is because formation of 2AP in rice grains is optimum at low to moderate nitrogen levels (Singh *et al*., 2010). Although the level of phosphorus and other micronutrients of Kibos soils (Table 3) were within the recommended ranges for optimal growth of NERICA varieties (IRRI, 2011), rice grains from the site still lacked important aroma influencing compounds such as 2AP. Soil humidity and sodium chloride concentration have strong correlation with rice fragrance factors (Gaur *et al*., 2010). On the other hand, the amount of rainfall (219 mm) received during grain filling stage at Maseno was comparatively high and the high environmental temperatures (Appendix 2) of the site could have led to low soil moisture which is ideal for accumulation of aroma compounds. Drought stress during grain formation increased 2-AP content (Yoshihashi *et al.* 2002).

Most aldehydes detected in this study were synthesized in higher concentrations in Oyani and Kibos than in Maseno site. Low concetration of aldehydes nonenal, (E, E) 2,4 –decadienal contribute to aroma (Buttery *et al*., 1988). In other studies, shading effects increased the concentration of 2-acetyl-1-pyrroline, (E)-2-hexenal, 1-hexanol, heptanal, octane, 1-heptanol, 1-

octen-3-ol, octanal, benzyl alcohol, benzene acetaldehyde, and 3, 8-dimethylundecane in Yuxiangyouzhan and Nongxiang 18 (Zhaowen Mo *et al.*, 2015). This is in agreement with the results obtained in Maseno and Oyani in which the experimental plots experienced shading effect (Plates 1 and 3). The overall aroma of rice is not the effect one compound but the presence of 2AP and other compounds especially aldehydes tridecen-1-al<2E>, 2E, 4E-nonadienal, hexanal, which were absent in Kibos site. Kibos site was therefore unsuitable for production of aromatic compounds.

Soil analysis results showed that soils in Kibos had a pH of 6.51 which was ideal for NERICA production (Oikeh *et al*., 2013), while the total nitrogen of 0.17- 0.21 % was below the required level (Nwilene *et al*., 2008). Addition of nitrogen to the soil by introducing DAP and CAN during planting and top dressing undertaken in this study increased the percentage of nitrates in the soil. Day/night temperatures of 25/15 ºC during ripening results in better aroma of basmati rice (Bhattacharjee *et al*., 2002), while early transplanting diminishes aroma (Ali *et al*., 1991). Maseno site had a mean temperature of 26.5^{o}C at planting, with maximum day temperatures of 30.5^{o}C and minimum diurnal temperature of 15^{o}C at the grain filling and ripening stages. At Kibos site, day and night temperatures of 31^{o}C and 15^{o}C at ripening were experienced. These temperatures were higher than the recommended day and night temperatures of 25^{o}C and 15^{o}C which favor accumulation of aroma compounds (WARDA, 2010). The concentration of volatile compounds in Japanese aromatic cultivars differed due to location of production and prevailing temperatures (Itani *et al*., 2004). Day and night temperatures of 32^{0} C /18^{0} C recorded in Oyani site during the grain filling, a stage during which most of volatiles accumulate and could easily be lost at elevated temperatures. The elevated temperatures of Oyani and Kibos could have reduced the concentration of VFCs in these sites.

Low soil moisture during grain filling stage enhance accumulation of the major aroma compound 2AP (Yoshihashi *et al*., 2002). Maseno site experienced a maximum rainfall of 61 mm during grain filling stage and a total annual rainfall of 1120.56mm within 117 days, which could have initiated drought stress that led to increased 2-AP content. The low rainfall received in Maseno site could have reduced the soil moisture content hence favoured the accumulation of VFCs. Further, Maseno site at an altidude of 1500m above sea level was suitable for production of aromatic varieties, since high altitude (Nakamura, 1998) and low soil moisture (Yang and Kao, 1999) increase 2-AP concentration in aromatic rice. For superior rice quality, cultivation at a high altitude was recommended (Itani *et al*., 2004). Environmental factors affecting aroma formation in

aromatic rice, such as cool weather during flowering and grain development; fertile soil, direct sowing, production on lighter soils and upland conditions, low soil moisture during grain filling, and manual deshelling were also observed (Singh *et al*., (1997). Light, well drained, slightly acidic soils have a potential for production of aromatic rice.

Soil phosphorus influences terpenoid production since terpenoid precursors (IPP: isopentenyl diphosphate and DMAPP: Dimethylallyl pyrophosphate) contain high-energy phosphate bonds and phosphorus is a key component of ATP and NADPH required for terpenoid synthesis (Ormeno and Fernandez, 2012). Yet, the soil chemical analysis results showed Kibos site had higher available P values than Maseno and Oyani. This explains the accumulation of tepenoids limonene, δ-cidanene, cedrol and β-Phellandrene at Kibos site compared to Maseno and Oyani. Since terpenoids are associated with indirect attraction of natural enemies of rice herbivores (Stam *et al*., 2015; Dicke *et al*., 2010; Hagenbucher *et al*., 2013; Liu *et al*., 2015), NERICA varieties produced in Kibos site have a higher potential for disease and pest resistance compared to those produced in Maseno and Oyani.

Table 10. Table showing the influence of sites on concentration of volatile compounds.

COMPOUND	SITES			MEAN	SD	%VAR	COMPOUND	SITES			MEAN	SD	%VAR
	MAS	KIB	OYA					MAS	KIB	OYA			
E,E-2,4-decadienal	0.049	0.439	0.105	0.1977	0.211	106.73	Furfural	0.013	0.039	0.039	0.0303	0.015	49.50
Geranyl acetone	0.052	0.349	0.082	0.1610	0.163	101.24	2,3-Octanedione	0.005	0.003	0.013	0.0070	0.005	71.43
β-ionone	0.045	0.033	0.090	0.0560	0.03	53.57	Pyrazine,2-ethyl-6-methyl	0.000	0.002	0.002	0.0013	0.001	76.92
2-hexanone	0.150	0.105	0.012	0.0890	0.07	78.65	Tridecen-1-al<2E>	0.001	0.000	0.000	0.0003	0.001	333.33
2- pentylfuran	0.083	0.224	0.076	0.1277	0.083	65.00	Dodecane,2,6,10,trimethyl	0.001	0.020	0.042	0.0210	0.021	100.00
Limonene	0.000	0.013	0.002	0.0050	0.007	140.00	E,E-2,4-decadienal	0.049	0.439	0.105	0.1977	0.211	106.73
6-methyl-5-hepten-2-one	0.009	0.018	0.029	0.0187	0.01	53.48	α-Copaene	0.020	0.046	0.047	0.0377	0.015	39.79
2- Acetyl-1-pyrroline	0.0005	0.000	0.001	0.0003	0.0003	100.00	Cubebene<alpha>	0.002	0.001	0.033	0.0120	0.018	150.00
Benzaldehyde	0.027	0.060	0.035	0.0407	0.018	44.23	Tetradecane	0.279	0.149	0.244	0.2240	0.067	29.91
Acetophenone	0.000	0.012	0.001	0.0043	0.007	162.79	Longifoline	0.022	0.176	0.042	0.0800	0.084	105.00
3-methyl pentanol	1.202	0.394	0.965	0.8537	0.415	48.61	b-Caryophyllene	0.040	0.047	0.056	0.0477	0.008	16.77
Cyclopentane, ethyl	0.003	0.006	0.005	0.0047	0.002	42.55	α-Cedrene	0.001	0.000	0.001	0.0007	0.001	142.86
1H-Pyrrole,1-methyl	0.001	0.001	0.001	0.0010	0.006	60.00	Nonadecane	0.013	0.100	0.000	0.0377	0.054	143.24
3-Methyl-butanal	0.008	0.012	0.004	0.0080	0.004	50.00	α-Humulene	0.044	0.073	0.101	0.0727	0.029	39.89
3-Methylhexane	0.019	0.015	0.008	0.0140	0.006	42.86	Dodecanol	0.660	0.016	0.029	0.2350	0.368	156.60
Cyclopentane,1,3-dimethyl	0.002	0.057	0.001	0.0200	0.032	160.00	γ-Muurolene	0.001	0.001	0.001	0.0010	0.000	0.00
2,2,7,7Tetramethyloctane	0.005	0.001	0.003	0.0030	0.002	66.67	α-Bisabolene	0.001	0.001	0.001	0.0010	0.000	0.00
2,2,3,5Tetramethyl heptane	0.006	0.008	0.000	0.0047	0.001	21.28	β-Bisabolene	0.002	0.001	0.001	0.0013	0.001	76.92
Cyclopentane,1,2-dimethyl	0.003	0.002	0.003	0.0027	0.006	22.22	δ-Cidanene	0.004	0.167	0.097	0.0893	0.082	91.83
Furan,2,5-dimethyl	0.012	0.010	0.000	0.0073	0.007	95.89	Naphthalene,1,6,7-trimethyl	0.001	0.001	0.002	0.0013	0.001	76.92
Furan,tetrahydro-2,5-dimethyl	0.015	0.012	0.005	0.0107	0.005	46.73	Amorphrene delta	0.040	0.003	0.002	0.0150	0.022	146.67
Vinylfuran	0.000	0.003	0.001	0.0013	0.001	76.92	Tridecane, 7-hexyl	0.002	0.000	0.000	0.0007	0.001	142.86
Heptane,2-methyl	0.007	0.073	0.007	0.0290	0.038	131.03	Tetratrioctane	0.002	0.000	0.000	0.0007	0.001	142.86
Heptane,3-methyl	0.004	0.327	0.006	0.1123	0.186	165.63	Hexadecene-1	0.046	0.105	0.074	0.0750	0.03	40.00
Heptane,4-methyl	0.012	0.007	0.001	0.0067	0.006	89.55	Hexadecane	0.032	0.133	0.018	0.0610	0.063	103.28
Isobutyl 2-methylbutanoate	0.004	0.004	0.005	0.0043	0.001	23.26	Cedrol	0.006	0.033	0.003	0.0140	0.017	121.43
1-Pentene	0.001	0.005	0.000	0.0020	0.003	150.00	p-Cymene	0.005	0.020	0.000	0.0083	0.011	132.53
3-hexanol	0.009	0.244	0.005	0.0860	0.137	159.30	Pyridine	0.003	0.000	0.000	0.0011	0.0014	127.27
Dimethyl trisulphide	0.000	0.001	0.000	0.0004	0.0005	125.00	o-Cymene	0.001	0.031	0.040	0.0240	0.021	87.50
Hexanal	0.500	0.000	0.414	0.3047	0.267	87.63	β-Phellandrene	0.002	0.014	0.014	0.0100	0.007	70.00
Pyrazine,methyl	0.003	0.003	0.009	0.0050	0.004	80.00	Octen-2-one<3E>	0.013	0.000	0.046	0.0197	0.024	121.83
Cyclotrisiloxan,hexamethyl	0.009	0.014	0.015	0.0127	0.004	31.50	Benzene acetaldehyde	0.010	0.040	0.032	0.0273	0.016	58.61

