STUDY OF GENETIC DIVERSITY OF SELECTED INDIGENOUS RICE VARIETIES OF MIZORAM

VANLALSANGA

DEPARTMENT OF BOTANY

MIZORAM UNIVERSITY

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BY

VANLALSANGA

DEPARTMENT OF BOTANY

SUBMITTED

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I have great pleasure in forwarding the thesis entitled **"Study of genetic diversity of selected indigenous rice varieties of Mizoram"** submitted by Mr. Vanlalsanga for the Ph. D degree of Mizoram University. Mr. Vanlalsanga has put in the prescribed number of terms of research work under my supervision. The data incorporated in the thesis are original based on his own independent observations.

Aizawl:27th September, 2019

(Dr. Y. TUNGINBA SINGH)

Supervisor

DECLARATION BY THE CANDIDATE

I, **Vanlalsanga**, hereby declare that the subject matter of this thesis entitled **"Study of genetic diversity of selected indigenous rice varieties of Mizoram"** is the original research work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the Mizoram University for degree of *Doctor of Philosophy* in Botany.

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PREFACE

Rice plays a very important role in the life of a large part of the World's human population as a staple food. It supplies most of the calorie intake and plays a crucial role to keep the body healthy. In other words, the health of human's body in many countries of the world directly depends on rice. The World's rice production is estimated at 650 million tonnes which needs to be increase to meet the ever-increasing demand. Though the utilization of high yielding varieties (HYV), developed and released during the Green Revolution between 1950 and the late 1960s, increase the production of rice, better improved varieties are still in need. The major sources for development and improvement of rice are landraces or indigenous rice varieties/cultivars. Indigenous rice varieties are confined to specific area, with historical origin on that particular area, genetically diverse and adapted to various biotic and abiotic stresses such as drought, salinity, blasts, high temperatures, pests, local environmental conditions such as farming practices, seed selection, sowing, and management, etc. They possess high genetic diversity and are thought to be an intermediate stage between wild species and cultivated varieties. A large number of indigenous rice varieties are reported but most of the rice cultivated in the World are HYVs developed scientifically. This trend implies a possible narrowing of the natural gene pool. But the farmers of the many rice growing countries still prefer and practice their own landrace cultivation that they inherit from their forefathers, which suit the local microclimate and adaptation. The genetic variability found within these varieties is believed to be able to adapt to local climate, changing environments, farming practices, etc. India is very rich in indigenous rice varieties especially West Bengal and the NE states of India are thought to be home for a large

number these varieties. The considerable content of genetic diversity of indigenous rice varieties can be employed as a source of germplasm for future rice improvement programmes to meet the ever-increasing demand. Many molecular markers such as AFLP, RFLP, RAPD, SNP, SSR, etc. have been developed and utilized for assessment of genetic diversity within and between populations. Of these markers, PCR-based simple sequence repeat (SSR) or microsatellite markers are the most preferred and widely used markers in population genetics studies, marker-assisted selection, gene duplication or deletion and DNA fingerprinting. The advantages of microsatellite markers include the requirement of only small amount of template DNA, detection of high level of allelic diversity (polymorphism), co-dominant, abundance and distributed throughout the genome, more variable and more informative than other molecular markers.

It is understood that the assessment of genetic diversity is important for crop management, utilization and conservation. My Ph.D dissertation "Study of genetic diversity of selected indigenous rice varieties of Mizoram" was undertaken to assess genetic diversity of rice varieties of Mizoram. The main objectives of my study are:

1. Collection of indigenous rice varieties from different parts of Mizoram.
2. Assessment of genetic diversity and population structure in the collected population.
3. Identification of diverged varieties for improved breeding programmes.
4. Designing strategy for conservation of indigenous varieties.

In order to achieve these objectives, 53 indigenous rice varieties from various villages covering all the districts of Mizoram were used to analyzed their genetic diversity using microsatellite markers. The results obtained indicated a high level of genetic diversity among the studied populations. My study will enrich tha knowledge base for rice of Mizoram and promote local conservation programmes and facilitate their effective use. The varieties with high genetic diversity observed in the present study will be helpful as a source of germplasm for future breeding programmes. At the same time, few cultivars showed very low levels of genetic diversity calling for necessary conservation strategies.

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Place: Aizawl Vanlalsanga

Date: 27th September, 2019

CONTENTS

Chapter 1
Introduction

1.1 Rice

Rice (*Oryza sativa* L.) is considered as one of the most important cereal crops of the world. It is also a major staple food for a large part of the world's human population. And maximum rice production is consumed in Asia alone, where more than 2 billion Asian population feed on rice. Diversity and adaptation by rice to a varied range of geographical, ecological and climatic conditions is already shown (Yadav et al. 2013), for example, below sea level in Kuttanad, Kerala to high elevation of 2000 meters above sea level in North East India, Jammu & Kashmir, Uttaranchal and Himachal Pradesh (NBPGR 2006). In India, rice-growing areas can be broadly grouped into five regions, viz., Northeastern (NE) region (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura), Eastern region (Bihar, Chhattisgarh, Madhya Pradesh, Orissa, Eastern Uttar Pradesh and West Bengal), Northern region (Haryana, Himachal Pradesh, Jammu & Kashmir, Punjab, Western Uttar Pradesh and Uttaranchal), Western region (Gujarat, Maharastra and Rajasthan) and Southern region (Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Pondicherry) (NBPGR 2006).

Archaeological evidence has suggested the domestication of rice in the Yangtze River valley region of China however, genetic evidence has shown that rice was originated in the Pearl River valley region of China (Molina et al. 2011; Huang et al. 2012). The genus *Oryza* can be divided into four species complexes, viz., *O. sativa*, *O. officinalis*, *O. ridelyi,* and *O. granulata* and the *O. sativa* complex consists of two domesticated species, viz., *O. sativa* and *O. glaberrima*, and six wild species, viz., *O. rufipogon*, *O. nivara*, *O. barthii*, *O. longistaminata*, *O. meridionalis* and *O. glumaepatula*. The *O. officinalis* complex consists of many species including *O. officinalis*, *O. minuta*, *O. rhizomatis*, etc. The *O. ridleyi* contains *O. ridleyi* and *O. longiglumis*. The *O. granulata* complex consists of *O. granulata*, *O. meyeriana* and *O.brachyantha* (Vaughan et al. 2003; Sweeney and McCough 2007). *O. sativa* is distributed globally with a higher concentration in Asia and is known as Asian rice. *O. glaberrima* is grown in tropical Africa, *O. rufipogon* is distributed throughout Asia and Oceania and is known as brownbeard rice. *O. barthii* is endemic to tropical Africa and *O. longistaminata* is found throughout Africa and these species are known as African species. *O. meridionalis* is native to Australia and *O. glumaepatula* is endemic in Central and South America (Sweeney and McCough 2007).

The Botanical Classification of rice (http://www.gramene.org/species/oryza/rice_taxonomy.html):

Kingdom : Plantae (Plants)

Subkingdom : Tracheobionta (Vascular plants)

Superdivision : Spermatophyta (Seed plants)

Division : Magnoliophyta (Flowering plants)

Class : Liliopsida (Monocotyledons)

Subclass : Commelinidae

Order : Cyperales

Family : Poaceae/Graminae (Grass family)

Genus : *Oryza*

Species : *sativa* L.

Rice grain can be broadly classified into three layers viz., bran (fibre rich outer layer), endosperm (middle part) and germ (embryo). The growth and development of rice plant can be divided into three phases – vegetative phase, reproductive phase and ripening phase (Vergara 1991).

Figure 1: Rice plant (Courtesy http://wikipedia.org/wiki/Rice).

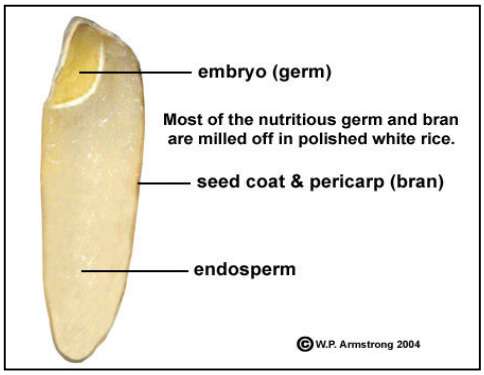

Figure 2: Rice grain (Courtesy http://www.palomar.edu).

Rice with husk is called paddy, while rice with husk removed and polished is called milled rice. Rice with husked removed but bran intact is called brown rice, and rice broken into small pieces during drying, transporting or milling is called broken rice and is known as lower quality rice (Ashfaq and Saleem 2015). Though cultivated rice is popularly known as an annual plant, it can also survive as a perennial, as a ratoon crop, producing new plant after harvesting over generations in some tropical areas (IRRI 2009). The estimated global rice production is about 650 million tones under the cultivation area of 156 million hectares (FAOSTAT 2008). FAO (2004) reported that about 90% of the world's rice production is produced and consumed by small scale farmers in the developing countries, about 60% of the cropland in developing countries in Asia is devoted to growing rice, and about 20-40% of their income is spent on rice. With the increase in human population, rice production needs to be increased to meet the rising demand. It has been reported that with improved varieties and proper crop management, rice production is increased to about double in the past few decades (Byerlee 1996), but the need for better-improved varieties still remain unchanged.

Rice being one of the most important staple crops, plays a very important role in delivering the nutrients to the human population to keep them healthy. The nutritional value of rice crops is determined by its types and quantities of metabolites content, which in turn is strongly influenced by environmental and genetic factors (Lemaux 2008).

1.2 Indigenous rice cultivars

Indigenous crop cultivars can be defined as the populations of cultivated plants confined to a specific area, with historical origin on that particular area, genetically diverse and adapted to local environmental conditions such as farming practices, seed selection, sowing and management, etc. based on local farmer's knowledge (Villa et al. 2006; Azeez et al. 2018). Although a large number of rice cultivars are available, most of the rice cultivated in the World is high yielding varieties (HYV) developed scientifically. This trend implies a possible narrowing of the natural gene pool. However, it is also surprising to know that the farmers of the hilly areas are still practicing their own landrace cultivation that they inherit from their forefathers, which suit the local microclimate and adaptation. The cultural importance of these local landraces is also depicted by these people. Similarly, landraces are preferred by local farmers in many regions for their better adaptability to local climate, and these varieties are chosen even though they require longer duration (Parzies et al. 2004; Pusadee et al. 2009; Roy et al. 2016). The genetic variability found within these varieties has the ability to adapt to local climate, changing environments, farming practices, etc.

