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Addis Ababa University

School of Graduate Studies

Inter Simple Sequence Repeats (ISSRs) Fingerprinting, Phenotypic Variability and Trait Associations in Released and Elite Rice (*Oryza sativa* L.) Genotypes of Ethiopia

A Thesis submitted to the School of Graduate Studies, Addis Ababa University,
in partial fulfillment of the requirements for the Degree of Master of Science in Biology (Applied Genetics)

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April, 2011

Dedicated to **Asfaw Mengistu**

Etaferahu Mekuria

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ACKNOWLEDGEMENTS

I would like to thank Professor Endashaw Bekele for accepting me as his student and allowing me to conduct my M.Sc research in his Genetics Research Laboratory. Dr. Kasahun Tesfaye, I have no more words to explain about your over all roles in bringing me to this moment and level, except saying thank you. Your patience and priceless assistance have backed me from falling down and made the study successful. In deed, the support and advising provided by both Professor Endashaw Bekele and Dr. Kasahun Tesfaye is highly appreciated. I am thankful to the support of Dr. Kifle Dagne and Dr. Yigzaw Dessalegne. The assistance from Mr. Gezehagne Girma and Mr. Abel Teshome in the laboratory during DNA extraction and subsequent laboratory activities has helped me in getting the out put easily. My brothers Abdukalic Mohammed, Abayneh Nigussie and Teklu Azene, you have special place in my heart for your unreserved all round assistance during the course of the study, without you the realization of this work would be questioned. I would like to pass my heart felt gratitude to my friends Tesfalem Chalew, Gezehagne Kebede, Samuel, Habitamu Setegne, Deacon Sindew Taddese, Ababaw Getachew, Shewangizaw ,Yeshitila and Kelemu Gebre for your great concern and assistance in planting, trial management and data collection of the field experiment. My families Bezawork Mekuria, Lomi Degefa, Mulatu Mekuria, Wubrist Asfaw, Birhanu Asfaw, Frehiwot Ararsa and Tigist Tekaw (Butany), your invaluable affection, care and considerations have played a great role in the completion of the study. Special thanks go to the management and staffs of Efratana Gidim Woreda Office of Agriculture and Rural Development for facilitating the field experiment. A lot of thanks go to Mikias for allowing me to use his PC for a long time. Above all, the help from my brother Yenealem Nibret in getting back up of the whole document from my laptop when the system ceased working, is unforgettable. The help, provisions, patience and collaboration from the staff and management of the head quarter (ARARI) and Adet Agricultural Research Center was crucial in accomplishing the study; hence you deserve a special acknowledgement. I also acknowledge all other individuals involved in the study directly or indirectly. Finally, I would like to thank the Department of Biology, Addis Ababa University for giving me the chance to study my M.Sc in Applied Genetics.

Mitiku Asfaw

LIST OF ACRONYMS

AARC	Adet Agricultural Research Center
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of Variance
ARARI	Amhara Regional Agricultural Research Institute
CSA	Central Statistics Authority
DMRT	Duncan's Multiple Range Test
FAO	Food and Agriculture Organization
GCV	Genotypic Coefficient of Variation
GD	Gene diversity
IAR	Institute of Agricultural Research,
IITA	International Institute of Tropical Agriculture
IRRI	International Rice Research Institute
ISSR	Inter Simple Sequence Repeats
MAS	Marker Assisted Selection
masl	meters above sea level
NERICA	New Rice for Africa
NJ	Neighbor Joining
NPL	Number of polymorphic loci
NRRDSE	National Rice Research and Development Strategy of Ethiopia
PARC	Pawe Agricultural Research Center
PCO	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
PP	Percent Polymorphism
RAPD	Random Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
RFLP	Restriction Fragment Length Polymorphism
SNNPR	Southern Nations, Nationalities and Peoples Region
SNP	Single Nucleotide Polymorphism
SoPAPRI	Somali Pastoral and Agro-pastoral Research Institute
SSR	Simple Sequence Repeats
UPGMA	The Un-weighted Pair Group Method with Arithmetic mean

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ABSTRACT

A study on the morphological and molecular characterization of rice (Oryza sativa L.) varieties and lines of Ethiopia was carried out in 2009/10 cropping season. A total of 18 released and elite rice varieties of Ethiopia were used. Inter simple sequence repeats (ISSR) was used as a molecular marker to assess genetic diversity within and among varieties using five ISSR primers (810, 824, 834, 873 and 878). A total of 75 clear and reproducible bands were amplified, out of which 62(82.67%) were observed to be polymorphic. The number of polymorphic loci ranged from 8 for primer-873 to 16 for primer-810. The lowland rice genotypes showed the highest polymorphism (74.67%), followed by upland (61.33%) and NERICA varieties (57.33%). In the same fashion, genetic diversity analysis indicated that lowland rice genotypes showed higher diversity (0.27) than both upland (0.25) and NERICA varieties (0.23). The over all genetic diversity of Ethiopian rice was found to be 0.29. The un-weighted pair group method using arithmetic mean (UPGMA) and neighbor joining trees clustered the genotypes in to their respective ecosystem Lack of purity in IREM-194, HO-04 and AD-12 was prevalent from the constructed trees and field observation. The principal coordinate analysis (PCO) also recovered the UPGMA and neighbor joining tree groups. Analysis of molecular variance (AMOVA) revealed that higher percentage of variation was attributed to variation within individuals (88.67) and the variation among groups was lower (11.33).In the morphological study, which was carried out in a randomized complete block design (RCBD) with three replications, 23 qualitative and 13 quantitative traits were evaluated based on the standard procedures given by International Rice Research Institute and Bioversity International. The result of all diversity parameters indicated that there exists a clear difference among the genotypes for all the traits considered. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) revealed the variability of the genotypes for each of the 13 quantitative traits. Phenotypic coefficient of variation (PCV) varied from 5.38% for number of days to heading to 20.49% for leaf length. Similarly, genotypic coefficient of variation (GCV) ranged from 5.36% for number of days to heading to 19.47% for leaf length. Higher heritability values were observed for all the 13 quantitative traits, out of which nine traits showed heritability percentage more than 90 %. It ranged from 98.98% for number of days to heading to 55.13% for grain yield. Correlation of traits showed that most of the traits were associated. Cluster analysis using morphological data grouped all the test materials in to two main clusters but failed to strictly cluster the genotypes in to their respective ecosystems. Principal component analysis (PCO) showed that the first three principal components explained about 86.81 % of the total variation. Generally, the study indicated the existence of narrower genetic diversity among the genotypes which calls for timely action to widen the genetic diversity of Ethiopian rice so as to combat the devastating outcome of having narrower genetic diversity in cultivated crops.

Key words: Rice (*Oryza Sativa* L.), ISSR, genetic diversity, trait association

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the leading cereal crops of the world and is the principal food crop of about half of the world's population. It is a major source of calories for them (Sasaki and Burr, 2000). In many regions, it is eaten with every meal and provides more calories than any other single food (Stoskopf, 1985). It can also be used in the manufacture of cosmetics and textiles; beer and wine are also made from it (Onwueme and Sinha, 1991). Besides its food value, it has high cultural and social values in rice consuming societies.

Rice production in Ethiopia started a few decades ago and now the country is proved to have reasonable potential to grow different types of rice for lowland rain fed, upland and irrigated ecosystems (NRRDSE 2010). As Shahi (1994) reported, in Ethiopia a single upland variety could be grown in the altitudinal range of 1000-2000masl. Berehe (2003) reported that rice is a potential emergency and food security crop for Ethiopia. In the last few decades several varieties have been released for production by national and different regional research institutes of the country. Knowledge on the extent and pattern of genetic diversity of the varieties so far released is rare and was not assessed systematically either by morphological and/or molecular studies.

Analysis of genetic diversity is important to examine differences between individuals of the same species and is also used to compare the genetic composition of members of different species over a wider taxonomic range (Dale and Schatz, 2002). Several research results indicated that studying the extent and patterns of distribution of genetic variation of a crop species is essential for effective utilization of germplasm in plant breeding programs (Bekele, 1983; Demissie and Bjornstrand, 1996; Demeke *et al*., 1992). Identification of different genotypes of crop species is essential when diverse accessions of crop germplasms are to be characterized, newly developed cultivar is to be registered and purity of the variety is to be determined (Malik *et al*., 1990).

Different genetic markers can be used to assess the extent and pattern of crop genetic diversity. A genetic marker is a variant allele that is used to label a biological structure or

process through the course of an experiment (Griffiths *et al*., 1996). Essentially, it is a 'signature' in the DNA that allows diagnostic detection of DNA sequence variation existing between species and/or varieties (Schulman *et al*., 2004).

There are three different classes of genetic markers: Morphological markers-which quantify variation at phenotype level, biochemical- at gene product level and molecular- at DNA level. Initially, phenotypic markers were used to analyze variation in plants; these were replaced by proteins (isozymes) which, in turn, have been replaced partly by molecular markers (Griffiths *et al*., 1996).

Molecular genetic markers using DNA polymorphism have been increasingly used to characterize and identify a novel germplasm for uses in the crop breeding programs (O' Neill *et al*., 2003). Unlike the morphological and biochemical markers which may be affected by environmental factors and growth practices, molecular markers can identify changes in the DNA sequence (Alan, 2007). Several works have been documented on their use in different diversity analyses studies. For instance, they allow rapid identification of breeding lines (Sajida *et al*., 2009; Seetharam *et al*., 2004), hybrids (Xin Ye-yun *et al*., 2005), cultivars and species (Malik *et al*., 1990; Thaura *et al*., 2008), facilitate genetic diversity and relatedness estimations in germplasm (Samarajeewa *et al*., 2004), and they allow phylogenetic relationships to be established with more accuracy than was previously possible with morphological and biochemical techniques (Sarla *et al*., 2005; Tesfaye, 2006; Geleta, 2007; Bekele and Shigeta, 2011).

Different molecular markers can be used for direct measurements of genetic variation. Comparison of complete genome sequence is the only way of detecting all possible type of variation that may occur. However, quicker methods targeted at certain types of changes in the genome, are needed for detecting the pattern and distribution of genetic variation (Dale and Schatz, 2002). With this regard there are several DNA-based molecular markers like Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), micro-satellites or SSR, Randomly Amplified

Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR) etc (Bornet *et al*., 2002)

Inter simple sequence repeats (ISSR), which involves PCR amplification of DNA using a single primer composed of a micro satellite sequence anchored at the 3' or 5' end by 2-4 arbitrary nucleotides, is one of DNA based molecular marker which could be used to assess genetic diversity (Qian *et al.,* 2001). It has been successfully employed to assess genetic diversity within and between populations in several plant species (Liu and Wendell 2001). As a dominant marker, ISSR targets simple sequence repeats (microsatellites) that are abundant throughout the eukaryotic genome and evolve rapidly. The technique does not require prior knowledge of DNA sequence for primer design (Kantety *et al*., 1995). It has been successfully used for estimating genetic diversity in rice (Sarla *et al*., 2005) and many other crops.

Morphological and molecular markers have been used complementarily for genetic diversity analysis of rice accessions. For instance, both markers were employed for genetic diversity analysis of wild rice collections (Song *et al*., 1999; Samarajeewa *et al*., 2004); mutant rice genotypes (Sajida *et al*., 2009), hybrids (Wang Sheng-jun, 2006), and cultivars (Seetharam *et al*., 2004).

Knowledge on the degree and pattern of genetic diversity of rice varieties so far released in Ethiopia is limited. Understanding the status of divergence or convergence of the genetic variability of the varieties is essential in order to devise/revise the rice breeding program towards attaining sustainable rice production in the country. Hence, the general objective of this study was to analyze the extent of genetic diversity and trait associations of released rice varieties in Ethiopia using both molecular (ISSR) and morphological markers so as to get a clue on status of genetic variability and trait associations for further rice breeding activities.

2. LITERATURE REVIEW

2.1 Distribution, Importance and Taxonomy *of Rice (Oryza sativa* L.*)*

Rice (*Oryza sativa* L.) is the most important staple food crop of about half of the world's population. It is the leading and one of the oldest cereal crops of South-east Asia (Stoskopf, 1985). It is also one of the most important crops that provide food for more than half of the world population and is a major source of calories for them (Sasaki and Burr, 2000). Scholars widen its importance to the entire world population. Rice serves as the main source of calories consumed by human and occupied almost one-fifth of the total land area covered under cereals during 1990 cropping season (Mickel *et al*., 1990).

Rice has been cultivated for several thousands of years as the principal crop in different parts of the world. It is the staple food and main source of income for over half of the world's population and it will continue to be a mainstay of life of future generations. Rice is one of the potential crops to meet the growing food demand of the ever growing world's population (Onwueme and Sinha, 1991).

Rice is a highly diverse crop species with wide geographic dispersal and eco-genetic differentiation, and hence, it is cultivated in a wide range of ecological environments (Malik *et al*., 1990). Rice is the only major crop that can be grown in the standing waters of vast areas of flat, low-lying tropical soils. It is uniquely adapted for growth in submerged soils (Daniel and Thulasidas, 1993).

Rice is grown in the tropical and subtropical regions of most continents. It grows from sea level to 3000 masl such as in Himalayas. It is grown even in the temperate regions of Northern China. Its cultivation under widely differing conditions is because of its great cultivar diversity (NRRDSE, 2010). In other words, because of its long history of cultivation and selection under diverse environments, rice has accumulated various pools of mutants with a broad range of adaptability and tolerance. Its cultivation extends over a wide range of environmental, soil, hydrological as well as climatic conditions. One of the main reasons for this wide range of environmental conditions is the genetic diversity of rice cultivars (Onwueme and Sinha, 1991).

Though the crop is grown in the tropical and sub tropical regions of all continents, much of the total rice crop is produced (90%) in South and East Asia. Africa accounts only for 2 % of the world production (NRRDSE, 2010; Onwueme and Sinha, 1991) of rice. Total production and area coverage of rice is increasing from time to time. FAO (2008) reported that more than 115 million hectares of land was covered with rice and yielded more than 220 million tones in 1961 which increased to more than 156 million hectares of area coverage and 662 million tones of production during 2007 cropping season.

The genus *Oryza* belongs to the tribe *Oryzeae* of the family *Poaceae*. There are 12 genera within the Oryzeae tribe (Vaughan, 1994). In Flora of Ethiopia and Eritrea, the genus *Oryza* was reported to have 20 species, of which two species (*O. sativa* and *O. glaberrima*) were cultivated ones and the rest wild species (Sylvia, 1995). The description of the genus on Flora of Ethiopia and Eritrea (Volume 7) was based on the earlier works of Tateoka around 1963 and Clayton around 1968 (Tateoka, 1963; Sylvia, 1995). But recent works on the taxonomy of the genus *Oryza* indicated that it consisted of more than 20 species (approximately 25 species) of which 23 are wild species and the two, *Oryza sativa* and *Oryza glaberrima*, are cultivated (Lu, 1999; Vaughan, 1994; Vaughan *et al.,* 2003).

Oryza sativa is the most widely grown of the two cultivated species. It is grown world wide, including in Asia, North and South America, Europe, Middle East and African countries. *Oryza glaberrima*, however, is grown solely in West African countries. *O. sativa* and glaberrima-sativa hybrids are replacing *O. glaberrima* in many parts of Africa due to higher yields (Linares, 2002). Such a trend of hybridization promotes the genetic erosion of *O. glaberrima* and calls for appropriate mode of conserving this species. Cultivated rice is generally considered a semi aquatic annual grass; although in the tropics it can survive as perennial, producing new tillers from nodes after harvest (ratooning) (Mc Donald, 1979). At maturity, the rice plant has a main stem and a number of tillers. Each productive tiller bears a terminal flowering head or panicle (OECD, 1994; Vaughan and Morishima, 2002).

2.2 Rice research and development in Ethiopia

Considering Ethiopia's elevation, terrain, and climate that make its agricultural system unique and allowing for multi-crop cultivation in small fragmented areas, the existence of vast land and water resources still waiting to be developed and the hot to warm moist climates which are potentially suitable for rice culture as they fulfill all the requirements of the crop, Shahi (1994) concluded that the time may not be too far for Ethiopia to be one of the major producers of rice in the world. However, such promotion of monocropping, unless it is corrected by strict conservation measures both at in situ and gene bank levels would result in a serious depletion of genetic resources both at the wild and domesticated level.

The genus *Oryza* was first described by Linnaeus in 1753 (Lu, 1999), who recognized only one species, *Oryza sativa*, based on the samples of cultivated rice from Ethiopia. On the other hand, a few domestic documentations on rice cultivation in the country traced back to only three to four decades. But this does not mean that the previous statement is wrong. Considering the publishing institute (i e. IRRI) and history as a basis, the situation is reasonably beyond doubt. But it is clear that there is a lack of record or inaccessibility of records on cultivation of rice in the country between 1753 and 1970s. Despite this historic event, the country was unable to exploit the huge potential from rice production until wild rice (*O. longistiminata* and *O. barthi*) was discovered in Fogera plain and Gambela areas (Afework, 2002). The Gambela and Didessa valley was studied in mid 1975 for its vegetation, soil and weed flora in order to see the relevance for rice production by Teweldebirhan Gebregziabher and recommendation was made (Endashaw Bekele- Personal communication).

The discovery of wild rice in Fogera plain and Gambela areas together with 1975 recommendation has initiated different governmental and non governmental organizations to start adaptation trials on cultivated rice in different parts of the country (Zegeye *et al.,* 2004). Afework (2002) documented that different governmental and non governmental organizations like the Americans, Japan Oversea Cooperation Volunteers

(JOCV), Institute of Agricultural Research (IAR), Agricultural Development Department (ADD) of the Ministry of Agriculture, Tana Beles Project (TBP), Ethiopian Water Construction Authority (EWCA), International Institute of Tropical Agriculture (IITA), Addis Ababa University Science Faculty and the North Korean agricultural experts were involved in rice research and development activity in Fogera plain, Chefa, Gambela, Melka Werer, Lante and Pawe areas up to 1980's and they came up with the encouraging results and recommendations.

Some improved varieties had been released informally and extended into the resettlement areas in Gambella and Pawe for demonstration and large scale production (Zegeye *et al.,* 2004). In the Fogera plain of the Amhara region the Jigina and Shaga Farmers' Producers' Cooperatives started large scale production of rice with the technical support of North Korean agricultural experts. The extension program of rice was very successful. However, due to the liquidation of farmer's producers' cooperatives and evacuation of rice producers from the resettlement areas around 1990's, the rice research, extension and production activities were weakened for a while (Afework, 2002).

In1994, the development activity was re-initiated based on new strategy and approach by proposing rice testing and development program. The program encouraged volunteer farmers to participate and a large number of households were involved in growing rice.

Currently, rice has received due emphasis, among other crops, in promotion of agricultural production, and is considered as the "Millennium crop" expected to contribute in ensuring food security in the country (NRRDSE, 2010). Different reports showed that, even though, introduced recently, rice has proven to be a crop that can assure food security in Ethiopia, (CSA, 2008; NRRDSE, 2010; Berhe, 2003; Zena *et al*., 2009) and if successful, initiatives to boost rice production in Ethiopia can help the country achieve food security (Berhe, 2005). However, although rice is considered as a 'Millinium crop' it should be seen as one of the packages of crops that ensures food security in Ethiopia. It is with this understanding that the Government of Ethiopia has prepared a National Rice Research and Development Strategy which envisages seeing the

existing limited area and subsistence dominated rice sub-sector transformed progressively into commercially profitable and viable production system with the goal of contributing to national food security, increased income and reduced poverty through establishment of a competitive and sustainable rice production and marketing system (NRRDSE, 2010).

2.3 Rice production trend, potential and consumption in Ethiopia

It is well known that tef, maize, wheat and sorghum are among the cereal crops used as staple food crops and are targets of most of the food security programs in the country. However, the recent trends in the area and production of rice along with its high compatibility in the traditional consumption habits shows that rice is becoming as one of the added staple foods and important for ensuring household food security (Berhe, 2003; NRRDSE, 2010).

Due to the growing demand for food security, coordinated efforts of different non governmental and governmental organizations and improving farmers' awareness, the importance of rice is increasing very fast. Since in almost all regions of the country upland, lowland rain fed and irrigated rice production systems are in place, the number of participating farmers and investors is alarmingly increasing. The area coverage under rice is also increasing from year to year (Zegeye *et al.,* 2004; Berhe, 2003; NRRDSE, 2010). The increasing trend both in area and production of rice in the country is presented in Table 1.

Ethiopia has a potential for both rain fed and irrigated rice cultivations. According to NRRDSE (2010), the production potential for rain-fed rice in the country was assessed using GIS techniques by considering ecological requirements of rice like rainfall, slope, soil texture, altitude, and temperature. Accordingly, there are about 30 million ha of land suitable for rain fed rice ecosystem in the country (NRRDSE, 2010). Similarly, as reported by Awulachew (2007), the country is endowed with irrigated rice potential which is estimated to be about 3.7 million ha of land. Suitability map of rain fed rice production of the country is presented in Figure 1. Similarly, size of potential area by

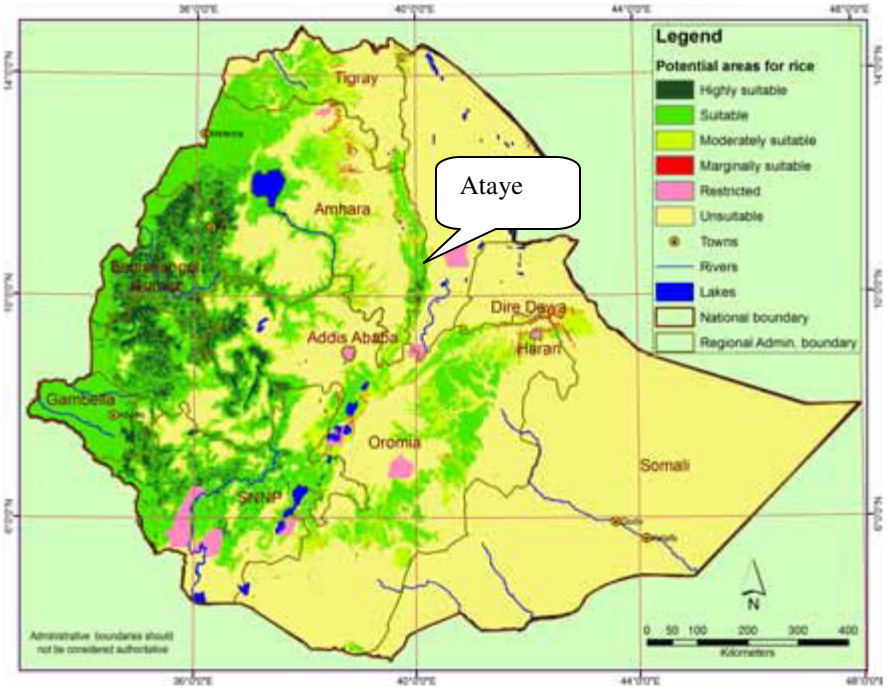
level of suitability for rain fed rice production and irrigation potential of the country presented hereunder in Tables 2.2 and 2.3, respectively.