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER STUDY

5.1 Summary

- NERICA 4 had the highest chlorophyll content at 140 KgN/ha, while Kibos site produced the highest plant height, chlorophyll content and number of tillers. NERICA 4 is therefore a potentially high yielding upland rice variety at Kibos site.
- NERICA 1 produced the highest number of volatile flavor compounds including 2-AP, which was not synthesized in NERICA 4 and 10. NERICA 1 is therefore potentially an aromatic variety.
- Nitrogen fertilizer rates of 60 to 100 KgN/ha favoured formation of aroma compound 2AP, among other volatile compounds.
- Maseno and Oyani sites had favourable moisture content during grain filling stage, a critical stage at which the aroma compound 2AP maximumly accumulates. This led to production of aromatic NERICA 1.

5.2 Conclusions

- Chlorophyll content of NERICA varieties may be used as a pointer to predict high yielding variety, especially NERICA 4 which showed potential for improved yield.
- Nitrogen fertilizer rate of 140 KgN/ha may be potentially suitable for improved yield of NERICA varieties.
- Kibos site showed a potential for improved yield of NERICA varieties.
- NERICA 1 is aromatic due to the presence of 2AP, while NERICAs 4 and 10 lacked 2AP.
- Maximum accumulation of volatile compounds occured at N-rates between 60 to 100 KgN/ha.
- Maseno and Oyani sites are suitable for production of aromatic NERICA varieties.

5.3 Recommendations

- NERICA 4 is recommended to farmers for cultivation in Lake Victoria basin.
- The recommended rate for cultivation of potentially high yielding NERICA varieties is 140 KgN/ha.

- Kibos site is recommended for increased production of NERICA varieties due to maximum growth parameters.
- Farmers in Lake Victoria basin should be encouraged to cultivate high quality NERICA 1 for improved consumption.
- Maseno and Oyani sites are recommended for cultivation of aromatic NERICA varieties.
- Nitrogen fertilizer rates of 60 to 100 KgN/ha is recommended for cultivation of aromatic NERICA varieties.

5.4 Suggestions for further study

- The correlation between leaf chlorophyll content, plant height and tiller number, and actual yield output need to be ascertained.
- The influence of site factors such as altitude, soil pH and soil moisture on accumulation of aroma compounds should be investigated further.

6.0 REFERENCES

Aduayi, N. (2002). Fertilizer Use and Management Practices for Crops in Nigeria. Federal Fertilizer. Department, Federal Ministry of Agriculture and Rural Development, Abuja Nigeria.

AfricaRice, (2010). New Breeding Directions at AfricaRice; Beyond NERICA. AfricaRice Centre, Cotonou Benin. www.AfricaRice.org (Access date: 17th April, 2013).

AfricaRice, (2015). Annual Report 2015: Investing in rice research and innovation for Africa. AfricaRice Centre, Cotonou Benin. www.AfricaRice.org (Access date: 20th December, 2016).

Agarwaal, O.P. (1998). Chemistry of Natural and Organic Products, 23rd Edn. Goel Publishing House, New Delhi.

Aharoni, A., Jongsma, M.A., Bouwmeester, H.J. (2005). Volatile science? Metabolic engineering of terpenoids in plants. *Trends in Plant Science*, 10: 594–602.

Akparobi, S.O. (2009). Effect of farmyard manures on the growth and yield of *Amaranthus cruentus*. *Agriculture Tropica et Subtropica*, 42: 1-4.

Ali, A., Karim, M.A., Ali, S.S., Majid, A. (1991). Relationship of transplanting time to grain quality in Basmati 385. *International Rice Research Newsletter,* 16: 11-15**.**

Anderson, D.N.S., Orlova, I., Gatto, N., Reichelt, M., Rhodes D., Boland W., Gershenzon, J., (2005). The non-mevalonate pathway supports both monoterpene and sesquiterpene formation in snapdragon flowers. *Proceedings of the National Academy of Sciences*, USA, 102: 933–938.

Anderson, J.M., Ingram, J.S.I. (1993). Tropical Soil Biology and Fertility. A hand book of methods. CAB International, UK. pp: 221

Anjum, S.A., Xie X., Wang, L-C., Saleem, L. F., Man, C., and Lei, W. (2011). Morphological physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, 6(9): 2026-2032.

Apaseku, J.A., Dowbe, N. (2013). Response of NERICA and *sativa* rice lines to nitrogen and phosphorus rates by number of tillers and shoot biomass yield. *African Journal of Food Agriculture, Nutrition and Development*. 13(5): 1-20.

Asante, M.D., Kovach, M.J., Huang, L., Harrington, S., Dartey, P.K., Akromah, R., Semon, M. McCouch, S. (2010). The genetic origin of fragrance in NERICA 1. *Molecular Breeding*, 26: 419–424.

Ashrafuzzaman, M., Islam, M.R., Ismail, M.R., Shahidullah, S.M., Hanafi, M.M. (2009). Evaluation of six aromatic rice varieties for yield and yield contributing characters. *International Journal of Agriculture and Biology,* 11: 616-620.

Atera, E., Kazuyuki, I., Tetsushi, A., Takashige, I. (2007). Response of NERICA Rice to *Striga hermonthica* infections in Western Kenya. *International Journal of Agriculture and Biology*, 2: 271–275.

Beck, H.C., Hansen, A.M., Lauritsen, F.R. (2002). Metabolite production and kinetics of branched-chain aldehyde oxidation in *Staphylococcus xylosus*. *Enzyme and Microbial Technology*, 31: 94–101.

Bekere, W., Urayama, H., Togashi, M. (2014). Growth pattern and yield of NERICA 1 and NERICA 4 rice varieties as a function of split nitrogen application at Tsukuba, *East Japan. Agricultural Forestry and Fisheries*, 3(1): 24–29.

