Doctoral Thesis

Molecular diversity analysis of Ethiopian rice genetic resources

（エチオピアイネ遺伝資源における遺伝的多様性解析に関わる研究）

by

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Acronyms and Abbreviations

Acp	Acid phosphatase
Afr	Africa
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
Cat	Catalase
CGIAR	Consultative Group for International Agricultural Research
cp	Chloroplast
CSA	Central Statistical Agency
CV	Coefficient of Variation
DNA	Deoxy Ribonucleic Acid
DV	Differential variety
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agriculture Organization
FAOSTAT	FAO Statistics
GAM	Genetic Advance as percent of Mean
GenAIEx	Genetic Analysis in Excel
GenStat	General Statistics
GCV	Genotypic Coefficient of Variation
ha	Hectare
HI	Harvest Index
INDEL	Insertion-Deletion
IRRI	International Rice Research Institute
JICA	Japan International Cooperation Agency

Acronyms and Abbreviations (Continued)

JIRCAS	Japan International Research Centre for Agricultural Sciences
MoARD	Ministry of Agriculture and Rural Development
NBRP	National BioResource Project
NCBI	National Centre for Biotechnology Information
NEB	New England Biolabs
NERICA	New Rice for Africa
NIAS	National Institute of Agrobiological Sciences
NJ	Neighbor-Joining
NNPR	Nations, Nationalities and Peoples Region
NNRTC	National Rice Research and Training Centre
ORF	Open Frame Reading
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
PIC	Polymorphism Information Content
Pgi	Phosphoglucoisomerase
RF	Resistance Frequency
SAS	Statistical Analysis System
SPSS	Statistical Package for Social Sciences
S	Supplementary
t	Ton
WARDA	West Africa Rice Development Association

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Abstract

Rice is an important staple crop worldwide with its origins in South and Southeast Asia (*O. sativa*) and in West Africa (*O. glaberrima)* where it has a large gene pool consisting of wild relatives and landraces in addition to improved varieties. In Ethiopia, the crop is also considered as one of the most important grain crops primarily due to its various uses; food, beverage, livestock feed (straw and bran), cooking (husk), source of employment and income. Despite a rapid expansion of rice cultivation, domestic production has not yet met the demand for rice. In order to supply the growing demand, efforts are underway, one of which is improving productivity through use of rice genetic resources in breeding. Genetic resources are quite important to improve cultivars for higher yield and diseases resistance. Since the first introduction, several rice germplasm have been introduced into Ethiopia. However, details of these genetic resources including native wild rice are not well studied to make use of them in breeding. In view of this, four research studies were conducted to investigate genetic characteristics of cultivars and wild rice for future breeding in Ethiopia with the following specific objectives: (i) to study genetic diversity and classify Ethiopian rice cultivars based on molecular markers and morpho-physiological characters, (ii) to evaluate Ethiopian rice cultivars for blast resistance based on differential system, (iii) to assess genetic variation among Ethiopian rice cultivars based on agronomic traits evaluated under lowland rain fed condition, and (iv) to elucidate maternal lineage, genetic diversity and population structure in wild rice populations from Ethiopia.

In the 1st experiment (Chapter 2), 79 rice accessions were analyzed using fifty SSR and four INDEL markers. They were also evaluated for phenol reaction of hulls and grain size. A total of 351 alleles with a mean of 7.02 alleles per locus, ranging from 2 to 13 alleles per locus of SSRs were observed. Improved and landrace populations separately showed

genetic diversity of 0.55 and 0.48, respectively. Accessions were classified into two major clusters corresponding to Japonica/Japonica like varieties including NERICA and Indica/Indica-like varieties. Phenol reaction and grain size were also corresponded to the classification. Chloroplast and nuclear INDEL markers identified true Japonica and Indica types with their recombinant types.

In the 2nd blast inoculation experiment (Chapter 3), a total of 92 accessions comprising 60 Ethiopian rice accessions, 28 differential varieties and four control accessions were evaluated using 20 blast isolates. Results showed that accessions were grouped into two major clusters corresponding to resistant and susceptible groups. Except for few landraces, the majority of Ethiopian accessions including NERICAs belonged to resistant group. But in terms of resistance frequency, about 78% of Ethiopian accessions showed intermediate blast resistance frequency while 17% of them showed high blast resistance frequency and the rest 5% which included X-Jigna were with low resistance frequency. Moreover, gene postulation in Ethiopian accessions compared to differential varieties indicated the involvement of several resistance genes in high frequency including *Pit*, *Pik-p*, *Pish*, *Pib*, *Pik-s*, *Pik-m*, *Pi7* (t), *Piz-t*, *Pi9* (t), *Pi12* (t), *Pi19* (t), and *Pi20* (t).

In the 3rd experiment (Chapter 4), a total of 60 Ethiopian rice cultivars for agronomic traits were compared between different environmental conditions at two sites Fogera and Pawe in Ethiopia. Highly significant differences were observed among cultivars for 90% of the traits. Most of the traits such as days to heading, days to maturity, panicle length, grain yield, thousand seed weight, biomass yield and harvest index showed high broad sense heritability. Hierarchical cluster analysis classified cultivars into four clusters (I, II, III and IV). Cluster I (22) and II (20) comprised the largest number of cultivars. About 77% of cultivars in Cluster I consist of improved varieties including NERICAs. Cultivars in Cluster I were relatively early in days to heading and days to maturity while cultivars in Cluster II

were intermediate in days to heading and days to maturity with higher mean values for grain yield, and biomass yield. Clusters III and IV were dominated by landraces with few improved cultivars and they showed late in days to heading and days to maturity. This information combined with results of molecular analysis and blast inoculation of cultivars can accelerate our efforts of identifying potential plant materials for rice crossbreeding in Ethiopia.

The 4th experiment (Chapter 5) focused assessing maternal lineage, genetic diversity and population structure of 163 wild rice accessions from Ethiopian compared to 52 control accessions representing O. *barthii* (20), *O. longistaminata* (19) and *O. glaberrima* (13). Eight chloroplast INDELs (cpINDELs) in addition to 16 SSRs were newly developed based on publicly available whole chloroplast genome data of *O. barthii* and *O. longistaminata*, and applied to 215 accessions. Twenty plastid type combinations were detected. Four out of them, Types 1, 2, 3 and 6 were found among five populations in Ethiopia. Type 6 was specific to north group (Amhara) and it was shared with control *O. longistaminata* population but three were unique to Ethiopia. Type 2 and 3 were unique to south group (Gambella). Type 1 was shared between north and south groups. Using 16 SSRs, total number of alleles amplified per locus ranged from 4 to 14 with mean value of 9.69, with 155 alleles in total. From five populations in Ethiopia, Fogera population showed the highest *He* (0.67), followed by Dera population (*He* = 0.62) while, Kera, Lare, and Abobo populations showed *He* of 0.57, 0.56, and 0.55, respectively. In fact, *He* of control *O. longistaminata* population (0.70) was the highest of all eight populations. Neighbor-joining method phylogenetic tree analysis classified accessions into five cluster groups, out of which Ethiopian wild rice accessions corresponded to only three clusters, III, IV and V with some admixtures. Population structure analysis at K=2 revealed that all populations from Ethiopia were clustered with control *O. longistaminata* while *O. bathii* and *O. glaberrima* belonged to another group. At K=5 showed that five natural populations were classified into three subpopulations with some admixtures

corresponding to phylogenetic tree analysis. Phylogenetic tree analysis and structure analysis K=5 suggested the presence of three groups of *O. longistaminata* natural populations in Ethiopia. These resources would be valuable resources for future breeding program to supply disease resistance or abiotic stress tolerance.

In conclusion, this study provides valuable information to better understand the genetic characteristics of rice genetic resources from Ethiopia that can be used to improve elite rice cultivars but susceptible to different stresses.

Key words: Genetic diversity, landrace, improved cultivar, wild rice, differential variety, DNA marker, blast, Ethiopia.

Chapter 1

General introduction

1.1 The crop rice: Overview

Rice (*Oryza sativa* L.) is one of the earliest domesticated grain crops and a primary food source for nearly half the world population (Khush *et al*. 2001). Two cultivated rice; *Oryza sativa*, Asian rice (grown worldwide) and *Oryza glaberrima*, African rice (limited to West Africa), are recognized globally in addition to many distantly related wild types (Oka 1988; Mondal *et al*. 2018). The genus *Oryza* to which cultivated rice belongs probably originated at least 130 million years ago and spread as a wild grass in Gondwanaland the super continent that eventually broke up and drifted apart to become Asia, Africa, the Americas, Australia and Antarctica (Chang 1976). Rice is grown every continent except for Antarctica and provides about 20% of the world's kilocalories supply and 15% of human's protein consumption. In some regions of Asia, up to 71% of the daily energy and 70% of the protein intake comes from rice (Fitzgerald *et al*. 2009). The crop is grown in diverse cropping systems and environments-from single crop systems in temperate and tropical regions in both rain fed and irrigated conditions, to intensive monoculture in irrigated areas in the tropics where rice is grown two or three times per year (Laborte *et al.* 2017).

According to FAO (2018), worldwide paddy production reached more than 759 million tons harvested from over 163 million ha in more than 100 countries. Global rice demand is estimated to rise from 723 million tons in 2015 to 852 million tons in 2035 (Brar and Khush 2018). On the other hand, estimates made by experts indicate that a rate of increase in a supply of rice is lower than rate of increase in the demand of rice consumers.

Arable land is diminishing for several reasons in addition to recurrent stresses on the crop such as diseases, cold, drought, and salinity which are also aggravated with changing environmental conditions (Brar and Khush 2018). The situation is more severe specifically in Africa where there is no technological advance coupled with high population growth which makes the demand for rice always many folds higher than domestic production. African annual rice production covers only 62% of the actual needs while the demand is growing faster (Yelome *et al*. 2018). Thus, many African countries including Ethiopia are investing a lot of money for rice import while they have untapped potential to produce enough. To cope with these constraints, new rice varieties that combine higher yield potential with superior stress resistance are needed.

1.2. Taxonomic relationship

Rice belongs to the genus *Oryza* and the tribe Oryzeae of the family Gramineae (Poaceae). The genus has been classified into four different species complexes (*sativa, officinalis, meyeriana,* and *ridleyi*) based on their distinct genome types (Brar and Khush 2003). It contains two cultivated and about 23 wild species (2n=24, 48 chromosomes) representing the genomes: AA, BB, CC, BBCC, CCDD, EE, FF, GG, and HHJJ (Morishima 1984; Brar and Khush 2003; Vaughan *et al*. 2003) of which the genomes AA, BB, BBCC, CC, and FF are reported to exist in Africa (Table 1. 1). The *O. sativa* complex belongs to the AA genome and contains two domesticated species, *O. sativa* and *O. glaberrima* and six wild species: *O. rufipogon, O. nivara, O.barthii, O. longistaminata, O. meridionalis,* and *O. glumaepatula* (Vaughan *et al*. 2003). The *O. rufipogon* (Asia) and *O. longistaminata* (Africa) are perennial types and believed to be distant ancestors of *O. sativa* and *O. glaberrima,* respectively; whereas *O. nivara* (annual) is immediate ancestor to *O. sativa* (which differentiated into two

major subgroups; Indica and Japonica types) while *O. barthii* (annual) is immediate ancestor to *O. glaberrima* (Fig. 1. 1) (Brar and Khush 2003; Vaughan *et al*. 2003).

Table 1. 1 Oryza species, chromosomes and genome types of rice in Africa

Species	2n	Genome type
Oryza sativa L.	24	AA
Oryza glaberrima	24	AA
Oryza barthii	24	AA
Oryza longistaminata A. Chev. et Roehr.	24	AA
Oryza punctata Kotschy ex Steud	24	BB
Oryza schweinfurthiana Prod.	48	BBCC
Oryza eichingeri A. Peter	24	CC
Oryza brachyantha A. Chev. et Roehr	24	FF

Source: Vaughan *et al.* 2008

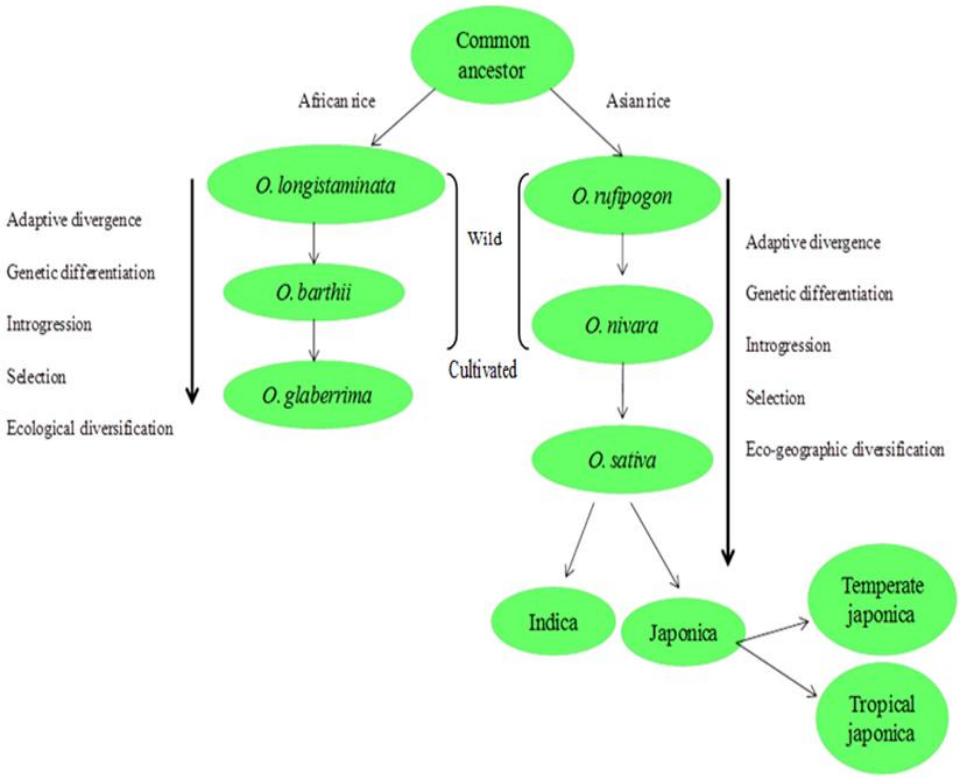

Fig. 1. 1 Simplified schematic representation of the phylogenetic evolution of AA genomes Asian and African rice (Modified from Nadir *et al.* 2018)

1.3 Ethiopia from rice crop production perspective

1.3.1 Potential and cultivation ecosystems

Although rice is one of the leading crops in the world (Khush *et al*. 2001) and Ethiopia is the center of diversity for major crop species (Vavilov 1951), rice was not known as a crop in Ethiopian farming system until the early 1980s. However, wild rice exists in different parts of Ethiopia since early times, mainly in Amhara and Gambella regions, and it was one of the drivers to introduce Asian rice into Ethiopia (Gebey *et al*. 2012). Addis *et al*. (2018) reported that the first rice introduction was started at Gambella and Pawe to support farmers' resettlement program and at Fogera for food security in 1970s and 1980s, respectively. According to MoARD (2010), Ethiopia has about 5 million hectares of highly suitable land for rice production, out of which less than 1.1% is under rice cultivation (Table 1. 2).

Despite variations among countries, globally five rice ecosystems exist; irrigated lowland, rainfed lowland, upland, deep water and tidal wetlands as characterized by water regimes, drainage, temperature, soil type, topography and location (Edirisinghe and Bambaradeniya 2006). In Ethiopia, three ecosystems are recognized; rain fed lowland, rain fed upland and intermittently irrigated types. Currently, large volume of production comes from rain fed lowland ecosystem which concentrated mainly in Fogera plains of the Amhara region.

1.3.2 Importance and production trend

Globally, rice has great economic importance (Bajaj and Mohanty 2005). In Ethiopia, its importance is increasing and it has significantly changed livelihood of farmers and other

stakeholders along the value chain specifically in Fogera, Libokemkem and Dera districts of Amhara region (Gebey *et al*. 2012). Rice is considered as a multipurpose crop. It is a major source of income not only for farmers but also many others and it is used as food in different preparations; as vegetable rice, bread, injera, porridge, cake, and local drinks. Its byproducts such as straw and husk are used as main source of cattle feed and as fuel, respectively (Gebey *et al*. 2012; Addis *et al*. 2018). Nationally, rice ranks second in productivity among major cereal crops (CSA 2017) and its overall trend shows an increase in the number of rice farmers, area, production and productivity (Table 1. 2).

Table 1. 2 Ten years rice production trend in Ethiopia

Year	No. of rice Farmers	Area (ha)	Production (ton)	Productivity (t/ ha)
2008	79,812	35,088	7,139.40	2.04
2009	126,432	47,739	10,312.80	2.16
2010	88,828	29,866	9,041.20	3.03
2011	121,059	30,649	8,861.90	2.89
2012	115,832	41,811	121,041.60	2.9
2013	119,497	33,820	92,362.73	2.73
2014	114,818	46,832	131,821.85	2.82
2015	134,363	45,454	126,806.45	2.79
2016	150,041	48,418	136,000.73	2.81
2017	161,376	53,107	151, 018.33	2.84

CSA 2008-2017

Following the release of improved rice varieties and promotion efforts, most regions start growing rice (MoARD 2010; CSA 2016; CSA 2017). Amhara, Oromia, South NNPR, Benishangul Gumize, Tigray and Gambella are major rice producing regions in Ethiopia

(Table 1.3). Currently, the largest production comes from Amhara region. Only in 2016 and 2017, 79.3% and 78.2% of total production of the country was obtained from this region harvested from over 36 thousand and 39 thousand hectares of land, respectively (Table 1.3). Despite an increase in domestic production, it cannot meet local demands and thus rice import has increased drastically from about 43,000 tons (2010) to 312,000 tons (2016) with values of about US$26 million and US$ 171 million, respectively (Addis *et al*. 2018).

Table 1. 3 Countrywide rice area, production and productivity in 2016 and 2017 by region in Ethiopia

Region	Area (ha)		Production (ton)		Yield (t /ha)	
	2016	2017	2016	2017	2016	2017
Amhara	36,413.62	39,829.58	107,911.23	118,030.94	2.96	2.96
Oromia	5,190.03	6,100.19	13,813.00	16,651.14	2.66	2.73
South NNPR	3,700.19	4,049.58	7,408.60	9,072.03	2.00	2.24
BenishangulGumize	2,507.61	2,556.45	5,435.47	5,877.42	2.17	2.30
Tigray	459.07	414.68	1,142.99	1,036.60	2.49	2.50
Gambella	147.57	156.31	289.44	350.19	1.96	2.24
Total	48,418.09	53,106.79	136,000.73	151,018.32	2.81	2.84

Source: CSA (2016 and 2017)

1.3.3 Rice production constraints in Ethiopia

Domestic rice production cannot meet local demands not only because of the increase in consumption habit but also the crop is suffering from several production constraints. For many years, however, rice was considered as healthy crop with regard to diseases except for other constraints such as termites in upland rice and weeds in all ecosystems. Currently, diseases such as blast, sheath rot, brown spot, and bacterial blight, insect pests (stem borer), and abiotic stresses (cold, drought, and soil nutrient deficiency) are becoming major challenges which in some extent varied among regions and many of which are aggravated by

the changing environmental conditions (MoARD 2010; Gebey *et al*. 2012; Tilahun *et al*. 2013; Mebratu *et al.* 2015; Wasihun and Flagot 2016; Tekalign *et al.* 2019).

Through breeding research efforts which entirely relied on the introduction of germplasms, more than 30 improved varieties have been released for different ecosystems in addition to accessions introduced in the past. However, the current national average productivity of the crop is estimated to be 2.84 tons/ ha (CSA 2017). This is much lower than world average of 4.6 tons /ha (FAOSTAT 2019) which may be attributed to blast infection, low yielding capacity of varieties, and other important constraints mentioned. Worldwide, different efforts have been followed to characterize and identify high yielding varieties that are resistant to blast and other stresses. Rice breeding in Ethiopia should aim at developing varieties that combine high yielding potential, blast resistance and tolerant to major stress of different ecosystems. This necessitates knowledge of the genetic diversity and relationship among rice genetic resources and their reaction to blast races in order to identify potential materials that can be utilized in crossbreeding programs.

1.4 Genetic diversity in rice genetic resources

Diversity in plant genetic resources provides opportunity for plant breeders to develop new and improved accessions with desirable characteristics, which include both farmer and breeder preferred traits (Govindaraj *et al.* 2014). Genetic resources of rice encompass commercial and obsolete varieties, landraces, breeding lines, natural hybrids and wild rice species, and all of which are genetic foundations for the breeding efforts needed to sustain the productivity of rice cultivation (Jackson and Lettington 2002). Probably far more than any other crop, rice can grow under diverse geographical, climatic and cultural condition which is attributed to its high genetic diversity (Rai 2002). As 2018 CGIAR Genebank platform

annual report indicated, a total of 130,154 and 21,300 rice germplasms exist at IRRI and AfricaRice, respectively, from which valuable rice genetic resources are distributed to several national programs including Ethiopia.

Ethiopian national rice research program has been introducing germplasms every year though many of which are lost as it deteriorates due limitation in germplasm management facilities such as lack of cold store to keep germplasms. Until 2018, about 3336 rice germplasms of different ecologies comprising improved varieties, advanced lines, and hybrids have been introduced into the country from various sources (Dessie *et al*. 2019). Before inception of formal rice research, different missionaries also introduced rice accessions with a range of sources and few are cultivated as landraces today. Currently, farmers and research centers play key role to conserving rice genetic resources in Ethiopia as landraces and improved accessions. Except for *O. glaberrima*, several *O. sativa* accessions and upland NERICAs have adapted to different production systems. It is anticipated that rice accessions in Ethiopia exhibit high genetic diversity attributed to their diverse origins. However, this needs to be investigated using some of the common crop characteristics including plant height, panicle length, maturity, apparent amylose content, seed size, diseases resistance, and yield related traits, and using different molecular techniques affiliated to rice genetic diversity analysis.

Ethiopia is also home to wild rice genetic resources, one of the drivers of Asian rice introduction into the country, covering different regions (Dadi and Engels 1986; Girma *et al*. 2010; Melaku *et al*. 2013). *Oryza longistaminata* is one the wild rice predominantly growing in Amhara and Gambella regions. Girma *et al*. (2010) and Jackson *et al*. (2010) also reported that Ethiopia is one of the distribution sites of *O. barthii* in Africa. It is reported that *O. longistaminata* has the highest genetic diversity of all previously in depth investigated African species such as *O. barthii* and *O. glaberrima* (Dadi and Engels 1986; Kiambi *et al*.

2005; Sharma 1983). This African wild rice also has important traits such as long anthers, strong rhizomes and bacterial leaf blight resistance (Sacks *et al*. 2003) and a large biomass production ability under low-input conditions which is an important trait for breeding low input adaptable rice (Yang *et al.* 2010). Thus, Ethiopian wild rice resource needs to be investigated for diversity and other traits to make use of its merits.

1.5 Genetic characterization and assessment of diversity in rice

Understanding and assessment of the crop genetic diversity is a prerequisite as well as fundamental step for proper utilization and conservation of genetic resources (Kumar *et al.* 2010). Genetic resources such as landraces, improved accessions and wild rice relatives are major sources of useful genes and these can be exploited to broaden genetic diversity and improve important agronomic traits of cultivated varieties. Characterization of accessions and investigation of their genetic diversity is the process by which variation among individuals or groups of individual or population is analyzed by a specific method or combination of methods (Mohammadi and Prasanna 2003).

1.5.1 Assessment of variation in morpho-physiological and agronomic traits

The traits of grain morphology are among morphological characteristics which can be used to characterize and classify rice accessions into Indica or Japonica (Oka 1958; Reflinur *et al*. 2018). Grain morphology, such as grain length, grain width and grain length-width ratio are known to be important indicators of the evolution of crop due to continues selection for large seeds during domestication (Konishii *et al*. 2008). Phenol reaction is also used as physiological trait to classify accession corresponding to Indica or Japonica (Oka 1958). In

addition, alkali digestibility and apparent amylose content are among important characters that are used to characterize rice accessions which help rice breeders in selection (Juliano 1992). Genetic variation of rice accessions for consumer and producer preferred agronomic traits, some of which vary across regions, should be precisely assessed in order to apply in the selection of parental materials and subsequent generations following crossbreeding (Abadassi 2016).

1.5.2. Characterization of accessions using isozymes as biochemical markers

Isozymes are enzymes that differ in amino acid sequence but catalyze the same reaction and they differ in electrophoresis mobility and are encoded by different genetic loci. These markers are co-dominant, cheap and relatively easy to use. According to Oka (1958), Nakagahra (1978), Endo and Morishima (1983), and Second (1982, 1984), isozymes have been used as biochemical characters to describe Asian *O. sativa* accessions into two main types, Indica and Japonica. Muto *et al.* (2016) also developed three nuclear INDEL markers (isozymes located at different chromosomes); Pgi 1-INDEL, Cat 1-INDEL, and Acp 1-INDEL to classify Laos accessions into Indica or Japonica on the bases of deletion (D) or non-deletion (ND) or insertion (INS) or non-insertion (Non-INS) on each genotype. They reported that Pgi 1 showed D, ND; Cat 1: ND, D; and Acp1: INS, Non-INS for Japonica and Indica type accessions, respectively. They also found that some accessions were not categorized to any of the group and reported as Indica-like types or Japonica-like types. Thus, application of these INDEL markers alone could not sufficiently classify accessions into two groups, corresponding to Japonica or Indica types. Hence, morpho-physiological characters such as seed morphology, phenol reaction of hulls, and starch digestibility using alkaline

solution could also be used as complement for the classification of rice accessions (Oka 1958).

1.5.3 Assessment of genetic diversity using molecular markers

Molecular markers are becoming a standard practice in the assessment of genetic diversity of plant genetic resources (Collard *et al*. 2005). They are identifiable DNA sequences at specific locations of the genome and are not influenced by environmental factors and so are more reliable than morphological markers (Collard *et al*. 2005). Molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeats (ISSR), Simple Sequence Repeats (SSR), and Single Nucleotide Polymorphism (SNP) have been applied in rice for taxonomic classification, phylogenetic, and diversity studies (Collard *et al.* 2005; Jones *et al*. 1997; Winter and Kahl 1995). This enabled detection of differences or relatedness among cultivated rice accessions and in wild rice germplasms.

Among different molecular markers, SSR markers are highly polymorphic, technically simple, informative and reproducible and thus, they are widely used in rice genetic diversity and population structure analysis studies (Garcia *et al*. 2004). Cytoplasm (chloroplast and mitochondrial) DNA markers are also widely used to study diversity and evolutionary relationship in rice genetic resources (Daniell *et al*. 2016; Gray 2015). Kaewcheenchai *et al.* (2018) examined the genetic structure of wild rice populations in Thailand using both chloroplast and nuclear genomes. The cpINDEL markers in their study revealed unique maternal lineages in wild rice populations in Thailand as compared to other Asian wild rice accessions. They also detected diverse genetic variation using SSR markers throughout the genome. Lam *et al.* (2019) also elucidated unique maternal lineage and

diversity among populations of wild rice in Vetinam with cpINDEL and SSR markers. Chloroplast INDELs such as ORF100 can also be employed to discriminate *O. sativa* rice accessions as Indica or Japonica types (Chen *et al*. 1993, 1994; Kanno *et al*. 1993; Muto *et al*. 2016).

1.6 Rice blast and its importance in Ethiopia

Rice blast disease, caused by the pathogen *Pyricularia oryzae* (syn. *Magnaporthe oryzae*), is the most devastating fungus that occurs in most rice growing areas of the world (Ou 1985). The disease is sporadic but could cause massive yield losses as high as 70-80% when predisposition factors favor its development such as, high relative humidity, optimum temperature (17-28^{o}C), long dew durations, cloudy weather, and excessive nitrogen fertilization (Piotti *et al*. 2005). In Africa, it is a widespread and destructive disease that can result in yield losses of up to 100% during an epidemic (Séré *et al*. 2011).

In Ethiopia, the disease has become very important recently and resulted in some cases a complete failure of rice fields. Survey reports by Mebratu *et al.* (2015) in Southwest of South NNPR, and Wasihun and Flagot (2016) in Pawe district of Benishangul Gumize region emphasized that blast is a major threat to rice cultivation. They reported that rice blast was observed in all assessed fields, and high incidence and severity of leaf blast and panicle blast was observed in some fields. Tekalign *et al*. (2019) also reported that blast is one of the most important diseases in Fogera, Dera, and Libokemkem districts of Amhara region. They mentioned that X-Jigna was more affected by blast and other diseases, followed by Gumara in the lowland production ecosystem while accessions from upland ecosystem were least affected. This is contrary to Séré *et al.* (2011) who reported that blast is more destructive in upland than lowland ecosystems. Currently, as research and promotion efforts proceed, rice is

expanding to new areas mainly of upland ecosystems of Ethiopia which often are more prone to blast (Séré *et al*. 2011). Some farmers also start applying high fertilizer rates to sustain high yield which may, on the other hand, aggravate blast diseases. Moreover, durability of many blast resistant rice accessions ranges only 2-3 growing seasons before disease resistance is overcome (Wilson and Talbot 2009). Thus, as a new threat, rice blast will continue as an important constraint to rice cultivation in Ethiopia which calls for breeding blast resistant varieties.

1.6.1 Pathogenesis and diseases cycle of *Magnaporthe oryzae*

The pathogen is highly adaptable to a wide range of environmental conditions and rice blast disease can be found in all rice production ecosystems (Sere *et al*. 2004). The fungus has a very wide host range infecting more than 50 plant species, most of which belong to grass family (gramineae) including rice, wheat, finger millet and barely (Talbot 2003). The fungus overwinters by means of mycelia and spores inside infected plant remains. It can also survive on infected seeds which can easily move across borders if proper safety measures are not in place. The fungus attacks rice plants at all stages of development and can infect leaves, stems, nodes, and panicles (Wilson and Talbot 2009). However, most infections occur on the leaves, causing diamond shaped lesions with a gray or white center to appear, or on the panicles which turn white and die before being filled with grain (Nutsugah *et al*. 2008). Different races of the fungus can infect different parts of rice plants and in some cases a single race can infect all aerial parts of the plant at the same time.

The disease cycle of rice blast involves three distinct phases; infection, colonization, and sporulation (Leung and Shi 1994). Once landing on a rice plant, conidia of the fungus germinate on a hydrophobic leaf surface (Fig. 1. 2). The spores undergo autophagy to allow

proper formation of specialized infection structure called the appressorium. Pressure generated in melanized appressorium ruptures the leaf surface, growing invasively into epidermal cells by means of invasive hyphae. After successful penetration, invasive hyphae colonize the entire host cell and grow biotrophically in host cells which further facilitate infection (Mosquera *et al.* 2009; Khang *et al.* 2010). Following post-inoculation, the fungus has become necrotrophic, producing thin, invasive hyphae followed by eventual development of lesions and production of more conidiophores (Wilson and Talbot 2009), from which several spores are produced. Spores can be carried readily through the air, by wind or rain onto neighboring plants (Nutsugah *et al*. 2008).

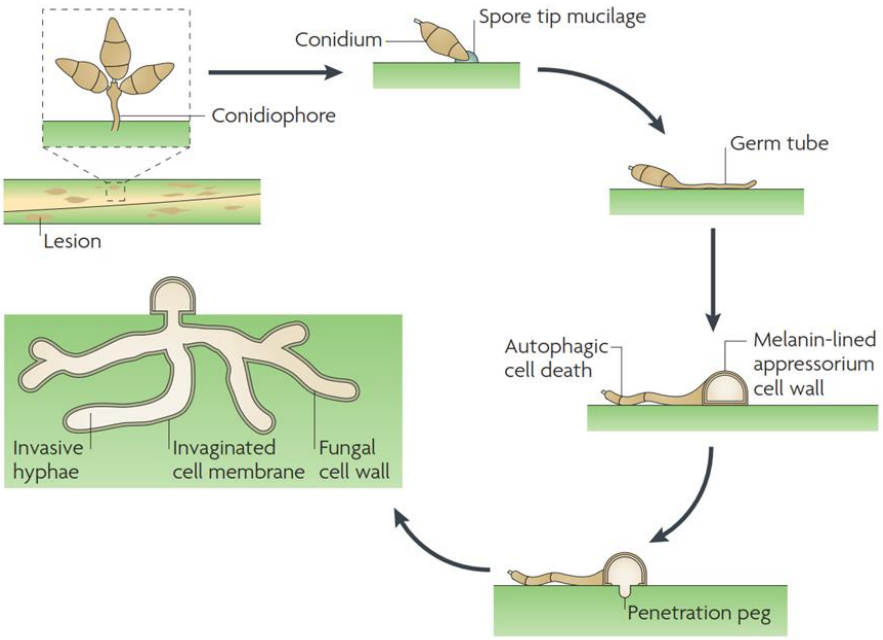

Fig. 1. 2 Life cycle of the rice blast fungus *Magnaporthe oryzae* (Wilson and Talbot 2009)

1.6.2 Genetics of resistance to rice blast and resistance genes

Genetic improvement of resistance against rice blast is a significant and primary target in rice breeding programs as it reduces the use of pesticides which is not affordable to poor farmers

and minimizes rice losses due to blast diseases (Song *et al.* 2014). Genetic resistance to blast is categorized into two types; 1) race-specific (complete, qualitative, true, or vertical) which is controlled by a single gene with major effect and it often breaks down easily with the occurrence of new races of the pathogen, and 2) race-nonspecific (partial, quantitative, field, or horizontal) which usually is complex and is based on the additive interaction of a few or several genes having minor to intermediate effects and show durable resistance (Ezuka 1972; Parlevlite 1979; Séré *et al*. 2011; Song *et al*. 2014; Fukuota and Okuno 2019). Since the first blast resistance gene *Pia* was identified in the *japonica* variety Aichi Asahi by Yamasaki and Kiyosawa (1966), about 100 resistance genes have been identified: 45% in *japonica* cultivars, 51% in *indica* cultivars, and the remaining 4% in wild species of rice (Ballini *et al*. 2008; Sharma *et al*. 2012). Over half of these genes were reported to be located in gene clusters on all rice chromosomes except chromosome 3, the three largest gene clusters being on chromosomes 6, 11, and 12 (Ballini *et al*. 2008; Ashkani *et al*. 2014). On chromosome 6, about 14 genes and /or alleles (*Pi2*, *Piz*, *Piz-t*, *Piz-5*, *Pi8*(t), *Pi9*, *Pi13*, *Pi13*(t), *Pi25*(t), *Pi26*(t), *Pi27*(t) *Pid2*, *Pigm*(t), and *Pi40*(t)) have been mapped (Qu *et al*. 2006). At least nine genes ((*Pi1*, *Pi7*, *Pi18*, *Pif*, *Pi34*, *Pi38*, *Pi44* (t), *PBR*, and *Pilm2*) and six alleles at the *Pik* locus (*Pik*, *Pik-s*, *Pik-p*, *Pik-m*, *Pik-h*, and *Pik-g*) have been mapped on the long arm of chromosome 11, and at least 17 resistance genes and/ or alleles (*Pita*, *Pita-2*, *Pitq6*, *Pi6*(t), *Pi12*(t), *Pi12*(t), *Pi19*(t), *Pi20*(t), *Pi21*(t), *Pi24*(t), *Pi31*(t), *Pi32*(t), *Pi39* (t), *Pi62* (t), *Pi157* (t) *IPi*, and *IPi3*) have been mapped in the region near the centromere of chromosome 12 (Koide *et al*. 2009).

Of 100 resistance genes, about 28 genes have been cloned and functionally validated and yet most of these cloned and characterized genes only confer resistance to one or a few blast isolates following the model of gene-for-gene interaction (Jia *et al*. 2000). Resistance of such genes tend to retain an effective level for only a short time, especially when the varieties

with resistance genes grown in large areas (Qu *et al*. 2006) and therefore, resistance genes with broad-spectrum resistance are more reliable in rice. Different broad-spectrum resistance genes have been documented and validated; *Piz* (Kiyosawa 1967), *Pi1* (Yu *et al*.1991), *Pi2* (Chen *et al*. 1996), *Pi9* (Liu *et al*. 2002), *Pigm* (Deng *et al*. 2006) and *Pi40* (Jeung *et al*. 2007). *Piz* was originally reported in the U. S. cultivar Zenith and has shown resistance to five U.S. races of blast (Roychowdhury *et al*. 2012). *Pi2* was first identified in a highly resistant *indica* rice cultivar 5173 (Zhou *et al*. 2006) and it showed resistance to 455 blast isolates collected from the Philippines and most of the 792 blast isolates from 13 major rice regions of China (Chen *et al.* 1996). *Pi9* was originally obtained from *Oryza minuta*, a tetraploid wild rice and the lines carrying *Pi9* were highly resistant to 43 blast isolates collected from 13 countries (Qu *et al*. 2006).

1.6.3 Phenotyping accessions for blast resistance using differential system

Genetic variation for blast resistance in rice based on differential system has been intensively investigated and reported. Differential system in blast screening test combines standard differential varieties which contain pre-define resistance gene and standard differential blast isolates with good pathogenecity to discriminate accessions. This system plays key role in screening new rice accessions for blast resistance, estimate blast resistance gene(s) each accession may have and investigate pathogencity of new blast races (Tsunematsu *et al*. 2000; Ebron *et al*. 2004; Kobayashi *et al*. 2007; Takehisa *et al*. 2009; Kawasaki-Tanaka and Fukuta 2014; Vasudevan *et al.* 2014; Khan *et al*. 2017; Odjo *et al.* 2017). Different researchers including Tsunematsu *et al*. (2000), Fukuta *et al* (2004), and Kobayashi *et al*. (2007) developed differential varieties (monogenic lines) that covered a total of 24 blast resistance genes; *Pia, Pii, Pik* (*Pik, Pik-s, Pik-m, Pik-h, Pik-p*), *Piz* (*Piz, Piz-5, Piz-t*), Pita, *Pita-2*, *Pib,*

Pit, Pish, Pi1, Pi3, Pi5(t), *Pi7*(t), *Pi9*(t), *Pi11, Pi12, Pi19* and *Pi20*(t). Telebanco-Yanoria *et al*. (2008) also developed a differential system comprising monogenetic lines and 20 standard blast isolates collected from the Philippines and they have been used to characterize several rice accessions in terms of blast resistance.

In phenotyping accessions for blast resistance, no single protocol is used by different researchers. Some researchers suggest two sets of screening at a time; natural infection in uniform nursery and controlled inoculation in green house while others focus on either of the two methods. Moreover, scoring of leaf blast reaction after inoculation in green house is not consistent among researchers. Standard evaluation system (SES) at IRRI (1996), for instance, used a scale of 0-9 i.e 0, 1, or 3: resistant; 5, 7, or 9: susceptible (Table 1.4). However, most previous reports showed a scoring scale of 0 to 5, where 0-2: resistant and 3-5: susceptible (Table 1. 4).

Table 1. 4 Variation in leaf blast reaction scoring under greenhouse at seedling stage of rice

Scale	Description	Reference
0 to 9	0, 1, or 3: resistant; 5, 7, or 9: susceptible	SES IRRI 1996; Vasudevan *et al* . 2014
0 to 5	0-3: resistant; 4-5: susceptible	Wu *et al* . 2015; Wu *et al* . 2017
	0-2: resistant; 3: moderate; 4-5: susceptible	Ebron *et al* . 2004; Song *et al* . 2014
0 to 5	0-2: resistant; 3-5: susceptible	Mackill and Bonmman 1992; Hayashi et al. 2009; Hayashi and Fukuta 2009; Takehisa *et al* . 2009; Kawasaki-Tanaka and Fukuta 2014; Khan *et al* . 2014, 2017

Takehisa *et al*. (2009) characterize two parental lines, Kasalath and Nipponbare before using them in crossing in greenhouse, using 21 standard blast isolates from Japan and the Philippines in comparison to 29 monogenic lines harboring 23 kinds of resistance genes and they found that Kasalath was susceptible to most isolates of the Philipines while Nipponbare

was susceptible to blast isolates from Japan but resistant to isolates of the Philippines. Similarly, Kawasaki-Tanaka and Fukuta (2014) classified 324 Japanese rice accessions into three resistance groups using 11 standard blast isolates in comparison to reactions of 23 monogenic lines under greenhouse. Odjo *et al.* 2017 also evaluated rice accessions from West Africa using 32 blast isolates of Japan and West Africa and 26 differential varieties in greenhouse and they classified accessions as susceptible, intermediate and resistant groups. Despite the tremendous information on variations for blast resistance in rice accessions and blast resistance genes they harbored in many countries, extent of rice blast resistance among rice accessions from Ethiopia is not understood.