Table 1: Rice production, area and the number of participating farmers by region (2006-2009)

Region	2006			2007			2008			2009		
	No. farmers	Area (ha)	Production (ton)	No. farmers	Area (ha)	Production (ton)	No. farmers	Area (ha)	Production (ton)	No. farmers	Area (ha)	Production (ton)
Amhara	46228	15392	34816	96300	32100	73280	211440	52985	140135	218375	68430	199761
Tigray	-	-	-	-	-	-	3600	1271	3286	6855	5150	14505
Ben-Gumz	-	-	-	-	-	-	1474	362	1181	3830	10080	32256
Oromiya	2061	683	1949	8865	2955	9465	22036	5875	20676	25065	12065	41408
Somali	150	28	84	13590	4530	13815	5154	9920	38120	8300	16780	67120
SNNPR	4863	2424	5976	31113	10371	25742	15741	18721	77723	22443	29156	92562
Gambella	-	-	-	-	-	-	657	1314	4456	na	13350	50720
Total	52302	18527	42825	149868	49956	122302	260102	90448	285577	284868	155886	498332

Source: NRRDSE-2010

NB: '-'indicates regions did not start rice production and/ or no data access for 2006 and 2007 cropping seasons.



Source: NRRDSE-2010

Figure 1: Suitability map of rain fed rice production in Ethiopia (Ataye is the site where the field experiment was conducted)

Table 2: Size of potential area by level of production potential for rain-fed rice

Region	**Highly suitable (ha)**	**Suitable(ha)**	**Moderately Suitable (ha)**	**Total (ha)**
Tigray	-	1278245	935565	2213810
Afar	2318	147084	124158	273560
Amhara	483839	5289945	2228180	8001964
Ben-Gmz	2053332	2817944	53235	4924511
Somali	-	69893	375222	445115
Oromia	2051787	8082388	3993068	14127243
Dire Dawa	-	601	38553	39154
Gambella	373848	2752345	38037	3164230
SNNPR	625771	4472184	1065648	6163603
Total	*5590895*	*24910629*	*8852666*	*39354190*

Source: NRRDSE-2010

'-' none at all and/ or non-significant

NB: Area of suitability for rice production must take into account the agricultural system in each region, the impact of monocropping on genetic resources and other packages of production for sustainable food production program.

Table 3: Irrigation potential in Ethiopia by basin

River basin	**Irrigable land (ha)**	**Region (s)**
Tekeze	83368	Tigray and Amhara
Abay	815581	Amhara, Oromia and Benishangul-Gumuz
Baro-Akobo	1019523	Benishangul-Gumuz, Gambella, Oromia & SNNPR
Omo-Gibe	67928	SNNPR and Oromia
Rift valley (lakes)	139300	Oromia and SNNPR
Mereb	76560	Tigray
Afar (Denakil)	158776	Afar, Tigray and Amhara
Awash	134121	Amhara, Oromia, Afar and Somali
Wabishebelle	237905	Oromia, Harari and Somali
Genale-Dawa	1074720	Oromia, SNNPR and Somali
Total(ha)	***3798782***	

Source: Awlachew (2007)

Urban and rural rice consumers of Ethiopia have different preferences. i.e. rural consumers in rice production areas, consume locally produced rice and urban consumers consume mainly imported rice (Zegeye *et al.,* 2004). In most of the production areas, rice is consumed through traditional ways of food preparation mainly mixing with "*tef*" or sole to make "*enjera*" and mixing with wheat or sole to make bread. Commonly, local liquor called "*areki*" and "*tela*" are also made of rice. In urban areas, the imported rice, that is commonly aromatic to non-aromatic and non-sticky after cooking is preferred (Berhe, 2003 and 2005; Zegeye *et al.,* 2004; NRRDSE, 2010).

2.4 Marker systems used in crop improvement strategies

Identification of different genotypes of crop species is essential in characterizing accessions of crop species, for registration of newly developed cultivars and to determine the purity of varieties (Malik *et al*., 1990) and this could be achieved through identification, characterization and quantification of traits or markers. Traits that serve as genetic markers are by definition polymorphic; the more polymorphic the trait, the greater its potential value to germplasm management (Seetharam *et al*., 2004). Markers are entities that are heritable as simple Mendelian traits and are easy to score (Schulman

et al., 2004). Three marker systems could be recognized- morphological, biochemical and molecular marker systems- in crop improvement strategies.

2.4.1 Morphological markers

Morphological characters are the earliest of all markers to assay, and their use is as old as breeding and selection itself. Morphological markers are a marker system based on phenotypic appearance. The morphological traits used as markers had several advantages. Firstly, they are easily describable and facilitate enumeration. Secondly, detection and use of morphological markers are simple and cheap (Chittaranjan and Albert, 2008; Bornet *et al*., 2002).

The main shortcomings of this approach are the number of characters that can be detected is few (lower polymorphism), and cover only a limited genetic fraction of the organism. The existence of inter-allelic interactions in many morphological characters may lead to deviation of dihybrid segregation ratios which in turn limits their application in linkage analysis. It is also impossible to distinguish homozygous dominant and heterozygous individuals because of dominant intra-allelic interactions (Chittaranjan and Albert, 2008). Moreover, some morphological traits are observed to be very plastic in nature, which could be easily influenced by environmental change and affect the exact relationships of plant species and/or populations (Vithanage *et al*., 1995).

Despite their limitations, morphological markers are well established tools for taxonomy, variety classification and breeding. And, the limitation to their application can be overcome by the application of biochemical and molecular markers.

2.4.2 Biochemical markers

Biochemical markers are markers based on protein polymorphisms through electrophoretic separation of protein molecules. The assays require the extraction of proteins from plant tissues, followed by their separation using gel electrophoresis (Schulman *et al*., 2004). Depending on the number of loci, their state of homo-or hetero-zygosity, and the enzyme configuration (i.e. the number of separable units), from one to

several bands are visualized. The position of these bands can be polymorphic and can be considered as informative loci. The main advantages of protein markers are their co-dominant inheritance and low cost of the assay (Weising *et al*., 2005).

The general utility of biochemical markers is hindered mainly by the relatively small number of genetic loci that can be assayed in any population (lower polymorphism), technical complexity (since each protein system requires its own electrophoretic and staining protocol) and problems associated with specificity to tissue type, physiological age or developmental state of the tissue (Chittaranjan and Albert, 2008). In other words, the major shortcomings of this marker system are lower polymorphism, lower genome coverage and technical difficulties (Schulman *et al*., 2004).

2.4.3 Molecular markers

Molecular markers are based on naturally occurring polymorphisms in DNA sequences (i.e. base pair deletion, substitutions, additions or patterns) (Gupta, 1999). They are superior to both morphological and biochemical markers because they are relatively, abundant through the genome even in a highly inbred cultivars, completely independent of environmental conditions and can be detected at virtually any stage of plant development (O' Neill *et al*., 2003). DNA based markers provide very effective and reliable tool for measuring genetic diversity in crop germplasm and studying evolutionary relationships than other available techniques for assessing the genetic variability and relatedness among crop germplasm (Malik *et al*., 1990). They can be applied in the identification of cultivars and clones, genetic mapping, marker assisted selection (MAS), population genetics, molecular systematics and etc (Weising *et al*., 2005).

According to Vithanage *et al* (1995) molecular markers are 'land marks' which can be identified on the genome and, therefore, offer the best possible means of identifying individuals from biological samples. In recent years, different marker systems such as RFLP, RAPD, ISSR, STS, AFLP, SSR, SNPs, and others have been developed and applied to a range of crops (FAO, 2003). In rice, molecular markers have been used to

identify accessions (Olufowote *et al*., 1997; Virk *et al*,. 1995), to determine the genetic structure and pattern of diversity for cultivars of interest (Junjian *et al*., 2002).

Molecular markers can be classified in to two major groups: those based on DNA-DNA hybridization between a DNA or RNA 'probe' and total genomic DNA (eg. RFLP and dot-blot assay) non- PCR based; and, those based on the PCR amplification of genomic DNA fragments (eg. RAPD, ISSR, SCAR, SSR, AFLP, SNP, etc). Some of the major and commonly used molecular markers are presented briefly below

A. Non-PCR based molecular markers

Restriction Fragment Length Polymorphism (RFLP)

RFLPs were the first widely used non-PCR based molecular markers for assessment of DNA variation in selected organisms (Chittaranjan and Albert, 2008). In RFLP analysis, DNA is extracted from organism of interest and digested with restriction enzymes that recognize specific DNA motifs and the resultant fragments are separated by gel electrophoresis. The separated DNA fragments are transferred to a membrane filter by capillary blotting, a procedure termed as 'Southern blotting'. Hybridization of 'probe' with the filter follows to get one or a set of bands (differences that may be present within the restriction digestion products) (Karp *et al*., 1996). Bands are visualized where the probe has hybridized to homologous DNA fragments: the pattern obtained is referred to as the restriction fragment pattern. Polymorphisms arise from sequence changes in the restriction site as well as from the detection of insertions/ deletions in the restriction fragments detected by the probe (Chittaranjan and Albert, 2008).

Abundant distribution in genome of most species, reproducibility and their co-dominance natures are the advantages of RFLP markers (Karp *et al*., 1996). However, technical limitations restrict the application of RFLPs. Its requirements for high quantity and quality of DNA, difficulty in automation due to blotting and hybridization steps are also the constraints that limit its wide application (Karp *et al*., 1996).

B. PCR based molecular markers

The advent of the polymerase chain reaction (PCR) (Mullis *et al*., 1986) allowed the development of techniques that overcome some of the difficulties associated with RFLPs and generated new molecular markers. Following is a short account on a few PCR based molecular markers, their advantages, limitations and importance in the study of genetic diversity.

i. Random Amplified Polymorphic DNA (RAPD)

RAPD is a PCR based technique that uses decamers, or other suitably short primers, of arbitrary sequence to amplify discrete fragments of the genome (Williams *et al*., 1990). The primers are random and arbitrarily selected to amplify random DNA segments flanked by the primers throughout the genome (Yin *et al*., 2001). The resulting amplification product is derived from a stretch of DNA that is bordered or flanked by primer annealing sites. The amplified products are separated on an agarose gel and normally visualized using ethidium bromide –staining under UV light. Polyacrylamide gels are sometimes used when higher resolution levels are required (Yin *et al*., 2001; Karp *et al*., 1996).

Polymorphisms are detected as the presence or absence of bands (Malik *et al*., 1990; Samarajeewa *et al*., 2004). The RAPD technique is useful because it requires no sequence information for primer design and no probe development. The technique is simple, fast and relatively cheap (Williams *et al*., 1990). It is a dominant marker and used for plant breeding purposes like the construction of genetic maps and the tagging of desirable traits for marker assisted selection; and also useful in phylogenetic and population studies (Staub *et al*., 1996).

Lower reproducibility because of low stringency condition is the main disadvantage of RAPD. Results can differ according to the source of the tissue analyzed, DNA extraction techniques and PCR protocols (Staub *et al*., 1996; Jones *et al*., 1997). Therefore, constant reaction conditions are crucial for reliable and reproducible results (Weising *et al*., 2005).

ii. Amplified Fragment Length Polymorphism (AFLP)

The AFLP is another PCR based molecular marker developed in mid 1990's and is essentially a combination of RFLP and PCR. i.e. the DNA digested with restriction enzymes is amplified with PCR (Chittaranjan and Albert, 2008). It allows selective amplification of restriction fragments giving rise to a large number of useful markers which can be located on the genome relatively, quickly and reliably (FAO, 2003).

Restriction fragments have adapters ligated to their ends allowing PCR amplification from primers derived from the adapters. Each band may be identified by its size and the selective bases used in the primers to generate them (Chittaranjan and Albert, 2008). Polymorphisms arise from sequence changes in the restriction sites or the selective bases used by the primers and insertions/deletions in the fragments. Bands are generally detected as presence or absence, making AFLPs a dominant marker system (Maughan *et al*., 1996).

Ability to generate higher polymorphism and no primer sequence requirement are the advantages of AFLP markers (Rafalski *et al.,* 1996). AFLPs have proved to be useful in assessing genetic relationships: its ease of use and high information level. AFLP is technically demanding and require high quality DNA which limits the application of the technique (Karp *et al*., 1996; Gedil *et al*., 2001).

iii. Simple Sequence Repeat (SSR)

SSRs (also termed microsatellites or sequence tagged microsatellite sites-STMS) are stretches of DNA, consisting of short tandomly repeated sequence motifs (Tautz and Schlotter., 1994). They are highly polymorphic DNA markers with discrete loci and co-dominant alleles (Chittaranjan and Albert, 2008). Microsatellites are ideal genetic markers for detecting differences between and within species. It consists of tandomly repeated 1-7 bp units, distributed widely throughout the genome. These are heritable, useful to monitor gene flow, excellent for parentage determination and ideally suitable for analysis via multiplexing with highly reproducible profiles (Farooq and Azam*,* 2002).

The variation in the number of tandomly repeated units results in highly polymorphic banding patterns (Junjian *et al*., 2002).

Microsatellites may even be used across species and genus boundaries. Microsatellites are not limited to the nuclear genome. They occur in chloroplast as well as in mitochondrial genome (Soranto *et al*., 1999). Despite their advantage, initial isolation of useful SSR loci can be a time consuming and expensive process (Chittaranjan and Albert, 2008; FAO, 2003).

Polymorphism is revealed by PCR-amplification from total genomic DNA, using two unique primers that flank and hence define the microsatellite locus. Amplification products obtained from different individuals can be resolved on gels to reveal polymorphism that are detected based on size differences due to differences in the number of repeats. Microsatellites have a wide range of applications: determination of paternity and kinship, studying the structure of populations, determination of hybridity etc (Brufood and Wayne, 1993; Schlotterer and Pemberton, 1994).

iv. Single Nucleotide Polymorphism (SNP)

Single base changes and short insertion/deletion represent the most abundant source of DNA polymorphisms in organisms (Kwok *et al*., 1996; Collins *et al*., 1997; Kruglyak, 1997) and they are both generally classified as single nucleotide polymorphisms or SNPs (Cho *et al*., 1999).

In principle, SNPs could be bi-, tri- or tetra-allelic polymorphism. However, tri allelic and tetra allelic SNPs are rare almost to the point of non-existence, and so SNPs are sometimes simply referred to as bi-allelic markers (di-allelic markers). These polymorphisms could be used as simple genetic markers that may be identified within or near every gene (Chittaranjan and Albert, 2008). There is also great potential for these SNPs in the detection of associations between allelic forms of a gene and phenotypes (Rafalski, 2002).

SNPs are single base pair positions in the genomes of two or more, at which different sequence alternatives (alleles) exist in populations (Weising *et al*., 2005). In recent years, SNPs have become an increasingly important class of molecular markers. The potential number of SNP markers is very high, meaning that it could be possible to find them in all parts of the genome and micro-array procedures have been developed for automatically scoring hundreds of SNP loci simultaneously at low cost per sample (FAO, 2003)

v. Inter Simple Sequence Repeats (ISSR)

The Inter Simple Sequence Repeats (ISSR) is a microsatellite based multi locus marker technique, which is simple and useful for estimating genetic diversity in several crop plants (Sarla *et al*., 2005). The technique has the advantage of RAPD and in addition shows higher level of polymorphism, reproducibility and cost effectiveness per polymorphism. ISSRs involve amplification of DNA by a single primer 16-18bp long composed of repeated sequence anchored or non anchored at the 3' or 5' end by 2-4 arbitrary nucleotides (Zietkiewicz *et al*., 1994).

It is possible to exploit SSRs without the need to characterize and develop primers. Primers composed of SSRs can be used to amplify regions between two nearby inversely oriented SSR (Zietkiewicz *et al*., 1994). Furthermore, the repeat length variability that makes SSR markers so polymorphic can be sampled by anchoring the SSR primers at the 5' end (Fisher *et al*., 1996). ISSR assays can be undertaken for any species that contains a sufficient number and distribution of SSR motifs and have the advantage that genomic sequence data are not required.

ISSRs are now being applied for cultivar identification, assessment of genetic diversity in various plant species (Hou *et al*., 2005; Pomper *et al*., 2003) and in determining genetic diversity and phylogenetic relationships in *Oryza* (Joshi *et al*., 2000; Girma *et al*., 2007). In rice, ISSR has been used for the analysis of microsatellite motif and frequency and fingerprinting of varieties, determining the phylogenetic relationships among *Oryza* species, in distinguishing basmati rice varieties as markers for restorer genes, and in studies on comparing effectiveness of different molecular markers (Sarla *et al*., 2005).

3. OBJECTIVES

3.1 General objective

The general objective of the study was to investigate the genetic variability of rice cultivars and elite lines of Ethiopia using inter simple sequence repeats and morphological markers; and trait associations in these rice genotypes for some morphological characters.

3.2 Specific objectives

- To investigate the genetic diversity among Ethiopian rice lines and varieties using inter simple sequence repeats,
- To observe variability of the varieties and lines with respect to qualitative and quantitative morphological traits,
- To identify morphological traits accounting to gross variability,
- To investigate trait associations that will help for further improvement of rice in Ethiopia.

4. MATERIALS AND METHODS

The investigation to assess the variability and trait associations of Ethiopian rice varieties and lines was carried out at two levels with two sets of experiments-morphological and molecular sets of diversity analyses. The morphological study was conducted at Ataye, a place in North eastern part of the country in Amhara Regional State. The experimental materials used were 18 released and elite rice genotypes of Ethiopia. The detailed descriptions of the study area for the morphological study and the test materials used are given under section 4.1 and 4.2, respectively. The molecular analysis was conducted in the Molecular Genetics Research Laboratory of the Department of Biology, Addis Ababa University.

4.1 Description of the study area

The field experiment was carried out at Ataye Agricultural Demonstration Site of the Office of Agriculture and Rural Development of the Efratana Gidim Woreda, North Shoa administrative zone of the Amhara National Regional State. The experiment was conducted during short rainy cropping season of 'Belg'. It was conducted on alluvial soil with supplemental irrigation. The site is elevated at 1461 masl and located at longitude and latitudes of 39° 55' 60 E and 10° 20' 60 N. The area is characterized by bimodal rainfall distribution. The main rainy season is from late June/early July to late September/early October; and the short rainy season mostly between January and April. The field experimental site is shown in Figure 1.

4.2 Experimental materials

The experimental materials of released and elite rice cultivars/lines of Ethiopia were supplied by the National Rice Project Coordinating Center, Adet Agricultural Research Center. A total of eighteen genotypes were used for the study. Based on their ecology, the test materials were classified as upland rain fed (eight), lowland rain fed (eight) and irrigated (two). The varieties were released by different regional and national agricultural research centers. List of the experimental materials, their local name, maintainer, ecosystem and year of release are given in Table 4. Year of release vary significantly from earliest 1992 to the latest 2008, it may have impact on segregation of the genotypes

in that earlier released one are highly segregate than the latest one. However, in order to minimize this problem the rice research program of the country had a basic and pre-basic seed maintenance activity each year since the release of each variety.

Table 4: List of the experimental materials/genotypes/, their local name, ecosystem, maintainer and year of release

No.	Genotypes	Local name	Ecosystem	Maintainer	Year of release
1	IAC-164	Gumara	Rain fed lowland	AARC	1992
2	IRAT-209	Kokit	Rain fed upland	AARC	1992
3	IREM-194	Tigabe	Rain fed upland	AARC	1992
4	AD-01	Getachew	Rain fed upland	AARC	2008
5	AD-12	Andasa	Rain fed upland	AARC	2008
6	AD-48	Tana	Rain fed upland	AARC	2008
7	NERICA-1	-	Upland-irrigated	SoPAPRI	2007
8	NERICA-2	-	Upland-irrigated	SoPAPRI	2007
9	NERICA-3	-	Rain fed upland	PARC	2007
10	NERICA-4	-	Rain fed upland	PARC	2007
11	NERICA-6	-	Rain fed upland	SoPAPRI	-
12	Suparica-1	-	Rain fed lowland	PARC	2007
13	X-Jigina	Local	Rain fed lowland	AARC	-
14	Demewoze	Local	Rain fed lowland	AARC	-
15	HO-O4	Elite	Rain fed lowland	AARC	-
16	HO-13	Elite	Rain fed lowland	AARC	-
17	FOFIFA-3730	Elite	Rain fed lowland	AARC	-
18	FOFIFA-3737	Elite	Rain fed lowland	AARC	-

Source: IAR, 2007; NRRDSE, 2010

4.3 Molecular Study

4.3.1 Plant materials

Rice leaf samples were collected from an experiment laid out in randomized complete block design in Ataye for the morphological characterization. A total of 41 leaf samples (individual rice cultivars/lines) from 18 genotypes were collected for molecular study. Two plants per genotypes (cultivar/line) were randomly selected for all varieties except for IREM-194 and HO-04-7-1-B-5, where four and five plants per genotype were randomly taken, respectively. This was intentionally done due to observed lack of plot uniformity on the two genotypes that could be due to seed admixture. However, four samples were missed and the analysis was carried out with 37 samples. From each

sample plants fresh leaves were taken at random and dried in silica gel for genomic DNA extraction.

