ओड़िशा प्रान्त की जैपुर एवं अन्य क्षेत्रों में धान (*Oryza sativa* L.) की पारंपरिक भू–प्रजातियों का परिवर्तनात्मक अध्ययन

Dynamics of on-farm management of rice (*Oryza sativa* L.) landrace populations in Jeypore tract and other parts of Odisha

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Dynamics of on-farm management of rice (*Oryza sativa* L.) landrace populations in Jeypore tract and other parts of Odisha

A Thesis

By

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CERTIFICATE

This is to certify that the thesis entitled **"Dynamics of on-farm management of rice (*Oryza sativa* L.) landrace populations in Jeypore tract and other parts of Odisha"** submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **PLANT GENETIC RESOURCES** is a record of *bona fide* research work carried out by **Mr. LOGA PRIYAN, M.** under my guidance and supervision. No part of this thesis has been submitted for any other Degree or Diploma.

I further certify that any help or information received during the work on this thesis has been duly acknowledged.

Place: New Delhi	**(I.S. Bisht)** Chairperson Advisory Committee
Date:	

DEDICATED

TO

My Beloved Parents, Teachers,

Friends and Farmers

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Date:

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ABBREVIATIONS

%	:	Percentage
°C	:	Degree Celsius
µl	:	Microliter
Bp	:	Base pair
C.D.	:	Critical Difference
CTAB	:	Cetyl Trimethyl Ammonium Bromide
DNA	:	Deoxyribose Nucleic Acid
dNTPs	:	Deoxy Nucleotide Tri Phosphate
EDTA	:	Ethylene Diamine Tetra Acetic acid
EtBr	:	Ethidium Bromide
Fig.	:	Figure
mM	:	Milli molar
$MgCl_2$	:	Magnesium chloride
ml	:	Milliliter
mm	:	Millimeter
NaCl	:	Sodium chloride
NaOAc	:	Sodium acetate
NBPGR	:	National Bureau Of Plant Genetic Resources
ng	:	Nanogram
PCR	:	Polymerase chain reaction
RM	:	Rice microsatellites
RFLP	:	Restricted Fragment Length Polymorphism
RAPD	:	Random Amplified Polymorphic DNA
RNA	:	Ribonucleic acid
RNase	:	Ribonuclease
STMS	:	Sequence-tagged microsatellite site
SSR	:	Simple Sequence Repeat
TAE	:	Tris-Acetate-EDTA
Taq	:	*Thermus aquaticus* DNA Polymerase
UPGMA	:	Unweighted Pair Group Method of Arithmetic Average
V	:	Volts

CHAPTER 1 INTRODUCTION

Evolution and domestication of crops were the two principal dynamic processes, unique towards its biology, space and time. The basic foundation for agriculture and global food security revolves around plant genetic resources (PGR), these genetically diverse collections of plant germplasms are the prime source material for the researchers in breeding them to new plant varieties, which in turn serves as a most powerful weapon for them in their battle against hunger. The survival of a plant species against environmental changes which encompass new pests and diseases and new climatic conditions, profoundly depends upon its genetic diversity. Genetic diversity is a responsible factor for adaptation and the rate of response to selective forces during evolution of a species (Hammer and Teklu, 2008). Traditional farmers acts both as the conservers and providers of these inestimable resources of genetic diversity in the form of locally adapted genotypes or landraces for most of the major and minor crops which have sustained from various natural and artificial selection forces for over thousands of years in the past (Myers, 1994). Landraces are geographically or ecologically distinctive populations which are conspicuously diverse in their genetic composition both between populations [i.e. between landraces] and within them (Brown, 1978). Being evolved through indigenous cultivation practices, landraces were not subjected to deliberate intensive selection which is rather a common practice in scientific plant breeding for producing a modern variety, thereby landraces represent a storehouse of genetic heterogeneity (Zeven, 1998). The diversity at genetic levels of a landrace can been studied under two dimensions, the first being **between site** and population variations (mainly generated by heterogeneity in space) and the second one is **within site** and population variations (generated by heterogeneity in space and time) (Frankel & Soulé, 1981).

Rice (*Oryza sativa* L.) the most vital staple food crop that feeds more than half of the global human population (Maclean and Hettel, 2006); the livelihoods and economies of several billion people around the world rely on this crop. In 2010, the global rice cultivation area was approximately 154 million ha, of which Asia shares 137 million ha (88 percent of the area). In specific Southeast Asia alone occupies 48 million ha (31 percent of the global rice area) of global rice cultivation area (FAOSTAT, 2012). Rice has been intermingled with the culture of the people for well over 4,000 years and it feeds about 557 million people in Southeast Asia (Manzanilla *et al*., 2011). Among the Southeast Asian countries, India in particular covers up enormous amount of high yielding varieties, diverse landraces and wild forms in rice cultivated under varied geographical conditions with different farming systems. In specific several researches identified the Jeypore tract of Odisha in India as the centre of origin and diversity of cultivated rice crop (*Oryza sativa*) (Ramiah and Ghose, 1951; Ramiah, 1953; Sharma *et al.,* 2000). This tract comprises the geographical area extending from parts of north-eastern Andhra Pradesh and covers the whole state of odisha and some regions of southern Bihar (Smita, 2009), and is acknowledged for the long historical practice of *in situ* on farm conservation under diverse environments for different crops including rice. On farm conservation has been considered as enhanced plant genetic resources (PGR) utilization at the local level and consistent with agricultural development in marginal environments and small farm situations (Maxted *et al*., 1997; Worede *et al*., 1999; Bisht *et al*., 2006, 2007; FAO, 2009).

The sampling and management procedures for rice genetic resources could not be built in an effective manner unless a clear knowledge of correlation and distinction among rice varieties from the targeted region is established. A cardinal parameter to be studied for effective genetic conservation is to discover the allelic richness of the population or samples which are targeted for conservation (Zhu Ming *et al.,* 2004). One of the most commanding tools employed to explore the genetic variation in rice varieties is molecular marker technology, which augments in framing out a strategic decision making for effective genetic resource conservation. Among the molecular markers, Microsatellite markers are presumed to be suitable for evaluating genetic diversity and relationships among closely related plant accessions or individuals, such as different rice cultivars (Tonapha *et al.,* 2009; Vanniarajan *et al.,* 2012). To date, innumerable SSR primer pairs have been developed in rice which renders a tremendous opportunity for gaining an insight into the genetic structure of the rice genome.

The global natural environments in the near past were assaulted by various human activities leading to the gradual loss in biodiversity in forms like genetic erosion; lack of proper attention to conserve biodiversity will lead to widespread degradation of many wild lands and their biota's (Frankel, 1974). Immediate concern is to collect and conserve these irreplaceable genetic resources by deploying *in situ* and *ex situ* method of conservation.

Ex situ conservation or Genebank accessions captures only a unique portion of species evolution and arrests it for future utilisation, thereby representing a pattern called static conservation. In contrast, the *in situ* and on farm conservation allows the genetic level modifications to take place in a plant population, so they are recognized as dynamic conservation method (Bretting and Duvick, 1997).

The large scale built up of *ex situ* collections of crop landraces across the globe results in safeguarding the landraces against genetic erosion from traditional farming and thereby protecting it for sustainable utilization in future, since it is a valuable source of resistance towards biotic and abiotic stress. However, adequacy of *ex situ* conservation strategy has been sometime questioned mainly on grounds of being static and for detaching the collection from local knowledge systems. *In situ* conservation on farm has now been considered as back-up and complementary strategy to *ex situ* conservation.

Despite the surge of support for on-farm conservation of PGR on global scale, no agreed set of scientific principles yet exists for its implementation. Further, not only the consequences of *ex situ* and on-farm conservation strategies have not been studied and resolved but also method of researching, understanding and quantifying the complementary role of these strategies have not been well developed. Even the baseline data on the original composition of landraces is not available to assess the trend of genetic change in space and time.

The present research problem, therefore, aimed at investigating the potentials and consequences of on-farm management of populations of some locally common rice landraces collected from Jeypore tract of Odisha.

The major objectives of the study are:

- To explore the Genetic similarity/distinctiveness of farmer-named varieties under dynamic and static management and diversity loss over time and space.
- To understand the dynamics of farmers management of different landrace populations under marginal management.
- Landrace diversity and sustainability of agricultural production under subsistence farming.

Rich diversity of rice crop and their wild relatives exits in India with respect to morphological and physiological characteristics. They vary in maturity duration from 60 to over 200 days and can be grown at varied elevations from sea level to 2300 m. They are also well adapted to different seasons of the year under both upland and deep water conditions. Due to high environmental heterogeneity, wide morphological variations in rice landraces exist in India. Jeypore tract in the Odisha state, considered as a centre of origin of rice, is home for a rich diversity of landraces. It is inhabited by ancient farming communities with intense belief in tradition including crop cultivation. Participatory Plant Breeding has been demonstrated in channelizing the peoples' initiative and enthusiasm towards conservation, cultivation, consumption and commercialization leading to accelerated poverty reduction (Arunachalam *et al*., 2006).

Rice has one of the largest *ex-situ* holdings in the world (Jackson and Juggan, 1993). This accessible collection of diverse cultivated and wild species of rice has made great contributions to rice breeding. *In situ* conservation on farm has now been considered as back-up and complementary strategy to *ex situ* conservation and models for its implementation has also been suggested (Maxted *et al.,* 1997, 2002). It has been considered as enhanced PGR utilization at the local level and consistent with agricultural development. This diversity benefits the farmers by expanding the choices they had available to them, which in turn increases the probability of exploring new varieties that can adapt to their specific paddy environments and economic circumstances.

Characterization of these valuable Plant genetic resources is considered to be the principle management tool for their efficient management and utilisation. The information generated helps in discriminating various accessions, detect redundancies and monitor genetic changes created during crop maintenance. Characterization should eventually lead to a system of recording and storing useful data that can be readily retrieved and made available to others and help in planning breeding programmes (Dabas *et al*., 1994).Several morphological and molecular methods of characterization have been introduced by many workers in order to identify, characterise and differentiate the genotype of interest. Compared to morphological analysis, molecular markers can reveal differences among accessions at the DNA level and thus providing a more direct, reliable and efficient tool for germplasm conservation and genetic diversity assessment.

2.1. Morphological characterisation of rice

The most conventional and age old practice of identifying a germplasm is based on morphology of different characters among several species including rice. One of the pioneer work in this regard were reported by Kato *et al*., (1928), where they identified two main varietal types in rice *viz., indica* and *japonica* through morphological, serological and inter-varietal hybrid fertility test. Later, a third type was identified by Matusuo (1952) on the basis of rice morphology. In a couple of years, these three types of rice crop were referred to as *indica, japonica* and *javanica* (Moringa, 1954).

Sharma *et al.* (2007) characterised 183 landraces of rice adapted to the lowland and hills in Nepal to determine the level of genetic richness among the landraces. They observed significant differences between landraces for all morphological traits. The analysis separated the lowland and hills adopted landraces into broad groups. Joshi and Bauer (2007) investigated the cultivation and loss of rice landraces in tarai region of Nepal and identify the factors influencing the probability of cultivating the most dominant landraces has declined substantially following the development and the extension of high yielding modern varieties in Nepal. Although the varietal diversity appeared too high, the number of landraces under cultivation is declining. The knowledge of agro-morphological diversity and the distribution pattern of variation among conserved accessions could be an invaluable aid in germplasm management and crop improvement strategies.

Sanni *et al.* (2008) evaluated 880 landrace rice (*O.sativa*) accessions in Cote d’Ivoire for 13 agro-morphological characters to study the geographical pattern of morphological variation. Result of the phenotypic frequency showed that, the landraces from the north and north-western zones were mostly tall, early heading and maturing, compared to those from the west and west-central which were mainly dwarf to medium height, late heading and maturing. This differential distribution of landraces with height, heading and maturity period reflected the distribution pattern of different *O.sativa* landraces in Cote d'Ivoire, which could be useful in germplasm management and breeding programmes.

Chakravorty and Ghosh (2012) characterised fifty one rice landraces using 46 agro-morphological traits following Distinctiveness, Uniformity and Stability test (DUS). They have also reported that the characters which are of qualitative in nature is less influenced by environmental fluctuations and can be considered as marker characters in the identification of rice landraces.

2.2. Molecular characterisation and identification

Molecular markers are specific sequences of DNA, which helps in distinguishing and identifying various crop species. The variations in the molecular markers may occur due to the changes in the sequences of bases in the DNA. These changes may lead to the formation or deletion of a restriction site or a random change in the base sequences or even changes within the simple repeat regions of a DNA or in its flanking regions. The occurrence of these variations may be due to deletion, duplication, point mutation, insertion, inversion or spontaneous mutation of a DNA segment.

A range of techniques that can detect polymorphism at the DNA level are available. Molecular markers have significant value in breeding programmes to characterise and evaluate genetic variability in germplasm and to identify varieties. They offer several advantages over biochemical markers. Their expressions are independent towards crop stage or organ, nor do they vary in any part of the plant organ (Morell *et al.,* 1995) and being unlimited in number, a thorough sampling of genome is possible (Helentjaris *et al.,* 1985). In rice, molecular markers have been used to identify accessions (Olufowote *et al.*, 1997), to determine the genetic structure and pattern of diversity in landraces of interest (Vanniarajan *et al*., 2012; Hassan *et al*., 2012 and Choudhury *et al*., 2013).

2.2.1. Simple sequence repeats (Microsatellites) as molecular markers

Simple sequence repeats (SSRs) commonly referred to as microsatellites, is tandemly repeated mono to hexa-nucleotide motifs that are ubiquitous in eukaryotic genomes and exhibit highly variable numbers of repeats at a locus. They are codominant in nature and are widely used in rice genetics to fingerprint accessions and analyse diversity. Their abundance and hyper variability make them valuable as genetic markers (Weber and May, 1989). SSRs consists of 2-6 nucleotide core units such as (CA), (ATT), or (ATGT) that are tandemly repeated in the genome (Litt and Luty, 1989). The regions flanking a micro satellite are generally conserved among genotypes of the same species and PCR primers to the flanking regions can be used to amplify the SSR-containing DNA fragment. Length polymorphisms is created when PCR products from different alleles vary in length as a result of variation in the number of repeat units in the SSR, and can then be analysed by acrylamide or agarose-gel electrophoresis to resolve contrasting alleles. The high level of informativeness and codominance of microsatellite markers, their widespread occurrence in eukaryotic genomes, and easy amplification via standard PCR technology, make SSRs the current marker of choice in many species. In rice, more than 500 microsatellite markers have been developed and used to construct genetic maps (Wu and Tanksley, 1993; Akagi *et al*., 1996; Panaud *et al.*, 1995, 1996; Chen *et al*., 1997 and Temnykh *et al*., 2000, 2001). These markers provide important co-dominant landmarks that are well distributed throughout the rice genome. SSRs have been widely used in rice genetics to fingerprint accessions, analyse diversity, identify introgressions in interspecific crosses, trace pedigrees, and locate genes and QTLs on rice chromosomes and in marker –assisted selection. In sequencing and genomics applications, SSRs are increasingly useful as sequence tag connectors (STCs) linking the physical and genetic maps of rice (McCouch *et al*., 2001).

Studies on microsatellites in plants were first published by Condit and Hubbell (1991), who reported abundant occurrence of microsatellites in plant species. Thereafter, SSR markers have been developed for several plant species including rice (Zhao and Kochert, 1992 and Wu and Tanksley, 1993). Wu and Tanksley showed the abundance of GA and GT repeats in rice genome with multiple alleles at all loci and when compared to RFLP heterozygosity, values were found to be significantly higher for microsatellites. Using the rice genome sequence information from the database, Akagi *et al.* (1996) developed *35* microsatellite DNA markers distributed on all chromosome regions. This could help to select plant carrying desired chromosomal regions for gene tagging purpose. Microsatellite loci were found to be distributed randomly in the genome which led to construction of a microsatellite map providing genome wide coverage in rice using 94 microsatellite markers (Chen *et al*., 1997). Screening rice genomic library indicated that there are estimated 5700-10000 microsatellites in rice, with the relative frequency of different repeats decreasing with increasing size of the motif (McCouch *et al*., 1997). They constructed a map consisting of 12 simple sequence length polymorphism (SSLP) markers evenly distributed throughout the 12 chromosomes of rice which is sufficient for genotype identification, gene and Quantitative Trait Locus (QTL) analysis, screening of large insert libraries and marker assisted selection in breeding.

2.2.2. Microsatellite markers for diversity analysis

Molecular markers assay genetic variation directly at the level of nucleotide sequence of DNA and thus remain unaffected by environmental influences. Among the various molecular markers, PCR-based microsatellite markers detect a significantly higher degree of polymorphism in rice (Wu and Tanksley, 1993; Yang *et al.,* 1994) and are especially suitable for evaluating genetic erosion among closely related rice landraces (Akagi *et al.,* 1996). Simple sequence repeats (SSRs) are versatile DNA markers that are readily assayed and highly informative. Non-targeted approaches to SSR development often leave large genomic regions without SSR markers. In rice, microsatellites are abundant and well distributed throughout the genome (Akagi *et al.,* 1996). They are valuable as genetic markers because they are codominant, detect high levels of allelic diversity and are assayed efficiently by PCR (Mc-couch *et al.,* 1997). The current level of average genome-wide coverage provided by microsatellites in rice has been one markers every 6 centimorgens (Temnykh *et al.,* 2000).

The growing number of rice microsatellite markers warrants a comprehensive comparison of allelic variability between the markers developed using different methods, with various sequence repeat motifs, and from coding and non-coding portions of the genome. Cho *et al*. (2000) performed a comparison over a set of 323 microsatellite markers; 194 were derived from genomic library screening and 129 were derived from the analysis of rice-expressed sequence tags (ESTs) available in public DNA databases. The frequency of polymorphism between parental pairs of six inter-subspecific crosses and one inter-specific cross widely used for mapping in rice was evaluated. Microsatellites derived from genomic libraries detected a higher level of polymorphism than those derived from ESTs contained in the Gen Bank database (83.8% versus 54.07%). Preferential location of specific motifs in coding versus non-coding regions of known genes was related to observed levels of microsatellite diversity. A strong positive correlation was observed between the maximum length of a microsatellite motif and the standard deviation of the molecular weight of amplified fragments.

Vanniyarajan *et al*. (2012) explored the genetic diversity structure of various rice landraces belonging to *indica* and *japonica* types using highly polymorphic microsatellite markers. The study revealed the pattern of molecular differentiation which separated *indica* and *japonica* genotypes; and in turn *indica* types had two subpopulations within the landraces.

SSR primers were used in assessing 59 rice genotypes of Bangladesh, for their pattern of genetic variation and population structure which in turn is very important for utilisation and efficient management of germplasms. A total of 114 alleles were detected and the mean gene diversity over all SSR loci was calculated, through which the diversity and unique features of rice landrace collections were examined (Hassan *et al.,* 2012).

Microsatellites markers were used for fingerprinting of hybrids, assessing variation within parental lines and testing the genetic purity of hybrid seed lot in rice. Ten Sequence Tagged Microsatellite Sites (STMS) markers were employed for fingerprinting 11 rice hybrids and their parental lines (Nandkumar *et al*., 2004). Nine STMS markers were found polymorphic across the hybrids and produced unique fingerprints for the 11 hybrids. A set of four markers (RM206, RM216, RM258 and RM263) differentiated all the hybrids from each other, which can be used as referral markers for unambiguous identification and protection of these hybrids. The analysis of plant to plant variation within the parental lines of the hybrid Pusa RH 10, using informative markers indicated residual heterozygosity at two marker loci. This highlights the importance of STMS markers in maintaining the genetic purity of the parental lines. The unique value of the restorer gene linked marker for testing the genetic purity of hybrid seeds was demonstrated for the first time.

The potential of DNA based markers such as sequence tagged microsatellite site (STMS) for establishing distinctness, uniformity and stability (DUS) also merits investigation. A set of 55 mapped STMS markers, selected from 12 linkage groups of rice genome, was used to examine distinctness of 23 aromatic rice genotypes including the commercially important Basmati varieties (Singh *et al*., 2004).Forty-one of these markers showed polymorphism between the varieties. The number of alleles per locus ranged from 2-4 with an average of 2.3. The Polymorphic Information Content (PIC) of the markers varied from 0.083 to 0.665 with an average of 0.338. The marker based clustering of the varieties corresponded with the known phenotypic classification, thereby providing confidence in the distinctness established by the mapped STMS markers. The utility of these markers to study uniformity and ability was analysed using a commercially important cross-bred Basmati rice variety, namely, Pusa Basmati. Genotyping of twenty individual plants, grown from the nucleus, breeder, foundation, certified and farmers saved seed samples using all the 55 markers revealed no variation among the plants.

Olufowote *et al*. (1997) evaluated 71 rice cultivars for within cultivars variation using a combination of phenotypic, RFLP and SSR markers. The study showed that landraces were more heterogeneous and displayed more number of RFLP and SSR alleles. Further SSRs detected a greater number of alleles and were able to discriminate between even closely related individuals more efficiently than RFLP.

The allelic variation detected by the sequence-tagged microsatellite sites (STMS) markers enables differential of the homozygotes from the heterozygotes and therefore is most useful in understanding varietal uniformity. Kohli *et al*. (2004) used STMS markers for a comparative study in revealing the genetic diversity among rice landraces with the commercially cultivated varieties.

2.2.3. Comparing diversity under static and dynamic management

In situ conservation has now been considered as back-up and complementary strategy to *ex situ* conservation and models for its implementation has also been suggested (Maxted *et al*., 1997, 2002). It has been considered as enhanced PGR utilization at the local level and consistent with agricultural development (Worede and Mekbib, 1993; Worede *et al*., 1999; Feyissa, 2000). As *in situ* conservation stands now there seems to be *de facto* conservation of landraces that farmers have been practicing for centuries as part of their farming system (Seboka and Van Hintum, 2006).

Soleri and smith (1995) have studied the consequence of *ex situ* and *in situ* conservation based on maize accessions by measuring variation in morphological and genetic structure between accessions of the same populations kept under static and dynamic condition. The occurrence of genetic shift and drift was reported in *ex situ* collections of maize.

An exploratory study was conducted by Tin *et al*. (2001) comparing gene bank-conserved (*ex situ*) and farmer managed (*in situ*) populations of the same farmers' varieties of rice, here the *ex situ* materials represents adaptation to pre-intensification conditions while the *in situ* populations have been exposed to the intensive production system for a number of years. The data on various morphological traits and biochemical (isozyme) markers were recorded. The results revealed that the populations maintained by farmers showed a general trend of later flowering and maturity time, more uniformity of grain quality, lower frequency of undesired off-types, and reduced drought stress tolerance compared with corresponding *ex situ* populations. They observed adaptability is at risk under on farm conservation due to natural and intentional selection pressure for rice.

The value of genetic resources depends upon two diversity aspects; the first being marker or genetic diversity, which exhibits the extent of differences between individual copies of genes. It provides the information about the lineage or breeding history of the populations. They are indicators of the level of gene flow between populations. The second aspect is the occurrence of gene combinations conferring adaptedness (Tin *et al.,* 2001). This indicates the degree to which populations are adapted to their environment and of their potential for continued performance or donors of characters in plant breeding. These above two sets of attributes were considered analogous to the characterization and evaluation data of genetic resources (Brown 2000).

CHAPTER 3 MATERIALS AND METHODS

3.1 Experimental materials

The experimental materials for sequence tagged microsatellites (STMS) analysis comprised of five named rice landraces assembled from parts of Odisha (Orissa) state of India, representing both static (*ex situ* in genebank) and dynamic (*in situ* on-farm) conservation . The static populations were assembled during 1986 and 1987 and conserved *ex situ* in the National Genebank at the National Bureau of Plant Genetic Resources, New Delhi, India. Whereas the on-farm managed accessions were collected during 2008 and these landraces are still under continuous traditional farming system. The area of collection comes under the Jeypore tract, which is known to represent the centre of origin and diversity in rice.

3.2 Molecular characterization

Thirty seedlings of each population were sown in pots and raised under net house. 4-6 weeks old fresh leaves of individual plant sample were taken for DNA extraction.