1.7 Problem statement

Although rice cultivation is expanding across most regions in Ethiopia, the productivity of the crop is quite low (<3 tons/ha; CSA 2018) compared to world average (4.6 tons/ha; FAOSTAT 2019). Domestic production cannot meet the growing demand for rice and huge amount of rice is imported every year amounting to 312,000 tons milled rice with values of about US$ 171 million (Addis *et al*. 2018). The crop is also constrained by several biotic and abiotic factors among which rice blast is one occurring in most rice growing areas of the country. Several improved rice cultivars have adapted to different production ecosystems in Ethiopia in addition to landraces introduced in the past. These cultivars have been cultivated for long time as healthy crop. However, continues cultivation of few kinds of rice cultivars has induced the outbreak of blast disease (Nyongesa *et al*. 2016). In order to supply sufficient rice and to overcome the challenges of blast outbreaks there is an urgent demand for improved varieties which are high yielding and blast resistant that can meet consumers' preference. Improving productivity and diseases resistance of cultivars through breeding

heavily depends on efficient utilization of available rice genetic resources including landraces, improved cultivars and wild rice following proper characterization. However, it is not still possible to exploit the variation in rice genetic resources because information on the genetic diversity of the plant and reaction to blast is very limited in Ethiopia. Therefore, studies on characterization and genetic diversity in Ethiopian rice genetic resources are imperative based on agronomic traits (Abadassi 2016; Anyaoha et al. 2018), morpho-physiological markers (Matsuo 1952; Morishima and Oka 1981; Oka 1953), biochemical and molecular markers (Chen *et al*. 1993; Garris *et al*. 2004; Ishikawa *et al.* 1991; Oka 1988; Pai *et al.* 1975; Second 1982). Thus, four different experiments were conducted in this study with the following objectives:

1.8 Objectives

The general objective of the study was genetic analysis of rice genetic resources from Ethiopia for future breeding program to know their genetic characteristics with the following specific objectives:

I. To study genetic diversity and classify Ethiopian rice cultivars based on molecular markers and morpho-physiological characters
II. To evaluate Ethiopian rice cultivars for blast resistance based on differential system by inoculation test under greenhouse
III. To assess genetic variation among Ethiopian rice cultivars based on agronomic traits evaluated under lowland rain fed condition in Ethiopia
IV. To investigate genetic diversity, maternal lineage and population structure in wild rice populations from Ethiopia

Chapter 2

Genetic diversity analysis and classification of Ethiopian rice cultivars based on molecular markers and morpho-physiological characters

Abstract

Despite extensive studies on cultivated rice globally, genetic diversity and structure of Ethiopian materials remain unclear. Landraces and improved accessions in Ethiopia were characterized using SSR markers, and they revealed high genetic diversity. Accessions were classified into two major clusters, I and II. Cluster I was further divided into two sub clusters, Ia and Ib. Cluster Ia corresponded to Japonica-like types and Cluster Ib to the Japonica types and Cluster II to Indica types with some Indica-like types. Many landraces and improved varieties belonged to Cluster Ia while a superior landrace, X-Jigna, corresponded Cluster Ib. Examined with diagnostic INDEL markers, all accessions in Cluster Ia were judged as recombinant types and those in Ib as Japonica types. Model-based clustering also classified accessions into three subgroups, Group 1, Group 2 and Group 3. Japonica types corresponded to Group 1 and Indica types to Group 3 while recombinant types to Group 2. Alkali digestibility and apparent amylose content tests classified most accessions into intermediate types for both characters. Results demonstrated that Ethiopian accessions exhibited high genetic diversity by molecular markers and reasonable variation for phenotypic traits. The DNA clustering information among accessions may be useful in breeding schemes for selection of counterparts in crossbreeding programs.

Key words: genetic diversity, rice, DNA marker, Indica-Japonica, Ethiopia

Introduction

Rice (*O. sativa* L.) is a crop of major economic and cultural importance in Asia, where over 90% of world rice is produced, feeding more than half of the world's population (Barker *et al.* 1985). Consumption of rice is growing faster than any other food crop in Africa and it is also a cash crop providing employment in African countries. Twenty-two of the 43 rice-producing countries in Africa are experiencing growing demand for rice, necessitating the importation of 10–90% of their needs, at an estimated cost of over US$5.5 billion per year (AfricaRice 2017). In Ethiopia, rice is an economically important and strategic food security grain crop and its production has doubled within a short time despite fluctuations over the years (MoARD 2010; CSA 2017). Currently, total annual paddy production and productivity have reached 151 thousand tons and 2.84 tons/ha, respectively, making it the second highest yielding cereal crop after maize (CSA 2017). Despite an increase in domestic production, rice importation has also increased drastically from ~43,000 tons (2010) to ~312,000 tons (2016) equating to values of ~US$26 million and ~US$ 171 million, respectively (Addis *et al*. 2018).

Although currently expanding, the exact timing of the initial introduction of rice to Ethiopia is unclear. According to Gebey *et al*. (2012), rice was introduced in the early 1970s, whereas EthioRice (2018) suggested that rice cultivation started in the early 1980s. Addis *et al*. (2018) also reported that the first rice introduction was started in Gambella and Pawe to address issues of food security and resettlement and in Fogera for food security, in the 1970s and 1980s, respectively. As rice cultivation expanded, rice research was initiated and rice breeding research in Ethiopia has focused on the introduction of germplasms for evaluation of adaptation to local conditions. Since then, numerous rice germplasms have been introduced, many of which were bred by AfricaRice and IRRI. NERICAs (New Rice for Africa) developed and released by the Africa Rice Center (then WARDA: West Africa Rice

Development Association) are one of these introduced improved varieties, which were developed by inter-species hybridization between Asian rice, *O. sativa*, and stress resistant African rice, *O. glaberrima* (Jones *et al*. 1997).

Despite the release of new varieties for upland, lowland rainfed, and intermittently irrigated conditions including NERICAs, farmers in some localities tended to continue cultivating landraces introduced in the past. Including landraces, several cultivars exist in Ethiopia. X-Jigna is one of the landraces preferred by several farmers because of its high productivity and chilling tolerance adapted to highlands such as Fogera, Dera and Libokemkem districts from which more than 75% of total annual production harvested. However, more than 20 years of cultivation has seen the emergence of various difficulties in cultivation, such as diseases. In order to improve rice, Ethiopian rice breeding program requires not only introduced material but also a cross-hybridization program to fine-tune rice cultivars depending upon local demands. Hence, genetic background of materials including landraces should be known. However, rice cultivars, both landraces and improved accessions in Ethiopia have not been characterized in terms of morpho-physiological traits such as seed size, phenol reaction, alkali digestibility, and apparent amylose content. Most importantly, they have never been investigated for their genetic diversity and population structure using molecular markers.

The genetic differentiation of rice accessions has been studied based on morphological and physiological characteristics (Matsuo 1952; Morishima and Oka 1981; Oka 1953). Other markers were developed, such as isozymes, simple sequence repeat (SSR), and chloroplast markers, for evaluation of the diversity of genetic resources (Chen *et al*. 1993; Garris *et al*. 2004; Ishikawa *et al.* 1991; Oka 1988; Pai *et al.* 1975; Second 1982). These classifications always distinguished two main varietal groups, namely Indica and Japonica. Cross-hybridization between these groups always induces high sterility as a

reproductive barrier (Oka 1988). In this study, we applied DNA markers to precisely classify Ethiopian materials and we also used phenol reaction, alkali digestibility, and apparent amaylose content tests to characterize rice accessions. Thus, morpho-physiological characterization and genetic diversity examined with DNA markers will provide valuable information to accelerate rice breeding program in Ethiopia.

Materials and Methods

Plant materials

In molecular diversity analysis, a total of 79 rice accessions were subjected to DNA genotyping (Tables S2. 1, S2. 2). As landraces, 27 local accessions were used, collected from five areas in four regions; Fogera in Amhara, Pawe and Assosa in Benshagulgumize, Guraferda in South NNPR, and Abobo in the Gambella region (Fig. 2. 1). These regions are different in terms of temperature, rainfall pattern and intensity, relative humidity, soil type, cropping duration, and environmental stress to which local accessions have adapted. In order to compare genetic diversity and relationships, 33 improved accessions that were released between 1998 and 2017 by different research centers in Ethiopia were included. The improved accessions comprised upland NERICAs, other upland rice, lowland rice, and intermittently irrigated rice. As controls, seven Indica and twelve Japonica varieties comprising six Tropical-Japonica (Tr-J) and six Temperate-Japonica (Tm-J) types were used, as characterized previously by Muto *et al*. (2016) (Table S2. 2). In addition, 42 Japanese Rice Core Collection (JRC; https://www.gene.affrc.go.jp/databases-core_collections_jr_en.php), and 63 World Rice Core Collection (WRC; https://www.gene.affrc.go.jp/databases-core_collections_wr_en.php) varieties were employed to clarify genetic similarity with

Ethiopian Japonica- and Indica-type accessions, respectively. Most accessions we used in this experiment from the JRC accessions were Japonica types, whereas the WRC includes Indica and Japonica types (Ebana *et al.* 2010; Ichitani *et al*. 2016). The JRC and WRC were provided by the National Institute of Agrobiological Sciences (NIAS*)*, Japan. In phenol reaction, seed morphology, alkali digestibility, and apparent amylose content tests, all 60 Ethiopian accessions (27 landraces and 33 improved accessions) were used in comparison to Indica (IR64) and Japonica (Mashigura) as control accessions.

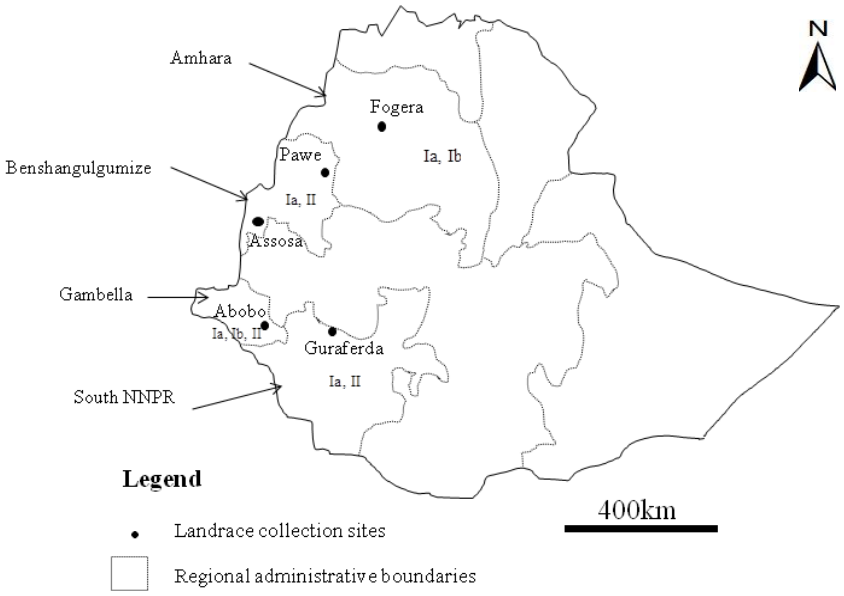

Fig. 2.1 Landrace collections sites from Ethiopia. DNA clades Ia, Ib and II are distributed across sites demonstrating relationship.

Molecular markers and DNA extraction

A total of 50 SSR markers showing polymorphic and reproducible amplifications (McCouch *et al*. 2002; Temnykh *et al*. 2000) (Table S2. 3) were used. These polymorphic markers were

applied to all 79 accessions. Ten primers pairs listed in Table S2. 3 were also applied to detect similarities between Ethiopian Japonica accessions and the JRC, and between Indica accessions and the WRC. Four INDEL markers; ORF100 (Chen *et al*. 1993), Cat1-INDEL, Pgi1-INDEL, and Acp2-INDEL (Muto *et al*. 2016) were applied to classify each accession as Indica or Japonica by genotype to confirm the Indica-Japonica classification based on SSR markers. Genomic DNA was extracted from fresh leaves of 2-week-old seedlings for each accession using the urea method as described by Chen and Dellaporta (1993) with minor modifications.

Polymerase chain reaction (PCR) and genotyping

PCR was carried out in a total volume of 20 µL per reaction containing 1.5 µL of template DNA, 2 µL 10× PCR buffer, 2 µL dNTPs (2 mM), 1 µL of forward and reverse primers, 0.1 µL *Taq* DNA polymerase (Takara Bio In., Shiga, Japan), and 12.4 µL sterile water. PCR amplification was performed using a thermal cycler (Bio-Rad Laboratories, Inc., California, USA) in the following PCR conditions: pre-heating at 94°C for 3 min, 30 cycles of 95°C for 10 s, 55°C for 30 s, and 72°C for 30s, and a final extension of 72°C for 1 min. The amplification products were separated on 6% polyacrylamide gels at 1500 V for 1: 30 to 2 h in 0.5×TBE and detected by silver staining, as described by Creste *et al*. (2001). ORF100, Cat1-INDEL, Pgi1-INDEL, and Acp2-INDEL were amplified with supplier-recommended reaction buffer with 0.25 U rTaq (NEB Inc., Tokyo, Japan). The PCR conditions were pre-heating at 94°C for 3 min, followed by 35 cycles of 95°C for 10 s, 55°C for 30 s, 72°C for 1 min, and post-heating at 72°C for 5 min. The amplified DNA fragments were electrophoresed on 1.5% agarose gels at 100 V for 1 h in 1×TAE to allow genotyping by their relative migration distances.

Phenol reaction of hulls

Accessions were characterized using phenol reaction test following the procedures of Oka (1958). Three dry seeds of each accession were soaked in 2mL 1.5% phenol solution (prepared as 100mL distilled water mixed with 1.5mL phenol) for six hours. After six hours, the seeds were dried gently at room temperature for 24 hours. Seeds hull were examined for color change. Seeds color changed into black were considered as positive (+) and those remain unchanged as negative (-). According to Oka (1958), Indica types show color change while Japonica type remain unchanged. Thus, based on the relative change in color of the hull of grains of rice, we broadly grouped accessions into two groups; as Indica or Japonica types by comparing to control accessions.

Seed morphology, alkali digestibility and apparent amylose content

To determine seed size of improved accessions and landraces, we measured grain length, grain width, and length-to-width ratio of 10 randomly selected seeds for each accession. Then, average value of each measure was used for comparison. Alkali digestibility of each accession was estimated according to Prathepha *et al*. (2005) and Wunna *et al*. 2015 with some modification. Five whole milled kernels without cracks were selected, cross cut at center and placed in petridish to which 10 mL of 2% (W/V) potassium hydroxide (KOH) solution was added. The peridishes were kept at room temperature for 23hr. The disintegration of the starch granules of each accession was compared to control accessions. The alkali spreading value of each sample was rated as 1, 2, and 3 for low, intermediate and high digestibility, respectively.

Apparent amylose content in the endosperm of each accession was determined by using the spectrophotometer method following procedures of Juliano (1971) and Wunna *et al*. (2015) with some modification. About 100 whole-grain rice of each accession was dehusked and polished, and then ground using laboratory mill. Then, 100mg of rice flour was put into a 50mL volumetric flask to which 1mL of 95% ethanol and 9mL of 1N sodium hydroxide were added. Flasks were vortexed for 1min and then boiled for 10 min to gelatinize the starch. Then, volume filled up to 50mL with distilled water and was shaken well. 5mL of starch solution was transferred into 50mL flask and treated with 1mL of 1N acetic acid and 2mL of iodine solution (2% KI and 0.2% I_2) and volume filled up with distilled water, then vortexed well and stand at 27^{o}C for 20 min to allow reaction. For each sample, about 2mL of solution was transferred into spectrophotometer cell and absorbance of the solution was measured at 620nm using spectrophotometer. Each accession was evaluated with three replications following the same procedures.

Data analysis

Diversity, cluster and population structure analysis

Molecular data of 79 rice accessions based on 50 SSR markers were subjected to statistical analysis using GenAlEX6.5 software (http://www.anu.edu.au/BoZo/GenAlEx/) to determine the number of alleles per locus, number of effective alleles per locus and genetic diversity (expected heterozygosity, *He*). Analysis of molecular variance was also performed using the same software. Polymorphism information content (*PIC*) and major allele frequency (MAF) were estimated using Power marker v3.25 (Liu and Muse 2005). *He* was calculated by the

formula, *He* = 1–$\sum_{i=1}^{n} xi^2$ where, *n* is the number of distinct alleles at a locus, and x_i (*i*=1, 2…*n*) is the frequency of allele *i* in the population (Nei 1973).

Cluster analysis of DNA data of 79 accessions was carried out to classify accessions by Ward's method (Ward, 1963) using JMP 14.0 software (JMP version 14.0; SAS Institute, Inc., Cary, NC, USA). Relationship among landrace collection regions was also illustrated by phylogenetic tree analysis using Populations ver. 1.2.32 and tree was edited using Mega ver. 7.0. GenStat ver.16 and SAS V9.0 (SAS 2002) were used in morpho-physiological data analysis. Population structure analysis was performed using the model-based clustering procedure implemented in the STRUCTURE 2.3.4 (Pritchard *et al*. 2000). The number of Markov Chain Monte Carlo (MCMC) repetitions was set to 200,000 iterations after a burn-in period of 100,000 for *K*=1–10 clusters. Selection of suitable *K* was determined using the ad hoc quantity *delta K* method (Evanno *et al.* 2005). Then, the optimum number of clusters was K=3. The membership probabilities (Q) calculated from STRUCTURE $\geq$ 0.80 were used to assign accessions to corresponding subgroups, and individuals with Q < 0.80 were considered as admixtures.

Results

SSR genetic diversity

Analysis of DNA polymorphism based on 50 SSR markers among 79 accessions showed multiple alleles ranging from 2 to 13 and an average of 7.02 alleles per locus with a total of 351 alleles. Number of effective alleles ranged from 1.33 to 8.56, with an average of 3.56 alleles per locus. Expected heterozygosity among 79 accessions varied from 0.23 to 0.88,

with an average of 0.65. PIC among the markers ranged from 0.12 (RM6313) to 0.68 (RM8137) and the major allelic frequency (MAF) varied from 0.51 to 0.90 (Table S2. 4). In addition, genetic diversity as expected heterozygosity (*He*) of landraces for Amhara (n=6), Gambella (n=4), Benshangul Gumize (n=15), and South NNPR (n=2), improved (n=33) and control accessions (n=19) were 0.62, 0.52, 0.35, 0.42, 0.55, and 0.68, respectively which revealed that landraces from Amhara had high genetic diversity, followed by Gambella while improved accessions were in between (Table 2. 1). However, control accessions showed the highest genetic diversity which could be attributed to their diverse origin (Table 2. 1).

Table 2. 1 Regional composition of DNA clusters

Variety types	Region	No. of accessions in each DNA cluster										Genetic diversity
		I						II		Total		
	Region	Ia	%	Ib	%	Sum	%	II	%		%	
Landrace	Amhara	2	33.3	2	33.3	4	66.7	2	33.3	6	100	0.62
	Gambella	2	50	1	25	3	75	1	25	4	100	0.52
	Benshangulgumize	13	86.7	0	0	13	86.7	2	13.3	15	100	0.35
	South NNPR	1	50	0	0	1	50	1	50	2	100	0.42
	Sum	18	66.7	3	11.1	21	77.8	6	22.2	27	100	0.48
Improved varieties	-	26	78.8	0	0	26	78.8	7	21.2	33	100	0.55
Control	-	1	5.3	11	57.9	12	63.2	7	36.8	19	100	0.68
Total		45	57	14	17.7	59	74.7	20	25.3	79	100	0.65
Genetic diversity		0.40		0.57		0.54		0.62		0.65		-

Genetic diversity was estimated in terms of expected heterozygosity (Nei 1973).

Analysis of molecular variance among 79 accessions of five populations revealed significant variation within populations ($P<0.001$) explaining 79% of the variation, whereas the remaining 21% was attributed to among population variation (data not shown). Similar result was reported by Aljumaili *et al*. (2018) who evaluated 50 rice accessions using 32 SSR markers and found that 89% of molecular variance was due within population variation, while 11% among populations with overall mean expected hetrozydosity (*He*) of 0.60.

Kaewcheenchai *et al* (2018) also reported multiple numbers of alleles among 118 rice accessions from Thailand ranging from 2.5 to 7.4 alleles per locus using 11 SSR markers and relatively high genetic diversity of *He*= 0.73.

DNA clusters and principal coordinate analysis and regional compositions

Cluster analysis based on 50 SSR data demonstrated two major groups, Clusters I and II (Fig. 2. 2). Cluster I included control Japonica accessions, upland NERICAs and most other improved upland and lowland accession including most landraces while Cluster II included the control Indica accessions with some improved accessions and landraces. Therefore, the two clusters were considered to correspond to Japonica with Japonica-like and Indica with Indica-like types, respectively. Cluster I was divided into two sub-clusters, Clusters Ia and Ib. Cluster Ia consisted of 45 accessions, including 18 landraces and 26 improved accessions including upland NERICAs, and one Japonica control, WAB56-104 (Table 2. 1). Popular and high yielding improved accessions such as NERICA-4, Ediget, Shaga, NERICA-12 and NERICA-13 also belonged to Cluster Ia. Although landraces were mainly upland rice, improved accessions in Cluster Ia originated from diverse production systems; upland NERICAs (n=4), lowland rice (n=7), other upland rice (n=9) and intermittently irrigated rice (n=6). Although they were obtained from different ecosystems, the relatively lower genetic diversity of Cluster Ia (expected heterozygosity=0.40) suggested their genetic similarity, whereas accessions in Clusters Ib and II showed higher expected heterozygosity values of 0.57 and 0.62, respectively (Table 2. 1). Landraces from Amhara (33.3%), Gambella (50%), Benshangul Gumize (86.7%), and South NNPR (50%), and the improved accessions (78.8%) belonged to Cluster Ia (Table 2. 1). Cluster II also comprised landraces and improved accessions, from upland and lowland rain fed production system. In total, 20 accessions

consisting of all Indica control accessions, 21% improved accessions and landraces originated from Amhara (33.3%), Gambella (25%), Benshangul Gumize (13.3%) and South NNPR (50%) belonged to Cluster II (Table 2. 1).

Relationship among landrace collections regions revealed that Gambella and Benishangul Gumize were more similar, both of which dominated by Japonica-like and Indica-like materials while South NNPR was dominated by Indica and Japinica-like materials and tended to be out grouped. Amhara region was in between where it comprised three different groups; Japonica, Japonica-like and Indica-like types. However, all regions showed close relationship with control Japonica compared to control Indica population (Fig. 2. 3). Principal coordinate analysis also demonstrated three separate groups in the plot. Indica and Indica-like accessions grouped with control Indica population, whereas Japonica type accessions with control Japonica population. However, all Japonica-like Ethiopian accessions were separated from the other two. One Japonica type control accession (WAB56-104), a recurrent NERICA parent, was grouped with Japonica-like accessions in which most improved accessions and landraces including NERICAs belonged (Fig. S2. 1).

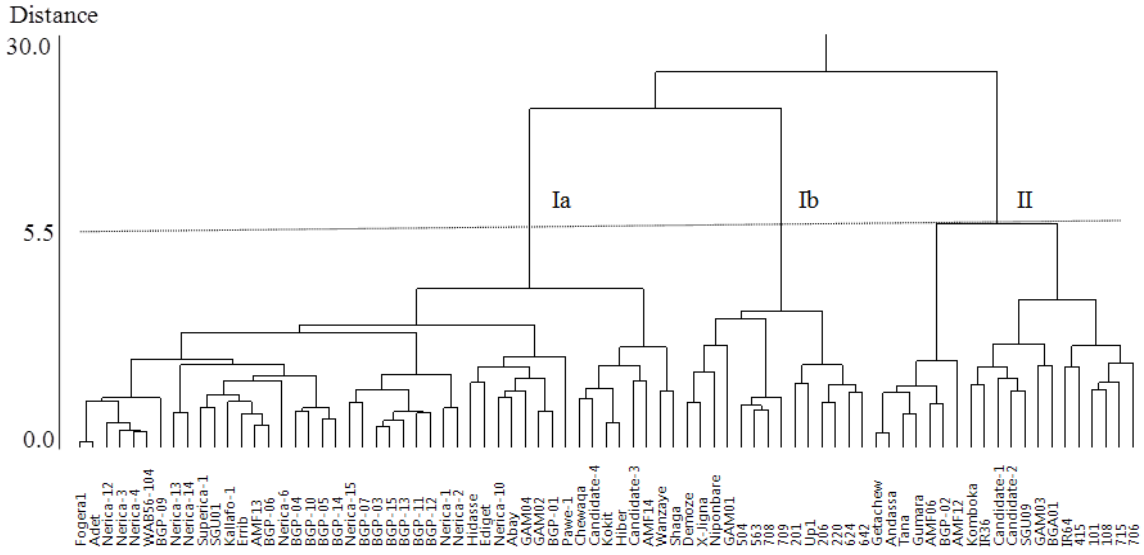

Fig. 2. 2 Phylogenetic tree demonstrating the genetic relationships among 79 rice accessions based on 50 SSR markers

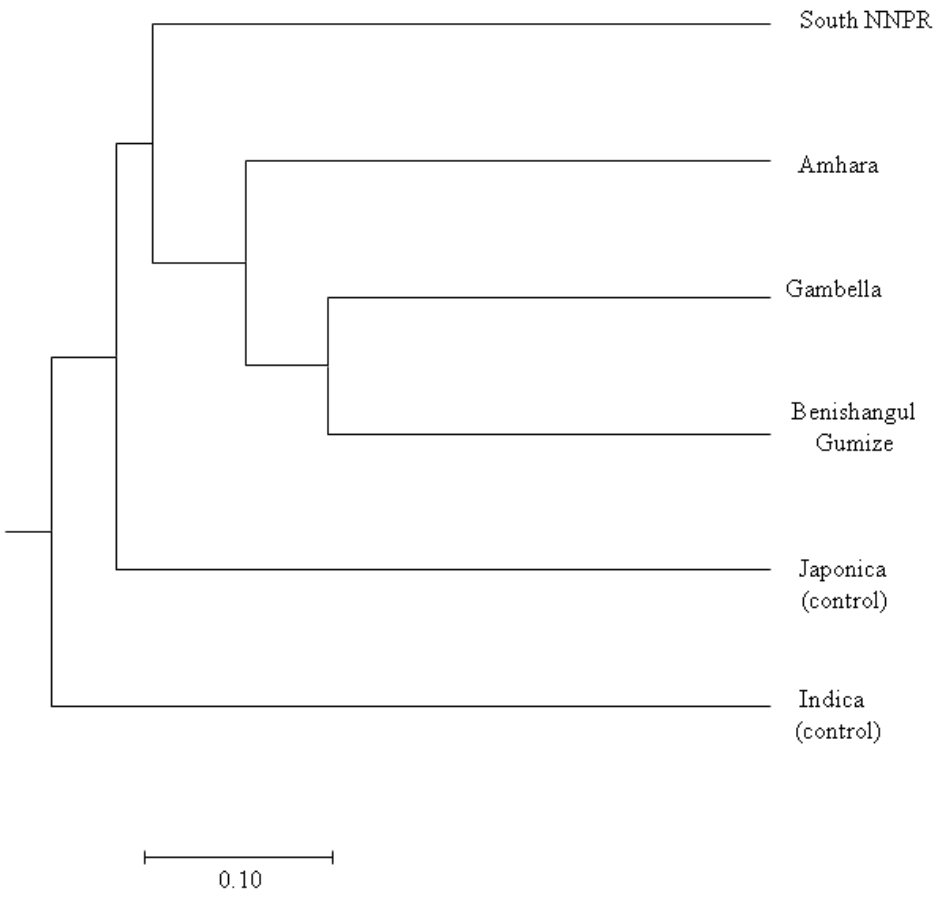

Fig. 2. 3 Relationship of landrace collection regions compared to control populations based on 50 SSR marks.

Indica-Japonica classification and phenol reaction

Cluster analysis based on 50 SSR data classified 79 accessions into two major groups, many of which were associated with Japonica and some others to Indica types. Several researchers used chloroplast INDELs (Chen *et al*. 1993, 1994; Kanno *et al*. 1993) and nuclear INDEL markers (Chen *et al*. 1994; Glaszmann 1987; Muto *et al.* 2016) to classifying rice accessions into Indica or Japonica type in addition to use of conventional classification based on morpho-physiological characters (Oka 1958; Second 1982). We applied one chloroplast marker and three nuclear INDEL markers to all accessions to further elucidate the Indica-Japonica classification. Thus, based on chloroplast INDEL (ORF100), 44 Ethiopian

accessions in Cluster Ia showed non-deletion type suggesting that the maternal donors of all accessions in this cluster were Japonica type, whereas the other three nuclear INDELs (Cat 1-INDEL, Pgi 1-INDEL, and Acp2-INDEL) showed inconsistency which carry alternative alleles specific to Indica or Japonica (Table S2. 1). All the Ethiopian accessions in Cluster Ia belonged to Japonica-like types because the alternative alleles were mixed but the maternal donor was Japonica. Eleven Ethiopian accessions of Cluster II belonged to Indica-like types because they carried Indica type deletion ORF100 but nuclear INDELs were mixed. These recombinant types between Indica and Japonica types may be attributed to their complex breeding history. The upland NERICA varieties in Cluster Ia, crossbreeds of *O. sativa* (Japonica) and *O. glaberrima* were also shown as Japonica-like types because of different alleles were introduced from *O. glaberrima* (Table S2. 1). Cluster Ib consisted of 14 accessions, including 11 control Japonica and three landraces (X-Jigna, GAM01 and Demoze). ORF100 and three nuclear INDELs also confirmed that these three landraces belonged to Japonica types (Table S2. 1).

Accessions in Cluster II comprised six landraces and seven improved accessions including Fogera 2 (Komboka) along with seven control Indica accessions. Komboka and SGU09 carried deletion type ORF100 as Indica type cytoplasm and all Indica type alleles for the three nuclear INDELs. The remaining five landraces and six improved accessions in Cluster II carried various mixtures of genotypes for the three INDELs**.** Their cytoplasm donor was Japonica type as revealed by ORF100. Thus, the two of the 13 Ethiopian accessions were identified as Indica types and the other eleven as Indica-like types (Table S2. 1).

Phenol reaction also classified accessions broadly into two groups corresponding to Japonica type (negative reaction) and Indica type (positive reaction). A total of 44 accessions in Cluster Ia and three accessions from Cluster Ib showed negative phenol reaction. As described by nuclear INDEL markers above, all Ethiopian accessions in Cluster Ia were

identified as Japonica-like types and those in Cluster Ib were identified as Japonica types which showed that both Japonica types and Japonica-like types including NERICAs showed negative reaction to phenol test (Table S2. 5). On the other hand, accessions in Cluster II responded differently in that 38.5% of accessions showed negative reaction and 61.5% of them showed positive reaction which suggested that only 61.5% were Indica types, which actually composed from Indica and Indica-like types (Table S2. 5).

Relationship of Ethiopian Japonica and Indica types with the JRC and WRC

In order to clarify trends, some Ethiopian landraces were compared with core collections developed by NARO, known as the JRC and WRC (Figs. S2. 2, S2. 3). Japonica type accessions from Ethiopia (X-Jigna, Demoze, and GAM01) were genotyped with the JRC, and Indica type accessions (Komboka and SGU09) with the WRC using 10 randomly selected SSR markers (Table S2. 3). We found that two accessions, X-Jigna and Demoze, showed a close genetic association with Dango (JRC 25), Rikutourikuu (JRC 49), Aikoku (JRC 26), and Ginbouzu (JRC 27) which originated from different localities in Japan; whereas GAM01 was closely associated with Wataribune (JRC 19) and Himenomochi (JRC 50) (Fig. S2. 2). These results demonstrated that the two landraces, X-Jigna and Demoze, had a closer genetic relationship to each other than was the case for GAM01. This may have been due to the uncertain origin of these landraces. GAM01 was collected from another area of Ethiopia with different environmental conditions. With regard to the Indica types, Komboka showed genetic similarity to Jena 035 (WRC 04), Beikhe (WRC 03), and Puluikarang (WRC 06), whereas SGU09 showed similarity to Naba (WRC 05), Tadukan (WRC 20), Kemasin (WRC 62), and Bingala (WRC 66) (Fig. S2. 3). These WRC accessions were classified as Indica

types but originated from different countries, indicating that Indica type accessions from Ethiopia may have been introduced from diverse origins.

Population structure and relationship with original populations

Model-based population structure analysis at the optimal K=3 showed that 79 accessions were clustered into three subgroups; Group 1, Group 2, and Group 3 (Fig. 2. 4a, b). Based on ancestry relationships of accessions, from five populations, 16.5%, 48.1%, and 25.3% of individuals belonged to Group 1, Group 2, and Group 3, respectively with ~10% as admixture (Table 2. 2). Nearly 60% of improved accessions clustered into Group 2 and 21% to Group 3 with 18% as admixture

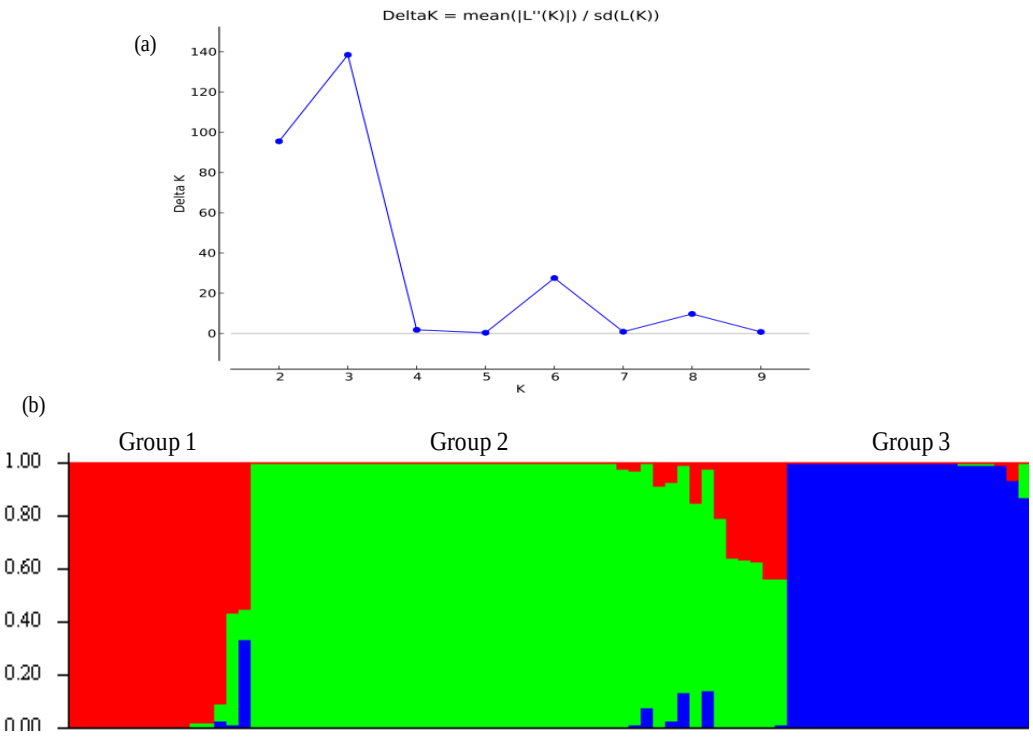

Fig. 2. 4 Model-based population structure of 79 rice accessions using 50 SSR markers by STRUCTURE. (a) Plot of Delta *K* values for each *K* (*K*= 1-10) based on the second order change of the likelihood function, (b) Graph for inferred ancestry of individuals at *K*= 3. Group1, Group 2 and Group 3 refers to Japonica, Japonica-like and Indica/Indica-like types, respectively.

Landraces were distributed across the three subgroups, many of which (~62%) belonging to Group 2 and ~ 22% to Group 2 while only ~ 7% corresponded to Group 1 or as admixture. With regard to control accessions, 100% of temperate Japonica and Indica types belonged to Group 1 and Group 3, respectively; whereas ~83% of tropical Japonica to Group 1 and the remaining ~17% to Group 2 (Table 2. 2). Group 3 showed the highest average genetic distance (*He*=0.64), followed by Group 1 (*He*=0.62), and Group 2 (*He*=0.35) which was in line with the previous results.

Table 2. 2 Relationship between original populations and model based subgroups at K=3 for 79 rice accessions based on 50 SSR markers

Original population	No. of cultivars	No. of accessions by structure subgroup (%)			
		Group 1	Group 2	Group 3	Adimixture
Improved cultivars	33	0 (0.0)	20 (60.6)	7 (21.2)	6 (18.2)
Landraces	27	2 (7.4)	17 (62.9)	6 (22.2)	2 (7.4)
Control					
Tr-Japonica	6	5 (83.3)	1 (16.7)	0 (0.0)	0 (0.0)
Tm-Japonica	6	6 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Indica	7	0 (0.0)	0 (0.0)	7 (100.0)	0 (0.0)
Total	79	13 (16.5)	38 (48.1)	20 (25.3)	8 (10.1)
Average distance (*He*)		0.62	0.35	0.64	

Population structure and relationship with distance based clusters

Relationship between model based subpopulation and distance based clusters were compared in Table 2. 3. About 84% of accessions belonging to Cluster Ia were distributed to Group 2 with about 16% as admixtures. On the other hand, nearly 93% of accessions in Cluster Ib belonged to Group 1and 100% of accessions from Cluster II corresponded to Group 3.

Table 2. 3 Relationship between distance-based DNA clusters and model-based subpopulations at K=3 for 79 rice accessions based on 50 SSR markers

Distance-based DNA cluster	No. of cultivars	No. of accessions by subpopulations (%)			
		Group 1	Group 2	Group 3	Adimixture
Ia	45	0 (0.0)	38 (84.4)	0 (0.0)	7 (15.6)
Ib	14	13 (92.9)	0 (0.0)	0 (0.0)	1 (7.1)
II	20	0 (0.0)	0 (0.0)	20 (100.0)	0 (0.0)
Total	79	13 (16.5)	38 (48.1)	20 (25.3)	8 (10.1)

This relationship demonstrated that accessions which showed close associated with Japonica and Indica types in distance-based clustering were clustered into Group 1 and Group 3, respectively while those related to recombinant type including NERICAs belonged to Group 2 with some admixtures (Table 2. 3). Thus, the results of the model-based grouping showed similar trend of relationship of accessions as it was illustrated by distance based clustering and this was also in line with nuclear INDEL markers discrimination of accessions.

Variation in morpho-physological traits and relationships with DNA clusters

Rice accessions showed wider variation for seed morphological traits with length (mm), width (mm), and length to width ratio ranging from 6.41 to 9.21, 2.31 to 3.27, and 2.08 to 3.84, respectively (Table S2. 5). The majority of improved accessions, most landraces from Gambella and all landraces of Benishangul Gumize region were dominated by medium size in seed length (Table S2. 5). About 50% of landraces from Amhara were short in seed length and the rest were medium types. Relationships between seed morphology and DNA clusters revealed that some accessions belonging to Cluster Ib showed shorter seed length and those in Cluster II showed relatively longer seed length while those accessions belonging to Cluster Ia tended to be intermediate between accessions of Ib and II (Table S2. 5, Fig. 2. 5).

Assessment of rice accessions for alkali digestibility showed that 73% of Ethiopian accessions revealed intermediate alkali degradation with only 25% as low and 5% as high degradation (Table S2. 5). Relationship between alkali digestibility of accessions and DNA clusters showed that nearly 98% of accessions in Cluster Ia were with intermediate alkali degradation while 67% accessions in Cluster Ib exhibited high digestibility. However, almost all accessions in Cluster II showed low alkali degradation. These results demonstrated that starch granules of accessions related to Indica were stronger than those related to Japonica to alkali digestibility (Table S2. 5).

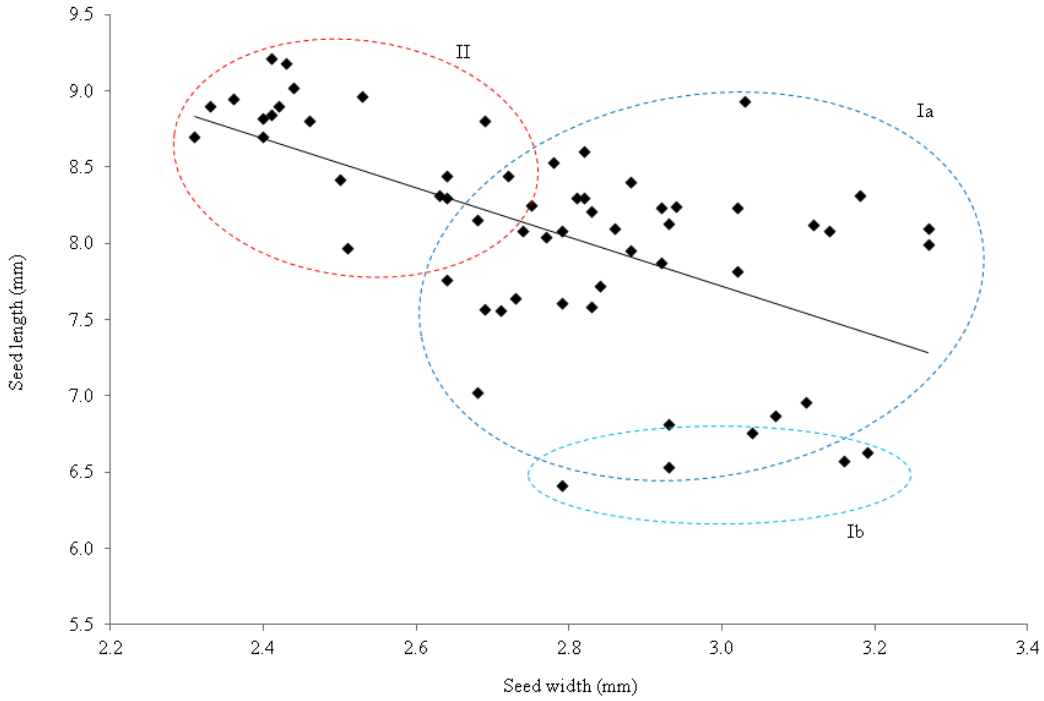

Fig. 2. 5 Scatter plot showing variation of seed size in 60 rice cultivars. DNA clusters; Ia, Ib and II refers to Japonica-like, Japonica , & Indica/Indica-like types of cultivars

Estimation of apparent amylose content among 60 Ethiopian accessions revealed that about 38 accessions (~63%), 16 accessions (~26.7%), and 6 accessions (10%) showed intermediate (21-25%), high (26-33%) and low (12-20%) amylose content, respectively (Table S2. 5). Five improved accessions; Chewaka, Kokit, Pawe-1, Ediget, and Nerica-15

showed low ranges of amylose content (18-20%) while most improved accessions including NERICAs and landraces showed intermediate amylose content (21-25%) (Table S2. 5). Relationships between apparent amylose content and DNA cluster revealed that about 68% of accessions in Cluster Ia, 100% accessions in Cluster Ib, and about 39% accessions in Cluster II showed intermediate apparent amylose content (21-25%). About 18% accessions in Ia and 62% of the accessions in II exhibited high apparent amylose content (26-33%). Results suggested that most Indica/Indica-like type accessions had intermediate to high amylose content while those Japonica/Japonica-like types exhibited wider range of apparent amylose content, from low to high, most of them showing intermediate content (Table S2. 5).

Discussion

SSR diversity

Since the first introduction of Asian rice into Ethiopia and after the release of the first improved rice accession (1998) through the research system, several rice accessions have become adapted to different rice production systems, notably; upland, rainfed lowland and intermittently irrigated rice in Ethiopia. More than 30 improved accessions and some landraces exist today. The success of crossbreeding depends on the presence of contrasting but compatible parental lines that are identified through proper characterization and selection (Bertan *et al*. 2007). In this study, we studied 60 Ethiopian rice accessions comprising 33 improved accessions and 27 presumed landraces collected from different localities in comparison to 19 controls accessions using molecular markers.