4.3.2 DNA extraction

Total genomic DNA was extracted from each rice leaf sample following modified CTAB method of Borsch *et al* (2003).Triple extraction was employed for each sample tissue so as to get optimum quantity and quality of genomic DNA.

4.3.3 Test gel and electrophoresis

Genomic DNA of 2µl from each sample with 6µl of loading dye (2%) was loaded on an agarose gel of 0.98% (100ml 1XTBE and 0.98g agarose, boiled in a micro-oven for 3 minutes and poured on to gel making apparatus-tray with comb) and electrophoresed at a constant voltage of 80V for about 45 minutes. Then the gel was stained with 50µl of ethidium bromide (10mg/ml) for about 30 minutes in 450ml of distilled water and de-stained for 30 minutes in 450ml of distilled water alone using stain-destainer apparatus.

The quality and quantity of DNA could be estimated using a UV spectrophotometer and checked visually by ethidium bromide staining of 0.8% agarose gel (Sarla *et al*., 2005). The second method was adopted in the present study and hence, gel picture was taken under UV trans-illuminator by Biodoc Analyse 2.0 with digital cannon camera to check the quality of sample DNA. Out of the three extractions, the one with high band intensity and lesser smear were selected from the picture for subsequent PCR analysis. High band intensity and low smear entailed high quality and quantity of the genomic DNA sample among the three extractions. Most samples selected for the polymerase chain reaction (PCR) were taken from the second extraction and followed by few samples from the first extraction. Based on the observed band intensity, the selected genomic DNA was further diluted to 1:5 and1:10; and then test gel was repeated to evaluate if the concentration of DNA used for ISSR-PCR was equal.

4.3.4 Primer selection and optimization

ISSR kit obtained from the University of British Columbia (Primer kit UBC 900) was used in this analysis. Inter simple sequence repeat primers described by Sujatha *et al*

(2004) and Girma *et al* (2010) were used for the initial variability and reproducibility test and finally five ISSR primers were selected for this study.

Representative samples from each genotype were selected to screen the primers with 1:5 or 1:10 dilution of the stock DNA. In this initial test for polymorphism and reproducibility, the five primers (Table 5) showed high variability and reproducibility and used for PCR in the final diversity study. The selected primers include 3 di-nucleotides and 2 tetra-nucleotides and they are high performance liquid chromatography purified and designated as 'H'. Table 5 shows the list of primers used, their annealing temperature with respective sequences and other properties.

Table 5: List of primers used and their annealing temperature with respective sequences

Primer	**Primer sequence**	**Melting Temperature**	**Annealing Temperature**	**% GC content**
810-H	GAGAGAGAGAGAGAGAT	50.4	45	47
824-H	TCTCTCTCTCTCTCTCG	52.8	48	53
834-H	AGAGAGAGAGAGAGAGYT	50.4	45	47
873-H	GACAGACAGACAGACA	49.2	45	50
878-H	GGATGGATGGATGGAT	49.2	45	50

Source: Primer kit 900 (UBC 900); Y= *Pyrimidines (C or T)*

4.3.5 PCR and gel electrophoresis

PCR reactions were carried out in Biometra 2000 T3 thermocycler. PCR amplification was carried out in a 25µl reaction mixture containing 1µl template DNA, 13.2µl ddH_2O, 5.6µl dNTP (125mM), 2.6µl taq buffer (10X Thermopol reaction buffer), 2.0µl $MgCl_2$ (2mM), 0.4µlprimer (20pmol/µl) and 0.2µl Taq polymerase(5u/µl).

The amplification program was 4 minutes pre heating and initial de-naturation at 94 ^oC, then 39*15seconds at 94^oC, 1 minutes primer annealing at (45/48 ^oC) based on primer used, 1.30 minutes extensions at 72 ^oC. The final extension for 7 minutes at 72 ^oC followed. The PCR products were stored at 4 ^oC until loaded on gel for electrophoresis. An agarose gel (1.67g agarose with 100 ml 1XTBE) was prepared and 8µl of ISSR amplification product of each sample with 2µl loading dye (6X) was loaded on gel (Girma *et al.*, 2010).

DNA marker of 100bp (molecular ladder) was used to estimate molecular weight of each PCR product. The electrophoresis was done for 2 hours at a constant voltage of 100V. Gel picture was taken after staining with ethidium bromide mixed with 450ml of distilled H_2O for 30 minutes and de-stained for 30minutes in 450ml of distilled H_2O.

4.3.6 Data scoring and analysis

Clearly resolved, unambiguous bands were scored visually for their presence or absence for each primer and sample (Seetharam *et al*., 2004). A low intensity for any particular fragment may be explained by the lesser representation of that particular sequence in the bulk sample (Malik *et al*., 1990). However, the intensity of the bands was not taken into account and the fragments with the identical mobility were considered to be identical fragments and treated as a unit character. Each fragment was scored independently as '1' for presence and '0' for absence. Bands with the same molecular weight and mobility were treated as identical fragments. The total number of bands, distribution of bands across rice ecosystems, number of polymorphic bands in a set of accessions, and genetic diversity of all individuals and across each primer were calculated (Sarla, *et al*., 2005). Similarity matrix was generated based on the simple-matching coefficient (Jaccard's), using the presence/absence data for individual ISSR fragment between pairs of rice cultivars/lines.

Different statistical analyses were performed on the ISSR data using several software packages. A computer statistical package, POPGENE version1.32 software (Yeh *et al.*, 1999), was used to calculate number of polymorphic loci and percent polymorphism so as to estimate genetic diversity for each rice variety and line. The non parametric Analysis of Molecular Variance (AMOVA) was used to apportion the variation among individuals within populations, among populations (upland, lowland and upland NERICA), and among genotypes within each population (Song *et al*., 1999). In other words, AMOVA procedure was used as described in Excoffier *et al* (2006) in order to describe population structure and variability among populations using Arlequin V2.0 (Amanda *et al*., 2005).

Principal Component Analysis (PCO) was performed by the NTSYS pc software package (Rohlf, 2005) in order to examine the patterns of variation among individual samples and highlight the resolving power of ordination (Seetharam *et al*., 2004). Jaccard's similarity coefficient (Jaccard, 1908) matrix was used for principal coordinate analysis. The calculation of Jaccard's coefficient was computed using PAST software version 1.18 (Hammer *et al.,* 2001). The first three axes were later used to plot a dendrogram with STATISTICA version 6.0 software (Hammer *et al.,* 2001; Statistica soft, Inc.2001). The un-weighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) was used to analyze and compare the individual genotypes and generates phenogram using NTSYS- pc version 2.02 (Rohlf, 2005). The neighbor joining (NJ) method (Saitou and Nei, 1987; Studier and Keppler, 1988) was used to compare individual genotypes and evaluate patterns of genotype clustering using Free Tree 0.9.1.50 software (Pavlicek *et al.,* 1999). UPGMA and Neighbor-joining methods were performed with the similarity matrix as input data in order to test the reliability of ISSR data for the possibility of classifying rice germplasm as compared to morphological data. The similarity analysis was plotted in the form of UPGMA and NJ dendrograms.

4.4 Morphological Study

4.4.1 Experimental design

The field experiment was laid out in a randomized complete block (RCB) design with three replications during 2009/10 cropping season. The plot was 5m long and 1.2 m wide ($6m^2$) with 0.2m and 0.5m inter row and between plots spacing, respectively. Each replication (block) was one meter apart. There were six rows per plot, out of which the first and sixth rows of each plot were used as border rows and data were taken from the middle four rows of each plot. As per the national recommendation, a seed rate of 60Kg/ha i.e. 36g per plot was used. Fertilizer was applied at a rate of 100/100Kg/ha Urea and DAP (i.e. 60/60 g/plot urea and DAP). All the DAP (60g/plot) and one-third urea (20g) was applied at planting. The second one-third of urea was applied as a basal application at tillering stage and the rest one-third urea was applied at panicle initiation stage of the crop. The experiment was weed free throughout its growing time. Hand

weeding was used for weed control. Since the experiment was conducted during the short rainy season (Belg), the experiment was supplemented with surface irrigation based on the start and onset of the belg rain of the locality. Similar experimental designs were used for genetic diversity analysis of rice genotypes (Barry *et al*., 2007; Seetharam *et al*., 2004; Wang Sheng-jun, 2006)

Figure 2. Field trial evaluation at the trial site, Ataye

4.4.2 Data collected

Based on the standard evaluation system for rice developed by International Rice Research Institute (IRRI, 2002) and Bioversity International (2007), heritable morphological and agronomic traits were collected from the middle four rows of each plot. The following qualitative and quantitative morphological and agronomic traits were collected.

i. ***Morphological Characteristics***

Auricle color: Score on the color type of small, paired ear-like appendages on either side of the base of the leaf blade (Auricles).

Awn color: Color of awn-a thorn-like extension from the keel of the lemma.

Awning: is the presence or absence of Awns

Basal leaf sheath color: score on the color type of leaf sheath-the lower part of the leaf, originating from a node and wrapped around the culm above the node.

Collar color: the color of the joint between the leaf sheath and leaf blade.

Culm angle: the inclination of the culm- the round, smooth-surfaced, ascending axis of the shoot consisting of hollow internodes joined by solid nodes.

Culm internode color: the color of the internode-the smooth, solid (when young), or hollow (when mature) part of the culm between two successive nodes.

Culm length: measure of the culm from the soil surface to panicle base in centimeters

Flag leaf angle: measured near the collar as the angle of attachment between the flag leaf blade and the main panicle axis

Grain width: the actual measurement of width in millimeters as the distance across the fertile lemma and the palea at the widest point of grain-the ripened ovary and its associated structures.

Leaf angle: the angle of openness of the blade tip measured against the culm of the leaf below the flag leaf.

Leaf blade color: score on the coloration of leaf blade- the flat and free portion of the leaf

Leaf blade pubescence: observation on the presence of hairs on the blade surface.

Leaf length: the actual measurement, in centimeters, of the leaf (the vegetative part of a tiller consisting of the blade and sheath.) just below the flag leaf

Leaf width: actual measurement, in centimeters, of the widest portion of the leaf blade just below the flag leaf

Lemma and palea color: observation on the coloration of lemma-the five-nerved bract of the floret and palea-the three-nerved bract of the floret.

Lemma and palea pubescence: observation on the presence of hairs on lemma and palea

Ligule color: score on the color type of ligule-a thin, upright, membranous structure attached to the base of the inside of the flower.

Ligule shape: observation on the shape variation of ligules

Panicle axis: observation on the orientation of panicle-the terminal component of the rice plant which bears the rice spikelets.

Panicle length: actual measurement in centimeters from panicle base to tip

Panicle type: observation on the type of panicles based on their mode of branching, angle of primary branches, and spikelet density as compact, intermediate and open.

Secondary branching of panicles: observation on the abundance and distribution of spikletes borne on secondary branches of the panicle at near maturity stage.

Sterile lemma color: observation on the color variation of sterile lemma

Sterile lemma length: actual measurement of sterile lemma on each grain

ii. Agronomic traits

Leaf senescence: estimation on retention of greenness by observing all the leaves below the flag leaf at harvest stage.

Heading: the number of days from seedling to 85% flowering

Maturity: the number of days from seedling to 85% grain ripening

Panicle exertion: observation on the ability of panicles to exert from the flag leaf sheath

Phenotypic acceptability: visual observation on the phenotypic performance of varieties.

Plant height: the actual measurement from the ground to the tip of the panicle.

Grain shape: computed as a ratio of grain length to width.

Grain yield: the amount of grain obtained from each plot (interior four rows) in grams adjusted to 14% moisture content

Grain filling period (GFP): the number of days elapsed between heading to maturity days.

Disease score on major rice diseases: no prevalence (occurrence) of diseases during experimentation.

NB: a remark on how to measure and take record on each of the above morphological and agronomic traits is given under appendix 2.

4.4.3 Data analysis

Morphological traits of each genotype were measured on five randomly chosen plants in each replication for genetic diversity analysis of rice accessions from Ethiopia. Homogeneity and normality of error variance was done mainly using relationship of predicted means and residuals for all quantitative traits. For quantitative traits, analysis of variance revealed significant genotypic differences, but non significant replication

difference, hence average of the three replications was taken for further analysis. Means of morphological traits were compared according to Duncan's Multiple Range Test (DMRT) (Sajida *et al*., 2009).

The quantitative data for all the parameters were subjected to analysis using SAS statistical computer package for ANOVA. Range, coefficients of phenotypic and genotypic variances and heritability were calculated from mean square value and grand mean for each trait. Estimation of phenotypic correlation coefficient was performed using SAS and Cropstat statistical softwares (IRRI, 2007). Average data taken from five sample plants in each plot (four middle rows) was used in the analyses for traits that required sampling. Total variation was partitioned in to known and unknown effects following the standard procedures of ANOVA given in Table 6 (Gomez and Gomez, 1984).

Table 6: Source of variation, degree of freedom and mean squares for RCB design

Source of Variation	**Degree of freedom**	**Mean square**	**Expected Mean square**
Replications	(r-1)	MS_r	$\sigma^2_e + g\,\sigma^2_r$
Genotypes	(g-1)	MS_g	$\sigma^2_e + \sigma^2_g$
Error	(r-1)(g-1)	MS_e	σ^2_e

r= number of replications, g= number of genotypes, MS_e= mean square of error, MS_r= mean square of replication, MS_g= mean square of genotypes.

Genotypic, environmental and phenotypic variances were estimated according to Falconer (1981) as:

Genotypic variance $(\sigma^2_g) = (MS_g - MS_e)/r$

Environmental variance $(\sigma^2_e) = MS_e/r$

Phenotypic variance $(\sigma^2_p) = \sigma^2_g + \sigma^2_e$

Heritability in broad sense was computed as a percentage of genotypic variance to phenotypic variance. Correlation analysis was performed following the procedure given by Gomez and Gomez (1984) in order to examine the degree of associations between quantitative traits. The cluster analysis was conducted on average taxonomic distance with UPGMA method and a dendrogram was generated based on the genetic distance matrix using SPSS statistical computer package (Samarajeewa *et al*., 2004; Seetharam *et al*., 2004). Principal component analysis (PCO) was performed in order to highlight the resolving power of the ordination (Seetharam *et al*., 2004).

5. RESULTS

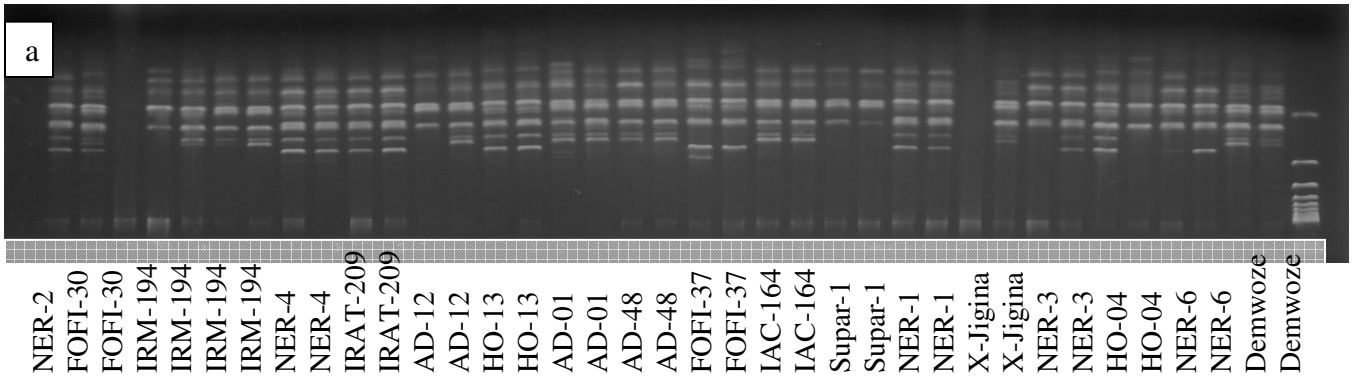
5.1 Molecular diversity analysis

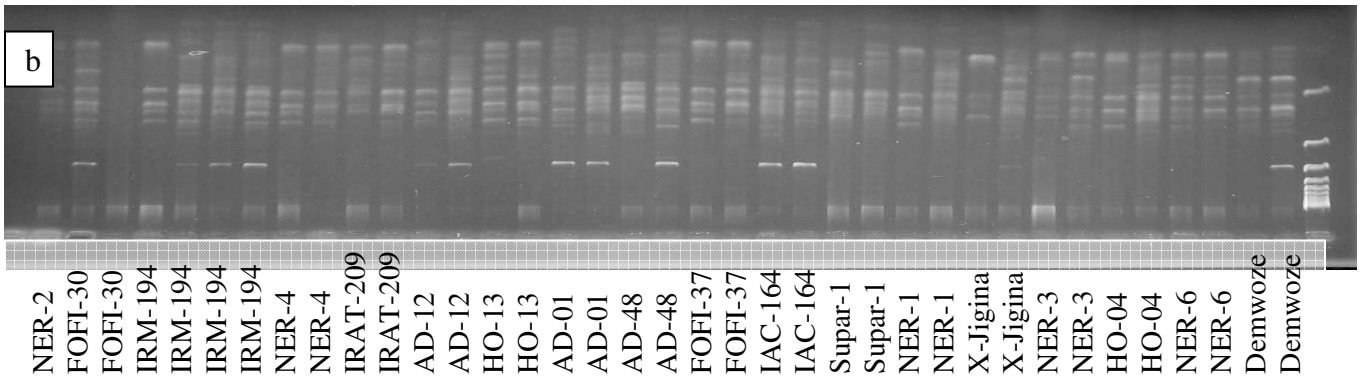
5.1.1 Banding patterns of the ISSR primers used

The banding patterns generated from the five primers used showed good amplification pattern (Table 7 and Figure 3). A total of 75 bands were scored from the five primers, out of which 62 were found to be polymorphic. The least polymorphic bands (eight) were scored from primer 873_H and the highest 16, were scored from primer 810_H.

Table 7. Banding patterns generated using the five primers, their repeat motifs, amplification patterns and number of scored bands.

Primers	Repeat motif	Amplification pattern	Number of scored bands
810_H	$(GA)_8T$	Good	16
824_H	$(TC)_8G$	Good	12
834_H	$(AG)_8YT$	Good	20
873_H	$(GACA)_4$	Good	12
878_H	$(GGAT)_4$	Good	15
		Total	75





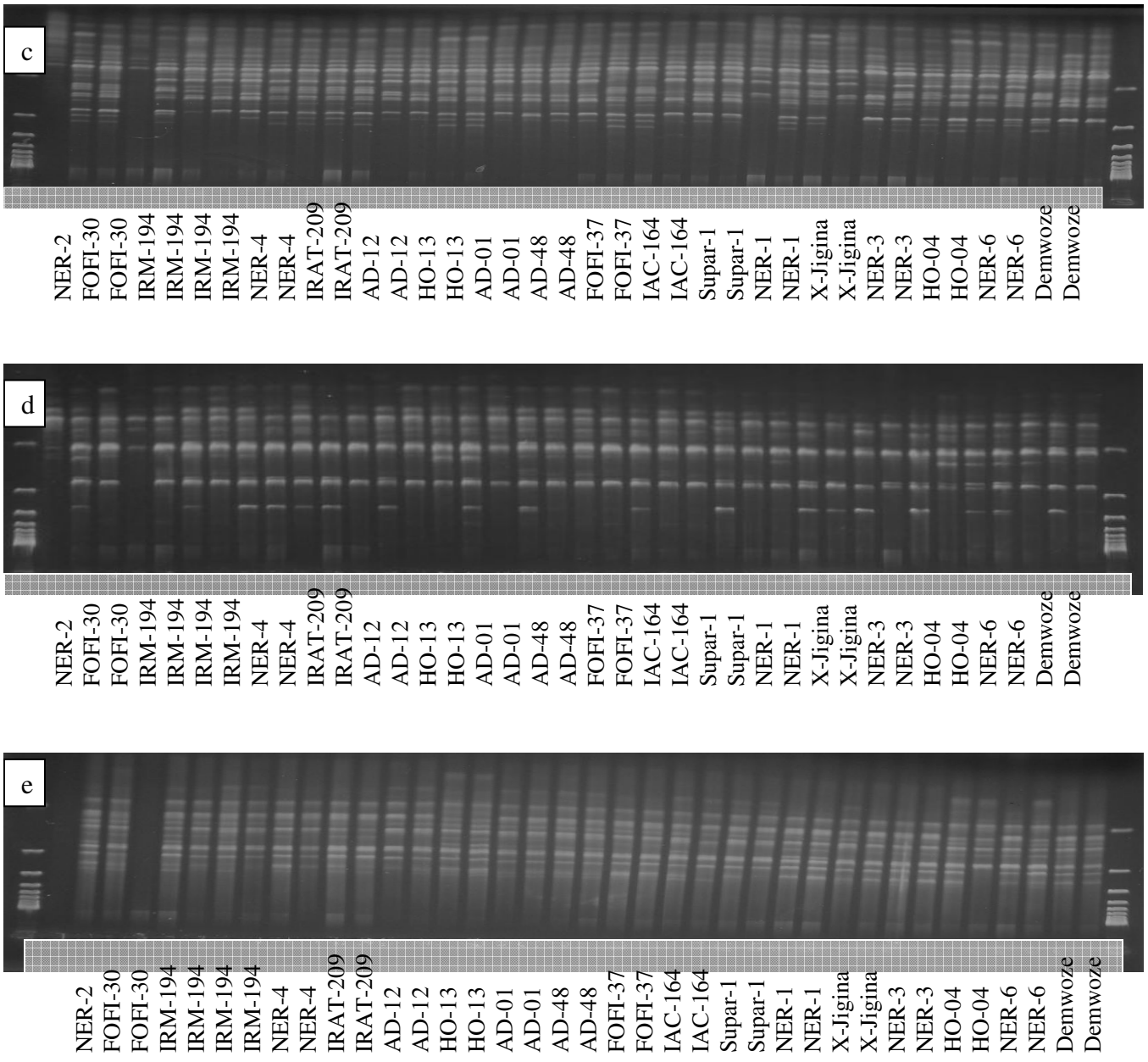


Figure 3: ISSR fingerprint generated from 37 individuals of 18 released and elite rice varieties of Ethiopia using primers (a) 810_H, (b) 824_H, (c) 834_H, (d) 873_H and (e) 878_H.