India has a rich rice germplasm collection that includes indigenous varieties, wild species, natural hybrids between the wild relatives and cultivar, and above that, the germplasm resulted from strong and robust breeding programmes by the Indian agricultural research system (Rai 1999). West Bengal and the NE states of India are thought to be home for a large number of indigenous rice varieties, hence a detailed investigation of these varieties is very essential with regard to morphology and genetics (Das et al. 2013; Choudhury et al. 2013). Increase in rice production to meet the ever-increasing human population is the need of the hour and an important factor for the crop improvement and productivity is the use of genetic variability in breeding programs (Babu et al. 2014). It has been suggested earlier that the indigenous varieties contain a considerable genetic diversity and can be employed as a source of germplasm for future rice improvement programmes. To use, conserve and manage such germplasm resources, an understanding of their genetic diversity is the basic requirement.

1.3 Genetic diversity

Diversity can be categorized into ecological diversity (diversity among different communities of species in an ecosystem), species diversity (diversity between species in a community), genetic diversity (diversity in genes and genotype present between and among individuals of a species) and genomic diversity (diversity between loci of an individual) (Rao and Hogdkin 2002; Bhandari et al. 2017). Genetic diversity can be defined as the total number of genetic characteristics in the genetic makeup between individuals within a population and between

populations (Gaston 2010). It plays the most important role in the survival and adaptability of a species (Frankham 2005). Populations need to adapt to changing environments and genetic diversity delivers that. More the variations more are the chance of possessing variations in alleles in some individuals in a population that are suited for the environment and those individuals are more likely to pass on that variant alleles to their offsprings (NBII 2011). These individuals possessing variant alleles are likely to continue for more generations (Groom et al. 2006). Populations with high heterozygosity, high heritability, and high polymorphic loci will adapt faster in certain environment than those populations with low genetic diversity (Lacy 1997). Inbreeding (mating between relatives) is known to cause heterozygosity depletion which leads to a probability of the two alleles at a locus being identical, and genetic drifts (random fluctuations in allele frequency) thus results in this depletion of heterozygosity (Lacy 1997). Better knowledge and understanding of plant genetic diversity (PGD) is essential for utilization, management, and conservation of plant genetic resources (PGR) (Mondini et al. 2009), and PGR can be stored in the gene bank, DNA library, etc (Govindaraj et al. 2015).

Genetic parameters such as heterozygosity (Nei 1973), expected heterozygosity (Nei 1973), polymorphism information content (PIC) (Botstein 1980) can be calculated as:

Heterozygosity, $$H = 1 - \sum_{i=1}^{l} P_i^2$$

where, P_i is the frequency of the i^{th} allele among the total number of alleles l.

Expected heterozygosity, $$H_E = 1 - \sum_i^n p_i^2$$

where, p_i is the frequency of the i^{th} allele, n_i is the total number of alleles at all loci.

PIC value, $\mathrm{PIC}_j = 1 - \sum_{i=1}^{n} P_i^2$

where, i is i^{th} allele of the j^{th} marker, n is the number of the j^{th} marker's alleles, P is allele frequency.

1.4 Molecular markers:

Molecular markers are genes or fragments of DNA with known chromosome locations which control particular regions of the genome (Semagn et al. 2006) and are very important in crop improvement programmes (Kebriyaee et al. 2012). Various markers such as morphological, biochemical and molecular markers, are used for assessment of genetic diversity within and between populations. Morphological markers are visually distinguishable important morphological traits such as seed structure, flower, growth habit, etc, and they are easy to use, do not require specific instruments (Nadeem et al. 2018). Biochemical markers are an allelic variation of enzymes in the analysis of genetic diversity, population structure, gene flow, etc. (Mateu-Andres and De Palco 2005). However, less polymorphism detection and influenced by the environment are the two main disadvantages of the biochemical marker (Modini et al. 2009). Among the marker systems, DNA or molecular markers are the most widely used type of marker (Govindaraj et al. 2015). Molecular markers are unlimited and are not affected by environmental issues or developmental stages of the plant unlike morphological and biochemical markers, so these points make molecular markers advantageous over other markers (Park 2009). Molecular markers can be classified into various groups based on the mode of gene

action (codominant or dominant), a method of detection (PCR and/or hybridization-based) and mode of transmission (maternal inheritance, paternal inheritance, nuclear inheritance, etc.) (Semagn et al. 2006). The first molecular marker system and the only marker based on hybridization is restriction fragment length polymorphism (RFLP). The technique involves the use of restriction enzyme which cuts DNA at a particular locus resulting in a huge number of fragments in different length (Williams 1989). RFLP is also known as genetic fingerprinting, profiling or testing marker. Random amplified polymorphic DNA (RAPD) is another technique used to analyze genetic diversity using random primers. It was developed by Williams et al. (1990) and is one of the most commonly and widely used technique in gene mapping, population genetics, molecular evolutionary and plant and animal breeding studies (Bardakci, 2001). It has some disadvantages including no information on the heterozygosity as it is a dominant marker and only detection of polymorphism as presence or absence of a band of a certain molecular weight (Brumlop and Finckh 2010). Another important technique based on both hybridization and PCR technology (digestion of DNA and then PCR) is amplified fragment length polymorphism (AFLP) (Vos et al. 1995). Being a dominant marker, it cannot distinguish dominant homozygous from heterozygous individuals. Other important molecular markers include single nucleotide polymorphism (SNP), inter-simple sequence repeat (ISSR), sequence-related amplified polymorphism (SRAP), mitochondrial microsatellites, random amplified microsatellite polymorphisms (RAMP), Simple Sequence Repeats (SSR), etc.

1.5 Simple sequence repeats markers

Simple Sequence Repeats (SSRs), also known as Microsatellites are repeating sequences of 1-6 or more base pairs of DNA (Turnpenny and Ellard 2005; Govindaraj et al. 2015). They are distributed throughout the genomes of eukaryotes and in some prokaryotes (Varshney et al. 2005), including chloroplasts and mitochondria genomes (Provan et al. 2001; Rajendrakumar et al. 2007). They can be classified as mononucleotide, dinucleotide, trinucleotide, tetranucleotide and hexanucleotide repeats and for molecular genetic studies, the sequences of dinucleotide, trinucleotide, and tetranucleotide repeats are the three most common choices (Selkoe and Toonen 2006). The number of nucleotide repeats in DNA varies within populations of a species as well as between the alleles of an individual (Al-Samarai and Al-Kazaz 2015).

Microsatellites are widely used as molecular markers in population genetics studies, marker-assisted selection, gene duplication or deletion and DNA fingerprinting. A large number of SSR markers have been developed, mapped and used as a genetic marker in rice (Temnykh et al. 2000; McCouch et al. 2002). The advantages of microsatellite markers include the requirement of only small amount of template DNA, detection of high level of allelic diversity (polymorphism), co-dominant, their abundance and distributed throughout the genome, more variable and more informative than other molecular markers like RFLP, RAPD and AFLP and easily assayed by PCR (Kanawapee et al. 2011; Li et al. 2014; Al-Samarai and Al-Kazaz 2015). On the other hand, these markers have some disadvantages including

the appearance of shadow or shutter bands, the presence of null alleles, homoplasy (alleles considering identical in the state are not necessarily identical by descent), laborious and time-consuming, etc (Miah et al. 2013; Abdul-Muneer 2014; Al-Samarai and Al-Kazaz 2015).

1.6 Mizoram State

The State of Mizoram lies in the extended Himalayan ranges in the NE region of India. It shares an International boundary with Bangladesh in the South West and Myanmar in the East and, and state boundaries of Assam in the North, Manipur in the North East and Tripura in the North West and falls in Indo-Burma hotspot region. It lies between 21°56'N to 24°31'N Latitude and 92°16'E to 93°26'E Longitude and has a geographical area of 21,087 sq. km. The State comprises eight districts – Aizawl, Lunglei, Champhai, Serchhip, Kolasib, Mamit, Siaha and Lawngtlai and three autonomous district councils in its Southern part – Lai, Mara, and Chakma Autonomous District Councils. It has a mild climate with summer temperature between 20ºC and 29ºC and winter temperature from 7ºC to 21ºC. The average rainfall in the State is 254 cm per year. Forest Survey of India (FSI 2003) has reported that the forest area of Northeast India is about 1,73,297 sq km which is 66.098% of the total geographic area of the region and Mizoram ranks second in highest forest cover of India at 86.27% of its total geographic area (FSI 2017).

Rice, in Mizo language, is called '*buh*', is the main crop and staple food for people of the state. Mizo people call cooked rice as 'chaw' meaning 'food'. This point clearly indicates the use and importance of rice as a staple food in Mizo

society. Besides rice, other popular crops cultivated in Mizoram include cabbage, chilli, ginger, mustard, turmeric, cowpea, brinjal, pumpkin, cucumber, banana, potato, chayote, Solanum, okra, wax gourd, bitter gourd, watermelon, soybean, pigeon pea, bitter bean, etc. About 90% of crop cultivation in Mizoram is rainfed and thus crops grow largely during the monsoon season (May to October). The hill rice or indigenous rice of the state are grown along with vegetables and fruits in upland areas such as jhumland and shifting cultivation sites where farmers directly seed the rice in these traditional farming areas. These jhum or shifting cultivation practices are the main economic sources in the rural areas of Mizoram (Singh et al. 2013) but this system of rice cultivation is slowly decreasing in the State and terrace/settled cultivation is gradually increasing. This change in agri-horticultural system from jhum to settled cultivation results in the preference of high yielding varieties to indigenous crop cultivars that leads to decrease in the number and diversity of indigenous crop varieties, since shifting cultivation also serves for conservation area of agrobiodiversity (Sati and Rinawma 2014).

