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Inter Simple Sequence Repeat (ISSR) analysis of wild and cultivated rice species from Ethiopia

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The genetic diversity of three wild rice populations of Ethiopia along with three cultivated rice populations were studied using Inter simple sequence repeats (ISSRs) as a molecular marker. A total of 93 clear and reproducible bands were generated using four dinucleotides and two tetra nucleotides primers. Both UPGMA and neighbor joining trees were constructed for each individual and population using Jaccard's similarity coefficient. The trees and PCO clearly indicated six distinct groups which are based on populations of origin. *Oryza glaberrima*, *Oryza sativa* and NERICA-3 clustered as a major group while *Oryza barthii* and *Oryza longistaminata* were clustered as the second major group. Such clustering of *O. glaberrima* with *O. sativa* and NERICA-3 is considered to be due to genetic admixture of *O. glaberrima* with *O. sativa*. The over all gene diversity and percent polymorphisms were found to be higher in wild rice (0.14; 38.3 respectively) than in cultivars (0.11; 28.3 respectively). The Shannon's diversity index also confirmed the existence of higher diversity in wild rice populations than cultivated species. Furthermore, partitioning of the Shannon's diversity showed that the majority of the variations were observed among population (63%). Similarly, AMOVA demonstrate highly significant (P = 0.00) genetic differences among populations (72.9) than within populations (27.1).

Key words: Genetic diversity, wild rice populations, cultivated rice, *Oryza sativa*, *Oryza glaberrima*, NERICA, ISSR.

INTRODUCTION

Rice is a unique crop of great antiquity and akin to progress in human civilization (Chang, 2002). It is an annual plant in the grass family, Poaceae. According to Onwueme and Sinha (1991) the genus *Oryza* comprises 25 species and of these only two species are cultivated, namely *Oryza sativa* and *Oryza glaberrima*. *O. sativa* is the common rice grown throughout the warmer regions of the world whereas *O. glaberrima* is grown to a limited extent in the flood plains of West Africa. The wild relatives of rice possess desirable genes which can be transferred to cultivate to enhance the agronomic performance of cultivars. Important traits such as resistance to brown plant hopper, white backed plant hopper from *Oryza eichingeri* Peter, resistance to brown plant hoper and tolerance for drought were transferred from *Oryza australiensis* Domin. In addition, resistance to brown

plant hopper and white backed plant hopper were transferred from *O. minuta* J.S.Presl ex C.B.Presel., and resistance to brown plant hopper, bacterial blight and bacterial leaf streak were transferred from *Oryza punctata* Kotschrex. Steud (Villegas, 1990). Moreover, Villegas (1990) indicated that introgression of genes from wild relatives to cultivated species will enhance the genetic variation essential to rice improvement. Rice production in Ethiopia started a few decades ago and now the country is proved to have reasonable potential to grow different rice types for lowland rain fed, upland and irrigated ecosystems. The Institute of Biodiversity Conservation of Ethiopia has various collections of wild rice species from both Fogera Plains and Gambella region around Baro River (Dadi and Engels, 1985) but these collected species are not yet characterized. Intensive collection and molecular marker based studies on wild rice species have been conducted in most of east, southern and other African countries (Kiambi et al., 2005; Ren et al., 2003). However, no similar studies have been carried in Ethiopia.

The study of genetic variation can help to examine

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differences between members of the same species. On the other hand, we can compare the genetic composition of member of different species even over a wider taxonomic range (Dale and Schantz, 2002). The analysis of genetic variation is one of the most important methods for conservation and sustainable use of genetic resources and different molecular markers can be used to evaluate and assess genetic variation within and among different species. Ultimately, comparison of complete genome sequences is the only way of detecting all the possible types of variation that may occur. However, quicker methods which target certain types of changes in the genome are the most preferred method to be use for routine screening and analysis (Dale and Schantz, 2002).

Molecular marker techniques, like Random amplifycation polymorphic DNA (RAPD) has been successfully employed to determine genetic diversity in different species including rice (Ge et al., 1999, Qian et al., 2001). However, RAPD has several limitations including dominance, uncertain locus homology, and sensitivity to the reaction conditions, and low reproducibility. In order to solve some of these problems, a new technique, inter-simple sequence repeat amplification (ISSR) could be used to assess genetic diversity (Qian et al., 2001). ISSR has been observed to be very useful in detecting genetic diversity and population structure of coffee (Tesfaye, 2006), tef (Assefa, 2003) and lentils (Fikru, 2006) collected from all over Ethiopia. Moreover, ISSR was also found better than RAPD to detect genetic diversity among barley accessions (Hou et al., 2005).

The aim of this study was therefore to analyze the genetic polymorphism and population genetic structure of wild populations of rice in Ethiopia and their relationship with cultivated rice species utilizing ISSR.

MATERIALS AND METHODS

Plant Material

A total of 60 samples representing six populations (10 individuals each) were collected from various areas in July 2006. Sampling was done randomly with approximately 10 m distance from each other. Two cultivars namely 'X-jigna' and NERICA-3 representing populations of one O. sativa, one NERICA respectively and two wild rice populations; O. barthii and O. longistaminata were collected from south Gonder, North west Ethiopia. In addition, one wild rice population (O. longistaminata) collected from Baro river side, Gambella (Figure 1) and O. glaberrima; a cultivated African rice species which was provided by Rice Regional Office, SG$_{2000}$, in Ethiopia were also included in the study. The leaf samples of wild rice populations, O. sativa and NERICA were collected and dried in silica gel for DNA extraction. However, the seed of O. glaberrima were grown in the greenhouse and the leaf samples were further collected and dried in silica gel for extraction of genomic DNA.

DNA extraction

Genomic DNA extraction was done based on the method used by (Borsch et al., 2003) which involves a modified CTAB method

employing triple extractions to yield optimal amounts of DNA.