5.1.2 Polymorphism based on ISSR analysis

In all populations or individuals the number of polymorphic loci ranges from eight for primer-873 to sixteen for primer-810. Of the total 75 loci scored, 62 (82.67%) were observed to be polymorphic. In the lowland rice varieties, the number of polymorphic

loci and per cent polymorphism were 56 and 74.67, respectively. Likewise, in the upland and NERICA rice varieties, the numbers of polymorphic loci were 46 and 43 and their polymorphism percentages were 63.32 and 57.33, respectively. Among the different groups of rice varieties and lines of Ethiopia the lowland ones showed the highest polymorphism, 74.67%, and the NERICAs showed the least percent polymorphism,57.33% (Table 8a and b). As to the primers used, two primers, namely, 810 and 824, showed 100 per cent polymorphism. The rest primers vis-à-vis, 834, 878 and 873 showed 75, 73.33 and 66.67 per cent polymorphism in the overall analysis. The least polymorphism was observed from primer-873. As compared to the results of Girma *et al* (2010), most primers gave lower NPL, except for primers 834 and 878. Lower percent polymorphism was recorded from primers 834, 873 and 878. Higher genetic diversity was revealed by primers 824 only; the rest primers showed lower GD as compared to the results of Girma *et al* (2010).

Table 8: (a) Number of polymorphic loci (NPL), percent polymorphism (PP), and genetic diversity (GD) of lowland, upland and NERICA rice varieties and genotypes of Ethiopia with all five primers; and (b) with each primer.

a

Rice ecology	**NPL**	**PP**	**GD**
Lowland	56	74.67	0.27
Upland	46	61.33	0.25
NERICA	43	57.33	0.23
Total	62	82.67	0.29

b

Primers	**Total no. of fragments**	**NPL**	**PP**	**GD**
810	16 (19)	16 (19)	100 (100)	0.35 (0.40)
824	12 (16)	12 (16)	100 (100)	0.36 (0.34)
834	20 (15)	15 (15)	75 (100)	0.27 (0.35)
873	12 (22)	8 (21)	66.67 (95.45)	0.20 (0.30)
878	15 (9)	11 (8)	73.33 (88.89)	0.26 (0.35)
Total	75 (93)	62 (90)	82.67 (96.77)	0.29 (0.34)

NB: Numbers in brackets are results obtained from Girma *et al.,* 2010 on wild and cultivated rice genotypes of Ethiopia.

5.1.3 Genetic diversity

Nei's gene diversity estimate was done for each rice ecology and overall rice varieties of Ethiopia. Among the rice ecology, lowland rice elite and released varieties have showed higher gene diversity estimate (0.27) than both upland (0.25) and NERICA (0.23) varieties of Ethiopia, both released and elite (Table5.2a). The over all genetic diversity of the Ethiopian rice varieties was observed to be 0.29. In other words the genotypes showed 0.71 similarity coefficient.

5.1.4 Analysis of molecular variance

Analysis of molecular variance was carried out on the over all ISSR data score of Ethiopian rice varieties with and without grouping. The grouping was constructed based on their ecology, upland vs lowland rice. As shown in Table 9a, analysis of molecular variance revealed that higher percentage of variation was attributed to variation within different ecological groups with 88.67 per cent and the rest 11.33 per cent variation was attributed to among groups variation. In other words, the variation among the different rice ecologies/populations vis-à-vis lowland, upland and upland NERICA was lower than the variation that exists within each population. It is also observed that variation among populations and within populations was found highly significant at P=0.00. Similarly with grouping (Table 9b), the AMOVA result showed that higher percentage of variation was attributed to variation within individuals (81.21%) and followed by variation among groups (16.46%) and variation among individuals within groups (2.33%). The grouping constituted two groups, that is, sativa rice- lowland and upland ecologies as one group and the upland NERICA of the inter- specific hybrid as the second group. The percentage of variation among groups, among individuals within groups and within individuals was also found to be highly significant at (P=0.00) level of significance.

Table 9: Analysis of molecular variance for elite and released rice varieties of Ethiopia (a) without grouping; and (b) with grouping.

a

Source of variation	Degrees of freedom	Sum of Squares	Variance components	Percentage of variation
Among groups	2	50.90	1.32	11.33
Within individuals	34	342.48	10.30	88.67
Total	36	393.38	11.61	

b

Source of variation	Degrees of freedom	Sum of Squares	Variance components	Percentage of variation
Among groups	1	36.22	2.08662	16.46
Among individuals within groups	1	14.67	0.30	2.33
Within individuals	34	342.48	10.30	81.21
Total	36	393.38	12.68	

5.1.5 Clustering analysis

UPGMA dendrogram clustering of 37 individuals based on 75 PCR bands amplified by three di-nucleotides (810, 824, and 834) and two tetra nucleotides (873 and 878) ISSR primers are shown in Figure 5. The dendrogram derived from the whole ISSR data showed two distinct clusters and sub-clusters within each main cluster as indicated on Figure 5. The first cluster consisted of all the lowland and most upland rice varieties of Ethiopia. Demewoze, a variety selected and maintained by an innovator rice farmer in Fogera plain, X-Jigina, a well adopted and produced local variety in all major rice producing areas of the country, Suparica-1, and IAC-164 (Gumara) were among the lowland varieties grouped with the first cluster. Upland varieties IREM-194 (Tigabe), AD-01 (Getachew), AD-048 (Tana) and AD-12 (Andassa) were grouped in the first cluster with the lowland varieties mentioned above.

At about 70% similarity coefficient, the first cluster forked into two sub-clusters- one consisting Demewoze and X-Jigina and the other consisting of IAC-164, Suparica-1, AD-01, AD-48 and IRM-194. X-Jigina and Demewoze showed more than 75% similarity. AD-01, AD-48 and IREM-194 clustered together very closely (88%). Suparica-1 was clustered with X-Jigina and Demewoze at about 73% similarity. It is interesting to note that more or less distinctness of lowland and upland sativa pure lines in the two sub-clusters of the first cluster indicating the rice ecological basis for genetic similarity/dissimilarity.

The second cluster was consisting of all the NERICA varieties vis-à-vis NERICA-6, NERICA-3, NERICA-1, NERICA-2, NERICA-4, and genotypes presumed to be lowlands in rice improvement strategy -FOFIFA-3730, FOFIFA-3737, HO-04 and HO-

13. This might be due to the fact that NERICAs are inter-specific hybrids of *Oryza sativa* and *Oryza glabberima* and hence the lines might have a very closer genetic similarity with NERICAs' sativa parent.

Around 70% similarity, two sub-clusters were distinct in the second cluster. The first sub-cluster consisted of NERICA-6 and HO-04 and the rest varieties and other genotypes of the second main cluster were grouped under the second sub-cluster. At around 80% of similarity, NERICA-1, FOFIFA-3737, IRAT-209, and NERICA-4 were closely related together and NERICA-3 was similar in more than 73% similarity with this sub-group. HO-13, NERICA-2 and FOFIFA-3730 were clustered together at more than 75% similarity. The clustering of IRAT-209 (Kokit) with the second group negates the expectation that it would be grouped in the first cluster. The genotype (IRAT-209) might have had a tight similarity with sativa parental lines of NERICAs.

Dendrogram developed from neighbor-joining analysis of 37 individuals (18 genotypes) based on 75 PCR bands amplified by three di nucleotide and two tetra nucleotide ISSR primers is given on Figure 4. The neighbor-joining algorithm is based on Jaccard's coefficients obtained after pair wise comparison of presence-absence finger print. From the dendrogram established by neighbor-joining method (Figure 4) and visual observation on field trial it is clearly noted that few varieties lacked purity (IREM-194, AD-12 and HO-04).

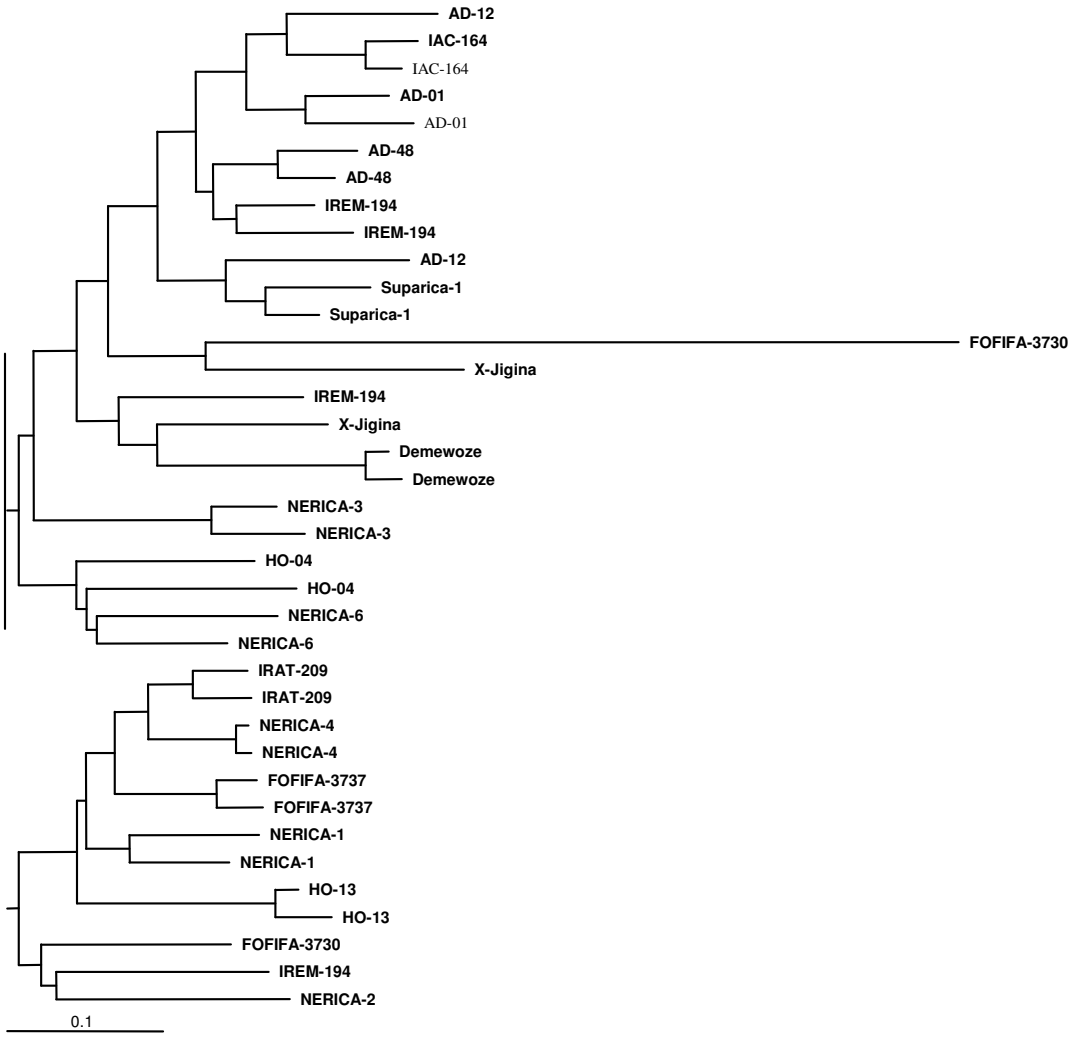


Figure 4: Neighbor-joining clustering of 37 individuals of 18 rice genotypes (released and elite) based on ISSR data generated from five primers.

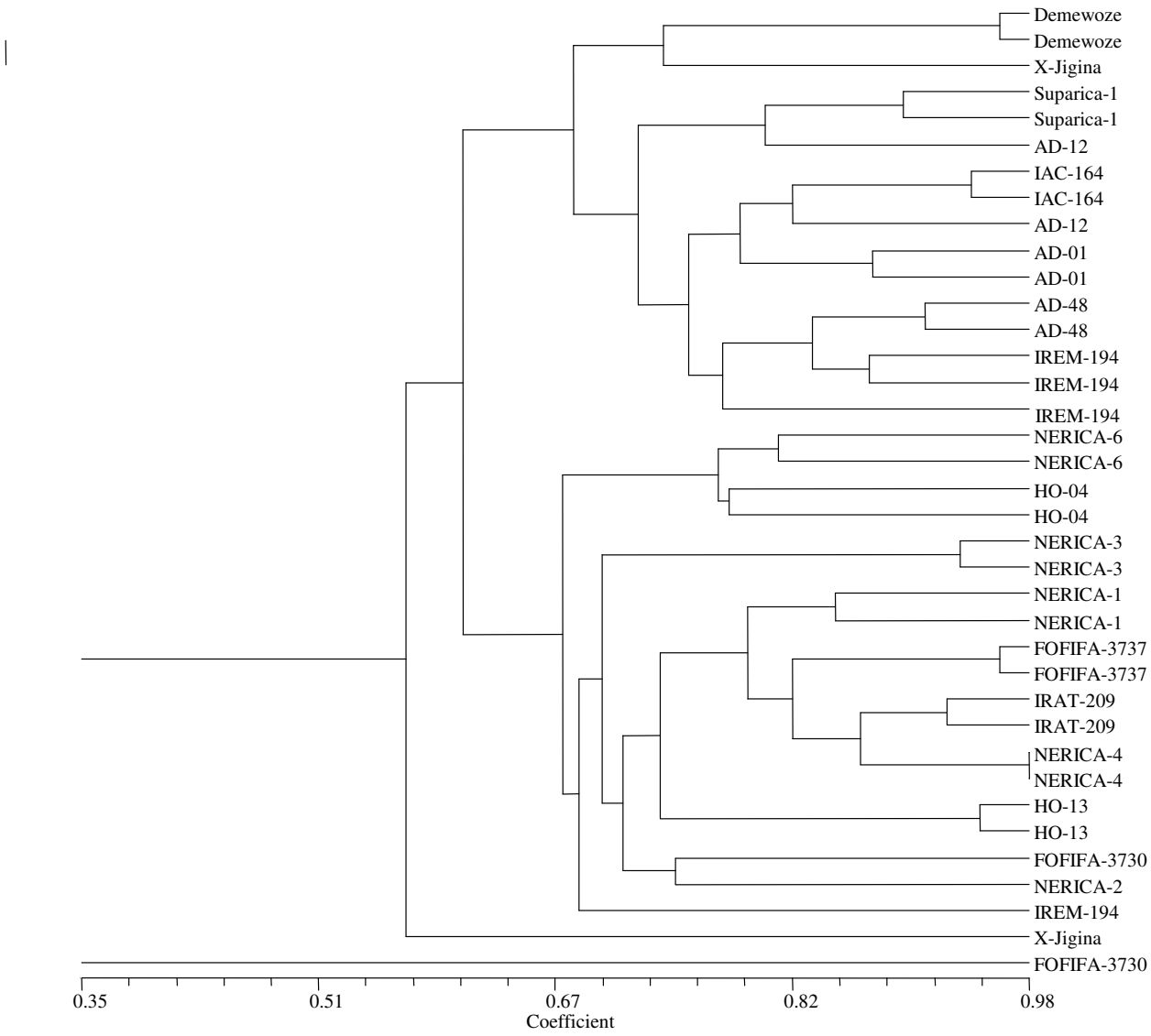


Figure 5: Dendrogram of 18 rice varieties and genotypes of Ethiopia generated from ISSR data using un-weighted pair group method of arithmetic means (UPGMA).

5.1.6 PCO analysis

PCO analysis was carried out on all the data obtained from five primers using Jaccard's coefficient of similarity. The first three coordinates of the PCO having Eigen values of 3.31, 1.64 and 1.53 with variance of 17.45%, 7.73% and 7.18%, respectively, were used to show the grouping of individuals using two and three coordinates (Figures 5.4 and 5.5). From Figure 6, it was depicted that all the test materials (rice genotypes) could be grouped into two major clusters. The first cluster roughly is comprised of all upland

sativa rice genotypes including IREM-194, IRAT-209, AD-01, AD-12, AD-48. The second cluster consisted of the rest NERICA rice varieties and a few lowland rice genotypes.

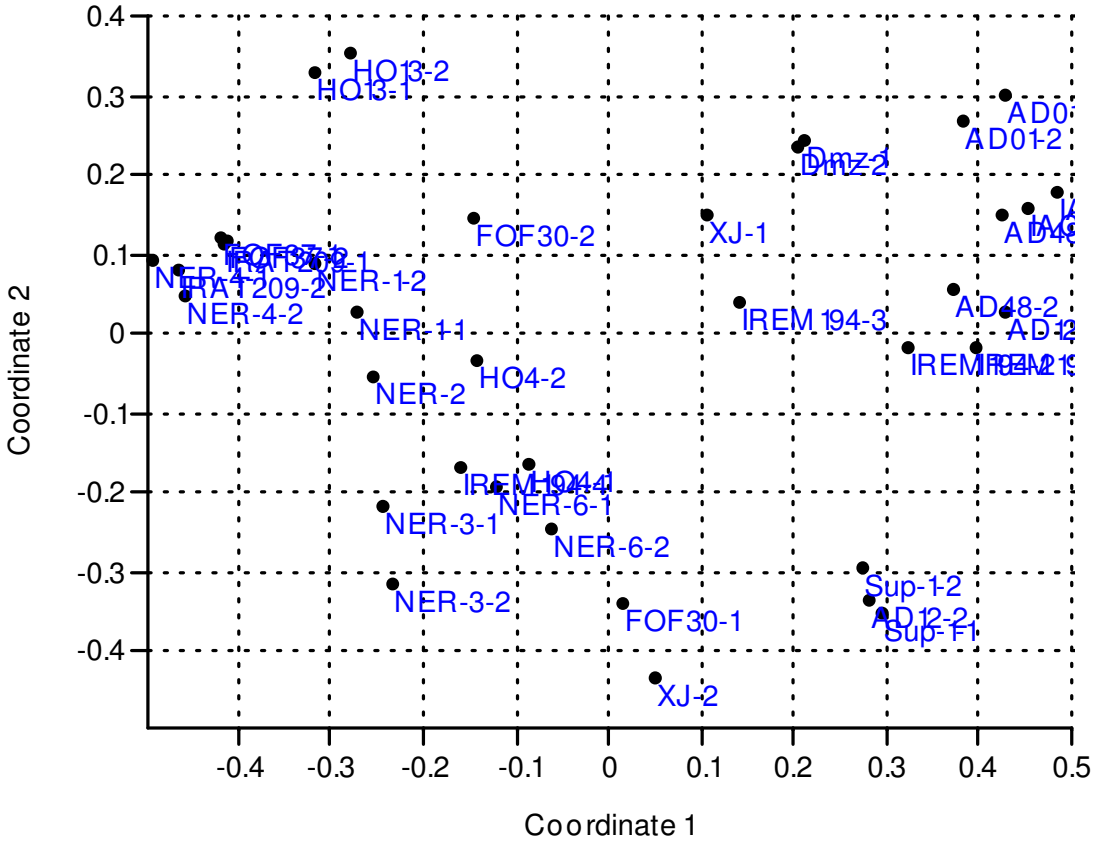


Figure 6: Two dimensional representations of PCO analysis of18 elite and released rice varieties of Ethiopia based on Jaccard's similarity coefficients using coordinates 1 and 2.

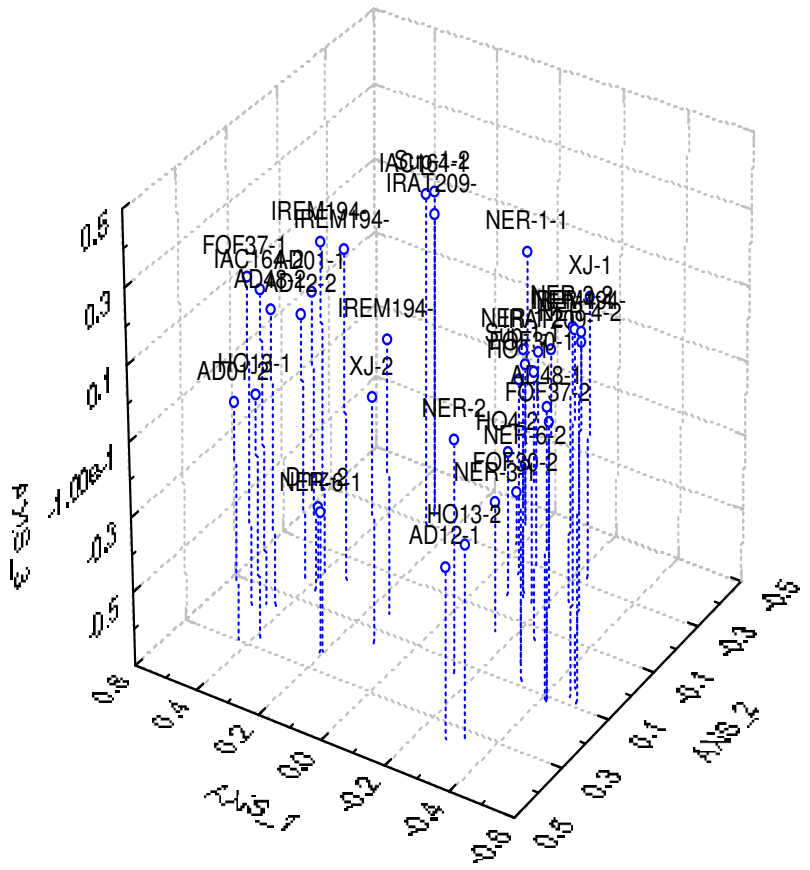


Figure 7: Three dimensional representation of principal coordinate analysis of genetic relationships among 37 individuals of 18 elite and released rice varieties of Ethiopia

5.2 Morphological diversity analysis

5.2.1 Analysis of variance

Analysis of variance was carried out for all (thirteen) quantitative traits of the test materials using SAS and cropstat statistical softwares following the procedures given by Gomez and Gomez (1984). Table 10 presents mean squares due to various sources of variation, coefficient of variation and coefficient of determination. The F-test showed that there was a highly significant (P=0.00) variation among the different rice varieties for all

the quantitative traits studied. The quantitative traits investigated in this study were leaf length, leaf width, culm length, plant height, panicle length, number of days to heading, number of days to maturity, grain filling period, grain length, grain width, grain shape, sterile lemma length and yield. Duncan's multiple range test (DMRT) carried out after the analysis of variance (ANOVA) showed that there was at least one variety superiorly significant from others for all the quantitative-agronomic and morphological traits investigated. The ANOVA and DMRT results are give on tables under Appendix 3 and 4, respectively. The results shown on Tables 10 and 11 are shortly presented below.