Shifting cultivation is believed to be originated during the Neolithic period in 7000 BC which have been practiced by millions of people all over the world, specifically confined to developing countries, and it is being practiced by about 60 million people in India particularly in NE region (Tripathi et al. 2018). With increase in human population and decrease in forest cover to be used for crop cultivation, the shifting cultivation cycle has decreased from about 20-30 years to about 4-5 years, leading to soil degradation as well as imbalanced natural ecosystem (Grogan et al. 2012; Singh et al. 2013; Tripathi et al. 2016). The cycle of shifting cultivation starts

with selecting the area, slashing the vegetations in the forest area and left for one or two months to dry, then burning and sowing. However, Government of India sponsored schemes such as Watershed Development Programme in Shifting Cultivation Areas (WDPSCA) is being implemented in some areas of Mizoram by the State Agriculture Department by making terrace, drainage line, creating water bodies for development of cropping and production system with an aim of management of natural resources, economic enhancement, poverty alleviation and eco-friendly living (agriculturemizoram.nic.in). Besides this, wet rice cultivation (WRC) is also practiced in few places of lowland (river banks and valley plains) areas of Mizoram (Lallianthanga et al. 2013). But, the main cropping system is still shifting cultivation.

Photo 1 A

Photo 1 B

Photo 1 C

Photo 1 D

Photo 1: A, Burned upland rice field. Photo taken by Dama Hmar, Zawlpal ram, Tachhip. Courtesy www.facebook.com, B, Shifting cultivation along with forest area. Courtesy http://www.akmindia.in, C, Farmers removing weeds in an upland rice field in Mizoram. Photo taken by Thlenga Ralte Courtesy http://azararalte.blogspot.com, D, Ripening rice in shifting cultivation in Mizoram. Courtesy http://buanga.blogspot.com

1.7 Objectives:

The present study focused on the assessment of genetic diversity of indigenous rice varieties of Mizoram using SSR markers. This study may provide a better understanding on the diversity of indigenous rice varieties of Mizoram, promote local conservation programmes and facilitate their effective use. The main objectives of my study are:

1. Collection of indigenous rice varieties from different parts of Mizoram.
2. Assessment of genetic diversity and population structure in the collected population.
3. Identification of diverged varieties for improved breeding programmes.
4. Designing strategy for conservation of indigenous varieties.

Chapter 2

Review of literature

Rice is a diploid plant, having a small genome size (430 Mb) and a significant level of genetic polymorphism and hence is one of the best model plants for the study of genetic structure and diversity (McCouch et al. 1988; Arumuganathan and Earle 1991; Wang et al. 1992; Latif et al. 2011; Sohrabi et al. 2012). Rice cultivation and its processing is the main economic source for about 2 billion people worldwide, especially in Asian countries (FAO 2004). Asian continent has dominated the world in production, consumption, and trade of rice where more than 92% of world's rice production, 90% of world's rice consumption, and 75% of world's rice surplus for exports are from Asia (Sriprasert 2005). About 100 million people in the World depend on upland rice as their food as well as their economic source (Arraudeau and de Valenfroid 1995). The production of direct sowing of rice in upland fields of many developing countries is quite low as compared with transplanting methods, but this type of sowing of rice is still very popular in many developing countries due to its low cost and energy saving (Ito 2005).

Rice also plays a central role in food security since it is a staple food for many countries of Asia, Latin America and Africa and is the main source of calorie

and protein intake for about half of the world's human population (FAO 2004). The United Nation (UN) defined food security as, "A situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (FAO 2003). Every year, the world's production of food is more than enough to feed all the human populations, but food insecurity remains to continue, especially in developing countries, where people cannot get enough food to obtain a healthy life, this implies the tons of food products are also wasted through various purposes (Tian et al. 2016).

Wild rice (*Oryza rufipogon* and *Oryza nivara*) are believed to be the progenitors of Asian rice (*Oryza sativa*) (Khush 1997; Yamanaka et al. 2003). There is two genera of wild rice – *Oryza* and *Zizania*. *Oryza rufipogon* also known as Asian wild rice is native to East, South and Southeast Asia (Londo et al. 2006). *Zizania aquatica* is known as Canadian rice or Indian rice or water rice which has grown naturally in North America (Wang et al. 1978). Wild rice possessed great genetic diversity which can be utilized in improvement programmes (Sun 2001; Brar 2003). Seed dormancy levels are higher in wild rice than in cultivated rice, which allows more duration of storing of viable seeds of wild rice for years before planting (Sweeney and McCough 2007). Genetic diversity analysis is important for germplasm utilization like breeding or improvement programmes and conservation of endangered species as it analyzes and identifies genetic relationship between populations, and is also influenced by different factors such as mutation, migration, selection, population size, gene flow, etc (Hedrick 2005; Ouborg et al. 2006; Park et

al. 2009; Rashmi et al. 2017). Genetic diversity analysis can be applied to study the variations between individuals of the same species as well as to compare the genetic composition of members of different species and even for a wider taxonomic range (Dale and Schantz, 2003). Plant biodiversity is often regarded as the greatest natural resource for human beings (Deka et al. 2014). Indigenous crop cultivars are believed to be originated as a result of time and natural selection (Azeez et al. 2018). Previous studies pointed out that indigenous crop cultivars traditionally cultivated and maintained by local farmers generally posses high level of genetic diversity and can be good candidates as germplasm resources for improvement or breeding programmes for better traits such as higher yield, resistance to drought, salt, pests and pathogens, etc. (Das et al. 2013; Choudhury et al. 2013). Indeed a large number of high yielding varieties, tolerance to biotic and abiotic stresses are also released every year in India (Singh et al. 2016). Though indigenous rice cultivars are known to possess high genetic diversity, only a small proportion of germplasms have been utilized in improvement programmes (Dhama et al. 2018). Sohrabi et al. (2012) pointed out that assessing and understanding of genetic diversity is important for crop improvement and management since it is the basis of plant breeding.

The NE India, comprising eight states viz., Arunachal Pradesh, Assam, Mizoram, Manipur, Meghalaya, Sikkim, Nagaland, and Tripura, is one of the mega biodiversity hotspot zone (Myers 1988). These states are rich in rice germplasm resources and crop diversity due to its high altitude, high rainfall, humidity, etc. (Anupam et al. 2017). It has been reported that more than 8,000 species out of 15,000 species of flowering plants found in India, including 500 out of 1,012 species

of Pteridophytes, 40 out of 54 species of Gymnosperms, 80 out of 90 species of Rhododendrons, 60 out of 110 species of bamboos, 825 out of 1,145 species of orchids and 25 out of 56 species of canes, are found in this region (Hegde 2000). Moreover, NE India is known to be a region of origin for at least 167 species of important agricultural and horticultural crops including rice, banana, citrus, etc. and 320 species of their wild relatives (Arora and Nayar 1984). That said, NE India is believed to have the richest plant diversity in India covering about 50% of the country's total biodiversity (Mao and Hynniewta 2000). About 10,000 indigenous rice cultivars are cultivated in NE India under upland, lowland and deep water conditions (Hore 2005). Therefore, NE India represents an important region for the conservation of genetic diversity of rice, since the region is also regarded as an origin and one of the zones for rice domestication. It is well noted that rice landraces of this region posses unique traits which can be used as a valuable resource for future rice breeding programmes. However, information on the molecular characterization of these germplasms is very limited (Choudhury et al. 2014). Some of the important qualities identified in NE rice landraces include adaptive traits for cold, salt, drought and flood tolerance, insect resistance, aroma, etc. These traits can be identified, accessed and cataloged, for a large number of this germplasm could be an important source for various rice improvement programmes (Pusadee et al. 2009; Roy et al. 2016). Genetic diversity also plays a vital role in characterizing traits responsible for species' survival and adaptation (Rao and Hodgkin 2002), hence identification and cataloging of superior varieties is essential.

Rice also possess nutritional value and health benefits such as richness in carbohydrates, cholesterol free, rich in vitamin and minerals like niacin, vitamin D, calcium, iron, etc., resistant starch, high blood pressure prevention due to its sodium-poor content, cancer prevention, skin care, dysentery prevention due to its content of diuretic properties, Alzheimer's disease prevention due to high content of neurotransmitter nutrients, heart disease prevention due to its antioxidant properties (Verma and Shukla 2011). Verma and Shukla (2011) have postulated that rice can protect the human body against many types of cancer because of its richness in insoluble fibre, which can protect the body against cancerous cells. It is opined that reports on colon and breast cancer are less in Asia, where rice is the main staple food than in the Western world since its phenol content interferes with the proliferation or colony forming ability of breast or colon cells (Hudson et al. 2000). Several compounds with antioxidant activity have been identified in rice, including phenolics (phenolic acids – p-coumaric syringic acids caffeic and ferulic and flavonoids – flavonols, flavones, anthocyanins and catechins), carotenoids (zeaxanthin, lutein, β-carotene and β-cryptoxanthin) and vitamin E (tocotrienols, tocopherols and γ-oryzanol) (Iqbal et al. 2005; Zhang et al. 2010). Rice is also rich in proteins, vitamins (vitamin B & E), minerals, lipids, phytin, trypsin inhibitor, phosphorous, silica, lipase and lectin (Rohman et al. 2014) but it contains very little vitamin A and C (Luh et al. 1991).