3.2.1 Extraction of genomic DNA

Genomic DNA from rice was isolated using CTAB method of Saghai-Maroof *et al.* (1984) with minor modifications. Thirty individual plants were taken for each landrace. Young actively grown fresh leaves of 30 days old rice plants were collected and used for DNA extraction. The healthy leaf material per accession was frozen in liquid nitrogen (–196^{0}C) and then crushed to a fine powder using mortar and pestle. The powdered tissue was transferred to a centrifuged tube (15 ml) and 10 ml of extraction buffer (60^{0}C) was added to it. This mixture was shaken well and incubated at 60^{0}C for one hour with intermittent shaking. Following incubation, equal volume of chloroform-isoamyl alcohol (24:1) was to the centrifuge tube. The contents were mixed gently by inverting the tubes for five minutes. Next, the tubes were centrifuged at 10,000 rpm (20,000 xg) for 10minutes in SS rotor in Sorval RC-5 centrifuge at 25^{0}C. This separated the mixture into two layers *viz.*, upper aqueous layer and lower organic layer. The aqueous layer was transferred to fresh centrifuge tubes and the DNA was spooled out carefully and excess chemical was drained out. DNA was placed in a 1.5 ml microtube, washed two times with 70% ethanol and dried under vacuum. Dried DNA pellet was dissolved in minimum volume of T:E (10:1) buffer.

3.2.2 Purification of genomic DNA

The dissolved DNA samples were treated with RNase A (10 mg/ml) at a concentration of 40 µl/ml of DNA and kept for incubation at 37^0C for one hour. After one hour equal volume of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added to the DNA solution and mixed by swirling for 5 minutes. This was spun at 10,000 rpm for 5 minutes and upper aqueous supernatant layer was removed. This was followed by two extractions with chloroform-isoamyl alcohol (24:1). The DNA was precipitated by adding 1/10 volume of 3M sodium acetate (pH 5.6) and 2.5 times (v/v) chilled ethanol (95 %). Extra salts were removed by further washing with 70% ethanol and DNA was pelleted and dried under vacuum. The pellet was dissolved in minimum volume of T: E (10: 0.1) buffer at room temperature and stored at – 20^0C.

3.2.3 DNA quantification

The DNA concentration was estimated using a Thermo Scientific Nanodrop 1000 Spectrophotometer. It measures 1 µl sample with high accuracy and reproducibility. The full spectrum (220 nm – 750 nm) spectrophotometer utilizes a patented sample retention technology that employs surface tension alone to hold the sample in place. For quantification 1 µl sample was pipetted onto the end of a fiber optic cable (the receiving fiber). A pulsed xenon flash lamp provides the light source and a spectrophotometer utilizing a linear CCD array was used to analyse the light after passing through the sample. For each sample three reading were taken and their average was used for calculating the dilution of samples. These estimates were confirmed by staining DNA with ethidium bromide after electrophoresis in 0.8% agarose gel at 100 V for I hour in TAE buffer (0.04 M Tris acetate, 0.001 M EDTA, pH 8.0) using known DNA concentration standards.

3.2.4 Dilution of DNA samples

A part of the DNA sample was diluted with appropriate amount of sterilized water to yield a working concentration of 20 ng/µl and stored at – 20^0C until used for Polymerase chain reaction (PCR) amplification.

3.3 Microsatellite (STMS) analysis

3.3.1 Optimization of PCR conditions STMS analysis.

The following procedure was used to carry out the PCR amplifications for STMS analysis. The PCR mixture consisted of *Taq* DNA polymerase, PCR buffer, dNTPs, $MgCl_{2,}$ microsatellite primers and genomic DNA. Optimization of PCR component concentrations were carried out for *Taq* DNA polymerase, $MgCl_{2,}$ primer and genomic DNA. Concentration of dNTPs (200 µM) and PCR buffer (10 x) was not varied. PCR reactions were carried out in a BioER^{TM} XP cycler. The thermocycling conditions followed were as follows,

1. Denaturation at 94^0C for 6 minutes.
2. 30 cycles of Denaturation at 94^0C for 1 minute, primer annealing at respective annealing temperature for 1 minute and primer extension at 72^0C for 1 minutes.
3. Final extension step at 72^0C for 10 minutes.
4. Holding of samples at 4^0C.

3.4 Selection of primer

The amplification regime as detailed in Cornell University website www.gramene.com was followed. Fourteen primer pairs were chosen for STMS analysis. These fourteen primers were representing whole rice genome. Primer pairs were chosen on the basis of reproducibility, quality of amplification and polymorphism. List of primers used for genotyping along with their sequences, chromosomal location and annealing temperature are given in Table 3.1.

Table 3.1. Details of STMS Primers, their chromosomal location, sequence and annealing temperature

Primer Name	**Chromosome Location**	**SSR motif**	**Primer Sequence 5' – 3'**	**A* (^oC)**
RM206(F)	11	$(GA)_{21}$	5'CCCATGCGTTTAACTATTCT3'	48
RM206(R)			5'CGTTCCATCGATCCGTATGG3'	
RM19(F)	12	$(ATC)_{10}$	5'CAAAAACAGAGCAGATGAC3'	46
RM19(R)			5'CTCAAGATGGACGCCAAGA3'	
RM218(F)	3	$(GA)_{24}$	5'TGGTCAAACCAAGGTCCTTC3'	52
RM218(R)			5'GACATACATTCTACCCCCGG3'	
RM233B(F)	2	$(GA)_{20}$	5'CCAAATGAACCTACATGTTG3'	48
RM233B(R)			5'GCATTGCAGACAGCTATTGA3'	
RM234(F)	7	$(GA)_{17}$	5'ACAGTATCCAAGGCCCTGG3'	52
RM234(R)			5'CACGTGAGACAAAGACGGAG3	
RM241(F)	4	$(GA)_{31}$	5'GAGCCAAATAAGATCGCTGA3'	50
RM241(R)			5'TGCAAGCAGCAGATTTAGTG3'	
RM249(F)	5	$(GA)_{14}$	5'GGCGTAAAGGTTTTGCATGT3'	50
RM249(R)			5'ATGATGCCATGAAGGTCAGC3'	
RM217(F)	6	$(GA)_{20}$	5'ATCGCAGCAATGCCTCGT3'	46
RM217(R)			5'TAGAAAGATGGAAGGAATGGTGAT3'	
RM11(F)	7	$(GA)_{17}$	5'TCTCCTCTTCCCCCGATC3'	55
RM11(R)			5'ATAGCGGGCGAGGCTTAG3'	
RM223(F)	8	$(GA)_{25}$	5'GAGTGAGCTTGGGCTGAAAC3'	54
RM223(R)			5'GAAGGCAAGTCTTGGCACTG3'	
RM242(F)	9	$(GA)_{26}$	5'GGCCAACGTGTGTATGTCTC3'	52
RM242(R)			5'TATGCCAAGACGGATGGG3'	
RM216(F)	10	$(GA)_{18}$	5'GCATGGCCGATGGTAAAG3'	48
RM216(R)			5'TGTATAAAACCACACACGGCCA3'	
RM222(F)	10	$(GA)_{18}$	5'CTTAAATGGGCCACATGCG3'	50
RM222(R)			5'CAAAGCTTCCGGCCAAAAG3'	
RM224(F)	11	$(GA)_{13}$	5'ATCGATCGATCTTCACGAGG3'	44
RM224(R)			5'TGCTATAAAAGGCATTCGGG3'	

A* = Annealing temperature

3.5 PCR Amplification

PCR reactions were carried out in a Bioer XP cycler (Bioer Technology Co. Ltd, Hangzhou, P.R. China) using PCR microplates (Axygen Inc., California, USA). The total PCR reaction volume was 25-µl, composed of 2.0 µl of 40 ng genomic DNA, 2.5 µl 10X PCR buffer (Tris with 15 mM MgCl2, Conc. 10X), 2.5 µl deoxynucleotides (dNTPs), 2.0 µl primer, 0.33 µl Taq DNA polymerase (conc. 3 U/µl) (GeNei™ Bangalore Genei, Bangalore, India) and 15.67 µl sterile deionized water. PCR tubes containing the above components were capped and given a pulse spin to allow proper settling of reaction mixture.

3.6 Agarose gel Electrophoresis

A 3.0 % agarose gel was prepared in 1X TAE buffer. The contents of the PCR tubes were loaded into the wells, after adding 2 µl loading dye (1X) to each tube. Electrophoresis was carried out in 1X TAE buffer at 110 V for one and half to two hours (till the bromophenol blue dye travelled less than 2/3 of the gel length). The gel was stained with ethidium bromide (10 µl/100ml). The resolved amplification products were visualized and photographed under a G:Box XT^4 gel documentation system (SYNGENE).

3.7 Data analysis

The amplification products were scored across the lanes comparing their respective molecular weights. Each band was treated as one STMS allele. Scoring of bands was done from photographs. Molecular weights of bands were estimated by using standard 50bp DNA ladder and the homology of bands was based on distance of migration in the gel.

STMS amplicon obtained from each entry were resolved as a single band on the agarose gel system and the data set were used to do the analysis. The polymorphism information content (PIC) value for each STMS locus was calculated using PIC calculator http://www.liv.ac.uk/~kempsj/pic.html (Jan, 2002). PopGene Version 1.32 software (Yeh *et al*., 2000) was used to estimate the various genetic diversity parameters.

The data were subjected for Analysis of Molecular Variance (AMOVA) among groups, among populations within groups and among individuals within populations using Arlequin 3.5 software (Excoffier and Lischer, 2010) to determine the distribution of variation at different hierarchical levels. Population structure by AMOVA is based on an analysis of variance of gene frequencies, taking into account the number of mutational differences between molecular haplotypes. Fixation indices (Weir and Cockerham, 1984) and population pairwise F_{ST} (pairwise estimates of the correlation of alleles between populations) values were also computed by using the above software. Rcmd (R software) was used in Arlequin version 3.5 software to generate a plot of pairwise F_{ST} values. Population average pairwise differences were also computed using the software.

The genetic structure of the populations was also studied by using the Bayesian Model-based approach proposed by Prichard *et al.* (2000) to assign the landraces into genetically structured groups. The model assumes K number of populations characterized with a set of allele frequencies at each locus that are in Hardy-Weinberg equilibrium. The application tests the presence of a population structure ($K > 1$) and assigns the individuals from the sample population into groups for a given number of populations (K) in a way Hardy-Weinberg disequilibrium and linkage disequilibrium (LD) is maximally explained. The software package STRUCTURE version 2.3.4 (Prichard *et al*., 2010) was used to perform this analysis. Optimum number of populations was inferred by running an admixture ancestry model with correlated allele frequencies starting from two populations $K = 1$ to $K = 10$, with 20 runs at each K. For each run, 5000 burn-ins followed by 50000 Markov chain Monte Carlo (MCMC) simulations were performed. The best value of K was determined by a calculation, which is based on the second order rate of change of the likelihood (ΔK) (Evanno *et al*., 2005). The ΔK shows a clear peak at the true value of K. This calculation was done by using an online software program called STRUCTURE HARVESTER, which is a python program with a web based front end for quickly parsing and summarising output data from STRUCTURE (Dent *et al.,* 2012). Inferred ancestry estimates of individuals (Q-matrix) were derived for the selected subpopulation (Pritchard *et al.*, 2000).

3.8 Morphological characterization

The rice landrace populations were also grown for morphological characterization in replicated field trials at NBPGR Base Centre, Cuttack (Odisha) during 2012 cropping season. Data were recorded on 24 qualitative and 17 quantitative traits (Table 3.2).

3.8.1 Data analysis and interpretation

Data on 17 quantitative traits were subjected to ANOVA and multivariate analyses (cluster and principal components analyses) using INDOSAT statistical package developed at the INDOSTAT Services, Hyderabad. The frequency distribution and comparative summary statistics of rice accessions for quantitative traits were computed. Twenty four descriptors for qualitative traits were recorded at different stages of growth in all the ten landraces of rice, as per Bioversity-IRRI guidelines (2007) (Table 3.3).

Ward's minimum variance clustering

Ward's minimum variance method was used to form clusters based on quantitative traits. Ward's method follows a series of clustering steps that begin with't' clusters, each containing one accession, and it ends with one cluster containing all traits. At each step it makes whichever merger of two clusters that will result in the smallest increase in the value of an index E, called sum of square (SS) index, or variance. This means that at each clustering step we must try all possible merger of two clusters, compute the value of E for each, and select that one whole value of E which is the smallest. Then we go on the next clustering step and repeat the process.

For each tentative set of clusters, E is computed as follows, (i) we calculate the mean of each cluster. The cluster mean is a fictitious accession whose descriptor values are the average of traits values for the traits in the given cluster, (ii) we compute the differences between each accession in a given cluster, (iii) for each cluster we square the differences computed above. Add those for each cluster, giving a SS for each cluster. Finally compute the value of 'E' by adding the SS for all clusters.

Principal component analysis

Principal components were analysed based on quantitative traits. Principal components Wi (i = 1,2,3,…..,n) were obtained as W = Zx where Z and x are column vectors of correlation ‘R’ among quantitative traits. The variance of Wi is the i^{th} characteristic root λ I of the correlation matrix ‘R’ with λ obtained by solving the characteristic equation (R – λ I) = 0. For each λ, the corresponding characteristic vector is obtained by solving the equation (R – λ I) = 0. Scatter plot was drawn using the first two principal components in order to identify the most distinct accession in different clusters.

Table 3.2. Various qualitative and quantitative traits recorded for morphological characterization of rice landraces

Qualitative traits				Quantitative traits	
1.	Basal Leaf sheath: Colour	18.	Panicle: exertion	1.	Plant height (cm)
2.	Leaf blade: Intensity of green colour	19.	Panicle threshability	2.	Leaf length (cm)
3.	Leaf blade: Attitude	20.	Lemma and palea: pubescence	3.	Leaf width (cm)
4.	Leaf blade: pubescence	21.	Lemma and palea: colour	4.	Ear bearing tillers (EBT)
5.	Auricle: colour	22.	Lemma: colour of apiculus	5.	Panicle length (cm)
6.	Collar: colour	23.	Caryopsis: Pericarp colour	6.	Ligule length (cm)
7.	Ligule: Shape	24.	Caryopsis scent	7.	100 grain weight (g)
8.	Ligule: colour			8.	Panicle weight (g)
9.	Culm: habit			9.	Days to 50% flowering
10.	Flag leaf: attitude			10.	No. of spikelets/panicle
11.	Culm: internode anthocyanin			11.	Filled grain/panicle
12.	Culm strength			12.	Unfilled grain/panicle
13.	Stigma: colour			13.	Sterility %
14.	Awns: presence			14.	Grain Length (mm)
15.	Panicle: number per plant			15.	Grain width (mm)
16.	Panicle: attitude of branches			16.	Yield/plant(g)
17.	Panicle: secondary branching			17.	Yield/m^2 (g)

Table 3.3. Various qualitative traits recorded in rice landraces using Bioversity International – IRRI rice descriptors

Sl. No.	**Qualitative trait descriptor**	**Note**	**State**	**Stage of observation**
1	Basal Leaf Sheath: Colour	1 2 3 4	Green Green with purple lines Light purple Purple	Late vegetative
2	Leaf blade: Intensity of green colour	0 3 5 7	No green colour visible due to anthocyanin Light Medium (green) Dark	Late vegetative
3	Leaf blade: Attitude	1 5 7	Erect Horizontal Drooping	Late vegetative prior to heading
4	Leaf blade: Pubescence	1 2 3	Glabrous Intermediate Pubescent	Late vegetative
5	Auricle: Colour	0 1 2 3 4 5	Absent (no auricles) Whitish Yellowish green Purple Light purple Purple lines	Late vegetative
6	Collar: Colour	0 1 2 3	Absent (collarless) Green Light green Purple	Late vegetative

		4	Purple lines	
7	Ligule: shape	0	Absent	Late vegetative
		1	Truncate	
		2	Acute to acuminate	
		3	2-cleft	
8	Ligule: colour	0	Absent (liguleless)	Late vegetative
		1	Whitish	
		2	Yellowish green	
		3	Purple	
		4	Light purple	
		5	Purple lines	
9	Culm: habit	1	Erect (<15°)	After flowering
		3	Semi-erect (intermediate) (~20°)	
		5	Open (~40°)	
		7	Spreading (>60–80°, culms not resting on the ground)	
		9	Procumbent (culm or its lower part rests on ground surface)	
10	Flag leaf: attitude (angle)	1	Erect	At anthesis
		3	Semi-erect (Intermediate)	
		5	Horizontal	
		7	Descending	
11	Culm: internode anthocyanin	0	Absent	Near colouration maturity.
		1	Purple	
		2	Purple lines	
12	Culm: strength	1	Very weak	At harvest
		3	Weak	
		5	Intermediate	
		7	Strong	

		9	Very strong	
13	Stigma: colour	1	White	At anthesis
		2	Light green	
		3	Yellow	
		4	Light purple	
		5	Purple	
14	Awns: presence	0	Absent	Flowering to maturity
		1	Partly awned	
		2	Fully awned	
15	Panicle: number per plant	3	Low	Early ripening
		5	Intermediate	
		7	High	
16	Panicle: attitude of branches	1	Erect (compact panicle)	Near maturity
		3	Semi-erect (semi-compact panicle)	
		5	Spreading (open panicle)	
		7	Horizontal	
		9	Drooping	
17	Panicle: secondary branching	0	Absent	Near maturity
		1	Sparse	
		2	Dense	
		3	Clustered	
18	Panicle: exertion	1	Enclosed	Near maturity
		3	Partly exerted	
		5	Just exerted	
		7	Moderately well exerted	
		9	Well exerted	
19	Panicle threshability	1	Difficult (few or no grains removed)	Determined by grasping the panicle with the hand, applying a slight rolling pressure with the palm and fingers,
		2	Intermediate (25–50% of grains removed)	
		3	Easy (>50% of grains removed)	

				and assessing the percentage of grains that are removed by the action
20	Lemma and palea: pubescence	1 2 3 4 5	Glabrous Hairs on lemma keel Hairs on upper portion Short hairs Long hairs (velvety)	Visual assessment of the presence and distribution of mature grains using hand lens.
21	Lemma and palea: colour	1 2 3 4 5 6 7 8 9 10 11	White Straw Gold and gold furrows Brown Brown spots Brown furrows Purple Reddish to light purple Purple spots Purple furrows Black	After anthesis to hard dough stage (pre-ripening stage)
22	Lemma: colour of apiculus	1 2 3 4 5 6 7 8 9	White Straw Brown Green Red Red apex Purple Purple apex Black	At maturity
23	Caryopsis: Pericarp colour	1 2	White Light brown	Post-harvest

		3 4 5 6 7	Speckled brown Brown Red Variable purple Purple	
24	Caryopsis scent	0 1 2	Non-scented Lightly scented Scented	From cooked kernel (freshly harvested grain)

Genetic diversity under static and dynamic management: A case of rice landraces from parts of Odisha in India

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Abstract

In the present study, Inter- and intra-population diversity of five named rice landraces from parts of Odisha state of India representing static and dynamic management were examined using 14 Sequence-tagged microsatellite site primer pairs. A total of 64 alleles were detected in 10 populations of the five named landraces. The number of alleles ranged from 2 to 7 with an average of 4.57 alleles per locus. Of the 64 alleles, 60 were common and four were rare. Moderate to low diversity in landrace populations was observed with number of alleles per population ranging from 16 to 25 and per cent polymorphism ranging from 14.29 to 64.29, respectively. The AMOVA indicated highest variation of 75.7% among populations within groups (static ***vs*** dynamic). A model based (structure analysis) method was also used for analyzing genetic structure of individuals showing the diversity pattern. Pair-wise estimates of F_{ST} revealed very high significant population differentiation which ranged from 0.68 to 0.89 indicating that the populations share limited genetic diversity among them. However, not many variations were observed in the phenotypes of populations representing static and dynamic management. This shows that adaptations of a population are apparently persisting over generations but the underlying genotypes have changed and new alleles or combinations may arise and increase in frequency at the expense of other alleles that have disappeared.

The importance of population biology research for *in situ* conservation, requires both descriptive and hypothesis testing, in order to guide technical improvement and management of landrace populations was emphasized.

Key words: Rice landraces, *Oryza sativa* L., static and dynamic management, population structure, adaptive variations

Introduction

The threats faced by crop landraces grown in traditional farming by large scale adoption of improved cultivars have led to the creation of conservation programs to preserve crop resources for future generations. One type of crop genetic resources conservation is *ex situ* (static) i.e. maintenance of genetic resources in gene banks under sub-zero temperatures (Plucknett *et al*., 1987) and another type is *in situ* (dynamic) i.e. maintenance on-farm under traditional productions (Brush 1991, 2000; Maxted *et al*., 1997). *Ex situ* conservation is well suited to capture and store alleles and genotypes, and is also well suited to users who need ready access. It also acts as a back-up to existing diversity that might otherwise be lost in human managed agricultural landscapes (Cohen *et al*., 1991). *Ex situ* conservation, however, is not suited to the conservation of the other components of the agroecosystem that generate crop genetic resources (Brush, 2000). *In situ* conservation is specifically intended to maintain those components in living, viable agroecosystems. Considering that *in situ* conservation is ecologically dynamic, it would fail if we stop change or preserve agroecosystem in a particular state (Brown, 2000). Sources of change that can be expected and must be tolerated include the introduction of new crops and crop varieties; exchange of varieties between farmers and localities; the use of inputs to improve the productivity of land and labour, such as fertilizers and pesticides; and commercialization (Brush, 2000).

There is now widespread recognition globally among the plant genetic resources community to turn its attention to *in situ* conservation of cultivated populations on farms. It has arisen from certain case studies of crop diversity that indicate substantial diversity still persists on farms in regions of crop origin and diversity, despite the advent of modem cultivars of crops to those areas (Brush 1995; Kumar *et al*., 2010; Pandey *et* *al*., 2011, 2012). There is now widespread recognition of the need to plan for *in situ* conservation to continue and indeed to improve its capacity to maintain genetic diversity as an adjunct to conservation in *ex situ* collections (Brown, 2000). There are, however, limited information available on scientific basis and the optimal procedures for on-farm conservation of crop landraces. As a basis for guiding the supporting research in population biology for on-farm conservation, the present studies on the genetic structure of traditional rice landraces from parts of Odisha state of India, both under static and dynamic management, were undertaken.

Two sets of attributes can be considered as an important indicator of genetic composition of rice landraces. The first set is *marker diversity* or the extent of differences between individual copies of genes at DNA level and is informative as to the ancestry or breeding history of the populations (Brown, 2000). They are indicators of the recency of bottlenecks in population size, the prevalence of outcrossing, the ease with which genes are recombined, and the level of gene flow between populations. The second set is *variation in adaptation*. This set comprises indicators of the degree to which populations are adapted to their environment and of their potential for continued performance or donors of characters in plant breeding. Both biotic and abiotic aspects of the environment are involved.

Materials and Methods

Five named rice landraces assembled from parts of Odisha (Orissa) state of India, representing both static (*ex situ* in genebank) and dynamic (*in situ* on-farm) conservation, were included in the present study (Table 4.1.1). The genebank conserved populations were assembled during 1986 and 1987 whereas the on-farm managed accessions were collected during 2008 as these landraces are still under continuous cultivation under traditional farming system. As none of these named landraces maintained on farm could be collected from the same household, all care was taken to explore and collect the landrace populations from the close-by areas of the populations assembled earlier and conserved *ex situ* in the National Genebank at the National Bureau of Plant Genetic Resources, New Delhi, India.

Table 4.1.1. Rice landraces studied for population genetic structure under static and dynamic management

Landrace	IC Number	Conservation status	Area of landrace collection	Frequency of occurrence
Asamchudi	98721	Static	Kalahandi	Locally common
	569071	Dynamic	Kalahandi	Locally common
Bhuta	86063	Static	Keonjhar	Locally common
	568854	Dynamic	Keonjhar	Locally common
Kalakadamba	85972	Static	Keonjhar	Locally common
	568859	Dynamic	Keonjhar	Locally common
Narada	85999	Static	Keonjhar	Locally common
	568856	Dynamic	Keonjhar	Locally common
Saleijhati	86055	Static	Keonjhar	Locally common
	568873	Dynamic	Keonjhar	Locally common

- **Molecular characterization**

Sample preparation

Thirty individual plants were taken for each landrace population. The seeds were selected randomly from the sample and planted in the field nursery for collecting leaf samples. Young actively growing leaves of 30-day-old plants were collected and used for DNA extraction. Leaves were harvested and immediately stored at -80ºC until total genomic DNA was extracted by using a modified cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof *et al*., 1984). The DNA quantification was done by using Nanodrop^{TM} 1000 Spectrophotometer (Thermo Scientific). These estimates were confirmed by staining DNA with ethidium bromide after electrophoresis in 0.8% agarose gel at 100 V for 1 h in TAE buffer (0.04 M Tris acetate, 0.001 M EDTA, pH 8.0) using known DNA concentration standards.