Test gel and electrophoresis

Extracted DNA was electrophoresed in 0.98% agarose gels at 80 V for 45 min, stained by soaking the gel in a 10 mg/ml ethidium bromide and photographed under UV transilluminator by Biodoc-Analyse 2.0 with digital canon camera. Following the protocol given by (Borsch et al., 2003) those with high band intensity and less smear were selected for PCR after further dilution (1:10).

Primer selection and optimization

A total of eight ISSR primers obtained from the University of British Columbia and primers used by (Sujatha et al., 2004) were used for the initial testing of variability and reproducibility test. Two individuals were selected from each population to screen the primers and finally; six primers were selected based on polymorphism level and reproducibility. Four of the ISSR primers were with dinucleotide repeat motifs where as two of the primers were based on tetranucleotide repeat motifs (Table 1)

PCR and gel electrophoresis

The polymerase chain reaction was conducted in a Biometra 2000 T3 Thermo cycler. PCR amplification was carried out in a 25 µl reaction mixture containing 1 µl template DNA, 13.2 µl H$_2$0, 5.6 µl dNTP (1.25 mM), 2.6 µl Taq buffer (10xThermopol reaction buffer), 2.0 µl Mgcl$_2$ (2mM), 0.4 µl primer (20 pmol/µl) and 0.2 µl Taq Polymerase (5 u/µl). The amplification program was 4 min preheating and initial denaturation at 94°C, then 39 cycles of 15 s at 94°C, 1 min primer annealing at (45°C/ 48°C) depending on primers used, and 1.30 min extension at 72°C; with a final extension of 7 min at 72°C. The PCR reactions were also stored at 4°C until gel electrophoresis.

The amplified DNA fragments were separated by electrophoresis on 1.67% agarose with 1xTBE buffer. DNA marker of 100 bp was used to estimate molecular weight. Electrophoresis was conducted for 2 h at constant voltage of 100 V. The gel picture was taken after staining with ethidium bromide (10 mg/ml) and destained as described above.

Data scoring and analysis

Each fragment that was amplified using ISSR primers, was treated as a binary unit character and scored as '0' for absence, '1' for presence or '?' for missing data. Different software programs were used for analysis. POPGENE version1.32 software (Yeh et al., 1999) was used to calculate genetic diversity for each population as number of polymorphic loci and percent polymorphism.

Analysis of molecular variance (AMOVA) was used to calculate variation among and within population using Areliquin version 3.01 (Excoffier et al., 2006).

Shannon–Weaver diversity index (H) was calculated as H= -∑pilog2pi where pi is the frequency of a given band for each population (Lewontin, 1972). Shannon's index of diversity was used to measure the total diversity (Hsp) as well as the mean intra – population diversity (Hpop). The proportion of diversity between populations was then calculated as (Hsp-Hpop/Hsp). NTSYS- pc version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek et al., 1999) software programs were used to calculate Jaccard's similarity coefficient which is calculated with the formula:
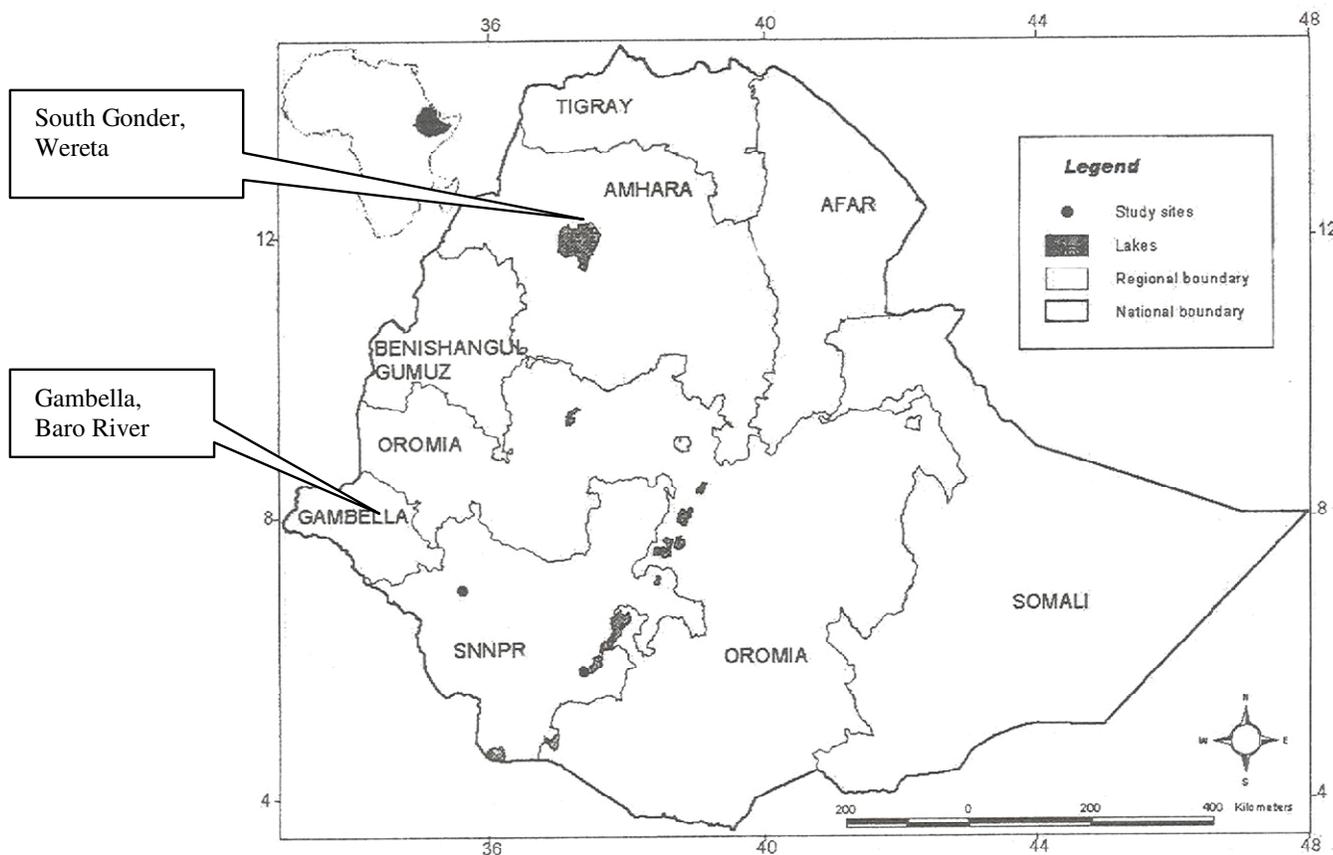
Figure 1. Map of Ethiopia showing sites from where samples of rice were collected, which are also among the potential areas for rice production.