Genetic markers can be broadly classified into three categories viz. morphological, biochemical and molecular markers. Variations in seed morphology (grain length, grain width, grain weight, etc.) of rice have been reported by previous

researchers (Jugran et al. 2010; Pachauri et al. 2013). Though seed storage protein of rice was low compared to other cereals (Wei-dong et al. 2006), it can be used as genetic markers in evolutionary and genetic diversity studies on rice (Bal and Bay 2010; Jugran et al. 2010; Tahir 2014). Previous researchers reported the similarity banding pattern of seed storage protein as well as variations in number and positions of bands as a unique and powerful tool in diversity studies (Ladizinsky and Hymowitz 1979; Jugran et al. 2010; Dhawale et al. 2015; Vithyashini and Wickramasinghe 2015). Many molecular markers have been developed for rice and used to detect genetic diversity within and between populations, including restriction fragment length polymorphisms (Sun et al. 2001), amplified fragment length polymorphisms (Bao et al. 2006), random amplified polymorphic DNA (Ge et al. 1999; Baishya et al. 2000), simple sequence repeats (Ravi et al. 2003; Lapitan et al. 2007), etc. Sun et al. (2001) compared the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*Oryza sativa* L.) using RFLP markers and they found that the genetic diversity of wild rice was much higher than that of cultivated rice. They concluded the fact that many alleles were lost during the evolution and development of wild to cultivated rice which leads to lowering their genetic diversity. Saker et al. (2005) compared three marker systems– RAPD, SSR, and AFLP in genetic diversity analysis of seven Egyptian rice genotypes. They have shown that among the three markers, SSRs gave the highest level of polymorphism which was followed by RAPD and then AFLP. Also, a high genetic diversity was reported among NE Indian rice based on morpho-physiological characters (Vairavan et al. 1973), enzymatic characters (Glaszmann et al. 1989), and molecular markers

including RAPD (Bhuyan et al. 2007; Ibemhal and Laishram 2012) and SSR (Das et al. 20013; Choudhury et al. 2014; Roy et al. 2015). It has been demonstrated that SSRs are well distributed throughout the genome and are one of the most preferred markers for crop improvement in many species because they are abundant, reliable, highly reproducible, widely distributed in genomes, easy to score, highly polymorphic genetic information contents, codominant in nature and require only a small amount of DNA and can detect a large number of DNA polymorphism (McCouch et al. 1997; Gupta and Varshney 2000; Park et al. 2009; Li et al. 2014; Shamim et al. 2016). Codominant markers can distinguish homozygotes from heterozygotes, that is why they are more informative than dominant markers (Govindaraj et al. 2015). Then, Das et al. (2013) studied genetic diversity of aromatic and non-aromatic accessions from West Bengal and some accessions from the NE States including aromatic and non-aromatic germplasm using SSR marker. They have found that the non-aromatic landraces from West Bengal were most diverged followed by the aromatic landraces from the same state while the NE accessions ranked third. Choudhury et al. (2013) have also compared the genetic diversity of indigenous rice varieties and agronomically improved varieties of Eastern Himalayan Region of NE India. They reported that the genetic diversity of indigenous varieties was higher than that of agronomically improved varieties. Similarly, Rathi and Sarma (2012) studied amylose content and the genetic diversity of 106 glutinous rice cultivars of Assam using 51 SSR markers, where they found a wide variation in amylose content and genetic diversity among the accessions studied. Then, Rathi et al. (2014) detected a considerable level of gene diversity

among 100 upland rice genotypes of Assam using SSR markers. Anupam et al. (2017) also analyzed 74 rice germplasms of Tripura comprising of local landraces, improved varieties, breeding lines and other varieties using drought and blast-linked SSR markers. They found out some varieties possessed blast and drought resistance genes but the level of genetic diversity in overall was low as compared to previous genetic diversity analysis of rice varieties of North East India. Ibemhal and Laisram (2012) studied indigenous rice varieties of Manipur using RAPD markers where they found a moderate level of genetic diversity within the collected rice cultivars. Roy et al. 2014 studied the diversity of 37 Chakhao landraces from Manipur using 47 microsatellite markers and found high genetic diversity within the studied populations. Diversity assessment of rice varieties of Mizoram have been attempted by a few workers (Choudhury et al. 2013; Das et al. 2013) where it was reported that Buh and Buhrimtui were 58.4% similar (Das et al. 2013) and Kawnglawng was interchanged between sub-species *indica* and *japonica* (Choudhury et al. 2013). However, the representation of varieties was very low ie. very few rice varieties were studied, which indicated that further investigation covering maximum varieties from the state was required. About 90-95 landraces of Mizoram were recorded by the previous researchers, most of which were grown in upland conditions, and few in terrace/settled/lowland conditions, which differ in growth duration, grain quality, yield and resistance (Sharma and Hore 1993).

Chapter 3

Methodology

3.1 Collection sites

Seeds of local rice varieties were directly collected from local farmers from different villages of all the eight districts of Mizoram (Figure 3). Collection was done from Darlawn, Khawruhlian, Phuaibuang and Sumsuih in Aizawl district; North Chaltlang in Kolasib district; Mimbung, NE Khawdungsei and Saichal in Champhai district; Mamit and Rawpuichhip in Mamit district; Chhingchhip in Serchhip district; Zobawk in Lunglei district; Vawmbuk in Siaha district and Lawngtlai, Diltlang and Mualbukawnpui in Lawngtlai district. Details of rice cultivars collected, village name, location, elevation, and rice types are presented in Table 1.

3.2 Collection and Planting

A total of 63 rice varieties including 53 local varieties, five *indica,* and five *japonica* were used in the current study. Seeds were brought, planted and grown on poly pots at Department of Botany, Mizoram University. After 15 days of planting, the leaves were cut just above the soil and kept at -20ºC for further applications.

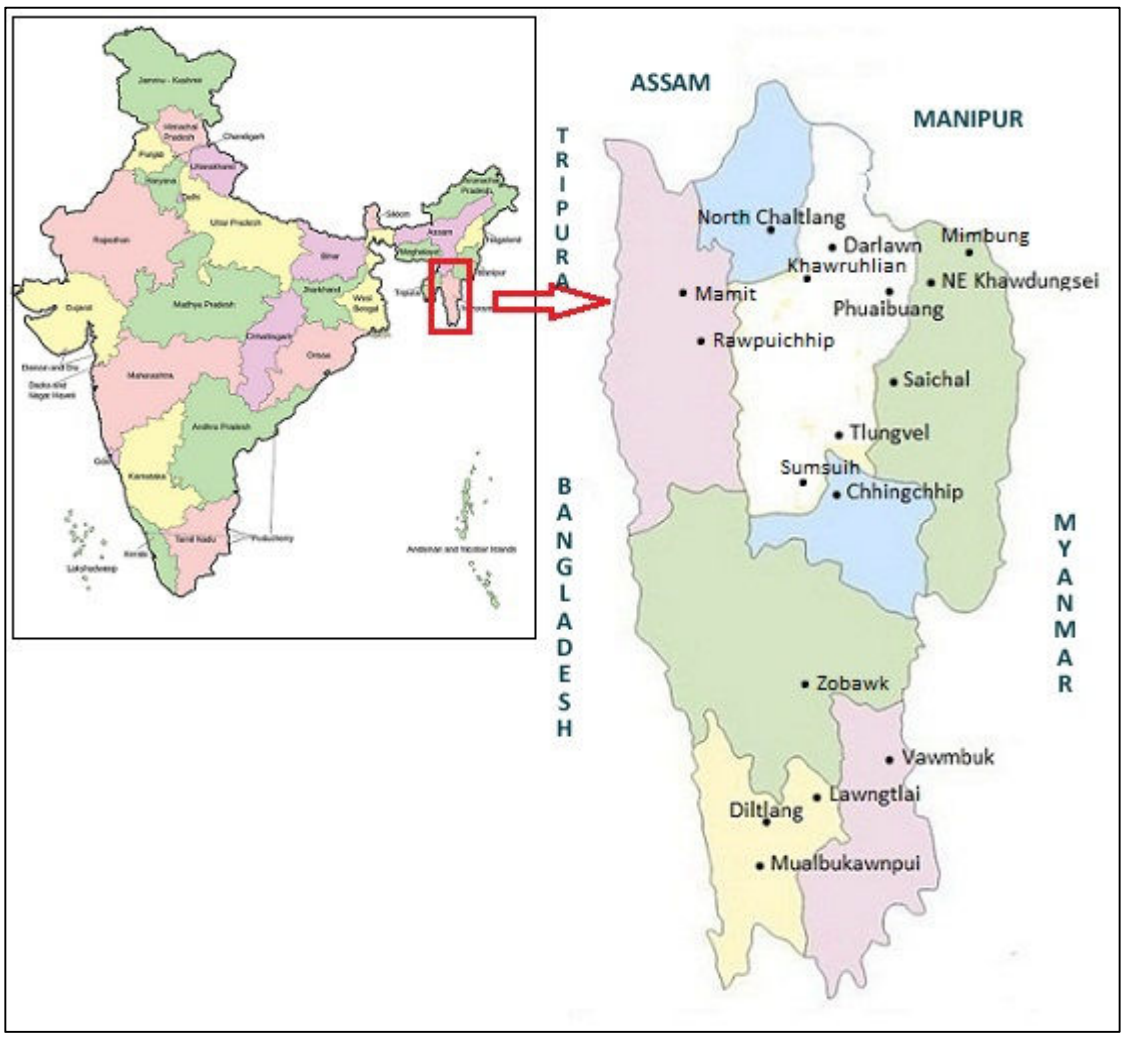

Figure 3: Map of India (inset) and Mizoram showing collection sites.