Bergman, C.J., Delgado, J.T., Bryant, R., Grimm, C., Cadwallader, K.R., Webb, B.D. (2000). Rapid gas chromatographic technique for quantifying 2-acetyl-1-pyrroline and hexanal in rice (*Oryza sativa* L.). *Cereal Chemistry*, 77: 454-458.

Bhattacharjee, P., Singhal, R.S., Kulkarni, P.R. (2002). Basmati rice: A review. *International Journal of Food Science and Technology*, 37: 1-12.

Bioversity International, IRRI and WARDA, (2007). Descriptors for wild and cultivated rice (*Oryza* spp.). Bioversity International, Rome, Italy; International Rice Research Institute, Los Banos, Philippines; WARDA, Africa Rice Center, Cotonou, Benin.

Borg-Karlson, A.K., Tengo, J., Valterova, I., Unelius, C.R., Taghizadelh, T., Tolash, T., Francke, W. (2003). (S)-(+)-Linalool, A mite attractant pheromone component in the bee *Colletes cunicularius. Journal of Chemical Ecology*, 29; 1-14

Bounphanousay, C.P., Jaisil, C.P., Fitzgerald, J.M., Hamilton, N.R.S. (2008). Chemical and molecular characterization of fragrance in black glutinous rice from Lao PDR. *Asian Journal of Plant Science*, 7: 1 – 7.

Brady, N.C., Weil, R.R. (2008). The nature and properties of soils. Revised 14th Ed. Pearson Prentice Hall. New Jersey, US.

Bredie, W.L.P., Mottram, D.S., Guy R.C.E. (1998). Aroma volatiles generated during extrusion cooking of maize flour. *Journal of Agricultural and Food Chemistry*, 46 (4) 1479–1487.

BRRI (Bangladesh Rice Research Institute), (2002). Annual Internal Review; Held on 22nd - 26th, December, Soil Science, Bangladesh Rice Research Institute: Gazipur. pp. 3-6, 25-26.

Bryant, R., McClung, A. (2011). Volatile profiles of aromatic and non-aromatic rice cultivars using SPME/GC–MS. *Food Chemistry*, 124 (2): 501-513.

Buttery, R.G., Ling, L.C., Juliano, B.O., Tumbaugh, J. (1983). Cooked rice aroma and 2-acetyl-1-pyrroline. *Journal of Agricultural and Food Chemistry*, 31: 823-826.

Buttery, R.G., Turnbaugh, J.G., Ling, L.C. (1988). Contribution of volatiles to rice aroma. *Journal of Agricultural and Food Chemistry*, 36: 1006–1009.

Champagne, E.T. (2008). Rice aroma and flavor: A literature review. *Cereal Chemistry*, 85: 445–454.

Champagne, E.T., Bett-Garber, K.L., Thomson, J.L., Shih, F.F., Lea, J., Daigle, K. (2008). Impact of pre-soaking on the flavor of cooked rice. *Cereal Chemistry*, 3 (1): 115 – 125

Champagne, E.T., Thompson, J.F., Bett-Garber, K.L., Mutters, R., Miller, J.A., Tan, E. (2004). Impact of storage of freshly harvested paddy rice on milled white rice flavor. *Cereal Chemistry*, 1: 444-449.

Chaunabasappa, K.S., Reddy, B.G.M., Patil, S.G., Kumar, D. (1998). Response of late transplanted rice (*Oryza sativa*) to age, number of seedlings and fertilizers levels. *Indian Journal of Agronomy*, 43(4): 636 – 638.

Charlwood, B.V., Charlwood, K.A. (1998). Monoterpenoids. In: *Methods in Plant Biochemistry*. (Editors; Dey, P.M., Harbonne, J.B.) Hercon Bruce Jovanovich Publishers, New York, pp 43-98.

Chen, S., Yang, Y., Shi, W., Ji, Q., He, F., Zhang, Z., Cheng, Z., Liu, X., Xu, M. (2008). Badh2, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance, *The Plant Cell*, 20: 1850–1861

Cho, S., Nuijten, E., Shewfelt, R.L., Kays S.J. (2014). Aroma chemistry of African *Oryza glaberrima* and *Oryza sativa* rice and their interspecific hybrids. *Journal of the Science of Food and Agriculture*. 94: 727–735.

Costello, P.J., Henschke, P.A. (2002). Mousy off-flavor of wine: Precursors and biosynthesis of the causative N-heterocycles 2-ethyltetrahydropyridine, 2-acetyltetrahydropyridine, and 2-acetyl-1-pyrroline by *Lactobacillus hilgardii* DSM 20176. *Journal of Agricultural and Food Chemistry*, 50: 7079–7087.

Dey, A.K., Sharma, M., Meshram, M.R. (2016). An analysis of leaf chlorophyll measurement method using chlorophyll meter and image processing technique. *Procedia Computer Science*, 85: 286 – 292.

Dicke, M, Baldwin, I.T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science*, 15(3): 167–175.

Dickinson, J.R., Harrison, S.J., Dickinson, J.A., Hewlins, M.J. (2000). An investigation of the metabolism of isoleucine to active amyl alcohol in Saccharomyces cerevisiae. *Journal of Biological Chemistry*, 275: 10937–10942

Domagalska, M.A., Leyser. O., (2011). Signal integration in the control of shoot branching. *National Review of Molecular and cell Biology*, 12(4): 211–221.

Dorward, A., Spencer, D., Abalu, G., Philip, D., Ogungbile, D. (2008). Evaluation of adoption of NERICA and other improved upland rice varieties following varietal promotion activities in Nigeria: A study for the Gates and Rockefeller Foundations, Final Report. School of Oriental and African Studies, University of London.

Dotterl, S., Wolfe, L., Jurgens, A. (2005). Qualitative and quantitative analyses of flower scent from *Silene laifolia*. *Phytochemistry*, 66: 203-213.

Dutta, K.K., Lahira, B.P., Mia, M.A.B. (1998). Characterization of some aromatic and fine rice cultivars in relation to their physico-chemical quality of grains. *Indian Journal,* 1: 61-64

Economic Review of Agriculture, (2010). Central Planning and Monitoring Unit. Ministry of Agriculture - Kenya.

Eller, F.J., Vander, M.R.K., Behle, R.W., Flor, W.L.B., Palmquist, D.E. (2014). Bioactivity of cedar-wood oil and cedrol against arthropods pest. *Environmental Entomology*, PMID: 2469-2522.

Erbilgin, N., Krokene, P., Kvamme, T., Christiansen, E. (2007). A host monoterpene influences Ips typographus (Coleoptera: *Curculionidae, Scolytinae*) responses to its aggregation pheromone. *Agricultural and Forest Entomology*, 9; 135-140.

Espino Luis, (2014). Rice yield components. UC rice blog. Carlifornia rice production. http://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=14826. (Accessed on 23rd Nov, 2015).

FAO. (2007). Rice market monitor. www.fao.org. (Accessed on 22nd October, 2016).

FAO, (2011). The state of food insecurity in the world; how does international price volatility affect domestic economies and food security? www.fao.org (Accessed on 3rd May 2016)

Feibert, E.B.G., Shock, C.C., Saunders, L.D. (1998). Nitrogen fertilizer requirements for potatoes using carefully scheduled sprinkler irrigation. *Horticultural Sciences*, 33(2): 262-265.

Fischer, A.J., Ramirez, H.V., Gibson, K.D., Da Silviers Pinheiro, B. (2001). Competitiveness of semi dwarf upland rice cultivars against Palisade grass (*Brachiaria brizantha*) and Signal grass (*B. decumbens*), *Journal of Agronomy*, 93(5): 967–973.

Fisher C., Scott R.T. (1997). Food flavours biology and chemistry. The Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 4WF, UK, pp 20-24.

Fukuta, Y., Konisho, K., Senoo-Namai, S., Yanagihara, S., Tsunematsu, H., Fukuo, A., Kumashiro, T. (2012). Genetic characterization of rainfed upland New Rice for Africa (NERICA) Varieties. *Breeding Science*, 62: 27–37

Gitonga K. (2016). Kenya Grain and Feed Annual; Corn Wheat and Rice Report. *USDA, Global Agricultural Information Network*, pp 1-6.

Goodwin, H.L., Rister, M.E., Branson, R.E., Stansel, J.W., Webb, B.D., Ward, J.B., Kunz, K. (1992). Market Potential for Domestic Rice Varieties among Asian Americans. TAMRC No. CPM-1- 92T (November). College Station, Texas: Department of Agricultural Economics, Texas A&M University.