Table 1. List of primers, annealing temperature and sequence. All the primers are high performance liquid chromatography purified and designated as 'H'.

Primers	Annealing temperature	Sequence
810- H	45°C	5'-GAG AGA GAG AGA GAG AT-3'
818- H	48°C	5'-CAC ACA CAC ACA CAC AG-3'
824- H	48°C	5'-TCT CTC TCT CTC TCT CG -3'
834- H	45°C	5'-AGA GAG AGA GAG AGA GYT -3'
854- H	48°C	5'-TCT CTC TCT CTC TCT CRG -3'
872- H*	35°C	5'-GAT AGA TAG ATA GAT A -3'
873- H*	45°C	5'-GAC AGA CAG ACA GAC A -3'
878- H*	45°C	5'-GGA TGG ATG GAT GGA T -3'

*Tetranucleotide primers and the rest are dinucleotides.

$$S_{ij} = \frac{a}{a+b+c}$$

Where 'a' is the total number of bands shared between individuals i and j, 'b' is the total number of bands presents in individual i but not in individual j and 'c' is the total number of bands present in individual j but not in individual i. The unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973)

was used to analyze and compare the individual genotypes and generates phenogram using NTSYS- pc version 2.02 (Rohlf, 2000). The neighbor joining (NJ) method (Saitou and Nei 1987; Studier and Keppler, 1988) was used to compare individual genotypes and evaluate patterns of genotype clustering using Free Tree 0.9.1.50 Software (Pavlicek et al., 1999). The major difference between the two algorithms is that UPGMA assumes equal rates of evolution (molecular clock assumption) along all branches, whereas the neighbor joining method assumes variations in the rate of change

Table 2. Fingerprint patterns generated using eight ISSR primers; six of them are selected for this study.

Primers used	Repeat motif	Amplification pattern	Amplification of specific bands	Number of scorable bands
810- H	$(GA)_8T$	Good	Good with smear	19
818- H	$(CA)_8G$	Smeared	-	-
824- H	$(TC)_8\,G$	Good	Good	16
834- H	$(AG)_8\,YT$	Good	Good with smear	15
854- H	$(TC)_8\,RG$	Good	Good	12
872- H	$(GACA)_4$	Few samples amplified	-	-
873- H	$(GACA)_4$	Good	Good	22
878- H	$(GGAT)_4$	Good but with smear	Good with smear	9

(Saitou and Nei, 1987; Studier and Keppler, 1988). To further examine the patterns of variation among individual samples, a principal coordinated analysis (PCO) was performed based on Jaccard's coefficient (Jaccard, 1908). The calculation of Jaccard's coefficient was made with PAST software version 1.18 (Hammer et al., 2001). The first three axes were later used to plot the three dimensional PCO with STATISTICA version 6.0 software (Hammer et al., 2001; Statistica soft, Inc.2001).

RESULTS

Banding patterns and ISSR primers

Out of the eight primers tested initially, six of them gave relatively clear banding pattern and they were selected and used in this study (Table 2). The molecular size of the bands amplified using six primers were in the range of 450 bp to 3kb (Figure 2). A total of 93 bands were scored from 6 primers. Among dinucleotides the fewest (12) bands were scored from 854_H and the most bands were scored from 810_ H (19). Across all primers, more bands (22) were scored from tetra nucleotides (873_H, Table 2).

Genetic diversity

Among all the populations studied *O. glaberrima* and *O. barthii* (wild Gonder-1) had the highest percentage of polymorphism among cultivated and wild rice species, respectively. Wild rice populations were observed to show higher percentage polymorphism as compared to cultivars (Table 3).

NERICA, the cultivated rice included in this study, had higher gene diversity (0.12) than both *O. sativa* (0.11) and *O. glaberrima* (0.11). *O. barthii* (wild Gonder-1) has higher gene diversity among wild rice populations in the present study with gene diversity value of 0.18 (Table 4). The gene diversity observed in wild rice populations (0.14) were found higher than cultivated rice populations with overall gene diversity of 0.11.

The overall analysis of Shannon's diversity index with both di and tetra nucleotide primers indicated that the

wild rice collected from Gonder (*O. barthii*) was found to be more diverse as compared to the other two, *O. longistaminata* from Gonder and Gambella. Among cultivated rice species *O. glaberrima,* African rice, showed higher Shannon's diversity index as compared to the other two cultivated rice populations with least diversity in *O. sativa* (0.20) as shown in (Table 4). The mean genetic diversity for populations and mean genetic variation for the entire data was higher with di-nucleotide primers as compared to tetranucleotide primers, but the mean Shannon diversity level revealed by dinucleotide (0.28) was not significantly higher than the Shannon diversity level of tetranucleotide primer (0.23) with 95% confidence interval at t(10) = 1.67, P-value = 0.156.