In our study, SSR polymorphism among 79 accessions showed multiple numbers of alleles per locus ranging from 2 to 13 with an average of 7.02 alleles per locus. The PIC

(Polymorphism Information Content) also varied from 0.12 to 0.68 for SSR markers RM6313 and RM8137, respectively, with an average of 0.51. These results were similar to those found by Brondani *et al.* (2006), who obtained 5.2 alleles per locus and *PIC* values of 0.61when they evaluated 30 rice cultivars using 25 SSR markers and those found by Roy *et al*. (2016) that obtained 8.49 alleles per locus and *PIC* of 0.62 using 35 SSR markers in 67 hill rice accessions. Our results also demonstrated that accessions had relatively high genetic diversity or expected heterozygosity (*He*=0.65), ranging from 0.23 to 0.88 and this value was a little higher than the previous study of 50 aromatic rice by using 32 SSRs in Malaysia (*He*=0.60; Aljumaili *et al*. 2018) and smaller than that of 85 accessions by using 29 SSRs in Korea (*He*=0.73; Yoon and Park 2015). Results in this study also showed that 79% of significant genetic variation was attributed to variation within populations with only 21% of the variation was accounted for among population variation. In our study, both landrace and improved populations from Ethiopia showed reasonably high genetic diversity which could be an opportunity for varietal improvement.

Genetic relationship and classification of rice accessions

Genetic relationship among accessions was illustrated using cluster analysis and principal coordinate analysis of accessions. In cluster analysis, we classified all accessions into two major clusters; Cluster I corresponded mainly to Japonica with Japonica-like types and Cluster II to Indica with Indica-like types. Principal coordinate analysis also revealed similar trend of relationship among accessions. This classification was again separated by chloroplast and nuclear INDEL markers. In all gene combinations for three nuclear INDELs, namely *Pgi1*, *Cat1*, and *Acp2*, the haplotype, D-D-INS is predominant (41.7%), followed by D-ND-(Non-INS) (25%) and ND-D-(Non-INS) (15%). This is due to the fact that improved

accessions have been established with various parental combinations including NERICA. Most improved accessions and landraces including X-Jigna tended to cluster with control Japonica types. These materials are predominantly distributed in northwestern Ethiopia, where low temperature frequently induces chilling stress because of high altitude. Because of hybrid sterility in the progeny of Indica-Japonica crosses, understanding of the genetic background is necessary to choose ideal counterparts to improve rice varieties further. Oka (1958) pointed out that a single approach alone cannot guarantee Indica-Japonica classification.

We further examined Ethiopian rice accessions using phenol reaction as reported by Oka (1958) and Second (1982). Accessions were classified into two major groups, many of which responding to phenol reaction similar to control Japonica types and some others similar to control Indica types. This classification by phenol reaction was to some extent corresponding to classification of accessions by INDEL markers. Most accessions belonging to Custer Ia and all in Ib were showed negative phenol reaction and thus classified as Japonica type which corresponded to classification by ORF100. Characterization of accessions by other approaches such as alkali digestibility and apparent amylose content demonstrated that majority of Ethiopian rice accessions are intermediate types for both characters.

Population structure and relationships with distance-based DNA clusters

Model based genetic structure was also applied to understand relationship among accessions. Effective conservation, management and utilization strategies for rice accessions need a basic understanding of their genetic structure (Yoon and Park 2015). Model-based population structure analysis classified 79 accessions into three subpopulations in which the first group

comprised Japonica types, the second group Japonica-like types and the third group Indica/Indica-like types. Accessions in the third group showed the highest genetic diversity, followed by that of the first group which included all control Japonica types along with three Ethiopian landraces, X-Jigna, GAM01 and Demoze. High genetic diversity of the two groups might be attributed to the diverse origins of control accessions, from different Asian countries. Majority of Ethiopian accessions were grouped to the second subgroup which tended to show similar trend of classification with distance-based clustering. The current results of genetic diversity, interrelationship and genetic structure based on molecular markers suggested that Ethiopian rice accessions comprised materials with contrasting relationship having overall high genetic diversity. Therefore, these materials are considered as important genetic resources with great potential to be exploited in future rice breeding.

Supplementary data

Table S2. 1 Details of accessions and their Indica-Japonica classification as presumed by four INDEL markers

Accession	Cultivation type	Type	Origin	Location/Country	DNA Cluster	INDEL[a]				Indica-Japonica[b] classification
						ORF100	Pgi 1	Cat 1	Acp2	
SGU01	Upland	Landrace	South NNPR	Ethiopia	Ia	ND	D	D	INS	Rec.
GAM02	Upland	Landrace	Gambella	Ethiopia	Ia	ND	D	ND	Non-INS	Rec.
AMF13	Lowland	Landrace	Amhara	Ethiopia	Ia	ND	D	D	INS	Rec.
GAM04	Upland	Landrace	Gambella	Ethiopia	Ia	ND	D	D	Non-INS	Rec.
BGP-01	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	ND	Non-INS	Rec.
BGP-03	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-04	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-05	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-06	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-07	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-09	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-10	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-11	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-12	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-13	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-14	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-15	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
AMF14	Lowland	Landrace	Amhara	Ethiopia	Ia	ND	D	D	INS	Rec.
Fogera 1	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	INS	Rec.
Adet	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-12	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-13	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	ND	ND	Non-INS	Rec.
Chewaqa	Upland	Improved	China	China	Ia	ND	D	D	INS	Rec.
Hiddasse	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-3	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	INS	Rec.
NERICA-4	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	INS	Rec.
SUPERICA-1	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	Non-INS	Rec.
Kokit	Upland	Improved	Amhara	Ethiopia	Ia	ND	D	D	Non-INS	Rec.
Pawe-1	Upland	Improved	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
Hiber	Lowland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	Non-INS	Rec.
Ediget	Lowland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	INS	Rec.
NERICA-15	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-6	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-14	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
Kallafo-1	Intermittent irrigated	Improved	Madagascar	Madagascar	Ia	ND	D	D	INS	Rec.
NERICA-1	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-2	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-10	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
Abay	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	INS	Rec.
Candidate 3	Lowland	Improved	International Rice Research Institute	Philippines	Ia	ND	D	ND	Non-INS	Rec.
Erib	Lowland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
Candidate 4	Lowland	Improved	Madagascar	Madagascar	Ia	ND	D	D	INS	Rec.
Wanzaye	Lowland	Improved	Madagascar	Madagascar	Ia	ND	D	ND	Non-INS	Rec.
Shaga	Lowland	Improved	Madagascar	Madagascar	Ia	ND	D	D	INS	Rec.
Demoze	Lowland	Landrace	Amhara	Ethiopia	Ib	ND	D	ND	INS	J
X-JIGNA	Lowland	Landrace	Amhara	Ethiopia	Ib	ND	D	ND	INS	J
GAM01	Upland	Landrace	Gambella	Ethiopia	Ib	ND	D	ND	INS	J
SGU09	Upland	Landrace	South NNPR	Ethiopia	II	D	ND	D	Non-INS	I
AMF06	Lowland	Landrace	Amhara	Ethiopia	II	ND	ND	ND	INS	Rec.
AMF12	Lowland	Landrace	Amhara	Ethiopia	II	ND	ND	ND	Non-INS	Rec.
GAM03	Upland	Landrace	Gambella	Ethiopia	II	ND	ND	D	Non-INS	Rec.
BGA01	Upland	Landrace	Benshangulgumize	Ethiopia	II	ND	ND	D	Non-INS	Rec.
BGP-02	Upland	Landrace	Benshangulgumize	Ethiopia	II	ND	ND	D	INS	Rec.
Getachew	Upland	Improved	Amhara	Ethiopia	II	ND	ND	D	Non-INS	Rec.
Andassa	Upland	Improved	Amhara	Ethiopia	II	ND	ND	D	Non-INS	Rec.
Tana	Upland	Improved	Amhara	Ethiopia	II	ND	ND	D	Non-INS	Rec.
Fogera2 (Komboka)	Lowland	Improved	International Rice Research Institute	Philippines	II	D	ND	D	Non-INS	I
Gumara	Lowland	Improved	Amhara	Ethiopia	II	ND	ND	D	Non-INS	Rec.
Candidate 1	Lowland	Improved	International Rice Research Institute	Philippines	II	ND	ND	D	Non-INS	Rec.
Candidate 2	Lowland	Improved	International Rice Research Institute	Philippines	II	ND	ND	D	Non-INS	Rec.

[a] Genotypes of ORF100, -Pgi 1-INDEL-Cat 1-INDEL-Acp2-INDEL: ND-D-ND-INS for Japonica, D-ND-D-Non-INS for Indica type.

[b] Classification into Indica (I) or Japonica (J) was based on Muto *et al.* (2016); Rec. refers to recombinant.

Table S2. 2 Accessions used as reference control in Indica-Japonica classification based on INDEL patterns

Accession	Origin	Location/Country	Known as	DNA Cluster	INDEL				Indica-Japonica classification
					ORF100	Pgi 1	Cat 1	Acp2	
IR36	International Rice Research Institute	Philippines	Indica	II	D	ND	D	Non-INS	I
IR64	International Rice Research Institute	Philippines	Indica	II	D	ND	D	Non-INS	I
101	Taiwan	Taiwan	Indica	II	D	ND	D	Non-INS	I
108	Taiwan	Taiwan	Indica	II	D	ND	D	Non-INS	I
415	India(Aduturai)	India	Indica	II	D	ND	D	Non-INS	I
706	North China	China	Indica	II	D	ND	D	Non-INS	I
715	Central China	China	Indica	II	D	ND	D	Non-INS	I
WAB56-104	Africarice	Côte d'Ivoire	Tropical Japonica	Ia	ND	D	D	INS	Rec.
201	Philippines	Philippines	Tropical Japonica	Ib	ND	D	ND	INS	J
206	Philippines	Philippines	Tropical Japonica	Ib	ND	D	ND	INS	J
220	Philippines	Philippines	Tropical Japonica	Ib	ND	D	ND	INS	J
624	Celebes island	Indonesia	Tropical Japonica	Ib	ND	D	ND	INS	J
642	Celebes island	Indonesia	Tropical Japonica	Ib	ND	D	ND	INS	J
Up1	Japan	Japan	Temprate Japonica	Ib	ND	D	ND	INS	J
504	Taiwan	Taiwan	Temprate Japonica	Ib	ND	D	ND	INS	J
563	Japan	Japan	Temprate Japonica	Ib	ND	D	ND	INS	J
708	North China	China	Temprate Japonica	Ib	ND	D	ND	INS	J
709	Central China	China	Temprate Japonica	Ib	ND	D	ND	INS	J
Nipponbare	Japan	Japan	Temprate Japonica	Ib	ND	D	ND	INS	J

Genotypes of ORF100, -Pgi 1-INDEL-Cat 1-INDEL-Acp2-INDEL: ND-D-ND-INS for Japonica, D-ND-D-Non-INS for Indica type.

Table S2. 3 List of molecular markers used in cultivarss evaluation

Marker type	Chr	Locus	Forward	Reverse	Repeat motif	Product size	Remark
SSR	1	RM495	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	(CTG)7	159	Temnykh *et al.*(2000)
SSR	1	RM3604	ATGTCAGACTCCGATCTGGG	TCTTGACCTTACCACCAGGC	(GA)13	153	McCouch *et a.*(2002)
SSR	1	RM259	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT	(CT)17	162	Temnykh *et al.*(2000)
SSR	1	RM6840	TACCAAGACTCCGCTATGGC	GAAGAAGGGATCATGGATCG	(TCT)17	191	McCouch *et a.*(2002)
SSR	1	RM8111	AGGTAACTAAGCTAGGTGTT	TAGGTACAGTAATACCAAGC	(CT)30	149	Temnykh *et al.*(2000)
SSR	1	RM8137	GTAATTGAATTTCACTGCTGCT	ACGTACGTGACGTGCTTATG	(AT)54	177	McCouch *et a.*(2002)
SSR	2	RM262	CATTCCGTCTCGGCTCAACT	CAGAGCAAGGTGGCTTGC	(CT)16	154	Temnykh *et al.*(2000)
SSR	2	RM1367	GTGTGTACGTAGGATCGGAG	TGCTACTCCTAGCTGCTACC	(AG)27	159	McCouch *et a.*(2002)
SSR	2	RM240	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC	(CT)21	132	Temnykh *et al.*(2000)
SSR	2	RM406	GAGGGAGAAAGGTGGACATG	TGTGCTCCTTGGGAAGAAAG	(GA)17	146	Temnykh *et al.*(2000)
SSR	2	RM3865	AACCATGGACAGTTGAACAC	CTCCGACAAGAACTTCCTC	(GA)29	223	McCouch *et a.*(2002)
SSR	2	RM6378	ATAGGGTGGGTGTGCTGAAC	TGCACAAAACTGCAGGTCTC	(GAA)19	167	McCouch *et a.*(2002)
SSR	2	RM324	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	(CAT)21	175	Temnykh *et al.*(2000)
SSR	3	RM8208	GCCCAAACTACACTCTCTTG	GTAAATGCCTGAGTGCCTAC	(AGA)12	142	McCouch *et a.*(2002)
SSR	3	RM168	TGCTGCTTGCCTGCTTCCTTT	GAAACGAATCAATCCACGGC	T15(GT)14	116	Temnykh *et al.*(2000)
SSR	3	RM8203	CATTGATAATGTCCAGTGACG	CTCCTGTTGTCATTCTTTGG	(GA)24	212	McCouch *et a.*(2002)
SSR	3	RM7389	AGCGACGGATGCATGATC	TTGAGCCGGAGGTAGTCTTG	(GATA)7	111	McCouch *et a.*(2002)
SSR	4	RM8213	AGCCCAGTGATACAAAGATG	GCGAGGAGATACCAAGAAAG	(TC)10	177	McCouch *et a.*(2002)
SSR	4	RM3317A	AGCAACCTGACAGAAGAATG	TCTCGTTGAGTTGGAAGAAG	(CT)14	138	McCouch *et a.*(2002)
SSR	4	RM5586	CTCCATAATCAAGGAAGCTA	ATGAGTTCTTTCGTCAGTGT	(TG)30	134	McCouch *et a.*(2002)
SSR	4	RM3524	CGGAGCTGGTCTAGCCATC	GTCTCCGTCTTCCTCACTCG	(CT)31	129	McCouch *et a.*(2002)
SSR	4	RM3367	GGATCCATCCATCCACTGAC	GGATATGTGCTGCTGTGTGC	(CT)16	126	McCouch *et a.*(2002)
SSR	4	RM3836	ACTGTGGAGTACAGGTCGGC	GAAACGGAAACGAAACCCTC	(GA)22	126	McCouch *et a.*(2002)
SSR	5	RM3663	CATCAACCTCCACGAACATG	CTCGGTGGTGATCCTCCTC	(GA)14	125	McCouch *et a.*(2002)
SSR	5	RM3790	TAATTGCGGTCTCGTGCC	AACCACCTCAACTACTGCCG	(GA)19	119	McCouch *et a.*(2002)
SSR	5	RM6313	ATCCAGATCCACTTTGACCG	GGAGGACTTCTACCATCCTTG	(CTT)11	107	McCouch *et a.*(2002)
SSR	5	RM405	TCACACACTGACAGTCTGAC	AATGTGGCACGTGAGGTAAG	(AC)14	110	Temnykh *et al.* 2000
SSR	6	RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC	(GA)15	122	Temnykh *et al.*(2000)
SSR	6	RM276	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA	(AG)8A3(GA)33	149	Temnykh *et al.*(2000)
SSR	6	RM162	GCCAGCAAAACCAGGGATCCGG	CAAGGTCTTGTGCGGCTTGCGG	(AC)20	229	Temnykh *et al.*(2000)
SSR	6	RM3138	TTGACAAGAGATCAAGGCGG	GTGAATGTTGAGCTGCATGG	(CA)16	105	McCouch *et a.*(2002)
SSR	6	RM508	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC	(AG)17	235	Temnykh *et al.*(2000)
SSR	7	RM1134	ACACCCAACTTTTCTCACGC	AGCTAGGGTTTCGATCTCCC	(AG)12	144	McCouch *et a.*(2002)
SSR	7	RM11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	(GA)17	140	McCouch *et a.*(2002)
SSR	7	RM234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	(CT)25	156	Temnykh *et al.*(2000)
SSR	8	RM408	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC	(CT)13	128	Temnykh *et al.*(2000)
SSR	8	RM152	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG	(CCG)9	152	Temnykh *et al.*(2000)
SSR	8	RM3395	ACCTCATGTCCAGGTGGAAG	AGATTAGTGCCATGGCAAGG	(CT)17	97	McCouch *et a.*(2002)
SSR	8	RM7356	CCAAGGACACATATGCATGC	GCAATTCATGGCGCTGTTC	(CTAT)6	158	McCouch *et a.*(2002)
SSR	8	RM6948	GGTAAGTTGTCGGTTGCCTC	ACGTCCATACCAGGTCAAGC	(TTC)8	94	McCouch *et a.*(2002)
SSR	9	RM7048	CAACCCCTAATTTCACGCTC	GACTTCACTGGCACTGGATG	(AATA)8	166	McCouch *et a.*(2002)
SSR	9	RM3164	TCCTCCTGCTAGCTGCCTAG	TCGCCTTCCTTTTCACTCAC	(CT)12	120	McCouch *et a.*(2002)
SSR	10	RM8201	TCTGTTTATAAGCGCAGCAC	GCCGGCGAGCTACTACTAC	(CT)13	163	McCouch *et a.*(2002)
SSR	10	RM258	TGCTGTATGTAGCTCGCACC	TGGCCTTTAAAGCTGTCGC	(GA)21(GGA)3	148	Temnykh *et al.*(2000)
SSR	10	RM171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	(GATA)5	311	Temnykh *et al.*(2000)
SSR	10	RM271	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC	(GA)15	101	Temnykh *et al.*(2000)
SSR	11	RM5704	AAAAGTTTTGAATAAAACGAATG	ATGTGATTCTCCAAGCAGAG	(AAT)20	210	McCouch *et a.*(2002)
SSR	11	RM3133	TCAATAGACACACGGGCATG	CGATTTTGCTCACTGCACAG	(CA)14	98	McCouch *et a.*(2002)
SSR	11	RM21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	(GA)18	157	McCouch *et a.*(2002)
SSR	12	RM7376	TCACCGTCACCTCTTAAGTC	GGTGGTTGTGTTCTGTTTGG	(GAAA)6	195	McCouch *et a.*(2002)
cpINDEL	Chloroplsat	ORF100	AGTCCACTCAGCCATCTC TC	CAAATAAATCATTTTCTTTAG	INDEL	1054/985	Chen *et al* . 1993
Nulceal INDEL	6	Cat 1	CATCGCGAAGGCTACTCTAC	CCAGAGACTGGGAAATCAGG	INDEL	338(J)/296(I)	Muto *et al* . 2016
Nulceal INDEL	3	Pgi 1	AGATCTTTCCCTAATATTCTTTAG	CCTGTCATTCAGATCATGAAAATT G	INDEL	1180(I)/1179(J)	Muto *et al* . 2016
Nulceal INDEL	12	Acp2 for Japonica	CACGTGGTTTTAATAATAATCCAC	TCATTTTCCTAGTAGTGGGGTG	INDEL	408nt (J)	Muto *et al* . 2016
		Acp2 for Indica	CACGTGGTTTTAATAATAATCCAC	TATCAGATTGGTCCATTTTTCAAG	INDEL	491nt (I)	Muto *et al* . 2016

Table S2. 4 Molucular statistics for number of allele (*Na*), number of effective alleles, expected heterozygosity (*He*), polymorphism information content (*PIC*) and major allele frequency (*MAF*) based on 50 SSR markers

SSR marker	*Na*	*Ne*	*He*	*PIC*	*MAF*
RM495	3	2.79	0.64	0.39	0.73
RM3604	3	2.57	0.61	0.47	0.62
RM259	8	4.43	0.78	0.64	0.52
RM6840	7	3.01	0.67	0.43	0.70
RM8111	6	2.79	0.64	0.47	0.63
RM8137	10	6.17	0.84	0.68	0.51
RM262	5	2.32	0.56	0.45	0.62
RM1367	11	5.36	0.81	0.66	0.52
RM240	5	1.56	0.34	0.18	0.90
RM406	8	2.18	0.53	0.29	0.84
RM3865	10	6.34	0.84	0.67	0.52
RM6378	11	6.46	0.84	0.67	0.52
RM324	6	1.87	0.47	0.25	0.85
RM8208	8	4.81	0.79	0.65	0.52
RM168	2	1.51	0.32	0.36	0.60
RM8203	7	4.07	0.75	0.60	0.55
RM7389	4	2.34	0.56	0.49	0.58
RM8213	12	4.31	0.76	0.62	0.54
RM3317A	4	2.08	0.52	0.44	0.61
RM5586	10	3.11	0.67	0.62	0.51
RM3524	10	5.85	0.83	0.62	0.56
RM3367	7	3.45	0.71	0.63	0.51
RM3836	8	3.17	0.69	0.57	0.56
RM3663	4	1.80	0.43	0.51	0.52
RM3790	7	2.66	0.62	0.57	0.53
RM6313	3	1.33	0.23	0.12	0.94
RM405	7	2.62	0.61	0.55	0.54
RM510	6	4.10	0.76	0.61	0.54
RM276	13	8.56	0.88	0.61	0.59
RM162	9	3.19	0.69	0.40	0.75
RM3138	6	1.96	0.50	0.54	0.51
RM508	7	4.56	0.78	0.65	0.51
RM1134	6	2.00	0.49	0.26	0.85
RM11	7	3.96	0.74	0.45	0.72
RM234	9	4.75	0.79	0.61	0.55
RM408	5	3.27	0.69	0.54	0.58
RM152	5	1.99	0.49	0.52	0.53
RM3395	7	4.13	0.75	0.59	0.56
RM7356	9	5.53	0.82	0.61	0.57
RM6948	4	2.39	0.58	0.49	0.58
RM7048	6	2.73	0.63	0.57	0.53
RM3164	4	2.40	0.58	0.50	0.58
RM8201	7	3.98	0.74	0.62	0.53
RM258	11	7.13	0.86	0.64	0.56
RM171	5	3.62	0.72	0.58	0.56
RM271	6	2.20	0.55	0.30	0.82
RM5704	8	2.76	0.64	0.59	0.52
RM3133	8	2.51	0.59	0.57	0.52
RM21	12	7.31	0.87	0.65	0.54
RM7376	5	1.76	0.42	0.22	0.87
Average	7.02	3.56	0.65	0.51	0.61

Table S2. 5 Charatctrization of Ethiopian rice accessions for seed morphology, phenol reaction, alkali digestibility and apparent amylose content

Accession	Phenol reaction[a]	Alkali digestibility[b]		Apparent amylose content[c]		Seed size		
		Scale	classification	%	Classification	Length (mm)	Width (mm)	Length/Width
SGU01	-	2	Intermediate	24	Intermediate	7.72	2.84	2.70
GAM02	-	2	Intermediate	27	high	8.04	2.77	2.93
AMF13	-	2	Intermediate	24	Intermediate	8.93	3.03	2.95
GAM04	-	2	Intermediate	26	high	8.30	2.82	2.92
BGP-01	-	2	Intermediate	24	Intermediate	8.08	2.74	2.94
BGP-03	-	2	Intermediate	22	Intermediate	8.40	2.88	2.92
BGP-04	-	2	Intermediate	22	Intermediate	7.97	2.51	3.19
BGP-05	-	2	Intermediate	20	low	7.82	3.02	2.59
BGP-06	-	2	Intermediate	26	high	8.13	2.93	2.76
BGP-07	-	2	Intermediate	22	Intermediate	7.57	2.69	2.82
BGP-09	-	2	Intermediate	26	high	8.53	2.78	3.06
BGP-10	-	2	Intermediate	23	Intermediate	8.21	2.83	2.91
BGP-11	-	2	Intermediate	25	Intermediate	8.23	3.02	2.73
BGP-12	-	2	Intermediate	21	Intermediate	7.61	2.79	2.72
BGP-13	-	2	Intermediate	25	Intermediate	8.23	2.92	2.83
BGP-14	-	2	Intermediate	25	Intermediate	7.76	2.64	2.93
BGP-15	-	2	Intermediate	24	Intermediate	7.56	2.71	2.77
AMF14	-	2	Intermediate	23	Intermediate	6.76	3.04	2.22
Fogera 1	-	2	Intermediate	22	Intermediate	8.80	2.69	3.27
Adet	-	2	Intermediate	24	Intermediate	8.15	2.68	3.04
NERICA-12	-	2	Intermediate	24	Intermediate	8.10	2.86	2.82
NERICA-13	-	2	Intermediate	24	Intermediate	8.08	2.79	2.89
Chewaqa	-	2	Intermediate	18	low	6.96	3.11	2.24
Hiddasse	-	2	Intermediate	23	Intermediate	8.60	2.82	3.06
NERICA-3	-	2	Intermediate	23	Intermediate	8.44	2.64	3.20
NERICA-4	-	2	Intermediate	24	Intermediate	8.25	2.75	3.06
SUPERICA-1	-	2	Intermediate	21	Intermediate	8.24	2.94	2.78
Kokit	-	2	Intermediate	20	low	8.08	3.14	2.58
Pawe-1	-	2	Intermediate	20	low	8.31	3.18	2.62
Hiber	-	2	Intermediate	25	Intermediate	8.12	3.12	2.66
Ediget	-	2	Intermediate	18	low	6.87	3.07	2.26
NERICA-15	-	2	Intermediate	19	low	8.44	2.72	3.17
NERICA-6	-	2	Intermediate	26	high	7.87	2.92	2.68
NERICA-14	-	2	Intermediate	24	Intermediate	8.30	2.81	2.95
Kallafo-1	-	2	Intermediate	24	Intermediate	7.58	2.83	2.65
NERICA-1	-	2	Intermediate	26	high	7.95	2.88	2.77
NERICA-2	-	2	Intermediate	25	Intermediate	7.02	2.68	2.63
NERICA-10	-	2	Intermediate	24	Intermediate	7.64	2.73	2.81
Abay	-	2	Intermediate	24	Intermediate	8.31	2.63	3.16
Candidate 3	-	1	low	21	Intermediate	6.81	2.93	2.35
Erib	-	2	Intermediate	27	high	8.30	2.64	3.13
Candidate 4	-	2	Intermediate	28	high	7.99	3.27	2.44
Wanzaye	-	2	Intermediate	22	Intermediate	8.10	3.27	2.48
Shaga	-	2	Intermediate	24	Intermediate	6.53	2.93	2.24
Demoze	-	2	Intermediate	25	Intermediate	6.50	3.10	2.09
X-JIGNA	-	3	high	24	Intermediate	6.63	3.19	2.08
GAM01	-	3	high	24	Intermediate	6.41	2.79	2.30
SGU09	+	1	low	28	high	9.21	2.41	3.83
AMF06	+	1	low	26	high	8.42	2.50	3.40
AMF12	+	1	low	25	Intermediate	8.96	2.53	3.55
GAM03	+	1	low	26	high	8.70	2.31	3.78
BGA01	+	1	low	29	high	8.90	2.42	3.69
BGP-02	-	1	low	28	high	8.70	2.40	3.67
Getachew	-	1	low	29	high	8.82	2.40	3.70
Andassa	-	1	low	28	high	8.84	2.41	3.68
Tana	-	1	low	23	Intermediate	9.02	2.44	3.72
Fogera2	+	1	low	26	high	8.90	2.33	3.84
Gumara	-	1	low	24	Intermediate	8.95	2.36	3.81
Candidate 1	+	1	low	24	Intermediate	9.18	2.43	3.78
Candidate 2	+	1	low	23	Intermediate	8.80	2.46	3.60

[a] (-) related to Japinica and (+) related to Indica (Oka 1958)

[b] scaling and classification according to Prathepha *et al*. (2005)

[c] 0.5% (waxy), 6-12% (very low), 13-20% (low), 21-25% (intermediate), and 26-33% (high) according to Juliano (1991)

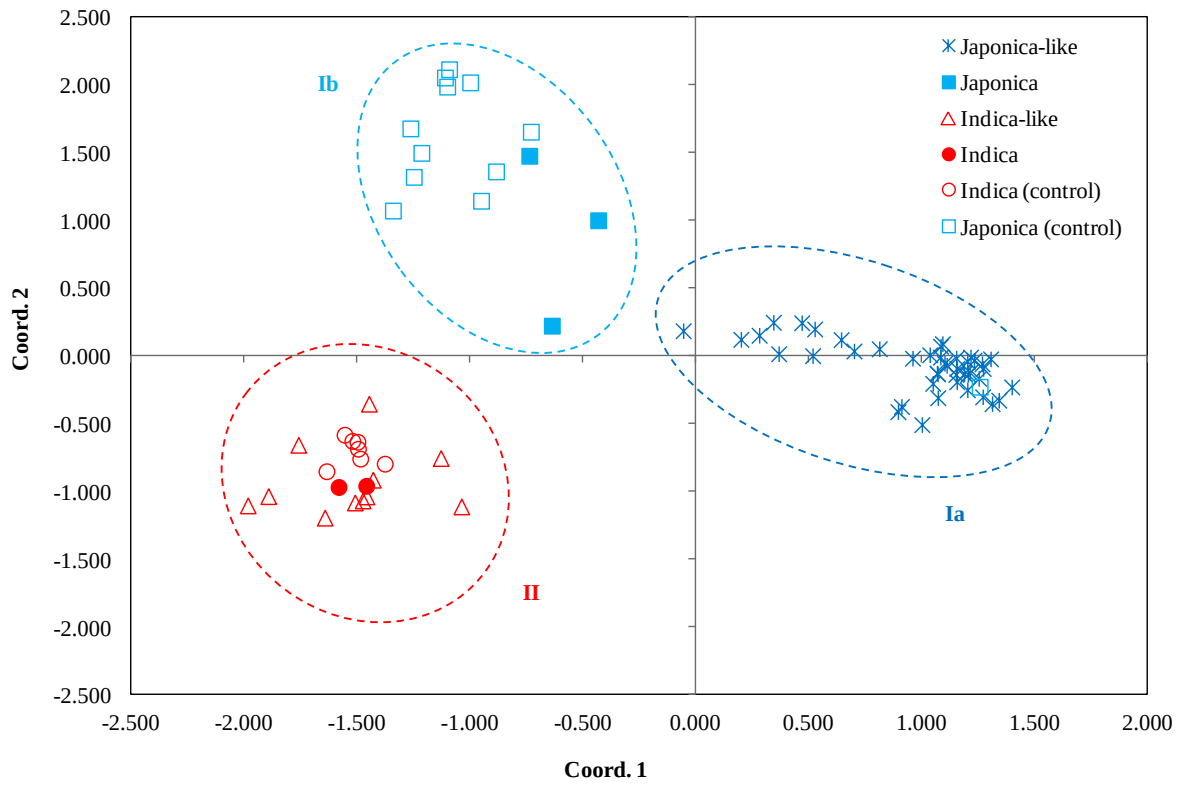

Fig. S2. 1 Principal coordinate analysis of 79 rice accessions based on 50 SSR markers. Ia, Ib and II refers to DNA clusters.

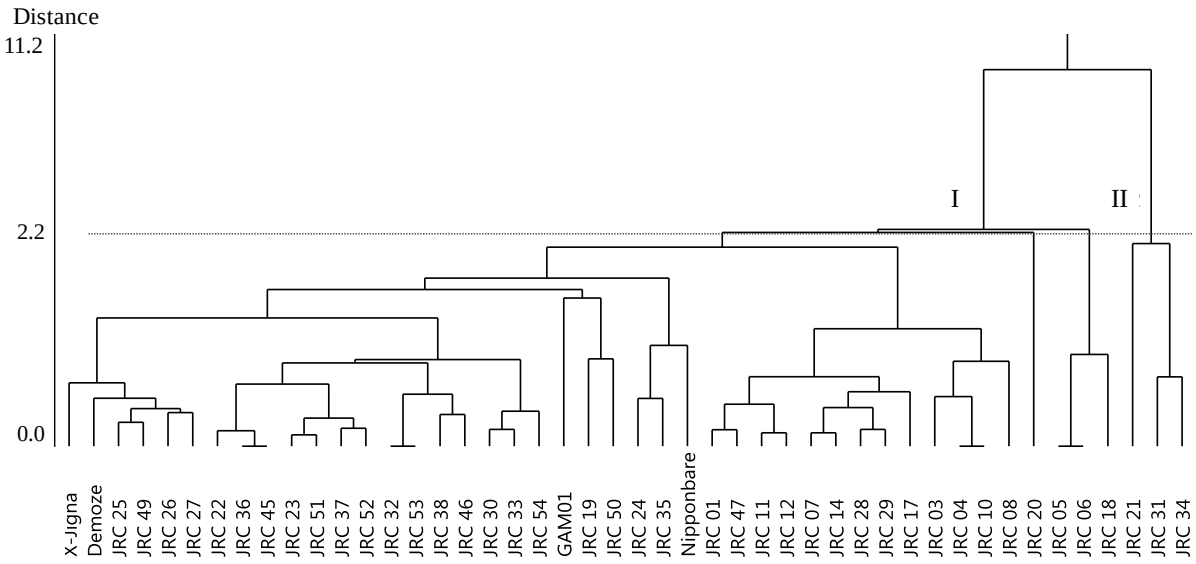

Fig. S2. 2 Relationship of Ethiopian Japonica-types rice with the JRC based on 10SSR polymorphism.

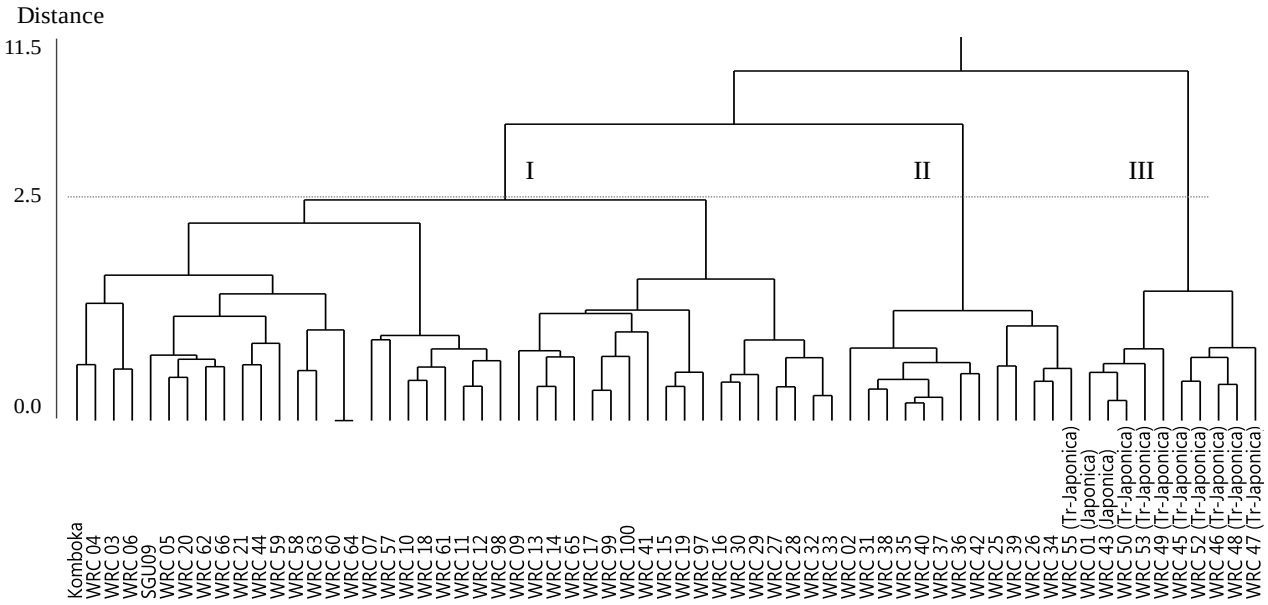

Fig. S2. 3 Relationship of Ethiopian Indica-types rice with the WRC based on 10SSR polymorphism.

Chapter 3

Phenotypic variation for blast resistance among Ethiopian rice cultivars using differential system

Abstract

Rice blast, caused by *Magnaporthe oryzae*, is regarded as one of the most seriously emerging rice diseases in Ethiopia. Varietal resistance is the most economical and safe option to manage this disease particularly for small scale farmers. In this study, 92 rice accessions comprising landraces, improved varieties, differential varieties and control cultivars were evaluated against 20 blast isolates of known pathogenecity to classify rice accessions into resistant and susceptible groups. Accessions were classified into three clusters; Cluster A, B1, and B2. Majority of the accessions that showed relatively high resistance belonged to Cluster A while Clusters B1 and B2 included intermediate to susceptible accessions. Except for two landraces, X-Jigna and BGA01, Ethiopian accessions showed high resistance and belonged to Cluster A. Most of them revealed resistance frequency ranging from 60 to 85%. Seven improved accessions; Shaga, Ediget, Gumara, NERICA-12, Adet, NERICA-1, and NERICA-2 showed resistance frequency of 80-85% and five others; NERICA-14, NERICA-4, NERICA-15, Pawe-1, and Erib had resistance frequency of 75%. As postulated using blast reaction phenotype, more than 80% of Ethiopian accessions harbored a combination of two or more genes for blast resistance. This specific study provided important information about variation in blast resistance and classified Ethiopian rice accessions into resistant and susceptible groups.

Key words: blast resistance, rice, blast isolates, Ethiopia

Introduction

Given rice crop does not have a long cultivation history in Ethiopia, it has been cultivated as healthy crop since its introduction except for some challenges such as weeds, termites, and cold stress. Recently, however, its cultivation faces several biotic and abiotic constraints causing a significant damage to the crop. Rice blast caused by the fungal pathogen *Magnaporthe oryzae* B. Couch (formerly *Magnaporthe grisea*) has become one of the most serious diseases of rice in Ethiopia (Mebratu *et al.* 2015; Wasihun and Flagot 2016; Tekalign *et al.* 2019). Globally, blast is the most devastating rice disease with a wide range of hosts and in Africa the disease infestation can result in yield losses of up to 100% (Sere *et al.* 2011).

In Ethiopia, though not yet quantified, a substantial amount of yield loss occurs every season due to this disease, sometimes leading to a complete filed failure in some localities. Survey reports by Mebratu *et al.* (2015) in South SNNPR and Wasihun and Flagot (2016) in Pawe district of Benishangul Gumize region indicated that blast is a major threat to rice cultivation. They reported that rice blast was observed in all assessed fields, and high incidence and severity of leaf blast and panicle blast was observed in some fields. Similarly, Tekalign *et al.* (2019) reported that blast is one of the most important diseases in Fogera, Dera, and Libokemkem districts of Amhara region. They found that X-Jigna, the most popular landrace, was most affected by blast and other diseases, followed by Gumara. The disease also affects rice fields of Saudi Star, a private rice production company located in Gambella region (company expert communication). Thus, minimizing the occurrence of the diseases epidemics and reducing yield losses are central to sustain rice productivity and production.

Host resistance and use of good cultural practices such as crop rotation, controlling the timing and amount of nitrogen applied, and managing water in the field are the most promising approach for blast diseases management (Emmanuel 2016). Use of fungicides is also another effective option in managing rice blast diseases; however, this approach is not accessible and affordable to subsistence rice farmers in Africa including Ethiopia. Thus, growing blast resistant cultivars remains as the most economical and effective option to manage rice blast diseases. The interactions between blast races and rice cultivars have been explained by the gene-for-gene theory (Flor 1956; Silue *et al.* 1992): variation of blast races is assumed to correspond with resistance gene (s) in rice cultivars. In our molecular based genetic diversity analysis of Ethiopian rice cultivars, we found that accessions possessed high genetic diversity. We also tried to classify accessions corresponding to Japonica/Japonica-like types or Indica/Indica-like types using INDEL markers. Both landraces and improved accessions have adapted to different production ecosystems in the country. Many of these accessions except some NERICAs showed symptoms of blast in Ethiopia. However, the variation in blast resistance among rice accessions has not been investigated yet in Ethiopia either in the field or under controlled environmental conditions.

Kawasaki-Tanaka and Fukuta (2014) studied 324 Japanese rice accessions using 16 blast isolates, and classified them into three resistance groups. They also postulated nine resistance genes among accessions including *Pik-s*, *Pish*, *Pia*, *Pii*, *Piz*, *Piz* (t), *Pik*, and *Pita* based on reaction patterns with different varieties. Odjo *et al.* (2017) also assessed genetic diversity and blast resistance using 61 SSR markers and 32 blast isolates among 195 rice accessions from West Africa, and found resistant and susceptible groups. Khan *et al*. (2017) classified 334 Bangladish rice accessions into four resistance groups using 20 standard blast isolates of Japan, Bangladish and Keneya origin. They postulated resistance genes and found that many accession harbored *Pik* allele, while *Piz* and *Pita* loci were in lowest frequency.

In this study, we evaluated Ethiopian rice accessions for blast resistance based on the differential system using 20 blast isolates originated from different countries under greenhouse condition. We compared the accessions with standard differential varieties which contained 23 resistance genes, and with susceptible control accessions, LTH and US-2. Therefore, the objective of this study was to evaluate phenotypic variation in blast resistance among Ethiopian rice cultivars. This study is the first report in rice accessions from Ethiopia in blast inoculation experiment and hence, the results could provide valuable information to complement rice breeding in Ethiopia.

Materials and Methods

Plant materials

In blast inoculation experiment, a total of 92 accessions were used (Table S3. 1). Plant materials comprised 60 accessions from Ethiopia, 28 differential varieties (DVs), and four control accessions. Ethiopian accessions included 27 landraces and 33 improved varieties. Landraces were collected from four regions, Amhara, Gambella, Benshangul Gumize, and South NNPR. Improved cultivars included upland NERICAs, other upland rice, lowland rain fed rice, and intermittently irrigated rice. IR64 as Indica type and WAB56-104 as Japonica type were considered as resistant control accessions. Two universal susceptible control accessions, a Japonica type, Lijiangxintuanheigu (LTH) and an Indica type, US-2 were used for comparison (Table S3. 1). Differential varieties and susceptible controls were used as characterized by Kawasaki-Tanaka and Fukuta (2014) and they were kindly provided by Dr Fukuta at JIRCAS TARF, Ishigaki Island, Okinawa, Japan. In order to get uniform seed

harvest, Ethiopian accessions were at first multiplied under greenhouse condition at Ishigaki in 2017 with kind cooperation of Dr Fukuta.