Sequence tagged microsatellite site (STMS) genotyping

A group of fourteen highly polymorphic and diverse primer pairs representing eleven rice chromosomes were chosen for STMS analysis. PCR reactions were carried out in a Bioer XP cycler (Bioer Technology Co. Ltd, Hangzhou, P.R. China) using PCR microplates (Axygen Inc., California, USA). The total PCR reaction volume was 25-µl, composed of 2.0 µl of 40 ng genomic DNA, 2.5 µl 10X PCR buffer (Tris with 15 mM MgCl2, Conc. 10X), 2.5 µl deoxynucleotides (dNTPs), 2.0 µl primer, 0.33 µl Taq DNA polymerase (conc. 3 U/µl) (GeNei™ Bangalore Genei, Bangalore, India) and 15.67 µl sterile deionized water. Samples were subjected to the following thermal profile for amplification in a thermocycler: initial extended step of denaturation at 94◦C for 6min followed by 32 cycles of denaturation at 94◦C for 1min, primer annealing at respective annealing temperature for 1 min, primer extension at 72◦C for 1min and a final extension at 72°C for 10min. After amplification, the PCR tube was stored at 4°C until electrophoresis. The amplified products were electrophoresed on 3% agarose gel at 110

V in 1X TAE buffer. Gels were stained with ethidium bromide and photographed using a G:Box XT^4 gel documentation system (SYNGENE).

Data analysis

Molecular weights of bands were estimated by using standard 50bp DNA ladder and the homology of bands was based on distance of migration in the gel. STMS amplicon obtained from each entry were resolved as a single band on the agarose gel system and the data set were used to do the analysis. The polymorphism information content (PIC) value for each STMS locus was calculated using PIC calculator http://www.liv.ac.uk/~kempsj/pic.html (Jan, 2002). PopGene Version 1.32 software (Yeh *et al.*, 2000) was used to estimate the various genetic diversity parameters. NTSYS-PC (ver. 2.02; Exeter Software, N.Y., Rohlf, 2000) was used to prepare the dendrogram based on Nei's genetic distance.

Analysis of Molecular Variance (AMOVA) among groups, among populations within groups and among individuals within populations was performed in Arlequin 3.5 (Excoffier and Lischer, 2010) to determine the distribution of variation at different hierarchical levels. Population structure by AMOVA is based on an analysis of variance of gene frequencies, taking into account the number of mutational differences between molecular haplotypes. Fixation indices (Weir and Cockerham, 1984) and population pairwise F_{ST} (pairwise estimates of the correlation of alleles between populations) values were also computed by using the above software. Rcmd (R software) was used in Arlequin version 3.5 software to generate a plot of pairwise F_{ST} values. Population average pairwise differences were also computed using the software.

The genetic structure of the populations was also studied by using a Bayesian model-based clustering approach proposed by (Prichard *et al.*, 2000) to assign the landraces into genetically structured groups. The software package STRUCTURE version 2.3.4 (Prichard *et al.*, 2010) was used to perform this analysis. STRUCTURE software was run for presumed populations (*K)* from 2 to 10, following the admixture ancestry model. Run length of 5000 burn-ins followed by 50000 Markov chain Monte Carlo (MCMC) simulations with 20 replications was performed. The best *K* value was calculated from STRUCTURE HARVESTER online software (Dent *et al.,* 2012). Inferred ancestry estimates of individuals (Q-matrix) were derived for the selected subpopulation (Pritchard *et al.,* 2000).

- **Morphological characterization**

The rice landrace populations were also grown for morphological characterization in replicated field trials at NBPGR Base Centre, Cuttack (Odisha) during 2012 cropping season and data were recorded on 24 qualitative and 17 quantitative traits (Table 4.1.2.). Data on quantitative traits were subjected to ANOVA and multivariate analyses (cluster and principal components analyses) using INDOSAT statistical package developed at the INDOSTAT Services, Hyderabad. Twenty four descriptors for qualitative traits were recorded at different stages of growth in all the ten landraces of rice, as per Bioversity-IRRI guidelines (2007).

Table 4.1.2. Various qualitative and quantitative traits recorded for morphological characterization of rice landraces

Qualitative traits				Quantitative traits	
1.	Basal Leaf sheath: Colour	18.	Panicle: exertion	1.	Plant height (cm)
2.	Leaf blade: Intensity of green colour	19.	Panicle threshability	2.	Leaf length (cm)
3.	Leaf blade: Attitude	20.	Lemma and palea: pubescence	3.	Leaf width (cm)
4.	Leaf blade: pubescence	21.	Lemma and palea: colour	4.	Ear bearing tillers (EBT)
5.	Auricle: colour	22.	Lemma: colour of apiculus	5.	Panicle length (cm)
6.	Collar: colour	23.	Caryopsis: Pericarp colour	6.	Ligule length (cm)
7.	Ligule: Shape	24.	Caryopsis scent	7.	100 grain weight (g)
8.	Ligule: colour			8.	Panicle weight (g)
9.	Culm: habit			9.	Days to 50% flowering
10.	Flag leaf: attitude			10.	No. of spikelets/panicle
11.	Culm: internode anthocyanin			11.	Filled grain/panicle
12.	Culm strength			12.	Unfilled grain/panicle
13.	Stigma: colour			13.	Sterility %
14.	Awns: presence			14.	Grain Length (mm)
15.	Panicle: number per plant			15.	Grain width (mm)
16.	Panicle: attitude of branches			16.	Yield/plant(g)
17.	Panicle: secondary branching			17.	Yield/m^2 (g)

Results

- **Molecular diversity analyses**

Fourteen STMS primer pairs were used for molecular diversity analyses of five named rice landraces, each representing both static and dynamic conservation (Table 4.1.3). A total of 64 alleles were detected in 10 populations of five named landraces. The number of alleles ranged from 2 to 7 with an average of 4.57 alleles per locus. Of the 64 alleles, 60 were common and four were rare. Summary diversity of each landrace populations is presented in Table 3. The effective number of alleles and expected heterozygosity ranged from 1.08 to 1.25 and 0.07 to 0.17, respectively, across populations. Total number of alleles per landrace population ranged from 16 to 25, and per cent of polymorphic loci ranged from 14.29 to 64.29 (Table 4.1.4).The corresponding allele frequencies for each locus have been listed in Table 4.1.5, from which polymorphic information content (PIC) has been computed and it ranged from 0.148 to 0.760 with an mean average of 0.519 (Table 4.1.3).

Individual landrace pairwise distribution of allele frequencies under static and dynamic management are presented in Table 4.1.6. The population structure of rice landrace populations under static and dynamic management have been strikingly different as revealed by the frequency of private alleles. The mean frequency of private alleles, both under static and dynamic management, was recorded to be more than 50 per cent (Table 4.1.6). The AMOVA indicated highest variation of 75.7% among populations within groups (static ***vs*** dynamic) followed by among individuals within populations (11.22%), among groups (6.66%) and within individuals (6.41%), respectively (Table 4.1.7).

Pair-wise estimates of F_{ST} revealed very high significant population differentiation which ranged between 0.68 to 0.89 (Fig. 4.1.1; Fig. 4.1.2). High population differentiation was observed even between populations of same named landrace under static and dynamic management. This shows that the populations share limited genetic diversity among them. Even no pairs of a named landrace population representing static and dynamic conservation grouped together in UPGMA dendrogram (Fig. 4.1.3) based on Nei’s genetic distances.

Table 4.1.3. The repeat motif, number of alleles per locus (n), polymorphism information content (PIC) and allele size range for all 14 sequence-tagged microsatellite (STMS) loci:

Locus	SSR motif	No. of Alleles (n)	PIC	Allele Size (bp)	
				Range	Difference
RM206	$(GA)_{21}$	5	0.674	140 – 180	40
RM19	$(ATC)_{10}$	4	0.557	210 – 250	40
RM218	$(GA)_{24}$	5	0.557	130 – 200	70
RM233B	$(GA)_{20}$	6	0.355	140 – 180	40
RM234	$(GA)_{17}$	3	0.442	140 – 160	20
RM241	$(GA)_{31}$	7	0.636	100 – 160	60
RM249	$(GA)_{14}$	3	0.254	110 – 150	40
RM217	$(GA)_{20}$	6	0.647	120 – 170	50
RM11	$(GA)_{17}$	2	0.148	130 – 150	20
RM223	$(GA)_{25}$	5	0.499	140 – 180	40
RM242	$(GA)_{26}$	4	0.572	150 – 240	90
RM216	$(GA)_{18}$	4	0.578	130 – 160	30
RM222	$(GA)_{18}$	4	0.582	200 – 240	40
RM224	$(GA)_{13}$	6	0.760	130 – 180	50
Total		**64**	**-**	**-**	**-**
Mean		**4.57**	**0.519**	**-**	**-**

Table 4.1.4. Summary diversity of rice landrace populations based on 14 STMS markers

Name of the Landrace	Accession	Na	Ne	I	He	Nei He)	n	P	P%
Asamchudi	98721 (S)	1.50 ± 0.52	1.22 ± 0.25	0.23 ± 0.25	0.15 ± 0.17	0.15 ± 0.16	21	7	50.00
	569071 (D)	1.64 ± 0.50	1.25 ±0.28	0.26 ± 0.25	0.17 ± 0.17	0.16 ± 0.17	23	9	64.29
Bhuta	86063 (S)	1.57 ± 0.51	1.10 ± 0.14	0.15 ± 0.17	0.08 ± 0.10	0.08 ± 0.10	22	8	57.14
	568854 (D)	1.29 ± 0.47	1.18 ± 0.34	0.15 ± 0.26	0.10 ± 0.18	0.10 ± 0.18	18	4	28.57
Kalakadamba	85972 (S)	1.14 ± 0.36	1.08 ± 0.27	0.06 ± 0.17	0.04 ± 0.14	0.04 ± 0.13	16	2	14.29
	568859 (D)	1.36 ± 0.50	1.11 ± 0.26	0.11 ± 0.20	0.07 ± 0.14	0.07 ± 0.14	19	5	35.71
Narada	85999 (S)	1.36 ± 0.50	1.19 ± 0.35	0.16 ± 0.27	0.11 ± 0.19	0.11 ± 0.19	19	5	35.71
	568856 (D)	1.36 ± 0.50	1.15 ± 0.26	0.15 ± 0.23	0.10 ± 0.16	0.10 ± 0.16	20	5	35.71
Saleijhati	86055 (S)	1.71 ± 0.61	1.09 ± 0.11	0.16 ± 0.15	0.08 ± 0.08	0.08 ± 0.08	24	9	64.29
	568873 (D)	1.79 ± 0.70	1.25 ± 0.27	0.28 ± 0.25	0.17 ± 0.16	0.17 ± 0.16	25	9	64.29

Na = Observed number of alleles**; Ne** = Effective number of alleles; **I** = Shannon's Information Index; **He** = Expected heterozygosity;

Nei He = Nei's expected heterozygosity; **n =** Total number of alleles present; **P =** Number of polymorphic loci; **P%** = % of polymorphic loci

S = Static; D = Dynamic

Table 4.1.5. Allele frequencies in 10 rice landrace populations

Locus	Fragment size	Asamchudi		Bhuta		Kalakadamba		Narada		Saleijhati	
		IC98721 (S)	IC569071 (D)	IC86063 (S)	IC568854 (D)	IC85972 (S)	IC568859 (D)	IC85999 (S)	IC568856 (D)	IC86055 (S)	IC568873 (D)
RM206	180			0.0833							
	175	0.2333	0.9667				0.0667	0.3333	1		0.8333
	160			0.9167						0.0333	
	150	0.7667			1						0.0667
	140		0.0333			1	0.9333	0.6667		0.9667	0.1000
RM19	250	0.7667	0.7667		1				0.0833	0.1333	0.9667
	240			0.9667		1	0.0333				
	230	0.2333	0.2333				0.9667	1	0.9167	0.8667	0.0333
	210			0.0333							
RM218	200							1			
	160										0.1000
	150		0.9333								
	140	1	0.0667		1						0.8333
	130			1		1	1		1	1	0.0667
RM233B	180					0.5000					
	175		0.3667						0.1333		
	170			0.0167							
	160						0.5000				
	150	1	0.6333	0.9833	1	0.5000		1	0.8667	1	1

	140						0.5000				
RM234	160	1			1			0.4000	1		1
	150		1	1		1	1			1	
	140							0.6000			
RM241	160										0.1333
	150		0.1333	0.0667						0.8667	
	140	0.2333							0.2667		0.8667
	130		0.8667	0.9333	1	1	1			0.1000	
	120	0.7667							0.7333		
	110							1			
	100									0.0333	
RM249	150		1		0.4500				0.5000		
	130	1		1	0.5500	1	1	1	0.5000	0.9667	1
	110									0.0333	
RM217	170			0.0667							0.4333
	160	0.2333			0.4667		1		1		
	150			0.9333		1				0.9333	0.5667
	140	0.7667	0.8333		0.5333			1			
	130									0.0667	
	120		0.1667								
RM11	150	1	1	1	1	1	1	0.0333	1	1	1
	130							0.9667			
RM223	180				0.1667						
	170					0.0333		0.3333		0.9667	
	160	1	1	1	0.8333		0.0667		0.9333		0.8667
	150					0.9667	0.9333	0.6667	0.0667	0.0333	
	140										0.1333
RM242	240		0.8333							0.0667	

	230	1			1		1		1		1
	220		0.1667	1		1				0.9333	
	150							1			
RM216	160	0.1000	0.0500								
	150			0.8333	1				0.6667	0.0333	0.1667
	140	0.9000	0.9500				1				
	130			0.1667		1		1	0.3333	0.9667	0.8333
RM222	240	0.8333					0.0667				0.1333
	230			0.1833							
	220	0.1667	1		0.3667		0.9333	1	1		0.8667
	200			0.8167	0.6333	1				1	
RM224	180	0.9000									
	170			0.0333		1					
	160	0.1000	0.7333				1				0.1333
	150			0.9667	1			0.0333	1	0.0667	
	140		0.2667					0.9667			0.8667
	130									0.9333	

S= Static, D=Dynamic

Table 4.1.6. Landrace pairwise distribution of allele frequencies under static and dynamic management

Landrace	Conservation status	Total no. of alleles (n)	Mono-morphic alleles	Poly-morphic alleles	Rare alleles	Private alleles*
Asamchudi	Static	21	7	14	-	9 (43.9)
	Dynamic	23	7	14	2	10 (43.5)
Bhuta	Static	22	9	10	3	14 (63.6)
	Dynamic	18	10	8	-	10 (55.6)
Kalakadamba	Static	16	13	2	1	8 (50.0)
	Dynamic	19	10	8	1	11 (57.9)
Narada	Static	19	11	6	2	10 (52.6)
	Dynamic	20	8	12	-	10 (50.0)
Saleijhati	Static	24	9	10	5	14 (58.3)
	Dynamic	25	6	18	1	15 (60.0)
Mean	***Static***	20.4	9.8	8.4	2.2	11 (53.49)
	Dynamic	21.0	8.2	12.0	0.8	11.2 (53.39)

* Percentage in parenthesis

Table 4.1.7. AMOVA design and results

Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among groups	1	207.00	0.30 Va	6.66
Among populations within groups	8	1653.35	3.42 Vb	75.70
Among individuals within populations	290	378.37	0.51 Vc	11.22
Within individuals	300	87.00	0.29 Vd	6.41
Total	599	2415.72	4.52	-

Fixation Indices: $\mathbf{F_{IS}}$ **:** 0.64; $\mathbf{F_{SC}}$ **:** 0.81; $\mathbf{F_{CT}}$ **:** 0.07; $\mathbf{F_{IT}}$ **:** 0.94

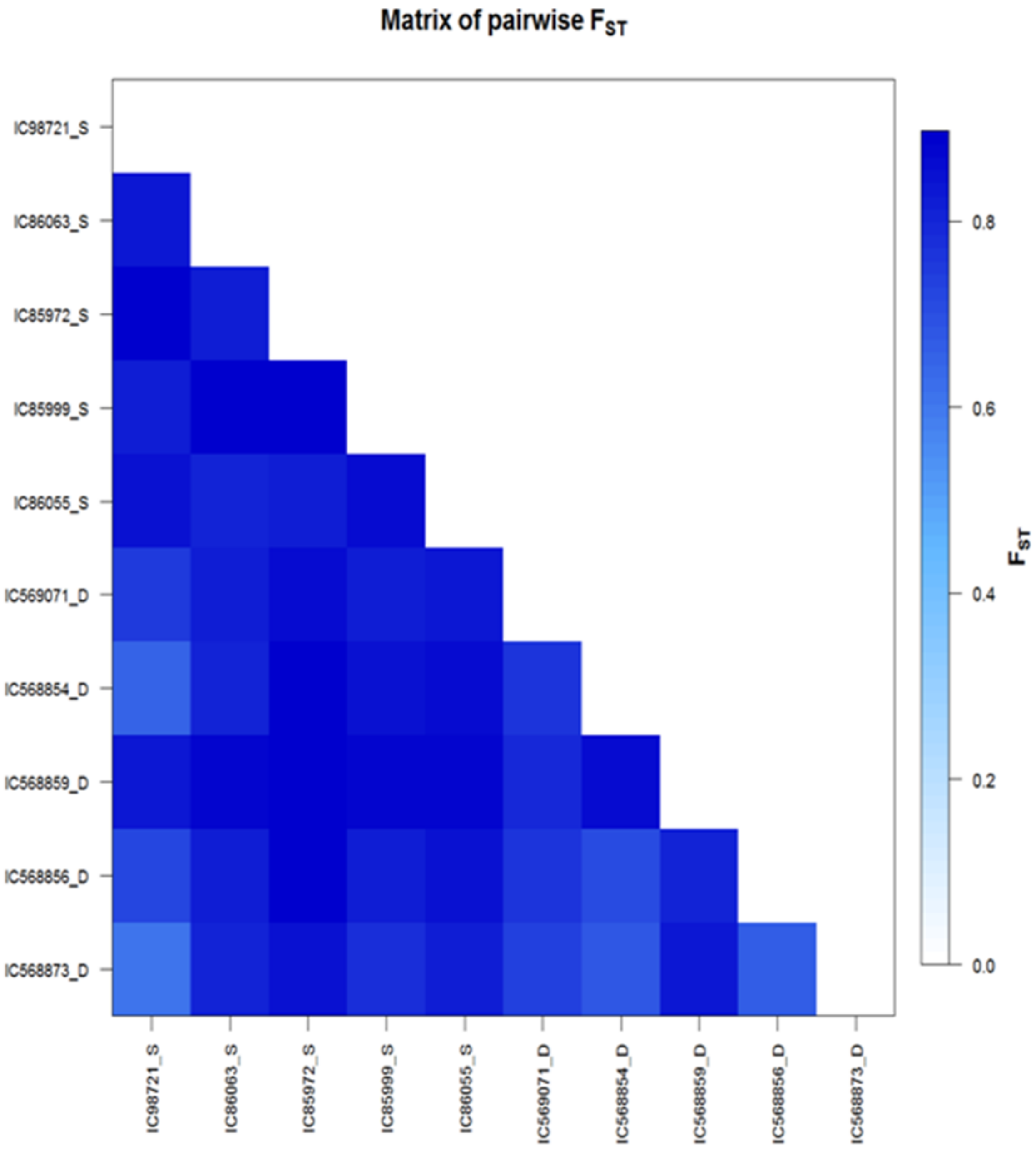

Fig. 4.1.1. Population pairwise F_{ST} of rice landraces (S and D indicates static and dynamic conservation status)

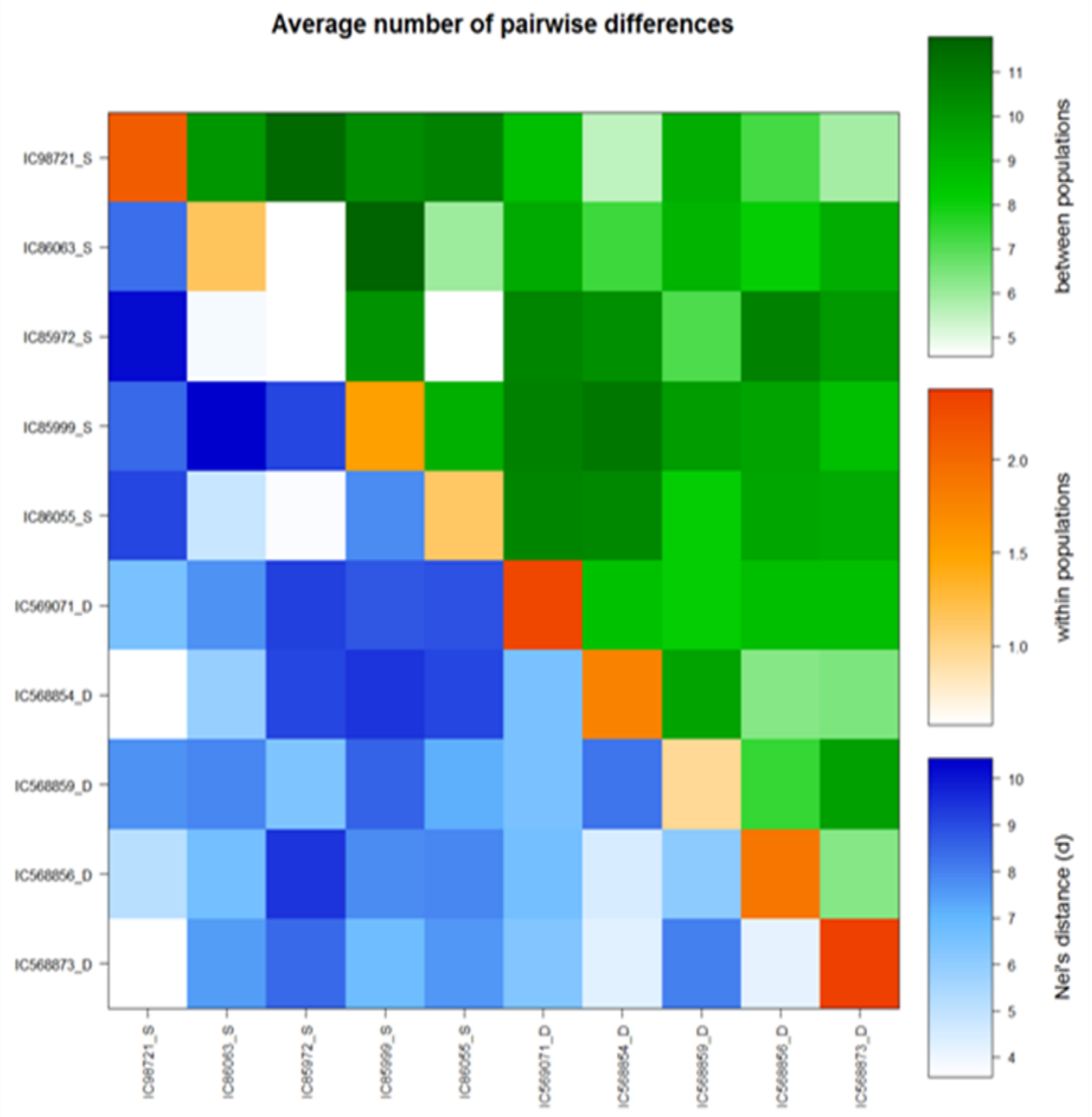
Average number of pairwise differences

Fig. 4.1.2. Population average pairwise differences of rice landraces

(S and D indicates static and dynamic conservation status)