Partitioning genetic diversity

Analysis of molecular variance revealed that a higher percentage of variation is attributed to variation among populations within groups (49.4%) followed by variation among groups and within populations with 26.4% and 24.2%, respectively (Table 5b). AMOVA also revealed that variation among populations within groups, variation among groups and within populations was found highly significant. Similarly without grouping (Table 5a) high variation was attributed to among the six rice populations with high fixation index (0.73) with least variation within populations. Moreover, partitioning the diversity using Shannon's index revealed higher genetic diversity among populations (63%) of rice as compared to within population diversity of 27% (Table 4).

Genetic similarity and clustering analysis

UPGMA based dendrogram for 6 rice populations shows that the wild and cultivated rice populations were distinctly separated (Figure 3). Among cultivated rice *O. sativa* and NERICA form a sub cluster. Similarly *O. longistaminata* from Gambella and Gonder were found in a sub cluster with *O. barthii* as an out group.

UPGMA was also used to construct a dendrogram for
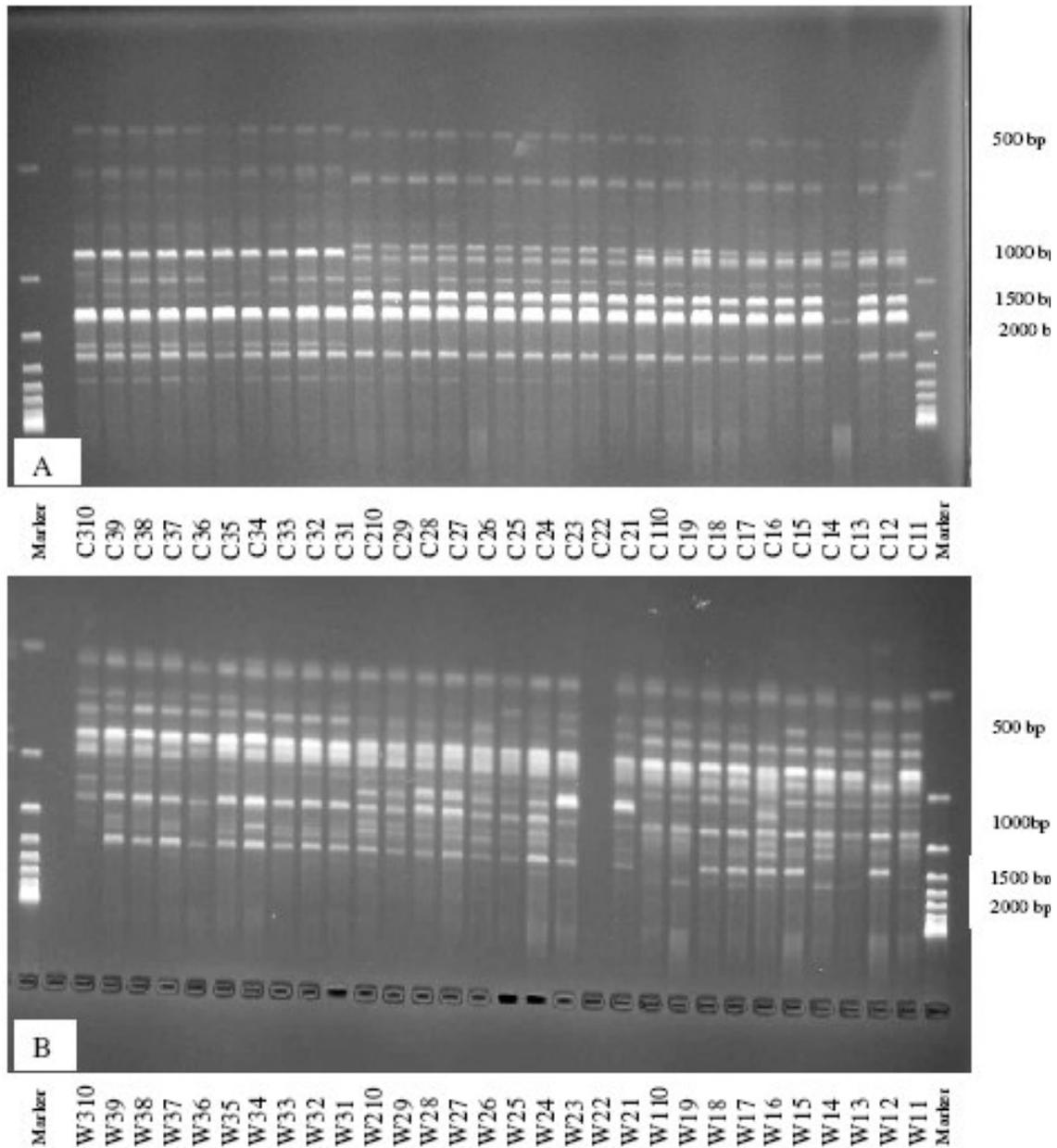
Figure 2. ISSR fingerprint generated from 30 cultivated rice (A) and 30 wild rice populations (B) using primer 873-H.

60 individuals, based on 93 bands. The six populations (NERICA, *O. sativa, O. glaberrima, O. barthii* (wild Gonder-1), *O. longistaminata* (wild Gonder-2) and *O. longistaminata* (wild Gambella)) appeared to each cluster separately (Figure 4). NERICA, a species of rice developed through interspecific hybridization of Asian and African rice, formed a cluster with Asian rice. Generally cultivated and wild species clearly observed to have separated groups in both UPGMA and neighbor joining analysis. The two methods showed almost the same tree topology with similar groupings, although few individuals appeared to escape from groups in case of neighbor-joining analysis

(data not shown).

Principal coordinate analysis (PCO)

All the data obtained using six ISSR primers were used in PCO analysis using Jaccard's coefficients of similarity. The first three coordinates of the PCO had Eigen values of 7.04, 5.23 and 4.10 with variance of 15.30, 11.36 and 8.91% respectively (Figure 5) used to show the grouping of individuals using three coordinates. All six populations formed separate clusters at different space in the three

Table 3. Number of polymorphic loci, percent polymorphism and gene diversity of both wild and cultivated rice with all primers.