Leaf length (cm): DMRT for leaf length (cm) grouped the varieties in to eight non-mutually exclusive groups. The highest records were observed from HO-13-5-3-B-4 followed by HO-04-7-1-B-5 with values of 43.93 and 41.73cm, respectively. The lowest leaf lengths were found to be 21.13 and 21.8cm for varieties NERICA-2 and NERICA-1, respectively. The mean leaf length for all the varieties under investigation was found to be 30.82cm.

Leaf width (mm): Seven non-mutually exclusive groups were found to exist from DMRT. The highest record was obtained from HO-13-5-3-B-4 with mean value of 13.67mm followed by NERICA-6 with mean value of 12.00mm. The lowest leaf width was found to be 10.27, 10.4 and 10.4 mm from NERICA-2, NERICA-4 and NERICA-1. All the varieties could be grouped in to one group in respect to leaf width as intermediate. The mean value of the varieties was recorded to be 10.80mm.

Plant height (cm): Six visible groups of Ethiopian rice varieties were found to exist after the test. The highest mean plant height values were recorded from HO-13-5-3-B-4 with value of 108.33cm followed by HO-04-7-1-B-5 with mean value of 104. 47cm. NERIA-4 and IRAT-209 were found to be the shortest varieties with mean values of 76.07 and 76.81cm, respectively. 88.10cm being the mean value of all genotypes tested.

Panicle length (cm): The test materials were grouped in to four distinct groups. The highest record was found to be 22.93cm for HO-13-5-3-B-4 and the least was obtained

from NERICA-1 with mean value of 17.87 cm. The overall mean value of the varieties was found to be 19.5cm.

Culm length (cm): The varieties were observed to be grouped in to six distinct groups. The highest culm length record was obtained from HO-13-5-3-B-4 with mean value of 85.4cm followed by HO-04-7-1-B-5 with mean value of 82.53cm. The lowest records were found to be 49.73cm for NERICA-1 and 49.87cm for NERICA-2. The average value for culm length of the whole varieties tested was 68.60cm. However, according to Bioversity International's guideline (2007) for plant description, the varieties could be grouped as: very short (NERICA-1 and NERICA-2); very short to short (X-Jigina, FOFIFA-3737, Suparica-1, FOFIFA-3730, IRAT-209, NERICA-3, NERICA-4 and IREM-194) and short (HO-13-5-3-B-4, HO-04-7-1-B-5, AD-12, IAC-164, Demewoze, NERICA-6, AD-01 and AD-48).

Number of days to heading: DMRT revealed ten mutually non-exclusive groups (the first and the last groups were found to be mutually exclusive) of varieties based on the number of days elapsed from seeding to heading. Elite variety HO-13-5-3-B-4 was too late as compared to others and took 124.67 days to head. NERICA-2 was found to be early to head with mean observed number of days of 98.33. The mean number of days to heading for all varieties tested was observed to be 107.13 days.

Number of days to maturity: Seven mutually exclusive and non exclusive groups were identified after DMRT. In a similar fashion with that of number of days to heading the latest variety to mature was HO-13-5-3-B-4 with mean value of 153.00 and the earliest to mature among the varieties was NERICA-2 which took 124.67 days to mature. The overall mean number of days to mature was found to be 136.67 days.

Grain filling period: The number of days taken by each variety to fill grain was in the range of 35.67 days for AD-48 to 24.00 for NERICA-4. Varieties AD-48, AD-12 and IAC-164 were found to take longer period to fill grain, with mean values of 35.67, 34.67 and 34.33 days, respectively. Where as, varieties NERICA-4 and NERICA-3 took the

shortest period among the other varieties to fill grain, with observed mean values of 24.00 and 24.33 days, respectively. The mean observed period for grain filling by all varieties was found to be 29.54 days.

Grain length (mm): Based on the mean grain length records of the varieties, the test grouped all the varieties in to seven distinct groups. The longest grained variety was found to be HO-13-5-3-B-4 with mean value of 10.12mm followed by FOFIFA-3737 with a mean record of 9.92mm. The shortest grained varieties among the other were X-Jigina and Demewoze with mean values of 7.36 and 7.61mm, respectively. The mean grain length of all genotypes was found to be 8.98mm.

Grain width (mm): The mean separation procedure, DMRT, put all the varieties in to five distinct groups based on their mean observed grain width values. The mean observed value of the genotypes foe the trait was found to be 3.27mm. The narrowest seeded varieties were found to be NERICA-2, NERICA-3 and NERICA-4 with mean observed values of 2.86, 2.90 and 2.91mm, respectively. In contrast the widest records for the trait were obtained from IRAT-209, Demewoze and X-Jigina with mean observed values of 3.59, 3.49 and 3.47 mm, respectively.

Grain shape: The trait is obtained as a ratio of grain length to grain width. The test grouped the varieties in eight different groups. NERICA-4 and NERICA-3 with mean observed values of 3.27 and 3.18, respectively, gave the highest grain length to grain width ratio. The lowest observed values for the trait were found to be 2.13 and 2.18 for the varieties X-Jigina and Demewoze, respectively.

Sterile lemma length (mm): DMRT showed five different groups of varieties based on their sterile lemma length variations with a mean value of 2.76mm. Varieties HO-13-5-3-B-4 and NERICA-4 gave the highest sterile lemma length records with mean values of 3.23 and 3.22mm, respectively. The shortest records for the trait were obtained from IAC-164, IREM-194 and HO-04-7-1-B-5 with mean observed values of 2.43, 2.45 and 2.49mm, respectively.

Grain yield (g/plot): All the test varieties, with mean observed value of 1556.98 g/plot adjusted at 14% moisture content, grouped in to three different groups. The highest yield record was obtained from variety AD-12 with mean grain yield value of 2161.4 g/plot. Varieties X-Jigina and NERICA-2 yielded the least with mean values of 1169.3 and 1174.2 g/plot.

Table 10: ANOVA mean square values, coefficient of variation (CV) and coefficient of determination (R^2) of 13 traits for 18 released and elite rice varieties of Ethiopia.

SOURCE	DF	MEAN SQUARES				
		Leaf length	Leaf width	Culm length	Plant height	Panicle length
Blocks	2	13.42	0.11	13.37	17.43	0.27
Genotypes	17	119.67	3.83	394.32	495.66	7.92
Error	34	11.62	0.52	24.97	33.48	1.07
CV (%)		11.06	6.70	7.28	6.57	5.31
R^2		0.84	0.79	0.89	0.88	0.79

SOURCE	DF	MEAN SQUARES				
		Grain filling period	Grain length	Grain width	Grain shape	Sterile lemma length
Blocks	2	9.41	0.07	0.01	0.002	0.24
Genotypes	17	42.12	1.67	0.12	0.35	0.16
Error	34	3.43	0.12	0.01	0.02	0.05
CV (%)		6.27	3.92	2.47	4.39	7.73
R^2		0.86	0.87	0.91	0.92	0.15

SOURCE	DF	MEAN SQUARES			
		Grain yield (g/plot)	Grain yield (Kg/ha)	Days to Heading	Days to Maturity
Blocks	2	8406.97	52543.53	0.96	4.67
Treatments	17	244288.59	1526803.66	99.73	189.92
Error	34	109618.89	685118.03	1.02	4.76
CV (%)		21.26	21.26	0.94	1.60
R^2		0.53	0.53	0.98	0.95

NB: The ANOVA and DMRT tables for each trait are given on appendix 3 and 4, respectively.

Table 11. Mean performance of 18 released and elite rice genotypes of Ethiopia for thirteen quantitative morphological traits during 2009/2010 cropping season at Ataye.

Genotypes	LL	LW	Ht	PanL	CL	Hed	Mat	GFP	GL	GW	GS	SLmL	Yield
AD-01	32.47	11.07	95.60	19.40	76.20	104.67	137.67	33.00	9.37	3.25	2.89	2.88	1817.35
AD-12	37.33	11.47	102.20	21.47	80.73	111.33	146.00	34.67	8.97	3.39	2.65	2.85	2161.43
AD-48	34.40	10.60	95.07	20.27	74.80	109.33	145.00	35.67	8.68	3.36	2.58	2.62	1832.99
Demewoze	31.07	9.67	97.33	19.07	78.27	106.00	135.00	29.00	7.61	3.49	2.18	2.71	1789.95
F-3730	32.27	11.00	81.80	18.47	63.33	104.67	131.33	26.67	9.72	3.31	2.94	2.95	1257.77
F-3737	28.33	11.73	87.60	18.07	69.53	106.00	132.33	26.33	9.92	3.30	3.03	2.91	1366.82
HO-04	41.73	11.80	104.47	21.93	82.53	115.33	148.00	32.67	8.43	3.39	2.49	2.49	2302.94
HO-13	43.93	13.67	108.33	22.93	85.40	124.67	153.00	28.33	10.12	3.31	3.06	3.23	1671.59
IAC-164	36.53	10.87	99.80	21.33	78.47	110.67	145.00	34.33	8.48	3.27	2.59	2.43	1567.20
IRAT-209	24.20	9.07	76.87	18.20	58.67	104.00	129.67	25.67	8.86	3.59	2.48	2.75	1252.15
IREM-194	26.80	9.47	74.20	18.20	56.00	105.33	137.33	32.00	8.42	3.38	2.50	2.45	1443.96
NERICA-1	21.80	10.40	67.60	17.87	49.73	102.67	129.00	26.33	9.74	3.08	3.16	2.64	1608.17
NERICA-2	21.13	10.27	67.93	18.07	49.87	98.33	124.67	26.33	8.99	2.86	3.15	2.65	1540.08
NERICA-3	25.67	10.53	75.93	18.20	57.73	104.33	128.67	24.33	9.21	2.90	3.18	2.86	1715.63
NERICA-4	26.07	10.40	76.07	18.73	57.33	104.00	128.00	24.00	9.52	2.91	3.27	3.22	1725.33
NERICA-6	31.80	12.00	99.33	21.53	77.80	106.33	139.00	32.67	8.92	3.26	2.74	2.60	1232.47
Suparica-1	29.47	11.33	87.47	18.73	68.73	107.00	136.33	29.33	9.32	3.34	2.79	2.71	1588.12
X-Jigina	29.80	9.20	88.27	18.53	69.73	103.67	134.00	30.33	7.36	3.47	2.13	2.66	1540.41
Mean	30.82	10.80	88.10	19.5	68.60	107.13	136.67	29.54	8.98	3.27	2.77	2.76	1556.98
CV (%)	11.06	6.70	6.57	5.31	7.28	0.94	1.60	6.27	3.92	2.47	4.39	7.73	21.26
R^2	0.84	0.79	0.88	0.79	0.89	0.98	0.95	0.86	0.87	0.91	0.92	0.15	0.53

LL= leaf length, LW= leaf width, Ht= plant height, PanL= panicle length, CL= culm length, Hed= number of days to heading, Mat= number of days to maturity, GFP= grain filling period, GL= grain length, GW= grain width, GS=grain shape, SLmL= sterile lemma length.

5.2.2 Estimates of variability parameters

Mean observed values of heritable thirteen morphological and agronomic quantitative traits were subjected to both descriptive as well as inferential statistics including mean, range of mean observed values, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and heritability in broad sense. This was so to trace the extent of genetic variability that exists among the different tested rice varieties and lines of Ethiopia (Table 12).

In a general overview, phenotypic coefficient of variation (PCV) varies from 5.38% for number of days to heading to 20.49% for leaf length. Among the traits considered, higher PCV value was observed for leaf length (20.49%). Leaf width, culm length, plant height, grain filling period, grain shape and grain yield were found in the range of 10.46% to 18.33% which could be considered as medium. The rest six traits vis-à-vis panicle length, number of days to heading and maturity, grain length, grain width and sterile lemma length showed the least PCV values. .

The values of genotypic coefficient of variation (GCV) ranged from 5.36% for number of days to heading to 19.47% for leaf length. No trait was observed to have value greater than 20%. Hence, traits with medium GCV value include leaf length, culm length, plant height, grain filling period, grain shape and grain yield, with values in the range of 12.14% to 19.47%. Leaf width, panicle length, number of days to heading and maturity, grain length, grain width and sterile lemma length showed the least GCV in the range of 5.36% to 9.71%.

Higher heritability values were observed for all the 13 quantitative traits considered. The highest heritable trait was found for number of days to heading (98.98%) followed by number of days to maturity (97.49%). The least heritability was observed for grain yield with value of 55.13%. Among all the traits considered, nine traits showed heritability above 90%. Sterile lemma length showed lower heritability percentage (71.22%) as compared to the above traits but it was found to be higher than that of grain yield heritability value (55.12%).

Table 12 Range, grand mean, phenotypic coefficient of variation, genotypic coefficient of variation and heritability for 13 traits in 18 released and elite rice varieties of Ethiopia.

Trait	Min.	Max.	Range	Mean	PCV(%)	GCV(%)	H(%)
Leaf length	21.13	49.93	28.80	30.82	20.49	19.47	90.29
Leaf width	9.07	13.67	4.6	10.81	10.46	9.71	86.32
Panicle length	17.87	22.93	5.06	19.50	8.33	7.75	86.48
Culm length	49.73	85.40	35.67	68.60	16.71	16.17	93.67
Plant ht.	67.6	108.33	40.73	88.10	14.59	14.09	93.25
Heading	98.33	124.67	26.34	107.1	5.38	5.36	98.98
Maturity	124.67	153	28.33	136.6	5.85	5.77	97.49
GFP	24	35.67	11.67	29.54	12.68	12.16	91.86
Grain length	7.36	10.12	2.76	8.98	8.30	7.99	92.57
Grain width	2.86	3.59	0.73	3.27	6.24	6.07	94.78
Grain shape	2.13	3.27	1.14	2.77	12.40	12.14	95.83
Ster. lemma length	2.43	3.23	0.8	2.76	8.32	7.02	71.22
Grain yield	1169.3	2161.4	992.1	1556.98	18.33	13.61	55.12

5.2.3 Trait-wise estimates of variability

Leaf characteristics variability

Leaf attributes considered were leaf length and leaf width. Leaf length ranges from 21.13cm to 49.93cm with mean value of 30.82 cm. It had high phenotypic variability (PCV=20.49%) and moderate genotypic variability (19.47%). Heritability of leaf length was observed to be very high (90.29%). Observed leaf width was in the range of 9.07-13.67mm with mean value of 10.81mm. It was observed that the trait had moderate PCV (10.46%) and lower GCV (9.71%) values. Overall, leaf width was found to highly heritable (86.32%) (Table 12).

Variability in culm characteristics

Culm length and plant height were considered in this investigation so as to get a sort information on how the varieties vary in their culm and plant height characteristics. Culm length ranged from 49.73cm to 85.40cm with mean culm length of 68.60cm. Both PCV and GCV were moderate 16.71% and 16.17%, respectively (Table 12). However, heritability of the trait was found to be high (93.67%). In the same fashion, plant height was in the range of 67.60cm to 108.33cmm with mean value of 88.10cm. Although lower than the values in culm length, PCV (14.59%) and GCV (14.09%) were found to be moderate in plant height. With almost in equal magnitude with that of culm length, plant height was also found to be highly heritable (93.25%).

Variability in grain characteristics

Grain length, grain width, grain shape, sterile lemma length and grain yield were considered so as to get a clue on how the test materials varied with regard to these important agronomic traits. The range of observed values for grain length and width were from 7.36mm to 10.12mm and 2.86mm to 3.59mm, respectively. Their mean observed values were 8.89mm for grain length and 3.27mm for grain width. Both traits showed lower trend of variability in PCV and GCV with values of 8.30% and 7.99% and 6.24% and 6.07% for grain length and grain width, respectively. Both grain traits were found to be highly heritable, 92.57% and 94.78% for grain length and grain width, respectively (Table 12).

Sterile lemma length was found to be in the range of 2.43mm to 3.23mm with mean observed value of 2.76mm. It showed lower variability in PCV and GCV with values of 8.32% and 7.02%, respectively, but higher heritability (71.22%). Grain yield showed wider range of observed values, these could be due to the crop improvement objective that yielded up in these varieties that mainly focused on grain yield advantage over the existing local and/or standard varieties. It ranged from 1169.30 to 2161.40gm with mean value of 1556.98g. It showed moderate variability in PCV and GCV with values of 18.33% and 13.61%, respectively. Though the value was lower than the other traits considered, the trait showed higher heritability (55.13%) in all the released and elite rice varieties of Ethiopia studied. Panicle length was in the range of 17.87cm to 22.93cm with mean observed value of 19.50cm. Lower variability was observed in panicle length, PCV (833%) and GCV (7.75%) values (Table 12).

Variability in duration of growing period

The test materials took 98.33 to 124.67 and 124.67 to 153 days to head and mature, respectively. On average, the varieties took 107.1 and 136.6 days to heading and maturity (Table 12). The variability in both traits was found to be lower. PCV values were 5.38% and 5.85% for number of days to heading and maturity, respectively. Where as 5.36% and 5.77% GCV for number of days to heading and maturity, respectively. But they

showed higher heritability, 98.98% and 97.47% for number of days to heading and maturity, respectively.

Grain filling period was in the range of 24 to 35.67 days with mean value of 29.54 days. In contrast to the number of days to heading and maturity, it showed moderate variability among the test materials with PCV (12.68%) and GCV (12.16%). Heritability was found to be high (91.86%) as other traits did (Table 12).

5.2.4 Association of traits and estimates of correlation parameters

Association of quantitative morphological traits was carried out to assess the strength of relationships amongst them. Pearson correlation coefficients of the traits are given on Table 13 and a short account on the result for each trait is presented as follows.

Leaf length: It was found out that the association of leaf width, plant height, panicle length, culm length, number of days to heading, number of days to maturity and grain filling period with leaf length was highly significant at 1% level of significance. Grain yield was found to be associated with the trait at 5%. At 10% level of significance, the correlation leaf length with grain shape and grain width was significant but that of sterile lemma length and grain length was non significant (Table 13).

It was highly and positively correlated with plant height (r=0.79), panicle length (r=0.75), culm length (r=0.77), number of days to heading (r=0.82) and number of days to maturity (r=0.84). Its correlation with grain filling period (r=0.51), leaf width (r=0.63), and grain yield -g/plot (r=0.41) was positive but moderate. Weak positive correlation was also observed with grain width (r=0.33). Though it was seen to be weak, the correlation with grain shape was negative (r=-0.24) (Table 13).

Leaf width: Correlations of the trait with grain filling period, grain yield and grain width were found to be non significant at 10% level of significance. Sterile lemma length and grain shape were found to be significantly correlated with leaf width at 5% level of significance while grain length and the rest traits were found to be highly correlated with

the trait at 1% level of significance. Moderate positive correlation with leaf length (r=0.63), panicle length (r=0.62), number of days to heading (r=0.63), plant height (r=0.57), culm length (r=0.55), number of days to maturity (r=0.53) and grain length (r=0.46) was observed to exist with leaf width. Weaker positive correlations were also pointed out with sterile lemma length (0.27) and grain shape (r=0.34) (Table 13). No strong positive or negative associations observed with the trait.

Plant height: Leaf length, leaf width, panicle length, culm length, number of days to heading, number of days to maturity and grain filling period showed a highly significant correlation with plant height at 1% level of significance. Similarly, the correlation of grain yield, grain shape and grain width with the trait were also found to be highly significant at 1%. The rest two traits, namely sterile lemma length and grain length, were found to have no correlation at 10% level of significance. Leaf length (r=0.79), panicle length (r=0.85), culm length (r=0.99), number of days to heading (r=73), and number of days to maturity(r=82) showed higher positive correlation with plant height. Moderate positive correlations were observed with leaf width (r=0.57), grain filling period (r=0.6), grain shape (r=0.44) and grain yield (r=0.43). Weaker negative correlation was observed with grain width (-0.35) (Table 13).

Panicle length: Correlations with leaf length, leaf width, plant height, culm length, number of days to heading, number of days to maturity and grain filling period were found to be highly significant at 1%. Association with that of grain yield was also highly significant at 1% level of significance. Sterile lemma length, grain shape, grain width and grain length were not significantly correlated with panicle length at 10%. Higher positive correlation with leaf length (r=0.75), plant height (r=0.85), culm length (r=0.81), number of days to heading (r=0.75) and number of days to maturity (r=0.80); moderate positive correlation with leaf width (r=0.62), grain filling period (r=0.52) and grain yield (r=0.42) were found to exist with the trait (Table 13).