Blast isolates and inoculums cultivation

A total of 20 standard differential blast isolates originating from Japan (n=9), the Philippines (n=6), China (n=1), Laos (n=1), Benin (n=1), Nigeria (n=1) and Kenya (n=1) were used to inoculate 92 accessions (Table S3. 1). These blast isolates were maintained by Dr Fukuta at JIRCAS, Ishigaki, Japan. Blast isolates were selected based on their reported differential diseases patterns on differential varieties carrying blast resistance genes. We expected that using these isolates for screening would facilitate the identification of potentially promising and broad-spectrum rice blast resistance sources. Stock blast isolates were re-cultured from storage on an oatmeal agar plate with streptomycin, and were grown for about 12-14 days at about 25°C. To induce sporulation, culture plates were scraped with a toothbrush and then put on a tray covered with wrapping film pitted with several holes to reduce humidity in the tray and left under a fluorescent light for 4-5 days. Conidia were dislodged from the surface of sporulated plates with a paintbrush into 10 to 20 mL of distilled water. Spore suspensions were filtered through 4 layers of gauze (cheese close) and spore concentration was adjusted to 10 x 10^4spores/mL using a hemacytometer (a counting-chamber device). Tween 20 was added to 0.01% just before inoculation (Hayashi *et al*. 2009).

Inoculation, diseases scoring and resistance gene postulation

After treating with fungicides, seeds of each accession were pre-germinated. Then, three pre-germinated seeds of each accession were sown in plastic cell trays (10 x 12 cells; cells 16mm

diameter, 25 mm deep) filled with peat soil. Ample amount of fertilizer was added at 2nd leaf stage as ammonium sulfate solution (Roumen 1992) per tray. The same amount of fertilizer was added before inoculation. For each blast isolate, the experiment was carried out with two replications in a greenhouse at 25-28 ^{o}C. Border lines of each seedling tray were sown with US-2 (a universal susceptible control) to avoid any border effect. The spore concentrations of each blast isolate was standardized to 10 x 10^{4}spores/mL in 0.01% Tween 20 and 80 mL of the fresh suspension was sprayed until run-off onto each tray with fine sprayer 21 days after sowing. After inoculation, the seedlings were kept in an incubator /dew chamber at 25 ^{o}C having a relative humidity more than 90% for 24 hours. Then, seedlings were transferred to the greenhouse with humidity of about 60% and temperature of 28 ^{o}C to 30 ^{o}C. The disease reaction score for each accession was assessed seven days after inoculation. The reaction scoring was based on the 0-5 scale as described by Hayashi and Fukuta (2009). Scores 0-2 and 3-5 corresponded to resistant (R) and susceptible (S) reactions, respectively.

In order to postulate resistant genes in Ethiopian accessions, reaction pattern of each accession to blast isolates were compared to reaction patterns of differential varieties and susceptible controls to corresponding blast isolates as reported by Ebron *et al*. (2004), Kawasaki-Tanaka and Fukuta (2014), and Khan *et al*. (2017). Among 28 DVs used, only 25 harbored 23 pre-determined resistance genes (Table S3.1) as reported by Kobayashi *et al*. (2007), Telebanco-Yanoria *et al*. (2010), and Tusnematsu *et al*. (2000) while three DVs are candidate monogenic lines for which resistance genes they are supposed to have are not yet confirmed (Personal communication with Dr Fukuta, JIRCAS, Ishigaki, Japan). The assumption in gene postulation was that the pattern of reaction of each rice accession was the result of at least a single resistance gene in one chromosome region. That is *Pit* or *Pish* on chromosome 1; *Pib* on chromosome 2; *Piz*, *Piz-t*, *Piz-5*, or *Pi9* on chromosome 6; *Pii*, *Pi3*, or

Pi5(t) on chromosome 9; *Pik-s, Pik-m, Pik, Pik-h, Pi1, Pik-p, Pi7*(t), or *Pia* on chromosome 11; or *Pita, Pita*-2, *Pi12*(t), *Pi19*(t), or *Pi20*(t) on chromosome 12.

Data analysis

For cluster analysis, mean score two replications of each accession was summarized and used for analysis to classify accessions using Ward's hierarchical method (Ward 1963) using the software JMP 14.0 (JMP version 14.0 for Windows, 2018; SAS Institute, Inc., Cary, NC, USA). The resistance frequency (RF) of accessions in relation to blast isolates was calculated as RF= (No. of incompatible isolates / total no. of isolates used) x100% (Wu *et al*. 2015). Statistical analysis of RF was performed using SAS ver. 9 (SAS Institute, Inc. 2002, USA). Based on resistance frequency, accessions were classified as high resistance frequency (RF>85%), intermediate (RF=50-85%) and low (RF<50%) (Wu *et al*. 2017).

Results

Blast resistance phenotypes and classification by resistance frequency

Blast races in Ethiopia have not been established yet so as to use in research for screening rice accessions by artificial inoculation. Thus, we applied known blast races from Japan, China, Laos and Africa (Keneya, Nigeria and Benin) and differential varieties carrying known resistance genes to evaluate Ethiopian rice cultivars. About 65% of accessions (60 out of 92) showed resistance reaction (scores of 0-2) to blast isolates while 32 accessions were moderate to susceptible (reaction score greater than 2) (Fig. 3. 1). Most accessions of Ethiopia exhibited resistance to blast isolates from Japan, but blast isolates from the

Philippines, Laos, Benin, China, and Kenya were relatively virulent to discriminate Ethiopian accessions (Table S3. 1).

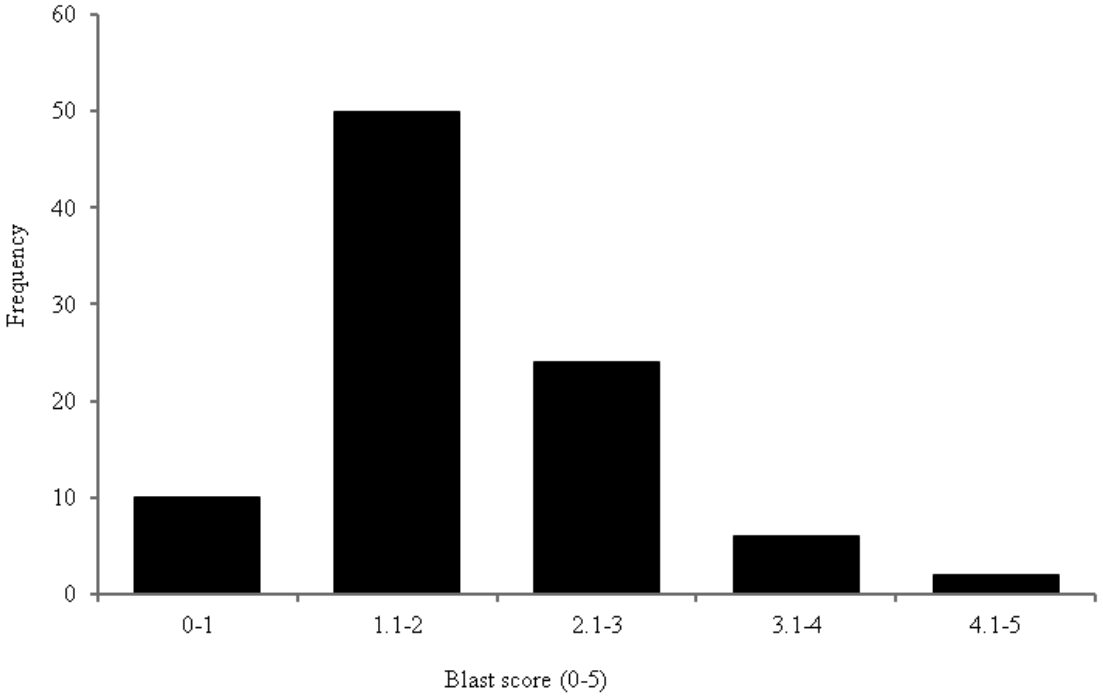

Fig. 3. 1 Distribution of 92 rice accessions based on mean blast reaction score as inoculated by 20 blast isolates

Wu *et al*. (2017) classified blast resistance frequency (RF) of rice accessions as low (RF<50%), intermediate (RF=50-85%) and high (RF>85%). Among 92 accessions used in our study, classification of accessions by RF revealed that about 22%, 65% and 12% of the accessions showed low (0-49%), intermediate (50-85%), and high RF (>85%), respectively (Table 3. 1). Out of 60 rice accessions from Ethiopia, about 78% showed intermediate RF while 17% and 5% of the accession showed high and low RF, respectively (data not shown). Improved accessions such as NERICA-12, Shaga, Adet, NERICA-1, NERICA-2, and NERICA-14 exhibited high RF. Only three landraces comprising BGA01, X-Jigna, and GAM03 showed low RF (Table 3. 1). The two susceptible control accessions (US-2 and LTH) also showed low RF while two resistant control accessions such as WAB56-104 and

IR64 showed intermediate and high RF, respectively. In addition, 16 differential varieties (out of 28) showed low RF and all the remaining differential varieties showed intermediate RF (Table 3. 1).

Table 3. 1 Classification of 92 rice accessions by resistance frequncey (RF) estimated from reaction patterns of 20 blast isolates

Group	Cultivars by RF (%)		
	Low (n=21) (0-49%)	Intermediate (n=60) (50-85%)	High (n=11) (>85%)
Improved		NERICA-6, Candidate 1, Chewaqa, Tana, SUPERICA-1,Kokit, Hiber, Kallafo-1, Abay, Getachew, Andassa, Candidate 3, Wanzaye, Fogera1, NERICA-13, Hiddasse, NERICA-10, NERICA-3, NERICA-4, Pawe-1, Fogera 2, Ediget, Gumara, NERICA-15, Candidate 2, Erib, Candidate 4	NERICA-14, NERICA-1, Shaga, Adet, NERICA-12, NERICA-2
Landrace	BGA01, X-Jigna, GAM03	BGP-07, Demoze, GAM01, GAM02, AMF12, BGP-01, BGP-09, BGP-13, AMF14, SGU09, AMF13, BGP-03, BGP-04, BGP-06, BGP-11, BGP-15, SGU01, BGP-12, AMF06, BGP-05	GAM04, BGP-02, BGP-10, BGP-14
Differential varieties	IRBLks-F5, IRBLsh-B, IRBLt-K59, IRBLi-F5, IRBL12-M[US], IRBLkp-K60, IRBL19-A, IRBLta-K1[LT], IRBLa-A, IRBL5-M[LT], IRBLta-CP1, IRBLta-K1[US], IRBLb-B, IRBLkm-Ts, IRBL7-M, IRBLz-Fu	IRBL3-CP4, IRBLk-Ka[LT], IRBL5-M[US], IRBLzt-T, IRBL12-M, IRBLta2-Pi[LT], IRBLta2-Re, IRBLkh-K3[LT], IRBLz5-CA-1,IRBL20-IR24, IRBL1-CL[LT], IRBL9-W	
Controls	US-2, LTH	WAB56-104	IR 64

Clustering of accessions and gene postulation

Cluster analysis based on the pattern of reaction classified accessions into two major clusters, A and B, and Cluster B was further divided into two sub-clusters, B1 and B2 (Fig.3. 2). Accessions in Cluster A (n=66, 71.7%) represented a resistant group with a RF of 76% (Table 3. 2). Most popular improved accessions such as NERICA-4, Ediget, Gumara, Pawe1, Kokit, NERICA-12 and NERICA-13 belonged to this cluster, along with the majority of landrace accessions (Table S3. 1). The two resistant controls, the Japonica accession (WAB56-104), and the Indica accession (IR64), were also grouped in Cluster A. Accessions in this cluster showed stronger resistance to blast isolates originating from Japan than to those from the Philippines, Laos, China, and Africa (Table S3. 1). The mean blast reaction for accessions in Cluster A ranged from 0.9 to 2.3 with an overall average score of 1.5 (data not

shown). Cluster A also included seven DVs possessing specific resistance genes, namely IRBL3-CP4 for *Pi3*, IRBL9-W for *Pi9* (t), IRBLz-Fu for *Piz*, IRBLz5-CA-1for *Piz-5*, IRBLta2-Pi [LT] and IRBLta2-Re for *Pita-2*, and IRBL20-IR24 for *Pi20* (t). Accessions clustered together with these DVs may carry the same genes as *Pi3*, *Pi9* (t), Piz, *Piz-5*, *Pita-2* and *Pi20* (t) along with *Pish*, *Pib*, *Pit*, and *Pia* in their genetic background. Resistance genes postulated based on Ebron *et al*. (2004) suggested that most of these resistance genes were carried by different number of Ethiopian rice accessions as *Pib* (n=24), *Pit* (n=19), *Pia* (n=8), *Pi3* (n=8), *Pita2* (n=5), *Piz* (n=5), *Pi20* (t) (n=4), and *Pi9* (t) (n=4) (Table S3. 1). Some accessions were resistance to a range of blast isolates while others were resistant to only few blast races. This may be due the fact that there were cultivars with more than one resistance gene with cumulative effect corresponding to different blast races.

Cluster B1 included one improved accession and 11 DVs, many of which showed relatively intermediate to high susceptibility. The reaction score for accessions in this cluster ranged from 1.9 to 3.0 with an overall mean of 2.5. Each of these 11 DVs possess specific resistance genes such as IRBLb-B for *Pib*, IRBLa-A for *Pia*, IRBL5-M[LT] for *Pi5*(t), IRBLkm-Ts for *Pik-m*, IRBL1-CL[LT] for *Pi1*, IRBLkh-K3[LT] for *Pik-h*, IRBLk-Ka[LT] for *Pik*, IRBLkp-K60 for *Pik-p*, IRBL7-M for *Pi7*(t), IRBLzt-T for *Piz-t*, and IRBL12-M for *Pi12*(t). As postulated, the improved accession (Candidate 1) belonging to this cluster was also found to have three resistance genes; *Pi7* (t), *Pik-m*, and *Pik-p*) in its genetic background (Tables S3. 1, S3. 3).

Cluster B2, on the other hand, comprised 14 accessions (15.2% of the total) including two landraces (X-Jigna and BGA01), two susceptible control accessions (LTH and US-2) and ten DVs, with mean reaction scores ranging from 2.5 to 4.4 and an overall average reaction score of 3.0 (data not shown). Most accessions in this cluster were highly susceptible to blast isolates from the Philippines, Benin, Nigeria, China, Laos, and Kenya, but less susceptible to

most of those from Japan. Out of ten DVs in this cluster, only seven DVs possess pre-determined specific resistance genes including IRBLsh-B for *Pish*, IRBLt-K59 for *Pit*, IRBLi-F5 for *Pii*, IRBLks-F5 for *Pik-s*, IRBLta-K1[LT] and IRBLta-CP1 for *Pita*, and IRBL19-A for *Pi19*(t). The two landraces, X-Jigna and BGA01, probably contain one or more of these resistance genes in their genetic background. Based on gene postulation, X-Jigna was found to have the resistance gene, *Pit* and some other unknown resistance genes, whereas the genetic background of BGA01 was found to include an unknown type of resistance gene (Tables S3. 1, S3. 3).

In this study, resistance gene postulation in most improved accessions and landraces showed the presence of *Pik-p*, *Pit*, *Pik-m*, *Pib*, *Pi7*(t), *Pik-s*, *Pii*, and *Pi19*(t) relatively in high frequency (Tables S3. 1, S3. 2, Fig. 3. 3). However, gene postulation was merely depending on blast phenotype comparison and therefore further investigation is needed using molecular techniques with the same set of differential varieties and susceptible control accessions to confirm the presence or absence of postulated resistance genes among Ethiopian rice accessions.

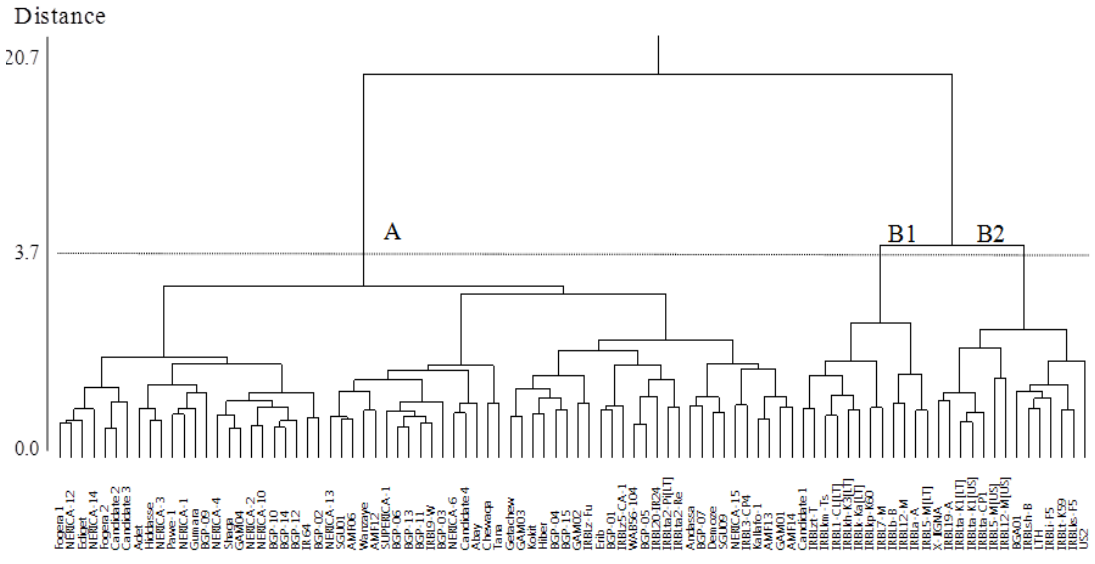

Fig. 3. 2 Ward's method clustering of 92 rice accessions based on reaction patterns to 20 blast isolates

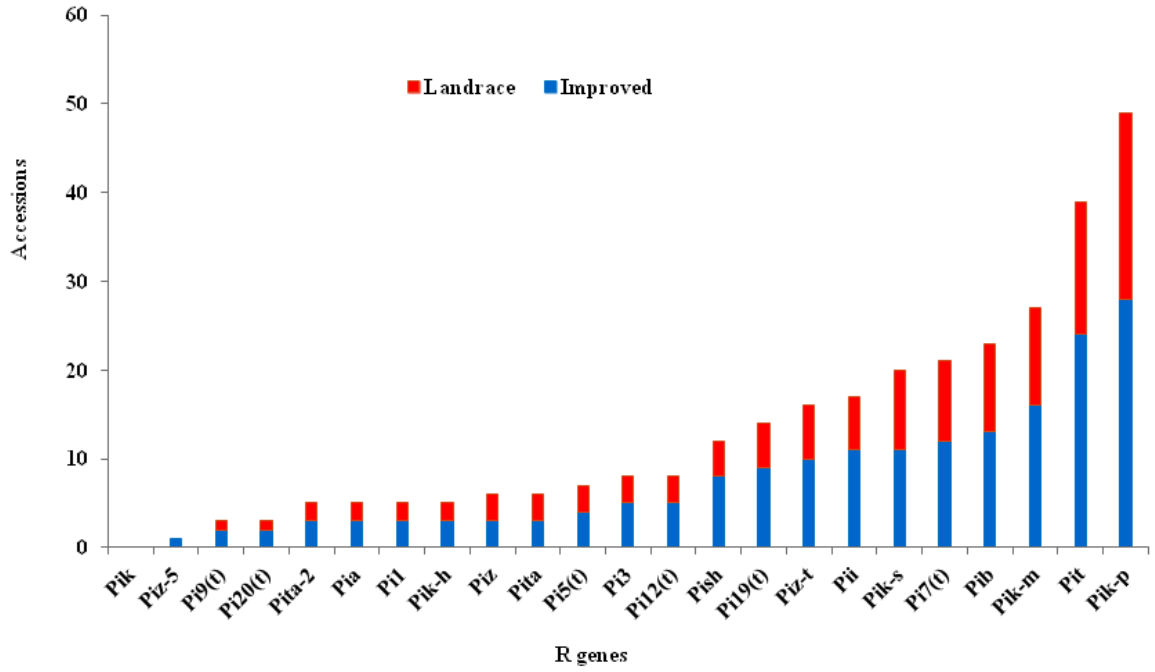

Fig. 3. 3 Distribution of genes for blast resistance as postulated among Ethiopian rice accessions by differential system

Relationships of blast phenotype clusters to regional compositions

Relationships between blast phenotype clusters and regional compositions of rice accessions were compared (Table 3. 2). Except for Candidate 1, which clustered to B1, most improved accessions (97% of 33) were grouped in Cluster A. Similarly, the majority of landraces from four regions-Amhara (83.3%), Gambella (100%), Benishangul Gumize (93.3%) and South NNPR (100%) belonged to Cluster A, but no landrace was associated with Cluster B1 (Fig. 3. 2). One landrace from Amhara (X-Jigna) and another from Benishangul Gumize (BGA01) belonged to the most susceptible group, Cluster B2. The RF of accessions in Clusters, A, B 1, and B2 were 76, 51.7, and 27%, respectively. On the other hand, landraces from Amhara, Gambella, Benshangul Gumize and South NNPR showed RF of 67.5, 66.3, 74.0 and 77.5%, respectively. Improved accessions also showed RF of 77.6%, whereas the control accessions showed RF of 50% (Table 3. 2). These results demonstrated that except for a couple of

landraces, improved accessions and most landraces tended to be resistant to blast isolates considered.

Table 3. 2 Regional composition of blast reaction clusters (A, B1, and B2) categorized by the phenotype of the blast reaction pattern

Varietal type	Region	No. of rice accessions by blast reaction cluster										RF (%) [a]
		A		B						Total		
		A	%	B1	%	B2	%	Sum	%		%	
Landrace	Amhara	5	83.3	0	0.0	1	16.7	1	16.7	6	100.0	67.5
	Gambella	4	100.0	0	0.0	0	0.0	0	0.0	4	100.0	66.3
	Benishangulgumize	14	93.3	0	0.0	1	6.7	1	6.7	15	100.0	74.0
	South NNPR	2	100.0	0	0.0	0	0.0	0	0.0	2	100.0	77.5
	Sum	25	92.6	0	0.0	2	7.4	2	7.4	27	100.0	71.3
Improved varieties	-	32	97.0	1	3.0	0	0.0	1	3.0	33	100.0	77.6
Control	-	9	32.1	11	39.3	8	28.6	19	67.9	28	100.0	50.0
Total		66	75.0	12	13.6	10	11.4	22	25.0	88	100.0	67.0
RF (%)		76.0		51.7		27.0		41		67.0		-

[a] Resistance frequency (RF)=(No. of incompatible isolates / total no. of isolates used) x100% (Wu *et al*., 2015).

Relationships between blast phenotype and DNA clusters

Blast phenotypes clusters A, B1, and B2, and DNA Clusters Ia, Ib, and II were compared (Table 3. 3). In this comparison, accessions that have been examined both in the DNA polymorphism and blast inoculation test were considered. Thus, most accessions in Clusters I and II belonged to Cluster A. Except for one accession that from Cluster Ib belonged to Cluster B2, all other accessions belonging to Clusters Ia and Ib were grouped into Cluster A (Table 3. 3). Out of 14 accessions in Cluster II, 12 accessions belonged to Cluster A and only two accessions belonged to Cluster B1 or B2. This result demonstrated that the majority of accessions corresponding to the Japonica/Japonica-like types (except for X-Jigna), and that the majority of the Indica/Indica-like types (except for BGA01) tended to belong to Cluster A. Overall, 92.6% of landraces and 97% of improved rice accessions collected from Ethiopia, the majority of which belonged to Custer A, showed high resistance to the blast races

considered here. These results suggested that the accessions had a narrow variation of blast resistance because most of them skewed to the resistant group. This was in contrary to the previous report (Kawasaki-Tanaka and Fukuta 2014), in which 324 accessions with 16 blast isolates were evaluated and clustered into two clusters, I and II, in which accessions in Cluster I showed a wider variation of resistance from susceptible to highly resistant.

Table 3. 3 Relationship between DNA and blast reaction clusters

DNA cluster	Blast reaction cluster[a]							
	A	(%)	B1	(%)	B2	(%)	Total	(%)
Ia	45	(100.0)	0	(0.0)	0	(0.0)	45	(100.0)
Ib	2	(66.7)	0	(0.0)	1	(33.3)	3	(100.0)
Sum	47	(97.9)	0	(0.0)	1	(2.1)	48	(100.0)
II	12	(85.7)	1	(7.1)	1	(7.1)	14	(100.0)
Total	59	(95.2)	1	(1.6)	2	(3.2)	62	(100.0)

[a] Accessions used in both DNA polymorphism and blast inoculation experiment were considered in here i.e all Ethiopian accessions (33 improved and 27 landraces) and two controls: IR64 and WAB56-104

Discussion

Blast phenotype variation

In order to improve blast resistance of local cultivars and to improve domestic productivity to curb the increasing import, breeding high-yielding and disease resistant varieties is essential. We examined landraces and improved accessions for blast resistance using 20 blast isolates. Accessions were compared with two susceptible controls (LTH and US-2), two accessions as resistant control (WAB56-104 and IR64), and 28 differential varieties carrying 23 different blast resistance genes. This is the first report of its kind to characterize Ethiopian rice accessions for blast resistance using standard blast isolates. Two landraces, X-Jigna and

BGA01, showed high susceptibility to most blast isolates used. However, the majority of improved accessions and landraces showed intermediate to high resistance to 20 most blast isolates. This was in contrary to Vasudevan *et al*. (2014) who screened 4246 IRRI accessions including six monogenic lines and susceptible controls (IR72 and CO39) against five blast isolates through artificial inoculation and they found that only 289 accessions exhibited broad-spectrum resistance to all five blast isolates while majority of the accessions were susceptible to most blast isolates.

In this study, the blast resistance of Ethiopian accessions was higher to blast isolates from Japan than those from the Philippines, China, Laos, and Africa. Similar result was reported by Khan *et al*. (2017) that evaluated 334 rice accessions from Bangladesh in comparison with 25 differential varieties and one susceptible control (LTH) against 20 blast isolates originated from Bangladesh, Kenya, and Japan and they found that the resistance potential of Bangladesh rice accessions was comparably higher against blast isolates from Japan than against those from Bangladesh and Kenya. Kawasaki-Tanaka and Fukuta (2014) also investigated 324 Japanese rice accessions including 23 monogenic lines and three control accessions against 16 blast isolates from Japan and the Philippines. They found that blast isolates from tropical countries such as the Philippines were significantly important for discriminating Japanese accessions as resistant or susceptible group.

In the current study, all Japonica-like and most Indica-like type accessions of Ethiopia were highly resistant to most 20 blast isolates. These accessions may contain multiple resistance genes and/or QTLs that are effective against different blast races. Crossing these resistant rice accessions, which have preferred agronomic traits, with highly susceptible but popular Ethiopian landrace such as X-Jigna will facilitate the breeding of blast resistance in Ethiopia.

Gene postulation and relationship between blast phenotype clusters and DNA clusters

Resistance genes in Ethiopian accessions were postulated by comparing reaction patterns in differential varieties to blast isolates as described by Ebron *et al*. (2004), Kawasaki-Tanaka and Fukuta (2014) and Khan *et al*. (2017). In this study, no single differential variety was susceptible to all 20 blast isolates used, and therefore resistance genes in our accessions were estimated for all 23 R genes by comparing to each differential variety's reaction. Kawasaki-Tanaka and Fukuta (2014) and Khan *et al*. (2017) reported that the differential variety for *Pi*19 (t) was susceptible to all blast isolates they used and they failed to estimate for *Pi*19 (t) in accessions for this reason. In contrast, a differential variety for *Pi*19 (t) in this study showed resistance to more than five blast isolates. Expected resistance genes varied markedly among accessions. Out of 60 accessions from Ethiopia, 50 accessions carried more than one resistance gene and many of these accessions were grouped in Cluster A. Nearly all landrace and improved Ethiopian accessions carry blast resistance genes *Pit* and *Pik-p*. Other resistance gene combinations; *Pish*, *Pib*, *Pik-s*, *Pik-m*, *Pi7* (t), *Piz-t*, *Pi9* (t), *Pi12* (t), *Pi19* (t), and *Pi20* (t) were found in high frequency in Ethiopian accessions. These accessions are adapted to different production systems; upland, lowland rainfed, and irrigated conditions which could, therefore, be potential sources for improving cultivars for blast resistance in specific production system.

Unique relationship was observed between blast phenotype clusters (A, B1, and B2) and DNA clusters (Ia, Ib, and II). The accessions in Cluster I comprised a wide range of accessions corresponding to resistant to highly resistant (Cluster A) to moderately to highly susceptible accessions (Clusters B1 and B2). In these results, blast resistance phenotype variation and its relationship with DNA classification suggested that Ethiopian rice accessions are good reservoirs of useful genetic resources to be used in rice breeding for

improving blast resistance. However, the current study was based on phenotype of blast reaction only under greenhouse condition. Hence, further study is needed combining greenhouse and field conditions, and using molecular markers confirm the presence of resistant genes. In addition, evaluation of rice accessions for blast resistance using local blast races is pertinent which may enable breeders to identify some specific blast resistant genes which can contribute to mitigate the effect of blast in rice growing localities in Ethiopia.

Supplementary data

Table S3. 1 Summary of pattern of blast reaction among 92 rice accessions inoculated by 20 blast isolates