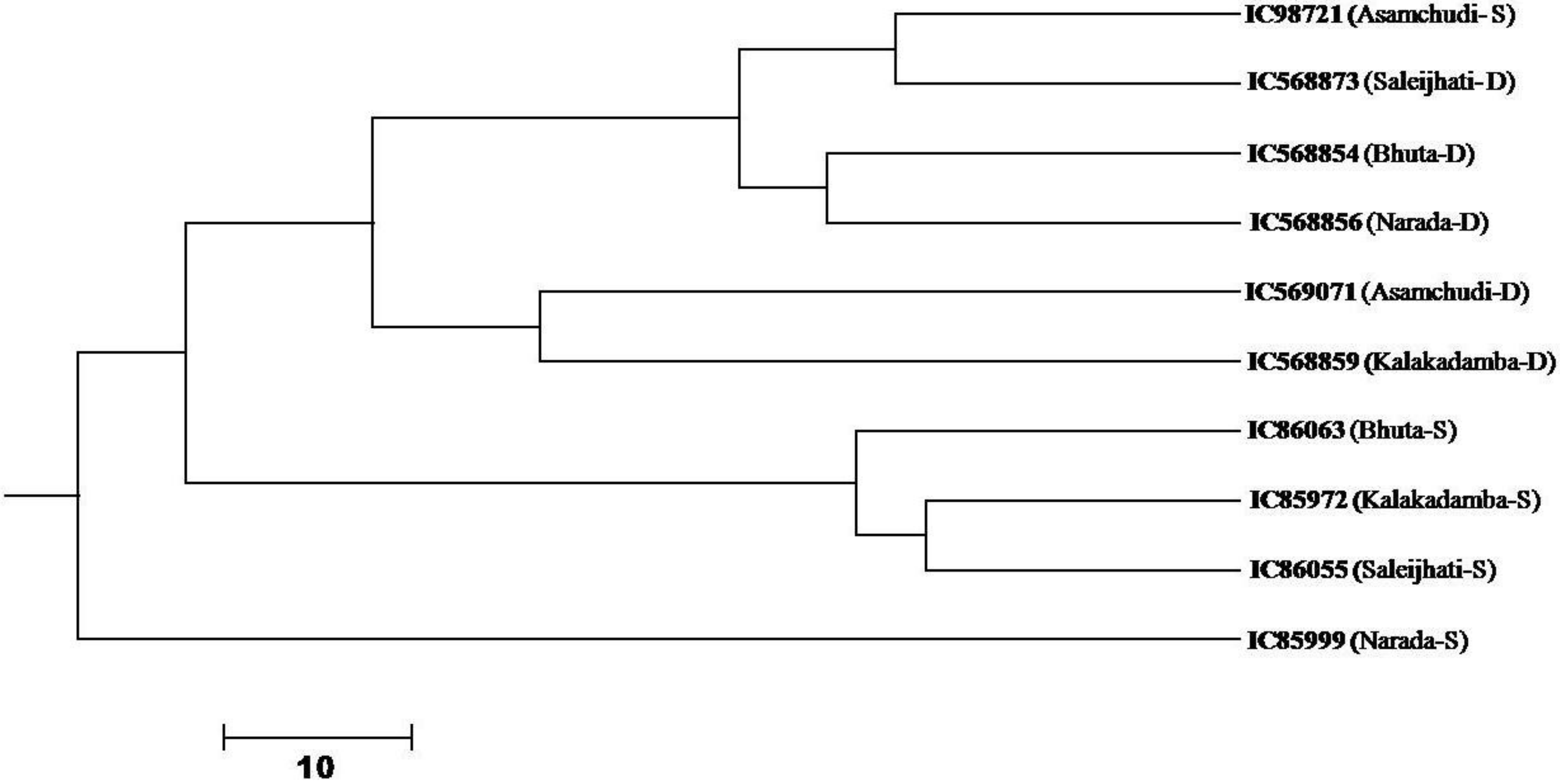

Fig. 4.1.3. UPGMA dendrogram based on Nei's genetic distance of rice landraces representing static (S) and dynamic (D) conservation

STRUCTURE software was used to perform model-based cluster analysis. In 20 replicated runs for different K values from 2 to 10 (presumed number of populations) based on the distribution of 64 different alleles at 14 SSR loci among 300 individuals (30 individuals for each 10 landraces). According to STRUCTURE documentation, the best value of K was determined by a calculation, which is based on the second order rate of change of the likelihood (ΔK). The ΔK shows a clear peak at the true value of K which in turn captures the major structure in the data is frequently the most appropriate choice. In present study the most appropriate number of clusters (K) was identified at K=4 (fig. 4.4 (a)), where the total samples were divided into four model clusters (fig. 4.4 (b)). Proportions of ancestries for all individuals are presented in fig. 4.5. All the ten rice landraces may be categorized as pure landraces as the average shared ancestry of 30 individuals of respective landrace for a model cluster is more than 90 %, among those landraces the dynamic conserved landraces showed more admixtures in comparison with their respective static conserved ones (Table 4.8).The model based clustering pattern was found similar to the UPGMA clustering pattern with some minor deviations. The landraces like IC 98721 and IC 568873 were grouped in the same clusters as it was grouped as in UPGMA method.

Table 4.1.8. Shared ancestry of rice landrace populations (average of 30 individual from each landrace)

Sl. no	Landrace	IC Number	Conservation status	Cluster I	Cluster II	Cluster III	Cluster IV
1	Asamchudi	98721	Static	0.991	0.001	0.006	0.002
2		569071	Dynamic	0.014	0.016	0.967	0.003
3	Bhuta	86063	Static	0.003	0.994	0.002	0.001
4		568854	Dynamic	0.993	0.003	0.003	0.001
5	Kalakadamba	85972	Static	0.001	0.995	0.002	0.002
6		568859	Dynamic	0.003	0.008	0.985	0.002
7	Narada	85999	Static	0.005	0.003	0.002	0.990
8		568856	Dynamic	0.976	0.003	0.014	0.007
9	Saleijhati	86055	Static	0.004	0.983	0.011	0.002
10		568873	Dynamic	0.975	0.007	0.011	0.007

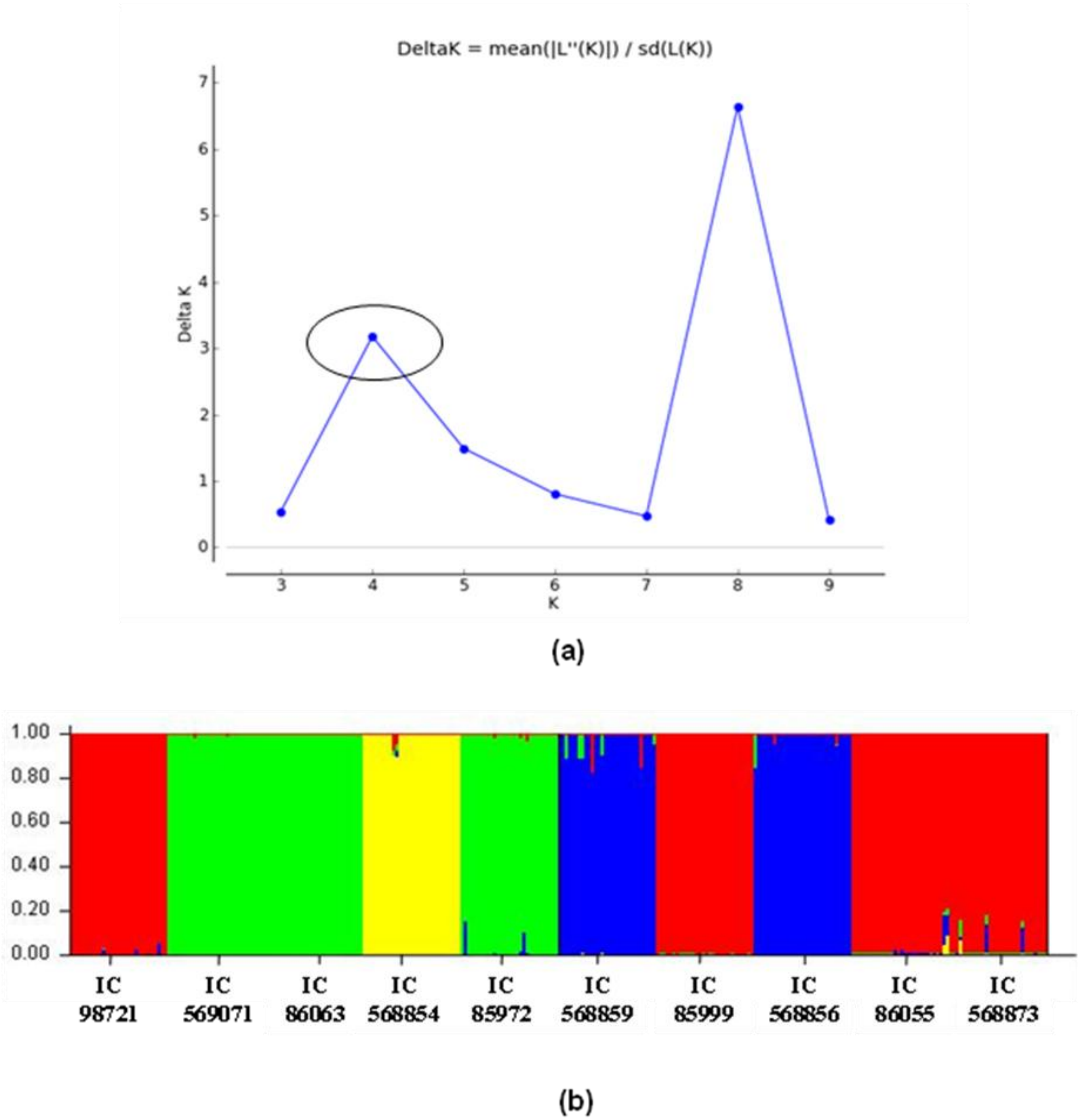

Fig. 4.1.4. Analysis of population structure showing (a) Values of ΔK for determining optimum number of subpopulations for total population (b) Estimated population structure of all 10 rice landrace populations

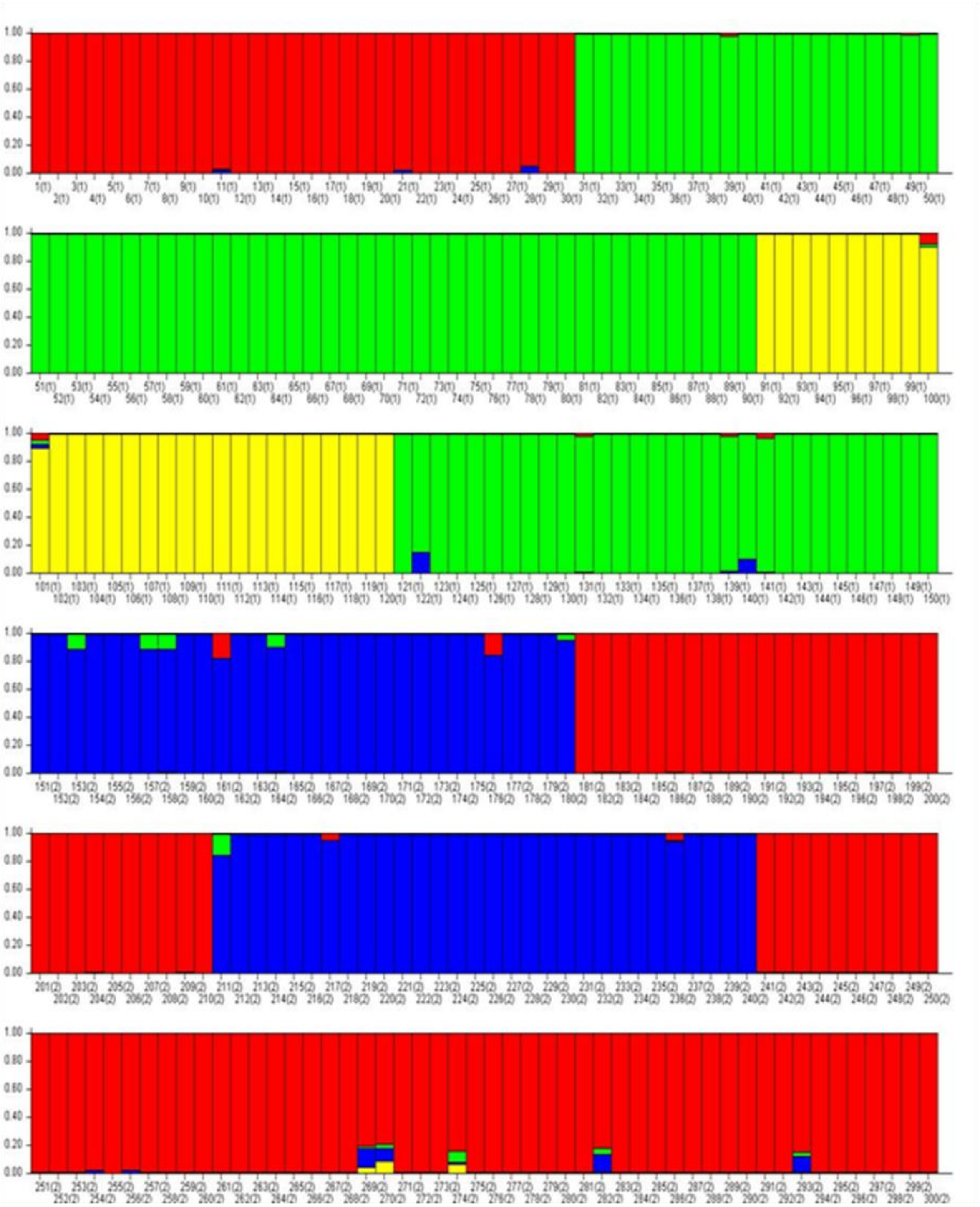

Fig. 4.1.5. Assignment of 300 individual from 10 rice landrace populations to four clusters by STRUCTURE software. Each individual bar represents an individual of a rice landrace. 1-30= IC 98721, **31-60=** IC 569071, **61-90=** IC 86063, **91-120=** IC 568854, **121-150=** IC 85972, **151-180=** IC 568859, **181-210=** IC 85999, **211-240=** IC 568856, **241-270=** IC 86055, **271-300=** IC 568873.

- **Analyses of adaptive variations**

Minor variations were observed both within and among landraces under static and dynamic management. All the ten landraces analysed for qualitative descriptors were non-scented and had medium green coloured leaf blade. They were monomorphic for the traits like shape of the ligule (2-cleft), attitude of branches in panicle (drooping) and panicle: secondary branching (sparse) (Table 4.1.9). There were distinguishable differences within and between landraces for the traits representing colour of basal leaf sheath, auricle, collar, stigma, lemma epiculus and pericarp. In case of panicle exertion, significant differences were seen between the landraces Asamchudi (just exerted) and Narada (well exerted). The traits like attitude of leaf blade and flag leaf which are very well correlated with interception of yield in rice displayed significant variations, which were helpful in distinguishing the diversity within landraces. Observations on the colour of the ligule ranged from white to purple which further showed up within landrace differences (Table 4.1.9). Regarding culm strength, an important trait for judging the lodging resistance of a plant exhibited differences within Kalakadamba landrace in which the accession representing static conservation had strong culm compared to on-farm managed population. The population representing dynamic management of Narada landrace showed the presence of awn whereas the other landraces were awnless (Table 4.1.9).

Descriptive statistics for important quantitative traits was analysed. Greater diversity was recorded for traits such as panicle weight, yield/plant, ear bearing tillers, 100-grain weight, etc. Significant differences among populations of different landraces for important quantitative traits were recorded. The variations were, however, greater for different landraces than for populations of same named landrace under static and dynamic management for majority of the traits (Table 4.1.10).

The data on quantitative traits were subjected to cluster and principal components analyses. In cluster analysis, the populations were broadly grouped into three clusters (Fig. 4.1.6).

Table 4.1.9. Characterization of the Landraces as per Bioversity – IRRI descriptors:

Landraces	IC No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Asamchudi	98721	LP	M	E	I	YG	LG	2C	P	I	E	A	S	P	A	I	D	Sp	Mwe	Ea	Hlk	St	Ra	W	NS
	569071	G	M	E	I	YG	G	2C	W	E	E	A	VS	W	A	I	D	Sp	Je	I	Hlk	St	St	LB	NS
Bhuta	86063	LP	M	H	I	P	P	2C	PL	I	H	PL	S	P	A	I	D	Sp	Mwe	Ea	Hlk	B	Ra	LB	NS
	568854	P	M	E	I	P	P	2C	PL	I	E	PL	S	P	A	I	D	Sp	Mwe	I	Hlk	St	Ra	B	NS
Kalakadamba	85972	G	M	E	I	YG	LG	2C	W	I	E	A	S	W	A	I	D	Sp	Mwe	Ea	Hlk	St	St	W	NS
	568859	P	M	H	I	P	P	2C	PL	I	H	PL	I	P	A	I	D	Sp	We	Ea	Hlk	Bf	P	LB	NS
Narada	85999	P	M	E	I	YG	P	2C	W	I	E	PL	S	LP	A	I	D	Sp	We	Ea	Hlk	Blk	Ra	LB	NS
	568856	P	M	H	I	P	LG	2C	PL	I	H	PL	S	P	PA	I	D	Sp	We	I	Hup	St	St	B	NS
Salejhati	86055	G	M	E	I	YG	G	2C	W	I	E	A	S	W	A	I	D	Sp	Mwe	Ea	Hlk	St	St	W	NS
	568873	G	M	H	I	YG	G	2C	W	I	H	A	VS	W	A	I	D	Sp	We	I	Hlk	St	St	R	NS

(1) Basal Leaf sheath: Colour, (2) Leaf blade: Intensity of green colour, (3) Leaf blade: Attitude, (4) Leaf blade: pubescence, (5) Auricle: colour, (6) Collar: colour, (7) Ligule: Shape, (8) Ligule: colour, (9) Culm: habit, (10) Flag leaf: attitude, (11) Culm: internode anthocyanin, (12) Culm strength, (13) Stigma: colour, (14) Awns: presence, (15) Panicle: number per plant, (16) Panicle: attitude of branches, (17) Panicle: secondary branching, (18) Panicle: exertion, (19)Panicle threshabiltiy, (20) Lemma and palea:pubescence, (21) Lemma and palea:colour, (22) Lemma: colour of apiculus, (23) Caryopsis: pericarp colour, (24) Caryopsis scent.

A=Absent, B=Brown, Bf=Brown furrows, Blk=Black D=Drooping, E=Erect, Ea=Easy, G=Green, H=Horizontal, Hlk=Hairs on lemma keel, Hup=Hairs on Upper portion, I=Intermediate, Je=Just exerted, LB=Light Brown, LP=Light Purple, LG=Light Green, M=Medium, Mwe=Moderately well exerted, NS=Non-Scented, P=Purple, PA=Partially Awned, PL=Purple lines, R=Red, Ra=Red apex, S=Strong, Sp=Sparse, St=Straw, VS=Very strong, W=White, We=Well exerted, YG=Yellowish green, 2C=2 – Cleft.

Table 4.1.10. Variations for important quantitative traits of different rice landrace populations under static and dynamic management

Landrace	Conservation status	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	EBT	Panicle length (cm)	100 grain weight (g)	Panicle weight (g)	Days to 50% flowering (days)	No. of spikelet/ panicle	Grain Length (mm)	Grain breadth (mm)	Yield/ plant (g)
Asamchudi	Static	160.75	53.18	1.41	7.46	28.90	2.17	3.38	114.80	162.60	6.21	1.96	12.20
	Dynamic	130.23	44.07	1.20	6.34	26.58	2.18	3.30	111.30	143.40	6.08	1.96	12.90
Bhuta	Static	147.92	47.94	0.87	6.73	27.37	2.63	3.16	114.80	143.40	6.07	2.27	13.27
	Dynamic	139.94	57.02	1.02	7.66	28.64	2.10	3.19	111.70	166.10	5.12	2.13	13.11
Kalakadamba	Static	137.95	48.11	1.05	6.14	28.98	3.20	6.98	116.10	235.40	6.08	2.77	16.62
	Dynamic	133.24	57.02	1.25	7.29	28.44	3.27	5.91	104.80	190.50	6.17	2.72	17.94
Narada	Static	125.70	35.47	0.88	7.29	21.76	2.63	3.91	107.60	190.50	5.12	2.20	8.60
	Dynamic	92.04	43.47	0.93	9.31	28.90	3.27	3.38	106.50	143.40	6.07	2.55	13.80
Saleijhati	Static	160.75	57.02	0.93	12.29	28.98	2.10	2.40	119.30	111.30	6.08	2.77	11.81
	Dynamic	147.92	57.02	1.05	9.30	28.90	2.46	3.38	114.80	148.60	6.17	2.72	13.45
Overall Mean		137.64	50.03	1.06	7.98	27.75	2.60	3.90	112.17	163.52	5.92	2.40	13.37
CV %		0.63	1.28	7.13	1.32	6.07	5.77	2.20	0.71	1.30	2.16	1.92	3.82
CD (5%)		0.77	0.57	0.07	0.09	1.50	0.13	0.08	0.71	1.89	0.11	0.04	0.44

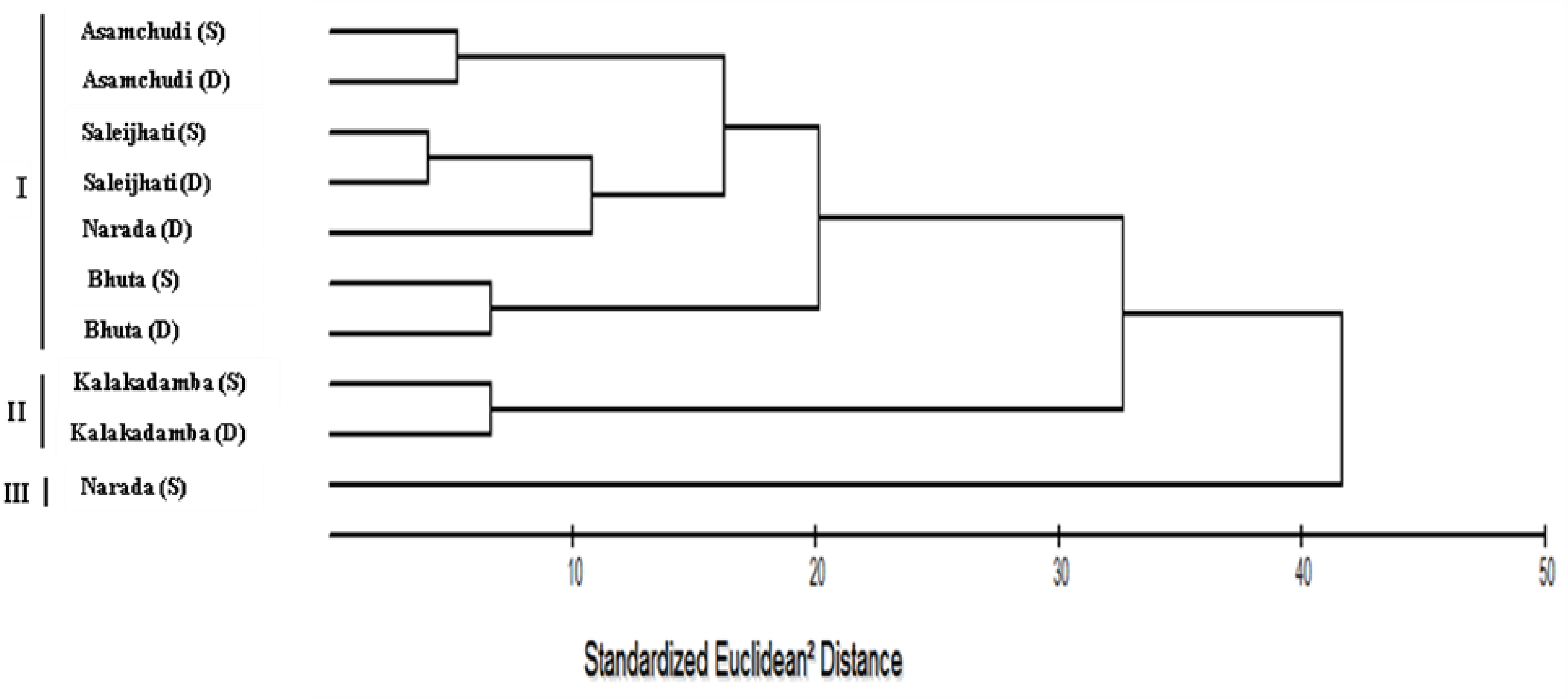

Fig. 4.1.6. Ward's minimum variance dendrogram of 10 rice landrace populations

Populations under static and dynamic management of four landraces grouped together except one landrace, Narada of which the populations representing static and dynamic conservation clustered separately. In principal components analysis, the first three most informative components explained 75.51% of the cumulative variance, and 35.97, 25.83 and 13.71 per cent variance individually. The important traits with greater weightings in first components were plant height, panicle length and ligule length; in second component the important traits were no. of filled grains per panicle, panicle weight, spikelets/panicle and 100-seed weight, and in third component the traits with greater weightings were unfilled grains per panicle, ear bearing tillers and percent sterility. In general, the PCA confirmed the groupings obtained through cluster analysis. The population representing static conservation of landrace Narada was distantly placed in PCA biplot as well (Fig. 4.1.7). The findings, therefore, indicate that not many variations observed in pattern of adaptive variations of rice landraces representing static and dynamic conservation.