Culm length: Leaf length, leaf width, plant height, panicle length, number of days to heading, number of days to maturity and grain filling period were found to be highly

correlated with culm length at 1%. Correlation coefficients of the trait with grain yield, grain shape and grain width (at 1% level of significance) were also found to be highly significant. Sterile lemma length and grain length showed a non significance correlation with culm length at 10% level of significance. The association of the trait with leaf length (0.77), plant height (r=0.99), panicle length (r=0.81), number of days to heading (r=0.70) and number of days to maturity (r=0.80) was observed to be higher and positive. Similarly, the correlation with grain filling period (r=0.59), grain width (r=0.47), grain yield (0.43) and leaf width (r=0.55) were positive but moderate. However, grain shape (-0.38) showed weaker and negative association with the trait (Table 13).

Heading: Correlation coefficients of number of days to heading with leaf length, leaf width, plant height, panicle length, culm length, number of days to maturity and grain yield at 1% were found to be highly significant. Correlations with that of grain filling period and grain width were also significant at 5%. The rest three traits showed non significant correlation with number of days to heading (Table 13).

Stronger positive association with leaf length (r=0.82), plant height (r=0.73), panicle length (r=0.75), culm length (r=0.70) and number of days to maturity (r=0.88); and, moderate positive correlation with leaf width (r=0.63) were found to exist with number of days to heading. There were also weaker but positive associations with grain yield (r=0.39), grain filling period (r=0.34) and grain width (r=0.32) (Table 13).

Maturity: Correlation of the trait with leaf length, leaf width, plant height, panicle length, culm length number of days to maturity, grain filling period, grain yield and grain width at 1% were found to be highly significant. Grain shape at 5% was significantly correlated with number of days to maturity. Correlations with that of grain length and sterile lemma length were found to be non significant at 10% (Table 13). There were observed stronger positive association with leaf length (r=0.85), plant height (r=0.82), panicle length (r=0.80), culm length (r=0.80) and number of days to heading (r=0.88) and grain filling period (r=0.74). Moderate positive correlation with leaf width (r=0.53), grain width (r=0.43) and grain yield (r=0.46) were found to exist with number of days to

maturity. However, weaker negative association was observed between number of days to maturity and grain shape (r=-0.31) (Table 13).

Grain filling period: Correlation coefficient of grain filling period with leaf width was non significant at 10% level of significance. Trait association with grain yield, grain width and grain shape at 1% were found to be highly significant. Similarly, correlations of the trait with leaf length, plant height, panicle length, culm length and number of days to maturity were found to be highly significant at 1%. Associations with number of days to heading and, grain length and sterile lemma length were also found to be significant at 5% (Table 13).

It was strongly and positively correlated with number of days to maturity only; and, moderate and positive associations were observed with leaf length (0.51), plant height (r=0.60), panicle length (r=0.51), culm length (r=0.59) and grain width (r=0.40). Correlations with number of days to heading (r=0.34) and grain yield (r=0.36) were found to be positive but weak. Weaker negative relationships were also seen with grain length (r=-0.34) and sterile lemma length (r=-0.30); and modest negative association with grain shape (r=-0.47) (Table 13).

Grain length: The correlation coefficients were non-significant at 10% significant level for leaf length, plant height, panicle length, culm length, number of days to heading, number of days to maturity and grain yield. But there were significant differences for grain width, grain shape and sterile lemma length at 1%, and for grain filling period at 5% level of significance. The association between grain length and grain shape was strong and positive (r=0.86). In addition sterile lemma length (r=0.57) showed a moderate association with grain length. Grain filling period (r=-0.34) and grain width (r=-0.37) have showed a weaker complementation with the trait (Table 13).

Grain width: Correlations of leaf width, plant height, culm length, panicle length and grain yield with grain width did not showed significant difference at 10% level of significance. At 5% level of significance, associations of leaf length and number of days

to heading with the trait showed a significant difference. The rest traits showed high significant correlation coefficients with grain width. The correlation was found to be strong and negative with grain shape(r=-0.79). Associations with number of days to maturity (r=0.43) and grain filling period (r=0.40) were found to be positive and moderate, but the associations with leaf length (r=0.33) and number of days to heading (r=0.32) were positive but weak. There was also a negative but weak complementation of grain width with grain length (r=-0.37) (Table 13).

Grain shape: correlation of grain shape with panicle length, number of days to heading and grain yield was found to be non-significant at 10% level of significance. Associations of the trait with leaf width and number of days to maturity at 5% and the rest traits, except leaf length, at 1% level of significances were found to be significant. Leaf length showed significant correlation with grain shape at 10% level of significance. There was a strong positive correlation of the trait with grain length (r=0.86) and strong negative correlation with grain width (r=-0.79). The association with sterile lemma length (r=0.48) was positive and moderate; where as, the association of the trait with grain filling period (r=-0.47) was found to be moderate but negative. Traits including leaf length (r=-0.24), plant height (r=-0.35), culm length (r=-0.38) and number of days to maturity (r=-0.31) showed a weak and negative relationships with grain shape. The only weak and positive complementation was observed between grain shape and leaf width (r=0.34) (Table 13).

Sterile lemma length: It was observed that the correlation of the trait with leaf length, plant height, culm length, panicle length, number of days to heading, number of days to maturity, grain width and grain yield was non-significant at 10% level of significance. Positive moderate associations of sterile lemma length were observed with grain length (r=0.57) and grain shape (r=0.48). Complementation of sterile lemma length with leaf width (r=0.27) and grain filling period (=-0.30) were found to be weak but in positive and negative directions, respectively (Table 13).

Grain yield: It is a very important trait on which several efforts were made so as to improve its potential. It is controlled by both genotypic and environmental factors. No

strongly and either positively or negatively associated trait found to exist in this study. This could happen probably due to several uncontrolled errors during the course of the experiment. However, there were moderate to weak positive associations with some traits. At 10% level of significance, leaf width, grain length, grain width, grain shape and sterile lemma length showed non-significant correlations with grain yield, but leaf length was significantly correlated at this level of significance. While the associations of leaf length, panicle length, culm length, number of days to heading and grain filling period with grain yield were found to be significant at 5% level of significance, the correlations of plant height and number of days to maturity with grain yield were found to be highly significant.

The correlation with leaf length (r=0.41), plant height (r=0.43), panicle length (r=0.42), culm length (r=0.43) and number of days to maturity (r=0.46) were found to be positive and moderate. Number of days to heading (r=0.39) also showed to complement the trait positively but in a weaker magnitude (Table 13).

Table 13: Pearson correlation coefficients of 13 quantitative morphological traits of 18 released and elite rice varieties of Ethiopia tested during 2009/10 short rainy season in Ataye.

	LL	LW	Ht	PnL	CL	Hed	Mat	GFP	GL	GW	GS	SLmL	Yield
LL	1.00												
LW	0.63***	1.00											
Ht	0.79***	0.57***	1.00										
PnL	0.75***	0.62***	0.85***	1.00									
CL	0.77***	0.54***	0.99***	0.81***	1.00								
Hed	0.82***	0.63***	0.73***	0.75***	0.70***	1.00							
Mat	0.84***	0.53***	0.82***	0.79***	0.80***	0.88***	1.00						
GFP	0.51***	0.16	0.60***	0.51***	0.59***	0.34**	0.74***	1.00					
GL	-0.07	0.46	-0.14	0.01	-0.15	0.13	-0.08	-0.34*	1.00				
GW	0.32	-0.10	0.44**	0.19	0.47**	0.32**	0.43**	0.40**	-0.37**	1.00			
GS	-0.24*	0.34**	-0.35**	-0.12	-0.38**	-0.10	-0.31*	-0.47**	0.86**	-0.79***	1.00		
SLmL	-0.04	0.27**	0.08	0.06	0.08	0.12	-0.06	-0.30*	0.57**	-0.16	0.48***	1.00	
Yield	0.40**	0.20	0.43**	0.41**	0.43**	0.39**	0.46**	0.36**	-0.16	-0.01	-0.11	0.05	1.00000

***= highly significant at 1 %; **=significant at 5% and *= significant at 10%; values with no marks are non-significant at 10%.

LL=Leaf length, LW=Leaf width, Ht=Plant height, PnL=Panicle length, CL=Culm length, Hed= no. of days to heading, Mat= no. of days to heading, GFP=Grain filling period, GL=Grain length, GW=Grain width, GS=Grain shape, SLmL=Sterile lemma length and Yield= Grain yield

5.2.5 Principal component analysis based on morphological traits

In the analysis made to estimate the relative contribution of the traits studied towards the overall phenotypic variation among the 18 varieties and genotypes of Ethiopia, the first three principal components with eigen values greater than unity explained about 86.81 % of the total variation. The first principal component (PC_1) alone explained about 53.11 % of the entire variability. The second and third principal components (PC_2 andPC_3) explained about 25.79 % and 7.91 %, respectively, of the entire variability among the test materials (Table 14).

Leaf length, plant height, culm length, panicle length, grain filling period, number of days to heading and maturity chiefly accounted for about 53.11 % of the gross variability among the genotypes through PC_1. The second PC, around 25.79 % of the variation, was largely originated from leaf width, grain length, grain shape and sterile lemma length. Grain width and sterile lemma length were the contributions to PC_3.

5.2.6 Cluster analysis

Dendrogram was generated for the 18 released and other rice varieties of Ethiopia using both qualitative and qualitative morphological traits. Figure 8 showed UPMGA dendrogram developed using qualitative and quantitative morphological traits of 18 rice genotypes of Ethiopia based on average distance between clusters.

Almost all varieties and other genotypes of the country were clustered in to two major groups. The exceptional case was the clustering of AD-12 and HO-04 different from the other varieties and other genotypes. The first cluster was comprised of AD-01, AD-48, Demewoze, HO-13, NERICA-3, NERICA-4, IAC-164, Suparica-1, X-Jigina and NERICA-1. This in turn splitted in to two sub clusters-the first comprising of AD-01, AD-48, Demewoze, HO-13, NERICA-3, NERICA-4 and the second sub cluster consisted of IAC-164, Suparica-1, X-Jigina and NERICA-1. The second major cluster was composed of the rest varieties and other genotypes vis-à-vis FOFIFA-3730, IRAT-209, NERICA-6, FOFIFA-3737 and IREM-194. It also had two sub clusters within it.

FOFIFA-3730, IRAT-209, NERICA-6 being grouped under the firs sub-cluster and FOFIFA-3737 and IREM-194 grouped under the second sub-cluster of the second main cluster. The clustering analysis using morphological traits failed to distinct the genotypes in to their respective ecologies-upland, lowland, irrigated- as compared to the result obtained from the molecular cluster analysis.

Table 14: Eigenvectors, egine values and variability explained by first three principal components for the 13 traits of 18 released and elite varieties of Ethiopia.

	Eigen value	**Difference**	**Proportion**	**Cumulative**
PC-1	6.90	3.55	0.53	0.53
PC-2	3.35	2.32	0.26	0.79
PC-3	1.03	0.29	0.08	0.87

Quantitative	**Eigen vectors**		
morphological Traits	**Prin-1**	**Prin-2**	**Prin-3**
Leaf length	**0.37**	0.08	0.04
Leaf width	0.23	**0.40**	0.02
Plant height	**0.37**	0.01	0.09
Panicle length	**0.35**	0.11	-.10
Culm length	**0.36**	-.003	0.11
Days to heading	**0.33**	0.19	0.11
Days to maturity	**0.37**	0.03	-.04
Grain filling period	**0.28**	-.23	-.25
Grain length	-.06	**0.49**	0.13
Grain width	0.20	-.31	**0.55**
Grain shape	-.16	**0.48**	-.20
Sterile lemma length	-.02	**0.41**	**0.31**
Grain yield	0.20	0.01	-.66

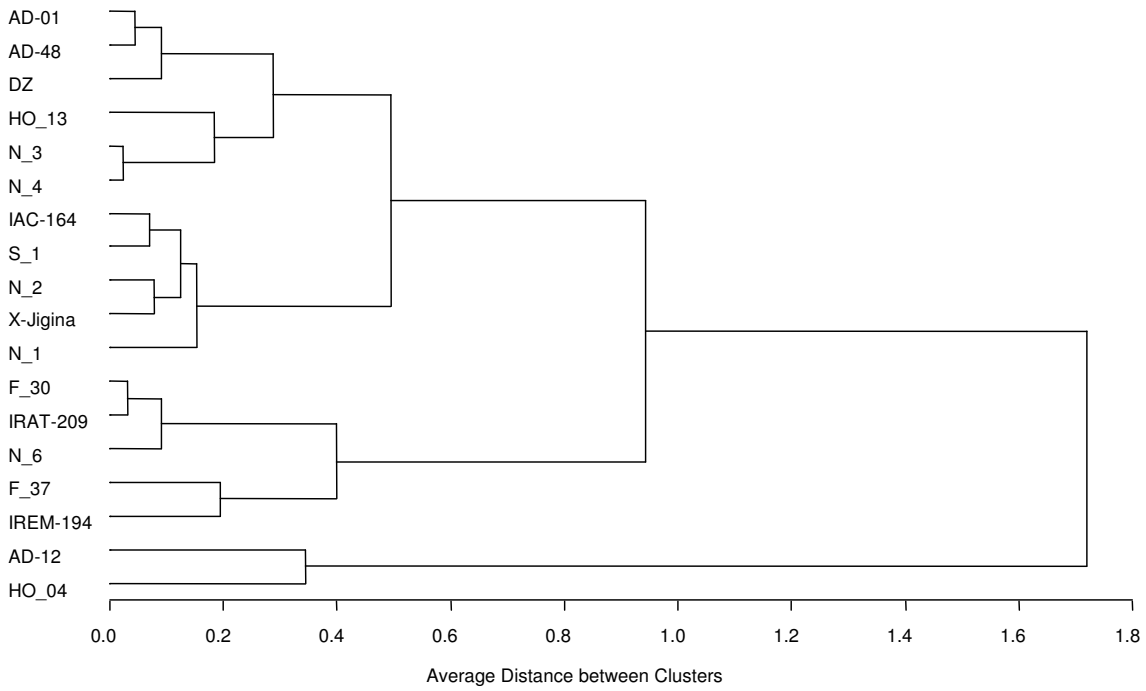


Figure 8. UPMGA dendrogram developed from morphological traits (both quantitative and qualitative) of 18 released and elite rice varieties of Ethiopia.

6. DISCUSSION

In this part, the results obtained from appropriate data analysis of ISSR and morphological studies are discussed shortly under the following sub topics: Utility of ISSR and morphological markers in assessment of genetic variability of the test varieties and genotypes, extent of genetic diversity of rice cultivars and implications for future breeding program of the country, degree of correlations of traits and implication for rice yield improvement and genetic diversity and relationships of genotypes.

6.1 Utility of ISSR and Morphological markers

The utility and comparative advantage of ISSR over morphological markers, indeed, is a known fact. It is discussed here, however, is to indicate that the result is in total agreement with the already established facts. In the past, the characterization of germplasm diversity was carried out by means of morphological and biochemical markers which, in many cases, did not have the resolution power for revealing polymorphisms in genetic analyses and/or for differentiating between closely related genotypes (Alan, 2007). Seetharam *et al* (2004) stated that genetic diversity analysis in plants has been traditionally assessed using morphological traits and argued that this assessment of phenotypic variation may not be a reliable measure of genetic differences as gene expressions are under environmental influence and called for the use of DNA based markers.

The present study considered the use of morphological and molecular (ISSR) markers to assess the extent of genetic variability that exist in different lowland, upland and NERICA rice varieties and lines of Ethiopia. The results revealed that both ISSR data and morphological traits quantified the available genetic variability among and within different rice ecologies in varying extent (magnitude). It was documented that both morphological and molecular markers could be used complementarily so as to get full-fledged information on the extent of genetic variation (Seetharam *et al*., 2004). Report by Samarajeewa *et al*., (2004) also noted the importance of employing both marker systems for characterization of locally collected wild rice germplasm in Sir Lanka. In Guinea, the genetic variability of the two cultivated rice species was assessed using morphological and molecular tools (Barry *et al*., 2007). Similarly there was also a report

by Sajida *et al* (2009) on the use of molecular markers and morphological traits for the study of genetic differentiation of rice mutants in Pakistan.

The present study clearly indicated that, compared with morphological characters, the molecular data are more powerful technique to study the relationships and variability among rice germplasm and the resolution is much higher to identify individual varieties, even to those with the same label. Therefore, as compared to morphological markers the ISSR finger printing is an effective mean for screening the rice germplasm, due to their independence from the effect of environment (Alan, 2007). In spite of the low variability, ISSR markers have distinguished cultivars and lines of Ethiopian rice distinctly. These markers can be used for the molecular identification/characterization of the Ethiopian germplasm. The UPGMA cluster analysis showed that all 18 rice cultivars could be easily distinguished based on the information generated by the five ISSR markers. The cultivars were clearly distinguished even though a high relatedness or similarity was measured between cultivar pairs. Where as, the morphological markers could differentiate all the 18 rice genotypes tested, but failed to differentiate the varieties in to their respective ecologies.

Actually, it is a fact that DNA markers are more reliable and convenient than morphological characterization for the identification and characterization of genetic variation (Zeng *et al*, 2004). Accordingly, several works have been documented on using molecular markers alone for variety identification, purity analysis, genetic diversity analysis etc. For instance, in rice molecular markers have been used to identify accessions (Oluflowote *et al* 1997), to determine the genetic structure and pattern of diversity for cultivars of interest (Akagi *et al* 1997), and several reports also showed that molecular markers have been used for assessing genetic variability that could exist among different wild relatives of rice world wide (Song *et al*., 1999, Wei Qian *et al*., 2006; George *et al*., 2001). It was also noted that compared to morphological analysis, molecular markers can reveal differences among accessions at DNA level and thus provide a more reliable and efficient tool for germplasm conservation and management (Junjian *et al*., 2002).

However, it should be understood that the importance of morphological traits is unquestionable and the best measure to analyze genetic diversity among genotypes would be with the use of all information, both from morphological characters and DNA based markers (Seetharam *et al*., 2004) like ISSR. Genotypes that are found to be diverse based on both morphological and molecular markers could be reliably used for further different breeding program. Kumar *et al* (2003) also noted that data from both morphological and molecular markers subjected to various numerical and taxonomic techniques could measure the relationship among genotypes effectively.

6.2 Genetic diversity of rice cultivars and implications for future breeding

Information on the amount of genetic variation in germplasm and genetic relationships between genotypes and lines are important considerations in designing effective breeding programs and also stabilize productivity through broadening the genetic base of rice varieties (Malik *et al*., 1990). The results derived from analyses of genetic diversity at the DNA and phenotypic level could be used for designing effective breeding programs aiming to conserving, identifying, characterizing and broadening the genetic bases of commercially grown varieties (Sajida *et al*., 2009; Malik *et al*., 1990).

In the present study, though all the diversity parameters quantified the available gene diversity among the cultivated rice varieties of Ethiopia, the total genetic variability is generally narrower. The low genetic diversity found among the Ethiopian cultivars evidence the narrow genetic bases used in the national breeding program of Ethiopia. In addition, it could be attributed to strong selection by breeders and local farmers for traits of their interest. The result is in agreement with the situation in other parts of the world. For Instance, Guimaraes *et al* (1995) stated that although differences in genetic diversity and relatedness are observed within rice germplasm of different countries, the general feature is a very close relationship among cultivars. The mean genetic similarity of the genotypes considered in the study was found to be 0.71. It is in agreement with different reports in different parts of the world. For instance, Sarla *et al* (2005) reported that the

mean genetic similarity among 20 rice varieties of India to be 0.71. Davierwala *et al* (2000) and Singh *et al* (1999) reported values of 0.70 and 0.80, respectively in Indian elite rice varieties; all of them recommended the need to widen the genetic base of the cultivars considered. Ethiopian rice breeding program is dependent solely on introduction of rice germplasm from abroad and despite the richness of genetic resources, only a small proportion of the world rice germplasm collections have been used in breeding programs of the source countries and institutes (Sajida *et al*., 2009). As a consequence a high genetic similarity is found within several commercial rice germplasm in different rice producing countries (Thaura *et al*., 2008) and in Ethiopia.

Therefore, it is important to take timely action to broaden the genetic base of the rice varieties cultivated in the country in order to reduce its vulnerability to diseases and insect pest outbreak. Recent studies carried out by the International Rice Research Institute showed that there is still a tremendous amount of unexploited genetic diversity in the primary gene pool of rice that can be used for enhancing the diversity in commercially grown varieties, elite lines and local germplasm (Thaura *et al*., 2008; Guimaraels *et al* 1995). Hence, the Ethiopian rice breeding program should revise its rice breeding program in a way that assists in broadening the available narrower genetic diversity of the varieties and elite lines.

6.3 Correlations of traits and implication for rice yield improvement

Determination of the association of morphological traits in rice varieties and genotypes of Ethiopia was also one of the major objectives of the study. The aim of investigating of morphological trait associations was to have some clue that may help for further improvement of the crop in the country. Though all pair wise associations were estimated and have shown variable degrees of associations ranging from strong to weak with positive or negative correlations, due emphasis here is given to associations of traits with grain yield, grain shape and growth duration parameters. Grain yield and growth duration parameters are considered for the breeding strategy mainly focused on developing rice varieties with higher yield and early maturing for different growing ecologies. Grain

shape on the other hand is considered on account of its market implications. As Mackill *et al* (1996) noted, grain shape has a great implication on the international rice markets, as long and cylindrical rice grains fetch high price in most markets.