Goufo, P., Duan, M., Wongpornchai, S., Tang, X. (2010). Some factors affecting concentration of the aroma compound 2-acetyl-1-pyrroline in two fragrant rice cultivars grown in South China, *Frontier Agriculture*, China, 4; 1–9.

Gu, X., Zhang, Z., Wan, X., Ning, J., Yao, C., Shao, W. (2009). Simultaneous distillation extraction of some volatile flavor components from Pu-erh tea samples - Comparison with steam distillation-liquid/liquid extraction and soxhlet extraction. *International Journal of Analytical Chemistry*, 10:1-6.

Grosch, W. (1982). Lipid degradation products and flavors, In: *Food Flavors. Part A.* (Editors; Morton, I.D., MacLeoad, A.J.) 1st Ed; Amsterdam: Elsevier, pp. 325–398.

Ha, T.Y., Park, S.H., Lee, C.H., Lee, S.H. (1999). Chemical composition of pigmented rice varieties. *Korean Journal of Food Science and Technology*, 31: 336-341

Hagenbucher, S., Olson, D.M., Ruberson, J,R., Wäckers, F.L, Romeis, J. (2013). Resistance mechanisms against arthropod herbivores in cotton and their interactions with natural enemies. *Critical Review of Plant Science*, 32 (6): 458–482.

Haque, M.A., Khaliq, Q.A., Karim, A., Ahmed, J.U., Rahman, M.H. (2004). Nitrogen fertilizer effect on dry matter production and grain growth and aromatic rice. *Journal of Sub-tropical Agricultural Resource Development,* 2(2): 15-19.

Hinge, V., Patil, H., Nadafl, A. (2016). Comparative characterization of aroma volatiles and related gene expression analysis at vegetative and mature stages in Basmati and non-Basmati Rice (*Oryza sativa* L.) Cultivars. *Journal of Applied Biochemistry and Biotechnology*, 178; 619-639.

Hofmann, T., Schieberle, P. (1998). 2-oxopropanal, hydroxy-2-propanone, and 1-pyrroline, important intermediates in the generation of the roast-smelling food flavor compounds 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine. *Journal of Agricultural and Food Chemistry*, 46:2270–2277.

Horner, D.S., Muñoz-Amatriaín, M., Muehlbauer, G.J., Rossini, L. (2014). Genetics of tillering in rice. (Editor; Hussien, B. A.), Elahe-Tavakol, India, pp. 1-50.

Hu, H., Liu, H.Q., Zhang, H., Zhu, J.H., Yao, X.G., Zhang, X.B., Zheng, K.F., (2010). Assessment of chlorophyll content based on image colour analysis. *Proceedings of 2nd International Conference on Information Engineering and Computer Science* (ICIECS), Wuhan, China. 185-190.

Huang, T.C., Huang, Y.W., Hung, H.J., Ho, C.T., Wu, M.L. (2007). Δ1-Pyrroline-5-carboxylic acid formed by proline dehydrogenase from the *Bacillus subtilis* ssp. natto expressed in Escherichia coli as a precursor for 2-acetyl-1-pyrroline. *Journal of Agricultural and Food Chemistry*, 55: 5097-5102.

Huang, T.C., Teng, C.S., Chang, J.L., Chuang, H.S., Ho, C.T., Wu, M.L. (2008). Biosynthetic mechanism of 2-acetyl-1-pyrroline and its relationship with pyrroline-5-carboxylic acid and methylglyoxal in aromatic rice callus. *Journal of Agricultural and Food Chemistry*, 56: 7399–7404.

IRRI (International Rice Research Institute). (2002). Annual report 2001. Los Baños (Philippines): International Rice Research Institute. pp. 1-24

IRRI (International Rice Research Institute). (2011). Annual report 2010. Los Baños (Philippines): International Rice Research Institute. pp. 1-44

Itani, T., Tamaki, M., Arai, E. Horino, T. (2004). Distribution of amylose, nitrogen, and minerals in rice kernels with various characters. *Journal of Agricultural and Food Chemistry*, 50: 5326-5332.

Jaenson, T.G., Palsson K., Borg-karlson A.K. (2007). Evaluation of extracts and oils of mosquito (Diptera: Culicidae) repellent plants from Sweden and Guinea Bissau. *Journal of Medical Entomology*, 43; 113-119.

Jaetzold, R., Schmidt, H., Hortnez B. and Shisanya, C. (2007). Ministry of Agriculture: Farm management handbook of Kenya Vol. 2 - Natural conditions and farm management information. 2nd Edition, Part A. West Kenya (Nyanza Province), pp 1 – 81.

Jezussek, M., Juliano, B.O., Schieberle, P. (2002). Comparison of key aroma compounds in cooked brown rice varieties based on aroma extract dilution analyses. *Journal of Agricultural and Food Chemistry*, 50: 1101–1105.

Jones, M., Dingkuhn, M., Aluko, G., Semon, M. (1997). Interspecific *Oryza Sativa* L. X *O. glaberrima* Steud. Progenies in upland rice improvement. *Euphytica*, 94: 237–246

Juliano, B.O., Onate, L.U., del Mundo, A.M. (1965). Relation of starch composition, protein content, and gelatinization temperature to cooking and eating qualities of milled rice. *Food Technology*, 19: 1006-1011.

Kamau, N., Mercy, B., Olwande, J., Githiku, J. (2010). *Consumption and Expenditures of Key Food Commodities and Food Security in Urban Households: The Case of Nairobi and Its Environs.* URL: http://www.tegemeo.org. Accessed on 21st October, 2016.

Karl-Georg, F., Franz-Josef, H., Johannes, P., Wilhelm, P., Dietmar, S., Kurt, B., Dorothea, G., Horst, S. (2003). Flavors and Fragrances - Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, 11-141.

Kebrom, T.H., Richards, R. (2013). Physiological perspectives of reduced tillering and stunting in the tiller inhibition (tin) mutant of wheat. *Functional Plant Biology*, 40 (10): 977 – 978.

Kebrom, T.H., Spielmeyer, W., Finnegan. E.J. (2013). Grasses provide new insights into regulation of shoot branching. *Trends in Plant Science,* 18 (1): 41–48.

Kennedy, G., Burlingame, B. (2003). Analysis of food composition data on rice from a plant genetic resources perspective. *Food Chemistry*, 80 (4): 589-596.

Khan, Z.R., Midega, C.A.O., Amudavi, D.M., Hassanali, A., Pickett, J.A. (2008). On farm evaluation of the push-pull technology for the control of stem borers and striga weed on maize in western Kenya. *Field Crops Research*, 106; 224-233.

Kimani, A.W., Amm, G., Kiarie, N., Ikahu, J.K., Kimani, J.M. (2010). Factors leading to the deterioration of aroma and grain quality in Kenyan Basmati rice. *Kenya Agricultural Research Institute, Mwea.* Newsletter 1 – 5.

Kinyumu, D.M., Maruyama, S., Hayashi, H., Kato M. (2009). Comparative study on the growth and yield of NERICA cultivated with organic and inorganic fertilizers: Participatory on-farm research at Marakwet District in Kenya. *Journal of Developments in Sustainable Agriculture,* 4; 106-117.

Knudsen, J.T., Eriksson, R., Gershenzon, J., Stahl, B. (2006). Diversity and distribution of floral scent. *Botanical Review*, 72: 1–120.

Kore, W.O., Onyango, G., Omuga, G. (2007). NERICA on-farm trials. *Proceedings of the 5th Workshop on the Promotion of New Rice for Africa (NERICA) in Kenya*, 26th -27th February. African Institute for Capacity Development (AICAD), pp 13-14.

Kramer, P.J., John S.B., (1995). Water Relations of Plant and Soils, 1st Edition, Academic Press, Elsevier. pp 1-495.

Krishnan, P., Ramakrishnan, B., Reddy, K.R., Reddy, V.R. (2011). High-temperature effects on rice growth, yield, and grain quality. In Editor; Sparks, D. L.) *Advances in Agronomy*. 111, Academic Press, Burlington. pp. 87-206.

Kumar, M., Singh, R.P., Rana, N.S. (2003). Effect of organic and inorganic sources of nutrition on productivity of rice *(Oryza sativa). Indian Journal of Agronomy*, 48 (3): 175-177

Kwach, B.O., Owuor, P.O., Kamau, D.M., Msomba S.W., Uwimana, M.A. (2016). Variations in the precursors of plain black tea quality parameters due to location of production and nitrogen fertilizer rates in Eastern African clonal tea leaves. *Experimental Agriculture,* 52 (2): 266–278.