Discussion

The genotypes of different rice landrace populations representing static and dynamic conservation were different for most of the STMS loci analysed in the present study. Population differentiation based on F_{ST} measures was very high even for the same named landrace. Some recent studies have assessed population genetic structure of named rice landraces (Pusadee *et al.*, 2009; Sreejayan *et al.*, 2011; Kumar *et al.*, 2010; Pandey *et al.*, 2012) and found very high partitioning of total variability among individual populations. However, for adaptive variations the diversity pattern for majority of the landraces was more or less similar and not many variations were observed in the phenotypes of populations representing static and dynamic management barring one landrace, Narada, where marked differences in phenotypes of both populations were observed.

Far fewer studies are available on the multilocus structure of landrace populations, that is, the extent to which genetic variants at one locus are correlated in occurrence with variants at another. Such structure arises from selection, genetic drift, or fragmentation of the population, and is retained through selection, isolation and the lack of migration, and restrictions on outcrossing and genetic recombination (Brown, 2000).

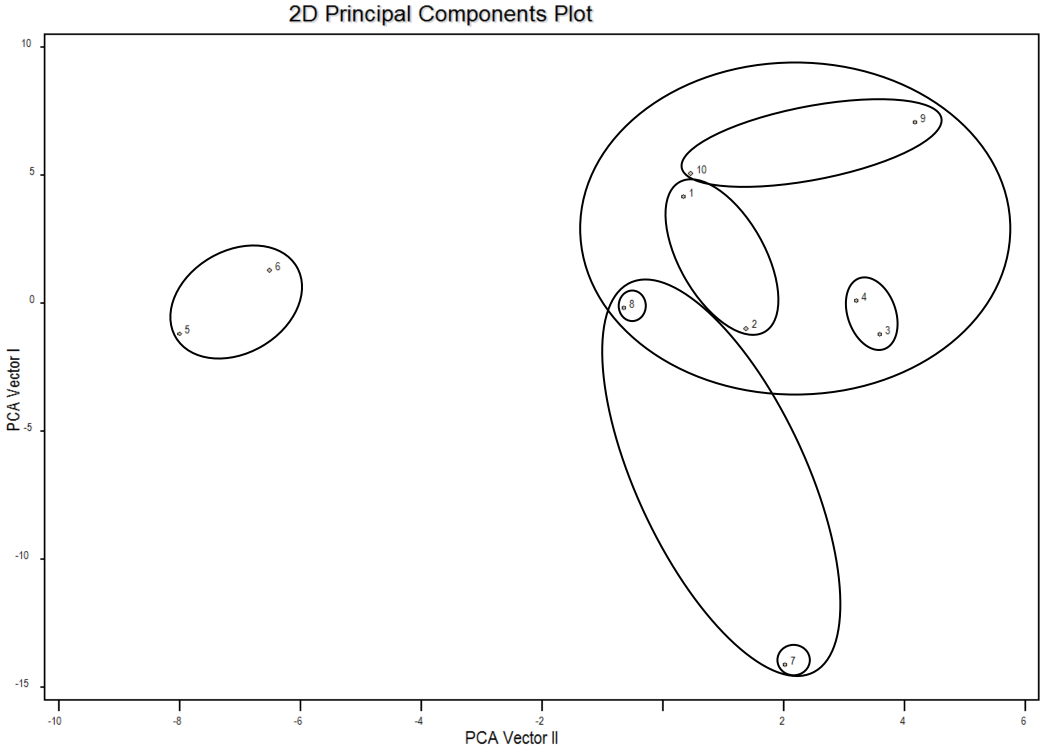

Fig. 4.1.7. Principal components scatterplot of 10 rice landrace populations (1&2: Asamchudi; 3&4: Bhuta; 5&6: Kalakadamba; 7&8: Narada; 9&10: Saleijhati under static and dynamic conservation, respectively)

In diversity assessment, the importance of variations due to adaptation and molecular diversity has been long argued. In the present study it has been observed that under on-farm management, while the particular attributes, characters, or adaptations of a population is apparently persisting over generations in different named landrace populations, the underlying genotypes have changed substantially. New alleles or combinations may arise and increase in frequency at the expense of other alleles that have disappeared (Table 4.1.6; Table 4.1.5). As has been argued by Brown (2000), *in situ* strategies fail to preserve all the extant biodiversity at the gene level. As better alleles or combinations arise and enjoy selective advantage, others thereby will be less fit and decline. This is the cost of evolutionary substitution and the price paid for allowing evolution to continue.

The above findings can also be argued in support of the neutral theory of molecular evolution (Kimura, 1968). Kimura's neutral theory claims that the overwhelming majority of evolutionary changes at the molecular level are not caused by selection acting on advantageous mutants, but by random fixation of selectively neutral or very nearly neutral mutants through the cumulative effect of sampling drift under continued input of new mutations (Kimura, 1991).

The *in situ* approach to conserving landraces undoubtedly holds several advantages over *ex situ* strategies. In view of the existing findings a few hypotheses could be formed to which research should be directed (Brown, 2000). The important areas of research on *in situ* conservation of landrace diversity on-farm in context of population structure may include allelic richness and genotypic diversity, specific adaptations, localized divergence, diversity to meet temporal and environmental adaptations, continuing crop evolutionary processes, etc. On-farm populations have the capacity to support a much greater number of rare alleles and of different (multilocus) genotypes than accessions in gene banks (Marshall 1989). Diversity itself confers long-term population fitness because it helps populations to cope with variable environments. Landrace populations of crops have survived centuries of selection for reliable production in subsistence agriculture, yielding a definite, known but probably limited benefit to the farmers that grow them (Frankel *et al.*, 1995). Presumably they are selected for resilience and stability though modest productivity. The *in situ* strategy conserves the crop evolutionary processes (mutation, migration, recombination, and selection). It provides scope for ongoing evolution, particularly in response to environmental changes and pathogen and pest pressures fluctuating in numbers and genetic composition. The key variables include (i) genetic diversity within populations, which is the essential raw material for evolution; (ii) breeding system variation (such as changes in outcrossing rate); (iii) variation in resistance in space and time, related to pest pressure and diversity; and (iv) the dynamics of seed systems, persistence, and migration. Further, regeneration of *ex situ* collections is currently considered a serious and enormous challenge (Brown *et al.*, 1997). Viability is inevitably lost at rates depending on the resources for and the management regimes of such collections. The task is to regenerate accessions without incurring genetic drift or genetic shift (Breese, 1989).

Diversity conserved on-farm is subject to a range of forces and is likely to be in a dynamic state. As yet, the data are far too limited to assess the various factors -

human, biological, edaphic, or climatic - to determine the requirements for optimal outcomes. The challenge is to plan for assessment of these factors in relation to changes in genetic structure over time. Population biology research for *in situ* conservation thus needs to be both descriptive and hypothesis testing in order to guide technical improvement and management of landrace populations (Brown, 2000).

Of the five rice landraces, two populations of the landrace Narada representing static and dynamic conservation grouped separately in UPGMA dendrogram and PCA analysis. One probable reason could be that farmers often are not consistent in naming and describing landraces. It is important to investigate across the area of study that same named landraces are genetically similar. As the populations of same named rice landraces in the present study were distinct genetically and highly differentiated, for population genetic parameters and also for monitoring allele loss/change over time and space one needs to collect these landraces at periodic intervals from the same household. The landrace also needs to be under continuous cultivation to make precise comparisons. Clarifications on what constitutes a landrace at each scale (village, community, region) is the first step toward defining the amount and distribution of crop diversity maintained by farmers (Jarvis *et al*., 2000).

It was noticed that all these rice landraces are under continuous cultivation for last several years. As long as farmers themselves find it in their own best interests to grow these populations, both farmers and society will benefit at no extra cost to anyone (Jarvis *et al*., 2000). Further, varietal dynamics based on average area and households growing the landrace are important for its *on-farm* management. The landraces can be categorized as common or rare based on average area and average number of households planting them. All populations in the present study were categorized as locally common as these were grown in more fields of above average sizes. Qualset *et al*. (1997) suggested that small land holdings isolate landrace populations from one another, thus reducing the generation of new genetic material by natural recombination. In common with biogeography theory (MacArthur and Wilson, 1967), they say that without human management, the genetic diversity in small 'patches' of crops would suffer genetic drift and inbreeding depression. They suggest that human inputs may offset these processes, as isolated populations can rely on seed exchange and farmer selection for the introduction of new genetic material (Louette *et al*., 1997). However, if two populations are isolated, they will diversify genetically to a greater extent and therefore inter-population diversity will increase.

The population structure of named landraces in traditional farming would help decide optimum number of landrace populations to be collected and conserved *ex situ* and level of adaptive variations in niche environments including biotic and abiotic stresses. The complementarity of *in situ* and *ex situ* conservation is based on the recognition that crop genetic resources involve more than the alleles and genotypes of crop populations. Beside the genetic raw material of landraces, crop genetic resources also comprise related species, agroecological interrelationships, and human factors (Brush, 2000). For the on-farm conservation of crop landraces, the traditional cultures and cropping systems that grow and use such populations are fundamental aspects of the habitats to which they are adapted. The systems shape their present genetic structure and determine the changes within landrace populations (Brown, 2000).

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Adaptive variations in different populations of some traditional rice landraces from parts of Odisha for morphological traits

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Abstract

Variations due to environmental adaptations among 20 populations of seven locally common rice landraces from parts of Odisha state were studied. Wide variations for various qualitative, and quantitative agro-morphological traits like grain yield per plant, number of grains per panicle, grain length and width, etc. among different landrace populations were recorded. A distinct pattern of agro-ecological adaptations was observed in populations of majority of the named landraces. Agro-morphological adaptations give users the opportunity to select and use landrace diversity in crop improvement.

Key words: Rice (*Oryza sativa* L.), adaptive variations, locally common landraces

Introduction

Seed-selection decisions of the farmers' were based on the wide range of agromorphological characteristics that their crops exhibit. These qualities include the phenology or morphological characteristics of the plant, unique aspects of the crop adaptation to a particular environment, or particular uses of the plant parts. These characteristics were helpful to the farmers, as well as scientists, in identifying and naming the precious landraces. Farmers hereby influence the population structure of a particular crop by determining the proximity of the crop population to potential breeding partners, and thus it shows how the genetic material is exchanged between and within fields (Jarvis *et al*., 2000).

Rice has been intermingled with the culture of the people for well over 4,000 years and it feeds about 557 million people in Southeast Asia (Manzanilla *et al*., 2011). Among the Southeast Asian countries, India in particular covers up enormous amount of high yielding varieties, diverse landraces and wild forms in rice cultivated under varied geographical conditions with different farming systems. In specific, several researches identified the Jeypore tract of Odisha in India as the centre of origin and diversity of cultivated rice crop (*Oryza sativa*) (Ramiah and Ghose, 1951; Ramiah, 1953; Sharma *et al.*, 2000). This tract specifically harbours numerous number of rice landraces which were known to be maintained by continuous traditional farming system. Among which the locally common landraces appears to be particularly important from farmers' point of view with certain specific objectives. One might expect them to possess a high proportion of locally common alleles of adaptive significance and therefore important for conservation and attracts the interests of users (Jarvis *et al*., 2000; Bisht *et al*., 2006, 2007; Kumar *et al*., 2010).

Adaptive variations of some rice locally common landrace populations over time have been investigated in this paper.

Materials and methods

Plant materials and sampling strategy

The experimental material comprised a total of 20 populations of seven named rice landraces from Kalahandi and Keonjhar district of Odisha state. Six populations of landrace Saleijhati; three populations each of Bhuta, Kalakadamba and Sarubhojni; two populations each of Asamchudi and Narada, and one population of Magura were included in the study. Landraces with more than one populations represented accessions assembled at different period and conserved *ex situ* in National Genebank at NBPGR, New Delhi. Accessions assembled during 2008 represented the on-farm managed populations of different named landraces. Each of these landraces was collected from niche environments of respective agro-ecologies following appropriate sampling strategy. The passport information on the origin of accessions is given in Table 4.2.1. The impact of improved modern varieties, in general, was low in the region these landraces under subsistence farming were collected at different point of time.

Field evaluation and characterization

The landraces were grown in *Kharif* season of 2011 and 2012 in the experimental farms of NBPGR Regional Station, CRRI Campus, Cuttack, Odisha (latitude: 28° 38' 7.11" N and longitude: 77° 13' 29.86" E). Standard field sowing practices for rice were followed. Each population was grown in three replications with complete randomized block design in four row plots. Each row comprised 24 plants transplanted under flooding condition. Observations on 34 characters, 15 quantitative and 19 qualitative were recorded as detailed in Table 3.3 of Chapter 3 on General Methodology. Data on quantitative traits were recorded on ten individuals per landrace population per replication and average values of two years were used in diversity analysis. Data on quantitative traits were statistically analyzed for range and pattern of variations using INDOSTAT statistical software developed at the INDOSTAT Services, Hyderabad. Classification (cluster analysis) and ordination (principal components analysis) analyses were performed. Skewed data on quantitative traits were transformed before multivariate analysis. Ward’s minimum variance clustering method was used to classify accessions in discrete clusters (Sneath and Sokal, 1973).

Table 4.2.1. Passport information on aromatic rice landrace populations from Odisha

S.No.	**Landrace name**	**Accession No.**	**District**	**Year of collection**	**Source of seed**
1	Asamchudi	IC-98721	Kalahandi	1987	NGB
2		IC-569071	Kalahandi	2008	On-farm
3	Bhuta	IC-86063	Keonjhar	1986	NGB
4		IC-462934	Keonjhar	2001	NGB
5		IC-568854	Keonjhar	2008	On-farm
6	Kalakadamba	IC-85952	Keonjhar	1986	NGB
7		IC-462962	Keonjhar	1998	NGB
8		IC-568859	Keonjhar	2008	On-farm
9	Magura	IC-569063	Kalahandi	2008	On-farm
10	Narada	IC-85999	Keonjhar	1987	NGB
11		IC-568856	Keonjhar	2008	On-farm
12	Saleijhati	IC-86055	Keonjhar	1986	NGB
13		IC-86072	Keonjhar	1986	NGB
14		IC-86077	Keonjhar	1986	NGB
15		IC-462950	Keonjhar	1998	NGB
16		IC-462964	Keonjhar	1998	NGB
17		IC-568873	Keonjhar	2008	On-farm
18	Sarubhojni	IC-86097	Keonjhar	1986	NGB
19		IC-86111	Keonjhar	1986	NGB
20		IC-568875	Keonjhar	2008	On-farm

NGB: Ex situ in National Genebank*;* On-farm: Original collection from farm house holds

Results

The range of variations for important quantitative traits has been presented in Table 4.2.2. Wide variations were recorded for various agronomic traits like grain yield per plant, panicle weight, number of ear bearing tillers/plant, number of spikelets per panicle, etc. Analysis of variance (ANOVA) studies of the landraces, presented in Table 4.2.3, have shown highly significant variations for all important quantitative characters studied. This indicates that there is substantial variability present among the landrace populations.

Table 4.2.4 presents the mean values of various quantitative traits of different landrace populations. Significant variations were also recorded even for populations of the same named landrace assembled at different time period. Overall not much temporal variations were recorded for quantitative traits (Table 4.2.5).

The correlation matrix of quantitative traits shows positively or negatively significant correlations between certain characters. Certain correlations like positive and significant correlation between panicle length and grain length, panicle length and grain weight, panicle length and days to flower are worth highlighting (Table 4.2.6).

Wide variations were also recorded for qualitative morphological traits among different named landraces. Among populations of the same named landrace variations were, however, low. For example, the trait leaf angle showed significant variations between and within the landraces of Asamchudi, Narada and Saleijhati (Table 4.2.7). The partial presence of awn was recorded in the on-farm conserved landraces of sarubhojni and Narada, whereas the NGB conserved types showed absence of awn. The trait grain threshability showed much variation among the landraces, while the variations recorded within landraces were less (Table 4.2.7).

Table 4.2.2. Range of variations for important quantitative traits

	Lowest	**Highest**	**Mean**	**C.V. %**
Plant height	86.6	199.0	140.77	15.4
Days to flower	79.0	141.0	110.5	10.7
Leaf length	32.6	69.1	51.7	16.5
Leaf width	0.8	1.9	1.0	23.4
Panicle length	18.6	50.0	28.2	16.4
EBT/ plant	3.0	15.0	7.1	30.3
Panicle weight	1.6	6.9	3.7	33.1
100- grain wt	1.9	3.6	2.5	17.4
Spikelet/ panicle	63.0	249.0	156.5	24.7
Grain length	4.8	6.8	5.9	9.3
Grain width	1.9	2.9	2.3	12.9
Yield/ plant	7.2	34.5	15.8	36.5
Yield/m^2	154.2	612.5	333.9	31.2

Table 4.2.3. Analysis of Variance (ANOVA) summary for important quantitative traits

	DF	Plant height	Days to flower	Leaf length	Leaf width	Panicle length	EBT/ plant	Panicle weight	100-grain wt.	Spikelet/ panicle	Grain length	Grain width	Yield/ plant	Yield/m^2
Replicates	2	353.2	0.8	43.7	0.01	28.5	1.2	0.1	0.01	235.5	0.01	0.01	3.8	1700.5
Treatment	19	945.0***	430.3***	130.3*	0.1*	38.3**	6.5*	4.6***	0.6***	4337.0***	0.9***	0.3***	85.2***	28932.9***
Error	38	245.7	0.9	45.4	0.05	12.7	3.8	0.05	0.01	135.4	0.01	0.01	8.9	2297.3
Total	59	474.5	139.2	72.7	0.06	21.5	4.6	1.5	0.2	1491.9	0.3	0.09	33.3	10854.7

Table 4.2.4. Variations in quantitative traits of rice landrace populations

Landrace	Landrace populations	Plant height	Days to flower	Leaf length	Leaf width	Panicle length	EBT/ plant	Panicle weight	100-grain wt.	Spikelet / panicle	Grain length	Grain width	Yield/ plant	Yield/m^2
Asamchudi	IC-98721	162.5	112.7	53.8	1.6	30.5	7.7	4.2	2.2	182.0	6.1	1.9	20.0	326.3
	IC-569071	130.2	114.7	44.1	1.0	26.4	5.7	3.3	2.1	160.0	6.2	2.1	13.2	378.4
Bhuta	IC-86063	139.7	113.0	47.9	0.9	27.3	6.7	3.6	2.7	124.0	6.0	2.3	14.1	243.3
	IC-462934	141.0	114.3	49.0	1.1	30.9	6.0	3.4	2.6	135.0	6.1	2.5	11.8	291.2
	IC-568854	147.9	114.0	52.2	1.0	28.9	6.3	3.6	2.2	179.3	5.1	2.1	11.5	241.5
Kalakadamba	IC-85952	134.4	116.3	47.3	1.0	30.9	6.3	6.3	3.2	238.7	6.1	2.7	30.9	575.2
	IC-462962	134.7	139.7	64.3	1.0	28.9	6.3	4.3	3.2	152.3	6.2	2.8	20.9	486.4
	IC-568859	138.6	104.7	56.8	1.0	28.7	6.3	5.7	3.2	170.3	6.1	2.7	17.8	441.8
Magura	IC-569063	139.0	115.7	54.4	1.0	27.8	7.3	2.3	2.1	138.0	4.9	2.6	10.2	322.8
Narada	IC-85999	91.5	98.7	35.5	0.9	19.2	7.3	5.6	2.4	187.3	4.8	2.2	25.9	510.0
	IC-568856	126.5	108.3	44.4	0.9	30.2	8.0	1.6	3.5	70.3	6.1	2.5	11.2	225.8
Saleijhati	IC-86055	162.0	114.3	58.2	0.9	25.5	12.0	2.4	2.1	120.7	6.2	2.0	18.1	321.6
	IC-86072	127.1	98.0	56.1	1.0	25.5	6.0	3.9	1.9	198.3	4.9	2.3	15.3	290.5
	IC-86077	159.7	115.7	50.1	1.1	26.2	5.7	3.6	2.3	164.3	6.1	2.7	12.5	232.9
	IC-462950	140.2	114.3	61.2	1.4	37.0	8.3	2.4	2.4	107.0	6.5	2.0	11.8	259.3
	IC-462964	170.2	112.3	55.7	1.3	30.1	8.0	4.9	2.7	163.7	6.7	2.0	16.6	341.2
	IC-568873	159.9	118.7	55.4	1.0	28.5	7.7	3.4	2.4	152.7	6.5	2.0	12.2	306.4

Sarubhojni	IC-86097	121.9	79.3	48.0	0.9	22.8	6.0	3.3	2.3	188.3	6.1	2.0	13.0	311.4
	IC-86111	147.7	113.3	49.7	1.0	28.9	5.7	4.6	2.7	182.7	6.4	2.0	12.5	247.1
	IC-568875	140.7	92.7	49.6	0.9	29.8	8.0	2.1	2.5	114.3	6.0	2.0	17.0	325.3
Overall Mean		**140.8**	**110.5**	**51.7**	**1.0**	**28.2**	**7.1**	**3.7**	**2.5**	**156.5**	**5.9**	**2.3**	**15.8**	**333.9**
CV%		**11.13**	**0.9**	**13.0**	**20.2**	**12.6**	**27.6**	**5.9**	**2.8**	**7.4**	**1.6**	**1.7**	**18.9**	**14.4**
CD (5%)		**25.91**	**1.6**	**11.1**	**0.3**	**5.9**	**3.2**	**0.4**	**0.1**	**19.2**	**0.2**	**0.1**	**4.9**	**79.2**

Table 4.2.5. Temporal pattern of adaptive variations of rice landrace populations

Period of collection	**Plant height**	**Days to flower**	**Leaf length**	**Leaf width**	**Panicle length**	**EBT/ plant**	**Panicle weight**	**100-grain wt.**	**Spikelet/ panicle**	**Grain length**	**Grain width**	**Yield/ plant**	**Yield/ m^2**
1980s (*Ex situ* NGB)	138.5	106.8	49.6	1.03	26.3	7.04	3.8	2.4	176.3	5.9	2.2	18.0	339.8
1990s (*Ex situ* NGB)	146.5	120.1	57.6	1.2	31.7	7.2	3.6	2.7	139.5	6.4	2.3	15.3	344.5
2000s (On-farm)	140.4	109.8	50.9	0.98	28.6	7	3.1	2.6	140.7	5.8	2.3	13.3	320.3
Mean	**141.8±4.2**	**112.2±6.9**	**52.7±4.3**	**1.1±0.1**	**28.9±2.7**	**7.1±0.1**	**3.5±0.4**	**2.6±0.2**	**152.2±20.9**	**6.0±0.3**	**2.3±0.05**	**15.5±2.4**	**334.9±12.8**