Population	NPL	PP (%)	GD
NERICA	25	26.9	0.12
O. sativa	26	28.0	0.11
O. glaberrima	28	30.1	0.11
Average (cultivated)		28.3	0.11
O. barthii (wild Gnder-1)	43	46.2	0.18
O. longistaminata (wild Gonder-2)	32	34.4	0.13
O. longistaminata (wild Gambella)	32	34.4	0.12
Average (wild)		38.3	0.14
Over all	90	96.77	0.34

NPL = number of polymorphic loci, PP (%) = percent polymorphism, and GD = gene diversity for each population and over all populations.

Table 4. Shannon's diversity index within and among rice populations with di and tetra nucleotide primers.

Popn/spps	Shannon's diversity index (H)		Over all
	Di- primers	Tetra– primers	H
NERICA	0.257	0.122	0.212
O. sativa	0.243	0.120	0.202
O. glaberrima	0.262	0.178	0.234
O. barthii (wild Gonder-1)	0.370	0.384	0.375
O. longistaminata (wild Gonder-2)	0.315	0.261	0.297
O. longistaminata (wild Gambella)	0.249	0.312	0.270
Hpopn	0.283	0.230	0.265
Hsp	0.799	0.734	0.777
Hpopn/Hsp	0.370	0.353	0.365
1-Hpopn/Hsp	0.630	0.615	0.625

Hpopn = mean genetic variation for popn, Hsp = mean genetic variation for the entire data, Hpopn/Hsp = proportion of genetic variations within rice populations, and 1-Hpopn/Hsp = proportion of genetic variations between rice populations.

Table 5 Analysis of Molecular Variance (AMOVA) of rice populations in Ethiopia Without grouping (A) and Analysis of Molecular Variance (AMOVA) of cultivated and wild rice groups (B).

A

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	Fixation Indices	P
Among Populations	5	643.083	12.4007Va	72.90	0.73	0.00
Within Populations	54	248.900	4.60926Vb	27.10		0.00
Total	59	891.983	17.010			

B

Source of variation	d.f	Sum of squares	Variance components	Percentage of Variation	P
Among Groups	1	248.950	5.01389 Va	26.37	0.00
Among populations within groups	4	394.133	9.39241 Vb	49.39	0.00
Within Populations	54	248.900	4.60926 Vc	24.24	0.00
Total	59	891.983	19.01556		
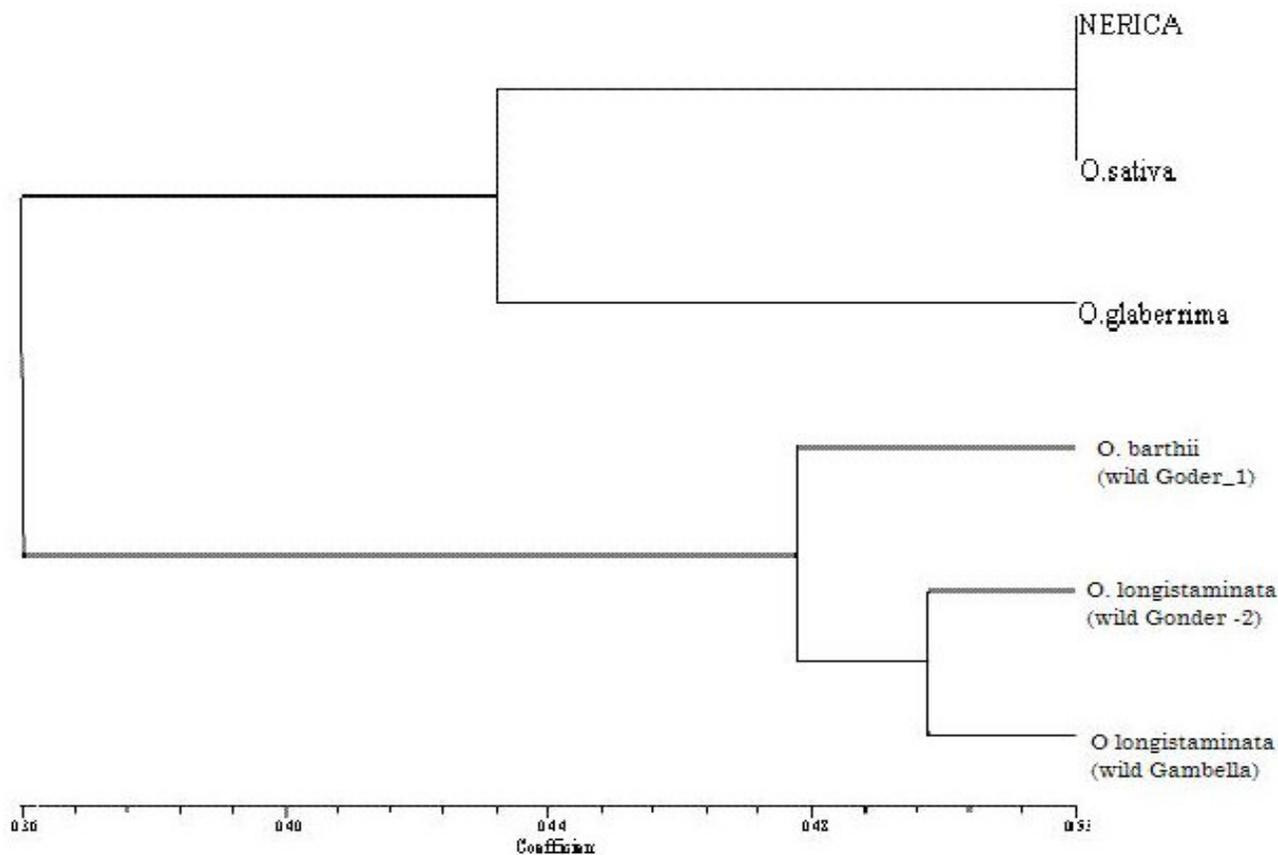
Figure 3. UPGMA based dendrogram for 6 rice populations using 6 ISSR (4 di and two tetra nucleotide) primers.

dimensions of the PCO. However, one individual sample from *O. longistaminata* (wild Gonder-2) observed to be intermixed with *O. barthii* (wild Gonder- 1) populations, possibly due to admixture.