In this study, all traits were hardly associated strongly with grain yield either positively or negatively. However, it was revealed that leaf length, panicle length, culm length, grain filling period, plant height, number of days to heading and maturity complement grain yield positively with moderate to weak degree of association. The relation of leaf length, plant height, panicle length and culm length with grain yield in this particular study is in agreement with the report of Nanda and Coffman (1979). The breeding objective, developing high yielder varieties, could be easier by simultaneously concentrating on those identified yield components than solely looking for a single trait that determines yield which is actually impractical (Mackill *et al*., 1996). Hence, it is suggested that consideration of leaf length, plant height, panicle length, culm length, number of days to heading and maturity in the yield improvement strategy may help in the identification and selection of promising lines.

Grain shape as a ratio of grain length to width has showed strong positive correlation with grain length and strong negative complementation with grain width. Likewise, its association with sterile lemma length was moderately positive and that of plant height, culm length, and number of days to maturity showed negative complementation. The concern of grain shape as a breeding objective is mainly due to market needs and consumer's preference (Mackill *et al*., 1996). This implies that the national breeding objective with regard to this trait should be inline with the demand and preference of local and international markets. Hence, consideration of the above mentioned traits which were associated with grain shape either positively or negatively will assist a breeder in addressing consumer preference and market needs with regard to the trait of interest.

One of the major rice breeding objectives so far dealt with and will continue to do so is development of early maturing improved varieties. This is a direct consequence of climate change that resulted in erratic rain fall that prevails in almost most rice growing

regions of the country. Mackill *et al* (1996) explained that growth duration parameters (number of days to heading and maturity) are an important agronomic trait crucial factor that determines the choice and success of rice varieties. The result indicated that leaf length, plant height, panicle length, culm length, number of days to heading, grain filling period, leaf width, grain width and grain yield had strong to moderate positive associations with number of days to maturity. Therefore, emphasis should be given to these traits during developing improved varieties suitable for different rice growing ecological situations.

6.4 Genetic diversity and relationships of genotypes

As discussed earlier the general situation of Ethiopian rice gene diversity is narrower and the varieties are closely related. It is in agreement with earlier studies that indicated the genetic diversity of commercially cultivated rice varieties became narrower in some parts of the world. For instance, in Latin America Perez *et al* (1992) tried to examine a total of 143 commercial varieties released in the region from 1971-1989, and found out that 101 different landraces were involved in the crosses that produced the varieties. However, only 14 ancient cultivars contributed much of the genes in the tested varieties. Similarly forty ancestors were involved in crossing to develop varieties, but only 11 of them accounted for 81% of the genes for the varieties released between 1971 and1993 (Guimarals *et al*, 1995). Similarly, Thaura *et al* (2008) indicated that despite breeding efforts, most of the varieties under current cultivation are closely related among them and even with cultivars released more than 20 years ago in Latin America.

A dendrogram derived from the whole ISSR data using UPMGA cluster analysis showed two distinct clusters and sub clusters within each main cluster. The first cluster consisted of all the lowland (Demewoze, X-Jigina, Suparica-1, and IAC-164) and most upland (IREM-194, AD-01, AD-048 and AD-12) rice varieties of Ethiopia. All NERICAs (NERICA-6, NERICA-3, NERICA-1, NERICA-2, and NERICA-4) and lowland genotypes (FOFIFA-3730, FOFIFA-3737, HO-04 and HO-13) grouped under the second major cluster. The clustering of these lowland genotypes with NERICAs mentioned above might be due to the fact that since NERICA are inter-specific hybrids of *Oryza*

sativa and *Oryza glabberima* and hence the lines might have a very closer genetic similarity with NERICAs' sativa parent.

Report on the use of ISSR data to construct a dendrogram, showed clear separation of Indian landraces and varieties in to different clusters and sub clusters (Sarla *et al*., 2005). Similarly, in an attempt to estimate genetic diversity of rice using SSR markers and morphological characters, Seetharam *et al* (2004) could generate a dendrogram based on UPGMA analysis and grouped thirty rice genotypes in to five distinct clusters at 66% of genetic similarity. Like wise, Sajida *et al* (2009) were able to separate different rice genotypes in to distinct groups and sub groups based on RAPD studies.

Kherdade *et al* (1985) considered PCV and GCV values greater than 20% as high, values between 10% and 20% as medium and values lower than 10% as low. Hence, among the traits considered, only leaf length was found to be classified as having high PCV value. Leaf width, culm length, plant height, grain filling period, grain shape and grain yield were considered as medium. The rest six traits vis-à-vis panicle length, number of days to heading and maturity, grain length, grain width and sterile lemma length showed the least PCV and considered as low. Similarly, traits with medium GCV value include leaf length, culm length, plant height, grain filling period, grain shape and grain yield. Leaf width, panicle length, number of days to heading and maturity, grain length, grain width and sterile lemma length showed the least GCV values and as a result were considered as low. No trait was observed to be considered to be high in GCV value.

According to Dubholkar (1992) and Robinson *et al* (1954), broad sense heritability in cultivated plants could be placed in the following category. Heritability estimates from 5-10% is low, values from 10-30% medium and values greater than or equal to 30% are considered to be high. Accordingly, all the traits investigated were found to be heritable, though there was variation amongst them.

From the dendrogram generated from both qualitative and quantitative morphological traits, it is depicted that almost all varieties and other genotypes of the country were

clustered into two major groups irrespective of rice ecological differentiation. Seetharam *et al*, (2004) noted clustering pattern clearly grouped rice genotypes based on morphological data in different clusters and sub-clusters splitted out from each main cluster. Samarajeewa *et al*, (2004) also reported a dendrogram that was constructed using morphological trait (both qualitative and quantitative traits) and indicated diversity among the species of wild rice in Pakistan. From the dendrograms it is clearly observed that genotypes IREM-194, AD-12 and HO-04 lacked purity. It could be attributed to seed admixture during planting, seed harvesting, storage and dispatching.

7. CONCLUSION AND RECOMMENDATIONS

Rice research and production in Ethiopia started a few decades ago and showed to have a huge potential for both rain fed and irrigated rice cultivation systems. In the last few decades, several varieties have been released for production by national and regional research institutes of the country. The present study was conducted with the main objective of assessing the extent of genetic diversity among the released and elite rice genotypes of Ethiopia using molecular (ISSR) and morphological traits.

Generally, both ISSR data and morphological traits quantified the available genetic variability among different rice ecologies and within each rice ecology in different magnitude. However, morphological characters alone cannot be regarded as critical indicators to identify individual rice genotypes for they are highly influenced by the environmental situations in which the genotypes are supposed to perform. It can be suggested that morphological trait analysis is a useful tool in studying the difference in ecological type. In contrast to morphological trait, the molecular markers revealed polymorphism at the DNA level, make it an ideal and a very powerful tool for characterization of genotype and estimation of genetic diversity in rice.

Despite the fact that the diversity analysis indicated the existence of variability among the 18 rice genotypes, the total genetic variability is generally narrower. The low genetic diversity found among the Ethiopian cultivars indicated that the genotypes are closely related. Therefore, the National Breeding Program should take cautious action to broaden the genetic base of the rice varieties cultivated in the country so as to reduce its vulnerability to diseases and insect pest outbreak. Hence, the Ethiopian Rice Breeding Program should revise its rice breeding approach in a way that assists in broadening the available narrower genetic variation of the varieties and genotypes. This could be achieved through gene transfer from wild relatives of rice, implementing crossing programs, enhancing variability through mutation breeding, utilize biotechnological tools in identifying, characterizing and transfer of noble genes from distant relatives of the species etc.

Few varieties lacked purity and should be purified to true to type so as to get the maximum performance from the varieties. Hence, variety purity analysis, breeder seed increase and maintenance activities should be carried out.

Determination of the association of morphological traits in rice varieties and lines with traits of interest is very important for further study and improvement of the crop. With this regard, it is suggested that consideration of leaf length, plant height, panicle length, culm length, number of days to heading and maturity in the yield improvement strategy may help in the identification and selection of promising lines and or cultivars. The concern of grain shape as a breeding objective is mainly due to market needs and consumer's preference, not because of any association with grain yield. This implies that our breeding objective with regard to this trait should be inline with the demand and preference of local and international markets. Hence, consideration of the above mentioned traits which were associated with grain shape either positively or negatively may assist breeding strategy dedicated to addressing consumer preference and or market needs with regard to the trait of interest. Similarly, one of the major rice breeding objectives so far dealt with and hopefully will continue to do so is development of early maturing improved varieties. This is a direct consequence unpredictable rain fall that prevails in almost most rice growing regions of the country. Number of days to maturity strongly and positively correlated traits were leaf length, plant height, panicle length, culm length, number of days to heading and grain filling period; and, leaf width, grain width and grain yield had moderate positive association. Therefore, it is important to consider these traits simultaneously so as to identify promising line with targeted growing duration (late, medium and early), depending on the target environmental situations. It may enable a breeder in providing farmers and investors with improved varieties that are suitable for different rice growing ecological situations.

It is hardly possible to get reports with reference to disease problems related to the 18 genotypes studied. Considering the elapsed time in rice production in the country, some argued that disease problem in Ethiopia is not economically important. That is to mean rice production has started a few decades ago and hence the time is not sufficient enough

for disease build up (personal experience). However, disease surveys in major rice producing areas of the country should be conducted and the problem should be quantified systematically. It is also important to apply plant quarantine procedures strictly while introducing rice germplasm so as to greatly reduce the possibility of disease infestation from abroad.

Genetic resource depletion resulting from several forces like promotion of monocropping should also be tackled by devising appropriate mode of conserving the genetic resources both at in situ and gene bank levels.

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9. APPENDICES

Appendix 1: ISSR data generated using five primers (810, 824, 834, 873 and 878) for 37 individuals of 18 rice accessions from Ethiopia.

Primer-810

Genotypes	No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Demewoze	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	0	0
Demewoze	2	1	1	0	1	1	1	1	0	0	1	1	1	1	1	0	0
NERICA-6	3	0	0	1	0	0	0	1	0	0	1	1	1	1	0	0	0
NERICA-6	4	0	0	1	0	0	0	1	0	0	1	1	1	1	0	1	1
HO-04-7-1-B-5	5	0	0	?	0	0	0	1	0	0	1	1	1	1	0	0	1
HO-04-7-1-B-5	6	1	1	1	?	1	0	1	0	?	1	1	1	1	0	0	1
NERICA-3	7	0	0	1	0	1	0	1	1	0	0	1	1	1	0	1	0
NERICA-3	8	0	0	0	0	?	0	1	1	0	0	1	1	1	0	1	0
X-Jigina	9	0	0	0	1	1	1	1	0	0	1	1	1	1	1	0	1
X-Jigina	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NERICA-1	11	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	0
NERICA-1	12	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	0
Suparica-1	13	0	0	0	0	0	0	1	0	0	1	1	0	1	0	1	0
Suparica-1	14	0	0	0	0	0	0	1	0	0	1	1	0	1	0	1	0
IAC-164	15	0	1	0	1	1	1	1	0	0	1	1	0	1	0	1	0
IAC-164	16	0	1	0	1	1	1	1	0	0	1	1	0	1	0	1	0
FOFIFA-3737	17	1	0	1	0	0	0	1	0	1	1	1	1	1	1	0	1
FOFIFA-3737	18	1	0	1	0	0	0	1	0	1	1	1	1	1	1	0	1
AD-48	19	0	0	0	1	1	1	1	0	0	1	1	1	1	0	1	1
AD-48	20	0	0	0	1	1	1	1	0	0	1	1	1	1	0	1	1
AD-01	21	1	1	0	1	1	1	1	0	0	1	1	1	1	0	1	1
AD-01	22	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1
HO-13	23	1	1	1	0	1	0	1	0	1	1	1	1	1	1	0	1
HO-13	24	1	1	1	0	1	0	1	0	1	1	1	1	0	1	0	1
AD-12	25	0	1	0	1	1	1	1	0	0	1	1	0	0	0	1	0
AD-12	26	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0
IRAT-209	27	0	0	1	0	1	0	1	1	1	0	1	1	1	1	1	0
IRAT-209	28	0	0	1	0	1	0	1	1	1	0	1	1	1	1	1	0
NERICA-4	29	1	0	1	0	1	0	1	1	1	0	1	1	1	1	1	1
NERICA-4	30	1	0	1	0	1	0	1	1	1	0	1	1	1	0	1	1
IREM-194	31	0	0	0	1	1	1	1	0	0	1	1	1	1	0	1	0
IREM-194	32	0	0	0	1	1	0	1	0	0	1	1	1	1	0	1	0
IREM-194	33	0	0	0	1	1	0	1	0	?	1	1	1	1	1	1	1
IREM-194	34	0	0	0	0	0	0	1	0	?	1	1	1	1	1	1	0
FOFIFA-3730	35	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
FOFIFA-3730	36	0	0	1	1	1	0	1	1	0	1	1	1	1	1	0	1
NERICA-2	37	0	0	1	0	1	0	1	1	0	0	1	0	1	1	1	0

Appendix 1- continued.

Primer-824

Genotypes	no.	1	2	3	4	5	6	7	8	9	10	11	12
Demewoze	1	1	1	1	1	1	1	1	0	0	1	0	0
Demewoze	2	1	0	1	1	1	1	1	0	0	1	0	0
NERICA-6	3	0	0	0	0	1	1	1	0	1	1	0	1
NERICA-6	4	0	0	0	1	1	1	1	0	1	1	0	1
HO-04-7-1-B-5	5	0	0	1	0	1	1	1	1	1	1	1	1
HO-04-7-1-B-5	6	0	0	0	1	1	1	1	0	0	0	0	1
NERICA-3	7	0	0	0	1	1	1	1	0	0	1	0	1
NERICA-3	8	0	0	0	0	1	1	1	0	0	0	0	1
X-Jigina	9	1	0	1	1	1	1	1	1	0	1	1	0
X-Jigina	10	0	0	0	0	1	0	1	0	0	0	1	0
NERICA-1	11	0	0	1	0	1	1	1	1	1	1	1	1
NERICA-1	12	0	0	0	1	1	1	1	0	0	0	1	1
Suparica-1	13	0	0	1	1	1	1	1	1	0	0	1	0
Suparica-1	14	0	0	1	1	1	1	1	1	0	1	1	0
IAC-164	15	1	1	1	1	0	1	1	0	1	0	1	0
IAC-164	16	1	1	1	1	0	1	1	1	1	0	1	0
FOFIFA-3737	17	0	0	0	0	1	1	1	0	1	1	1	1
FOFIFA-3737	18	0	0	0	0	1	1	1	0	?	0	1	1
AD-48	19	1	1	0	1	1	1	1	1	1	0	1	0
AD-48	20	0	0	1	1	1	1	1	1	0	0	1	0
AD-01	21	1	1	1	1	0	1	1	0	1	1	1	0
AD-01	22	1	1	0	1	1	1	1	0	0	1	1	0
HO-13-5-3-B-4	23	0	0	1	1	0	0	1	0	1	1	1	1
HO-13-5-3-B-4	24	0	0	1	1	0	0	1	0	1	1	1	1
AD-12	25	1	1	1	0	1	1	1	0	1	1	0	0
AD-12	26	1	0	1	0	1	1	1	0	0	0	0	0
IRAT-209	27	0	0	1	0	1	1	1	0	1	0	1	1
IRAT-209	28	0	0	0	0	0	1	1	0	1	0	1	1
NERICA-4	29	0	0	0	0	1	1	1	0	0	0	0	1
NERICA-4	30	0	0	0	0	1	1	1	0	0	0	0	1
IREM-194	31	1	1	0	1	1	1	1	1	0	?	1	0
IREM-194	32	0	1	0	1	0	1	1	1	1	0	1	0
IREM-194	33	0	1	1	1	1	1	1	1	0	0	1	0
IREM-194	34	0	0	0	0	1	1	1	0	0	0	0	1
FOFIFA-3730	35	?	?	?	?	?	?	?	?	?	?	?	?
FOFIFA-3730	36	1	1	0	0	1	1	1	0	1	0	1	1
NERICA-2	37	0	0	0	0	0	1	0	0	0	0	0	0

Appendix 1- continued.

Primer-834

Genotypes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Demewoze	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1
Demewoze	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1
NERICA-6	1	1	1	1	0	1	1	1	0	1	0	1	1	1	1	1	0	1	1	1
NERICA-6	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
HO-04	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1
HO-04-	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1
NERICA-3	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
NERICA-3	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
X-Jigina	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1
X-Jigina	?	0	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
NERICA-1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
NERICA-1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Suparica-1	0	0	0	0	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
Suparica-1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
IAC-164	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1
IAC-164	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1
FOFIFA-37	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1
FOFIFA-37	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1
AD-48	0	1	0	1	1	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1
AD-48	0	1	0	1	1	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1
AD-01	0	1	0	1	1	1	0	1	1	0	1	1	1	1	1	1	0	0	1	1
AD-01	0	1	0	1	1	1	0	1	1	0	1	1	1	1	1	1	0	0	1	1
HO-13	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1
HO-13	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1
AD-12	0	1	0	1	0	1	0	1	1	0	1	1	1	1	1	1	0	0	1	1
AD-12	0	1	0	1	0	1	0	1	1	0	1	1	1	1	1	1	0	0	1	1
IRAT-209	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1
IRAT-209	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1
NERICA-4	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1
NERICA-4	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1
IREM-194	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
IREM-194	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
IREM-194	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1
IREM-194	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	0	1
FOFIFA-30	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	1
FOFIFA-30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1
NERICA-2	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	0	1

Appendix 1- continued.

Primer-873

Genotypes	no.	1	2	3	4	5	6	7	8	9	10	11	12
Demewoze	1	1	1	0	1	1	1	1	1	1	1	1	0
Demewoze	2	1	1	0	1	1	1	1	1	1	1	1	0
NERICA-6	3	?	1	0	1	1	1	1	1	0	1	1	0
NERICA-6	4	1	1	1	1	1	1	1	1	0	1	1	0
HO-04-7-1-B-5	5	1	1	1	1	1	1	1	1	0	1	1	0
HO-04-7-1-B-5	6	?	1	1	1	1	1	1	1	1	1	1	0
NERICA-3	7	1	1	1	0	1	1	0	1	0	1	1	0
NERICA-3	8	?	1	1	0	1	1	0	1	0	1	1	0
X-Jigina	9	1	1	0	0	1	1	1	1	0	1	1	0
X-Jigina	10	1	1	1	0	1	1	1	1	0	1	1	0
NERICA-1	11	1	1	1	0	1	1	1	1	0	1	1	1
NERICA-1	12	1	1	0	1	1	1	1	1	0	1	1	1
Suparica-1	13	1	1	0	0	1	1	1	1	0	1	1	1
Suparica-1	14	1	1	1	0	1	1	1	1	0	1	1	1
IAC-164	15	0	1	0	0	1	1	1	1	0	1	1	1
IAC-164	16	0	1	0	0	1	1	1	1	0	1	1	1
FOFIFA-3737	17	1	1	1	0	1	1	1	1	0	1	1	1
FOFIFA-3737	18	1	1	0	0	1	1	1	1	0	1	1	1
AD-48	19	0	1	0	1	1	1	1	1	0	1	1	1
AD-48	20	1	1	0	1	1	1	1	1	0	1	1	1
AD-01	21	1	1	0	1	1	1	1	1	0	1	1	0
AD-01	22	0	1	0	0	1	0	1	1	0	1	1	0
HO-13-5-3-B-4	23	1	1	1	1	1	1	1	1	0	1	1	1
HO-13-5-3-B-4	24	0	1	0	1	1	1	1	1	0	1	1	1
AD-12	25	0	1	0	0	1	1	1	1	0	1	1	1
AD-12	26	1	1	1	0	1	1	1	1	0	1	1	1
IRAT-209	27	0	1	0	0	1	1	1	1	0	1	1	0
IRAT-209	28	1	1	1	0	1	1	1	1	0	1	1	0
NERICA-4	29	1	1	1	0	1	1	1	1	0	1	1	1
NERICA-4	30	1	1	1	0	1	1	1	1	0	1	1	1
IREM-194	31	1	1	1	1	1	1	1	1	0	1	1	1
IREM-194	32	0	1	1	1	1	1	1	1	0	1	1	1
IREM-194	33	1	1	1	1	1	1	1	1	0	1	1	0
IREM-194	34	0	1	0	0	1	1	1	1	0	1	1	0
FOFIFA-3730	35	0	0	0	0	1	0	1	1	0	1	1	0
FOFIFA-3730	36	0	1	0	1	1	1	1	1	0	1	1	0
NERICA-2	37	1	1	1	1	1	1	1	1	0	1	1	0

Appendix 1- continued.

Primer-878

Genotypes	no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Demewoze	1	0	1	1	1	0	1	1	1	1	1	1	0	0	0	0
Demewoze	2	0	1	1	1	0	1	1	1	1	1	1	0	0	0	0
NERICA-6	3	0	1	1	1	0	1	1	1	1	0	1	1	1	0	1
NERICA-6	4	0	1	1	1	0	1	1	0	1	1	1	1	1	0	0
HO-04-7-1-B-5	5	0	0	0	1	0	1	1	0	1	0	1	1	1	0	1
HO-04-7-1-B-5	6	0	1	1	1	0	1	1	0	1	0	1	1	1	0	1
NERICA-3	7	0	1	1	1	0	1	1	0	1	1	1	0	0	1	0
NERICA-3	8	0	1	1	1	0	1	1	0	1	1	1	0	0	1	0
X-Jigina	9	0	1	1	1	0	1	1	0	1	1	1	1	0	1	0
X-Jigina	10	0	1	1	1	0	0	1	1	1	1	1	1	1	1	0
NERICA-1	11	0	1	1	1	1	1	1	0	1	1	1	1	0	1	0
NERICA-1	12	0	1	1	1	1	1	1	0	1	1	1	1	0	1	0
Suparica-1	13	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0
Suparica-1	14	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0
IAC-164	15	1	0	1	1	0	0	1	1	1	1	1	1	1	0	0
IAC-164	16	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0
FOFIFA-3737	17	0	1	1	1	0	1	1	1	1	1	1	1	0	1	0
FOFIFA-3737	18	0	1	1	1	0	1	1	1	1	1	1	1	0	1	0
AD-48	19	0	1	1	1	1	0	1	1	1	1	1	1	1	0	1
AD-48	20	0	1	1	1	1	0	1	1	1	1	1	1	1	0	1
AD-01	21	0	1	1	1	1	0	1	1	1	1	1	1	1	0	0
AD-01	22	0	1	1	1	1	0	1	1	1	1	1	1	1	0	0
HO-13-5-3-B-4	23	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1
HO-13-5-3-B-4	24	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1
AD-12	25	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0
AD-12	26	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0
IRAT-209	27	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0
IRAT-209	28	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0
NERICA-4	29	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0
NERICA-4	30	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0
IREM-194	31	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0
IREM-194	32	1	1	1	1	0	0	1	1	1	1	1	1	1	0	0
IREM-194	33	1	1	1	1	0	1	1	1	1	1	1	0	1	0	0
IREM-194	34	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0
FOFIFA-3730	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FOFIFA-3730	36	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
NERICA-2	37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

NB: ‘1’ stands for present, ‘0’ for absent and ‘?’ for missed/ ambiguous bands

Appendix 2: Raw data of the morphological and agronomic qualitative traits for each of the 18 rice genotypes grown during 2009/2010 cropping season at Ataye.