Lam, H. S., Proctor, A. (2003). Milled rice oxidation volatiles and odor development. *Journal of Food Science,* 68: 2676–2681.

Li, X.Y., Qian, Q., Fu, Z.M., Wang, Y.H., Xiong, G.S., Zeng D.L., Wang X.Q., Liu, X.F., Teng, S., Hiroshi, F., Yuan, M., Luo, D., Han B., Li, J.Y. (2003). Control of tillering in rice. *Nature*, 422(6932): 618–621.

Lindsay, W.L.; Norvell, W.A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, 42: 421-428.

Linquist, B., Sengxua, P. (2001). Nutrient management in rainfed lowland rice in the Lao PDR. International Rice Research Institute, Los Banos (Philippines). In (Editors; Hardy B). pp 60.

Liu, Q., Romeis, J., Yu, H., Zhang, Y., Li, Y., Peng, Y. (2015). *Bt* rice does not disrupt the host-searching behavior of the parasitoid *Cotesia chilonis*. *Scientific Reports*. 5; 15295-15296

Lou, Y., Zhang, G., Zhang, W., Hu, Y., Zhang, J. (2013). Biological control of rice insect pests in China. *Biological Control*, 67 (1): 8–20

Lorieux, M., Petrov, M., Huang, N., Guiderdoni, E., Ghesquiere, A. (1996). Aroma in rice: Genetic analysis of quantitative trait. *Theoretical and Applied Genetics,* 93; 1145-1151.

Maga, J.A. (1984). Rice product volatiles: A review. *Journal of Agricultural and Food Chemistry*, 32; 964-970.

Mahanta P.K., Baruah, S., Owuor, P.O., Murai T., (1988). Flavour volatiles of Assam black teas manufactured from different plucking standards and orthodox teas manufactured

from different altitudes of Darjeeling. *Journal of Science Food and Agriculture*, 45; 317-324.

Mahattanatawee, K., Rouseff, R.L. (2010). 2-acetyl-2-thiazoline; a new character impact volatile in Jasmine rice. *In Expression of Multidisciplinary Flavour Science,* 475-478.

Mann, J. (1978). Secondary Metabolism. Oxford University Press, New York, pp. 241.

Maraval, I., Mestres, C., Pernin, K., Ribeyre, F., Boulanger, R., Guichard, E., Gunata, Z. (2008). Odor-active compounds in cooked rice cultivars from Camargue (France) analyzed by GC-O and GC-MS. *Journal of Agricultural and Food Chemistry,* 56; 5291–5298.

Marsili, R. (2007). Application of sensory-directed flavor-analysis techniques. In: (Editor. Marsili, R.) *Sensory-Directed Flavor Analysis*. Boca Raton, FL: CRC/Taylor & Francis. pp. 55-80.

Martinez-Valverde, I., Periago, M.J. Ros, G. (2000). Nutritional importance of phenolic compounds in the diet. *Archives of Latinoam Nutraceuticals*. 2000, 50; 5-18.

Mc'Garvey, D.J., Croteau, R. (1995) Terpenoid metabolism. *Plant cell.* 7; 1015-1026.

Metcalf, R.L., Kogon, M. (1987). Plant volatiles as insect attractants. *Critical Reviews in Plant Sciences,* 5: 251-301.

Van Moerkercke, A., Galvan-Ampudia, C.S., Verdonk, J.C., Haring, M.A., Schuurink, R.C. (2012). Regulators of floral fragrance production and their target genes in petunia are not exclusively active in the epidermal cells of petals. *Journal of Experimental Botany,* 63: 3157–3171.

Mohsen, Z.H., Ali, Y., Al-Chalabi, B.M. (1995). Insecticidal effects of acetophenone against *Culex quinquefasciatus* (Diptera: Culicidae). *Japanese Journal of Sanitary Zoology,* 46: 405-408.

Mokuwa, A., Nuijten, E., Okry, F., Teeken, B., Maat, H., Richards, P. and Struik, P.C. (2012). Robustness and strategies of adaptation within farmer varieties of African rice (*Oryza glaberrima*) and Asian rice (*Oryza sativa*) across West Africa. *PLoS ONE,* 8(3); 34801-34802.

Moriwaki, T. (1999). On the ideal culm elongation in rice plants (3). *Nogyogijyutu,* 54; 133-138.

Mwai, G.N., (2001). Growth responses of spiderplant (*Cleome gynandra L.*) to salinity. Msc Thesis, Maseno University, Kenya.

Nakamura, A. (1998). Breeding and cultivation of aromatic rice and brewer rice in Kochi Prefecture. *Nogyooyobi Engei,* 73; 887-895.

Newmark, H.L. (1996). Plant phenolics as potential cancer prevention agents. *Advanced Experiments on Medical Biology,* 401; 25-34.

NIST/EPA/NIH. (2008). NIST Mass Spectral Library. National Institute of Standard Technology, US Secretary of Commerce, U.S.A.

Nwilene, F.C., Oikeh, S.O., Agunbiade, T., Oladimeji, O., Ajayi, O., Sie, M., Gregorio, G.B., Togola, A., Toure, A.D. (2008). Growing lowland rice. Production hand book Africa Rice Center. Cotonou, Benin. 40: pp 670-701.

Odak, J.A., Owuor, P.O., Manguro, L. A. O., Cheramgoi, E., Wachira, F.N. (2016). Variability of overhead volatile organic compounds in clonal tea (*Camellia sinensis*) and their influence on red crevice mite (*Brevipalpus phoenicis Geijskes*) infestations**.** *International Journal of Tea Science*, 12 (1&2); 1-14.

Oikeh, S., Touré, A., Sidibé, B., Niang, A., Semon, M., Sokei, Y., Mariko, M. (2009) Responses of upland NERICA rice varieties to nitrogen and plant density, *Archives of Agronomy and Soil Science,* 55(3); 301-314.

Oikeh, S.O., Moussa, S., Diata, S., Osiname, O., Toure A., Nwiline, F., Okeleye A. (2008). Responses of upland NERICA rice to nitrogen and phosphorus in forest agro-ecosystems. *Journal of Agronomy,* 3; 100 – 101.

Ojiem, J.O., Vanlauwe, B., de Ridder, N. and Giller, K.E. (2001). Niche based assessment on contribution of legumes to the nitrogen economy in Western Kenya smallholder farms. *Plant and Soil,* 292; 119 – 135.

Okalebo, J.R., Gathua, K.W., Woomer, P.C. (2002), Laboratory methods of soil and plant analysis: a working manual. TSBF Programme, Nairobi, pp 36-37.

Olembo, N., M'mboyi, F., Oyugi, K. (2010). Success Stories in Crop Improvement in Africa: The case of NERICA in sub-Saharan Africa. African Biotechnology Stakeholders Forum (ABSF), Nairobi.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A. (1954). Estimation of Available phosphorus in soils by extraction with sodium bicarbonate, USDA, Washington DC.

Owuor, P.O., Obanda, M. (1997). The effects of some agronomic and processing practices and clones on the relative composition of the individual theaflavins in black tea. *International Food Science and Technology*, 3; 344–347.

Owuor, P.O., Kamau, D.M., Kamunya, S.M., Msomba, S.W., Uwimana, M. A., Okal, A.W., Kwach, B.O. (2011). Effects of genotype environment and management on yields and quality of black tea. In. *Genetics Biofuels and Local Farming Systems: Sustainable Agriculture Reviews*, (Editors; Lichthuse, E.), S*pring*, Heidelberg, pp 277-307.

Owuor, P.O., Obaga, S. O., Othieno, C.O. (1990). Effects of altitude on the chemical composition of black tea. *Journal of the Science of Food and Agriculture*, 50; 9-17.

Owuor, P.O., Tsushida, T., Aorita, H., Murai, T. (1988). Effects of geographical areas of production on the composition of volatile flavour compounds of Kenyan clonal black CTC teas. *Experimental Agriculture*, 24; 227-235.

Pang, J., Cuin, T., Shabala L., Zhou, M., Mendham, N., Shabala S. (2007). Effect of secondary metabolites associated with anaerobic soil conditions on ion fluxes and electrophysiology in barley roots. *Plant Physiology*, 145(1); 266–276.

Park, J.K., Kim, S.S., Kim, K.O. (2001). Effect of milling ratio on sensory properties of cooked rice and on physicochemical properties of milled and cooked rice. *Cereal Chemistry*, 78; 151-156.