DISCUSSION

Genetic diversity and utility of ISSR marker in rice

ISSR primers have high resolution power in fingerprinting and diversity analysis of rice (Joshi et al., 2000). In addition to the advantages (inexpensive, easy to gene-rate), ISSRs are powerful in detecting polymorphisms. They can detect even more polymorphism than RFLPs in maize (Kantety et al*.,* 1995) and more than AFLPs in rice (Blair et al*.,* 1999). The present study also confirms that ISSR markers are efficient in detecting polymorphism within and among populations and/or species of rice. The study also revealed the existence of higher diversity among wild rice species in Ethiopia. Thus, ISSR marker systems will provide a useful tool in the future design of collection strategies for conservation and use of wild rice species in Ethiopia. In the present study all the diversity parameters confirm higher genetic diversity in wild rice

than cultivated rice species.

Furthermore, O*. barthii* has been found to be a more diverse population as compared to other wild population studied. The comparative analysis of Asian cultivated rice (*O. sativa* ssp *indica* and ssp *japonica* with its close relatives; *O. rufipogon* and *O. nivara*) showed that the cultivars retained only 10 - 20% of the diversity in the wild species (Zhu et al., 2007). This is further observed in sunflower (*Helianthus annus*), where only 40 - 50% of the diversity in the wild are maintained in the cultivars (Liu and Burke, 2006). Moreover, cultivated maize maintained approximately 80% of the diversity found in its wild ancestor (Wright and Gaut, 2005). The evaluation of SSR polymorphisms of sorghum indicated that landraces retained 86% of the diversity observed in the wild (Casa et al*.,* 2005). The drop in diversity is substantially greater for genes involved in domestication than neutral and random markers (Buckler et al., 2001). The reason could be a severe bottleneck during domestication and founding effect of crop cultivars.

O.glaberrima is observed to show higher percent polymorphism and Shannon's index as compared to other cultivated rice samples studied here. This could be either the seed is admixed which is further supported by Semon et al. (2005) where the majority of accessions of
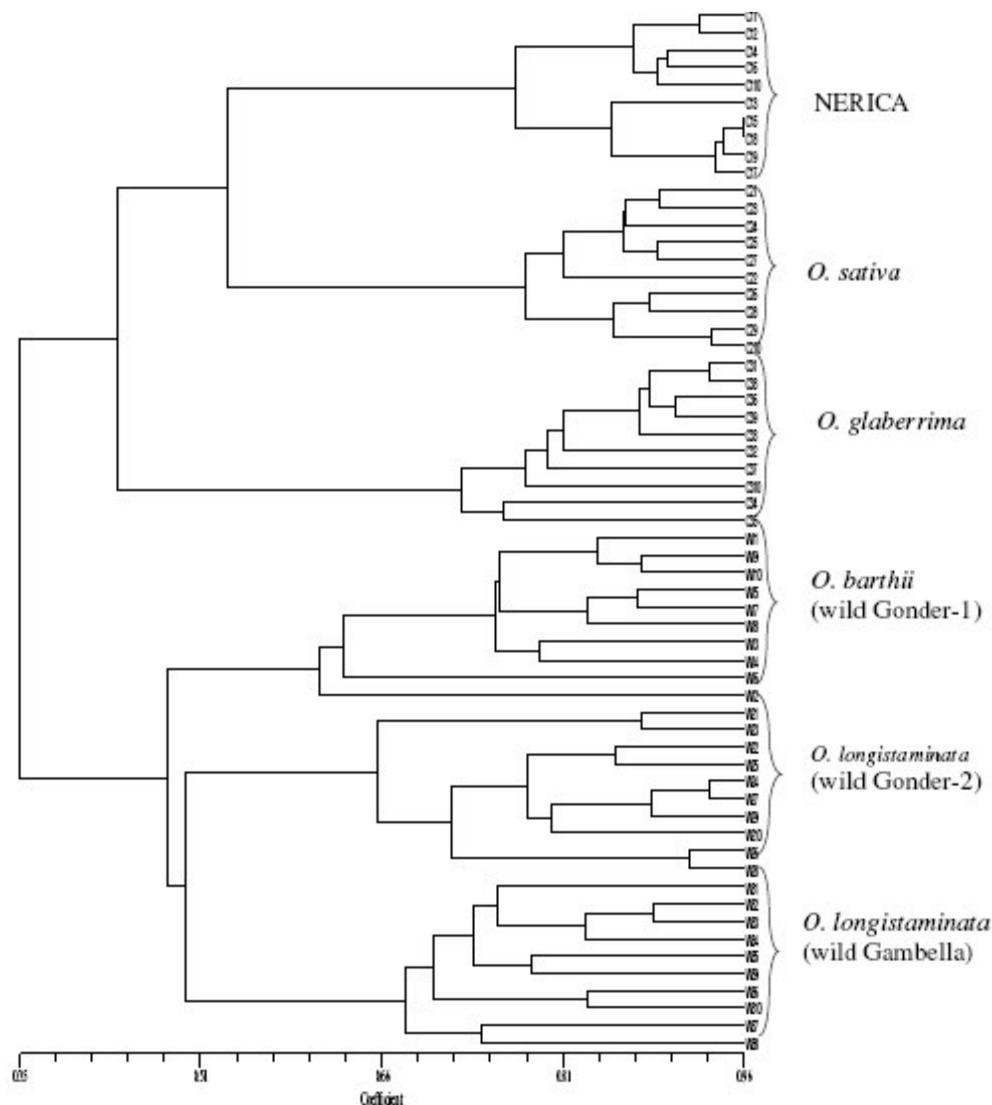
Figure 4. Dendrogram for 60 individuals of 6 rice populations obtained using the UPGMA of 93 PCR bands amplified by four di nucleotide (810,824,834 and 854) and two tetra nucleotide (873 and 878) primers.