Table 4.2.6. Correlation matrix for the quantitative traits

	Plant height	**Days to flower**	**Leaf length**	**Leaf width**	**Panicle length**	**EBT/ plant**	**Panicle weight**	**100-grain wt.**	**Spikelet/ panicle**	**Grain length**	**Grain width**	**Yield/ plant**	**Yield/m^2**
Plant height	1.00	0.32*	0.28*	0.41**	0.13	-0.13	-0.17	-0.09	-0.12	0.40**	-0.16	-0.18	-0.26*
Days to flower		1.00	0.34**	0.18	0.30*	0.02	0.07	0.25*	-0.12	0.16	0.37**	0.12	0.22
Leaf length			1.00	0.17	0.23	-0.04	-0.11	-0.01	-0.05	0.23	0.08	-0.21	-0.14
Leaf width				1.00	0.22	0.09	-0.002	-0.11	0.04	0.24	-0.19	-0.01	-0.05
Panicle length					1.00	0.09	-0.18	0.25*	0.21	0.34**	0.03	-0.15	-0.08
EBT/ plant						1.00	-0.28*	-0.10	-0.35**	-0.14	-0.24	0.18	0.09
Panicle weight							1.00	0.29*	0.79**	-0.13	0.26	0.73**	0.67**
100- grain wt								1.00	-0.13	0.32*	0.53**	0.29*	0.34**
Spikelet/ panicle									1.00	-0.24	0.05	0.51**	0.45**
Grain length										1.00	-0.19	0.07	-0.10
Grain width											1.00	0.17	0.32
Yield/ plant												1.00	0.84**
Yield/m^2													1.00

* Significant at 0.05; ** significant at 0.01

Table 4.2.7. Variations in rice landrace populations for important qualitative traits

Accession no.	Landrace	Leaf angle	Flag leaf angle	Ligule Colour	Collar colour	Auricle colour	Internode colour	Secondary branching	Awning	Stigma colour	Lemma palea colour	Seed coat colour	Threshability
IC-98721	Asamchudi	Horizontal	Erect	White	Green	Pale Green	Green	Light	Absent	White	Straw	Brown	Intermediate
IC-569071	Asamchudi	Erect	Erect	White	Green	Pale Green	Green	Light	Absent	White	Straw	Light brown	Intermediate
IC-86063	Bhuta	Horizontal	Horizontal	Purple lines	Purple	Purple	Purple lines	Light	Absent	Purple	Brown	Light brown	Easy
IC-462934	Bhuta	Horizontal	Erect	Purple lines	Purple	Purple	Purple lines	Light	Short & partly.aw.	Purple	Black	Red	Easy
IC-568854	Bhuta	Horizontal	Erect	Purple lines	Purple	Purple	Purple lines	Light	Absent	Purple	Straw	Brown	Intermediate
IC-85972	Kalakadamba	Horizontal	Intermediate	Purple lines	Purple	Purple	Purple lines	Light	Absent	Purple	Black	Red	Easy
IC-462962	Kalakadamba	Horizontal	Horizontal	Purple lines	Purple	Purple	Purple lines	Light	Absent	Purple	Black	Light brown	Easy
IC-568859	Kalakadamba	Horizontal	Horizontal	Purple lines	Purple	Purple	Purple lines	Light	Absent	Purple	Br.furrows	Light brown	Easy
IC-569063	Magura	Horizontal	Horizontal	White	Green	Pale Green	Green	Light	Absent	Purple	Reddish to Purple Red	White	Intermediate
IC-85999	Narada	Erect	Erect	White	Purple	Pale Green	Purple lines	Light	Absent	Light purple	Black	Light brown	Easy
IC-568856	Narada	Horizontal	Horizontal	Purple lines	Pale green	Purple	Purple lines	Light	Short & partly.aw.	Purple	Straw	Brown	Intermediate
IC-86055	Saleijhati	Horizontal	Erect	White	Green	Pale Green	Green	Light	Absent	White	Straw	White	Easy
IC-86072	Saleijhati	Horizontal	Erect	White	Pale green	Pale Green	Green	Light	Absent	White	Straw	White	Easy
IC-98677	Saleijhati	Erect	Erect	Purple	Pale green	Pale Green	Green	Light	Absent	Purple	Straw	White	Easy
IC-462950	Saleijhati	Horizontal	Erect	White	Green	Pale Green	Green	Light	Absent	White	Straw	Red	Easy
IC-462964	Saleijhati	Horizontal	Erect	White	Green	Pale Green	Green	Light	Absent	White	Straw	White	Easy
IC-568873	Saleijhati	Horizontal	Horizontal	White	Green	Pale Green	Green	Light	Absent	White	Straw	Red	Intermediate
IC-86097	Sarubhojni	Horizontal	Erect	Purple lines	Green	Pale Green	Purple lines	Light	Absent	Purple	Brown	Light brown	Intermediate
IC-86111	Sarubhojni	Horizontal	Horizontal	White	Green	Pale Green	Green	Light	Absent	Light purple	Brown	White	Intermediate
IC-568875	Sarubhojni	Horizontal	Horizontal	White	Green	Pale Green	Purple lines	Light	Short & partly.aw.	White	Straw	Red	Intermediate

In clustering pattern, landrace populations were grouped into three major clusters (Fig 4.2.1). Certain populations of the same named landrace, for example, Kalakadmaba, Sarubhojini, Saleijhati and Bhuta clustered together indicating limited adaptive variations over time and space for quantitative traits. Substantial variations in populations of some landraces, for example, Narada and Asamchudi was observed the individual populations of the same named landrace clustered separately.

The principal components analysis (PCA) revealed that the first three most informative principal components explained more than 64% variation (Table 4.2.8). Traits with greater weightings were number of spikelets/ plant, yield/plant and panicle length for PC axes 1, plant height, days to flowering, ligule length and panicle weight for PC axes 2, and grain width and 100-grain weight for PC axes 3. The PCA scatter plot of rice landrace populations is presented in Fig 4.2.2. In general, the PCA analysis confirmed the grouping obtained through cluster analysis.

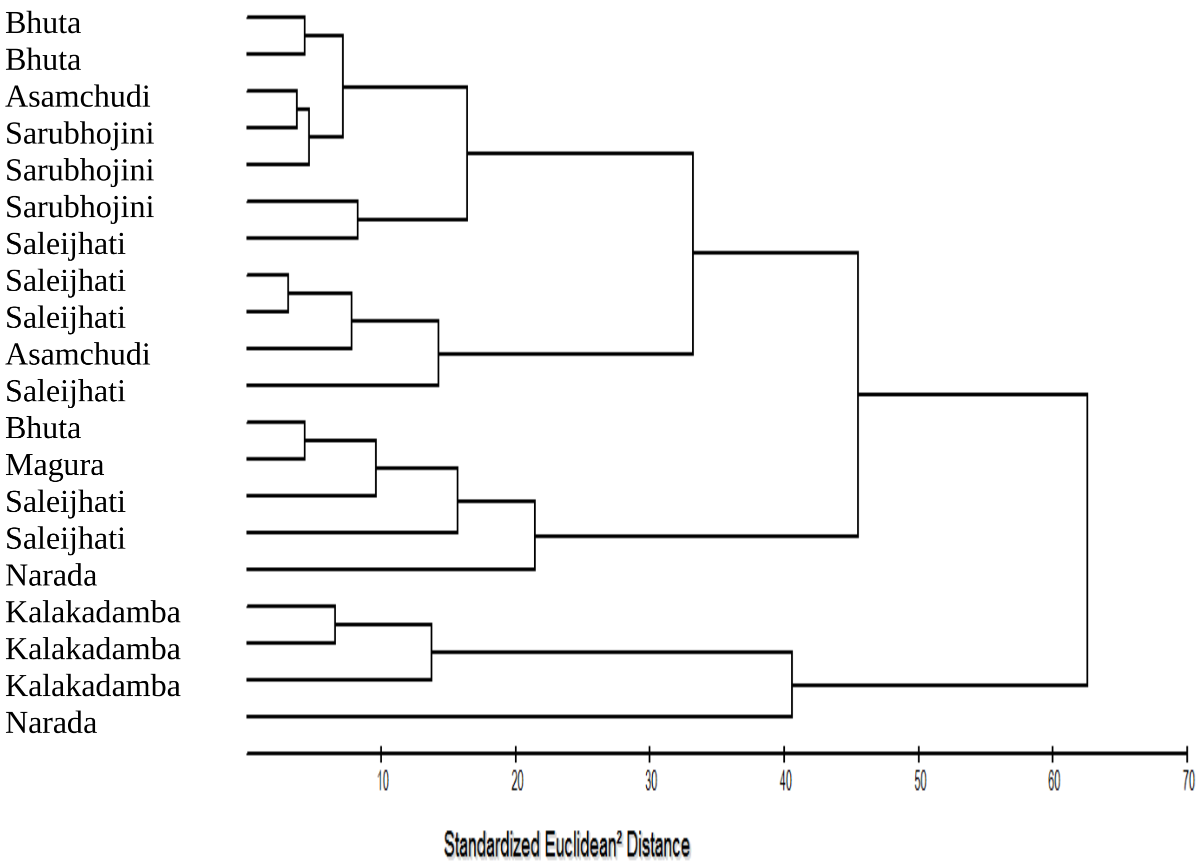

Fig. 4.2.1 Ward's minimum variance dendrogram of 20 rice landrace populations

Table 4.2.8. Matrix of principal components for 15 quantitative variables of 20 landrace populations

	PC I	PC II	PC III
Eigen value (Root)	4.88	3.77	2.25
% Variance explained	28.73	22.18	13.35
Cumulative variance	28.73	50.91	64.16
Traits with greater weightings	Spikelets/ plant, Yield/plant, Panicle length, Plant height	Days to flowering, Legule length, Panicle weight	Grain width, 100-Grain weight

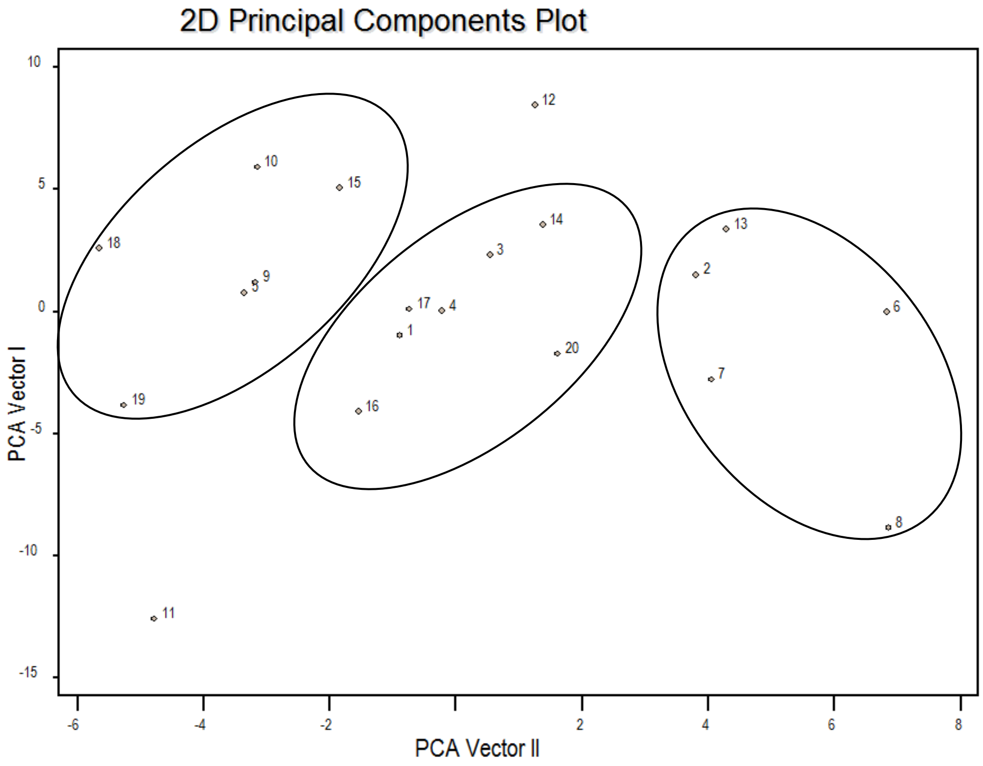

Fig. 4.2.2.Principal component scatter-plot of 20 rice landrace population

Discussion

In India, every landrace of rice at farm household level is invariably named and "farmer-named variety" is the unit that farmers manage and select over time. Farmers, however, may or may not be consistent in naming and describing landraces. Clarification of what constitutes a landrace at each scale (village, community, and region) is the first step toward defining the amount and distribution of crop diversity maintained by farmers. In the present study the samples are different but represent different populations some named landraces collected at different time period from the same area but not from the same household (the ideal situation). It is presumed that, under on-farm management, while the particular attributes, characters, or adaptations of a population may persist over generations, the underlying genotypes will change. New alleles or combinations are expected to arise and increase in frequency at the expense of other alleles that may well disappear. Strictly speaking, *in situ* strategies fail to preserve all the extant biodiversity at the gene level. As better alleles or combinations arise and enjoy selective advantage, others thereby will be less fit and decline. This is the cost of evolutionary substitution and the price paid for allowing evolution to continue (Brown, 2000).

Landrace populations are often highly variable in appearance, but they are each identifiable and usually have local names. A landrace has particular properties or characteristics. All components of the landrace population are adapted to local climatic conditions, cultural practices, and disease and pests (Harlan, 1975). Populations maintained *in situ* often shows minor phenotypic differences when compared to gene-bank conserved samples of the same named landrace. Both *ex situ* and *in situ* materials of the same landraces were derived from the same original source or locality where they are grown under continuous farming system, the observed differences could be due to (1) Random effects under *ex situ* conditions, e.g. sampling errors and differential survival in connection with collection; (2) Random effects under *in situ* conditions, e.g. genetic drift at unselected loci due to limited mass or single plant selection by farmers in a population of nearly homozygous lines; (3) Introgression from other sources under *in* *situ* conditions; or (4) Directional evolutionary changes under on-farm cultivation (Tin *et al.*, 2001).

From the comparison of *in situ* and *ex situ* conserved landraces made in this study, the plant materials from the same named landraces showed lesser variations compared to the between landrace deviations which is evident from the grouping pattern. This can be due to the fact that the particular type of landrace gets adapted to a specific environment and farmers practice in a locality which in turn shows lesser agro-morphological deviations. According to Smale *et al.* (2004), due to on-farm conservation of plant genetic resources a lot of genetic diversity is created which makes a substantial contribution to farmers' livelihood. The lacuna lies in using agro-morphological traits as a sole marker for distinguishing the landraces. The genetic diversity of the landrace could also be substantiated with these morphological traits for further more precise decision making in conserving these valuable resources.

In the present study, both the qualitative and quantitative characters from the landrace populations showed the existence of variations between the landraces. These variations might be well spread under these diverse agro-ecological situations, or else it would have been eradicated by now. This clearly indicates that these landraces are under farmers management because they sustained various risk factors involved in agriculture and rather gets optimized with different water, soil regimes, other environmental and economic factors relating to different uses (Chakravorthy *et al.*, 2013). These variations meant for adaptations indicate the degree to which these populations are adapted to their environment and rather highlights their potential for continued performance or donors of characters in plant breeding (Pandey *et al.*, 2011). Variations created due to genotype x environment interactions therefore stays more vital to the breeders for selecting parents as donors of desired traits and as a result it could very well be exploited in crop improvement program.

Morphological adaptive traits are generally believed to be subject to natural selection and their expression is partly under the influence of environmental factors. The findings of the present study suggest that from breeders'/users perspective adaptive variations are rather more important. At farmers' level stabilizing selection is normally operative and majority of the populations of the same named landraces under both *ex situ* and on-farm situations grouped together for adaptive quantitative variations (both Ward's Minimum Variance Dendrogram and PCA). One population each of landrace Narada (static), and Bhuta (static), however, clustered separately from their on-farm managed populations. This could be attributed to the fact that farmers may sometime are non-consistent in naming a landrace.

Further, as we could not collect the on-farm managed landrace populations from the same household, efforts were made to collect the named landrace from nearby areas following proper sampling strategies and it was a composite landrace sample of the entire village/region that may now serve as a reference material for periodic monitoring of changes in changes in future adaptive variations and also the allelic frequencies over time and space for later studies as well. The choice of the material and design is therefore a limitation and is a common phenomenon with every genebank worldwide.

Genetic diversity and divergence, in fact, require assessment for two sets of attributes, analogous to the characterization and evaluation data of genetic resources (Brown, 2000). The first set is marker diversity, or the extent of differences between individual copies of genes. This set of attributes is informative as to the ancestry or breeding history of the populations. They are indicators of the recency of bottlenecks in population size, the prevalence of outcrossing, the ease with which genes are recombined, and the level of gene flow between populations (Brown, 2000; Kumar *et al.*, 2010; Logapriyan et al., 2013). The second set is variation in adaptation. This set comprises indicators of the degree to which populations are adapted to their environment and of their potential for continued performance or donors of characters in plant breeding. Both biotic and abiotic aspects of the environment are involved. Variations due to environmental adaptations are, therefore, useful to the breeders for selecting parents as donors of desired traits.

It has been generally accepted that correlation between different characters represents a coordination of physiological processes which is often achieved through gene linkages (Mather and Jinks, 1977). Knowledge of the strength and type of association is an important pre-requisite for the formulation of breeding procedure.

Grain yield is a complex character influenced by a large number of other component characters. Knowledge of the association between grain yield and other biometrical traits and also among the component traits helps in improving the efficiency of selection. This idea about the nature of association will be useful to identify the key characters for which selection can be fruitfully made.

Almost all of these landraces were under continuous cultivation for last several decades. As long as farmers themselves find it in their own best interests to grow these populations, both farmers and society will benefit at no extra cost to anyone (Jarvis *et al*., 2000). But to what extent do farmers have an "incentive" to keep growing them? is a pressing concern. Since most small farmers produce food crops for their own consumption, these traits often include not only agronomic characteristics such as tolerance of biotic and abiotic stress, but also some consumption characteristics - such as their suitability for the preparation of special dishes that are "valued" in local communities. When markets are not well developed, the value of varieties is directly related to the extent to which they meet the needs of farm households. Even when markets develop, there may still be a number of attributes over which farmers define their needs that cannot be obtained through the market (Jarvis *et al*., 2000).

Any single landrace typically has both desirable and undesirable attributes, and no landrace alone satisfies all of the farmers' concerns (Bellon and Brush 1994). However, in many parts of the world, farmers still need to satisfy many of their requirements for food and feed from their own production. The present study also demonstrated the importance of several other variety traits in a variety choice analysis. Some varieties produce higher ratios of fodder to grain than others, while others produce grain that is more suitable for on-farm processing or specialized dishes than others. Smale *et al.* (2004) aptly attempted to incorporate variety traits in a variety choice analysis.

Maintenance of diversity for long term ecological balance and agricultural sustainability is primarily based on agro-climatic conditions, assured market and economic viability of farming. Under tremendous agricultural intensification pressures, the scope for *on-farm* conservation of different crops and their landrace diversity will directly depend upon continuity of rituals/ customs/ cultural practices and also their *sinequa-non* role in the livelihood/ nutritional security of the ethnic groups only. The findings could also help optimize the number of populations of a particular named landrace to be collected and conserved *ex-situ* in gene banks, it being a capital intensive exercise. The research findings would also help suggest PGR researchers the need of periodic sampling of landrace populations as farmers deliberately introduce new diversity in production systems through informal seed exchange.

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Traditional subsistence farming in Odisha state: exploratory studies on rice landraces and sustainability in agricultural productivity

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Abstract

Subsistence farming still predominates in many parts of the country and landrace cultivation is largely practiced, particularly by small holder farmers. A precise documentation of rice based traditional landrace agriculture with overall objectives of their conservation and showcasing their role in addressing livelihood security of farmers was done in the present study. The exploratory study was undertaken in four districts of western Odisha. The inventory of valuable lessons learned would help develop a policy framework for bringing sustainable agricultural intensification in subsistence farming systems, and exploring the potential of various “add-value” interventions to native agro-biodiversity in addressing livelihood security of farmers.

Key words: Subsistence farming, Rice landrace diversity, Sustainable agriculture

Introduction

Traditional varieties of crops are the result of their continuous selection process over the ages. Farmers’ have meticulously selected varieties to suit each land type and soil type under different climatic conditions. It appears that the selection of varieties has been done by many farmers in many villages and that is the reason why after some distance the varieties change. Informal seed exchange at community level may also have taken place and thus each village has more than one variety for each land type (Pandey *et al*., 2011). This has kept genetic diversity at higher level and ensured the sustainability of their agricultural system.

Odisha has been considered as the centre of origin and genetic diversity for cultivated rice. Thousands of rice varieties were cultivated in Odisha earlier and, even after the spread of high-yielding rice varieties, farmers still cultivate hundreds of these traditional types. A majority of these traditional cultivars are, however, low yielders but are largely grown for specific traits such as maturity duration, plant stature, panicle features, yield potential, tolerance of biotic and abiotic stresses, and other elusive traits such as aroma, cooking quality, and grain quality (Das, 2012).

The development and spread of semi-dwarf high yielding rice varieties in past three to four decades have replaced fast the diversity of the locally adapted landraces developed by farmers over generations. However, many of these traditional types are still favoured by farmers in rainfed uplands, semi-deepwater and deepwater situations, and saline areas of the state. Despite their low yield potential, they are preferred for favourable traits such as early seedling vigour, rapid germination, deep root system with higher proportion of thick roots, superior grain quality, better yield stability, and tolerance of moisture stress, submergence, and salinity (Patra and Dhua, 2003; Khatana *et al*., 2004; Das, 2012).

The National Bureau of Plant Genetic Resources, New Delhi has collected over 5,000 to 6,000 rice germplasm accessions mainly from different parts of the Odisha state. However, limited information is available on the potential of traditional rice landraces addressing livelihood security of native farm households under subsistence farming.

An exploratory survey visit to traditional production areas in the districts of Bolangir, Naupada, Kalahandi and Kandhmal in Odisha state was, therefore, made in the present study to document information on traditional rice varieties and related socio-economic aspects.

Methodology

The study was based on an exploratory visit to randomly chosen farm households of four districts of Odisha, Bolangir, Nuapada, Kalahandi and Kandhamal during 2012. A structured questionnaire for data collection was designed after it was ascertained that the present study is going to be unique as so far limited systematic studies have been conducted in the study districts on landrace documentation and livelihood security. The information was documented based on interactions with knowledgeable elderly farmers. It was ensured to get participation of farmers from all socio-economic strata, beside some landless labours. Information collected in the men dominated group discussions was ascertained with separately organised group meetings with women farmers as well. Data on traditional rice landraces and other socio-economic variables were documented for five to six villages, randomly chosen per district with 10 per cent households per village. Womens' perspectives were specially sought in case of seed storage, weeding, harvesting, threshing, storability, cooking quality and taste etc. of traditional rice landraces.

Information was documented on variables, such as, crop diversification and changes in cropping pattern over time; impact of commercialization and diversification of small farms on agro-biodiversity and farmer livelihood; income shares of farmers of the study districts from different farm and off-farm sectors, and potential of native rice landraces for crop improvement, etc.

Results

Land holding size and its distribution

Majority of land holdings in the study villages of four districts were either marginal or small with landholdings of less than two hectares per household. The proportion of land holdings less than two hectares is about 54% (Table 4.3.1).

Table 4.3.1. Land distribution pattern (%) in different districts

Districts	Large farmer (4 ha and above)	Medium farmer (2-4 ha)	Small farmer (1-2 ha)	Marginal farmer (Below 1 ha)	Landless
Bolangir	3.4	6.4	23.6	14.9	51.7
Nuapada	0.5	2.2	26.2	38.3	32.8
Kalahandi	0.4	6.2	13.2	52.9	27.3
Kandhmal	2.4	9.1	27.1	23.3	38.1
Mean	**1.8**	**5.9**	**22.5**	**32.3**	**37.5**

Impact of commercialization and diversification of small farms on agro-biodiversity and farmer livelihood

The impact of commercialisation and diversification of small farms on agro-biodiversity in view of various technological, infrastructural, institutional and policy constraints was investigated. The available data (Table 4.3.2), however, shows that about 72 per cent of the gross household income originates from the agricultural sector and nearly 78 per cent of the total household labour force is primarily engaged in agriculture. There has been very marginal difference between contribution of agriculture sector to gross income of farm households over time, between 1980s (79 per cent) and 2011-12 (72 per cent). The agricultural labour force to total labour force, however, decreased marginally from about 85 per cent during 1980s to 78 per cent in 2011-12.