Table 1: Details of rice collection:

Sl. No.	Variety name	Place of collection	Location	Elevation (ft)	District	Type
1.	Kungtawi sen	Lawngtlai	22º31'42.40" N 92º53'33.48" E	2377	Lawngtlai	Landrace
2.	Vaiphei	Lawngtlai	22º31'42.40" N 92º53'33.48" E	2377	Lawngtlai	Landrace
3.	Kawnglawng	Diltlang South	22º29'33.48" N 92º43'32.64" E	2321	Lawngtlai	Landrace
4.	Biruchuk	Lawngtlai	22º31'42.40" N 92º53'33.48" E	2377	Lawngtlai	Landrace

5.	Tuikuk buh	Lawngtlai	22º31’42.40” N 92º53’33.48” E	2377	Lawngtlai	Landrace
6.	Fare	Diltlang South	22º29’33.48” N 92º43’32.64” E	2321	Lawngtlai	Landrace
7.	Kawnglawng tial	Mualbukawnpui	22º20’00.78” N 92º42’43.41” E	1601	Lawngtlai	Landrace
8.	Kawnglawng var	Mualbukawnpui	22º20’00.78” N 92º42’43.41” E	1601	Lawngtlai	Landrace
9.	Buhban Langakthou	Vawmbuk	22º35’52.75” N 93º04’35.06” E	4195	Siaha	Landrace
10.	Buhbial	Vawmbuk	22º35’52.75” N 93º04’35.06” E	4195	Siaha	Landrace
11	Fazai	Vawmbuk	22º35’52.75” N 93º04’35.06” E	4195	Siaha	Landrace
12.	Laithangnu	Darlawn	24º00’51.63” N 92º55’28.06” E	3591	Aizawl	Landrace
13.	Tai sanghar	Darlawn	24º00’51.63” N 92º55’28.06” E	3591	Aizawl	Landrace
14.	Tai te	Darlawn	24º00’51.63” N 92º55’28.06” E	3591	Aizawl	Landrace
15.	Zawngin buh	Darlawn	24º00’51.63” N 92º55’28.06” E	3591	Aizawl	Landrace
16.	Baimasa	Phuaibuang	23º55’35.59” N 93º07’17.46” E	4571	Aizawl	Landrace
17.	Bialte	NE Khawdungsei	23º58’30.53” N 93º12’51.77” E	3802	Champhai	Landrace
18.	Buhban hmui	NE Khawdungsei	23º58’30.53” N 93º12’51.77” E	3802	Champhai	Landrace
19.	Buhngat	NE Khawdungsei	23º58’30.53” N 93º12’51.77” E	3802	Champhai	Landrace
20	Fazai ban	NE Khawdungsei	23º58’30.53” N 93º12’51.77” E	3802	Champhai	Landrace
21.	San	Saichal	23º43’07.92” N 93º04’05.75” E	3649	Champhai	Landrace
22.	Idaw	Tlungvel	23º36’17.20” N 92º51’14.29” E	3780	Aizawl	Landrace
23.	Mangbuh	Chhingchhip	23º28’15.32” N 92º51’23.27” E	3526	Serchhip	Landrace
24.	Buhpui	North Chaltlang	24º01’20.21” N 92º46’18.44” E	2668	Kolasib	Landrace
25.	Naga	Tlungvel	23º36’17.20” N 92º51’14.29” E	3780	Aizawl	Landrace
26.	Fazu	Saichal	23º43’07.92” N 93º04’05.75” E	3649	Champhai	Landrace
27.	Phodum	NE Khawdungsei	23º58’30.53” N	3802	Champhai	Landrace

			93º12’51.77” E			
28.	Vaibuh	NE Khawdungsei	23º58’30.53” N 93º12’51.77” E	3802	Champhai	Landrace
29.	Varsiama	NE Khawdungsei	23º58’30.53” N 93º12’51.77” E	3802	Champhai	Landrace
30.	Dengchungnunga	NE Khawdungsei	23º58’30.53” N 93º12’51.77” E	3802	Champhai	Landrace
31.	Dumte	Phuaibuang	23º55’35.59” N 93º07’17.46” E	4571	Aizawl	Landrace
32.	Mawitawi	Phuaibuang	23º55’35.59” N 93º07’17.46” E	4571	Aizawl	Landrace
33.	Zongam	Phuaibuang	23º55’35.59” N 93º07’17.46” E	4571	Aizawl	Landrace
34.	Fazupui	Zobawk	22º51’31.14” N 92º49’17.64” E	3534	Lunglei	Landrace
35.	Fangsin	Zobawk	22º51’31.14” N 92º49’17.64” E	3534	Lunglei	Landrace
36.	Tai buhpui	Rawpuichhip	23º47’07.03” N 92º33’38.38” E	2527	Mamit	Landrace
37.	Kungrei	Mamit	23º38’50.57” N 92º32’22.57” E	1527	Mamit	Landrace
38.	Tailuaia hmui	Khawruhlian	23º52’18.78” N 92º52’35.10” E	2911	Aizawl	Landrace
39.	Tailuaia hmui lo	Khawruhlian	23º52’18.78” N 92º52’35.10” E	2911	Aizawl	Landrace
40.	Buhban sen	Mimbung	23º59’58.70” N 93º17’07.44” E	4668	Champhai	Landrace
41.	Buhban zam	Mimbung	23º59’58.70” N 93º17’07.44” E	4668	Champhai	Landrace
42.	Zaitlai	Mimbung	23º59’58.70” N 93º17’07.44” E	4668	Champhai	Landrace
43.	Khawzawl buh	Mimbung	23º59’58.70” N 93º17’07.44” E	4668	Champhai	Landrace
44.	Bahipui	Mamit	23º38’50.57” N 92º32’22.57” E	1527	Mamit	Landrace
45.	Pawnbuh	NE Khawdungsei	23º58’30.53” N 93º12’51.77” E	3802	Champhai	Landrace
46.	Malcheng	North Chaltlang	24º01’20.21” N 92º46’18.44” E	2668	Aizawl	Landrace
47.	Taibial	North Chaltlang	24º01’20.21” N 92º46’18.44” E	2668	Aizawl	Landrace
48.	BPL	NE Khawdungsei	23º58’30.53” N 93º12’51.77” E	3802	Champhai	Landrace
49.	Pana	NE Khawdungsei	23º58’30.53” N 93º12’51.77” E	3802	Champhai	Landrace

50.	Robula	NE Khawdungsei	23º58'30.53" N 93º12'51.77" E	3802	Champhai	Landrace
51.	Zamzathanga	NE Khawdungsei	23º58'30.53" N 93º12'51.77" E	3802	Champhai	Landrace
52.	Roenga	Sumsuih	23º28'33.08" N 92º44'48.75" E	4353	Aizawl	Landrace
53.	Pi Hui buh	Chhingchhip	23º28'15.32" N 92º51'23.27" E	3526	Serchhip	Landrace
54.	BM71[a]	ABF, Hyderabad	17º23'06.16" N 78º29'12.02" E	-	Hyderabad	Improved
55.	IR71033-121-15B[a]	ABF, Hyderabad	17º23'06.16" N 78º29'12.02" E	-	Hyderabad	Improved
56.	MO1[a]	ABF, Hyderabad	17º23'06.16" N 78º29'12.02" E	-	Hyderabad	Improved
57.	PTB33[a]	ABF, Hyderabad	17º23'06.16" N 78º29'12.02" E	-	Hyderabad	Improved
58.	TN1[a]	ICGEB, New Delhi	28º31'47.21" N 77º10'05.37" E	-	New Delhi	Improved
59.	CAUR1[b]	ICAR, Kolasib	24º12'44.00" N 92º40'32.69" E	2057	Kolasib	Improved
60.	Gomati[b]	ICAR, Kolasib	24º12'44.00" N 92º40'32.69" E	2057	Kolasib	Improved
61.	RCM9[b]	ICAR, Kolasib	24º12'44.00" N 92º40'32.69" E	2057	Kolasib	Improved
62.	RCM10[b]	ICAR, Kolasib	24º12'44.00" N 92º40'32.69" E	2057	Kolasib	Improved
63.	RCM13[b]	ICAR, Kolasib	24º12'44.00" N 92º40'32.69" E	2057	Kolasib	Improved

[a]*Indica* varieties, [b]*Japonica* varieties. ABF = Agri Biotech Foundation, Hyderabad; ICGEB = International Centre for Genetic Engineering and Biotechnology, New Delhi; ICAR = Indian Council of Agricultural Research, Mizoram

3.3 Estimation of seed morphology

Grain quality traits were measured for grain length (mm), grain width (mm), 1000-grain weight (g) and grain length/width ratio and recorded. Five seeds per variety in triplicates were investigated.

3.4 Seed storage protein extraction and SDS-PAGE

Seed storage protein was extracted by following by Jugran et al. (2010). Briefly, the 0.25g powdered seed was extracted using 500 µl of buffer (0.5 M Tris pH 6.8, 20% glycerol, 10% SDS, 0.1% bromophenol blue and 2-mercaptoethanol) followed by vortexing for 2 minutes. After a brief denaturation, the sample was centrifuged at 7000 rpm for 12 minutes. Then, the supernatant containing storage protein was loaded and run on a 10% SDS-PAGE (Laemmli 1970). The gel was stained following standard protocol.

3.5 SDS-PAGE data analysis

Presence (1) or absence (0) of bands was scored for all the samples analyzed using Image Lab 5.0 software (Bio-Rad Laboratories, USA). Band sizes were ascertained using Protein Molecular Weight Marker (Genei, India). Jaccard's similarity coefficient between rice varieties was calculated, and a UPGMA (The unweighted pair group method with an arithmetic mean) tree constructed using online software, DendroUPGMA (http://genomes.urv.cat/UPGMA) (Garcia-Vallvé and Puigbo, 2002).

3.6 Isolation of Genomic DNA

Genomic DNA was isolated as per Edwards et al. (1991). Briefly, a 15-day-old seedling was macerated in a 2 ml centrifuge tube and 400 µl of extraction buffer (200 mM Tris-HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, 0.5 % SDS) was added. The sample was vortex vigorously for 1 minute. The mixture was left at room

temperature until all the samples were extracted. The extract was centrifuged at 13000 rpm for 5 minutes. Then, 300 µl of the supernatant was transferred to a fresh centrifuge tube, an equal volume of isopropanol was added and left at room temperature for 2 minutes and then centrifuged at 13,000 rpm for 5 minutes. The resulting pellet was air dried and dissolved in 100 µl TE (10mM Tris, 1 mM EDTA) buffer. The quanlity and quantity of DNA was estimated by 260/280 absorbance ratio and agarose gel electrophoresis.

3.9 Primer selection

Twelve SSR/microsatellite primers RM1, RM154, RM131, RM135, RM153, RM190, RM72, RM125, RM278, RM171, RM287 and RM117 were chosen according to their location on the rice chromosomes (Panaud et al. 1996; Temnykh et al. 2000) and used in the present study to amplify genomic DNA. The primer sequences, repeat motifs, chromosomal positions, expected amplicon size and annealing temperature for these markers were obtained from the rice genome database GRAMENE (http://www.gramene.org). Details of SSR primers used are presented in Table 2.