Accessions	Cultivation type	Type[a]	Origin	Reaction scores of accessions to 20 blast isolates[b]																				Blast Cluster	R genes predicted in the genetic gackground
				Japan									Philippines						China	Laos	Africa[c]				
				JPF 494	JPF500	JPF506	JPF507	JPF509	JPF510	JPF513	JPF514	JPF517	PHL2	PHL4	PHL8	PHL14	PHL15	PHL16	CHN125	LAO12	BEN43	NIG1	KNY135		
LTH	Lowland	Susceptible control	China	3.5	3.0	2.5	1.5	3.0	4.0	1.0	4.0	2.0	3.0	3.5	4.5	3.5	3.5	5.0	4.5	4.5	5.0	4.0	5.0	B2	none
US2	Lowland	Susceptible control	Japan	4.0	3.5	4.5	2.5	4.0	3.5	4.0	5.0	5.0	4.5	4.5	3.5	4.5	5.0	5.0	5.0	4.5	5.0	4.5	5.0	B2	none
IRBLsh-B	Lowland	Differential variety	International Rice Research Institute	3.0	2.5	3.5	2.5	3.0	3.5	0.5	4.0	1.0	2.5	2.0	3.0	3.0	3.0	4.0	4.5	4.0	2.5	4.5	2.0	B2	*Pish*
IRBLb-B	Lowland	Differential variety	International Rice Research Institute	3.5	3.0	2.5	1.0	1.5	2.0	2.0	0.5	1.0	0.0	4.5	3.5	4.0	5.0	4.5	5.0	1.0	5.0	4.0	1.0	B1	*Pib*
IRBLt-K59	Lowland	Differential variety	International Rice Research Institute	3.5	1.0	2.5	1.5	3.0	3.0	2.0	3.0	2.5	3.5	3.5	3.0	4.0	5.0	3.5	5.0	2.5	5.0	1.5	5.0	B2	*Pit*
IRBLa-A	Lowland	Differential variety	International Rice Research Institute	2.5	3.0	3.5	0.5	1.5	3.0	4.0	2.0	2.0	0.0	3.5	2.0	1.0	3.5	3.5	5.0	3.5	4.5	4.5	1.5	B1	*Pia*
IRBLi-F5	Lowland	Differential variety	International Rice Research Institute	2.5	2.5	3.0	1.0	1.5	3.0	3.0	3.0	2.5	2.5	2.0	4.5	2.5	2.0	4.0	5.0	3.0	4.5	4.5	5.0	B2	*Pii*
IRBL3-CP4	Lowland	Differential variety	International Rice Research Institute	1.0	1.5	2.5	0.5	1.0	1.0	2.0	1.5	0.0	2.0	1.0	1.5	3.5	2.5	3.5	4.5	2.0	4.0	3.0	5.0	A	*Pi3*
IRBL5-M[LT]	Lowland	Differential variety	International Rice Research Institute	2.5	3.0	4.0	0.5	2.0	1.5	3.0	2.0	0.5	0.5	2.5	2.0	4.5	3.0	5.0	5.0	3.0	5.0	4.5	1.5	B1	*Pi5* (t)
IRBLks-F5	Lowland	Differential variety	International Rice Research Institute	3.0	2.5	4.5	2.0	2.5	2.5	2.0	4.0	3.0	3.5	4.5	3.5	2.5	4.0	2.0	5.0	3.0	4.0	3.0	5.0	B2	*Pik-s*
IRBLkm-Ts	Lowland	Differential variety	International Rice Research Institute	2.0	1.5	2.5	3.0	2.0	1.0	2.0	2.5	0.5	0.0	3.0	3.5	4.5	3.5	4.0	1.5	2.0	4.5	5.0	5.0	B1	*Pik-m*
IRBL1-CL[LT]	Lowland	Differential variety	International Rice Research Institute	2.0	1.5	2.0	2.0	2.0	1.0	1.0	1.5	1.0	0.0	1.5	3.0	2.0	3.0	5.0	1.0	1.0	5.0	4.0	5.0	B1	*Pi1*
IRBLkh-K3[LT]	Lowland	Differential variety	International Rice Research Institute	2.0	0.0	0.0	1.0	1.5	1.0	0.5	1.0	0.0	1.0	2.0	4.5	4.0	3.5	4.5	1.0	1.0	5.0	5.0	5.0	B1	*Pik-h*
IRBLk-Ka[LT]	Lowland	Differential variety	International Rice Research Institute	1.0	1.0	1.5	2.5	1.0	2.0	0.0	2.0	1.0	1.0	1.0	5.0	4.5	4.5	5.0	2.0	1.5	4.5	5.0	5.0	B1	*Pik*
IRBLkp-K60	Lowland	Differential variety	International Rice Research Institute	1.5	2.5	1.0	2.5	1.0	1.5	3.5	3.0	0.0	0.0	2.5	3.0	5.0	4.5	5.0	4.5	4.0	5.0	5.0	5.0	B1	*Pik-p*
IRBL7-M	Lowland	Differential variety	International Rice Research Institute	1.0	1.5	2.0	1.0	1.5	0.0	2.5	4.5	1.0	1.0	2.0	4.0	4.0	3.5	5.0	5.0	4.0	5.0	5.0	5.0	B1	*Pi7* (t)
IRBL9-W	Lowland	Differential variety	International Rice Research Institute	1.0	0.5	1.0	0.5	1.0	1.0	1.0	0.0	0.0	1.5	2.0	3.5	4.0	2.5	1.5	1.0	2.0	1.5	0.5	1.0	A	*Pi9* (t)
IRBLz-Fu	Lowland	Differential variety	International Rice Research Institute	2.5	1.0	3.5	2.5	2.5	3.5	1.5	2.5	1.5	2.0	2.5	2.0	1.5	2.0	3.5	4.5	2.5	2.5	1.0	0.5	A	*Piz*
IRBLz5-CA-1	Lowland	Differential variety	International Rice Research Institute	1.0	2.5	2.0	1.5	2.5	2.0	0.5	2.0	0.0	1.0	2.0	2.5	3.0	2.0	2.0	4.5	1.5	2.0	2.0	2.5	A	*Piz-5*
IRBLzt-T	Lowland	Differential variety	International Rice Research Institute	1.0	1.0	1.0	0.5	2.0	0.5	0.5	1.0	0.0	0.0	3.5	2.5	3.0	3.5	2.5	0.5	2.5	4.5	5.0	1.0	B1	*Piz-t*
IRBLta2-Pi[LT]	Lowland	Differential variety	International Rice Research Institute	1.5	1.0	1.0	1.0	2.0	3.5	1.0	0.0	2.5	1.0	2.5	3.0	1.5	5.0	2.0	2.0	0.5	5.0	0.0	5.0	A	*Pita-2*
IRBLta2-Re	Lowland	Differential variety	International Rice Research Institute	1.0	1.5	1.0	0.0	3.0	3.0	0.0	0.0	0.0	1.0	3.5	2.5	2.0	3.0	1.0	1.0	0.5	3.5	0.0	5.0	A	*Pita-2*
IRBL12-M	Lowland	Differential variety	International Rice Research Institute	3.0	2.0	0.5	0.0	1.5	1.0	0.5	0.0	1.0	1.5	4.5	2.0	2.5	4.5	4.0	3.5	0.5	5.0	5.0	1.5	B1	*Pi12* (t)
IRBLta-K1[LT]	Lowland	Differential variety	International Rice Research Institute	3.5	1.5	2.5	1.0	3.0	4.0	3.0	1.0	3.5	1.5	2.0	2.5	2.5	5.0	2.0	5.0	5.0	5.0	0.0	5.0	B2	*Pita*
IRBLta-CP1	Lowland	Differential variety	International Rice Research Institute	3.0	1.5	1.0	1.0	3.5	3.5	3.5	1.0	3.0	2.0	2.5	1.5	4.5	5.0	1.5	5.0	4.5	4.5	1.5	5.0	B2	*Pita*
IRBL19-A	Lowland	Differential variety	International Rice Research Institute	3.5	1.5	2.5	1.5	3.5	3.0	1.0	1.5	3.0	3.0	1.0	2.5	2.0	4.5	3.0	4.5	4.0	4.5	4.5	5.0	B2	*Pi19* (t)
IRBL20-IR24	Lowland	Differential variety	International Rice Research Institute	1.0	1.0	1.0	0.0	2.5	3.5	1.0	1.0	2.0	0.5	1.0	1.0	0.5	4.5	2.5	2.5	0.5	1.5	2.5	0.5	A	*Pi20* (t)
IRBL5-M[US]	Lowland	Differential variety		1.0	1.5	1.5	0.0	2.5	3.0	3.0	5.0	1.5	2.0	0.0	2.5	0.0	1.0	4.0	5.0	0.5	4.5	3.5	5.0	B2	none
IRBL12-M[US]	Lowland	Differential variety		4.0	2.5	2.0	0.0	3.0	4.0	3.5	5.0	5.0	2.5	2.5	0.0	1.5	3.5	3.0	5.0	2.0	5.0	5.0	5.0	B2	none
IRBLta-K1[US]	Lowland	Differential variety		3.5	1.0	3.0	1.0	2.5	3.0	2.0	1.5	3.5	0.5	3.5	2.0	2.5	4.0	2.0	5.0	4.5	4.5	2.0	5.0	B2	none
IR64	Lowland	Resistant control	International Rice Research Institute	0.0	1.5	0.5	1.0	1.0	2.0	0.5	2.0	0.0	1.0	1.0	2.0	1.0	2.0	1.0	1.0	0.5	2.0	0.0	0.5	A	unknown
WAB56-104	Lowland	Resistant control	Africa Rice Center	2.0	2.0	0.5	0.0	1.0	2.5	0.0	1.0	1.5	1.5	3.5	1.5	2.0	2.5	1.5	2.0	1.0	0.5	1.5	1.0	A	*Pita-2*, *Pita*, *Pia* ,one of *Pit*, *Pik-s* , or unknown
SGU01	Upland	Landrace	South NNPR	0.5	0.0	0.5	1.5	1.0	0.0	0.0	1.0	0.0	2.0	4.5	2.5	1.5	2.0	3.0	1.5	1.0	2.0	0.5	3.5	A	one of *Pik-m*, *Pik-p*, *Pit* , or unknown
Demoze	Lowland	Landrace	Amhara	0.5	1.0	2.0	0.0	0.0	2.0	2.5	2.0	1.5	1.0	3.5	1.5	0.0	1.5	5.0	4.0	3.5	4.5	2.5	0.5	A	one of *Pia*, *Pi5* (t), *Pik-p* ,or unknown
SGU09	Upland	Landrace	South NNPR	1.5	1.5	1.0	0.0	0.0	1.0	1.5	3.0	1.5	1.0	3.0	2.0	0.0	1.0	2.0	2.5	2.5	3.0	2.0	1.0	A	*Piz*, *Pik-p*, *Pit*, one of *Pik-s*, or unknown
X-JIGNA	Lowland	Landrace	Amhara	2.5	2.0	1.5	0.5	2.5	1.5	2.0	3.0	2.0	1.5	2.5	1.0	2.5	5.0	3.5	4.5	3.0	3.0	2.5	4.5	B2	*Pit*, or unknown
AMF06	Lowland	Landrace	Amhara	1.0	1.5	1.0	1.0	1.0	0.0	1.5	1.5	0.0	1.0	4.0	2.0	2.0	2.0	2.5	1.5	1.0	2.5	0.0	1.5	A	*Pi12* (t), *Piz-t*, *Piz*, one of *Pik-m*, *Pi5* (t), *Pib*, or *Pia*
AMF12	Lowland	Landrace	Amhara	1.0	2.0	1.5	0.5	1.0	1.5	2.0	1.5	1.5	1.0	3.5	3.5	2.5	3.5	3.5	0.0	0.5	1.5	2.5	2.0	A	*Piz-t*, *Pik-m*, *Pib*, one of *Pik-p* , or unknown
GAM01	Upland	Landrace	Gambella	0.5	1.0	1.0	1.0	0.5	2.5	0.5	3.0	1.5	1.5	4.5	1.0	2.5	2.0	1.0	3.5	2.0	4.5	1.5	3.0	A	one of *Pit*, *Pik-s* , or unknown
GAM02	Upland	Landrace	Gambella	3.0	1.0	2.5	1.5	1.0	3.5	0.5	1.5	3.0	0.0	2.5	2.0	1.0	0.0	3.0	3.0	1.5	2.0	2.0	0.0	A	*Pit*,*or* unknown
GAM03	Upland	Landrace	Gambella	2.5	0.0	1.5	1.0	1.0	3.0	1.0	3.0	1.0	1.0	2.5	2.5	2.0	2.0	2.5	2.5	3.5	2.5	2.5	3.0	A	unknown
AMF13	Lowland	Landrace	Amhara	1.0	0.0	1.0	1.5	0.5	1.0	0.0	0.0	2.0	2.5	3.0	2.5	1.5	3.0	0.0	2.5	1.0	2.0	1.5	0.5	A	one of *Pit*, *Pik-s* , or unknown
GAM04	Upland	Landrace	Gambella	0.0	1.5	1.0	0.0	1.5	1.0	0.0	2.0	1.0	0.5	2.0	2.0	2.0	2.5	2.5	1.0	1.0	1.0	0.5	0.0	A	*Pi20(t)*, *Pi1*, *Pik-h*, one of *Pik*, *Pi3*, *Piz-t*, *Pi12* (t), *Pib*, *Pik-m*, *Pi7* (t), *Pia*, *Pi* 5(t), *Pi19* (t), *Pik-p*, *Pit*, or *Pish*
BGA01	Upland	Landrace	Benshangulgumize	1.5	2.5	1.0	1.0	2.5	4.0	1.5	4.0	0.5	3.0	4.0	3.0	2.5	2.0	3.0	3.0	2.5	4.0	4.0	1.0	B2	unknown
BGP-01	Upland	Landrace	Benshangulgumize	1.5	2.0	1.0	2.0	2.5	3.0	0.0	3.0	0.5	0.5	2.0	2.0	3.0	3.0	0.0	1.5	2.0	0.0	2.5	0.0	A	one of *Pish*, *Pik-s* , or unknown
BGP-02	Upland	Landrace	Benshangulgumize	0.0	1.0	0.5	1.0	1.0	1.0	0.5	1.0	0.0	2.0	0.0	1.0	3.0	0.0	0.0	1.0	1.0	2.5	1.0	1.0	A	*Pik-h*, *Pik*, *Piz-t*, one of *Pi12* (t), *Pi3*, *Pi7* (t), or *Pik-m*
BGP-03	Upland	Landrace	Benshangulgumize	1.0	0.0	0.0	1.5	0.5	0.0	0.0	0.0	0.0	0.0	4.0	3.5	5.0	4.5	4.5	1.0	1.0	1.5	1.0	1.0	A	*Piz-t*, *Pik-m*, *Pib*, one of *Pik-p*, *Pit* , or unknown
BGP-04	Upland	Landrace	Benshangulgumize	2.5	1.0	1.5	1.5	0.5	1.5	0.5	0.5	0.5	0.0	4.5	3.0	1.5	3.0	3.0	2.0	0.5	2.0	0.5	0.5	A	one of *Pib*, *Pit* , or unknown
BGP-05	Upland	Landrace	Benshangulgumize	3.0	2.0	1.0	0.5	1.0	3.0	0.0	0.5	1.5	2.0	1.5	2.0	2.0	2.5	1.0	1.5	1.5	1.5	0.0	0.5	A	*Pita*, *Pia*, *Pil9* (t), one of *Pit*, *Pik-s*, or unknown
BGP-06	Upland	Landrace	Benshangulgumize	1.5	1.0	1.5	1.0	1.0	1.0	1.0	0.5	0.5	1.5	3.5	3.5	3.5	4.0	3.0	2.0	1.0	1.5	0.5	1.5	A	*Piz-t*, *Pik-m*, *Pib* , one of *Pik-p*, *Pit* , or unknown
BGP-07	Upland	Landrace	Benshangulgumize	1.0	2.0	2.5	1.0	0.5	1.0	2.0	3.5	1.0	2.5	4.0	3.0	2.5	2.0	2.5	3.0	1.0	3.5	1.0	0.0	A	*Pit*, or unknown
BGP-09	Upland	Landrace	Benshangulgumize	2.0	0.0	2.0	1.0	2.5	1.5	0.0	3.0	1.0	3.5	2.5	3.5	3.5	1.0	2.0	2.0	1.5	1.0	1.0	0.0	A	one of *Pit*, *Pik-s*,*or* unknown
BGP-10	Upland	Landrace	Benshangulgumize	0.5	0.0	1.0	0.0	1.0	2.0	0.0	1.0	0.0	1.5	2.0	2.5	1.0	3.0	0.0	0.5	1.0	1.0	0.0	1.0	A	*Pi9* (t), *Pi1*, *Pik-h*,*one of Pita-2*, *Pik*, *Piz-t*, Pi7(t), *Pik-m*, *Pib*, *Pik-p* , or *Pi19* (t)
BGP-11	Upland	Landrace	Benshangulgumize	0.0	1.0	1.0	1.0	1.0	1.0	0.0	0.5	0.0	0.5	2.5	4.5	3.5	3.5	2.5	2.0	1.0	2.0	0.5	1.0	A	*Piz-t*, *Pik-m*, *Pib* , one of *Pik-p*, *Pit*, or unknown
BGP-12	Upland	Landrace	Benshangulgumize	0.0	0.0	1.0	0.0	1.5	1.0	0.0	0.0	0.0	0.5	2.0	2.5	3.0	3.0	2.0	2.5	0.5	1.0	0.0	0.0	A	*Pi7* (t), *Pib*, *Pik-p* , one of *Pit*, *Pish*, *Pik-s*, *or* unknown
BGP-13	Upland	Landrace	Benshangulgumize	0.5	0.0	1.5	1.0	1.0	1.5	0.5	2.0	0.5	1.5	3.5	4.0	4.5	4.0	3.5	3.5	1.5	1.0	0.5	2.0	A	one of *Pib*, *Pik-p*, *Pit*, or unknown
BGP-14	Upland	Landrace	Benshangulgumize	0.0	1.0	1.0	0.5	1.0	1.0	0.5	0.5	0.0	0.5	2.0	2.5	1.0	3.5	1.5	1.0	1.0	0.5	0.0	1.0	A	*Pi9* (t), *Pi1*, *Pik-h*,*one* of *Pita-2*, *Pik*, *Piz-t*, *Pi7* (t), *Pita*, *Pik-m*, *Pib*,*or Pik-p*
BGP-15	Upland	Landrace	Benshangulgumize	3.5	1.0	1.0	1.0	1.0	2.0	1.0	1.5	1.5	0.5	1.5	3.0	2.0	2.5	2.0	4.5	0.5	2.5	0.5	0.5	A	*Pib*, *Pita*, *Pi19* (t), one *Pit*, *Pish*, *or Pik-s*, or unknown
AMF14	Lowland	Landrace	Amhara	0.5	0.5	0.5	0.0	0.5	2.0	0.5	2.5	1.5	1.5	2.0	3.5	2.0	3.5	0.5	3.0	2.0	3.5	0.5	4.0	A	*Pi7* (t), *Pik-p*, *Pit*, one of *Pik-s*, or unknown
Fogera 1	Upland	Improved	Africa Rice Center	0.5	0.5	1.0	0.5	0.5	0.0	0.5	3.0	0.0	0.5	1.5	2.5	0.0	1.5	2.5	3.0	0.5	0.5	1.0	0.0	A	*Pi7* (t), *Pik-p*, *Pii*, one of *Pit*, *Pish* , or unknown
Adet	Upland	Improved	Africa Rice Center	2.5	1.0	1.0	1.5	1.5	0.5	1.5	1.0	1.5	0.5	1.0	2.0	0.0	0.0	2.0	2.0	1.5	1.0	1.0	0.0	A	*Pi12* (t), *Piz*, *Pib*, one *Pita*, *Pia*, *Pi5* (t), *Pi19* (t), *Pish*, *Pit*, *Pii* , *Pik-s* ,or unknown
NERICA-12	Upland	Improved	Africa Rice Center	0.5	0.0	1.0	1.0	0.0	0.5	0.0	1.5	0.5	1.0	2.0	2.0	1.0	2.0	3.0	2.0	2.0	0.5	1.0	2.0	A	*Pi20* (t), *Pi1*, *Pik-h*, one of *Pik*, *Pi3*, *Piz-t*, *Pi12* (t), *Pib*, *Pik-m*, *Pi7* (t), *Piz*, *Pia*, *Pi5* (t), *Pi19* (t), *Pik-p*, *Pii*, *Pit*
NERICA-13	Upland	Improved	Africa Rice Center	0.0	0.5	1.5	0.5	1.0	1.0	0.5	0.5	0.5	2.0	3.5	2.0	1.5	3.5	4.5	1.0	1.0	2.5	0.5	1.5	A	*Pi12* (t), *Piz-t*, *Pi3*,*one* of *Pib*, *Pik-m*, *Pia*, *Pik-p*, *Pii*, *Pi* t, or unknown
Chewaqa	Upland	Improved	China	0.0	0.0	1.0	0.5	2.5	0.5	0.5	4.0	1.0	0.5	4.0	5.0	5.0	4.5	5.0	2.0	1.0	1.5	0.5	1.0	A	*Pit*, or unknown
Hiddasse	Upland	Improved	Africa Rice Center	0.0	0.5	1.0	1.0	1.5	1.5	0.5	2.5	0.0	0.5	3.5	2.5	0.5	1.0	2.5	1.5	1.0	0.5	2.0	0.5	A	one of *Pik-m*, *Pik-p*, *Pit*, or unknown
Getachew	Upland	Improved	Amhara	1.5	0.0	2.0	1.5	1.0	1.0	0.0	3.5	0.0	0.5	3.0	2.5	1.0	4.0	2.0	2.0	3.0	2.5	2.0	2.0	A	one of *Pik-p*, *Pit*, *Pik-s* , or unknown
Andassa	Upland	Improved	Amhara	1.0	1.0	1.0	0.5	0.5	2.0	2.0	4.0	0.0	1.0	4.0	1.0	1.5	2.5	3.5	0.5	1.0	2.5	1.5	1.0	A	one of *Pik-m*, *Pik-p*, *Pit*,*or* unknown
Tana	Upland	Improved	Amhara	0.0	0.0	1.0	2.0	1.0	1.5	1.0	4.5	0.5	0.5	5.0	3.5	3.0	3.5	5.0	1.5	3.5	1.0	1.0	1.5	A	one of *Pik-p*, *Pit*, or unknown
NERICA-3	Upland	Improved	Africa Rice Center	1.0	1.0	1.0	2.0	1.5	2.0	1.0	1.5	1.5	0.0	2.5	3.5	0.0	0.5	2.5	1.5	1.0	0.0	1.5	1.5	A	*Piz-t*, *Pik-m*, *Pib*, one of *Pik-p*, *Pit*, or unknown
NERICA-4	Upland	Improved	Africa Rice Center	1.5	1.5	0.5	0.0	1.5	2.0	0.0	2.5	1.5	0.5	0.0	2.5	2.5	1.0	1.0	2.0	0.5	1.0	0.0	0.0	A	*Pi7* (t), *Pik-m*, *Pik-p* , one of *Pii*, *Pik-s* , or unknown
SUPERICA-1	Upland	Improved	Africa Rice Center	0.0	0.0	0.5	1.0	0.5	0.5	1.0	2.5	0.0	2.5	2.5	3.0	4.0	3.5	2.5	1.0	0.5	1.5	0.5	0.0	A	*Pit*, or unknown
Kokit	Upland	Improved	Amhara	2.0	0.0	0.5	1.0	1.5	2.0	0.5	3.0	1.0	0.5	3.0	3.5	3.5	3.5	3.0	5.0	2.0	0.0	1.5	0.0	A	one of *Pik-m*, *Pik-p*, *Pi* t or unknown
Pawe-1	Upland	Improved	Benshangulgumize	1.0	0.5	1.0	1.0	1.0	0.0	0.0	0.5	0.5	2.0	2.5	4.5	0.0	2.5	1.5	1.0	1.0	0.5	1.0	0.5	A	*Pita-2*, *Piz-t*, *Pik-m*, one of *Pib*, *Pik-p*, *Pit*, *Pik-s* , or unknown
Fogera2	Lowland	Improved	International Rice Research Institute	0.5	0.0	0.0	1.0	0.0	0.0	0.5	0.0	0.0	0.0	3.0	2.5	1.0	2.0	2.5	1.5	0.5	2.0	0.0	0.5	A	*Piz-t*, *Pik-m*, *Pib* , one of *Pik-p*, *Pit*, or unknown
Hiber	Lowland	Improved	Africa Rice Center	2.5	1.0	0.5	0.5	2.0	3.0	2.5	3.0	0.5	1.0	3.5	2.0	2.0	3.0	2.0	4.0	1.5	0.5	1.0	0.0	A	unknown
Ediget	Lowland	Improved	Africa Rice Center	0.0	1.0	1.0	0.0	0.0	1.0	0.0	1.5	1.0	0.5	1.0	2.0	1.5	1.0	3.5	3.5	1.0	1.0	0.0	2.5	A	*Pi3*, *Pi7* (t), *Pi19* (t), one of *Pik-p*, *Pii*, *Pit*, or unknown
Gumara	Lowland	Improved	Amhara	1.5	0.0	2.0	0.5	1.0	1.0	0.5	0.5	1.5	1.0	2.0	4.5	2.0	1.0	2.5	1.5	2.5	2.0	0.0	2.0	A	*Piz-t*, *Pi7* (t), *Pi19* (t), one of *Pik-p*, *Pii*, *Pit*, *Pish* , or unknown
NERICA-15	intermittent irrigated	Improved	Africa Rice Center	0.0	0.5	2.0	1.0	2.0	0.0	1.0	0.5	0.0	1.5	1.0	0.5	1.5	2.0	3.0	3.5	1.0	1.0	1.0	3.0	A	*Pi3*, *Pi7* (t), *Pi19* (t), one of *Pik-p*, *Pii*, *Pi* t, or unknown
NERICA-6	intermittent irrigated	Improved	Africa Rice Center	0.5	1.5	0.5	0.5	0.0	1.0	0.5	1.5	0.5	1.0	4.0	4.5	5.0	4.0	4.0	4.5	0.5	3.0	1.0	4.0	A	one of *Pik-p*, *Pit* , or unknown
NERICA-14	intermittent irrigated	Improved	Africa Rice Center	0.0	1.5	0.5	0.0	0.5	1.0	0.0	2.0	0.5	0.0	0.0	2.0	0.0	0.0	2.0	4.0	0.5	0.5	3.0	1.0	A	*Pi20* (t), *Pi12* (t), *Pi3*, *one of Pib*, *Pi7* (t), *Pia*, *Pi5* (t), *Pi19* (t), *Pik-p*, *Pii*, *Pish*, *Pik-s*, or unknown
Kallafo-1	intermittent irrigated	Improved	Madagascar	0.5	0.0	0.5	0.5	0.5	0.5	0.0	1.0	1.5	2.5	3.0	2.0	2.5	2.5	2.0	3.0	1.0	1.5	2.5	3.0	A	*Pik-* s, or unknown
NERICA-1	intermittent irrigated	Improved	Africa Rice Center	0.5	0.0	0.5	0.0	1.5	0.0	0.5	2.5	0.5	1.5	1.5	3.5	0.0	0.0	1.5	0.0	1.0	1.5	0.5	0.5	A	*Pi7* (t), *Pik-m*, *Pik-p* , one of *Pii*, *Pit*, *Pik-s* , or unknown
NERICA-2	intermittent irrigated	Improved	Africa Rice Center	1.0	1.5	1.0	0.0	2.0	0.0	1.0	1.5	0.5	0.5	2.0	3.5	1.0	2.0	0.0	0.5	1.5	1.5	1.0	0.0	A	*Pi9* (t), *Piz-5*, *Pi1* ,one of *Pik-h*, *Pita-2*, *Pik*, *Piz-t*, *Pi7* (t), *Pik-m*, *Pib*, *Pi19* (t), *Pik-p*, *Pii*, *Pit*, *Pish* ,or Pik-s
NERICA-10	intermittent irrigated	Improved	Africa Rice Center	0.5	1.0	1.5	0.0	1.0	1.5	0.5	1.5	0.5	0.5	2.0	3.0	1.0	2.5	0.0	2.5	1.5	2.5	1.0	0.0	A	*Pi7* (t), *Pib*, *Pita* , one of *Pi19* (t), *Pik-p*, *Pit*, *Pish*, *Pik-s*, or unknown
Candidate 1	Lowland	Improved	International Rice Research Institute	0.0	0.0	1.0	0.5	1.0	0.0	0.5	3.0	0.0	1.0	1.5	2.5	4.0	5.0	4.0	1.0	1.5	3.5	4.5	2.5	B1	one of *Pi7* (t), *Pik-m*, *Pik-p* , or unknown
Abay	Upland	Improved	Africa Rice Center	1.0	2.5	1.5	0.0	0.0	1.0	0.0	2.5	1.0	1.0	5.0	4.0	4.5	3.5	1.0	1.0	2.0	2.0	0.5	2.5	A	one *Pik-p*, *Pik-s*, or unknown
Candidate 2	Lowland	Improved	International Rice Research Institute	0.5	0.5	0.5	1.0	0.5	0.0	0.0	0.5	0.5	0.0	2.5	2.0	2.0	0.0	2.5	1.5	1.0	3.0	0.0	0.0	A	*Pi12* (t), *Piz-t*, *Piz*, one of *Pik-m*, *Pib*, *Pia*, *Pik-p*, *Pit* , or unknown
Candidate 3	Lowland	Improved	International Rice Research Institute	0.0	0.0	1.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	2.5	4.5	0.0	0.0	5.0	3.5	0.0	2.5	1.5	1.0	A	one of *Pib*, *Pik-p*, *Pit* , or unknown
Erib	Lowland	Improved	Africa Rice Center	0.0	2.0	0.0	1.5	2.0	1.5	0.0	1.5	0.0	0.5	3.5	2.0	5.0	3.5	1.5	2.0	0.5	0.5	1.5	0.5	A	*Pi12* (t), *Piz-t*, *Pik-m* , one of *Pib*, *Pi5* (t), *Pita*, *Pik-p*, *Pit*, *Pish*, *Pik-s*, or unknown
Candidate 4	Lowland	Improved	Madagascar	0.5	2.5	2.0	0.5	0.5	0.0	1.0	0.5	0.0	1.0	3.0	4.5	4.5	3.5	2.5	3.5	2.5	4.0	1.5	2.5	A	*Pik-p* , or unknown
Wanzaye	Lowland	Improved	Madagascar	0.5	2.0	0.0	0.0	1.0	1.0	2.0	0.0	0.5	0.0	5.0	3.0	3.0	2.0	3.5	0.0	0.5	2.5	0.0	0.0	A	*Piz-t*, *Pik-m*, *Pib* , one *Pik-p*, *Pit* , or unknown
Shaga	Lowland	Improved	Madagascar	1.0	2.0	1.0	0.0	1.5	1.5	1.0	1.0	1.5	1.0	2.0	3.0	2.0	2.5	2.0	0.0	0.5	0.5	0.0	0.5	A	*Pi9* (t), *Pi1*, *Pik-h*, *one* of *Pita-2*, *Pik*, *Piz-t*, *Pi7* (t), *Pik-m*, *Pib*, *Pik-p*, *Pi19* (t), *Pit*, *Pish*, *Pik-s*

[a] A total of 92 accessions including, landraces(n=27), improved varieties (n=33), differential varieties (n=28), two susceptible checks (LTH and US2), and two other controls IR64 as Indica and WAB56-104 as Japonca types.

[b] Standard blast isolates were kindly provided by JIRCAS at Ishigaki, Japan and reaction scores for each isolate was aveaged of two replications.

[c] BEN: Benin, NIG: Nigeria, and KNY: Keneya.

Table S3. 2 Reaction patterns of blast isolates among 92 rice accessions as explained by resistance (R) and susceptible (S) and estimated resistance frequency (RF)

Variety	Type	JPF494	JPF500	JPF506	JPF507	JPF509	JPF510	JPF513	JPF514	JPF517	PHL2	PHL4	PHL8	PHL14	PHL15	PHL16	BEN43	NIG1	CHN125	LAO12	KNY135	No. of S	No. of R	RF (%)
LTH	Susceptible control	S	S	S	R	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S	17	3	15
US2	Susceptible control	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	20	0	0
IRBLsh-B	DV	S	S	S	S	S	S	R	S	R	S	R	S	S	S	S	S	S	S	S	R	16	3	15
IRBLb-B	DV	S	S	S	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	R	R	11	7	35
IRBLr-K59	DV	S	R	S	R	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S	16	4	20
IRBLa-A	DV	S	S	S	R	R	S	S	R	R	R	S	R	R	S	S	S	S	S	S	R	12	7	35
IRBLi-F5	DV	S	S	S	R	R	S	S	S	S	S	R	S	S	R	S	S	S	S	S	S	16	4	20
IRBL3-CP4	DV	R	R	S	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	R	S	8	11	55
IRBL5-M[LT]	DV	S	S	S	R	R	R	S	R	R	R	S	R	S	S	S	S	S	S	S	R	12	7	35
IRBLks-F5	DV	S	S	S	R	S	S	R	S	S	S	S	S	S	S	R	S	S	S	S	S	17	3	15
IRBLkm-Ts	DV	R	R	S	S	R	R	R	S	R	R	S	S	S	S	S	S	S	R	R	S	11	8	40
IRBL1-CL[LT]	DV	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	R	R	S	6	13	65
IRBLkh-K3[LT]	DV	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	R	R	S	7	12	60
IRBLk-Ka[LT]	DV	R	R	R	S	R	R	R	R	R	R	R	S	S	S	S	S	S	R	R	S	8	11	55
IRBLkp-K60	DV	R	S	R	S	R	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	14	6	30
IRBL7-M	DV	R	R	R	R	R	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	11	9	45
IRBL9-W	DV	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	3	15	75
IRBLz-Fu	DV	S	R	R	S	S	S	R	S	R	R	S	R	R	R	S	S	R	S	S	R	10	9	45
IRBLz5-CA-1	DV	R	S	R	R	S	R	R	R	R	R	R	S	S	R	R	R	R	S	R	S	6	13	65
IRBLzt-T	DV	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	R	S	S	R	8	11	55
IRBLta2-Pi[LT]	DV	R	R	R	R	R	S	R	R	S	R	S	S	R	S	R	S	R	R	R	S	7	12	60
IRBLta2-Re	DV	R	R	R	R	S	S	R	R	R	R	S	S	R	S	R	S	R	R	R	S	7	12	60
IRBL12-M	DV	S	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	R	R	8	10	50
IRBLta-K1[LT]	DV	S	R	S	R	S	S	S	R	S	R	R	S	S	S	R	S	R	S	S	S	13	7	35
IRBLta-CP1	DV	S	R	R	R	S	S	S	R	S	R	S	R	S	S	R	S	R	S	S	S	12	8	40
IRBL19-A	DV	S	R	S	R	S	S	R	R	S	S	R	S	R	S	S	S	S	S	S	S	14	6	30
IRBL20-IR24	DV	R	R	R	R	S	S	R	R	R	R	R	R	R	S	S	R	S	S	R	R	6	12	60
IRBL5-M[US]	DV	R	R	R	R	S	S	S	S	R	R	R	S	R	R	S	S	S	S	R	S	10	9	45
IRBL12-M[US]	DV	S	S	R	R	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	S	15	4	20
IRBLta-K1[US]	DV	S	R	S	R	S	S	R	R	S	R	S	R	S	S	R	S	R	S	S	S	12	8	40
IR 64	Resistant control	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0	18	90
WAB56-104	Resistant control	R	R	R	R	R	S	R	R	R	R	S	R	R	S	R	R	R	R	R	R	3	15	75
Fogera 1	Improved	R	R	R	R	R	R	R	S	R	R	R	S	R	R	S	R	R	S	R	R	4	14	70
Adet	Improved	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	1	17	85
NERICA-12	Improved	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	1	17	85
NERICA-13	Improved	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	R	R	R	R	4	14	70
Chewaqa	Improved	R	R	R	R	S	R	R	S	R	R	S	S	S	S	S	R	R	R	R	R	7	11	55
Hiddasse	Improved	R	R	R	R	R	R	R	S	R	R	S	S	R	R	S	R	R	R	R	R	4	14	70
Getachew	Improved	R	R	R	R	R	R	R	S	R	R	S	S	R	S	R	S	R	R	S	R	6	13	65
Andassa	Improved	R	R	R	R	R	R	R	S	R	R	S	R	R	S	S	S	R	R	R	R	5	13	65
Tana	Improved	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S	R	R	R	S	R	7	12	60
NERICA-3	Improved	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	R	R	R	R	R	3	15	75
NERICA-4	Improved	R	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	R	R	3	15	75
SUPERICA-1	Improved	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	R	R	R	R	R	7	11	55
Kokit	Improved	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S	R	R	S	R	R	7	11	55
Pawe-1	Improved	R	R	R	R	R	R	R	R	R	R	S	S	R	S	R	R	R	R	R	R	3	15	75
Fogera 2	Improved	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	R	R	R	R	R	3	15	75
Hiber	Improved	S	R	R	R	R	S	S	S	R	R	S	R	R	S	R	R	R	S	R	R	7	11	55
Ediget	Improved	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	S	3	16	80
Gumara	Improved	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	R	S	R	3	16	80
NERICA-15	Improved	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	S	3	16	80
NERICA-6	Improved	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R	S	R	S	R	8	11	55
NERICA-14	Improved	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	2	16	80
Kallafo-1	Improved	R	R	R	R	R	R	R	R	R	S	S	R	S	S	R	R	S	S	R	S	7	12	60
NERICA-1	Improved	R	R	R	R	R	R	R	S	R	R	R	S	R	R	R	R	R	R	R	R	2	16	80
NERICA-2	Improved	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	1	17	85
NERICA-10	Improved	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	S	R	S	R	R	4	14	70
Candidate 1	Improved	R	R	R	R	R	R	R	S	R	R	R	S	S	S	S	S	S	R	R	S	8	11	55
Abay	Improved	R	S	R	R	R	R	R	S	R	R	S	S	S	S	R	R	R	R	R	S	7	12	60
Candidate 2	Improved	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	3	15	75
Candidate 3	Improved	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	R	S	R	R	5	13	65
Erib	Improved	R	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	R	R	R	R	3	15	75
Candidate 4	Improved	R	S	R	R	R	R	R	R	R	R	S	S	S	S	S	S	R	S	S	S	10	10	50
Wanzaye	Improved	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	R	R	R	R	5	13	65
Shaga	Improved	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	R	R	2	16	80
SGU01	Landrace	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	R	R	R	R	S	4	15	75
Demoze	Landrace	R	R	R	R	R	R	S	R	R	R	S	R	R	R	S	S	S	S	S	R	7	12	60
SGU09	Landrace	R	R	R	R	R	R	R	S	R	R	S	R	R	R	R	S	R	S	S	R	5	14	70
X-JIGNA	Landrace	S	R	R	R	S	R	R	S	R	R	S	R	S	S	S	S	S	S	S	S	12	8	40
AMF06	Landrace	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	3	15	75
AMF12	Landrace	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R	S	R	R	R	6	12	60
GAM01	Landrace	R	R	R	R	R	S	R	S	R	R	S	R	S	R	R	S	R	S	R	S	7	12	60
GAM02	Landrace	S	R	S	R	R	S	R	R	S	R	S	R	R	R	S	R	R	S	R	R	7	11	55
GAM03	Landrace	S	R	R	R	R	S	R	S	R	R	S	S	R	R	S	S	S	S	S	S	11	9	45
AMF13	Landrace	R	R	R	R	R	R	R	R	R	S	S	S	R	S	R	R	R	S	R	R	5	13	65
GAM04	Landrace	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	2	16	80
BGA01	Landrace	R	S	R	R	S	S	R	S	R	S	S	S	S	R	S	S	S	S	S	R	13	6	30
BGP-01	Landrace	R	R	R	R	S	S	R	S	R	R	R	R	S	S	R	R	S	R	R	R	6	12	60
BGP-02	Landrace	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	R	R	2	16	80
BGP-03	Landrace	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R	R	R	R	R	5	13	65
BGP-04	Landrace	S	R	R	R	R	R	R	R	R	R	S	S	R	S	S	R	R	R	R	R	5	13	65
BGP-05	Landrace	S	R	R	R	R	S	R	R	R	R	R	R	S	R	R	R	R	R	R	R	3	15	75
BGP-06	Landrace	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R	R	R	R	R	5	13	65
BGP-07	Landrace	R	R	S	R	R	R	R	S	R	S	S	S	S	R	S	S	R	S	R	R	9	9	45
BGP-09	Landrace	R	R	R	R	S	R	R	S	R	S	S	S	S	R	R	R	R	R	R	R	6	12	60
BGP-10	Landrace	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	R	R	2	16	80
BGP-11	Landrace	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R	R	R	R	R	5	13	65
BGP-12	Landrace	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	S	R	R	4	14	70
BGP-13	Landrace	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R	R	S	R	R	6	12	60
BGP-14	Landrace	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	R	R	2	16	80
BGP-15	Landrace	S	R	R	R	R	R	R	R	R	R	R	S	R	S	R	S	R	S	R	R	5	13	65
AMF14	Landrace	R	R	R	R	R	R	R	S	R	R	R	S	R	S	R	S	R	S	R	S	6	13	65

Table S3. 3 Distribution of 23 resistance genes containd by 25 differential varieties and as postulated among 60 accessions from Ethiopia compared to blast reaction patterns of differential varieties

Accessions	Distribution of resistance genes/chromosome by accessions																							Remak
	Pish	*Pib*	*Pit*	*Pia*	*Pii*	*Pi3*	*Pi5* (t)	*Pik-s*	*Pik-m*	*Pi1*	*Pik-h*	*Pik*	*Pik-p*	*Pi7* (t)	*Pi9* (t)	*Piz*	*Piz-5*	*Piz-t*	*Pita-2*	*Pi12* (t)	*Pita*	*Pi19* (t)	*Pi20* (t)	
Chromosome	1	2	1	11	9	9	9	11	11	11	11	11	11	11	6	6	6	6	12	12	12	12	12	
IRBLsh-B	O																							
IRBLb-B		O																						
IRBLt-K59			O																					
IRBLa-A				O																				
IRBLi-F5					O																			
IRBL3-CP4						O																		
IRBL5-M[LT]							O																	
IRBLks-F5								O																
IRBLkm-Ts									O															
IRBL1-CL[LT]										O														
IRBLkh-K3[LT]											O													
IRBLk-Ka[LT]												O												
IRBLkp-K60													O											
IRBL7-M														O										
IRBL9-W															O									
IRBLz-Fu																O								
IRBLz5-CA-1																	O							
IRBLzt-T																		O						
IRBLta2-Pi[LT]																			O					
IRBLta2-Re																			O					
IRBL12-M																				O				
IRBLta-K1[LT]																					O			
IRBLta-CP1																					O			
IRBL19-A																						O		
IRBL20-IR24																							O	
SGU01			O						O				O											
Demoze				O			O						O											
SGU09			O					O					O			O								
X-JIGNA			O																					
AMF06		O		O			O		O							O		O		O				
AMF12		O							O				O					O						
GAM01			O					O																
GAM02			O																					
GAM03																								unknown
AMF13			O					O																
GAM04	O	O	O	O		O	O		O	O	O	O	O	O				O		O		O	O	
BGA01																								unknown
BGP-01	O							O																
BGP-02						O			O		O	O		O				O		O				
BGP-03		O	O						O				O					O						
BGP-04		O	O																					
BGP-05			O	O				O													O	O		
BGP-06		O	O						O				O					O						
BGP-07			O																					
BGP-09			O					O																
BGP-10		O							O	O	O	O	O	O	O			O	O			O		
BGP-11		O	O						O				O					O						
BGP-12	O	O	O					O					O	O										
BGP-13		O	O										O											
BGP-14		O							O	O	O	O	O	O	O			O	O		O			
BGP-15	O	O	O					O													O	O		
AMF14			O					O					O	O										
Fogera 1	O		O		O								O	O										
Adet		O		O			O									O				O	O			
NERICA-12		O	O	O	O	O	O		O	O	O	O	O	O		O		O		O		O	O	
NERICA-13		O	O	O	O	O			O				O					O		O				
Chewaqa			O																					
Hiddasse			O						O				O											
Getachew			O					O					O											
Andassa			O						O				O											
Tana			O										O											
NERICA-3		O	O						O				O					O						
NERICA-4					O			O	O				O	O										
SUPERICA-1			O																					
Kokit			O						O				O											
Pawe-1		O	O					O	O				O					O	O					
Fogera2		O	O						O				O					O						
Hiber																								unknown
Ediget			O		O	O							O	O								O		
Gumara	O		O		O								O	O				O				O		
NERICA-15			O		O	O							O	O								O		
NERICA-6			O										O											
NERICA-14	O	O		O	O	O	O	O					O	O						O		O	O	
Kallafo-1								O																
NERICA-1			O		O			O	O				O	O										
NERICA-2	O	O	O		O			O	O	O	O	O	O	O	O		O	O	O			O		
NERICA-10	O	O	O					O					O	O							O	O		
Candidate 1									O				O	O										
Abay								O					O											
Candidate 2		O	O	O					O				O			O		O		O				
Candidate 3		O	O										O											
Erib	O	O	O				O	O	O				O					O		O	O			
Candidate 4													O											
Wanzaye		O	O						O				O					O						
Shaga	O	O	O					O	O	O	O	O	O	O	O			O	O			O		

Chapter 4

Genetic variation and traits relationship among rice cultivars based on agronomic traits evaluated under lowland rain fed condition in Ethiopia

Abstract

Sixty rice accessions comprising 33 improved accessions and 27 landraces were evaluated under rainfed lowland conditions during 2018 wet season at Fogera and Pawe research stations using alpha lattice design of three replications to estimate genetic variation and traits relationship. Highly significant ($P<0.01$) differences were observed among accessions at both sites and the interaction across sites for almost all agronomic traits considered. Most accessions performed better at Pawe compared to that of Fogera. Including grain yield, most traits exhibited relatively high broad sense heritability. Four principal components presented about 81 and 75% information of overall variation at Fogera and Pawe, respectively. Days to heading, days to maturity, plant height, panicle length, filled grains/panicle and grain yield were the most discriminate traits for explaining the total variation at each location. The accessions were grouped into four clusters irrespective of their initial presumed groups as improved and landrace. Nearly 48% of improved accessions containing the desired traits adapted predominantly to upland production system belonged to Cluster I. Clusters II and IV included both upland and lowland accessions while Cluster III included exclusively of upland accessions mainly of landraces. Thus, future crossing program between accessions of different cluster groups could possibly result in better heterosis in the first generation and thereby high variability in the subsequent generations for selection.

Key words: Genetic variation, rice, cluster, principal component, Ethiopia

Introduction

Rice is an important cereal crop cultivated and consumed globally to meet the daily calorie needs of ever increasing world population mainly in Asia and Sub-Saharan Africa (Anyaoha *et al.* 2018). This important cereal is cultivated and consumed across Africa but unable to meet local demands resulting huge annual import. In Ethiopia, since its introduction, the crop is increasingly expanding and its consumption has sharply increased. Mainly rainfed upland and lowland and partly intimately irrigated rice are produced by over 150 thousand households on 48 thousand ha of land, out of more than 20 million ha suitable land (MoARD, 2010; CSA 2017). It is the second high yielding crop, next to maize, constituting 4.7 % of the total area covered by cereals (CSA 2017).

Despite the huge potential in the country, the national average yield of the crop, which is about 2.89 t/ ha, is very low compared to most rice producing countries and to that of world average yield, 4.60 t/ ha (FAOSTAT 2019). This is characterized as poor yield which could be attributed to cultivation of low yielding varieties and use of low agricultural inputs, among other things. Thus, there is a need to further improve grain yield of rice for different production systems in Ethiopia. For yield and other important traits improvement, genetic diversity needs to be investigated for successful utilization of genotypes in breeding programs (Qamar *et al*. 2012). In Ethiopia, through introduction of germplasms, more than 30 improved rice varieties have been released for upland, lowland rainfed and intermittently irrigated conditions. In addition, some previously introduced accessions are also cultivated by farmers as landraces. Information about genetic diversity facilitates the selection of parental materials from existing accessions. Analysis of genetic diversity in improved and landrace collections can also facilitate reliable classification of accessions and identification of accessions with possible use for specific breeding. Such evidence is particularly useful to

assess the potential of heterotic combinations before attempting crosses and hence saving time and resources (Hallauer and Miranda 1988). Phenotypic variability and yield performance of some advanced candidate accessions (Abebe *et al*. 2017; Girma *et al*. 2018) and of some selected improved rice accessions of Ethiopia have been reported (Bitew *et al*. 2016, 2018). They found significant differences and reasonable variability for important characters such as plant height, panicle length, filled grains per panicle, days to heading and maturity, and grain yield. However, there is no information about genetic variation and traits relationship of Ethiopian rice accessions comprising improved accessions and presumed landraces. To assess the extent of genetic variation and traits relationship in improved accessions and presumed landraces, it is essential to evaluate these materials together under the same experimental procedure. In this study, we focused on the differential response of selected quantitative traits under two contrasting environments and analyze the pattern of genetic variation and relationship among different traits. This report will complement our efforts of identifying potential materials and suitable traits that might contribute to future rice breeding program.

Materials and methods

Plant materials

This study was carried out using a total of 60 rice accessions comprising 33 improved accessions and 27 presumed landraces. All these plant materials were included in our molecular diversity analysis and blast inoculation test studies (Chapters 2 and 3). Presumed landraces were collected from five different districts of four regional states in Ethiopia; Fogera in Amhara region, Pawe and Assosa in Benshangul Gumize region, Guraferda in

South NNPR, and Abobo in Gambella region with kind cooperation of researchers at Fogera, Pawe, Assosa, Bonga, and Gambella research centers. Improved rice accessions were released from 1999 to 2017 by different research centers in Ethiopia except for NERICA-10. They are from diverse production systems; upland, lowland rain fed, and intermittently irrigated condition. Each of intermittently irrigated and upland condition included different upland NERICAs.

Experimental sites

The experiment was carried out at two rice research stations, Fogera National Rice Research and Training centre station, Fogera, and Pawe research centre station, Pawe (Fig. 4. 1). Fogera is characterized by high elevation and low temperature. It also faces moisture stress.

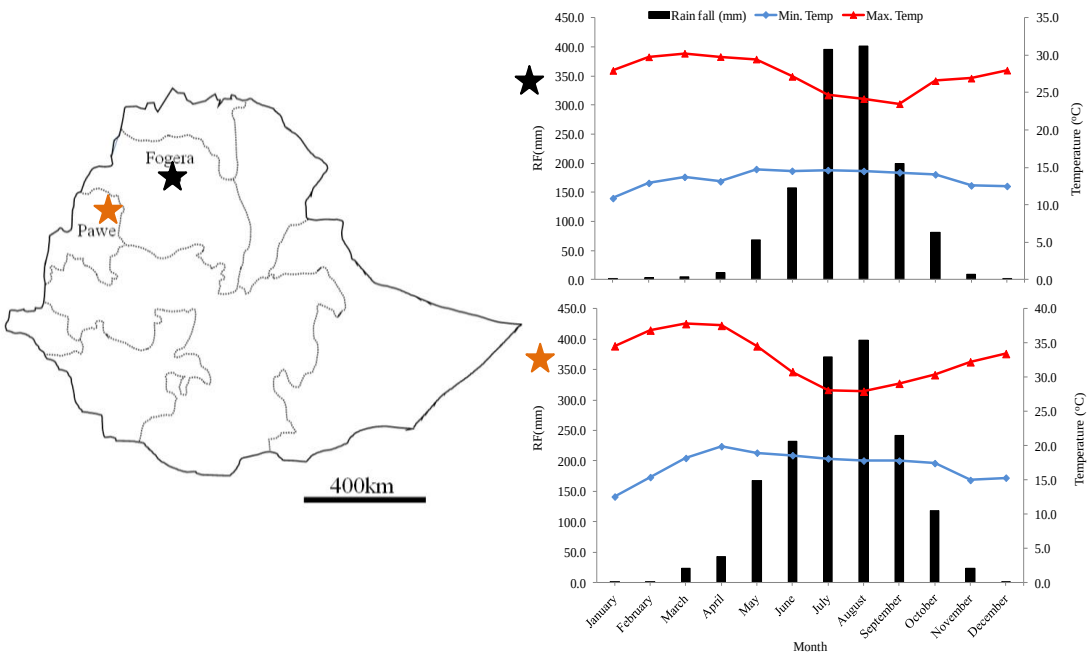

Fig. 4. 1 Experimental sites and monthly temperature and rainfall distribution at Fogera and Pawe (2005-2016). Max T and Min T: maximum and minimum temperatures, respectively.

Fogera station is positioned at 11^0 58'N, and 37^0 41'E at elevation of 1810 meter above sea level. The soil is vertisol with slightly acidic pH of 5.90 (Tilahun *et al*. 2013). Pawe station is located at 11^0 19'N and 34^0 24'E, at elevation of 1120 meter above sea level. This station has relatively high temperature and long rainy season. Average of eleven years (2005-2016) weather data of the two sites were indicated on Fig. 4. 1. Rainfall of the areas is uni-model, mainly from April to October at Pawe and June to October at Fogera, amounting to 1570.3 and 1234.5mm, respectively. Important rain fall months at Pawe ranges from late April to October and at Fogera from mid-June to early November which covers more than 90% of annual rainfall.

Experimental design and data collection

The experimental was laid down in a 15 x 4 alpha lattice design of three replications, each replication with four blocks. Fifteen accessions were planted per block. Each accession was sown in six rows of plot size 7.5 m^2 (5m x 1.5m). Spacing between replications, blocks, plots, and rows was 1.5m, 1m, 50cm, and 25cm, respectively. Fertilizer was applied as per to the local recommendation. Weeding and other field management operations were effective accordingly uniformly across experimental plots as required. Data were collected from the middle four rows excluding two border rows. Eleven quantitative traits were recorded for each accession per block per replication: Days to heading (DTH, days), days to maturity (DTM, days), plant height (PH, cm), panicle length (PL, cm), total grains per panicle (TGP, count), filled grains per panicle (FGP, count), fertility rate (FR, %), thousand seed weight (TSW, g), grain yield (Gy, t/ha), biomass yield (By, t/ha), and harvest index (HI, %), following the guidelines developed by IRRI (1996). Except for DTH, DTM, Gy, By, HI, and TSW, for the other traits, data were collected from ten randomly selected plants per plot.

Grain yield and biomass yield per plot was sampled from the middle four rows by cutting all plants from the bottom and sundried within bags. Weight of dried sample for each plot was measured to obtain above ground biomass and converted in tonnes per hectare (t/ha). For grain yield, each sample was threshed and weighed and adjusted at 14% moisture content in t/ha. Harvest index (HI, %) was calculated a ratio of grain yield to total above ground biomass yield.