Pascual, V.J., Wang, Y.M. (2016)**.** Utilizing rainfall and alternate wetting and drying irrigation for high water productivity in irrigated lowland paddy rice in Southern Taiwan. *Plant Productivity in Science*, 19; 1–12.

Perez, C.M., Juliano, B.O., Liboon, S.P., Alcantara, J.M., Cassman, K.G. (1996). Effect of late nitrogen fertilizer application on head rice yield, protein content and grain quality of rice. *Cereal Chemistry*, 73; 556-560.

Porteres, R. (1950). Homologous intraspecific articulation and monophylctic origin of *Oryza sativa* and *O. glaberrima*. *Reviue Botanique Applique Agronomie Tropicale*, 30; 147–157.

Rahman, M.M., Amano, T., Shiraiwa, T. (2009). Nitrogen use efficiency and recovery from N fertilizer under rice based cropping systems. *Australian Journal of Crop Science*, 3(6); 336-351.

Reineccius, G. (2006). *Flavor Chemistry and Technology*. Taylor & Francis Group, Boca Raton, Florida, pp. 391–418.

Reineccius, G.A. (2000). Recent developments in academic flavour research**.** In: Flavour Chemistry: Industrial and Academic research. (Editors; Risch, S.J., Ho, C.T.) ACS Symposium Series No**.** 756, *American chemical society:* Washington D.C. pp 13-23.

Rister, M.E., Goodwin, H.L., Branson, R.E., Stansel, J.W., Webb, B.D. (1992). The U.S. aromatic rice market: Results of an Asian-American household test. In: Proceedings of the 24th Rice Technology Work Group, Little Rock, Ark College Station, Texas: Texas A&M University. 23-26 February 1992, pp. 22-31.

Robinson, T.M., Owuor, P.O. (1997). Tea aroma. In *Tea: Cultivation to Consumption.* (Editors: Wilson. K.C., Clifford, M.N.) Chapman and Hall, London, 1st Edition chapter 18 pp 603-647.

Rohilla, R., Singh, V.P., Singh, U.S., Singh, R.K., Khush, G.S. (2000). Crop husbandry and environmental factors affecting aroma and other quality traits. pp. 201-216. In: *Aromatic Rices,* (Editors: Singh, R.K., Singh, U.S., Khush, G.S.) *Science Publishers, Inc*. Enfield. NH.

Ronoubigouwa, A.A.M. (2008). Characteristics of NERICA in Two Different Ecosystems (Upland and Lowland). A Master Degree Dissertation, Presented to Utsunomiya University Graduate School of Agricultural Science, pp 1-78.

Russell, G.B., Hunt, M.B., Bowers, W.S., Blunt, J.W. (1994). A sesquiterpenoid and relellent from *Dysoxylum spectable*. *Phytochemisty,* 35; 1455-1456.

Sakthivel, K., Sundaram, R.M., Shobha, N.R., Balachandran, S.M., Neeraja C.N. (2009). Genetic and molecular basis of fragrance in rice. *Biotechnology Advances,* 27; 468–473.

Salam, M.A., Ali, F., Anwar, M.P., Bhuiya, M.S.U. (2004). Effect of level of nitrogen and date of transplanting on the yield and yield attributes of transplanted *Aman*rice under SRI method. *Journal of Bangladesh Agricultural University,* 2(1): 31-36.

Sampaio, B.L., Edrada-Ebel, R., Da Costa, F.B. (2016). Effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*: a model for environmental metabolomics of plants. *Scientific Reports,* 6; 29265- 29331.

Sarkar, S.K., Sarkar, M.A.R., Islam N., Paul S. K., (2014). Yield and quality of aromatic fine rice as affected by variety and nutrient management. *Journal of Bangladesh Agriculture University,* 12(2); 279–284, ISSN 1810-3030.

Schieberle, P. (1990). The role of free amino acids present in yeast as precursors of the odorants 2-acetyl-1-pyrroline and 2acetyltetrahydropyridine in wheat bread crust. *Zeitschrift für LebensmittelUntersuchung und Forschung*, 191; 206-209.

Schirack, A.V., Drake, M.A., Sanders, T.H., Sandeep, K.P, (2006). Characterization of aroma-active compounds in microwave blanched peanuts. *Journal of Food Science,* 71; 513–520.

Sharma, A.R., Singh, D.P. (1999). Rice crop yield physiology and process. Springerverlag Berlin. Heidelberg: Editors. Smith, D.L., Hamel, C. pp. 109-138.

Shivay, Y.S., Singh, S. (2003). Effect of planting geometry and nitrogen level on growth, yield and nitrogen use efficiency of scented hybrid rice *(Oryza sativa). Indian Journal of Agronomy*. 48(1); 42-44.

Sides, A., Robards, K., Helliwell, S. (2000). Developments in extraction techniques and their application to analysis of volatiles in foods. *Tracking-Trends in Analytical Chemistry*, 19(5); 322-329.

Sidhue, M.S. Sikka, R., Singh, T. (2004). Performance of transplanted Basmati rice in different cropping systems as affected by N application. *International Rice Resource Notes*, 29(1); 63-65.

Sikuku, P.A., Kimani, J.M., Kamau, J.W., Njinju, S. (2016). Influence of nitrogen supply on photosynthesis, chlorophyll content and yield of improved rice varieties under upland conditions in Western Kenya**.** *American Journal of Experimental Agriculture*, 13(2); *1-14.*

Sikuku, P.A., Netondo, G.W., Onyango, J.C., Musyimi, D.M. (2010). Chlorophyll fluorescence, protein and chlorophyll content of three NERICA rain-fed rice varieties under varying irrigation regime. *Agricultural and Biological Science,* 5(2); 19-25.

Singh, R.K. Singh, R.U. (1997). 'Indigenous scented rice; farmers' perceptions and commitment'. *Paper presented at International Conference on Creativity and Innovation at Grassroots*, January 14-17, 1997 at IIM, Hyderabad.

Solomons, G.T.W. (1997). Fundamentals of Organic Chemistry, 5th Edn. John Wiley & Sons, New York, pp. 950-970.

Srivong, P., Waisalong, P., Wangsomnuk, P., Pongdontri, P. (2007). Comparison of aroma level of Khao Dok Mali-105 and its mutants by expression level of partial *Badh2* gene. *Poster Presentation in 1st BMB conference*: Biochemistry and Molecular Biology for the integration of life. May, 2007.

Suwanarit, A., Kreetapirom, S., Buranakarn, S., Varanyanond, W., Tungtrakul, P., Somboonpong, S., Rattapat, S., Ratanasupa, S., Romyen, P., Wattanapryapkul, S., Naklang, K., Rotjanakusal, S., Pornurisnit, P. (1996). Effect of nitrogen fertilizer on grain qualities of KhaoDawk Mali-105. *Kasetsart Journal of National Science*, 30; 458-474.

Stam, J.M., Kroes, A., Li, Y., Gols, R., van Loon, J.J., Poelman, E.H., Dicke, M. (2014). Plant interactions with multiple insect herbivores: from community to genes. *Annual Review of Plant Biology*, 65(1); 689–713.

Suwansri, S., Meullenet, J.F., Hankins, J. A., Griffin, K. (2002). Preference mapping of domestic/imported Jasmine rice for U.S.-Asian consumers. *Journal of Food Science*, 67; 2420-2431.

Tabo, A.R., Gweyi J.O., Thagana W. (2015). Effects of varietal differences, plant spacing and weeding regimes on weed density and yields of upland rice in Uganda, *NARO Bulletin*, pp 30-39.

Terashi, R., Saima, K. (1993). Bioactive Volatile Compounds from Plants: An overview: *American Chemical Society Symposium Series*, 525; 1-2.

Thomas, G.W., Peaslee, D.W., (1996). Testing soils for phosphorus. Editors, Walsh, L.M., Beaten J.D. *Soil Testing and Plant Analysis*, Book 3. SSSA Madison. pp. 115-132.

Tisdale, S.L., Nelson, W.L., Beaton, J.D., Halvin, J.L. (1993). *Soil Fertility and Fertilizers* 5th Edition. Prentice Hall Inc. Upper Saddle River, New Jersey. pp 634.

Tok S. Y., (2007). Rice flavor chemistry. A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy. University of Georgia. pp 121

Tsugita, T. (1986). Aroma of cooked rice. *Food Review International*, 1; 497-520.

Tsuzuki, E., Tanaka, K., Shida, S. (1981). Aromatic rices. *Bulletin for Faculty of Agriculture, Miyazaki University*, 28; 31-37.