Further, income derived through the sale of surplus farm produce is fairly supplemented by non-farm income. It was estimated (Table 4.3.3) that farming activities generate about 49 to 74 per cent of total household incomes, while non-farm sources account for the remainder, including remittances and wages. The average household income of the surveyed villages ranged from Rs. 63,333 (Kandhmal) to Rs. 1,76,666 (Kalahandi). The remittances from non-farm sector were relatively high from the study villages in Bolangir district resulting in high share of average gross non-farm income. Farming sector, however, is still the major source of income particularly in study villages of other three districts.

Further, a precise estimate on household basis was made on contribution of farm and non-farm sector in four districts. Wide variations in the income pattern were observed and creation of non-farm job opportunities in these districts can positively impact subsistence agriculture of small holder farmers.

Table 4.3.2. Levels of vertical diversification in different districts

District	Per cent share of agriculture in gross household income per household		Percentage of average household agricultural force to total labour force	
	1980s	2011-12	1980s	2011-12
Bolangir	62	48.8	73	66.7
Naupada	82	74.6	90	83.0
Kalahandi	94	90.6	92	88.7
Kandhmal	79	73.7	83	73.3
Mean	**79.3**	**72.0**	**84.5**	**77.9**

Table 4.3.3. Share of household income from farm and non-farm sectors in different districts

District	Average household income (Rs.)	Share of farm income	Share of non-farm income	% share of farm income to total income per household
Bolangir	1,33,333	65,000	68,333	48.8
Naupada	1,28,000	95,500	42,500	74.6
Kalahandi	1,76,666	1,30,000	46,666	73.6
Kandhmal	63,333	46,666	16,667	73.7
Mean	**125,333.0**	**89,291.5**	**43,541.5**	**67.7**

Traditional rice varieties in the study area

Most villages of the study area have undulated terrain and have many type of lands ranging from upland to water logged area.

Farmers have meticulously selected the varieties to suit different land types. They have varieties to mature in as short a period as upto 70 days to 180 days long duration. In a broad pattern, it was observed that the varieties were selected in such a way that the duration of the varieties planted increases gradually as one goes down towards lowland. The varieties planted on the upland are shortest in duration (70 days) and the ones planted in low lands and in water-logged areas have longest duration of 180 days (nearly three times more in duration than short duration). It can be said that there exist inverse relationship between the duration of variety and elevation of the land. Most villages in the study area have undulated terrain. A village situated at higher elevation in the hills also has uplands and lowlands and a village situated in the valley also has highlands and lowlands.

More than half of the total traditional rice varieties cultivated presently in the study area are suitable for lowland followed by upland and midland with a growing period of about 90 days (Fig. 4.3.1, Table 4.3.4). Availability of low number of varieties for medium land was noticed. The growing condition in upland is so difficult that it has short growing period with less moisture and poor soils. This growing condition restricts the selection and choice of varieties. On the other hand a large number of varieties have been selected and grown in low lands, where growing period is longer, soils are fertile and moisture situation is better.

- **Major characteristics of traditional rice varieties**

Traditional rice varieties cultivated in the study area have different durations, which vary from 70 to 180 days (Table 4.3.4). Short duration varieties meant for planting in upland are highly tolerant to drought, pest and diseases and have medium resistance to water logging conditions. The yield varies from 10 to 16 q/ha depending on the land fertility, rainfall pattern and crop management by the farmers. Those planted in lowlands have poor drought resistance capacity but high resistance to water logging conditions. These varieties have average resistance to pest and diseases. Their yield varies from 25-35q/ha depending on land fertility, rainfall and management practices. Table 4.3.4 also indicates that traditional rice varieties are more popular in upland and low lands because of their higher tolerance to drought and water logging conditions.

- **Trends in area under traditional varieties- space and time**

More and more uplands are being converted to midlands having growing period of 90-100 days. Land levelling and field bunds are required to convert upland into midland and gradually it can be converted to medium lands (land between midlands and low lands with a growing period of about 120-135 days) after some years. Change in land type is also effecting changes in cropping pattern and varietal preferences. Gradually the area under traditional varieties is decreasing in low lands, where high yielding cultivars of rice can be grown. This is done to expect higher yields with the help of chemical fertilizers.

There are some traditional rice varieties, which are more popular than others. About 10 landraces (common) are grown on more than 10% of the area on their respective land types. There are, however, about 35 rare traditional landraces cultivated on small farms in the study villages, relatively more landraces in low lands. Thus the genetic diversity in the low lands is quite high and helps in managing the pest and disease incidence below economic threshold level.

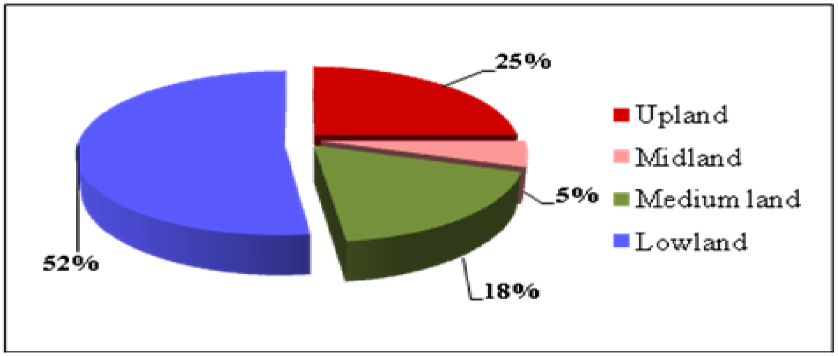
When analyzed in terms of total area under traditional rice landraces in the study villages, it was found that the varieties with 120-150 days of duration, which are grown mainly in medium and low lands accounts for half of the area. Long duration varieties, of more than 150 days, were cultivated only on about 20 % of the total area under traditional varieties of all durations. The figure clearly point towards farmers’ preference for medium duration varieties.

Table 4.3.4. Number of traditional rice varieties grown on different types of land in the study area and their important characteristics

Land types	**No of varieties under cultivation***	**% area under traditional rice landraces on different land types**	**Duration (days)**	**Level of tolerance/ resistance to**			**Range of Yield (q/ha)**
				Drought	Water logging	Pest & diseases	
Upland	16	25	Up to 70	High	Medium	High	10-15
Midland	12	5	70-120	High	Medium	Medium	12-30
Medium land	22	18	120-150	Medium	High	Medium	15-30
Lowland	36	52	150-180	Low	High	Medium	25-40

** Some varieties are cultivated both in midland and medium land and are included in respective land types*

Fig. 4.3.1. Percentage area under traditional landraces under different land types



- **Scented traditional rice varieties**

Aromatic traditional rice varieties are grown on about two to three percent of the total area under all traditional rice varieties in the study villages. About 10 traditional scented rice varieties are grown in the study villages. Variety wise analysis showed that Dubaraj followed by Kalikaranji are the major traditional scented rice varieties covering more than three fourth of the area under traditional scented rice varieties in the study villages. The aromatic varieties are considered delicacy and are used on special occasions. Farmers with greater holdings mainly grow aromatic varieties as their yield is relatively low. Aromatics rice, however, fetch premium price in the market, almost double the price of coarse grained landraces.

- **Some popular traditional rice varieties and their characteristics-Farmers' perspectives**

The exploratory survey has reported a large number of traditional varieties in the study villages. There was, however, least consistency in farmer naming landraces. In some cases the landrace is same but is called by different names in different villages and sometimes same landrace is known by more than one name in the same village by some farmers. It also appears, in some cases, that different varieties are known by same name in different villages. There are villages where more than 90% of the total rice area is under traditional varieties and on the other hand there are villages where traditional varieties are grown only on about 10% of the total rice area. On an average more than 62 per cent area was under improved cultivars and about 38 per cent area was devoted to traditional landraces (Table 4.3.5). Farmers normally keep their own seed of all the traditional varieties and several of the varieties are under continuous cultivation for last several decades. Farmers recall loss of certain named landraces over time but there has been periodic introduction of new landraces through informal seed exchange at community level.

Almost all traditional varieties have tolerance to pest and diseases and no pesticides are used. However, in case of incidence of some pest and/or disease traditional methods of pest management were used.

A few popular (common) varieties of wider adaptation for different land types and their main characteristics are presented in Table 4.3.6.

Table 4.3.5. Average landholding size, and area under traditional landraces and improved rice cultivars in different districts

District	Average landholding size per household (ha)	Area under rice landraces per household (%)	Area under improved varieties per household (%)
Bolangir	1.3	75.0	25.0
Nuapada	1.4	34.8	65.2
Kalahandi	1.5	19.7	80.3
Kandhmal	1.1	21.2	78.8
Mean	**1.3**	**37.7**	**62.3**

Table 4.3.6. Popular varieties of different land types

Land types and landraces	**Distinguishing characteristics**
Upland	
Saria	One of the most preferred traditional rice varieties for uplands grown in majority of the villages surveyed. It is sown by broadcasting the seeds. It is a 70 days variety and has inbuilt drought tolerance, and pest and disease resistance. It responds well to FYM application. The yield during years of normal rainfall ranges from 15-18 q/ha. Grain is short and bold, and brownish in colour. It is good in taste.
Setka	Setka is the other most popular variety of the uplands of 70-75 days duration. It is grown on more than one fourth of the total area under traditional rice varieties in the uplands of study villages. The variety has good pest and disease resistance. Reportedly this variety has excellent drought tolerance quality and can withstand few days of water logging as well. Average yield is 15-18 q/acre. This variety is rated as average in terms of ease of threshing. The grains were brownish in colour and easy to cook. Cooking Setka takes comparatively lesser time. It is mainly cultivated for own consumption and small quantities are sold when surplus but this does not attract good price. The storability is very good and can be easily stored for periods upto 3 years. Overall the farmers rated this variety as better for uplands.
Midland	
Lahadel	It is a 90 days duration variety grown in unirrigated conditions in midlands. More area is sown by broadcasting than transplanting. It has good tolerance to drought and average tolerance to water logged conditions. Resistance to pest and disease is also good. It is easy to thresh and processing losses are very less. Its grains are longer in size and reddish brown in colour. The yield is 12-15 q/ha. It is grown mainly for own consumption.
Latia	This variety is grown on 6-7% of the total area under traditional rice varieties on midlands. Some farmers also grow it on medium lands. It is longer in duration than Lahadel and has 100-120 days duration depending on the agricultural practice. It has good tolerance to drought. It has good cooking quality and swells well after boiling. It is mainly produced for own consumption.
Medium lands	
Kusuma	Variety Kusuma is grown on midlands to medium lands but the area under midlands is very small. It is grown on about 15-20% of the total area under traditional rice varieties on medium lands. Crop duration is about 110 days. It is sown by broadcasting the seed and taken as unirrigated crop. This variety with medium plant height has good tolerance to drought conditions. It has good pest and disease resistance. The yield is 15-22 q/ha. It can be easily harvested and breakage during processing is very low. The grains are coarse and blackish in colour. It takes comparatively longer time in cooking and expansion after cooking is also relatively low. It becomes sticky after cooking and is considered good for making cake and flattened rice. It is mainly used for home consumption.
Cheregudi	This variety is cultivated on about 15% of the total area under traditional rice varieties on middle lands in the study villages. It is 120 days in duration. Plant height is medium and has good tolerance to drought and very good resistance against pests and diseases. Yield low and on an average 15-20 q/ha. Grains, which become sticky on cooking, are whitish in colour. It is grown for own consumption and considered good for flattened rice.
Harisankar	Variety Harisankar is cultivated on about 8% of the total area under traditional rice varieties on medium land in the study villages. Its duration varies from 90-120 days. Its seeds are broadcasted and it is taken as a rainfed crop. The average plant height is about 75 cm. It has good drought and water logging tolerance and is highly resistant to pests and diseases. Its yield is 18-25 q/ha and threshing is an easy operation. Percentage of broken rice during processing is quite low. Grains are long slender is shape and brown to red in colour. It takes comparatively lesser time in cooking and is considered to have good cooking quality. It is used for own consumption. It is also used for preparing flattened rice and as an ingredient in making biscuits. It sells at slightly higher price than other coarse varieties.

Low lands	
Jhilli	This variety is grown on 20% of the total area under traditional rice varieties in low land of the study villages. It is of 150 days duration and has plant height of about 150 cm. It is highly tolerant to both drought as well as water logging conditions and high resistance against pest and diseases. However, it is grown as an irrigated crop. Yield varies between 20-30 q/ha. The grain type is fine and white in colour and it is grown for consumption as well as market.
Dubaraj	This is a long duration (150 days) variety with 120 cm long plant types. It is grown on about 20% of the total area under all scented traditional varieties on the low lands in the study villages. Both broadcasting and transplanting methods are used for planting. It has good resistance to pest and diseases and average tolerance to drought conditions. The yield varies from 15-20 q/ha in case of broadcasting to 25-30 q/ha in case of transplanted crop. Processing easily. Its cooking quality is considered good and cooking time less. Reportedly the increase in volume after boiling is high. It is grown for market as well as own consumption and sells at slightly better than improved cultivars.
Asamchudi	Asamchudi is cultivated on about 15% of the total low land area under traditional rice varieties in the study villages. It seems that there are two variants, which are known as Asamchudi in the study area as the duration and yield varies significantly in different areas. This variety has wider adaptation and can be can under irrigated conditions as well as unirrigated crop. Its duration is reported as 110 days in some areas and 140-150 days in other some other places Reported estimates for plant height varies from 100-150 cm. The yield estimates are also highly variable in the different areas ranging from 15-20 q/ha for broadcasted fields and 35-50 q/ha for transplanted fields. The grains are fine and whitish in color. The cooking is easy and takes less time. The grains swell significantly after cooking. Rice of this variety is easily digestible. This variety is also considered good for making puffed rice and thus has better commercial value.
Sapuri	Variety Sapuri is grown on about 12% of the total area under traditional rice varieties on low land of the study villages. Farmers in Nuapada study villages reported to grow it as an irrigated crop. It is of 150 days duration variety with excellent drought tolerance and disease resistance. Farmers keep their own seed. Grains are white in colour and good in taste. Rice of this variety is easy to cook and swells after boiling.
Sankri (SankariBanko)	Sankri is cultivated on about 4% of the total area under traditional rice varieties in the low land of study villages. It is grown on medium low lands and has duration of 120-150 days. It is grown in unirrigated as well as irrigated conditions. Transplanting gives better yield. Its tolerance to drought and water logging is rated as good. It responds well to FYM application Variety has good pest and disease resistance. It is very easy to thresh and processed in mills. It is good when processed as parboiled rice. The yield is 30-35 q/ha. Grain is medium in size and brownish in colour. It takes less time to cook and swells more after boiling.

Discussion

To understand the dynamics of local crop diversity in farming systems, we need to relate farmers' decision making to the pool of varieties available for planting. Bellon (1996) outlines a framework to accomplish this. The framework assumes that the farmer has several concerns, including adverse climate, soils, labour or fertilizer shortage, poor yield or storage life, and lack of appeal for home use or lack of marketability. The farmer's experience enables him to rank the populations or varieties available for planting for meeting these concerns. In population genetic terms, the farmer's concerns generate a multiniche model with different populations being differentially adapted to each niche. Bellon hypothesizes that the farmer retains the variety that best meets each concern. A variety is discarded if it no longer ranks first in meeting anyone of the concerns. Overall, a suite of varieties is needed to me*et al*l of a farmer's concerns. The concerns themselves are dynamic, changing with new market structures, technology, and government policies. Bellon's model thus suggests that the focusing or narrowing of concerns at the farm level may be the trigger for loss of diversity. A challenge for this model, however, is the relative size of each niche, and the integration of concerns across the whole farm when survival - for example, during drought - becomes overriding.

In context of deploying traditional landraces through informal seed system, little information is available about landrace diversity loss over time and space (Pandey *et al*., 2011). Genetic diversity has been reported to be maintained even when landraces are displaced by modern varieties (Steele *et al*., 2009). Further, in India, every landrace of rice at farm household level is invariably named and “farmer-named variety” is the unit that farmers manage and select over time. Farmers, however, may or may not be consistent in naming and describing landraces. Clarification of what constitutes a landrace at each scale (village, community, and region) is the first step toward defining the amount and distribution of crop diversity maintained by farmers. In diversity assessment, the importance of variations due to adaptation and genetic (DNA level) diversity, however, needs to be ascertained from users’ perspective. *In situ* strategies, however, fail to preserve all the extant biodiversity at the gene level. As better alleles or combinations arise and enjoy selective advantage, others thereby will be less fit and decline. This is the cost of evolutionary substitution and the price paid for allowing evolution to continue (Brown, 2000; Logapriyan *et al*., 2013).

It was noticed, in every study villages, that there are certain landraces which are under continuous cultivation for last several years. As long as farmers themselves find it in their own best interests to grow these populations, both farmers and society will benefit at no extra cost to anyone (Jarvis *et al*., 2000). Since most small farmers produce food crops for their own consumption, these traits often include not only agronomic characteristics such as tolerance of biotic and abiotic stresses, but also some consumption characteristics - such as their suitability for the preparation of special dishes that are "valued" in local communities. When markets are not well developed, the value of varieties is directly related to the extent to which they meet the needs of farm households. Even when markets develop, there may still be a number of attributes over which farmers define their needs that cannot be obtained through the market (Jarvis *et al*., 2000).

Some of the local landraces have the potential to be marketed in towns and cities. The gustatory preferences of the urban consumers who purchase them for food play a big role in determining the price premium. Urban consumer preferences determine the demand for the crop in local and distant market and the farmer has an incentive to grow the variety that earns the premium. The characteristics for which consumers pay a premium (aroma, colour, cooking quality) requires the knowledge inputs from nutrition and crop scientists. Other related economic analyses might include assessments of processing or handling costs, and market efficiency.

In an advanced, industrialized economy, and in the absence of special government programmes, it may be argued that landraces will only be grown when they have unique qualities that urban consumers or export markets value, and only if these same qualities cannot be easily transferred into modern varieties. Where genetic diversity is considered to be important in a target area but farmers are revealed to have few social, cultural or market-based incentives to maintain it, then specific publicly funded initiatives may be needed. Economists generally believe that these forms of interventions are more “costly” to society than market-based incentives.

It is common knowledge now that modern intensive agroecosystems that rely on monocultures and genetic homogeneity make the crops more susceptible to disease and pests, and to climatic variations. The modern improved varieties tend to be high yielding only when supplied with intensive inputs, ideal growing conditions and improved management. Traditional varieties are of value to us because they embody characteristics that are potentially valuable, but not yet exploited. In the future, new varieties will be needed that can survive in adverse environmental conditions such as saline or acidic soils or drought, etc. The genetic resources needed to develop these new strains will probably come from the diversity of plants stored in traditional agroecosystems (Wood, 1993).

There is enhanced emphasis world over on *in situ* on-farm conservation of traditional varieties now. It leads to productive genetic diversification; the development of diversity that is useful and appropriate for traditional farmers and adapted to local growing conditions. *In situ* conservation also ensures that control remains in the hands of the farmers who will be managing the crops and is a constant stimulus to more productive agriculture. It is estimated that 20% of current world food production is still produced from traditional multi-cropping systems (FAO, 2010).

The pace of non-farm diversification, in present study, has been relatively low compared to advanced agriculture systems where much of the labour force is absorbed outside agriculture. Probably India is one of the very few Asian countries with high shares of agricultural sector in GDP as well as total employment. This does not compare well with a country like Japan or Korea where agriculture contributes little to GDP and employs small percentage of the total labour force.

The recognition, dissemination and wider use of land race varieties may be both more relevant to the needs of poor farmers and also less damaging to the environment than the use of improved varieties. They are adapted to the diverse physical stresses and biological problems of local areas where they are grown under low inputs and low densities, sometimes in mixed cropping systems (Buddenhagen, 1983). In surveyed villages, many of the rice landraces have local ecosystem adaptation in subsistence agriculture in which they have evolved.

Continuous cultivation of traditional crop landraces offer promising lessons for sustainability because the traditional systems maintain biodiversity, thrive without agrochemicals, and sustain year round yields even under marginal environmental conditions and management. The local knowledge and wisdom accumulated during century of farming comprise a Neolithic legacy of fundamental value for the future of human kind.

In a global survey of crop varietal diversity on farms involving 27 crops, researchers found that considerable crop genetic diversity continues to be maintained on-farm in the form of traditional crop varieties, especially of major staple crops (Jarvis *et al*., 2008). In most cases, farmers maintain diversity as insurance in the face of social, economic and environmental unpredictability. Many researchers have concluded that this varietal richness enhances productivity and reduces overall yield variability. For example studies by plant pathologists provide evidence that mixing of crop species and/or varieties can delay the onset of diseases by reducing the onset spread of disease carrying spores and by modifying environmental conditions so that they are less favourable to the spread of certain pathogens (Altieri, 2004). Recent research in China found that four different mixtures of rice varieties grown by farmers from 15 different townships over 3,000 ha suffered 44% less blast incidence and exhibited 89% greater yield than homogeneous fields-without the need to use chemicals (Zhu *et al*., 2000).

The traditional agricultural development policies in India have not been favourable to diversified agricultural growth, particularly in the context of small farms and backward under-privileged regions. Therefore, if our objective is to promote small farm diversification and promoting ecologically managed farms in various agro-climatic regions of the country, a wide range of policies can be envisioned that would reward small farmers for sustaining agricultural biodiversity. These include, as Boyce (2004) suggested, a) Removal of anti-small farmer policy distortions, b) Social recognition by promoting ‘non-economic’ rewards for the conservation of agricultural biodiversity and also help to create public awareness of the need for policies to provide economic rewards, c) Market development- there is scope for development of domestic and international markets in which traditional varieties command a price premium, this could not only provide direct rewards to growers, but also help to raise public consciousness of the importance of diversity and the need for public policies to sustain it, d) Provision of local public goods, e) Payments for environmental services- payments to farmers who provide the environmental service of *in situ* conservation, and f) Policies to encourage part-time farming- policies that help to generate part-time, off-farm employment opportunities in rural areas can help to sustain small farms.

Such policies would both strengthen rural livelihood security and provide incentives for continued *in situ* on-farm conservation of agricultural biodiversity. These policies are not mutually exclusive, nor are any one policy alone likely to be sufficient. Taken together, however, these policies could do a great deal to support small farms and stewardship of agricultural biodiversity.

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CHAPTER 5 GENERAL DISCUSSION

Adaptations to diverse agro-ecologies and consequent genetic variations have resulted in built up of substantial landrace diversity across all rice growing regions globally. About 1, 20,000 rice accessions including crop landraces have been conserved *ex situ* in gene banks worldwide (Das *et al*., 2013). The National Genebank at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi holds about 95,000 accessions of rice which is a vital resource for various agronomic and quality traits, and also for monitoring loss of diversity over time and space. In India, the rice landraces originating from states of Assam and other parts of North-eastern region, West Bengal, Odisha, Chhattisgarh, North-western Himalayas, etc. all constitute an ideal resource for assessment of its genetic architecture and work out modalities for rational conservation plan for landrace diversity.

In rice, there are certain well-known landraces/farmer varieties reported in every production systems across specific agro-ecosystems in India. It is presumed that the adaptive variations even of different populations of the same named landrace under farmer management in niche environments often reflect in their genetic portfolios (Kumar *et al*., 2010). There have also been reports of stabilizing selections operating in these known local landraces operating at farm household level leading to greater population differentiation (Pandey *et al.*, 2010; Sreejayan *et al.*, 2011; Pandey *et al.*, 2012). More case studies, therefore, need to be undertaken to determine the population structure of named landraces and optimum populations (accessions) of same named landrace to be collected and conserved *ex situ* in Genebanks.

In every traditional production systems, there are certain landraces which are common and some are rare. One might expect these landraces to have a high proportion of locally common alleles of adaptive significance and therefore to be particularly important for conservation and particularly interesting for users. There is, however, no information available whether rare landraces are derivatives of common ones or they are distinct genetic entities. It could be that the differences are related much more to character differences (and possibly to the distribution of diversity) and all varieties have about the same allelic richness (Jarvis *et al*., 2000). Neither is it known whether increasing richness of farmer varieties or number of farmer varieties and increasing genetic diversity (allele richness) are positively correlated. To make rational conservation plans, it is important to test whether areas with a few varieties are conserving as much diversity as areas with many varieties.