Genotypes	Auricle Color	Collar Color	Leaf Angle	Leaf Blade Color	Leaf Blade Pubescence	Flag Leaf Angle	Ligule Color	Ligule Shape
X-Jigina	2	2	1	2	3	3	4	2
HO-13	2	2	1	2	3	1	1	2
HO-04	2	2	1	2	3	1	1	2
IREM-194	2	2	1	2	3	1	1	2
NERICA-1	4	3	1	3	3	3	3	2
AD-48	2	2	1	1	3	1	1	2
F-3737	4	2	1	2	1	3	4	2
Suparica-1	4	2	2	2	3	1	4	2
NERICA-3	2	2	1	3	1	1	4	2
NERICA-4	2	2	1	3	1	1	4	2
IRAT-209	4	2	2	3	2	3	1	2
NERICA-2	2	2	1	3	1	1	4	2
NERICA-6	2	2	1	1	1	1	4	2
IAC-164	2	2	1	2	3	1	1	2
AD-01	2	2	1	2	3	3	4	2
Demewoze	2	5	1	2	3	3	4	2
AD-12	2	2	1	1	3	1	4	2
F-3730	2	2	2	2	1	5	1	2

Genotypes	Leaf Senescence	Basal Leaf Sheath color	Awn color	Awning	Culm angle	Internode color	Panicle Axis	Panicle type
X-Jigina	7	1	0	0	3	3	4	1
HO-13	7	1	0	0	1	2	4	1
HO-04	7	1	0	0	5	2	2	2
IREM-194	7	1	0	0	1	2	4	1
NERICA-1	9	4	0	0	1	4	3	1
AD-48	7	1	0	0	5	2	2	2
F-3737	7	1	0	0	1	2	3	1
Suparica-1	7	1	0	0	5	2	3	1
NERICA-3	9	1	0	0	1	2	3	1
NERICA-4	9	1	0	0	1	2	3	1
IRAT-209	7	4	2	1	1	2	4	1
NERICA-2	7	2	4	7	1	4	3	1
NERICA-6	9	4	0	0	1	2	3	1
IAC-164	7	1	0	0	5	2	2	2
AD-01	7	1	0	0	3	2	2	2
Demewoze	7	3	2	9	3	2	4	1
AD-12	7	4	0	0	5	2	2	2
F-3730	7	1	0	0	3	2	4	1

Appendix 2- continued.

Genotypes	Lodging Incidence	Panicle Exertion	Phenotypic Acceptability	Lemma shape	Lemma & palea color	Lemma& palea pubescence	Sterile lemma color	Sec. Branching of panicles
X-Jigina	5	1	3	2	2	5	2	2
HO-13	9	3	1	1	1	5	1	2
HO-04	7	1	3	2	1	3	1	2
IREM-194	9	1	3	2	2	3	1	1
NERICA-1	9	3	5	1	3	0	4	1
AD-48	7	1	5	2	1	3	2	2
F-3737	7	3	1	1	2	0	1	2
Suparica-1	7	1	3	1	2	0	2	2
NERICA-3	9	3	1	1	3	0	1	2
NERICA-4	9	1	1	1	3	0	2	2
IRAT-209	7	3	3	2	2	3	1	2
NERICA-2	5	3	7	1	3	0	2	2
NERICA-6	9	3	1	1	2	0	2	2
IAC-164	7	1	3	2	1	3	1	2
AD-01	7	1	3	2	2	0	1	2
Demewoze	7	1	1	2	2	4	1	2
AD-12	7	1	5	2	1	3	1	2
F-3730	5	3	5	1	2	0	2	2

Remark: The scores given in the table are based on the standard evaluation system given by IRRI (2002) and Bioversity International (2007) and denoted as follows:
Auricle color (2= yellowish green, 4= light purple), Collar color (2= light green), Leaf angle (1= erect, 2= horizontal), Leaf blade color (1= light green, 2= green, 3= dark green), Leaf blade pubescence (1= glabrous, 2= intermediate, 3= pubescent), Flag leaf angle (1= erect, 3= intermediate, 5= horizontal), Ligule color (1= whitish, 3= purple, 4= light purple), Ligule shape (2=acute to acuminate), Leaf senescence (7= late, 9= very late), Basal leaf sheath color (1= green, 2= green with purple lines, 3= light purple, 4= purple), Awn color (0= awn less, 2= gold, 4= red), Awning (0= absent, 1= short and partly awned, 7= long and partly awned, 9= long and fully awned), Culm angle (1= erect, 3= intermediate, 5= open), Internode color (2= light gold, 3= purple lines, 4= purple), Panicle axis (1= upright, 2= semi-upright, 3= slightly dropping, 4= strongly dropping), Panicle type (1= compact, 2= intermediate), Lodging incidence (5= intermediate, 7= strong, 9= very strong), Panicle exertion (1= well exerted, 3= moderately well exerted), Phenotypic acceptability (1= excellent, 3= good, 5= fair, 7= poor), Lemma shape (1= pointed, 2= curved), Lemma and palea color (1= white, 2= straw, 3= gold and gold furrows), Lemma and palea pubescence (0= glabrous, 3= hairs on upper portion, 5= long hairs), Sterile lemma color (1= straw/yellow, 2= gold, 4= purple) and Secondary branching of panicles (1= sparse, 2= dense).

Appendix 3 . ANOVA tables for 13 quantitative traits

Leaf length (cm)

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	26.8311	13.4156		
GENOTYPES	17	2034.45	119.674	10.29	0.000
ERROR	34	395.249	11.6250		
TOTAL	53	2456.53			

Leaf width (mm)

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	0.223704	0.111852		
GENOTYPES	17	65.1437	3.83198	7.31	0.000
ERROR	34	17.8296	0.524401		
TOTAL	53	83.1970			

Plant height (cm)

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	34.8548	17.4274		
GENOTYPES	17	8426.17	495.657	14.80	0.000
ERROR	34	1138.32	33.4800		
TOTAL	53	9599.34			

Panicle length (cm)

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	0.537777	0.268889		
GENOTYPES	17	134.647	7.92039	7.40	0.000
ERROR	34	36.3956	1.07046		
TOTAL	53	171.580			

Culm length (cm)

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	26.7393	13.3696		
GENOTYPES	17	6703.45	394.321	15.79	0.000
ERROR	34	848.887	24.9673		
TOTAL	53	7579.08			

Days to heading

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	1.92593	0.962963		
GENOTYPES	17	1695.43	99.7309	97.60	0.000
ERROR	34	34.7407	1.02179		
TOTAL	53	1732.09			

Days to maturity

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	9.33333	4.66667		
GENOTYPES	17	3228.67	189.922	39.86	0.000
ERROR	34	162.000	4.76471		
TOTAL	53	3400.00			

Appendix-3 continued

Grain filling period

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	18.8148	9.40741		
GENOTYPES	17	716.093	42.1231	12.29	0.000
ERROR	34	116.519	3.42702		
TOTAL	53	851.426			

Grain length (mm)

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	0.133330	0.666650E-01		
GENOTYPES	17	28.3396	1.66703	13.45	0.000
ERROR	34	4.21367	0.123932		
TOTAL	53	32.6866			

Grain width (mm)

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	0.225951E-01	0.112976E-01		
GENOTYPES	17	2.12269	0.124864	19.14	0.000
ERROR	34	0.221768	0.652258E-02		
TOTAL	53	2.36705			

Grain shape

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	0.380134E-02	0.190067E-02		
GENOTYPES	17	6.00318	0.353128	23.97	0.000
ERROR	34	0.500797	0.147293E-01		
TOTAL	53	6.50778			

Sterile lemma length (mm)

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	0.473816	0.236908		
GENOTYPES	17	2.68237	0.157786	3.47	0.001
ERROR	34	1.54382	0.454064E-01		
TOTAL	53	4.70000			

Grain yield (g/plot)

SOURCE	D.F.	S.S.	M.S.	F	FPROB
BLOCKS	2	16813.930	8406.965		
GENOTYPES	17	4152905.956	244288.586	2.23	0.02
ERROR	34	3727042.085	109618.885		
TOTAL	53	7896761.972			

Appendix-4. Duncan's Multiple Range Test (DMRT) for 13 quantitative traits (at 0.05)

Leaf length (cm)	Duncan Grouping			Mean	N	Genotypes
		A		43.933	3	HO-13-5-3-B-4
	B	A		41.733	3	HO-04-7-1-B-5
	B	C		37.333	3	AD-12
	B	C		36.533	3	IAC-164
	D	C		34.400	3	AD-48
	D	C	E	32.467	3	AD-01
	D	C	E	32.267	3	FOFIFA-3730
	D	F	C E	31.800	3	NERICA-6
	D	F	C E	31.067	3	Demewoze
	D	F	G E	29.800	3	X-Jigina
	D	F	G E	29.467	3	Suparica-1
	D	F	G E	28.333	3	FOFIFA-3737
	H	F	G E	26.800	3	IREM-194
	H	F	G E	26.067	3	NERICA-4
	H	F	G	25.667	3	NERICA-3
	H		G	24.200	3	IRAT-209
	H			21.800	3	NERICA-1
	H			21.133	3	NERICA-2

Leaf width (cm)	Duncan Grouping				Mean	N	Genotypes
			A		13.6667	3	HO-13-5-3-B-4
			B		12.0000	3	NERICA-6
	C		B		11.8000	3	HO-04-7-1-B-5
	C		B		11.7333	3	FOFIFA-3737
	C		B	D	11.4667	3	AD-12
	C		B	D	11.3333	3	Suparica-1
	C		B	D	11.0667	3	AD-01
	C	E	B	D	11.0000	3	FOFIFA-3730
	C	E	B	D	10.8667	3	IAC-164
	C	E	F	D	10.6000	3	AD-48
G	C	E	F	D	10.5333	3	NERICA-3
G	C	E	F	D H	10.4000	3	NERICA-1
G	C	E	F	D H	10.4000	3	NERICA-4
G		E	F	D H	10.2667	3	NERICA-2
G		E	F	H	9.6667	3	Demewoze
G			F	H	9.4667	3	IREM-194
G				H	9.2000	3	X-Jigina
				H	9.0667	3	IRAT-209

Plant height (cm)	Duncan Grouping		Mean	N	Genotypes
		A	108.333	3	HO-13-5-3-B-4
	B	A	104.467	3	HO-04-7-1-B-5
	B	A	102.200	3	AD-12
	B	A	99.800	3	IAC-164
	B	A	99.333	3	NERICA-6
	B	C	97.333	3	Demewoze
	B	C	95.600	3	AD-01
	B	C	95.067	3	AD-48
	D	C	88.267	3	X-Jigina
	D	C	87.600	3	FOFIFA-3737
	D	C	87.467	3	Suparica-1
	D	E	81.800	3	FOFIFA-3730
	F	E	76.867	3	IRAT-209
	F	E	76.067	3	NERICA-4
	F	E	75.933	3	NERICA-3
	F	E	74.200	3	IREM-194
	F		67.933	3	NERICA-2
	F		67.600	3	NERICA-1

Appendix-4 continued

Panicle length (cm)	Duncan Grouping		Mean	N	Genotypes
		A	22.9333	3	HO-13-5-3-B-4
	B	A	21.9333	3	HO-04-7-1-B-5
	B	A	21.5333	3	NERICA-6
	B	A	21.4667	3	AD-12
	B	A	21.3333	3	IAC-164
	B	C	20.2667	3	AD-48
	D	C	19.4000	3	AD-01
	D	C	19.0667	3	Demewoze
	D	C	18.7333	3	Suparica-1

		D	C	18.7333	3	NERICA-4
		D	C	18.5333	3	X-Jigina
		D	C	18.4667	3	FOFIFA-3730
		D		18.2000	3	NERICA-3
		D		18.2000	3	IRAT-209
		D		18.2000	3	IREM-194
		D		18.0667	3	FOFIFA-3737
		D		18.0667	3	NERICA-2
		D		17.8667	3	NERICA-1

Culm length (cm)	Duncan Grouping			Mean	N	Genotypes
		A		85.400	3	HO-13-5-3-B-4
	B	A		82.533	3	HO-04-7-1-B-5
	B	A		80.733	3	AD-12
	B	A	C	78.467	3	IAC-164
	B	A	C	78.267	3	Demewoze
	B	D	A	C	77.800	3	NERICA-6
	B	D	A	C	76.200	3	AD-01
	B	D		C	74.800	3	AD-48
		D	E	C	69.733	3	X-Jigina
		D	E	C	69.533	3	FOFIFA-3737
		D	E		68.733	3	Suparica-1
	F		E		63.333	3	FOFIFA-3730
	F		G		58.667	3	IRAT-209
	F		G		57.733	3	NERICA-3
	F		G		57.333	3	NERICA-4
	F		G		56.000	3	IREM-194
			G		49.867	3	NERICA-2
			G		49.733	3	NERICA-1

Days to heading	Duncan Grouping				Mean	N	Genotypes
			A		124.6667	3	HO-13-5-3-B-4
			B		115.3333	3	HO-04-7-1-B-5
			C		111.3333	3	AD-12
	D		C		110.6667	3	IAC-164
	D				109.3333	3	AD-48
			E		107.0000	3	Suparica-1
	F		E		106.3333	3	NERICA-6
	F		E	G	106.0000	3	FOFIFA-3737
	F		E	G	106.0000	3	Demewoze
	F	H	E	G	105.3333	3	IREM-194
	F	H		G	104.6667	3	FOFIFA-3730
	F	H		G	104.6667	3	AD-01
		H	I	G	104.3333	3	NERICA-3
		H	I		104.0000	3	IRAT-209
		H	I		104.0000	3	NERICA-4
		H	I		103.6667	3	X-Jigina
			I		102.6667	3	NERICA-1
			J		98.3333	3	NERICA-2

Appendix-4 continued

Days to Maturity	Duncan Grouping				Mean	N	Genotypes
			A		153.000	3	HO-13-5-3-B-4
			B		148.000	3	HO-04-7-1-B-5
			B		146.000	3	AD-12
			B		145.000	3	IAC-164
			B		145.000	3	AD-48
			C		139.000	3	NERICA-6
		D	C		137.667	3	AD-01
		D	C		137.333	3	IREM-194
		D	C		136.333	3	Suparica-1
		D	C	E	135.000	3	Demewoze
		D		E	134.000	3	X-Jigina
			F	E	132.333	3	FOFIFA-3737
		G	F	E	131.333	3	FOFIFA-3730
		G	F		129.667	3	IRAT-209
		G	F		129.000	3	NERICA-1
		G	F		128.667	3	NERICA-3
		G	H		128.000	3	NERICA-4
			H		124.667	3	NERICA-2

Grain filling period	Duncan Grouping		Mean	N	Genotypes
		A	35.667	3	AD-48

	B		A		34.667	3	AD-12
	B		A		34.333	3	IAC-164
	B		A	C	33.000	3	AD-01
	B	D	A	C	32.667	3	NERICA-6
	B	D	A	C	32.667	3	HO-04-7-1-B-5
	B	D	E	C	32.000	3	IREM-194
	F	D	E	C	30.333	3	X-Jigina
	F	D	E	G	29.333	3	Suparica-1
	F	H	E	G	29.000	3	Demewoze
	F	H		G	28.333	3	HO-13-5-3-B-4
		H	I	G	26.667	3	FOFIFA-3730
		H	I	G	26.333	3	FOFIFA-3737
		H	I	G	26.333	3	NERICA-1
		H	I	G	26.333	3	NERICA-2
		H	I		25.667	3	IRAT-209
			I		24.333	3	NERICA-3
			I		24.000	3	NERICA-4

Grain length (cm)	**Duncan Grouping**			**Mean**	**N**	**Genotypes**
		A		10.1237	3	HO-13-5-3-B-4
	B	A		9.9220	3	FOFIFA-3737
	B	A	C	9.7433	3	NERICA-1
	B	A	C	9.7203	3	FOFIFA-3730
	B	D	A	C	9.5163	3	NERICA-4
	B	D		C	9.3690	3	AD-01
	B	D	E	C	9.3237	3	Suparica-1
		D	E	C	9.2097	3	NERICA-3
	F	D	E		8.9903	3	NERICA-2
	F	D	E		8.9650	3	AD-12
	F	D	E		8.9200	3	NERICA-6
	F	D	E		8.8583	3	IRAT-209
	F		E		8.6750	3	AD-48
	F				8.4763	3	IAC-164
	F				8.4253	3	HO-04-7-1-B-5
	F				8.4180	3	IREM-194
			G		7.6053	3	Demewoze
			G		7.3577	3	X-Jigina

Appendix-4 continued

Grain width (cm)	**Duncan Grouping**			**Mean**	**N**	**Genotypes**
			A	3.58533	3	IRAT-209
		B	A	3.48967	3	Demewoze
		B	A	3.46667	3	X-Jigina
		B	C	3.39033	3	AD-12
		B	C	3.39000	3	HO-04-7-1-B-5
		B	C	3.37500	3	IREM-194
		B	C	3.36400	3	AD-48
		B	C	3.34033	3	Suparica-1
			C	3.30733	3	HO-13-5-3-B-4
			C	3.30633	3	FOFIFA-3730
			C	3.29800	3	FOFIFA-3737
			C	3.27467	3	IAC-164
			C	3.25833	3	NERICA-6
			C	3.24833	3	AD-01
			D	3.08167	3	NERICA-1
			E	2.91400	3	NERICA-4
			E	2.90333	3	NERICA-3
			E	2.86167	3	NERICA-2

Grain shape	**Duncan Grouping**				**Mean**	**N**	**Genotypes**
			A		3.27173	3	NERICA-4
	B		A		3.17746	3	NERICA-3
	B		A	C	3.16399	3	NERICA-1
	B		A	C	3.15213	3	NERICA-2
	B	D	A	C	3.06379	3	HO-13-5-3-B-4
	B	D		C	3.02787	3	FOFIFA-3737
		D	E	C	2.94451	3	FOFIFA-3730
		D	E		2.88582	3	AD-01
	F		E		2.79484	3	Suparica-1
	F		E		2.74124	3	NERICA-6
	F		G		2.64948	3	AD-12
	F		G		2.59336	3	IAC-164

	F		G	2.58036	3	AD-48
			G	2.49678	3	IREM-194
			G	2.48869	3	HO-04-7-1-B-5
			G	2.47565	3	IRAT-209
			H	2.18183	3	Demewoze
			H	2.12503	3	X-Jigina

Sterile lemma length (mm)

Duncan Grouping				Mean	N	Genotypes
		A		3.2327	3	HO-13-5-3-B-4
		A		3.2227	3	NERICA-4
	B	A		2.9533	3	FOFIFA-3730
	B	A	C	2.9067	3	FOFIFA-3737
	B	A	C	2.8773	3	AD-01
	B	D	A	C	2.8607	3	NERICA-3
	B	D	A	C	2.8473	3	AD-12
	B	D	E	C	2.7487	3	IRAT-209
	B	D	E	C	2.7147	3	Suparica-1
	B	D	E	C	2.7113	3	Demewoze
	B	D	E	C	2.6613	3	X-Jigina
	B	D	E	C	2.6533	3	NERICA-2
	B	D	E	C	2.6387	3	NERICA-1
	B	D	E	C	2.6187	3	AD-48
	B	D	E	C	2.6033	3	NERICA-6
		D	E	C	2.4920	3	HO-04-7-1-B-5
		D	E		2.4467	3	IREM-194
			E		2.4260	3	IAC-164

Appendix-4 continued

Grain yield (g/plot)

Duncan Grouping			Mean	N	Genotypes
	A		2161.4	3	AD-12
B	A		1863.0	3	HO-04-7-1-B-5
B	A		1833.0	3	AD-48
B	A	C	1817.3	3	AD-01
B	A	C	1789.9	3	Demewoze
B	A	C	1725.3	3	NERICA-4
B	A	C	1715.6	3	NERICA-3
B	A	C	1671.6	3	HO-13-5-3-B-4
B	A	C	1588.1	3	Suparica-1
B	A	C	1567.2	3	IAC-164
B		C	1444.0	3	IREM-194
B		C	1396.4	3	NERICA-1
B		C	1366.8	3	FOFIFA-3737
B		C	1257.8	3	FOFIFA-3730
B		C	1252.1	3	IRAT-209
B		C	1232.5	3	NERICA-6
		C	1174.2	3	NERICA-2
		C	1169.3	3	X-Jigina

Declaration:

I, the undersigned, hereby declare that this thesis is my original work and that all sources of materials used for the thesis have been duly acknowledged.

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The thesis has been submitted with our approval as a supervisor

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Date: _____

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Date: _____