United States Department of Agriculture, Foreign Agricultural Service (2016). Grains: world production, markets and trade. www.fas.usda.gov/data/world-grain-markets. Accessed on 9th June, 2016.

Vanavichit, A., Yoshihashi, T., Wanchana, S., Areekit, S., Saengsraku, D., Kamolsukyunyong, W., Lanceras, J., Toojinda, T., Tragoonrung, S. (2006). Positional cloning of *Os2AP*, the aromatic gene controlling the biosynthetic switch of 2-acetyl-1-pyrroline and gamma aminobutyric acid (GABA) in rice (abstract). In: 5th *International Rice Genetics Symposium*. Manila, Philippines. Newsletter, 10: 15.

Van Moerkercke, A., Galvan-Ampudia, C.S., Verdonk, J.C., Haring, M.A, Schuurink, R.C. (2012). Regulators of floral fragrance production and their target genes in petunia are not exclusively active in the epidermal cells of petals. *Journal of Experimental Botany,* 63; 3157–3171.

Wang, Y., Li. J., (2008). Molecular basis of plant architecture. *Annual Review of Plant Biology,* 59; 253–279.

WARDA FAO/SAA. NERICA the New Rice for Africa – A Compendium. Cotonou Sasakawa Africa Association. Tokyo, Japan. p 210.

West Africa Rice Development Association (WARDA), (2008). NERICA the New Rice for Africa. Rice Production Hand book, Cotonou, Benin. pp 72

West African Development Association (WARDA), (2001). 'NERICA rice for life'. http://www.warda.org/ publications/NERICA 8.pdf, (Date accessed; October, 2015).

Widjaja, R., Craske, J.D., Wootton, M. (1996). Comparative studies on volatile components of non-fragrant and fragrant rices. *Journal of Food Science and Agriculture*, 70(2); 151-161

Wongpornchai, S., Dumri, K., Jongkaewwattana, S., Siri, B. (2004). Effects of drying methods and storage time on the aroma and milling quality of rice (*Oryza sativa L.*) cv. KhaoDawk Mali 105. *Food Chemistry*, 87; 407– 414.

Yajima, I., Yanai, T., Nakamura, M., Sakakibara, H., Habu, T. (1978). Volatile flavor components of cooked rice. *Agricultural and Biological Chemistry*, 42(6); 1229-1233

Yang, C.W., Kao, C.H. (1999). Importance of ornithine-amino transferase to proline accumulation caused by water stress in detached rice leaves. *Plant Growth Regulation,* 27; 189-192.

Yang, D.S., Lee, K. S., Jeong, O. Y., Kim, K. J., Kays, S. J. (2008). Characterization of volatile aroma compounds in cooked black rice. *Journal of Agricultural and Food Chemistry,* 56; 235–240.

Yang, D.S., Lee, K.S., Jeong, O.Y., Kim, K.J., Kays, S. J. (2007). Volatile flavor chemistry of black rice using dynamic headspace, multivariate analysis and GC-O. *Journal of Agriculture and Food Chemistry,* 31; 411 -423

Yang, D.S., Shewfelt, R.L., Lee, K.S., Kays, S.J. (2008). Comparison of odor-active compounds from six distinctly different rice flavor types. *Journal of Agricultural and Food Chemistry*, 56; 2780–2787.

Yang, L. S., Zhang, X. W., Li, Y. H., Li, H. R., Wang, Y., Wang, W. Y. (2012). Bio-accessibility and Risk Assessment of Cadmium from Uncooked rice using an in vitro digestion model. *Biological Trace Element Research*, 145 (1); 81-86.

Yoshida, S. (1981). *Fundamentals of Rice Crop Science*. International Rice Research Institute, Los Banos, Philippines. pp 41-248.

Yoshihashi, T., Nguyen, T.T.H., Kabaki, N. (2004). Area dependency of 2-acetyl-1-pyrroline content in an aromatic rice variety, KhaoDawk Mali 105. *Japanese Agriculture Research Quarterly,* 38; 105 - 109.

Zhang, C.F., Peng, S.B., Bennett, J. (2000). Glutamine synthetase and its isoforms in rice spikelets and rachis during grain development. *Journal of Plant Physiology,* 156(2); 230-233.

Zhang, Y.R., Wang, R., Lyn, F.Y., Lu, P.F., Luo, Y.Q. (2015). Identification of caragana plant volatiles, overlapping profiles, and olfactory attraction to *Chlorophrus caragana* in the laboratory. *Journal of Plant Interractions*, 10; 41-50

Zhenwei, S., Hongjun, G., Cheng, P., Aixing, D., Chengyan, Z., Mannaf, M.D.A., Islam, M.N.M., Weijan, Z. (2015). Organic amendments increase corn yield by enhancing soil resilience to climate change. *The Crop Journal*, 3; 110-117.

Zhou, Z., Robards, K., Helliwell, S., Blanchard, C. (2003). Composition and functional properties of rice. *International Journal of Food Science and Technology,* 37; 849-868.

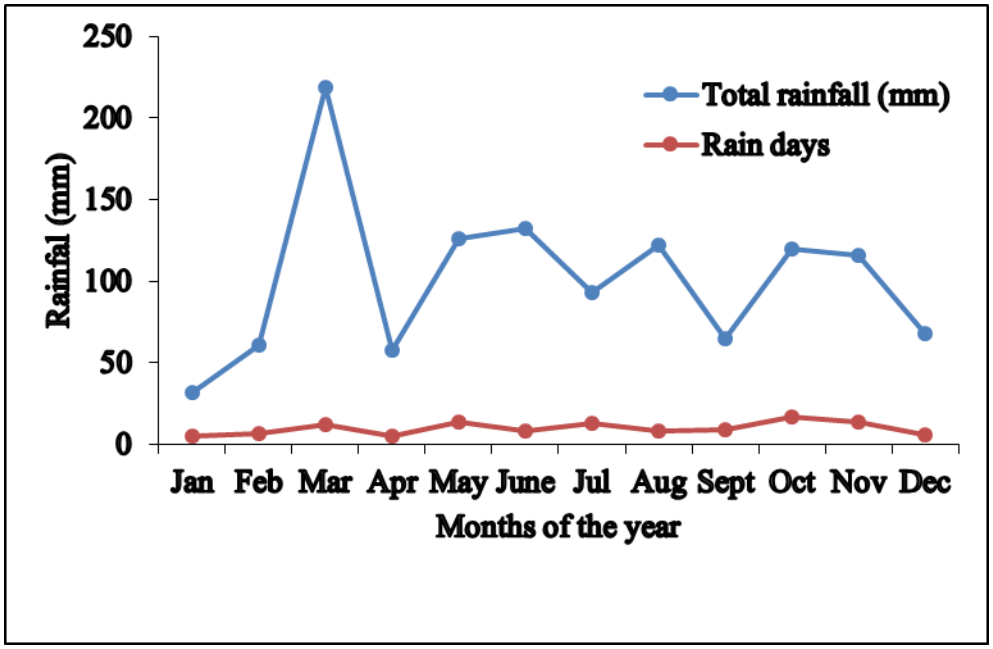
Zhou, G., Wang, X., Yan, F., Wang, X., Li, R., Cheng, J., Lou, Y. (2011). Genome-wide transcriptional changes and defence-related chemical profiling of rice in response to

infestation by the rice striped stem borer *Chilo suppressalis*. *Journal of Plant physiology*, 143(1); 21–40.