In India, every landrace of rice at farm household level is invariably named and the farmer-named variety is the unit that farmers manage and select over time. Farmers, however, may or may not be consistent in naming and describing landraces. Clarification of what constitutes a landrace at each scale (village, community, and region) is the first step toward defining the amount and distribution of crop diversity maintained by farmers. Considering large scale local level informal seed exchange, the genetic constitution particularly of several homonyms/ homophones also needs to be investigated for their genetic similarity and distinctness.

Often serious concerns have been expressed by Civil Society Organizations and conservationists that there has been large scale erosion of local landraces from traditional production systems on-farm. The precise documentation of diversity loss, however, needs to be documented and quantified in terms of loss of alleles and genotypes rather than merely the named landraces which is often misleading. In an interesting recent study, rice genetic diversity from high altitude region of Nepal, however, has been predicted to increase even when number of varieties decreases. Genetic diversity has been reported to be maintained even when landraces are displaced by modern varieties (Steele *et al*., 2009). Evidence from different parts of the world has also shown that certain traditional agricultural activities increased the number of species present rather than decreased them (FAO, 2006, 2010).

In diversity assessment, the importance of variations due to adaptation and molecular diversity needs to be ascertained from users' perspective. Under on-farm management, while the particular attributes, characters, or adaptations of a population may persist over generations, the underlying genotypes will change. New alleles or combinations are expected to arise and increase in frequency at the expense of other alleles that may well disappear (Brown, 2000). Strictly speaking, *in situ* strategies fail to preserve all the extant biodiversity at the gene level. As better alleles or combinations arise and enjoy selective advantage, others thereby will be less fit and decline. This is the cost of evolutionary substitution and the price paid for allowing evolution to continue.

The above-mentioned findings can also be argued in support of the neutral theory of molecular evolution (Kimura, 1968). Kimura’s neutral theory claims that the overwhelming majority of evolutionary changes at the molecular level are caused not by selection acting on advantageous mutants, but by random fixation of selectively neutral or very nearly neutral mutants through the cumulative effect of sampling drift under continued input of new mutations (Kimura, 1991). The *in situ* approach to conserving landraces undoubtedly holds several advantages over *ex situ* strategies.

The landraces conserved in the genebank (static) are often not suitable for population genetic studies and suffer from several deficiencies. It might be possible that the number of individuals taken from the collection sites has been very small and the potentially useful alleles may have been missed in the original samples. When breeders’ are involved in exploration programmes, single panicle selection for specific morphotype cannot be ruled out. On the other hand, the on farm (dynamic) managed populations for the present study were collected following proper sampling strategies and it was a composite landrace sample of the entire village and may serve as a reference material for periodic monitoring of changes in allelic frequencies over time and space for later studies. Farmers in the study area are usually not making conscious selections. Since majority of these native landraces were grown in small plots, we expect genetic drift occurring in these populations under continuous cultivation. As we consider that many of the alleles, in molecular diversity analysis, are selectively neutral they may not be contributing much to the adaptive phenotypic variations. Phenotypic adaptive variations from breeders’ perspective are, therefore, more important than molecular diversity alone and needs due consideration while deciding on number of populations collected and conserved per named landrace from a given village/region for *ex situ* conservation. The present study suggests for specific case studies to be initiated on deciding the optimum number of populations of a given named landrace to be collected and stored *ex situ* in genebanks.

The genotypes of different rice landrace populations were different for most of the STMS loci analysed in the present study. Population differentiations, based on F_{ST} measures, were very high even for the populations of same named landraces. Some recent studies have assessed the population genetic structure of named rice landraces (Pusadee *et al.*, 2009; Kumar *et al.*, 2010, Pandey *et al.*, 2010; Sreejayan *et al.*, 2011; Pandey *et al.*, 2012; Logapriyan *et al.*, 2013; Gayacharan *et al.*, 2014) and found very high partitioning of total variability among individual populations. Variation in the diversity pattern even for the same named landrace under dynamic and static conservation was also observed even for major gene governed qualitative morphological traits for some landraces.

The greater population differentiation in the molecular diversity analyses could be attributed to genetic drift. Far fewer studies have been carried out on the multilocus structure of landrace populations, i.e. the extent to which genetic variants at one locus are correlated in occurrence with variants at another locus. Such a structure arises from selection, genetic drift or fragmentation of the population and is retained through selection, isolation and the lack of migration, and restrictions on outcrossing and genetic recombination (Brown, 2000).

No significant correlation was observed between the observed pattern of molecular variations and the morphological adaptive variations. Even the variation pattern based on qualitative traits did not match the molecular variations. Only probable explanations could be that the microsatellites are considered to be neutral and thus provide no assessment of fitness. The forces causing high molecular differentiation could be due to genetic drift and no selection. Conversely, morphological traits are generally believed to be subject to natural selection and their expression is partly under the influence of environmental factors. Further, in contrast to morphological traits, microsatellite variation is based on DNA sequence variation. Variations for qualitative traits could be attributed to sampling variations.

As the molecular markers chosen for diversity analyses were not specifically linked with any morphological traits, the differences in diversity pattern were obvious. The present study highlights that adaptive variations are rather more important from users' perspective and farmers operate stabilizing selections so that populations of the same named landrace do not differ much for quantitative morphological traits. Random drift occurring in these landrace populations, however, make the populations genotypically distinct.

Diversity conserved on farm is subject to a range of forces and is likely to be in a dynamic state. As yet, the data are far too limited to assess the various factors – human, biological, edaphic or climatic – to determine the requirements for optimal outcomes. The challenge is to plan for the assessment of these factors in relation to changes in genetic structure over time. Population biology research for *in situ* conservation thus needs to be both descriptive and hypothesis testing in order to guide technical improvement and management of landrace populations (Brown, 2000).

A negative relationship between the presence of modern varieties and crop genetic diversity is typically assumed, but empirical examples suggest that the relationship is more complex. Some researchers have found that when modern varieties serve for generating cash, they can support the production of more traditional varieties which satisfy other consumption needs in farm households (Zimmerer, 1998). Testing the relationship between modern varieties and crop genetic diversity in other cases, with a good statistical design and sound economic and biological principles, would advance scientific knowledge of these issues.

As stated above, there are many crop landraces which farmers in these agro-ecologies produce and have potential to be marketed in towns and cities, the tastes and preferences of the urban consumers who purchase them for food play a big role in determining the price premium. These tastes and preferences determine the demand for the crop in local and distant market and the farmer has an "incentive" to grow the variety that earns the premium. The characteristics for which consumers pay a premium (aroma, colour, cooking quality) to physical attributes of the seed requires the knowledge of nutritionists, chemists and crop scientists. Other related economic analyses might include assessments of processing or handling costs, and market efficiency.

There is enhanced emphasis world over on *in situ* on-farm conservation of traditional varieties now. It leads to productive genetic diversification; the development of diversity that is useful and appropriate for traditional farmers and adapted to local growing conditions. *In situ* conservation also ensures that control remains in the hands of the farmers who will be managing the crops and is a constant stimulus to more productive agriculture.

There is increasing recognition that the diverse needs of resource-poor farmers cannot be addressed by the breeding of a restricted range of high-yielding, high-input varieties. Yields of improved varieties in favourable conditions have reached a plateau in many countries (Win and Win, 1990) or even subsequently declined. It has been suggested that a range of varieties are needed to fulfil specific socio-economic as well as agro ecological needs in the small farm system and that the breeding methods need to be reassessed to increase the ability of formal sector agricultural research to produce varieties useful to small farmers (Cromwell, 1990). It has been rightly argued that sophisticated and advanced methodologies are quite out of place on most breeding programmes in the developing world, where the basic strategies of local collection, introduction and selection have yet to be fully exploited (Smartt, 1990; Wood, 1993). In a broad review of plant improvement for sustainable agriculture, Ceccaralli *et al*. (1992) noted that plant breeders must adopt an overall strategy that differs from present strategies in national and international breeding programmes. Requirements include concerted efforts on plant genetic resource management, evaluation under farmer conditions, adaptation to unfavourable conditions, and re-examining the role of diversity (mixed cropping and genetic variability within crops) to achieve production stability.

Many researchers have concluded that this varietal richness enhances productivity and reduces overall yield variability. For example studies by plant pathologists provide evidence that mixing of crop species and/or varieties can delay the onset of diseases by reducing the onset spread of disease carrying spores and by modifying environmental conditions so that they are less favourable to the spread of certain pathogens (Altieri, 2008). Recent research in China found that four different mixtures of rice varieties grown by farmers from 15 different townships over 3,000 ha suffered 44% less blast incidence and exhibited 89% greater yield than homogeneous fields-without the need to use chemicals (Zhu *et al*., 2000).

To understand the dynamics of local crop diversity in farming systems, we need to relate farmers' decision making to the pool of varieties available for planting. Bellon (1996) outlines a framework to accomplish this. The framework assumes that the farmer has several concerns, including adverse climate, soils, labour or fertilizer shortage, poor yield or storage life, and lack of appeal for home use or lack of marketability. The farmer's experience enables him to rank the populations or varieties available for planting for meeting these concerns. In population genetic terms, the farmer's concerns generate a multiniche model with different populations being differentially adapted to each niche. Bellon hypothesizes that the farmer retains the variety that best meets each concern. A variety is discarded if it no longer ranks first in meeting anyone of the concerns. Overall, a suite of varieties is needed to me*et al*l of a farmer's concerns. The concerns themselves are dynamic, changing with new market structures, technology, and government policies. Bellon's model thus suggests that the focusing or narrowing of concerns at the farm level may be the trigger for loss of diversity. A challenge for this model, however, is the relative size of each niche, and the integration of concerns across the whole farm when survival - for example, during drought - becomes overriding.

The population structure of named landraces in traditional farming would help decide the optimum number of landrace populations to be collected and conserved *ex situ* and level of adaptive variations in niche environments including biotic and abiotic stresses. The complementarity of *in situ* and *ex situ* conservation is based on the recognition that crop genetic resources involve more than the alleles and genotypes of crop populations. Besides the genetic raw material of landraces, crop genetic resources also comprise related species, agroecological interrelationships and human factors. For the on-farm conservation of crop landraces, the traditional cultures and cropping systems that grow and use such populations are fundamental aspects of the habitats to which they are adapted. The systems shape their present genetic structure and determine the changes within landrace populations.

Maintenance of diversity for long term ecological balance and agricultural sustainability is primarily based on agro-climatic conditions, assured market and economic viability of farming. Under tremendous agricultural intensification pressures, the scope for *on-farm* conservation of different crops and their landrace diversity will directly depend upon continuity of rituals/ customs/ cultural practices and also aspects related to the livelihood/ nutritional security of particular ethnic groups. Land races, developed over thousands of years of zero application of agrochemicals, are pre-adapted to grow without chemical inputs, and therefore of value to the future needs of all crop improvement programmes. Traditional knowledge of adaptation to low nutrients or high disease pressure may be of global value.

CHAPTER 6 SUMMARY AND CONCLUSION

The National Genebank at National Bureau of Plant Genetic Resources (NBPGR), New Delhi holds about 95,000 accessions of rice including traditional landraces under *ex situ* storage. This kind of conservation, however, does not allow the rice varieties to adapt to changing environmental settings and changing agricultural practices. In contrast, on-farm conservation of diverse rice landraces is dynamic, i.e. the varieties are subjected to continuous selection by the farmers and are thus allowed to develop and evolve. Despite reduction in area under cultivation of traditional landraces and loss of landrace diversity, landrace cultivation still predominates in subsistence farming. Local rice landraces should, therefore, be seen as the products of careful selection rather than an unchanging embodiment of ancient germplasm.

Rice is not only the dominant staple food, but also an integral part of rural culture in India. It can therefore be attributed a cultural value, which is the most evident in areas that still maintain a large diversity of rice varieties. Many varieties are characterized by a very specific taste, and seeds of different varieties are informally exchanged among neighbours and relatives or given as presents. Considering these aspects, loss of biodiversity also implies a fading rural culture.

Inter- and intra-population diversity of five named rice landraces from parts of Odisha state of India representing static and dynamic management were studied using 14 STMS primer pairs. Moderate to low diversity in landrace populations was observed with number of alleles per population ranging from 16 to 25 and per cent polymorphism ranging from 14.29 to 64.29, respectively. The AMOVA indicated highest variation of 75.7% among populations within groups (static ***vs*** dynamic). Pair-wise estimates of F_{ST} revealed very high significant population differentiation which ranged from 0.68 to 0.89 indicating that the populations share limited genetic diversity among them. However, not many variations were observed in the phenotypes of populations representing static and dynamic management. This shows that adaptations of a population are apparently persisting over generations but the underlying genotypes have changed and new alleles or combinations may arise and increase in frequency at the expense of other alleles that have disappeared. The importance of population biology research for *in situ* conservation, requires both descriptive and hypothesis testing, in order to guide technical improvement and management of landrace populations was emphasized. Further, the complementarity of *in situ* and *ex situ* conservation is based on the recognition that crop genetic resources involve more than the alleles and genotypes of crop populations. Beside the genetic raw material of landraces, crop genetic resources also comprise related species, agroecological interrelationships, and human factors.

Variations due to environmental adaptations among 20 populations of seven locally common rice landraces from parts of Odisha state were studied. Wide variations for various qualitative, and quantitative agro-morphological traits like grain yield per plant, number of grains per panicle, grain length and width, etc. among different landrace populations were recorded. A distinct pattern of agro-ecological adaptations was observed in populations of majority of the named landraces. Agro-morphological adaptations give users the opportunity to select and use landrace diversity in crop improvement. Beside variation for various qualitative traits, the landrace populations varied greatly for grain weight - as characterized by the yield per plant-varied between 7.2 and 34.2g, the small grain varieties being the most popular ones. Another aspect that makes rice landraces attractive is the high quality of the grain. This refers to the palatability, the texture, and particularly the nutritional value. Moreover, the quality of the rice straw as an animal feed may gain increasing importance in the future due to the aggravating scarcity of feed resources. Rice straw is suitable as a feed for ruminants such as cows or buffaloes, which in turn are used in food production or serve as draught animals. On the one hand, landraces deliver relatively more straw than high yielding varieties. On the other hand, their straw tends to have higher crude protein content, which is often the most important limiting factor in ruminant nutrition in tropical areas.

Further, a precise documentation of rice based traditional landrace agriculture with overall objectives of their conservation and showcasing their role in addressing livelihood security of farmers was done in parts of western Odisha. The inventory of valuable lessons learned are expected to help develop a policy framework for bringing sustainable agricultural intensification in subsistence farming systems, and exploring the potential of various “add-value” interventions to native agro-biodiversity in addressing livelihood security of farmers.

In view of the existing findings, a few hypotheses towards which research should be directed could be made. The important areas of research on *in situ* conservation of landrace diversity on farm in the context of population structure may include allelic richness and genotypic diversity, specific adaptations, localized divergence, diversity to meet temporal and environmental adaptations, and continuance of crop evolutionary processes. On-farm populations have the capacity to support a much greater number of rare alleles and of different (multilocus) genotypes than accessions in gene banks. Diversity itself confers long-term population fitness because it helps populations to cope with variable environments. Landrace populations of crops have survived centuries of selection for reliable production in subsistence agriculture, yielding a definite, known but probably limited benefit to the farmers who grow them. Presumably, they are selected for resilience and stability, though of modest productivity. The *in situ* strategy conserves the crop evolutionary processes (mutation, migration, recombination and selection). It provides scope for ongoing evolution, particularly in response to environmental changes and pathogen and pest pressures fluctuating in numbers and genetic composition. The key variables include (i) genetic diversity within populations, which is the essential raw material for evolution; (ii) breeding system variation (such as changes in outcrossing rate); (iii) variation in resistance in space and time, related to pest pressure and diversity; and (iv)the dynamics of seed systems, persistence and migration. Furthermore, regeneration of *ex situ* collections is currently considered a serious and enormous challenge.

In an advanced, industrialized economy, and in the absence of special government programmes, it may be argued that landraces will only be grown when they have unique qualities that urban consumers or export markets value, and only if these same qualities cannot be easily transferred into modern varieties. Where genetic diversity is considered to be important in a target area but farmers are revealed to have few social, cultural or market-based incentives to maintain it, then specific publicly funded initiatives may be needed. Economists generally believe that these forms of interventions are more “costly” to society than market-based incentives.

ABSTRACT

Large scale abandonment of traditional landraces calls for systematic combing of all diversity rich areas so that important diversity is collected and safely conserved *ex-situ* in gene banks before lost from production systems. Further, as climate change is leading to the erosion of world's biological diversity, severe implications are predicted in agriculture and food supply notably in subsistence farming. As a consequence, a two-pronged strategy for mitigation and adaptation to climate change can be advocated. Agro-biodiversity plays a key role in this and calls for a revision of the present conservation approaches. Instead of *ex-situ* conservation in gene banks a broader concept needs to be envisaged through which emphasis is on *in-situ* on-farm conservation complemented by gene banks. It has two fold advantages as the future needs are unknown, maximum genetic resources could be conserved at the lowest possible public cost and as the genetic resources get exposed to environmental changes, well adapted material gets evolved instead of being stored in a gene bank. The observed pattern of molecular variations and the morphological adaptive variations of native landraces was investigated both under static (*ex-situ*) and dynamic (on-farm) management. The findings suggest that from breeders'/users perspective adaptive variations are rather more important. Often the morphological adaptive variations and even the variation pattern based on major gene controlled qualitative traits did not match the molecular variations. The microsatellites are normally provide limited assessment of fitness. The forces causing high molecular differentiation could be due to genetic drift and no selection. Conversely, morphological adaptive traits are generally believed to be subject to natural selection and their expression is partly under the influence of environmental factors. At farmers' level stabilizing selection is normally operative and no substantial loss of adaptation to past conditions and adaptations to new was noticed for majority of the named landraces. Further, u**nder tremendous agricultural intensification** pressures, the scope for on-farm conservation of rice landrace diversity will directly depend upon continuity of rituals/ customs/ cultural practices and also its enhanced role in the livelihood/ nutritional security of the ethnic groups.

ओड़िशा प्रान्त की जैपुर एवं अन्य क्षेत्रों में धान (*Oryza sativa* L.) की पारंपरिक भू–प्रजातियों का परिवर्तनात्मक अध्ययन

सार

धान की उन्नत प्रजातियों की अधिकाधिक खेती की वजह से पिछले कई दशकों में विश्व भर से इनकी भू–प्रजातियों (**Landrace**) का क्षरण होता रहा है। हालाँकि पारंपरिक भू–प्रजातियों को एकत्रित कर इन्हें जीन बैंकों (**Seed Genebanks**) में संरक्षित किया गया है, लेकन वर्तमान में इनके ऑन–फार्म (**On-farm**) संरक्षण को भी अत्यन्त महत्वपूर्ण समझा जा रहा है। वर्तमान शोध के अन्तर्गत ओड़िशा प्रान्त की कुछ भू–प्रजातियों के जीन बैक (**Static**) संरक्षित एवं ऑन–फार्म (**Dynamic**) प्रवन्धित नमूनों की आनुवंशिक विविधता का अध्ययन किया गया। इसके अतिरिक्त इन भू–प्रजातियों की वातावरण–जनित विविधता का भी अध्ययन किया गया। भू–प्रजातियों द्वारा पर्याप्त विविधता दर्शित की गई। पश्चिमी ओड़िशा प्रान्त के कुछ क्षेत्रों की धान की पारंपरित भू–प्रजातियों की खेती एवं कृषि परिवारों की आजीविका सुरक्षा में इनके योगदान सम्बन्धित सामाजिक–आर्थिक अध्ययन भी वर्तमान शोध में प्रस्तुत है।

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SOLUTION CHEMICALS AND REAGENTS USED FOR DNA EXTRACTION

1. Liquid Nitrogen

2. Cetyl Trimethyl Ammonium Bromide (C-TAB 10%)

10g C-TAB was dissolved in 60 ml of sterile distilled water and kept for 15 min. at 60˚C in water bath and finally volume was made up to 100 ml with sterile distilled water and autoclaved.

3. Sodium Chloride (Nacl, 4M)

292.2 g of Nacl was dissolved in H_2O and volume was made up to 100 ml. The solution was autoclaved prior to use.

4. Tris HCL buffer (pH 8.0, 1M)

12.11 g of Tris salt was dissolved in distilled H_2O and volume was made up to 100ml and pH was adjusted to 8.0 using 1N HCL. The solution was autoclaved prior to use.

5. Ethylene Diamine Tetra Acetic acid (EDTA, 0.5 M)

18.62 g EDTA was dissolved in sterile distilled H_2O. The pH of the solution was adjusted to 8.0 using 1 B NaOH. The volume was made up to 100 ml using sterile distilled H_2O and the solution was autoclaved.

6. β- Mercaptoethanol 2 percent solution

2 per cent of the total solution (v/v) was added in the CTAB extraction buffer

7. DNA extraction buffer

DNA extraction buffer was prepared afresh as and when necessary using stock solutions of the individual components.

Extraction Buffer composition

Component	Stock solution	Working buffer	Vol. of stock taken to prepare 200 ml buffer
C-TAB	10%	1.5%	40 ml
Nacl	4 M	1.4 M	70 ml
Tris	1 M	100 mM	20 ml
EDTA	0.5 M	20 mM	8 ml
β - Mercaptoethanol	100%	2%	4 ml
Distilled H_2O	-	-	58ml
			200 ml

8. Isopropanol

9. Sodium Acetate 3M, pH 5.6

30.75 g sodium acetates was dissolved in sterile distilled H_2O, pH was adjusted to 5.6 with glacial acetic acid and volume made up to 50 ml. The solution was autoclaved and stored till use.

10. Chloroform: Isoamyl alcohol (24 : 1) mixture

96 ml of chloroform was mixed with 4 ml of isoamyl alcohol. It was stored in amber coloured bottle.

11. 70% Ethanol

70 ml of absolute ethanol was mixed well with 30 ml of sterile water and stored in a stopper bottle till use.

DNA PURIFICATION

12. Phenol: Chloroform: Isoamyl alcohol (25:24:1) mixture.

100 ml of Tris saturated phenol was added to a mixture of 96 ml chloroform and 4 ml isoamyl alcohol. The mixture was mixed well prior to use and stored in amber coloured bottle.

13. RNase (20 mg/ml) solution

RNase	20mg
Tris-Cl (pH 7.5)	10 mM
NaCl	1.5 mM

Sterile water was added to make the volume to 1 ml. The solution was heated at 100˚C for 15 minutes to inactivate any DNase presented then stored in aliquots at -20˚C.

SOLVENT FOR DNA

14. Tris: EDTA (TE) buffer (10 mM Tris: 1 mM EDTA, pH 8.0)

10 ml of Tris (1M) Buffer, pH 8.0 and 0.2 ml of 0.5 M EDTA, pH 8.0 was mixed with sterilized H_2O and volume made up to 100 ml. The solution was autoclaved prior to use.

15. 10 X TAE buffer stock solution (100 mM Tris: 10 mM EDTA: 1M NaCl, pH 7.4)

12.11 g Tris, 3.72 g EDTA and 58.44 g of Nacl were dissolved in sterile distilled water and volume was made up to 1000 ml using distilled water. The pH was adjusted to 7.4 with HCL solution, filtered before use and stored at 4˚C.

GEL ELECTROPHORESIS

16. Agarose gel (3.0%)

6.0 g of agarose was made up to 200 ml with 1 X TAE buffer; the contents were mixed thoroughly for 3-4 min. to dissolve the contents. The mixture was cooled down to 40˚C. The molten was casted in a gel tray with a comb containing 30 teeth to produce the veil.

17. Ethidium bromide

10 mg of Ethidium bromide was dissolved in sterile water and volume was made upto 1 ml. The solution was stored in an amber coloured bottle at 4^{o}C.

18. Loading dye (10 X solution)

Bromophenol blue	0.25%
Xylene cyanol FF	0.25%
Glycerol	50%
TAE	1 X

Sterile water was added to the above compound to make volume to 100 ml.