O. glaberrima carry genetic evidence of admixtures due to introgression from *O. sativa* or because of possible gene flow between wild rice populations in the surrounding areas (Semon et al., 2005). This can be further supported with presence of close relatives of cultivated rice, which are commonly found or coexist in rice farming system. Moreover, the AA-genome of weedy and wild relatives is highly compatible sexually with cultivated rice (Chen et al., 2004). Hence, gene flow between wild and cultivated rice could be the main factor that could sometimes result in higher diversity of cultivars.

Distinction of different species of rice

All the clustering analysis (UPGMA, NJ and PCOs) have

clearly shown that Ethiopian wild rice are distinctly different from cultivated form of rice. Ren et al. (2003) with RAPD marker observed distinct group of the African *O. longistaminata* accessions and placed apart on UPGMA cluster from the Asian, Australian and other African group. But *O. barthii* and *O. glaberrima* accessions which are African wild and cultivated rice, respectively were randomly mixed with each other with relatively low variation since *O. barthii* is an immediate ancestor of *O. glaberrima* (Bautista et al., 2001). In our result, however, *O. glaberrima* clustered with cultivars unlike the reports of (Ren et al., 2003) which could be either due to the capacity of ISSR not illustrating true genetic relationship (Ren et al., 2003) rather complicated genetic variation or the seed used in this study might not represent the true African rice, *O. glaberrima.* The latter seems to hold since
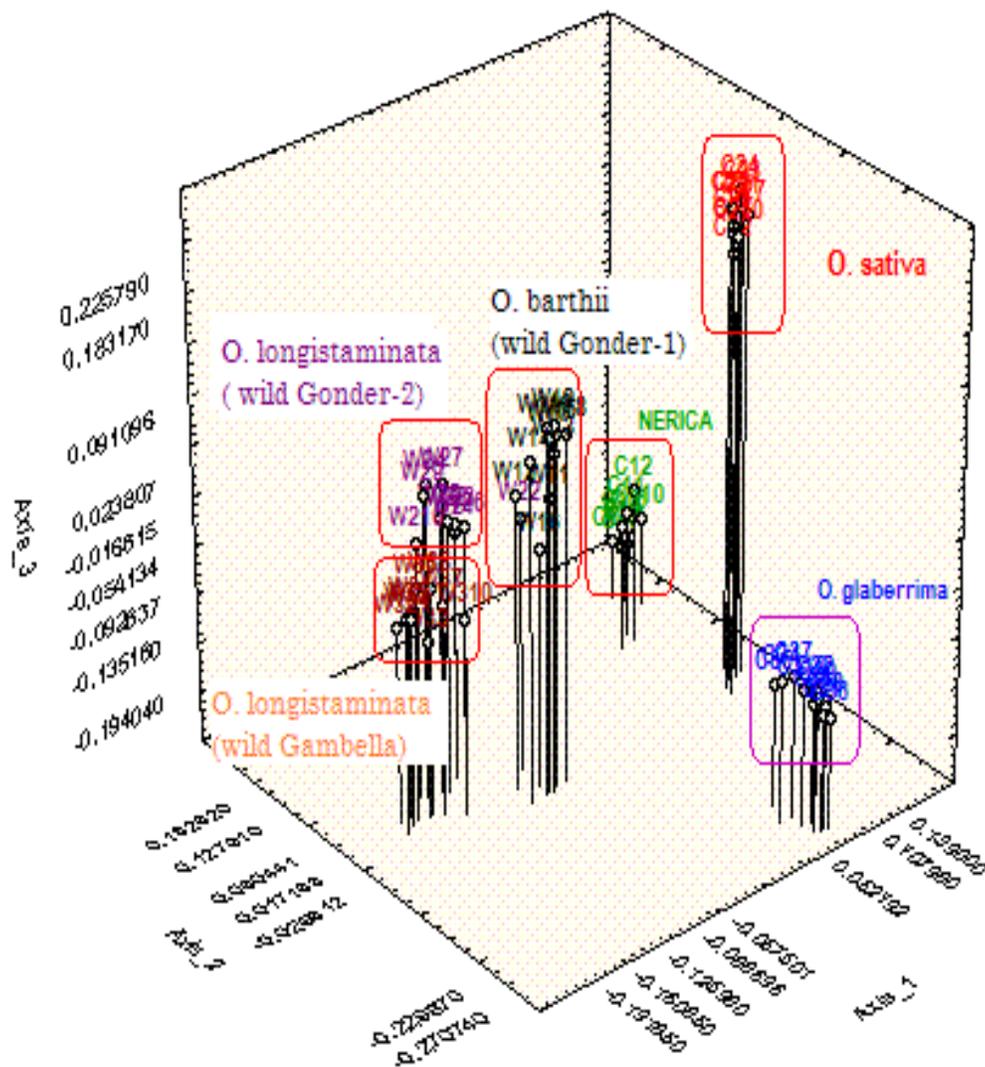
Figure 5. Three dimensional representation of principal coordinate analysis of genetic relationships among 60 individuals of 6 populations of wild and cultivated rice inferred from similarity matrix using the Jaccard's index.

about 67% accessions of *O. glaberrima* studied are found to have admixed to genes of *O. sativa* (Semon et al., 2005). *O. sativa* was introduced to West Africa by Arab traders and Portuguese navigators between 15 and 17th century. Since its introduction, it often grows in mixture with *O. glaberrima*. The present study supports the findings of Semon et al (2005) where the introgression of *O. sativa* into *O. glaberrima* germplasm are reported to have created intermediate types that cannot easily be distinguished at the phenotypic level from native cultivar of *O.glaberrima*.