Table 2: Details of SSR loci used in the present study:

Sl. No.	Primer name	Primer Sequence	Loca-tion	Expected amplicon size (bp)	Anneal. Temp. (ºC)
1.	RM1	Fp – 5'-GCGAAAACACAATGCAAAAA-3' Rp – 5'-GCGTTGGTTGGACCTGAC-3'	1	113	55
2.	RM154	Fp – 5'-ACCCTCTCCGCCTCGCCTCCTC-3' Rp – 5'-CTCCTCCTCCTGCGACCGCTCC-3'	2	183	61
3.	RM131	Fp – 5'-TCCTCCCTCCCTTCGCCCACTG-3' Rp – 5'-CGATGTTCGCCATGGCGTCTCC-3'	4	215	61
4.	RM135	Fp – 5'-CTCTGTCTCCTCCCCCGCGTCG-3'	3	131	55

		Rp – 5'-TCAGCTTCTGGCCGGCCTCCTC-3'			
5.	RM153	Fp – 5'-GCCTCGAGCATCATCATCAG-3' Rp – 5'-ATCAACCTGCACTTGCCTGG-3'	5	201	55
6.	RM190	Fp – 5'-CTTTGTCTATCTCAAGACAC-3' Rp – 5'-TTGCAGATGTTCCTGATG-3'	6	124	55
7.	RM125	Fp – 5'-ATCAGCAGCCATGGCAGCGACC-3' Rp – 5'-AGGGGATCATGTGCCGAAGGCC-3'	7	127	55
8.	RM72	Fp – 5'-CCGGCGATAAAACAATGAG-3' Rp – 5'-GCATCGGTACTAACTAAGGG-3'	8	166	55
9.	RM278	Fp – 5'-GTAGTGAGCCTATCAATAATC-3' Rp – 5'-TCAACTCAGCATCTCTGTCC-3'	9	141	55
10.	RM171	Fp – 5'-CGATCCATTCCTGCTGCTCGCG-3' Rp – 5'-CGCCCCCATGCATGAGAAGACG-3'	10	328	55
11.	RM287	Fp – 5'-TTCCCTGTTAAGAGAGAAATC-3' Rp – 5'-GTGTATTTGGTGAAAGCAAC-3'	11	118	55
12.	RM117	Fp – 5'-CGCCCCCATGCATGAGAAGACG-3' Rp – 5'-CGATCCATTCCTGCTGCTCGCG-3'	12	208	55

Location = Chromosome number, Anneal. temp. = annealing temperature.

3.8 PCR amplification

PCR amplification was performed in a 25 µl reaction containing 1X PCR buffer, 200µM dNTP mixture (Promega, USA), 3mM $MgCl_2$, 0.5U Taq polymerase (Genei, India), 50 ng of each primer and 30ng template DNA in an ABI Veriti 96 well Thermal cycler (ABI, USA). The amplification conditions followed was- initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing temperature (primer specific) for 30 seconds, extension at 72°C for 1 minute and a final extension at 72°C for 7 minutes. The amplified products were subjected to 2.5 % agarose gel electrophoresis and visualized by ethidium bromide staining in an AlphaImager Mini (Protein Simple, USA) (Sambrook et al. 2001). Sizes of alleles were ascertained using molecular weight marker (Genei, India) by Alpha View software (Protein Simple, USA).

3.9 SSR Analysis

Genetic diversity parameters such as the number of alleles, number of polymorphic loci, Fst, expected heterozygosity, Nei's gene diversity were estimated using genetic analysis software POPGENE 1.31 (Yeh et al. 1999). Arlequin 3.5 (Excoffier and Lischer 2010) was used to calculate population-wise diversity. Principal coordinate analysis (PCoA), Analysis of molecular variance (AMOVA) and pairwise population matrix were performed in GenAlEx 6.5 (Peakall and Smouse 2012). PowerMarker 3.25 (Liu and Muse 2005) was used to calculate Major allele frequency (MAF) and polymorphism information content (PIC) and MEGA 6 (Tamura et al. 2013) was used to construct Unweight Pair Group Method with an Arithmetic Mean (UPGMA) tree based on Nei's genetic distance. Bayesian model-based STRUCTURE 2.3.4 (Pritchard et al. 2000) was used to detect the possible population structure, where a 100,000 burn-in periods and 100,000 Markov Chain Monte Carlo (MCMC) repeats after burn-in was set. A possible number of clusters or K was determined with K=1 to K=10 and 10 replicate runs per K value (Evanno et al. 2005). Structure Harvester was then used to identify the final K value (Earl and von Holdt 2012).

Chapter 4

Results

4.1 Seed morphology

Seeds morphology data showed distinguished variation in grain quality traits among the studied varieties (Table 3). The longest grain length was observed in Kawnglawng (11.4 mm) while the shortest grain length was recorded in Tai bial (6.88 mm) with a mean population grain length of 9.01 mm. And grain width ranged from 2.21 mm (BM71) to 3.82 mm (Buhban langakthou) averaging 3.04 mm. The highest length/width ratio was observed in IR71033-121-15B (4.82) while the least was observed in Roenga (1.93). Average length to width ratio was found to be 3.02. A 1000-grain weight ranged from Gomati (18.61g) to Buban sen (46.8g) with an average of 27.5g.

Table 3: Grain quality traits of rice varieties of Mizoram.

Sl No	Name	Grain length (mm)	Grain width (mm)	1000-grain weight (g)	L/W ratio	State*
1.	Kungtawi sen	10.14	3	23.08	3.38	ELS
2.	Vaiphei	9.3	3.36	30.12	2.77	LB
3.	Kawnglawng	11.4	3.36	38.84	3.40	ELS
4.	Biruchuk	10.02	2.66	26.92	3.77	ELS
5.	Tuikuk buh	10	3.16	26.44	3.16	ELS
6.	Fare	9.14	3.04	27.32	3	ELS

7.	Kawnglawng tial	10.22	3.36	33.09	3.04	ELS
8.	Kawnglawng var	9.12	3.62	30	2.52	LB
9.	Buhban Langakthou	10.6	3.82	37.23	2.77	LB
10.	Buhbial	7.28	3.5	26.05	2.08	LB
11.	Fazai	9.2	3	27.12	3.06	ELS
12.	Laithangnu	9.68	3.38	28.57	2.86	LB
13.	Tai sanghar	10.46	3.02	27.16	3.46	ELS
14.	Tai te	8.92	2.7	23.25	3.3	ELS
15.	Zawngin buh	7.16	3.08	24.91	2.32	LB
16.	Baimasa	9	2.98	26.7	3.02	ELS
17.	Bialte	7.72	3.46	25.42	2.23	LB
18.	Buhban hmui	10.92	2.96	31.23	3.69	ELS
19.	Buhngat	9.1	3.12	29.15	2.92	LB
20.	Fazai ban	10.74	3.06	30.88	3.51	ELS
21.	San	9.02	3.04	26.98	2.97	LB
22.	Idaw	10.24	2.98	30.04	3.44	ELS
23.	Mangbuh	8.28	3.02	24.61	2.74	LB
24.	Buhpui	8.3	3.06	25.25	2.71	LB
25.	Naga	9.18	3.16	30.42	2.9	LB
26.	Fazu	9.16	3.56	30.89	2.58	LB
27.	Phodum	9.26	3.12	29.17	2.96	LB
28.	Vaibuh	8.08	3.28	25.98	2.46	LB
29.	Varsiama	8.9	3.5	30.11	2.54	LB
30.	Dengchungnunga	9.06	2.76	22.87	3.28	ELS
31.	Dumte	9.78	3.08	26.77	3.18	ELS
32.	Mawitawi	8.36	3.12	28.27	2.68	LB
33.	Zongam	9.46	2.9	25.83	3.26	ELS
34.	Fazupui	9.26	2.96	24.63	3.13	ELS
35.	Fangsin	8	2.7	23.91	2.96	LB
36.	Tai buhpui	7.78	2.58	19.29	3.02	ELS
37.	Kungrei	10.42	2.88	29.76	3.62	ELS
38.	Tailuaia hmui	7.1	3.44	27.66	2.06	LB
39.	Tailuaia hmui lo	8.14	3.2	31.48	2.54	LB
40.	Buhban sen	10.24	3.64	46.80	2.81	LB
41.	Buhban zam	8.8	3.1	23.75	2.84	LB
42.	Zaitlai	7.9	2.5	19.88	3.16	ELS
43.	Khawzawl buh	9.9	2.5	26.55	3.96	ELS
44.	Bahipui	9.74	3.28	32.88	2.97	LB
45.	Pawnbuh	7.62	3.26	22.42	2.34	LB
46.	Malcheng	7.06	3.12	26.95	2.26	LB
47.	Tai bial	6.88	3.44	24.04	2	LB

48.	BPL	8.32	3.22	27.30	2.58	LB
49.	Pana	8.6	2.94	24.17	2.93	LB
50.	Robula	9.32	3.18	32.26	2.93	LB
51.	Zamzathanga	10.02	2.8	26.23	3.58	ELS
52.	Roenga	7.26	3.76	25.29	1.93	LB
53.	Pi Hui buh	7.9	3.14	29.74	2.52	LB
54.	BM71	8.78	2.12	21.43	4.14	ELS
55.	IR71033-121-15B	10.7	2.22	27.87	4.82	ELS
56.	MO1	8.8	3.16	33.14	2.78	LB
57.	PTB33	7.6	2.94	21.38	2.58	LB
58.	TN1	8.16	2.74	27.36	2.98	LB
59.	CAUR1	8.76	2.4	24.65	3.65	ELS
60.	Gomati	8.34	2.28	18.61	3.66	ELS
61.	RCM9	9.48	2.24	24.72	4.23	ELS
62.	RCM10	10.16	2.62	28.48	3.88	ELS
63.	RCM13	9.3	2.9	29.33	3.21	ELS
	Mean	9.01	3.04	27.5	3.02	
	SE	0.14	0.05	0.58	0.07	

* As referred in Rice Research in India: ICAR Publication, 1985. ELS = Extra long slender, LB = Long bold. Red colour indicated the highest value and green colour indicated the lowest value.