Data analysis

Statistical analysis was carried out for each site and for the combined data. All collected data were subjected to analysis of variance using SAS software version 9.0 (SAS Institute 2002). Mean squares for grain yield and other related traits were used to estimate genotypic and phenotypic variances according to Burton and de Vane (1953) as follows:

For single site:

Genotypic variance (σ_g^2) = (MSg-MSe)/r and

Environmental variance= MSe

For combined data:

Genotypic variance (σ_g^2) = (MSg-MS_I)/lr;

Interaction variance (σ^2_I) = (MS_I-MSe)/r;

Environmental variance (σ_e^2) = MSe/r; and

Phenotypic variance (σ^2_p) = σ_g^2+ σ^2_I /l+ σ_e^2/lr, where r = number of replication, g = number of genotypes, l = number of locations, I = interaction, MSe = mean square of error, MSg = mean square of genotypes, MS_I =mean square of interaction. Thus, the variance components were used to compute genotypic coefficients of variation (GCV, %) and phenotypic

coefficient of variation (PCV, %), broad sense heritability (H^2b) (Allard 1960), and genetic advance (GA) (Johnson *et al.* 1955) as follows:

GCV= $[(\sigma_g^2)^{1/2}$/grand mean] x 100;

PCV= $[(\sigma^2_p)^{1/2}$/grand mean] x 100;

Heritability (H^2b) = $\sigma_g^2/\sigma^2_{p;}$

Expected genetic advance (GA) = (σ_g^2/σ^2_p) x k x σ_p and

Genetic advance as percent of mean (GAM) = (GA/Grand mean) x 100, where k (2.063) refers to a constant at 5% selection intensity for a particular trait considered whereas σ_p refers to phenotypic standard deviation. Phenotypic correlations coefficients were computed to elucidate relationships between traits by using PROC CORR procedure in SAS. Cluster analysis was also carried out using SPSS Software version 16 (SPSS Inc. 2007), respectively. Principal component analysis (PCA) was performed using GenStat software version 16 (GenStat 2013). PCA was employed to identify the different quantitative traits that contributed to the most variance in the measured variables.

Results

Analysis of variance and phenotypic performance

The results of analysis of variance for individual site and combined data showing mean squares of quantitative traits for 60 rice accessions were summarized in Table 4.1a-c. Accessions showed highly significant variations for all traits at Fogera (Table 4. 1a), and at Pawe except for fertility rate (Table 4. 1b). In combined data, the interaction effect showed significant difference($P<0.05$) for thousand seed weight and highly significant ($P<0.01$) for

days to heading, days to maturity, panicle length, filled grains per panicle, total grains per panicle, fertility rate, grain yield and harvest index but not significant for plant height (Table 4. 1c).

Table 4. 1 Analysis of variance for quatitative traits among 60 rice accessions (a-c)

(a). Fogera

Source of variation	Df	Mean square										
		DTH	DTM	PL	PH	FGP	TGP	FR	Gy	TSW	By	HI
Replication	2	13.9	13.7	24.7	445.2	685.7	790.7	1.1	2.5	27.5	10.8	85.6
Block(Replication)	9	354.7	257.0	6.7	131.5	239.1	345.4	63.1	1.6	5.3	6.6	67.1
Cultivar	59	418.7**	440.6**	6.9***	339.2**	294.9**	245.5**	93.8**	2.2**	26.6**	7.2**	215.9**
Error	109	51.8	38.6	1.5	27.2	132.3	128.8	34.8	0.36	4.8	1.5	36.6
CV(%)		8.1	4.6	6.6	6.2	14.0	12.2	6.7	21.5	8.2	16.8	15.7
R2		0.83	0.87	0.76	0.88	0.60	0.58	0.62	0.79	0.76	0.76	0.77

(b). Pawe

Source of variation	Df	Mean square										
		DTH	DTM	PL	PH	FGP	TGP	FR	Gy	TSW	By	HI
Replication	2	138.5	336.8	12.7	961.6	773.1	849.9	62.4	2.9	47.0	189.7	611.8
Block(Replication)	9	415.4	136.6	9.1	379.5	1277.3	1441.1	8.3	0.88	11.4	24.0	200.1
Cultivar	59	191.4**	112.9**	5.4**	257.2*	630.8**	645.6**	7.0ns	2.1**	13.7**	17.3**	107.4**
Error	109	45.8	26.8	1.8	166.0	342.2	380.1	8.7	0.45	5.5	6.8	37.7
CV(%)		8.1	4.3	6.8	13.3	15.8	15.8	3.1	21.6	8.8	27.4	18.3
R2		0.75	0.75	0.67	0.53	0.57	0.56	0.40	0.73	0.62	0.68	0.69

(c). Combined

Source of variation	Df	Mean square										
		DTH	DTM	PL	PH	FGP	TGP	FR	Gy	TSW	By	HI
Replication	2	108.8	195.5	31.9	1287.4	1940	2444.8	42.8	5.4	2.9	145.4	408.7
Block(Replication)	9	478.6	342	9.9	355.2	1445.6	1801.8	30.4	1.7	8.7	19.8	175.8
Cultivar (C)	59	530.7**	461.3**	9.3**	461.8**	701.2**	857.4**	49.5**	2.2***	31.3**	14.8**	185.2**
Location (L)	1	2073.6**	19462.8**	214.7**	12508.0*	113308.9*	90088.6*	3499.4**	4.5**	0.47ns	451.6**	2242.0**
C x L	59	103.1**	95.4**	3.2**	133.3ns	648.7**	496.8**	59.5**	2.1**	8.8*	10.3**	137.1**
Error	229	52.1	33.8	1.8	99.4	274.8	294.4	22.3	0.38	5.9	4.7	41.7
CV(%)		8.4	4.5	7	10.9	16.6	15.8	5.2	21.4	9.1	25.8	18
R2		0.78	0.87	0.73	0.70	0.77	0.74	0.67	0.76	0.64	0.69	0.71

Df: degree of freedom, DTH: days to heading, DTM: days to maturity, PL: panicle length(cm), PH: plant height(cm), FGP: filled grains/panicle(no), TGP: total grains/panicle (no), FR: fertility rate(%), Gy: grain yield(t/ha), TSW: 1000 seed weight(g), By: biomass yield(t/ha), HI: harvest index(%).

*, **, ns: refers to significnat at P<0.05, P<0.01 and non-significant, respectively

The majority of improved accessions, mainly of Japonica/Japonica-like and few Indica/Indica-like, showed early heading both at Fogera and Pawe (Fig. 4. 2). Most landraces (Japonica/Japonica-like) were relatively late heading at both sites (Figs.4. 2, 4. 3). However, almost all accessions tended to be earlier at Pawe than at Fogera which could be attributed to the high temperature at Pawe which favor rice crop to establish early and enhance early

heading (Figs. 4. 2, 4. 3). The minimum night temperature at Pawe is always higher than 15^{o}C while at Fogera it is always less than 15^{o}C which might prolong days to heading. Improved upland rice accessions included some the earliest heading types while upland landraces had very late heading types at both sites (Fig. 4. 3).

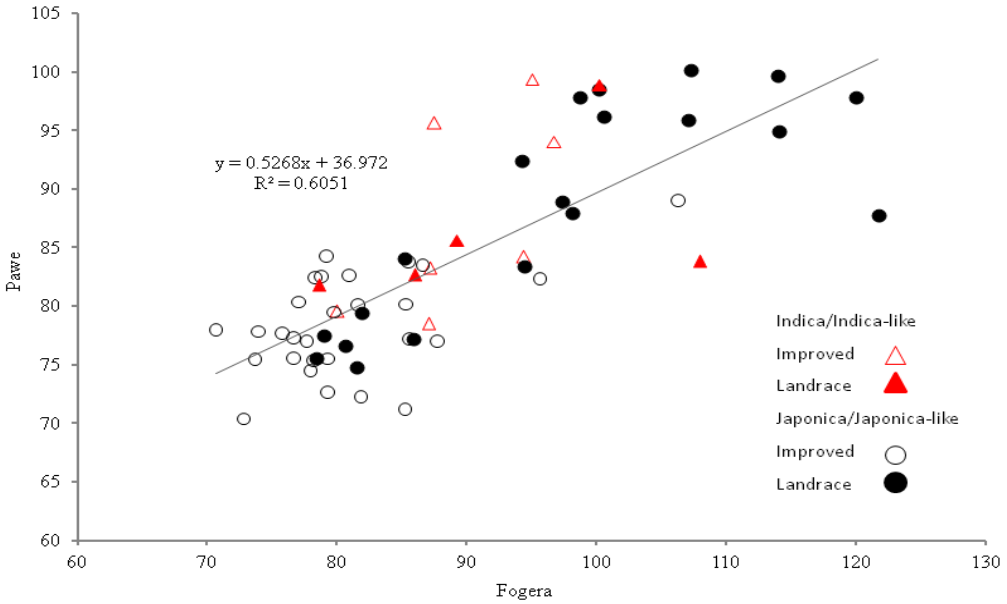

Fig.4. 2 Scatter plot showing days to heading variation among rice accessions grown at Fogera and Pawe. Accessions were classified into two groups based on molecular markers in previous study i.e some as Indica/Indica-like and most as Japonica/Japonica-like genetic background

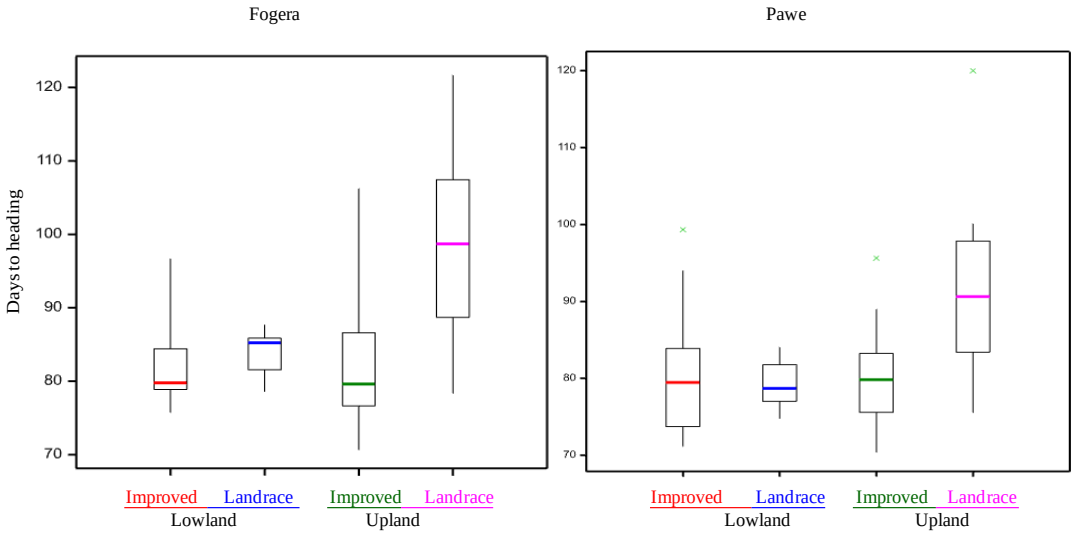

Fig. 4. 3 Box-plot showing variation in days to heading among groups of rice accessions at Fogera and Pawe

Lowland landraces showed narrow variation of heading days while upland landraces revealed wider variation at both sites (Fig. 4. 3). In terms of grain yield, some accessions from each group showed relatively higher performance at Pawe than at Fogera. Lowland improved accessions which comprised Indica/Indica-like and Japonica/Japonica-like types revealed the highest grain yield performance while upland landrace groups were the lowest at both sites (Fig. 4. 4). Lowland landrace groups which included X-Jigna were intermediate in grain yield at both sites but relatively higher at Pawe. Majority of upland improved accessions including NERICAS which comprised entirely of Japonica-like and Indica-like types showed relatively similar performance at Fogera and Pawe, with some performed significantly higher at Pawe (Fig. 4. 4).

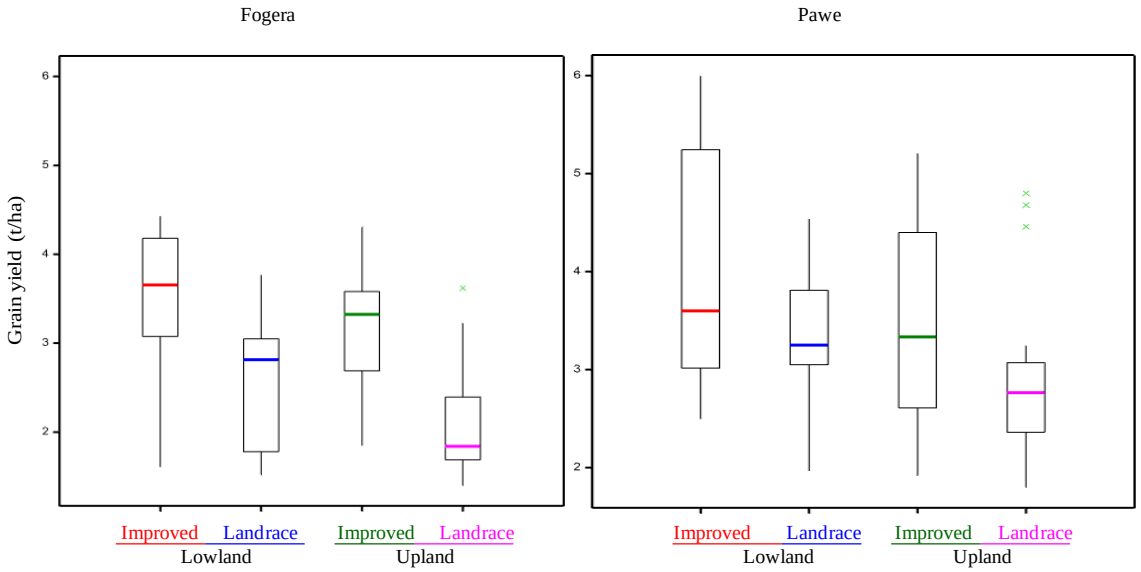

Fig. 4. 4 Box-plot showing variation in grain yield among groups of rice accessions at Fogera and Pawe

Estimates of mean, variances, heritability and genetic advance as percent of mean

Estimates of means, variances, heritability in broad sense (H) and genetic advance as percent of mean (GAM) were presented for selected quantitative traits among 60 rice accessions

(Tables 4. 2, 4. 3). Most traits showed relatively higher mean value at Pawe than at Fogera except for days to heading, days to maturity and harvest index. PCV was relatively higher than GCV for all traits at both sites. Deshmukh *et al*. (1986) classified PCV and GCV values as high (>20%), medium (10 to 20%), and low (<10%). At Fogera, high PCV was obtained for grain yield, followed by harvest index and biomass yield, and the rest ranged from low (fertility rate, 8.4%) to medium (filled grains per panicle, 16.63%) (Table 4. 2). GCV also ranged from (5.0%) for fertility rate to (28.0%) for grain yield. At Pawe, PCV ranged from 3.48% (fertility rate) to 33.71% (biomass yield); and GCV from 0.39% to 23.60% for fertility rate and grain yield, respectively (Table 4. 3). Dabholkar (1992) generally classified heritability in broad sense estimates as low (5-10%), medium (10-30%) and high (>30). Estimates of heritability at Fogera was high, ranging from 54.85% to 79.26% for most traits except two traits, filled grains per panicle (29.07%) and total grains per panicle (23.37%), both of which showed medium heritability estimate (Table 4. 2).

Table 4. 2 Estimates of mean, variances, heritability and genetic adavance as percent of mean for quantitative traits at Fogera

Traits	Mean	σ_e^2	σ_g^2	σ^2_p	H	GCV	PCV	GAM
Days to heading	88.28	51.76	122.32	174.08	70.27	12.53	14.95	21.67
Days to maturity	135.55	38.62	134.01	172.63	77.63	8.54	9.69	15.52
Panicle length (cm)	18.52	1.49	1.81	3.30	54.85	7.26	9.81	11.10
Plant height (cm)	84.78	27.22	104.00	131.22	79.26	12.03	13.51	22.09
Filled grains/panicle	82.13	132.31	54.23	186.54	29.07	8.97	16.63	9.97
Total grains/panicle	93.10	128.18	39.10	167.28	23.37	6.72	13.89	6.70
Fertility rate (%)	88.07	34.85	19.64	54.49	36.05	5.03	8.38	6.23
Grain yield (t/ha)	2.78	0.36	0.61	0.96	62.86	28.00	35.32	45.80
1000 seed weight (g)	26.60	4.77	7.28	12.05	60.43	10.15	13.05	16.27
Biomass yield (t/ha)	7.26	1.49	1.92	3.41	56.40	19.09	25.42	29.58
Harvest index (%)	38.48	36.59	59.80	96.39	62.04	20.10	25.51	32.65

σ_e^2: environmental variance, σ_g^2: genotypic variance, σ^2_p: phenotypic variance, H^2b: heritability (broad sense), GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, GAM: genetic advance as percent of mean.

Similar pattern of heritability estimates were obtained at Pawe (Table 4. 3). Except for plant height, filed grains per panicle, total grains per panicle, and fertility rate, all remaining

traits exhibited high heritability estimate at Pawe. Johnson *et al*. (1955) classified genetic advance as percent of mean (GAM) low with values from 0-10%, moderate 10-20%, and high 20% and above. Thus, the highest genetic advance as percent of mean was obtained from grain yield both at Fogera (45.80%) and Pawe (35.96%), followed by harvest index at Fogera (32.65%) and biomass yield at Pawe (Tables 4. 2, 4. 3).

Table 4. 3 Estimates of mean, variances, heritability and genetic advance as percent of mean for quantitative traits at Pawe

Traits	Mean	σ_e^2	σ_g^2	σ^2_p	H	GCV	PCV	GAM
Days to heading	83.48	45.77	48.53	94.30	51.46	8.34	11.63	12.35
Days to maturity	120.84	26.87	28.70	55.57	51.65	4.43	6.17	6.57
Panicle length (cm)	20.06	1.89	1.18	3.07	38.44	5.42	8.73	6.93
Plant height (cm)	96.57	165.96	30.42	196.38	15.49	5.71	14.51	4.64
Filled grains/panicle	117.62	426.07	179.54	605.61	29.65	11.39	20.92	12.80
Total grains/panicle	124.74	464.42	190.40	654.82	29.08	11.06	20.51	12.31
Fertility rate (%)	94.30	10.63	0.14	10.77	1.27	0.39	3.48	0.09
Grain yield (t/ha)	3.14	0.46	0.55	1.01	54.53	23.60	31.96	35.96
1000 seed weight (g)	26.67	5.55	2.72	8.27	32.86	6.18	10.78	7.31
Biomass yield (t/ha)	9.50	6.77	3.50	10.27	34.11	19.69	33.71	23.72
Harvest index (%)	33.49	37.66	23.24	60.90	38.16	14.39	23.30	18.34

σ_e^2: environmental variance, σ_g^2: genotypic variance, σ^2_p: phenotypic variance, H^2b: heritability (broad sense), GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, GAM: genetic advance as percent of mean.

Traits relationship

Relationships between quantitative traits were summarized as Pearson correlation coefficients (Tables S4. 1, S4. 2, S4. 3; Figs. 4. 5, 4. 6, 4. 7, and 4. 8). At Fogera, days to heading showed negative and significant correlation coefficients with grain and biomass yield (r= -0.34*, -0.25*) (Figs. 4. 5, 4. 6). Early heading types tended to show higher grain and biomass yields at Fogera than late types which could be attributed to the occurrence of moisture stress which caused high panicle sterility in late type varieties and result in low grain yield. On the contrary, at Pawe, grain yield and biomass yield showed positive correlation coefficients with days to heading (r=0.27*, 0.19). Some late type accessions tended to show high grain and

biomass yields (Figs. 4. 7, 4. 8). Moisture stress is not common at Pawe which allowed late varieties to gain high yields. Moreover, grain yield showed positive and significant correlation coefficients with other traits including panicle length, plant height, filed grains per panicle, and fertility rate at both Fogera and Pawe, and in combined data as well (Tables S4. 1, S4. 2, S4. 3). Positive correlation coefficients were also observed between panicle length, and filled grains per panicle (r= 0.41*), plant height (r= 0.60**), total grains per panicle (r= 0.33*), fertility (r= 0. 31*), biomass yield (r= 0.41*), and harvest index (r= 0.36*) at Fogera and in combined data (Tables S4. 1, S4. 3).

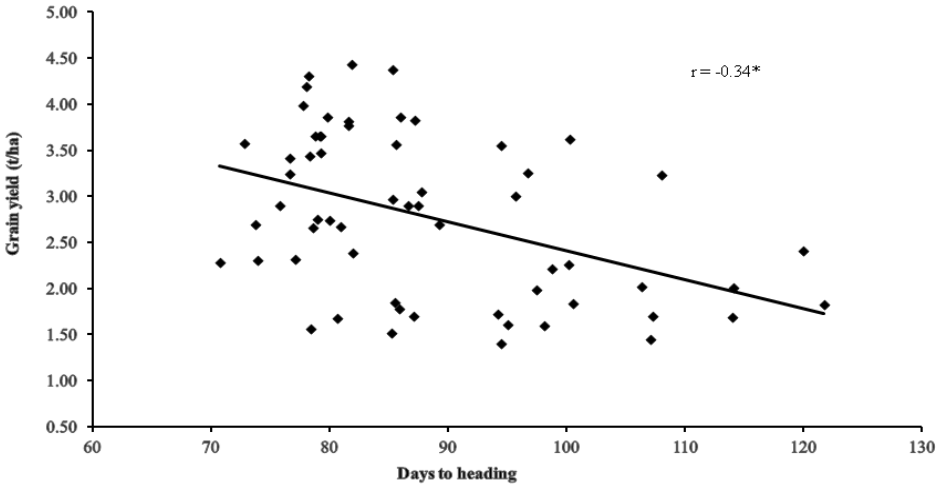

Fig. 4. 5 Relationship of days to heading and grain yield (t/ha) among 60 rice accessions evaluated at Fogera

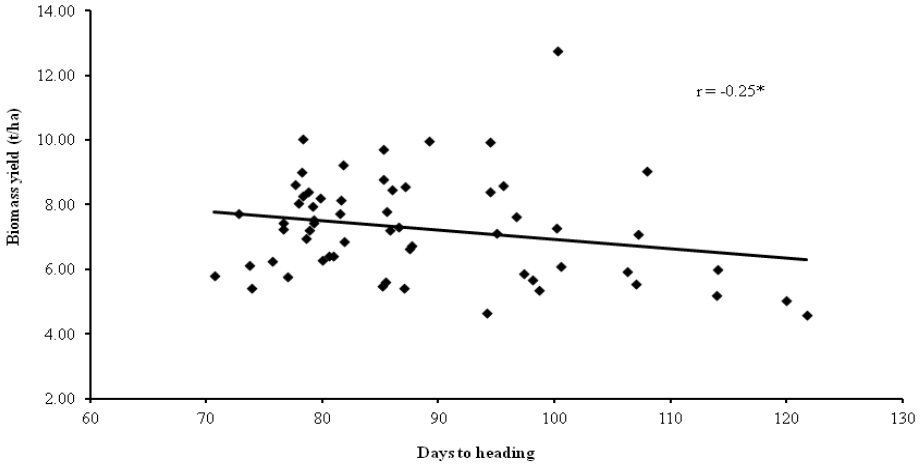

Fig. 4. 6 Relationship of days to heading and biomass yield (t/ha) among 60 rice accessions evaluated at Fogera

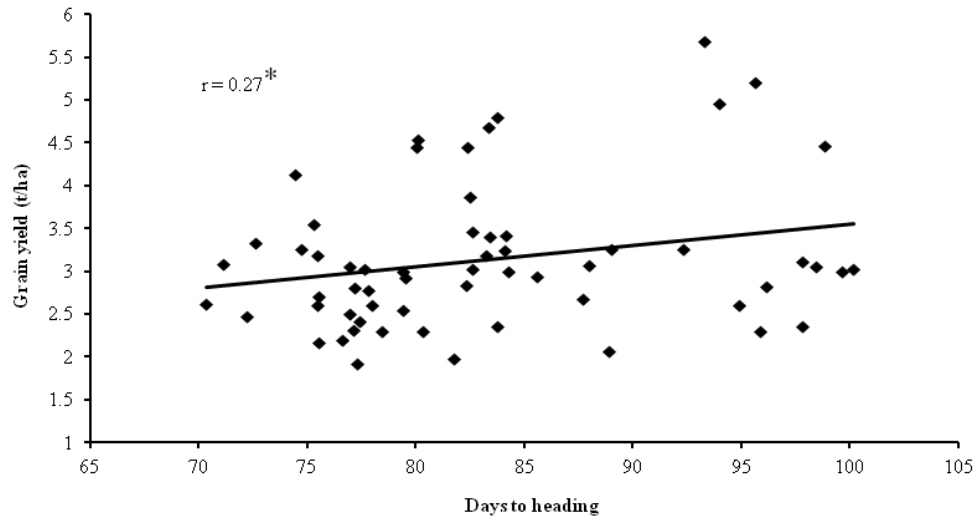

Fig. 4. 7 Relationship of days to heading and grain yield (t/ha) among 60 rice accessions evaluated at Pawe

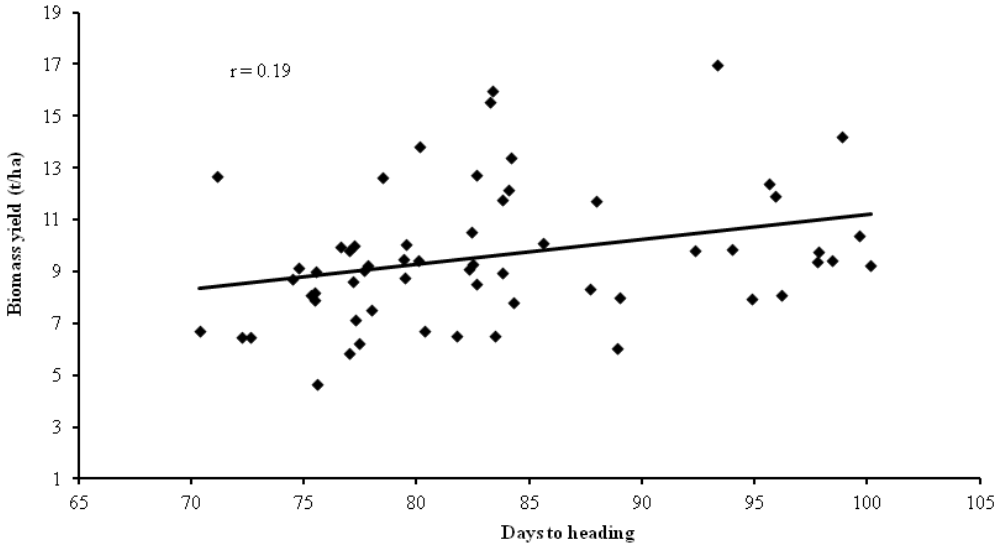

Fig. 4. 8 Relationship of days to heading and biomass yield (t/ha) among 60 rice accessions evaluated at Pawe

Biomass yield also showed positive correlation coefficients with plant height (r= 0.52**), filled grains per panicle (r= 0.29*), total grains per panicle (r= 0.20*), fertility rate (r= 0.30*), grain yield (r= 0.81***) at Fogera (Table S4. 1), and with days to panicle length (r= 0.20*), plant height (r= 0.48**), and grain yield (r= 0.59**) at Pawe (Table S4. 2), and with panicle length (r= 0.37*), plant height (r= 0.57**), filled grains per panicle (r= 0.28*), total grains per panicle (r= 0.23*), and grain yield (r= 0.57**) in combined data (Table S4. 3). The

degree of correlation among different traits is very important point in dealing with complex traits such as grain yield and others which are controlled by many genes and highly influenced by the environment (Amyaoha *et al*. 2018). Positive and significant correlation coefficients between grain yield and other traits suggested that selection in favor of these traits may lead to positive indirect selection for grain yield (Ahmadikhah 2010). Hence, selection for high grain yield can be achieved through selection of accessions for important traits that showed significant correlation coefficients with grain yield at both Fogera and Pawe.

Principal component analysis

Eigen values, Eigen vectors and percentage of variation explained through principal component analysis was presented in Table 4. 4. Principal components were computed from the correlation matrix and genotypic scores obtained for the first component and succeeding components with Eigen values greater than unity (Jeger *et al*. 1983). PCA measures the contribution of each component or independent impact of a particular trait to the total variance observed in a given population in relation to the traits of interest to the breeder (Anyaoha *et al* 2018). The results revealed that the first four significant principal component axes accounted for 81.07%, 74.95%, and 80.63% of total phenotypic variation at Fogera, Pawe and the combined data, respectively (Table 4. 4). PC1 only showed 43.67%, 31.30% and 28.97% of the variation at Fogera, Pawe, and the combined data, respectively in which filled grains per panicle at Fogera and Pawe, and days to maturity in combined data contributed for maximum variation.

Table 4. 4 Principal component analysis of quantitative traits for 60 rice accessions at Fogera, Pawe and combined data

Parameter	Fogera				Pawe				Combined			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Eigen value	4.804	1.858	1.217	1.038	3.44	1.96	1.73	1.11	3.186	2.579	1.848	1.255
Variation (%)	43.67	16.89	11.07	9.44	31.30	17.83	15.73	10.09	28.97	23.45	16.8	11.41
Comulative (%)	43.67	60.56	71.63	81.07	31.30	49.13	64.86	74.95	28.97	52.42	69.22	80.63
Eigen vector												
Days to heading	-0.33	0.29	-0.10	0.35	0.35	0.03	-0.51	0.04	-0.46	0.11	0.22	0.26
Days to maturity	-0.35	0.32	-0.05	0.23	0.35	0.07	-0.46	0.01	-0.47	0.11	0.19	0.22
Plant height	0.28	0.28	0.41	0.32	0.09	0.51	0.16	0.03	0.24	0.28	0.40	-0.19
Panicle length	0.33	0.26	0.10	0.15	0.17	0.26	0.45	-0.41	0.25	0.46	0.03	-0.15
Filled grains/panicle	0.37	0.24	-0.24	-0.31	0.48	-0.13	0.18	-0.11	-0.14	0.48	-0.33	-0.25
Fertility	0.36	-0.04	0.26	-0.05	0.22	-0.27	-0.20	0.08	0.41	0.00	-0.06	0.01
Total grains/panicle	0.26	0.33	-0.44	-0.37	0.47	-0.10	0.21	-0.12	-0.21	0.47	-0.32	-0.25
Grain yield	0.34	-0.24	-0.29	0.46	0.40	0.11	0.19	0.35	0.30	0.29	-0.01	0.58
1000 grain weight	0.13	-0.29	0.52	-0.18	-0.06	-0.05	0.25	0.79	0.22	-0.24	0.07	-0.37
Biomass yield	0.30	0.30	0.02	0.36	0.16	0.57	-0.07	0.21	0.18	0.30	0.51	0.17
Harvest index	0.18	-0.52	-0.37	0.30	0.20	-0.48	0.28	0.08	0.21	-0.01	-0.52	0.45

Cluster analysis and relationships with DNA clusters

Ward's method of hierarchical clustering based on 11 quantitative traits combined across two sites classified 60 rice accessions into four clusters (Fig. 4. 9). The largest number of accessions was found in Cluster I (22), followed by Cluster II (20). About 77% of accessions in Cluster I consist of improved accessions including NERICAs. A Japonica type and popular lowland landrace, X-Jigna, and other four landraces also belonged to this cluster (Table 4. 5). Accessions in Cluster I were relatively early heading types with intermediate mean values for plant height, panicle length, grain yield, and biomass yield while accessions in Cluster II were intermediate heading types with higher mean values for grain yield, and biomass yield (Table 4. 6). Nearly, 95% of accessions in Cluster I belonged to a DNA cluster Ia which comprised entirely Japonica-like accessions. Accessions in Cluster II comprised 50% Ia, 5% Ib and 45% II (Table 4. 5).

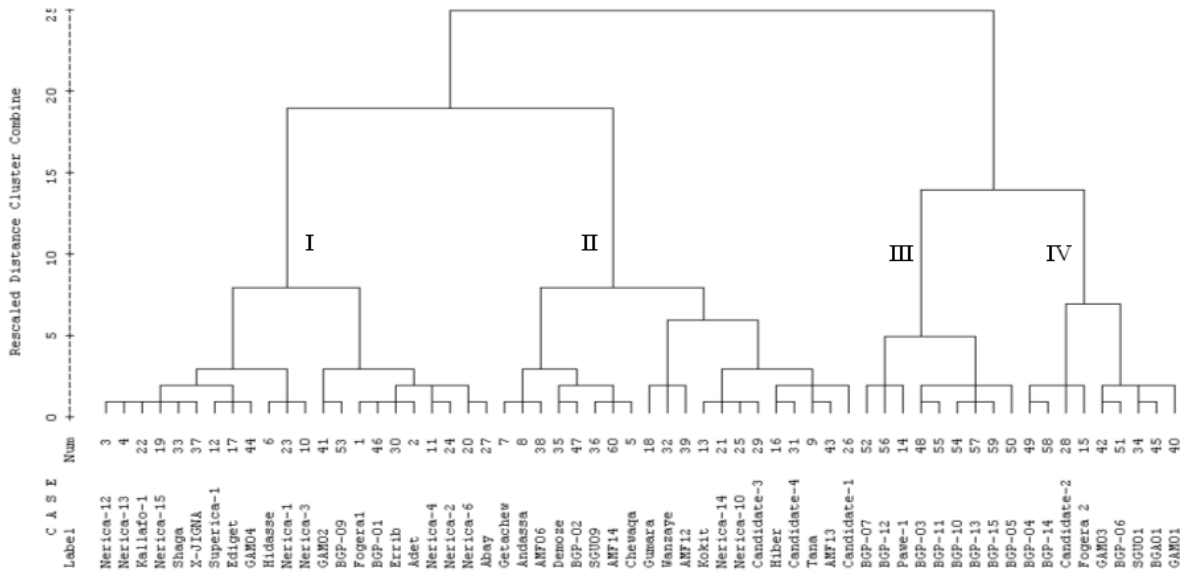

Fig. 4. 9 Hierarchical clustering of 60 rice accessions using Ward's method constructed based on Euclidean distance estimated for selected quantitative traits.

Table 4. 5 Distribution of 60 rice accessions by phenotype clusters and relationships with DNA clusters

Phenotype Cluster	No. of accessions (%)	Accessions by DNA clusters (n, %)[a]		
		Ia (44, 73.3%)	Ib (3, 5%)	II (13, 21.7%)
I	22 (36.7)	Fogera1, Adet, Nerica-12, Nerica-13, Hidasse, Nerica-3, Nerica-4, Superica-1, Ediget, Nerica-15, Nerica-6, Kallafo-1, Nerica-1, Nerica-2, Abay, Errib, Shaga, GAM02, GAM04, BGP-01, BGP-09	X-Jigna	-
II	20 (33.3)	Chewaqa, Kokit, Hiber, Nerica-14, Nerica-10, Candidate-3, Candidate-4, Wanzaye, AMF13, AMF14	Demoze	Getachew, Andassa, Tana, Gumara, Candidate-1, AMF06, AMF12, SGU09, BGP-02
III	9 (15.0)	Pawe-1, BGP-03, BGP-05, BGP-07, BGP-10, BGP-11, BGP-12, BGP-13, BGP-15	-	-
IV	9 (15.0)	SGU01, BGP-04, BGP-06, BGP-14	GAM01	Fogera 2, Candidate-2, BGA01, GAM03

[a] n: refers to number of accessions by each DNA cluster. Ethiopian accessions were clustered into three clusters, Ia, Ib and II (Chapter 2).

Clusters III and IV each comprised nine accessions dominated by landraces (Table 4. 5). Cluster III comprised rice accessions entirely from DNA cluster Ia while Cluster IV were composed from landraces and improved accessions of upland and lowland rice which were clustered into Ia, Ib and II (Table 4. 5). Accessions belonging to Cluster III were late heading

types and they had the lowest mean values for plant height, panicle length, fertility rate, grain yield, biomass yield, and harvest index (Table 4. 6). Thus, accessions grouped to contrasting clusters can be used for cross breeding to improved cultivars for different traits.

Table 4. 6 Mean values of group of accessions for selected quantitative traits by cluster

Cluster	Trait										
	DTH	DTM	PH	PL	FGP	FR	TGP	Gy	By	HI	TSW
I	79.2	121.5	90.3	19.5	105.9	92.0	114.9	2.9	8.0	37.9	27.0
II	81.8	124.0	93.5	19.5	89.3	92.5	96.5	3.1	9.1	36.2	27.3
III	99.8	141.6	79.7	17.4	92.9	86.9	104.8	2.3	7.4	32.9	26.1
IV	97.3	140.4	96.1	20.2	115.5	90.6	126.2	3.1	8.8	34.7	24.9
Mean	89.5	131.9	89.9	19.2	100.9	90.5	110.6	2.8	8.3	35.4	26.3

DTH: days to heading, DTM: days to maturity, PH: plant height, PL: panicle length, FGP: filled grains per panicle, FR: fertility rate, TGP: total grains per panicle, Gy: grain yield, By: biomass yield, HI: harvest index, TSW: thousand seed weight

Discussion

The rice growing conditions at Fogera and Pawe are different with fluctuations over years at both locations. Fogera has short rainfall season and mostly faces moisture stress. It has low temperature effect on rice especially for NERICAs. Under good years, lowland rice field gets sufficient water at Fogera resulting high crop performance. However, Pawe has relatively long rainy season of better distribution with high temperature. Overall, the total rainfall for rice-growing season at Pawe is always higher than at Fogera. Different rice accessions have adapted to these areas. Investigating phenotypic diversity and traits relationship among these rice accessions play important role to identifying potential parental materials for varietal improvement towards certain traits of interest. In variety improvement, breeding gain requires heritable variation in important agronomic traits of the crop. Therefore, the available

genetic variation, heritability, and expected genetic gain in such important agronomic traits are useful to design effective breeding strategies (Jalata *et al.* 2011).

The wide range of significant genetic variation among 60 rice accessions for the traits considered suggest that there are opportunities to improve agronomic and yield traits in Ethiopian rice accessions. Accession by location interaction revealed significant variations for almost all traits which indicated that accessions showed inconsistent performance at the two locations for several traits including days to heading, days to maturity, panicle length, filled grains per panicle, total grains per panicle, fertility rate, grain yield, biomass yield, 1000 seed weight and harvest index. Grain yield performance of accessions showed relatively higher at Pawe than at Fogera and improved accessions were also better than landraces. In addition, most improved accessions and landraces were early heading at Pawe compared to at Fogera. This may be attributed to the favorable environmental conditions at Pawe such as high temperature and sufficient rainfall with high relative humidity. Most traits showed relatively high heritability estimates at both sites. They also exhibited high genetic advance. Most researchers agree that high heritability alone is not enough; but both high heritability and high genetic advance are needed (Sohrabi *et al*. 2012). In this report, days to heading, days to maturity, plant height, grain yield, and harvest index had relatively high heritability and high genetic advance.

Correlation analysis of quantitative traits at both locations and in combined data showed similar trend of relationship between most traits and different for some others. Correlation between traits is important as it helps breeder to select important characters from the traits studied (Sohrabi *et al.* 2012). Most of the traits such as yield and yield component traits are influenced by interaction of genotype and environment, and, therefore, selection based on correlation coefficient makes it easy for plant breeders (Ahmadikhah *et al*. 2008). In contrary to Fogera, at Pawe, days to heading and days to maturity showed positive and

significant correlation coefficients with most traits indicating that relatively late and high yielding varieties can be selected for Pawe due to the presence of long rainy season with optimum temperature. Earliness is not farmers' important criterion for rice at Pawe. Most farmers preferred high yielding and medium to late maturing type varieties. But early type varieties often face sprouting due to rainfall in addition to severe birds attack. However, at Fogera, negative and significant correlation coefficients of days to heading with grain yield and most other traits indicated the importance of early type varieties in Fogera. At this site, high yielding and early type varieties are preferred by most farmers due to shortage of rainfall for late heading type varieties.

Principal component analysis showed high diversity among 60 rice accessions as revealed by Eigen vectors of 11 quantitative traits. Most traits revealed high contribution to total phenotypic variation including days to heading, days to maturity, plant height, panicle length, total grains per panicle, filled grains per panicle, and grain yield at Fogera, and Pawe including in combined data. These traits also exhibited relatively high heritability estimates. Hierarchical cluster analysis based on 11 quantitative traits of combined data classified 60 accessions into four different clusters. Clusters I and III comprised accessions with entirely of Japonica/Japonica-like genetic background as described by our previous study. Clusters II and IV contained a mixture of accessions from different genetic background including Indica/Indica-like types and some Japonica/Japonica-like types. Results in this study demonstrated availability of genetic variation among Ethiopian rice accessions for quantitative traits to be exploited differently for the two contrasting environments.

Supplementary data

Table S4. 1 Correlation coefficients among 11 quantitative traits in 60 rice accessions evaluated at Fogera

Traits	DTH	DTM	PL	PH	FGP	TGP	FR	Gy	TSW	By	HI
DTH											
DTM	0.73***										
PL	-0.34*	-0.31*									
PH	-0.24*	-0.21*	0.60**								
FGP	-0.34*	-0.30*	0.41**	0.34*							
TGP	-0.17	-0.12	0.33*	0.22*	0.92***						
FR	-0.46**	-0.44**	0.31*	0.368	0.53**	0.14					
Gy	-0.34*	-0.38*	0.41*	0.45**	0.25*	0.14	0.30*				
TSW	-0.25*	-0.27*	0.09	0.25*	0.05	-0.01	0.13	0.16			
By	-0.25*	-0.25*	0.41**	0.52**	0.29*	0.2	0.30*	0.81***	0.07		
HI	-0.51**	-0.57**	0.36*	0.29*	0.23*	0.09	0.34*	0.66**	0.17	-0.27*	

*, **, *** significant at P<0.05, 0.01, and 0.001, respectively. DTH: days to heading, DTM: days to maturity, PH: plant height, PL: panicle length, FGP: filled grains per panicle, TGP: total grains per panicle, FR: fertility rate, Gy: grain yield, By: biomass yield , TSW: thousand seed weight, and HI: harvest index

Table S4. 2 Correlation coefficients among 11 quantitative traits in 60 rice accessions evaluated at Pawe

Traits	DTH	DTM	PL	PH	FGP	TGP	FR	Gy	TSW	By	HI
DTH											
DTM	0.71***										
PL	0.11	0.12									
PH	0.17	0.13	0.49**								
FGP	0.37*	0.37*	0.45**	0.1							
TGP	0.35*	0.33*	0.45**	0.1	0.98***						
FR	0.12	0.21*	0.01	0.03	0.16	-0.02					
Gy	0.268	0.29*	0.24*	0.34*	0.04	0.03	0.1				
TSW	-0.23*	-0.24*	-0.19	-0.06	-0.08	-0.07	-0.09	0.22*			
By	0.19	0.19	0.20*	0.48**	-0.02	-0.04	0.14	0.59**	0.28*		
HI	-0.02	0.03	0.05	-0.07	0.15	0.15	0.02	0.16	0.04	-0.31*	

*, **, *** significant at P<0.05, 0.01, and 0.001, respectively. DTH: days to heading, DTM: days to maturity, PH: plant height, PL: panicle length, FGP: filled grains per panicle, TGP: total grains per panicle, FR: fertility rate, Gy: grain yield, By: biomass yield , TSW: thousand seed weight, and HI: harvest index

Table S4. 3 Correlation coefficients among 11 quantitative traits in 60 rice accessions based on combined data at two sites

Traits	DTH	DTM	PL	PH	FGP	TGP	FR	Gy	TSW	By	HI
DTH											
DTM	0.69**										
PL	-0.2	-0.33*									
PH	-0.15	-0.33*	0.67**								
FGP	-0.07	-0.33*	0.54**	0.43**							
TGP	-0.01	-0.26*	0.52**	0.38*	0.98***						
FR	-0.35*	-0.49**	0.35*	0.42**	0.50**	0.32*					
Gy	-0.07	-0.08	0.28*	0.32*	0.054	0.02	0.17				
TSW	-0.23*	-0.19	-0.06	0.07	-0.05	-0.06	0.04	0.02			
By	-0.06	-0.22*	0.37*	0.57**	0.28*	0.23*	0.32*	0.57**	0.15		
HI	-0.13	-0.06	0.06	-0.04	0.03	0.03	0.04	0.298	0.08	-0.25*	

*, **, *** significant at P<0.05, 0.01, and 0.001, respectively. DTH: days to heading, DTM: days to maturity, PH: plant height, PL: panicle length, FGP: filled grains per panicle, TGP: total grains per panicle, FR: fertility rate, Gy: grain yield, By: biomass yield , TSW: thousand seed weight, and HI: harvest index.