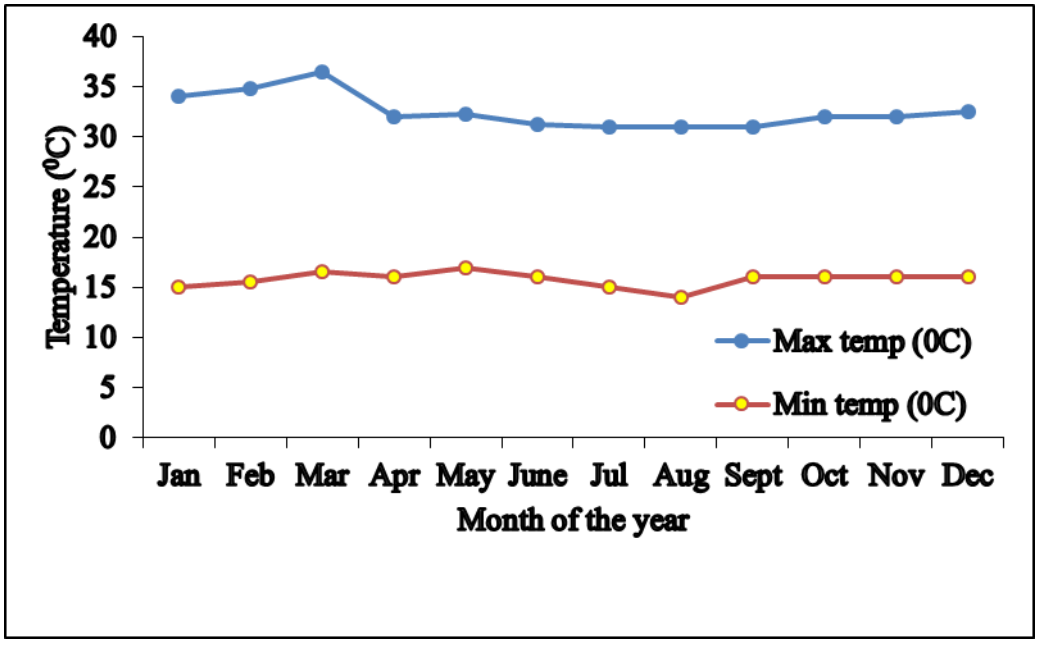
APPENDICES

Appendix 1: Weather data for the Kibos, Oyani and Maseno agro-ecological sites in which NERICA 1, 4 and 10 were cultivated.

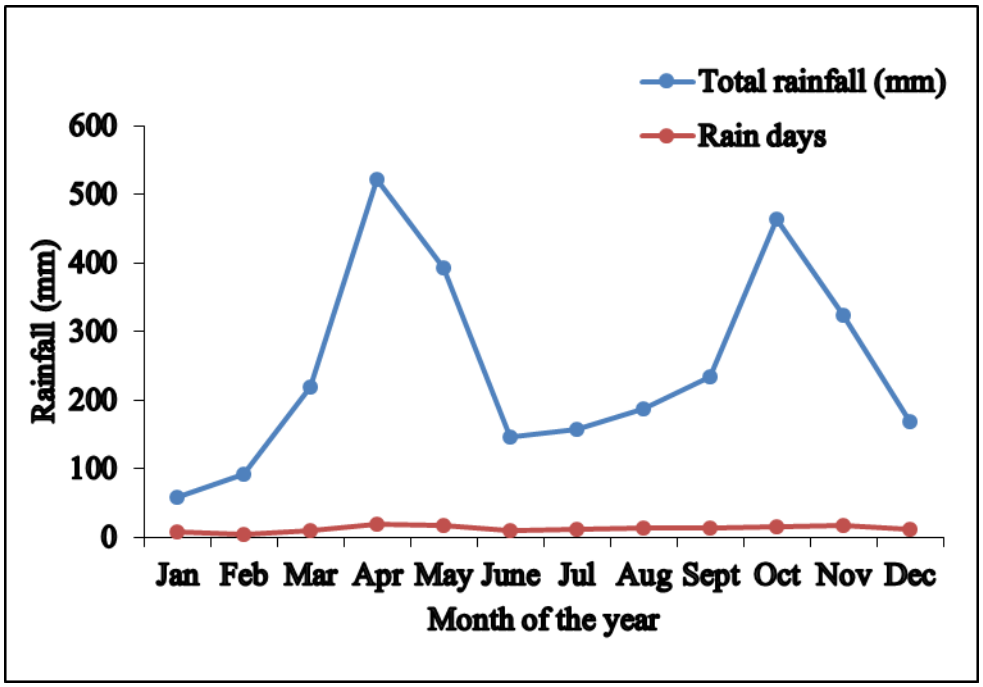
Appendix 1a: Average precipitation at Kibos in the year 2014



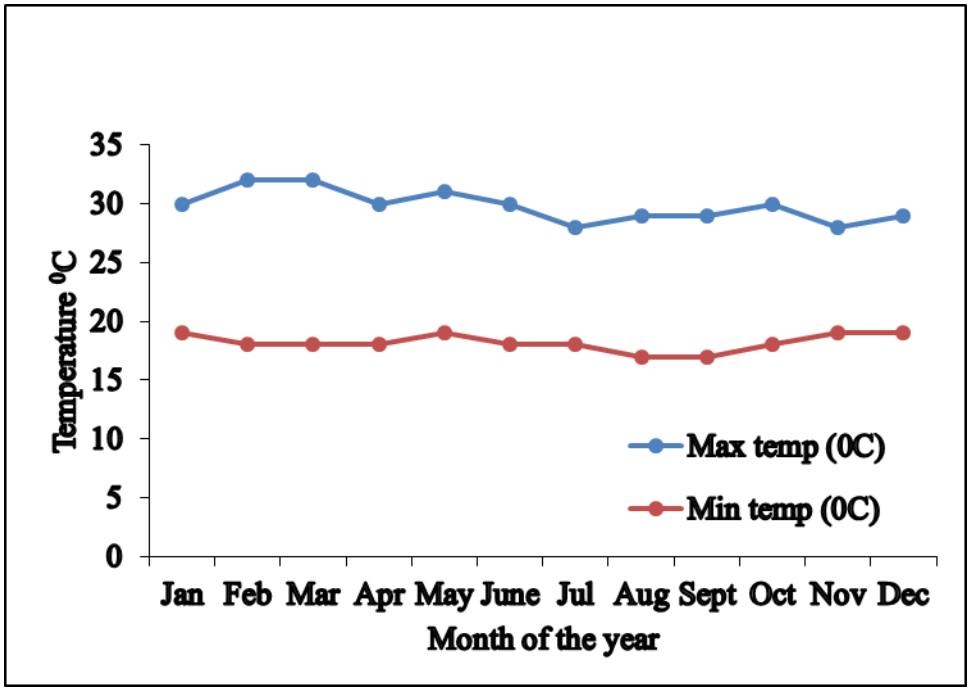
Appendix 1b: Average temperature at Kibos in the year 2014



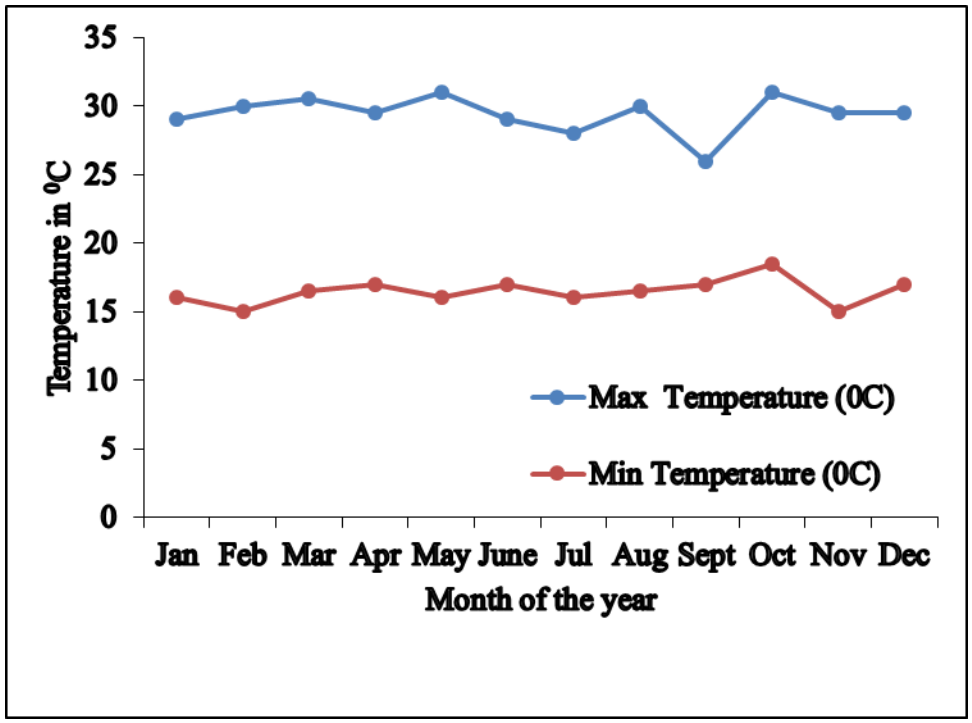
Appendix 1c: Average precipitation at Oyani in the year 2014



Appendix 1d: Average temperature at Oyani in the year 2014



Appendix 1e: Average temperature at Maseno in the year 2014



Appendix 1f: Average precipitation at Maseno in the year 2014