4.2 Protein profiling

Protein profiling of seed storage proteins of indigenous rice varieties of Mizoram showed very little variation among the populations. The number of polypeptide bands per variety ranged from 5 to 10. Two varieties viz., Dumte and Tailuaia hmui possessed 5 bands, eight varieties viz., Fangsin, Tailuaia hmui lo, Buhban sen, Buhban zam, BPL, Pana, Robula, and Zamzathanga exhibited 6 bands. Twenty five varieties i.e, Kawnglawng, Tuikuk buh, Kawnglawng tial, Fare, Buhbial, Buhban Langakthou, Fazai, Laithangnu, Zawngin buh, Tai sanghar, Bialte, Fazai ban, Idaw, Fazu, Mawitawi, Zongam, Fangsin, Tai buhpui, Zaitlai, Khawzwl buh, Bahipui, Pawnbuh, Malcheng, Tai bial and Roenga exhibited 7 bands, sixteen varieties i.e, Kungtawi sen, Vaiphei, Kawnglawng var, Tai te, Baimasa, Buhban

hmui, San, Buhngat, Vaibuh, Varsiama, Fazupui, Pi Hui buh, MO1, CAUR1, RCM10 and RCM13 exhibited 8 bands, eleven varieties i.e, Buhngat, Mangbuh, Phodum, Naga, Dengchungnunga, BM71, PTB33, IR71033-121-15B, Gomati, RCM9 and TN1 exhibited 9 bands and while Biruchuk possessed 10 bands. A dendrogram (UPGMA) constructed based on Jaccard's similarity coefficient showed three clear clusters. Cluster I was consists of 9 cultivars, cluster II 28 cultivars and Cluster III comprised of 26 cultivars.

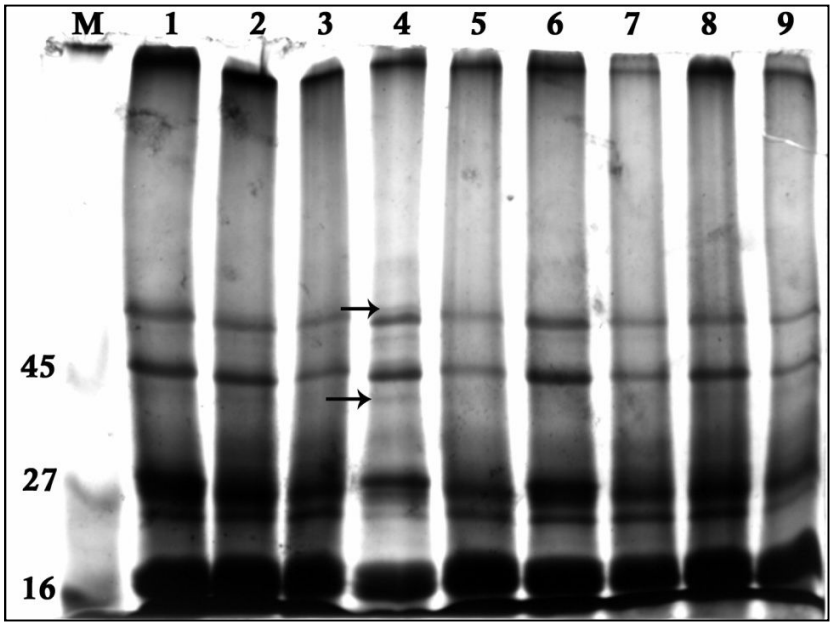

Figure 4: Protein profile of rice varieties of Mizoram. (Lanes 1 to 9 indicated individuals from Kungtawi sen, Vaiphei, Kawnglawng, Biruchuk, Tuikuk buh, Fare, Kawnglawng tial, Kawnglawng var, and Buhban langakthou.) Lane M represents protein marker (in KDa). Arrows indicated polymorphic bands (Vanlalsanga and Singh 2019).

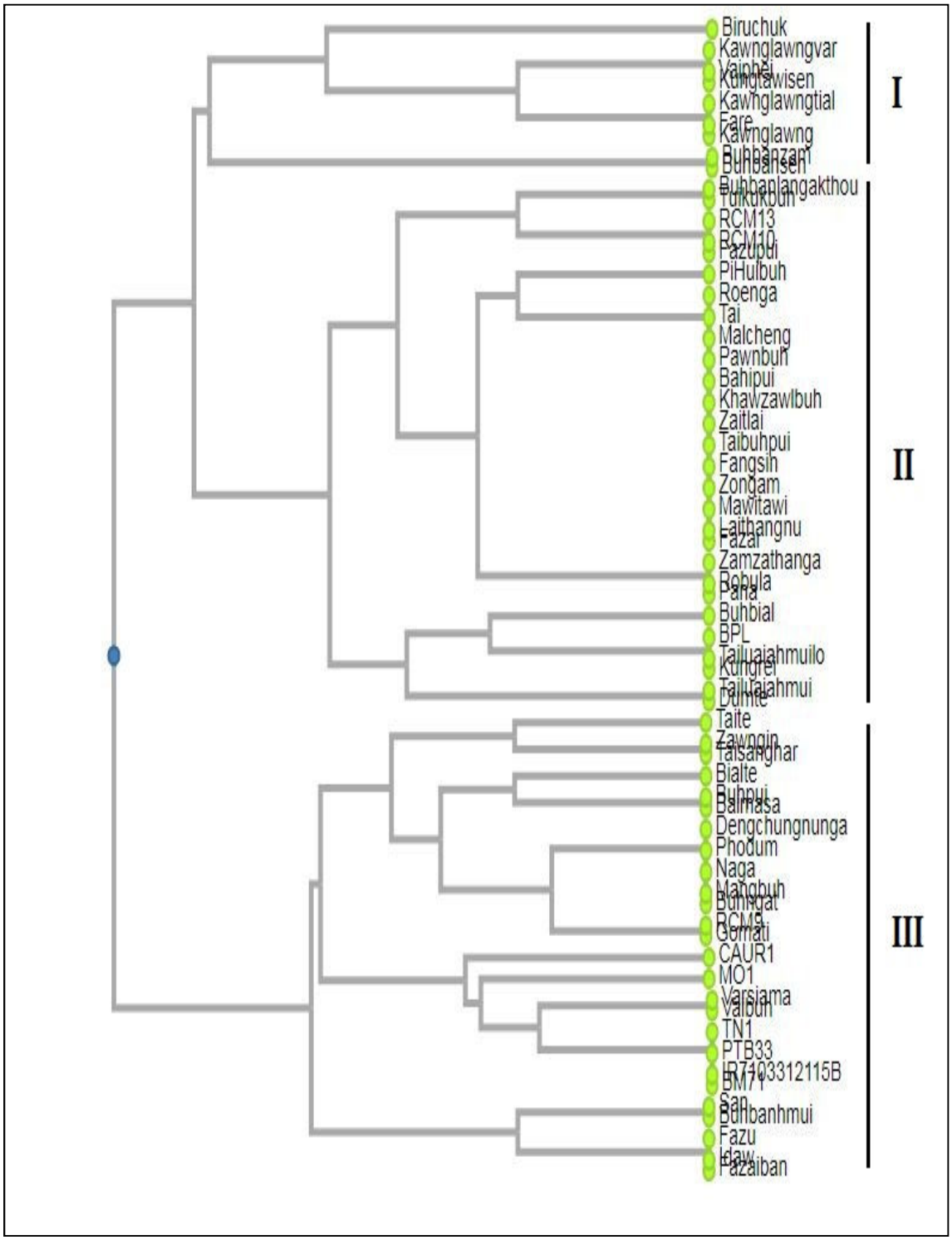

Figure 5: Dendrogram constructed using Jaccard's similarity coefficient based on seed storage protein.

4.3 Analysis of SSR diversity

4.3.1 Number of alleles

Eleven (11) of the 12 SSR primers were found to be polymorphic (91.67% polymorphism) and 63 bands were detected using these primers. The number of alleles ranged from 1 to 10 among the studied rice varieties. Out of 11 polymorphic alleles, maximum number of allele was amplified by primer RM135 followed by RM131 (9 alleles) and RM1, RM154 and RM72 (seven alleles each) while the minimum number of allele was generated by RM153, RM125 and RM287 (three alleles each) across all the cultivars screened (Table 3). The average number of alleles per polymorphic locus was found to be 5.6363. The number of effective alleles ranged from 1.0849 (RM153) to 5.7514 (RM135) with an average of 3.3379. MAF varied from 0.2722 (RM135) to 0.9984 (RM278) with an average of 0.5689.

4.3.2 Fst and gene flow

Genetic differentiation (Fst) ranged from 0.5736 to 0.9418 with an average of 0.7599. The maximum Fst value was found in RM287 while the minimum was found in RM135. Gene flow estimated from estimated from Fst = 0.25(1 – Fst)/Fst ranged from 0.0155 to 0.1859. The maximum gene flow was found in RM135 while minimum gene flow was found in RM287.

4.3.3 Genetic diversity

Expected heterozygosity ranged from 0.0783 to 0.8268 with averages of 0.6100. The maximum expected heterozygosity was found in RM135, followed by

RM1 (0.8191), RM72 (0.7853) and RM131 (0.7775). The minimum expected heterozygosity was found in RM153. Nei's gene diversity varied from 0.0783 to 0.8261 with an average of 0.6095. The maximum Nei's gene diversity was found in RM 135 while the minimum was found in RM153. The value of all the genetic diversity measures should be ranged from zero (no heterozygosity) to 1 (a maximum indicator of heterozygosity).

4.3.4 Polymorphism information content

The PIC value ranged from 0.0640 to 0.8004 with an average of 0.5517. The maximum PIC value was found in RM135, followed by RM72 (0.7531), RM131 (0.7442) and RM1 (0.7289) while the minimum PIC value was found in RM153 followed by RM125 (0.3066), RM287 (0.4077) and RM117 (0.4258). The average PIC value of polymorphic locus was found to be 0.5517.