Chapter 5

Genetic diversity, maternal lineage, and population structure analysis of wild rice from Ethiopia as revealed by chloroplast INDELs and SSR markers

Abstract

Oryza longistaminata, a perennial species of wild rice, is originated and widely distributed in Africa including Ethiopia. To elucidate maternal lineage, genetic diversity, and population structure, a total of 163 wild rice accessions in five natural populations (two in the north and three in the south) were collected from Ethiopia and characterized using chloroplast (cp) and SSR markers. Maternally inherited cpINDELs were developed and applied to clarify maternal lineages. *O. longistaminata* (n=19), *O. barthii* (n=20) and *O. glaberrima*(n=13) were used as control. Twenty plastid type combinations were detected. Materials in north group were dominated by plastid types, Type 1, and 6 while the south group carried only Types 1, 2 and 3. Parts of wild rice in north group shared Type 6 with control *O. longistaminata* and Type 1 was shared between the north and south groups. Types 2, and 3 were unique to the south group while Type 6 to the north group. SSR markers showed that Ethiopian populations had high genetic diversity and overall genetic variation within populations was significantly higher ($P<0.001$) than between populations. Phylogenetic tree analysis and model-based population structure analysis (K=5) showed similar trends of relationship among accessions. In this study, the variation in these natural wild rice populations suggested that this wild species is regarded as valuable genetic resource for future rice breeding. Thus, this wild rice should be conserved both in ex suit and as natural population to maintain its genetic diversity.

Keywords Wild rice, Chloroplast, *O. longistaminata*, *O. barthii*, *O. glaberrima*, Ethiopia

Introduction

While Asian rice, *O. sativa*, has been domesticated from *O. nivara,* African rice belonging to *O. glaberrima* has been domesticated from its wild ancestor, *O. barthii* (formerly known as *O. breviligulata)* (Linares 2002; Oka 1988). *O. longistaminata* is another AA genome type related to *O. glaberrima* and widely distributed in Africa. Africa is also home to other wild rice species of *Oryza* including; *O. punctata* Kotschy ex Steud. (2n=24), *O. schweinfurthiana* Prod. (4n=48), *O. eichingeri* A. Peter. (2n=24), and *O. brachyantha* A. Chev. Et Roehr.(2n=24), with BB, BBCC, CC and FF genomes, respectively (Wambugu *et al*. 2013). Though these wild relatives are phenotypically inferior to cultivated rice, with respect to agronomic traits, they are regarded as vast reservoir of genes for biotic and abiotic stresses resistance (Jena 2010; Sanchez *et al*. 2014). They offer genes for diseases resistance, weeds suppression ability, high nitrogen-use efficiency and tolerance to drought and soil toxicity (Girma *et al*. 2010; Kaewcheenchai *et al*. 2018; Ndjiondjop *et al*. 2018; Orn *et al*. 2015; Thomas *et al*. 2017).

In previous studies, notable attempts have been made to identify and transfer useful genes to accessions from AA genomes of African wild rice, *O. longistaminata* and *O. barthii*. Khush *et al*. (1990) identified a broad-spectrum resistance gene (*Xa-21*) for bacterial blight (BB) from *O. longistaminata* and they successfully transferred this gene to a susceptible *O. sativa* improved accession, IR24, through crossing and following four subsequent backcrossing of F1 with the recurrent parent (IR24). Several other improved varieties carrying the *Xa-21* gene, through marker-assisted breeding, have subsequently been released in different countries (Wambugu *et al*. 2013). Brar and Khush (2002) and Sanchez *et al*. (2014) also reported that *O. barthii* possesses resistance genes for green leaf hopper, bacterial blight, and tolerance to heat and drought. Despite these benefits, wild rice resources are

increasingly threatened due to human pressure and climate changes (Kaewcheenchai *et al.* 2018; Ndjiondjop *et al.* 2018; Thomas *et al*. 2017).

In Ethiopia, among the two AA genome African wild rice, *O. longistaminata* and *O. barthii*, the former is predominantly growing as natural population in the Amhara (Northwestern) and Gambella (Southwestern) regions since early times though no sufficient information is available with regard to their extent of genetic variation and relationship (Dadi and Engels 1986; Girma *et al*. 2010; Melaku *et al*. 2013). Asian cultivated rice was introduced into Ethiopia by foreign experts. At that time, wild rice species were used as main source of cattle feed in Fogera plains of Amhara region at times when farmers' major livelihood was based on cattle rearing. But now, it is considered as one of noxious weeds to rice and hence is regularly being destroyed, mainly during the cropping season.

Wild rice has not been the focus of research in the national rice breeding program and hence details of genetic diversity and evolutionary relationships in wild rice populations of Ethiopia remain less investigated. Reports indicated that *O. longistaminata* has higher genetic diversity than *O. barthii* mainly because of its dual nature of perpetuation; perenniality and seed reproduction by out crossing (Dadi and Engels 1986; Kiambi *et al*. 2005; Sharma 1983). Park *et al*. (2003) also compared genetic variation among African rice (*O. longistaminata, O. glaberrima,* and *O. barthii*), Asian rice (*O. sativa, O. rufipogon, and O. nivara*) and Australian wild rice (*O. meridionalis*) groups based on MITE-AFLP (miniature inverted-repeated transposable elements-amplified fragment length polymorphism) techniques and they found that *O. longistaminata* showed the highest genetic variation.

Under Ethiopian condition, the natural habitats of wild rice have been shrinking very rapidly due to changes in farming systems, expansion of agricultural practices, urbanization, and other human disturbances (Dadi and Engels 1986; Melaku *et al*. 2013). Such human disturbances combined with the lack of in situ conservation programs exposes genetic

resources to possible genetic erosion or extinction (Wambugu *et al*. 2013). In order to obtain maximum benefits from these genetic resources, it is imperative that they are collected, efficiently conserved and optimally utilized (Wambugu *et al*. 2013). However, so far there is not any visible effort towards *in situ* conservation plan of wild rice natural populations in Ethiopia. Moreover, information is lacking with regard to their maternal lineages, genetic diversity, and population structures based on different molecular markers in comparison to other wild rice accessions of Africa origin.

In the present report, we developed chloroplast INDEL markers by using publicly available genomes of *O. longistaminata* and *O. barthii.* The maternally inherited markers can give how much diverse genetic resources existed in Ethiopia. Thus, we applied these chloroplast INDELs and simple sequence repeat (SSR) markers to clarify, maternal lineages, genetic diversity and phylogenetic relationships and population structure in Ethiopian wild rice accessions. This study will provide recommendations for strategic conservation and utilization of wild relatives in future rice breeding program.

Materials and Methods

Plant materials

Wild rice accessions were collected from natural populations of two regions in Ethiopia, Amhara and Gambella regions from November to December in 2016 (Fig. 5. 1). The two regions have contrasting environmental conditions. Collection sites from Amhara region (Fogera and Dera) are relatively with low temperature and frequently face terminal moisture stresses. In contrast, the collection sites in Gambella region (Abobo, Kera and Lare) are characterized with lower elevation, high temperature and high rainfall of high humidity. In

Gambella, wild rice grows in a wide range of open swampy fields and/ or river banks and relatively less disturbed. However, in Amhara region, it grows mainly along and /or in the vicinity of cultivated rice and bordering small ditches and marshy pocket plots. Wild rice in this region experiences much disturbance. In total, 163 accessions were collected in five populations; Fogera population (n = 65, Fogera1- 65) and Dera population (n = 35, Dera1-35) in Amhara region, and Abobo population (n=5, Abobo1-5), Kera population (n=25, Kera1-25) and Lare population (n=33, Lare1-33) in Gambella region in Ethiopia (Table S5. 1, Fig. 5. 1). As control, *O. barthii* (n=20) and *O. longistaminata* (n=19) were applied which are Core collections of the National BioResource Project in Japan (Nonomura *et al.* 2010). Accessions in the core collection originated most from West African countries and only three accessions from in East Africa (Table S5. 1). *O. glaberrima* (n=13) accessions were provided from Africa Rice Center as another control population. Details of the plant materials are indicated in Table S5. 1.

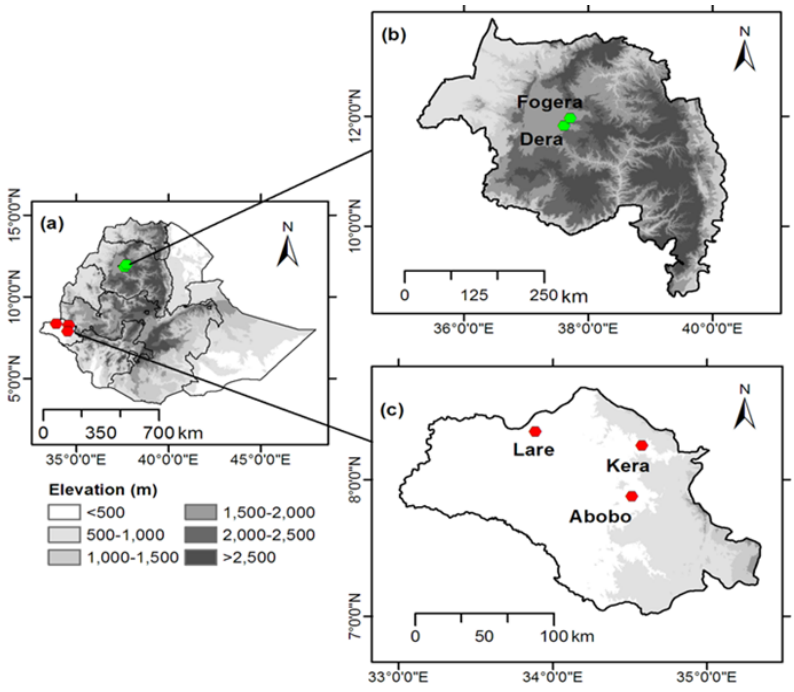

Fig. 5. 1 Wild rice collection sites in Ethiopia. (a) Ethiopia, (b) Amhara region and (c) Gambella region

Molecular markers

For investigating maternal lineages, eight sequences as whole chloroplast genome were used to develop chloroplast INDEL markers. Publicly available genome sequences of *Oryza barthii* and *Oryza longistaminata* were aligned to detect insertion/ deletion nucleotide from the pubMed-NCBI database (https://ww.ncbi.nlm.nih.gov/pubmed/). A total of 12 Afr-cpINDEL (African rice chloroplast INDEL) markers were developed. We evaluated their polymorphism using 39 wild rice core collections. Eventually, only eight of them showed stable and polymorphic banding patterns. Thus, the eight were applied for further analysis (Table S5. 2). A total of 16 polymorphic SSR markers were selected from our previous study of genetic diversity in cultivated rice varieties. These SSR markers were applied to assess genetic diversity of wild rice (Table S5. 2).

DNA extraction and genotyping

Genomic DNA was extracted from fresh leaves of two-week-old seedlings for each individual using urea method as described by Chen and Dellaporta (1993) with minor modifications. The SSR markers were amplified using a basic PCR cycle of preheating at 94ºC for 3 min, followed by 30-34 rounds at 95ºC for 10s, 50-55ºC (depending on primer type) for 30s, and 72ºC for 30s, and post-heating at 72ºC for 5 min with Thermopol *Taq* polymerase (NEB Ltd., Japan). Afr-cpINDEL markers were also amplified with supplier-recommended reaction buffer with 0.25 U rTaq (NEB Ltd, Japan). The PCR procedures were 95 ºC pre-heating for 3 min, followed by 35 cycles of 96 ºC for 30 s, 72 ºC for 1 min, and 75 ºC for 5 min. The amplifed DNA fragments were electrophoresed on 6% denaturing

polyacrylamide gel at 1500 V for 1:30 to 2 h in 0.5xTBE. The gels were stained with silver nitrate for visualizing DNA fragments.

Data analysis

Molecular data were subjected to statistical analysis using different analysis tools. The software GenAlEX6.5 (Peakall and Smouse 2012) was used to estimate the *Na* (number of different alleles per locus), *Ne* (effective alleles per locus), *Ho* (observed heterozygosity), and *He* (expected heterozygosity). The polymorphic information content (PIC) and Major allele frequency (MAF) were estimated by Power Marker v3.25 (Liu and Muse 2005). Phylogenetic tree analysis of accessions was performed using the neighbour-joing method based on Nei's unbiased genetic distances among accessions (Nei 1978) using Populations v1.2.32 and the tree was visualized and edited by tree explore of MEGA 7.0 (Kumar *et al.* 2016).

For population structure analysis, the whole data set of 215 accessions based on 16 SSR markers was used for investigating population structure using model-based software, Structure v2.3.4 (Pritchard *et al*. 2000). Five independent runs for each K (from 1 to 20) were performed using 100,000 Markov Chain Carlo (MCMC) repetitions and 50, 000 burn-in periods with the selection of admixture and correlated allele frequency models. The number of clusters (K) was estimated by computing the ad-hoc statistic ΔK, based on the rate of change in the log probability of the data between successive K values. The Evanno's ΔK method (Evano *et al*. 2005) was used for estimating LnP(D) values and the best K-value based on, on-line tool, Structure Harvester (Earl 2012) and hence the optimal K was two (K=2). Using the same software set up, a final run was carried out at 200,000 MCMC repetitions and 100, 000 burn-in periods. Based on the output, percentage of membership of

each individual to the corresponding K cluster was summarized. Population structure analysis was further performed K=5 using the same software set up.

Results

Maternal lineage and diversity in chloroplast genome

For discriminating wild rice accessions from Ethiopia based on their chloroplast genomes, eight cpINDELs developed using chloroplast genomes of two African wild species, *O. barthii* and *O. longistaminata* were applied to 163 Ethiopian accessions and 52 control accessions. Thirteen *O. glaberrima* accessions and 39 core collections representing 20 *O. barthii* and 19 *O. longistaminata* accessions were treated as controls. In total, 20 plastid types were detected among 215 accessions with different cpINDEL combinations (Tables 5. 1, 5. 2). Alternative genotypes were detected except for Afr-cpINDEL9, which showed multiple alleles (Table 5. 1). Multiple alleles resulted from single nucleotide repeats.

Table 5. 1 Plastid types detected among 215 rice accessions based on eight Afr-cpINDEL markers

cp-INDEL	Plastid type																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Afr-cpINDEL 1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
Afr-cpINDEL 3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1
Afr-cpINDEL 4	1	1	1	1	1	1	1	1	1	2	2	2	2	2	1	1	1	1	1	2
Afr-cpINDEL 5	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	2	1
Afr-cpINDEL 6	1	1	1	1	1	1	2	2	1	1	1	1	1	2	2	1	1	2	1	1
Afr-cpINDEL 7	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Afr-cpINDEL 9	1	2	3	1	1	2	1	1	3	1	1	2	3	1	1	1	3	1	3	1
Afr-cpINDEL 12	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	2	1	1	1	1

The control *O. barthii* population revealed the most diverse plastid types, followed by control *O. longistaminata* (Table 5. 2). The control *O. barthii* population did not share any plastid types with the control *O. longistaminata* population and with Ethiopian wild rice, but it shared one plastid type (Type 10) with *O. glaberrima* (Table 5. 2). *O. glaberrima* carried only two plastid types, Types 10 and 11, and neither of them was shared with Ethiopian accessions (Table 5. 2). These types detected in *O. glaberrima* differed only at Afr-cpINDEL12 to Type 11 in *O. barthii*, which was carried by three accessions, two from Guinea and one from Guinea-Bissau in West Africa (Table S5. 1).

Among 163 Ethiopian wild rice accessions, four plastid types were detected (Types 1, 2, 3, and 6), out of which three (Types 1, 2, and 3) were unique to Ethiopia (Table 5. 2). In the north group, accessions in the Dera population carried only Type 6, whereas the Fogera population carried two types, Types 1 and 6 (Table 5. 2). On the other hand, populations such as Kera and Lare carried two unique plastid types, Types 2 and 3, and Abobo population carried Types 1 and 2 (Table 5. 2).

Table 5. 2 Plastid types detected among Ethiopian wild rice populations as compared to control populations based on eight Afr-cpINDEL markers

Group	Population	No. of accessions	No. of plastid types	Plastid types (%)																				
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
North	Dera	35	1	-	-	-	-	-	35 (100)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Fogera	65	2	54 (83)	-	-	-	-	11 (17)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
South	Abobo	5	2	4 (75)	1 (25)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Kera	25	2		24 (96)	1 (4)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Lare	33	2		13 (39)	20 (61)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control	*O. barthii*	20	10	-	-	-	-	-	-	1 (5)	-	1 (5)	1 (5)	-	1 (5)	1 (5)	-	-	1 (5)	9 (45)	3 (15)	1 (5)	1 (5)
	O. longistaminata	19	6	-	-	-	6 (32)	1 (5)	5 (26)	-	2 (11)	-	-	-	-	-	1 (5)	4 (21)	-	-	-	-	-
	O. glaberrima	13	2	-	-	-	-	-	-	-	-	-	10 (77)	3 (23)	-	-	-	-	-	-	-	-	-

Type 6 was widely distributed in Tanzania, Mali, Cameroon, Ivory Coast, and Nigeria (Table S5. 1). This plastid type (Type 6) from north group shared with control *O. longistaminata*

(Table 5. 1). The plastid types in Ethiopia differed from Type 6 only at two cpINDELs, Afr-cpINDEL7 and 9 comprising from single nucleotide repeats. Types 1, 2, and 3 differed only at a single locus, Afr-cpINDEL9 (Table S5. 1) and they all were not shared with any control group. Maternal lineage of wild rice accessions from Ethiopia was not investigated and reported before. Hence, this result could be useful information for further investigation in wild rice in Ethiopia.

Genetic diversity using SSR markers

A detail of genetic diversity statistics based on 16 SSR markers genotyped across the whole population and for individual population is presented in Table 5. 3. The whole population revealed a total of 155 alleles, with an average of 9.69 alleles per locus (*Na*), ranging from 4 (RM508) to 14 (RM3138). The estimates of effective alleles per locus (*Ne*) and major allele frequency (MAF) ranged from 1.52 to 9.06 (average, 5.03) and from 0.17 to 0.81 (average, 0.39), respectively. The number of mean multiple alleles per locus (*Na*) from Ethiopian populations tended to be relatively lower than in control *O. longistaminata* population but higher than in control *O. barthii* population and *O. glaberrima* population except for Abobo and Kera. In Ethiopian populations, the highest total number of alleles was observed in Fogera population (100), followed by Lare (98), Dera (96), Kera (81), and Abobo (46) (Table 5. 3). Among five Ethiopian populations, Fogera, Lare, and Dera showed diverse alleles per locus with 6.25, 6.13, and 6.00 alleles, respectively (Table 5. 3).

The observed heterozygosity (*Ho*) in the whole population ranged from 0.04 to 0.97 with an average of 0.24 while genetic diversity or expected heterozygosity (*He*) ranged from 0.34 to 0.89 with an average of 0.73 (Table 5. 3). The mean observed herterozygosity (*Ho)* across 16 SSR markers in five Ethiopian populations ranged from 0.21 (Kera) to 0.29

(Fogera), relatively lower than in control *O. longistaminata* population but higher than that of control *O.barthii* and *O. glaberrima* populations (Table 5. 3). Fogera population revealed the highest genetic diversity (*He* = 0.67), followed by Dera population (*He* = 0.62) while Abobo population showed the lowest genetic diversity (*He* = 0.55). The lower genetic diversity at Abobo may be attributed small number of samples and yet, it showed higher genetic diversity than the control populations, *O. barthii* and *O. glaberrima* (Table 5. 3). Compared to the control populations which were originated from diverse countries in Africa, natural populations of Ethiopia maintain high genetic diversity. Only one core collection, *O. longistaminata,* showed relatively higher genetic diversity (*He* = 0.70) than populations from Ethiopia while other control populations exhibited the lowest genetic diversity (Table 5. 3).

Table 5. 3. Genetic diversity statistics of five Ethiopian wild rice populations, three control populations and the whole population based on 16 SSR markers

Locus	Chr.	Whole population (n=215)					Control populations									Ethiopian populations													
							O. barthii (n=20)			*O. longistaminata* (n=19)			*O. glaberrima* (n=13)			Dera (n=35)			Fogera (n=65)			Abobo (n=5)			Kera (n=25)			Lare (n=33)		
		Na	*Ne*	*Ho*	*He*	*MAF*	*Na*	*Ho*	*He*	*Na*	*Ho*	*He*	*Na*	*Ho*	*He*	*Na*	*Ho*	*He*	*Na*	*Ho*	*He*	*Na*	*Ho*	*He*	*Na*	*Ho*	*He*	*Na*	*Ho*	*He*
RM3604	1	5	2.21	0.25	0.55	0.59	2	0.00	0.10	4	0.21	0.37	1	0.00	0.00	4	0.43	0.47	2	0.34	0.39	2	0.40	0.36	2	0.16	0.37	2	0.18	0.17
RM406	2	6	1.95	0.19	0.49	0.70	3	0.00	0.46	5	0.16	0.51	2	0.00	0.15	5	0.31	0.37	3	0.31	0.36	2	0.40	0.36	2	0.12	0.12	4	0.06	0.12
RM3865	2	13	8.07	0.34	0.88	0.25	2	0.05	0.05	12	0.58	0.88	1	0.00	0.00	11	0.40	0.87	13	0.48	0.88	4	0.20	0.78	9	0.24	0.78	13	0.30	0.90
RM8208	3	10	7.62	0.07	0.87	0.18	5	0.00	0.78	5	0.00	0.77	1	0.00	0.00	7	0.11	0.76	8	0.05	0.86	5	0.60	0.76	8	0.08	0.87	10	0.06	0.89
RM168	3	10	4.46	0.18	0.78	0.37	3	0.05	0.30	10	0.16	0.87	1	0.00	0.00	6	0.23	0.49	9	0.29	0.82	4	0.20	0.78	5	0.16	0.69	4	0.06	0.57
RM3367	4	10	4.05	0.11	0.76	0.42	4	0.10	0.76	10	0.42	0.81	2	0.00	0.44	5	0.09	0.66	4	0.08	0.70	2	0.00	0.53	3	0.16	0.49	2	0.06	0.06
RM3663	5	12	6.21	0.97	0.84	0.30	6	1.00	0.67	7	1.00	0.82	2	1.00	0.52	8	1.00	0.73	7	0.95	0.79	3	1.00	0.69	4	1.00	0.69	7	0.91	0.78
RM3138	6	14	9.05	0.12	0.89	0.20	7	0.05	0.76	10	0.16	0.91	2	0.00	0.37	11	0.29	0.88	11	0.08	0.89	4	0.00	0.80	7	0.08	0.79	13	0.12	0.91
RM508	6	4	3.11	0.08	0.68	0.38	4	0.05	0.57	2	0.05	0.15	1	0.00	0.00	4	0.06	0.68	4	0.12	0.62	3	0.00	0.62	3	0.08	0.58	3	0.09	0.62
RM7121	7	11	5.49	0.30	0.82	0.32	4	0.00	0.59	7	0.47	0.83	2	0.00	0.49	6	0.46	0.77	6	0.42	0.78	3	0.40	0.71	5	0.20	0.49	3	0.18	0.27
RM3395	8	9	5.68	0.21	0.83	0.29	9	0.00	0.87	6	0.00	0.80	3	0.00	0.39	5	0.37	0.74	7	0.26	0.71	1	0.00	0.00	6	0.12	0.74	8	0.39	0.84
RM7356	8	10	1.85	0.40	0.46	0.25	6	0.20	0.32	4	0.58	0.67	4	0.54	0.44	4	0.09	0.21	2	0.52	0.39	2	0.20	0.20	3	0.16	0.22	3	0.67	0.67
RM7048	9	11	5.99	0.36	0.84	0.81	3	0.05	0.37	8	0.68	0.67	1	0.00	0.00	6	0.17	0.76	8	0.45	0.83	2	0.20	0.56	8	0.16	0.79	9	0.70	0.83
RM8201	10	10	4.24	0.01	0.77	0.71	1	0.00	0.00	7	0.00	0.84	1	0.00	0.00	5	0.09	0.67	5	0.00	0.65	3	0.00	0.71	4	0.00	0.69	4	0.00	0.44
RM5704	11	13	8.95	0.20	0.89	0.17	3	0.00	0.46	8	0.16	0.83	1	0.00	0.00	8	0.11	0.79	9	0.12	0.87	4	0.40	0.78	10	0.64	0.82	12	0.30	0.86
RM7376	12	7	1.52	0.07	0.34	0.32	10	0.05	0.89	4	0.16	0.42	6	0.08	0.81	1	0.00	0.00	2	0.12	0.12	2	0.20	0.20	2	0.04	0.04	1	0.00	0.00
Total		155					72			109			31			96			100			46			81			98		
Mean		9.69	5.03	0.24	0.73	0.39	4.50	0.10	0.50	6.81	0.30	0.70	1.94	0.10	0.23	6.00	0.26	0.62	6.25	0.29	0.67	2.88	0.26	0.55	5.06	0.21	0.57	6.13	0.26	0.56

Na : number of alleles, *Ne* : nunmber of effective alleles, *Ho* : observed heterozygosity, *He* : expected heterozygosity, *MAF* : major allele frequency

Analysis of molecular variance based on genetic distance between populations revealed significantly higher variation ($p<0.001$) within populations than between populations

explaining 74% and 26% of the total variation, respectively which indicated that individuals from different populations shared common alleles (more similar) while individuals within populations harbored less common alleles (less similar) (Table 5. 4).

Table 5. 4 Analysis of molecular variance (AMOVA) in wild rice accessions using 16 SSR markers

Source of variation	Degree of freedom	Sum square	Mean square	Variances estimated	Explained vaiance	P value
Among populations	7	1030.67	147.24	5.22	26%	
Within population	207	3145.22	15.19	15.19	74%	0.001
Total	214	4175.89		20.41	100%	

Phylogenetic relationship

The neighbor-joining tree constructed using genomic data of 16 SSR markers classified 215 accessions into five clusters (Fig. 5. 2). Cluster I corresponds to *O. barthii* and *O. glaberrima* control populations, Cluster II to *O. longistaminata* conrol population, Cluster III to Fogera and parts of Dera populations with few admixtures from Abobo and Kera, Cluster IV to Kera, Lare and parts of Abobo populations, and Cluster V to Dera and parts of Fogera populations (Fig. 5. 2). Accessions from the north group (Fogera and Dera) were classified into two clusters, Cluster III and V, whereas the south group fell between the two except for Abobo population and some accessions from Kera which distributed to Clusters III and IV. This suggested that accessions in the north group comprised genetically divergent individuals compared to the south group. The control *O. barthii* and *O. glaberrima* populations were distinctly grouped, whereas the control *O. longistaminata* population tended to be more closely related to parts of accessions from the north group (Fig. 5. 2).

A relationship among five wild rice collection sites and their relation to control populations was illustrated (Fig. 5. 3). Dera and Fogera were closely grouped. Similarly, two

sites from Gambella region, Abobo and Kera showed closer relationship while Lare tended to be out grouped. Among three control populations, *O. longistaminata* generally showed closer relationship to all five collection sites. However, the other two control populations, *O. barthii* and *O. glaberrima* were clustered together and they were distantly related to Ethiopian wild rice collection sites (Fig. 5. 3).

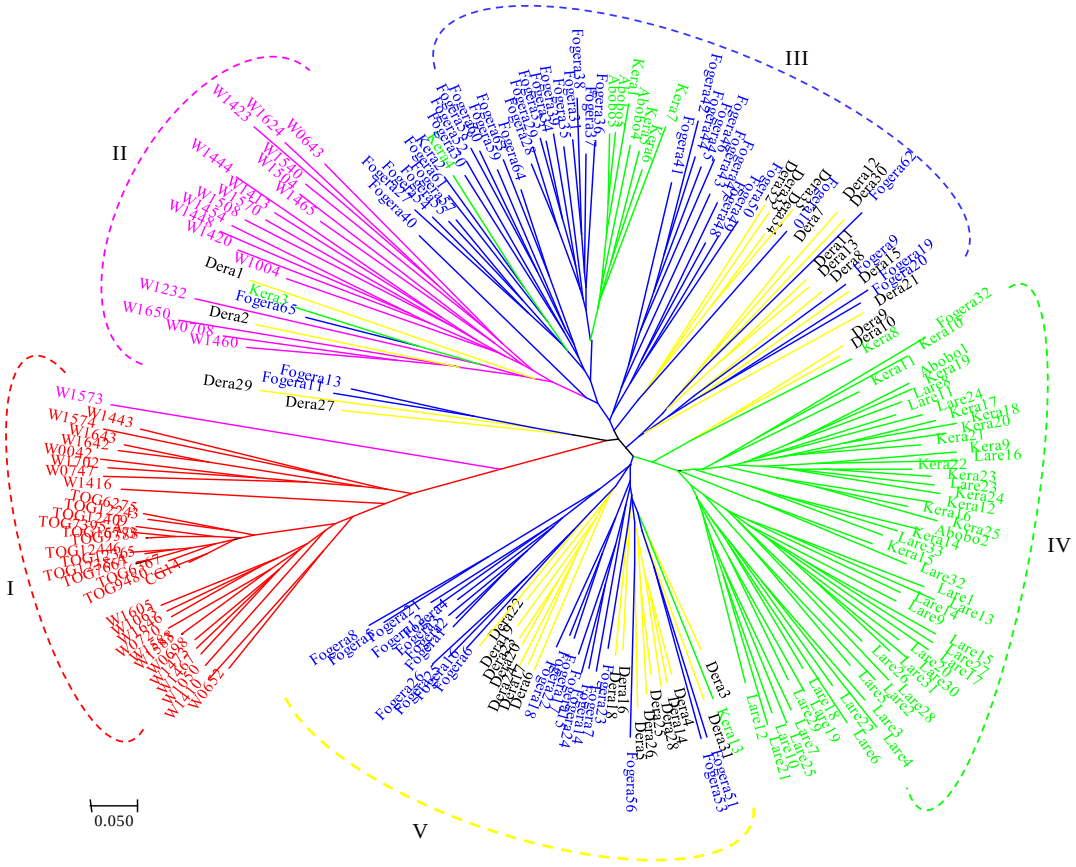

Fig. 5. 2 NJ method phylogenetic tree analysis of 215 accessions based on 16 SSR markers. I: *O. barhii* & *O. glaberrima*, II: *O. longistaminata*, III: Fogera and parts of Dera, IV: Kera and Lare, V: Dera and parts of Fogera.

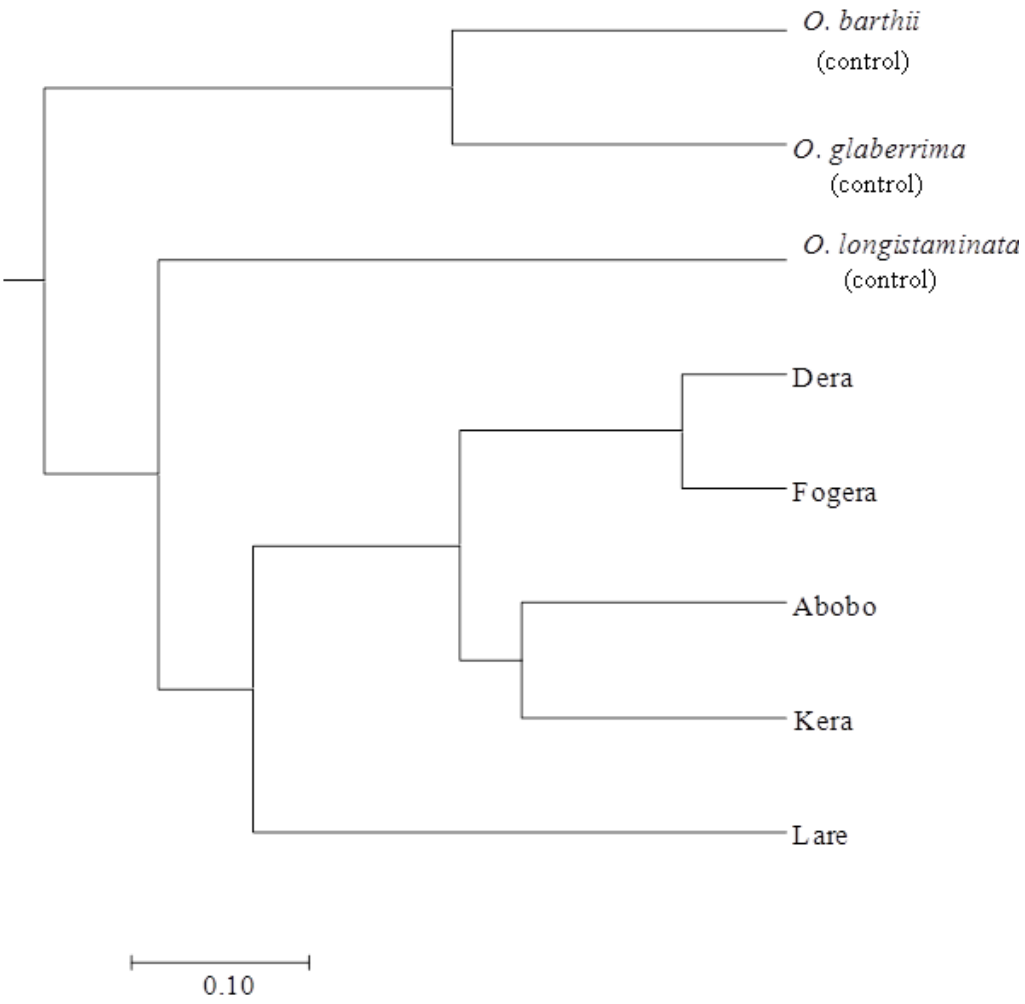

Fig. 5. 3 Relationship of wild rice collection sites compared to control populations

Population structure

The Bayesian model-based population structure analysis at the optimal K=2 showed that the 215 accessions were clustered into two subgroups, Group A and Group B (Fig. 5. 4A, B, C). Membership of individuals from each pre-defined population to the corresponding subgroup was estimated based on Pritchard *et al*. (2000) at the inferred ancestry probability cut-off point, $Q \geq 0.8$. Individuals with $Q < 0.8$ at the corresponding inferred cluster were grouped as admixture. From the whole accessions of eight pre-defined populations, 15.4% and 83.3% of

individuals clustered into subgroups, Group A and Group B, respectively with remaining 1.4% accessions identified as admixtures (Table 5. 5). Individuals from both the north and south groups clustered to Group B with control *O. longistaminata* population; whereas *O. barthii* and *O. glaberrima* to Group A (Table 5. 5, Fig. 5. 4C).

The LnP(D) score for the number of populations (K) continued increasing up to K=5 (Fig. 5. 4A). However, the Evano's ΔK value decreased to zero at K= 3 and then relatively increased at K= 4 and K=5 and then dropped to zero suggesting Group B was likely to be subdivided into other sub-groups at K=4 or K=5 (Fig. 5. 4B). Five subgroups were produced using structure analysis of 215 accessions at K=5 (Fig. 5. 4D). Out of 215 accessions, 163 Ethiopian accessions were classified into three subgroups; G2, G3, and G4, with the remaining 25 accessions identified as admixtures (Table 5. 6).

The first subgroup (G1) comprised exclusively of control *O. barthii* and *O. glaberrima* populations, and the fifth subgroup (G5) included control *O. longistaminata* population with four accessions as admixtures (Table 5. 6). The second subgroup (G2) comprised 48 accessions mainly from the south group, Lare (28), Kera (16), and Abobo (2), and two accessions from the north group. The third subgroup (G3) was composed of 44 accessions, the majority of which were from Fogera with a few accessions from Dera and; also 10 accessions from the south group, Abobo and Kera. Similarly, the fourth subgroup (G4) included 46 accessions, all of which were from Dera and Fogera (Table 5. 6). The results of model-based clustering at K=5 tended to show similar trend of grouping accessions as that of distance-based clustering. Both suggested the presence of three groups of wild rice gene pools in the natural populations of *O. longistaminata* in Ethiopia.

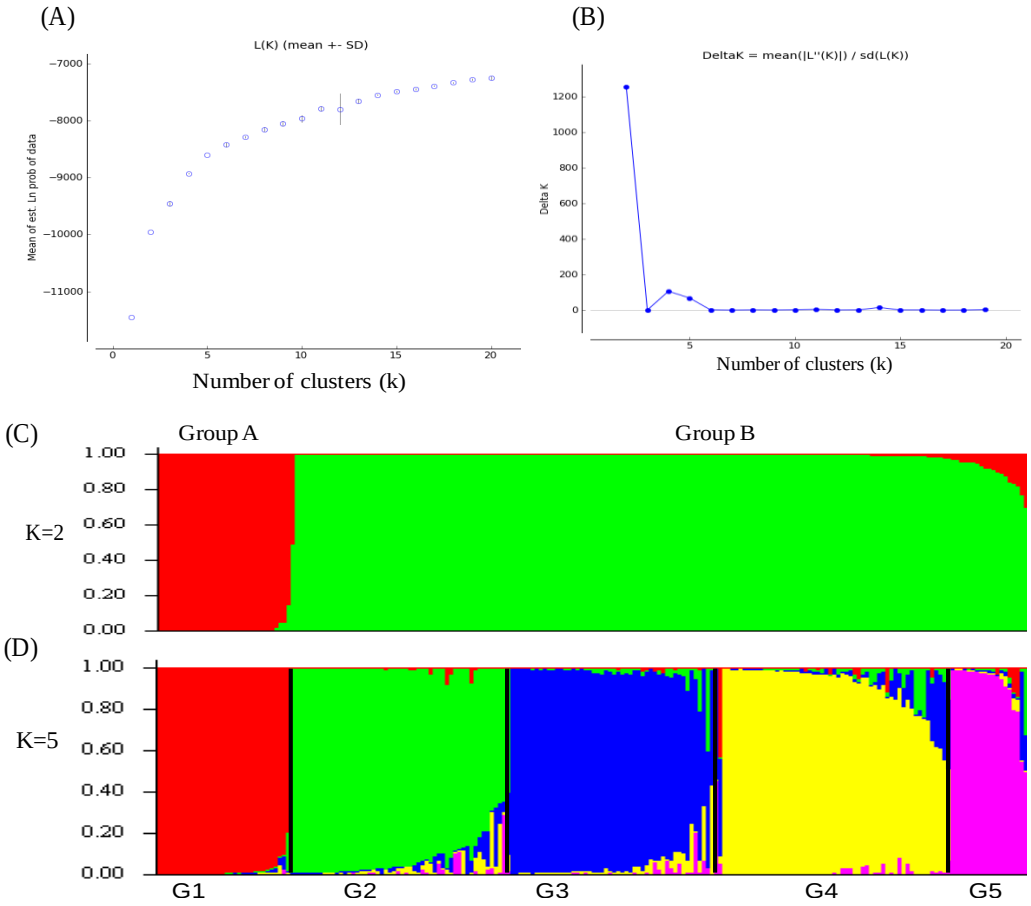

Fig. 5. 4 Model based population structure of 215 wild rice accessions based on 16 SSR markers by STRUCTURE. (A) Mean values of LnP(*K*) for 20 independent runs for each *K*, (B) Plot of Delta *K* values for each *K* (*K*= 1-20) based on the second order change of the likelihood function, (C) & (D) Graphs for inferred ancestry of individuals at *K*= 2 & K=5, respectively where Group A refers to *O. barthii* & *O. glaberrima*, Group B to control *O. longistaminata* & Ethiopian accessions; and G1 refers to *O. barthii* & *O. glaberrima*, G 2 to Kera & Lare, G 3 to Foger & Dera, G 4 to Dera & Fogera, and G 5 to *O. longistamnata*

Table 5. 5 Proportion of pre-defined population in each group after structure analysis at the best K (k=2)

Group	Population	No. of accessions	No. of accessions by subgroup (%)					
			Group A		Group B		Adimisxtutre	
			No.of accession	%	No.of accession	%	No.of accession	%
North	Dera	35	-	-	34	97.14	1	2.86
	Fogera	65	-	-	65	100	-	-
South	Abobo	5	-	-	5	100	-	-
	Kera	25	-	-	25	100	-	-
	Lare	33	-	-	33	100	-	-
Control	*O. barthii*	20	20	100	-	-	-	-
	O. longistaminata	19	-	-	17	89.47	2	10.53
	O. glaberrima	13	13	100	-	-	-	-
Total		215	33	15.4	179	83.3	3	1.4

Group A: control populations *O. barthii* and *O. glaberrima* ; and Group B: all accessions from Ethiopian and control population, *O. longistaminata.*

Table 5. 6 Relationships among pre-defined populations, plastid types, and population structure subgroups at K=5 for 215 rice accessions

Group	Population	No. of accessions	Plastid type	Subgroup[a]					
				G1	G2	G3	G4	G5	Admixtutre
North	Dera	35	6	-	1 (2.8)	3 (8.6)	21 (60.0)	-	10 (28.6)
	Fogera	65	1	-	1 (1.5)	31 (47.7)	16 (24.6)	-	6 (9.2)
			6	-	-	-	9 (13.8)	-	2 (3.1)
South	Abobo	5	1	-	2 (40.0)	2 (40.0)	-	-	-
			2	-	-	1 (20.0)	-	-	-
	Kera	25	2	-	16 (64.0)	7 (28.0)	-	-	1 (4.0)
			3	-	-	-	-	-	1 (4.0)
	Lare	33	2	-	10 (30.3)	-	-	-	3 (9.1)
			3	-	18 (54.5)	-	-	-	2 (6.1)
Control									
	O. barthii	20		20 (100.0)		-	-	-	-
	O. longistaminat	19		-		-	-	15 (78.9)	4 (21.1)
	O. glaberrima	13		13 (100.0)	-	-	-	-	-
	Total	215		33 (15.35)	48 (22.33)	44 (20.47)	46 (21.40)	15 (6.97)	29 (13.49)

[a] G1: *O. barthii* & *O. glaberrima* , G2: Kera & Lare, G3: Fogera & Dera, G4: Dera & Fogera, G5: *O. longistaminata*

Discussion

Maternal lineages and diversity in chloroplast genome

The study and conservation of wild relative populations should be a priority to secure genetic resources for future breeding programs (Fuchs *et al*. 2016), and this is particularly important for critical food crops such as rice (Fuchs *et al*. 2016; Thomas *et al*. 2017; Kaewcheenchai *et al.* 2018; Sandama *et al*. 2018). In Ethiopia, the presence and distribution of wild rice relatives and their genetic diversity has not been reported well except for limited reports (Dadi and Engels 1986; Girma *et al.* 2010; Melaku *et al*. 2013, 2018). Detailed evaluation of the genetic diversity of wild rice resources is essential in Ethiopia, which is considered as the edge of the distribution of *O. longistaminata* in East Africa. We presumed that unique genetic resources could be found compared with other areas in Africa. For the characterization of wild rice populations, newly developed chloroplast markers were applied to in order to trace

maternal lineages. In fact, the control *O. barthii*, and *O. longistaminata* populations exhibited diverse plastid types compared to wild rice populations from Ethiopia which could be attributed to their diverse origins in Africa. Only one control population, *O. longistaminata* shared only one plastid type with parts of Ethiopian population. *O. glaberrima* shared one of the two plastid types with *O. barthii*. Another plastid, Type 11, differed in a single cpINDEL, Afr-cpINDEL12, which might be caused by mutation after domestication of *O. glaberrima*. Both *O. glaberrima* and *O. barthii* populations never shared plastid types with wild rice populations from Ethiopia. When comparing natural populations in Ethiopia, different plastid types were detected between the north and south groups, some of them are unique to particular regions. However, these differences depended on just a few loci, which might indicate divergence through its dispersal to the edge of the distribution.