Distinctions of wild genotype from cultivated were also evident in ensete with RAPD profile (Birmeta et al., 2004). The recent analysis by Tesfaye (2006) also showed a clear distinction of wild *C. arabica* from landraces and cultivars. In sorghum, using SSR marker, it was observed that wild accessions from the same geographic origin tend to form the same group than distant locations and clustered by race. Moreover, the neighbor-joining analysis showed that wild sorghums generally formed a distinct group than cultivars (Casa et al., 2005).

Genetic differentiation and population structure

Analysis of molecular variance (AMOVA) using RAPD markers for wild rice species showed less variation attributed to differences between individuals within a population and population difference within region (Ge et al., 1999). In another observation using AFLP analysis of *Oryza* species, genetic variation increased from within species to between species (Aggarwal et al., 1999). Similarly this result is also in agreement with the above RAPD and AFLP analysis. The AMOVA analysis showed

highly significant genetic differences among populations within groups, among groups (cultivated and wild) and within populations. Of the total variation 49.39% attributable to among populations within groups, 26.37% to among groups and the least, 24.24% to within populations. This is further supported with partitioning genetic diversity analysis of Shannon's and the majority of the genetic diversity (62.5%) reside among populations. The UPGMA and neighbor joining tree also confirm a clear grouping and differentiation based on populations of origin. The result is in line with the mating system of rice. Inbreeding species generally shows lower levels of diversity but greater inter populational variation (Hamrick and Godt, 1990).

Relationships between wild and cultivated rice populations in Ethiopia

Wild rice species of Ethiopia and cultivated rice clustered separately and this study confirms the classification of *Oryza* taxonomy based on morphology and different molecular marker analysis (Joshi et al., 2000; Aggarwal et al., 1999). Although *O.glaberrima* is not expected to form cluster with cultivar of *O.sativa* since the wild populations used in this study are immediate ancestors of this species, due to admixturing of genes and introgression from *sativa* to *glaberrima* during the last 300 - 500 years of *O.sativa* introduction such clustering can result.

A cluster analysis (UPGMA and neighbor joining) was used to construct a dendrogram in order to see relationships among all individuals (Figure 4). Each population forms a discrete cluster similar to the dendrogram constructed based on population of origin. Similarly PCO analysis based three coordinates also show similar result except some observation in which a sample from *O. barthii* (wild Gonder-1) intermixed with *O. longistaminata* (wild Gonder-2) which might be due to their geographic proximity. PCO also show better the hybrid nature of NERICA as a hybrid between the two cultivated rice species.

NERICA cultivar (NERICA-3), a hybrid developed through interspecific hybridization of the two known cultivated species of Asian and African origin (*O. glaberrima* and *O. sativa*) is expected to be intermediate between the two species, however, it is observed to be close to *O. sativa*, Asian rice, than the African rice species since it might shared most traits from its parent of Asian race due to successive back crossing of the hybrid to *O. sativa.* This fact is also indicated by Semagn et al (2006) that NERICA rice developed through F1 progenies derived from interspecific hybridization between *O.sativa* and *O. glaberrima* then backcrossed with their *O. sativa* parents.

The wild rice species collected from Gonder tend to be more similar to *O. sativa species* than *O. glaberrima* (data not shown). The main reason could be gene flow

from cultivated to wild and/or vise-versa since this particular area is an area where rice cultivation has been going on for the last 3 decades. Moreover, the cultivated and wild species are growing together as a crop and weed in the south Gonder. Hence, gene flow occurs with a noticeable frequency from cultivated rice to its weedy and wild relatives (Chen et al., 2004). In addition, among the wild rice species reported to be found in Ethiopia (Phillips, 1995), two of them (*O. longistaminata* and *O. barthii*) are with AA-genome and are sexually compatible with cultivated species having the same genome (AA). Their interspecific F1 hybrids could form complete pairing in meiosis thus resulting in gene flow between them. But in wild Gambella, since there is no cultivated rice near the vicinity where wild rice is found, a wild rice population from Gambella (*O. longistaminata*) has less genetic similarity to *O. sativa* (Chen et al., 2004).

Wang et al. (1992), in their study on relationships in the genus *Oryza* using analysis of nuclear RFLPs, the cultivated African rice (*O. glaberrima)*, clustered with African wild species, *O. barthii*, suggesting that *O. barthii* is probably the ancestor of African cultivated rice, *O. glaberrima*. A wild species from Gonder (wild Gonder-1) which resembles more *O. glaberrima* than the other two wild species in our result is *O. barthii*, which is immediate ancestor of *O. glaberrima* and a species from which African rice, *O. glaberrima* is originated (Bautista et al., 2001). Moreover another wild species of rice, *O. punctata* (a species with two cytogenic groups:-BB, BBCC) which was genetically a bit distant from cultivated rice is also reported to be found in Ethiopia (Joshi et al., 2000). It is also indicated by Phillips (1995) that in Ethiopia there is a genus *Leersia* in many parts of the country including where both cultivated and wild rice is being found. Within the tribe Oryzeae, where also the genus *Oryza* belongs, *Leersia* is most closely related to *Oryza.* Some species of both genera are even appearing to be intermediate between both genera (Vaughan and Morishima, 2002).

Conclusion

Generally, this study demonstrated the existence of immense diversity of wild rice in Ethiopia. However, the current demands for additional land with population pressure, the majorities of river banks and wetlands which are the main reservoir for wild rice gene pool are transformed to farmland (personal observation).

Hence, there is urgent need for large scale collection mission on wild rice and related genera. Collection, taxonomy and molecular phylogenetic analysis of wild rice in Ethiopia should be carried out to survey and confirm the occurrence and distribution of wild rice species. Furthermore, detailed survey and studies are needed to delimit the species as well as occurrence and distribution of *Leersia* and *Oryza* genera found in Ethiopia so as to better understand their evolutionary relationships.

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