Genetic diversity and phylogenetic relationships based SSR markers

We applied 16 SSR markers to assess diversity and genetic structure in wild rice populations. Comparably, wild rice populations in Ethiopia showed higher genetic diversity compared to control *O. barthii* and *O. glaberrima* populations. Previous studies also indicated that *O. longistaminata* has higher genetic diversity than *O. barthii* (Sharma 1983; Dadi and Engels 1986; Kiambi *et al.* 2005). Particularly, populations in Amhara region, northern Ethiopia, exhibited higher genetic diversity which is in contrary to Melaku *et al*. (2013) who reported higher genetic diversity in wild rice accessions from Gambella, southern Ethiopia, than those from Amhara region.

Relationships as illustrated by neighbor-joining tree analysis based on Nei's genetic distance (Nei 1978) yielded five clusters in which accessions from Ethiopia were classified into three groups. In addition, relationships among collection sites and control populations

showed that control *O. longistaminata* population was closely related to Ethiopian sites while *O. barthii* and *O. glaberrima* were isolated and grouped together. These results suggested that these five sites considered in this study from Ethiopia are predominated by *O. longistaminata*. However, further investigation is needed covering large number of collections in wider areas and through application of different molecular markers such as simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) or combination of different molecular markers along with chloroplast markers to properly differentiate wild rice species and their diversity in Ethiopia.

Population structure and relationship with maternal lineages

The population structure of Ethiopian wild rice indicated that they actually belong to *O. longistaminata.* In combination with plastid types, it was suggested that unique maternal types were generated and dispersed through its dispersal process. The detailed structure also showed that there are four types based on population structure analysis. Among the four types, plastid Type 1 was carried by G2, G3, and G4. The subgroup, G2 with plastid Type 3 is only detected in Lare, in the south group. Subgroups, G2 and G3 with Type 2 were also unique to the south group. G4 was unique to the north group as it carried Type 6, which was not shared by the south group.

In this report, maternal lineage, genetic diversity, and population structure analysis showed that wild rice populations in Ethiopia are remarkably diverse. This is a good opportunity to proceed for more detailed analyses of the species to generate comprehensive information which could help how to protect this unique genetic resource in addition to searching for other wild rice species in Ethiopia. The distribution of *O. longistaminata* in northwestern and southwestern Ethiopia, which differed in environmental conditions,

revealed a wide range of adaptability of this species that can be utilized for the improvement of agricultural varieties (Dadi and Engels 1986). A small number of wild rice samples have been preserved at the Ethiopian Biodiversity Institute genebank for *ex situ* conservation (Girma *et al*. 2010; Wambugu *et al*. 2013), and very small number of samples from Ethiopia are also found at IRRI and AfricaRice gene banks (personal communication). However, the wide range of genetic variation in the country cannot be covered by these limited efforts. Moreover, as reported by Girma *et al*. (2010) and Jackson *et al*. (2010), Ethiopia is also one of the distribution sites of another African wild rice, *O. barthii*. However, its area of distribution and its availability is not properly known. Wild rice genetic resources are also diminishing increasingly. Therefore, in order to maintain the rich genetic diversity of wild rice genetic resources for use in breeding, natural populations should be protected from genetic erosion through *in situ* conservation, which can also promote evolutionary development and, more coverage of samples for ex situ conservation is also needed.

Supplementary data

Table S5. 1 Summary of wild rice accessions from Ethiopia and African wild rice core collections including *O. glaberrima* accessions used in this study

Accession	Type	Origin	Source or country[a]	Plastid type
W0652	*O. barthii*	Sierra Leone	NBRP	Type 18
W0747	*O. barthii*	Mali	NBRP	Type 18
W1050	*O. barthii*	Gambia	NBRP	Type 18
W1410	*O. barthii*	Sierra Leone	NBRP	Type 16
W1416	*O. barthii*	Sierra Leone	NBRP	Type 17
W1467	*O. barthii*	Cameroon	NBRP	Type 19
W1473	*O. barthii*	Chad	NBRP	Type 17
W1574	*O. barthii*	Nigeria	NBRP	Type 17
W1583	*O. barthii*	Chad	NBRP	Type 17
W1588	*O. barthii*	Cameroon	NBRP	Type 17
W1642	*O. barthii*	Botswana	NBRP	Type 17
W1643	*O. barthii*	Botswana	NBRP	Type 17
W1646	*O. barthii*	Tanzania	NBRP	Type 17
W1702	*O. barthii*	Mali	NBRP	Type 17
W0698	*O. barthii*	Guinea	NBRP	Type 9
W0720	*O. barthii*	Mali	NBRP	Type 13
W1443	*O. barthii*	Mali	NBRP	Type 10
W1605	*O. barthii*	Nigeria	NBRP	Type 12
W0042	*O. barthii*	No description	NBRP	Type 20
W1063	*O. barthii*	No description	NBRP	Type 7
W1460	*O. longistaminata*	Dahomey	NBRP	Type 14
W0708	*O. longistaminata*	Guinea	NBRP	Type 8
W1004	*O. longistaminata*	Ghana	NBRP	Type 8
W1232	*O. longistaminata*	Unknown	NBRP	Type 15
W1420	*O. longistaminata*	Mali	NBRP	Type 4
W1448	*O. longistaminata*	Ivory Coast	NBRP	Type 4
W1454	*O. longistaminata*	Burkina Faso	NBRP	Type 15
W1465	*O. longistaminata*	Nigeria	NBRP	Type 15
W1504	*O. longistaminata*	Tanzania	NBRP	Type 15
W1508	*O. longistaminata*	Unknown	NBRP	Type 4
W1540	*O. longistaminata*	Congo	NBRP	Type 5
W1570	*O. longistaminata*	Nigeria	NBRP	Type 4
W1413	*O. longistaminata*	Sierra Leone	NBRP	Type 4
W0643	*O. longistaminata*	Gambia	NBRP	Type 4
W1423	*O. longistaminata*	Mali	NBRP	Type 6
W1624	*O. longistaminata*	Cameroon	NBRP	Type 6
W1650	*O. longistaminata*	Tanzania	NBRP	Type 6
W1444	*O. longistaminata*	Ivory Coast	NBRP	Type 6
W1573	*O. longistaminata*	Nigeria	NBRP	Type 6
CG14	*O. glaberrima*	AfricaRice	AfricaRice	Type 10
TOG 12243	*O. glaberrima*	Africa Rice	AfricaRice	Type 10
TOG 6367	*O. glaberrima*	Africa Rice	AfricaRice	Type 10
TOG 7667	*O. glaberrima*	Africa Rice	AfricaRice	Type 10
TOG 9388	*O. glaberrima*	Nigeria	AfricaRice	Type 10
TOG 1238-A	*O. glaberrima*	Guinea-Bissau	AfricaRice	Type 10
TOG 12365	*O. glaberrima*	Guinea-Bissau	AfricaRice	Type 11
TOG 16773	*O. glaberrima*	Guinea	AfricaRice	Type 11
TOG 7395-A	*O. glaberrima*	Nigeria	AfricaRice	Type 10
TOG 12409	*O. glaberrima*	Guinea	AfricaRice	Type 11
TOG 6275	*O. glaberrima*	Liberia	AfricaRice	Type 10
TOG 12446	*O. glaberrima*	Chad	AfricaRice	Type 10
TOG 9480	*O. glaberrima*	Côte d'Ivoire	AfricaRice	Type 10
Dera1	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera2	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera3	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera4	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera5	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera6	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera7	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera8	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera9	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera10	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera11	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera12	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera13	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera14	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera15	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera16	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera17	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera18	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera19	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera20	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6

[a] NBRP: National BioResource Project, Japan.

Table S5. 1 (Continued)

Accession	Type	Origin	Source or country[a]	Plastid type
Dera21	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera22	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera23	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera24	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera25	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera26	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera27	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera28	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera29	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera30	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera31	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera32	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera33	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera34	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Dera35	*O. longistaminata*	Dera	Amhara, Ethiopia	Type 6
Fogera1	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera2	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera3	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera4	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera5	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera6	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera7	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera8	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera9	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera10	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera11	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 6
Fogera12	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera13	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera14	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera15	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera16	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera17	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera18	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera19	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera20	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera21	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera22	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera23	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera24	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera25	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera26	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera27	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera28	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera29	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera30	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera31	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera32	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera33	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera34	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera35	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera36	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera37	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera38	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera39	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera40	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera41	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera42	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera43	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera44	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera45	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera46	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera47	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera48	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera49	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera50	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera51	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera52	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera53	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera54	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera55	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera56	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera57	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1

Table S5. 1 (Continued)

Accession	Type	Origin	Source or country[a]	Plastid type
Fogera58	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera59	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera60	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera61	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera62	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera63	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera64	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Fogera65	*O. longistaminata*	Fogera	Amhara, Ethiopia	Type 1
Abobo1	*O. longistaminata*	Abobo	Gambella, Ethiopia	Type 1
Abobo2	*O. longistaminata*	Abobo	Gambella, Ethiopia	Type 1
Abobo3	*O. longistaminata*	Abobo	Gambella, Ethiopia	Type 1
Abobo4	*O. longistaminata*	Abobo	Gambella, Ethiopia	Type 1
Abobo5	*O. longistaminata*	Abobo	Gambella, Ethiopia	Type 2
Kera1	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera2	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera3	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera4	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera5	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera6	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera7	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera8	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera9	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera10	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera11	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera12	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera13	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 3
Kera14	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera15	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera16	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera17	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera18	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera19	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera20	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera21	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera22	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera23	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera24	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Kera25	*O. longistaminata*	Kera	Gambella, Ethiopia	Type 2
Lare1	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare2	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare3	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare4	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare5	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare6	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare7	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare8	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare9	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare10	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare11	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare12	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare13	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare14	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare15	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare16	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare17	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare18	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare19	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare20	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare21	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare22	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare23	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare24	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare25	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare26	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare27	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare28	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare29	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 2
Lare30	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare31	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare32	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3
Lare33	*O. longistaminata*	Lare	Gambella, Ethiopia	Type 3

Table S5. 2 List of molecular markers used in wild rice study

Marker	Chromosome/ genome	Primer sequence		Motif/type	Genome position[a]	Reference
		Forward	Reverse			
Nuclear SSR markers						
RM3604	Chromosome 1	ATGTCAGACTCCGATCTGGG	TCTTGACCTTACCACCAGGC	(GA)13	5140439	McCouch (2002)
RM406	Chromosome 2	GAGGGAGAAAGGTGGACATG	TGTGCTCCTTGGGAAGAAAG	(GA)17	35242091	McCouch (2002)
RM3865	Chromosome 2	AACCATGGACAGTTGAACAC	CTCCGACAAGAACTTCCTC	(GA)29	4414275	McCouch (2002)
RM8208	Chromosome 3	GCCCAAACTACACTCTCTTG	GTAAATGCCTGAGTGCCTAC	(AGA)12	22402431	McCouch (2002)
RM168	Chromosome 3	TGCTGCTTGCCTGCTTCCTTT	GAAACGAATCAATCCACGGC	T15(GT)14	28098678	McCouch (2002)
RM3367	Chromosome 4	GGATCCATCCATCCACTGAC	GGATATGTGCTGCTGTGTGC	(CT)16	24285096	McCouch (2002)
RM3663	Chromosome 5	CATCAACCTCCACGAACATG	CTCGGTGGTGATCCTCCTC	(GA)14	21426081	McCouch (2002)
RM3138	Chromosome 6	TTGACAAGAGATCAAGGCGG	GTGAATGTTGAGCTGCATGG	(CA)16	28470186	McCouch (2002)
RM508	Chromosome 6	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC	(AG)17	442849	McCouch (2002)
RM7121	Chromosome 7	GGAGATGGCACACGTCAAAC	AGGATCCCGTTTTGTAGCAG	(ATAA)6	5624074	McCouch (2002)
RM3395	Chromosome 8	ACCTCATGTCCAGGTGGAAG	AGATTAGTGCCATGGCAAGG	(CT)17	10294908	McCouch (2002)
RM7356	Chromosome 8	CCAAGGACACATATGCATGC	GCAATTCATGGCGCTGTTC	(CTAT)6	21282849	McCouch (2002)
RM7048	Chromosome 9	CAACCCCTAATTTCACGCTC	GACTTCACTGGCACTGGATG	(AATA)8	16936371	McCouch (2002)
RM8201	Chromosome 10	TCTGTTTATAAGCGCAGCAC	GCCGGCGAGCTACTACTAC	(CT)13	13833457	McCouch (2002)
RM5704	Chromosome 11	AAAAGTTTTGAATAAAACGAATG	ATGTGATTCTCCAAGCAGAG	(AAT)20	5481615	McCouch (2002)
RM7376	Chromosome 12	TCACCGTCACCTCTTAAGTC	GGTGGTTGTGTTCTGTTTGG	(GAAA)6	23477179	McCouch (2002)
Chloroplast INDEL markers				(Indel)		
Afr-cpINDEL1	Chloroplast	TTTCCGCTCCTTTTCTATCC	TGGATTGAAAAGGATGTTATG	AAAGA	3398^3399	KM088023
Afr-cpINDEL3	Chloroplast	TCAGTCCCGAACTAGGGTTC	CGCTATCAACCAGAAGTAG	T	16345^16355	KM103381
Afr-cpINDEL4	Chloroplast	AGTGAACCTTTGAAAGATAG	GTTACGTGGAGAAATCCAAG	T	4173^41374	KF359904
Afr-cpINDEL5	Chloroplast	CAAATAGAATTGCTTGACTTG	GAGGAAGTCTCTTGTAAATC	AACAAAAA	61081^61082	KM881642
Afr-cpINDEL6	Chloroplast	GGTTGGCTACTAAAATGAAAGG	ACTTAACTTAATTCTTCTAC	23bp	65067^65068	KM103371
Afr-cpINDEL7	Chloroplast	TTGTCGTAAGCATACGATTC	TAGATGAATACCCTCGATAC	13bp	65457^65458	KM088023
Afr-cpINDEL9	Chloroplast	TCCTTCCTTTTATCTACATC	AATAACCAACCTATTGCTTC	T	78966	KF359907
Afr-cpINDEL12	Chloroplast	AACTAAAAGATTCAAGGAAG	TAGAATTTTTTTGTTAGAATC	TTCT	109875^109876	KF359904

[a] Insertion sites are shown with ^ between the two sites

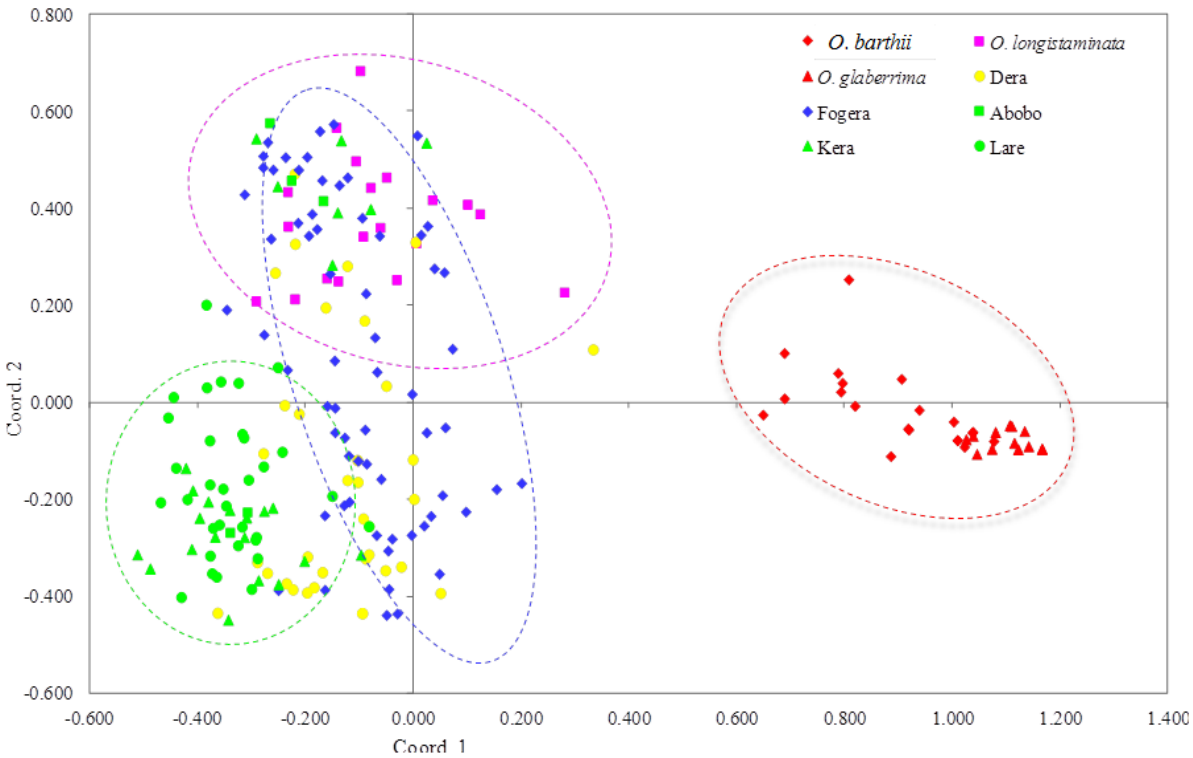

Fig. S5. 1 Principal coordinate analysis of 215 accessions based on 16 SSR markers

Chapter 6

General discussion

Introduction

After some years of stagnation following its first introduction into Ethiopia, rice has now become the second high yielding grain crop, after maize. Through research efforts and supported by different key actors, rice has significantly contributed to improve the livelihood of farmers and many others involved in rice business (Addis *et al*. 2018; Gebey *et al*. 2012; MoARD 2010). By informal introduction at first and following the inception of formal rice research, several rice germplasms are made available into Ethiopia and many of these have adapted to different production ecologies; rain fed lowland, upland, and intermittently irrigated systems. Ethiopia has now many cultivated rice genetic resources many of which are conserved by farmers and research system unlike wild rice genetic resources which exist as natural populations. However, improper management of cultivated rice genetic resources and continues human disturbances to wild rice resources coupled with environmental changes affect the diversity of rice genetic resources in Ethiopia. These resources should be collected and /or conserved for future use in breeding. For efficient utilization, conservation, and management of these genetic resources, understanding their genetic diversity and population structure is a fundamental pre-breeding undertaking. On the other hand, cultivated rice accessions have been affected by blast diseases causing significant yield losses in different regions of Ethiopia which necessitates searching for varieties resistant to blast with better agronomic performance. Thus, rice breeding program should aim at developing high yielding and blast resistant rice varieties while at the same time conserving and utilizing wild rice

genetic resources. The objectives of this study, therefore, were (1) to assess genetic diversity and classify Ethiopian rice cultivars based on molecular markers and morpho-physiological characters (2) to evaluate rice cultivars for blast resistance using differential system (3) to assess genetic variation among rice cultivars based on agronomic traits and (4) to investigate maternal lineage, genetic diversity and population structure in wild rice from Ethiopia using chloroplast and nuclear DNA markers.

Genetic diversity, classification and characterization of Ethiopian rice cultivars

In genetic diversity analysis using 50 SSR markers, accessions from Ethiopia showed relatively high genetic diversity and they were broadly classified into two major groups, based on their relationship with control Japoanica and Indica populations. Majority of landrace and improved accessions clustered with control Japonica showed negative phenol reaction while most of those clustered with control Indica showed positive phenol reaction (Chen *et al*. 1993; Ishikawa *et al.* 1991; Matsuo 1952; Morishima and Oka 1981; Oka 1953, 1988; Pai *et al.* 1975; Second 1982). In addition, chloroplast INDEL (ORF100) revealed that maternal donors of all Ethiopian accessions were Japonica genetic back group as they showed non-deletion type except for two accessions which showed deletion type; whereas nuclear INDEL markers discriminated most accessions as recombinant type which could be associated with their complex breeding history as they originated from diverse sources and only few accessions were judged as Japonica or Indica types. This Indica-Japonica classification based on a combination of parameters would enable breeders to select compatible parental lines to avoid the occurrence of reproductive barriers. However, the *indica–japonica* hybrid rice may have great potential in rice production, given that the problems of between-subspecies sterility can be resolved (Khush 2001; Peng *et al*. 2004). In

Ethiopia, improved rice accessions have been released mainly based on their yield performance and their overall disease resistance to address the primary needs of local farmers. Such improved accessions have not been evaluated for other morpho-physiological characters. In this study, characterization of Ethiopian rice accessions for alkali digestibility using potassium hydroxide (KOH) and apparent amylose content test revealed that most accessions, both improved cultivars and landraces, are intermediate type for both characters. According to Juliano (1992), apparent amylose content of rice can be classified as waxy (0-5%), very low (6-12%), low (13-20%), intermediate (21-25%) and high (26-33%). About 68% of Ethiopian rice accessions showed intermediate apparent amylose content with only few accessions in the two extremes, low or high.

Variation for blast resistance

Rice blast disease, caused by the fungus *Magnaporthe oryzae* B. Couch is among the leading diseases of rice in most of the rice growing regions of the world (Talbot 2003) and therefore use of resistant varieties to this devastating diseases is the most economical and feasible approach for growing rice (Ebron *et al*. 2004; Kawasaki-Tanaga and Fukuta 2014; Wu *et al*. 2017). We screened Ethiopian rice accessions for blast resistance and this is the first report of its kind to characterize Ethiopian rice accessions for blast resistance using differential blast isolates. We found that including NERICAs most rice accessions from Ethiopia revealed resistance (score less than 2) to blast isolates used here except for two landraces, X-Jigna and BGA01, which showed susceptibility to blast isolates compared to control accessions. The two controls, IR64 and WAB56-104 were resistant to blast isolates especially the former was not affected by any of the 20 blast isolates used while the latter was affected by few isolates. Most Ethiopian accessions were clustered with this highly resistant mega variety, IR64, to

which a highly resistant differential variety, IRBL9-W with a resistance gene *Pi9* (t) was also clustered. Emmanuel *et al.* (2016) also reported that this differential variety was highly resistant to most blast races they studied. However, very few Ethiopian accessions are postulated to have this resistance gene in their genetic background.

Even though accession from Ethiopia revealed resistance to most blast isolates, the resistance gene or combination of resistance genes each accession contained was not known. Ebron *et al*. (2004), Kawasaki-Tanaka and Fukuta (2014), and Khan *et al*. (2017) described how to postulate resistance genes in test materials compared to reaction patterns of differential varieties to blast races used. We postulated resistance genes following previous procedures based on phenotype data and we found that resistance genes varied among accessions. About 83% of accessions from Ethiopia harbored more than one resistance gene and many of these accessions were clustered with resistant controls and with the differential variety, IRBL9-W. However, resistance gene estimation was based on solely phenotype of blast reaction which needs further clarification using molecular markers. Thus, this should be investigated using molecular markers developed for blast research along with the same set of differential varieties and susceptible controls. Moreover, further screening of Ethiopian rice accessions using local blast races is indispensable which could help identify specific genes for blast resistance that could be useful to mitigate blast incidence in rice growing localities in Ethiopia.

Genetic variation in rice cultivars for agronomic traits

Rice accessions comprising improved accessions and landraces adapted to different ecologies were evaluated at two contrasting testing sites under lowland rain fed condition in Ethiopia. Significant genetic variation in agronomic traits among accessions was observed in field

experiments conducted at both sites, Fogera and Pawe. The present observed significant differentiation among accessions for agronomic traits is consistent with previous reports of Abebe *et al*. (2017), Bitew *et al*. (2018), and Girma *et al*. (2018) who used different set of materials, entirely improved varieties and/ or candidate varieties. With respect to heading and maturation time at both sites, most improved accessions were earlier than landraces. However, almost all materials were relatively late at Fogera compared to Pawe. This could be attributed to the high temperature and sufficient moisture with high relative humidity at Pawe. Grain yield performance of accessions showed relatively higher at Pawe than at Fogera and improved accessions were also better than landraces. Fogera 2, Candidate 1 and Candidate 2, were the three high yielding accessions at Pawe. Except Fogera 2 which was identified as Indica, the two showed mixed INDEL genotypes. But at Fogera, Candidate 3, Gumara, Fogera 1 and NERICA-4 were the four high yielding accessions and all were discriminated as recombinant type by nuclear INDEL markers.

Most of the traits such as yield and yield component traits are influenced by interaction of genotype and environment, and, therefore, selection based on correlation coefficient makes it easy for plant breeders (Ahmadikhah *et al*. 2008). At Pawe, days to heading and days to maturity showed a positive and significant correlation with grain yield. This suggested that relatively late and high yielding varieties can be selected for Pawe due to the presence of long rainy season with optimum temperature for rice. Actually, earliness is not farmers' top priority at Pawe. In many of the cases, farmers at Pawe do not prefer very early varieties as it can be subjected to sprouting and attacked by birds. Most farmers in this area preferred high yielding and medium to late maturing type varieties. However, at Fogera, days to heading and maturity were negatively correlated with grain yield. At this site, high yielding and early type varieties are most preferred by farmers due to recurrent terminal moisture stress which caused significant yield loss. In principal component analysis,

agronomic traits such as days to heading, days to maturity, plant height, panicle length, total grains per panicle, filled grains per panicle, and grain yield contributed significantly to the total phenotypic variation explained. In addition, all these traits exhibited intermediate to high broad sense heritability and hence, selection based on these traits could be preferred. Overall, this study demonstrated availability of genetic variation among Ethiopian rice accessions for quantitative traits to be exploited differently for the two contrasting environments.

Maternal lineage, diversity and genetic structure in wild rice from Ethiopia

Wild plant genetic resources are important reservoirs of the genetic diversity necessary to improve resistance/tolerance to biotic and abiotic stress as well as yield and related potential of modern accessions (Fuchs *et al*. 2016). The study and conservation of these wild relative populations should be, therefore, a priority to secure genetic resources for future breeding programs and this is also important for rice (Thomas *et al.* 2017; Kaewcheenchai *et al*. 2018; Sandama *et al*. 2018). However, details of wild rice maternal lineage, genetic diversity, and population structure in Ethiopia are less investigated. It is assumed that Ethiopia is one of centers of diversity for *O. longistaminata*, a perennial wild rice, at the age of distribution in East Africa. In order to study maternal lineage, genetic diversity and population structure of this species in Ethiopia, we used populations from two regions, Amhara and Gambella, we applied chloroplast INDELs and SSR markers. We presumed that unique genetic resources could be found compared with populations from other areas in Africa.

Application of newly developed chloroplast markers revealed that, unlike populations in Ethiopia, control *O. barthii*, and *O. longistaminata* populations had diverse plastid types which could be attributed to their diverse origins in Africa. Three plastid types, Type 1, 2, and 3 were unique to Ethiopian population. Only Type 6 was shared with control *O.*

longistaminata population. Control *O. glaberrima* and *O. barthii* populations never shared any plastid types with wild rice populations in Ethiopia. Moreover, different plastid types were detected between the north and south groups of Ethiopia which in fact resulted from variation in a few loci.

Using nuclear DNA markers, we found that *O. longistaminata* populations in Ethiopia had higher genetic diversity compared to *O. barthii* and *O. glaberrima*. Previous studies also indicated that *O. longistaminata* has higher genetic diversity than *O. barthii* (Sharma 1983; Dadi and Engels 1986; Kiambi *et al*. 2005). Particularly, Fogera and Dera populations from Amhara region, exhibited higher genetic diversity which is in contrary to Melaku *et al*. (2013) who reported higher genetic diversity from wild rice accessions in Gambella, southern Ethiopia, than those from Amhara region. Phylogenetic tree, principal coordinate, and population structure analysis suggested that Ethiopian populations are related to control *O. longistaminata* population that originated from other African counties, mainly West Africa and few from East Africa.

As reported in previous studies, *O. longistaminata* has a great role for use in breeding. Khush *et al*. (1990) identified a broad-spectrum resistance gene (Xa-21) for bacterial blight (BB) from *O. longistaminata* and transferred this gene to a susceptible *O. sativa* improved accession, IR24, through crossing and following subsequent backcrossing of F1 with the recurrent parent (IR24). Iwamoto *et al*. (1998) pointed out that *O. longistaminata* could easily be crossed with *O. sativa* to harvest fertile seed. In Kenya, *O. longistaminata* was successfully crossed with *O. sativa* and they produced seeds with which they developed progeny through self-fertilized and selection at a non-fertilized paddy field (Gichuhi *et al*. 2016) as an important approach of breeding for low input environment. Moreover, Ramos *et al*. (2016) successfully bred chromosome segment substitution lines (CSSLs) of *O. longistaminata* in *O. sativa* cv. Taichung 65 background. Despite these benefits, wild rice

resources including *O. longistaminata* are increasingly threatened due to human pressure and climate changes (Thomas *et al.* 2017; Kaewcheenchai *et al.* 2018; Ndjiondjop *et al*. 2018). This situation is more severe in Ethiopia as wild rice genetic resources are diminishing increasingly and no conservation effort is in place. Therefore, in order to maintain the rich genetic diversity of wild rice genetic resources for use in breeding, natural populations should be protected from genetic erosion through *in situ* conservation in addition to ex situ conservation.

Summary and conclusion

This study was undertaken within the framework of investigating rice genetic resources as the first step towards identifying potential plant materials which can be utilized in rice breeding programs in Ethiopia. Four experiments were executed with the following objectives; study genetic diversity and characterize rice cultivars, evaluate Ethiopian rice cultivars for blast resistance, assess genetic variation in rice cultivars based on agronomic traits and investigate maternal lineage, genetic diversity and population structure in wild rice from Ethiopia using molecular markers.

Genetic diversity analysis and classification of Ethiopian rice cultivars with SSR markers and INDEL markers complemented with characterization based morpho-physiological characters was the first experiment to be undertaken. Diverse 60 rice accessions (improved and landrace) from Ethiopia with 19 control accessions (twelve Japonica and seven Indica) were genotyped using 50 SSR markers and four INDEL markers. They were also evaluated for phenol reaction, alkali digestibility and apparent amylose content. A total 351 alleles with an average of 7.02 alleles per locus, ranging from 2 to 13 alleles per locus was observed. Expected heterozygosity (*He*) also covered 79 accessions from 0.23 to 0.88, an

average of 0.65. Landraces and improved accessions separately showed *He* of 0.48 and 0.55, respectively which indicated relatively their high genetic diversity. Two major cluster groups were produced corresponding to Japonica and Indica. Phenol reaction also showed similar trend of classification. However, further analysis using nuclear INDEL markers revealed that most accessions are recombinant type with only few identified as Japonica and Indica. Genetic structure analysis also yielded three subpopulations corresponding Japonica, Indica and recombinant type. Evaluation for alkali digestibility and apparent amylose content revealed that most Ethiopian accessions showed intermediate value for both characters while some others showed high or low estimated values. In this experiment, relatively high genetic diversity coupled with clustering information of accessions (landraces and improved varieties) are pertinent evidences in future rice breeding efforts.

The diverse 60 rice accessions from Ethiopia were also evaluated in the greenhouse for seedling blast resistance against 20 standard blast isolates originated from Japan (9), Phillippines(6), China (1), Laos (1) and Africa (3) in comparison to two resistant (IR64 and WAB56-104) and two susceptible (US-2 and LTH) control accessions along with 28 differential varieties containing 23 pre-defined blast resistance genes. We observed that nearly 65% of 92 accessions showed resistance reaction (scores of 0-2) to blast isolates. We also observed that most Ethiopian accessions were resistant to blast isolates from Japan but less resistant to other blast isolates. While 17% of Ethiopian accessions showed high blast resistance frequency (86-100%), the other about 78% of them showed intermediate blast resistance frequency (50-85%) and the remaining 5% showed low resistance frequency (0-49%). Including upland NERICAs popular improved varieties and some landraces were highly resistant to all blast isolates used. Resistance gene postulation based on blast phenotype in Ethiopian accessions indicated the involvement of several resistance genes, many of which being *Pit*, *Pik-p*, *Pish*, *Pib*, *Pik-s*, *Pik-m*, *Pi7* (t), *Piz-t*, *Pi9* (t), *Pi12* (t), *Pi19*

(t), and *Pi20* (t). More than 50% of Ethiopian rice accessions were found to have a combination of more than one gene for blast resistance in their genetic background. And yet more studies need to be carried out using local blast races to fully understand the resistance genes that are responsible for high blast resistance in Ethiopian rice accessions. Thus, accessions with better blast resistance can be used to improve elite rice cultivars with good agronomic characteristics but lack resistance to blast pathogen.

We also studied genetic variation in 60 Ethiopian rice cultivars adapted to different production ecosystems based on agronomic traits evaluated under lowland rain fed condition in Ethiopia. Analysis of variance procedure revealed significant differences among accessions for 90% of the traits at Fogera and Pawe, and on combined data as well indicating that traits of interest for selection at the two sites tended to be different. Most of the traits such as days to heading, days to maturity, panicle length, grain yield, thousand seed weight, biomass yield and harvest index showed high broad sense heritability at both sites and all showed significant correlation coefficients with grain yield. Principal component analysis extracted four components contributing to more than 81%, 74%, and 80% of total variation at Fogera, Pawe and the combined data, respectively. Days to heading, days to maturity, plant height, panicle length, fertility rate, grains per panicle, thousand seed weight, biomass yield, harvest index, and grain yield showed high contribution at one or all of the components and many of them showed significant correlation coefficients with grain yield and had relatively high heritability. Ward's method hierarchical cluster analysis produced four clusters; I, II, III and IV, with the largest number of accessions belonging to Clusters I (22) and II (20). About 77% of accessions in Cluster I consist of improved varieties including NERICAs. Accessions in Cluster I were relatively early in days to heading and days to maturity with intermediate mean values for plant height, panicle length, grain yield, and biomass yield while accessions in Cluster II were intermediate in days to heading and days to maturity with higher mean

values for grain yield, and biomass yield. Clusters III and IV were dominated by landraces with few improved accessions and they showed late in days to heading and maturity. This information combined with molecular analysis and blast inoculation data of cultivars can accelerate our efforts of identifying potential plant materials for rice crossbreeding in Ethiopia.

To elucidate maternal lineage, genetic diversity and population structure eight chloroplast INDELs (cpINDELs) and sixteen SSR markers were applied to 163 wild rice accessions from Ethiopia compared to 52 control accessions; *O. barthii* (20), *O. longistaminata* (19) and *O. glaberrima* (13). Among 20 combinations of plastid types identified in 215 accessions using cpINDELs, only four plastid types; Type1, 2, 3 and 6 were detected in five wild rice populations of Ethiopia. Only Type 6 was shared with control *O. longistaminata* population while three were unique to Ethiopia. Type 6 was specific the north Ethiopia (Amhara) while Types 2 and 3 to south Ethiopia (Gambella) but Type 1 was shared between Fogera (Amhara) and Abobo (Gambella) populations.

Total number of alleles amplified per locus ranged from 4 to 14 with an average of 9.69 with 155 alleles, in total amplified from 215 accessions using 16 SSRs. The mean observed (*Ho*) and expected (*He*) heterozygosity was 0. 24 and 0. 73, respectively. *He* of Fogera population was the highest (*He* = 0.67), followed by Dera population (*He* = 0.62) among five Ethiopian populations. In fact, *He* of control *O. longistaminata* population (*He* = 0.70) was the highest of all which could be attributed to its diverse origin in Africa while control *O. barthii* and *O. glaberrima* populations showed the lowest of all eight populations with *He* = 0.50 and 0.23, respectively. NJ method phylogenetic tree analysis classified the whole accessions into five cluster groups, out of which Ethiopian wild rice accessions corresponded to only three clusters with some admixture types. Population structure analysis at K=2 revealed that all populations from Ethiopia were clustered with control *O.*

longistaminata while *O. bathii* and *O. glaberrima* clustered together as separate group which confirmed wild rice type that predominated five districts of wild rice collection in Ethiopia. However, further analysis at K=5 showed that five natural populations were classified into three subpopulations with some admixtures corresponding to phylogenetic tree analysis. Thus, results of phylogenetic tree analysis and model based clustering at K=5 suggested the presence of three groups of *O. longistaminata* natural populations in Ethiopia. In conclusion, genetic diversity analysis and characterization of rice cultivars using molecular markers and morpho-physiological traits revealed high genetic diversity in cultivars and they were classified into different groups, mainly corresponding to Japonica, Indica and recombinant type which help selecting counterparts for cross breeding. Furthermore, blast resistance screening and genetic variations in agronomic traits classified rice cultivars in such a way that it complements selection of potential parents for breeding. The study on wild rice also showed the presence of high genetic diversity indicating their usefulness as genetic resource and thus, proper in-situ and ex-situ conservation should be in place for future use in breeding.

Recommendation

In the course of this study, the following are among the most important points noted to look into under the national rice breeding program in Ethiopia:

- Establishing crossing program primarily focusing to improve X-Jigna for diseases resistance, earliness, and other important rice plant characteristics
- Characterization of Ethiopian rice cultivars in terms cooking and eating quality attributes and stratify rice consumers in collaboration with food science/grain quality research laboratory to fine tune breeding targets
- Study on weedy rice, wild rice, and cultivated rice from Fogera plains to elucidate gene flow and interrelationship for use in breeding
- Comprehensive collection of wild rice accessions from all possible areas/regions in Ethiopia to clarify distribution, species types, evolutionary relationship, gene flow, and genetic structure in comparison to globally established core collections of wild rice, mainly of African origin.
- Crossing *O. longistaminata* with elite cultivars with subsequent backcrossing to improve resistance to different stresses
- Develop strategic plan for conservation and utilization of wild rice genetic resources in Ethiopia in collaboration with key stakeholders
- In collaboration with plant pathology researchers, characterization and identification of rice blast races in Ethiopia and if possible establishment of differential system in the long run.

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List of Publications

Journal

Taddesse L, Fukuta Y, and Ishikawa R (2020). Genetic study of diversity and blast resistance in Ethiopian rice cultivars adapted to different ecosystems. (**Accepted**, Breeding Science).

Workshop papers/posters

Taddesse Lakew, Ryuji Ishikawa and Katsunori Tanaka (2019). Three groups detected in wild rice populations from Ethiopia as revealed by chloroplast INDEL and SSR markers. 14th Tohuku regional breeding research annual workshop, 30 Nov.2019, Iwate University, Morioka, Japan. (Poster).

Taddesse Lakew, Yoshimichi Fukuta, and Ryuji ISHIKAWA (2018). Genetic diversity and variation for blast resistance in rice cultivars from Ethiopia. Innovation in Plant and Food Sciences, 3rd International Symposium. 15-18, 2018, Fujian Agricultural and Forestry University, Fuzhou, China. (Poster).

Taddesse Lakew, Yoshimichi Fukuta and Ryuuji Ishikawa (2018). Assessing genetic variation for blast resistance in rice cultivars from Ethiopia using differential system. UGAS International symposium, 02 Dec, 2018, Hirosaki University, Hirosaki, Japan. (Poster).

Taddesse Lakew and Ryuji Ishikawa (2018). Evaluation of genetic diversity of Ethiopian rice cultivars based on nuclear and cytoplasmic DNA molecular markers. Japanese Breeding Society annual workshop, 25-26 March 2018, Kyushi University, Kushu, Japan. (Oral presentation).

Taddesse Lakew and Ryuji Ishikawa (2017). Surveying genetic diversity in Ethiopian rice cultivars as inferred by SSR markers. 12th Tohuku regional breeding research annual workshop, 23 Nov.2017, Akita University, Akita, Japan. (Poster).

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