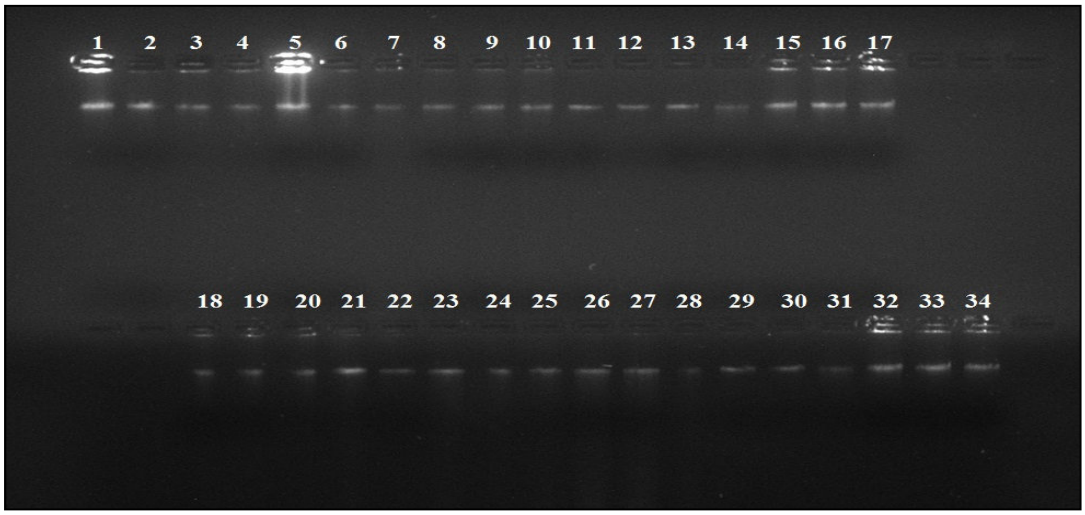

Figure 6: A 0.8% agarose gel showing genomic DNA of indigenous rice varieties of Mizoram, Numbers correspond to cultivar number in Table 1.

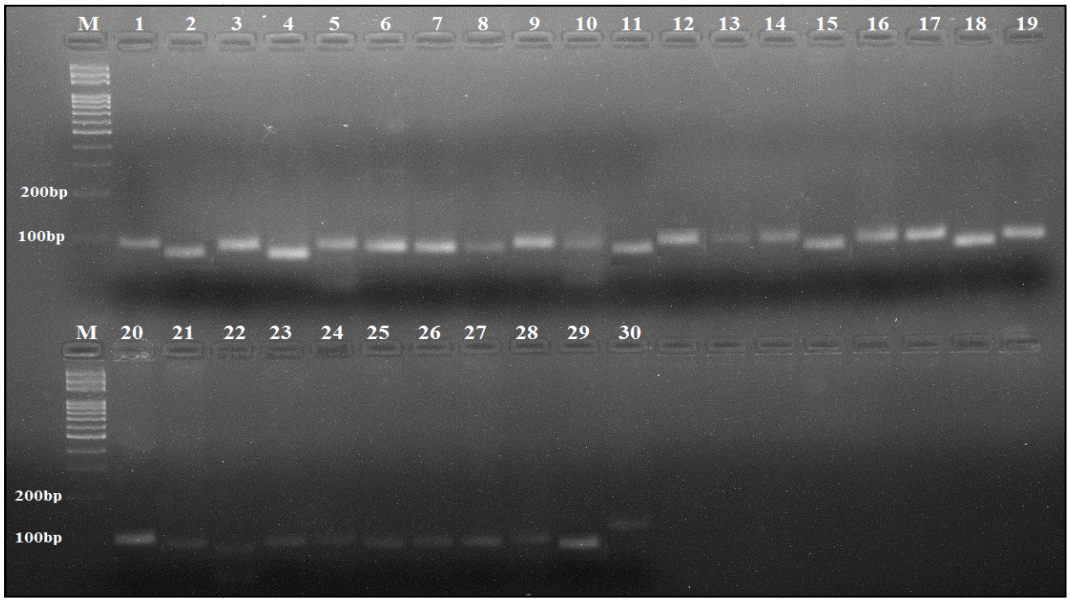

Figure 7: A 2.5% agarose gel showing the banding pattern of indigenous rice varieties of Mizoram generated by RM1. M represents a 100bp DNA ladder. Numbers correspond to cultivar number in Table 1.

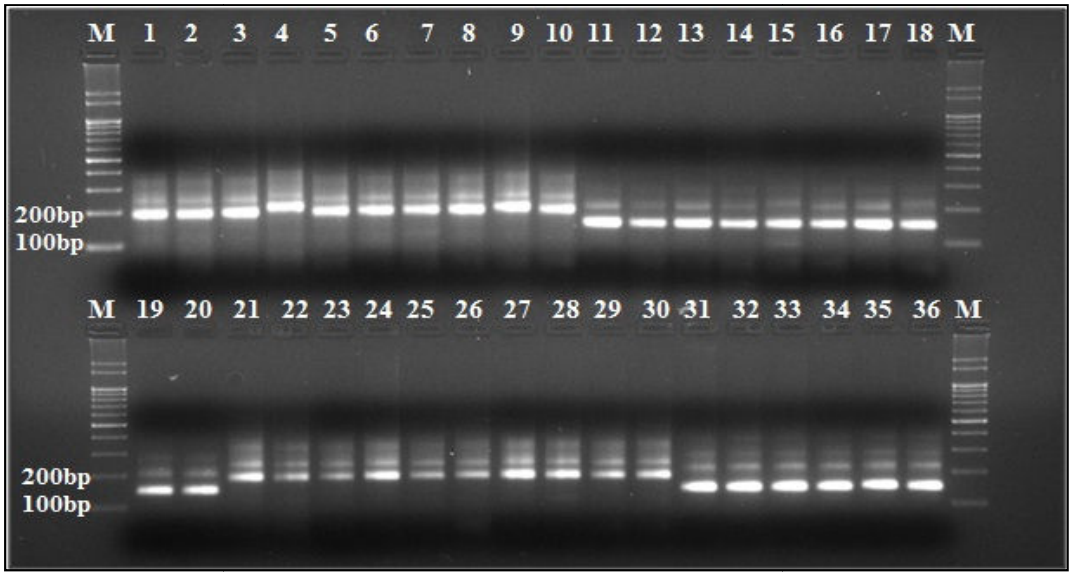

Figure 8: A 2.5% agarose gel showing the banding pattern of indigenous rice varieties of Mizoram generated by RM72. M represents a 100bp DNA ladder. Numbers correspond to cultivar number in Table 1.

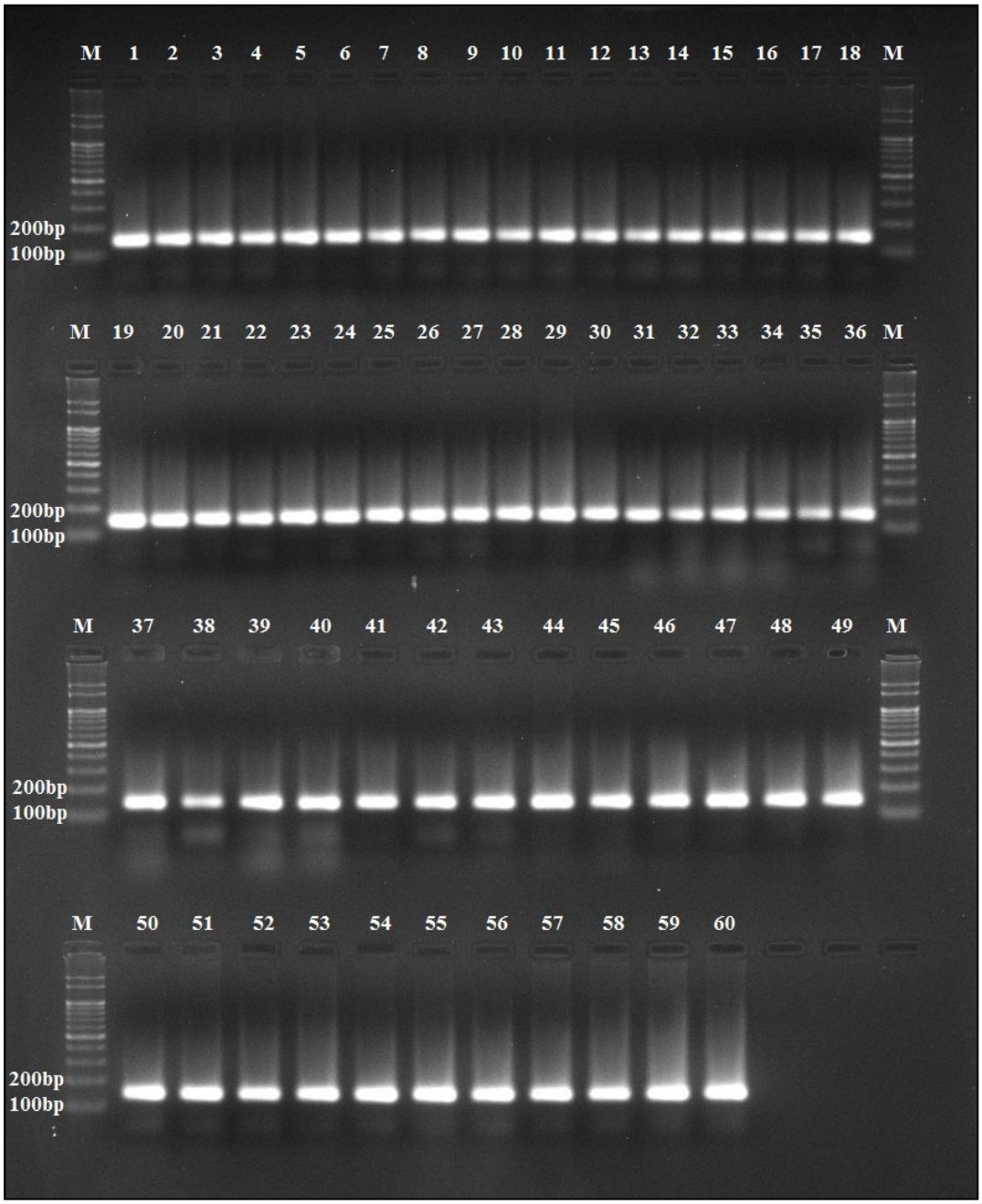

Figure 9: A 2.5% agarose gel showing the banding pattern (monomorphic) of indigenous rice varieties of Mizoram generated by RM278. M represents a 100bp DNA ladder. Numbers correspond to cultivar number in Table 1.

Table 4: Genetic parameters as revealed by polymorphic SSR markers.

Locus	na	ne	MAF	Fst	Nm	H_E	Nei	PIC
RM1	7.0000	5.5083	0.3278	0.8482	0.0447	0.8191	0.8185	0.7289
RM154	7.0000	3.3945	0.4452	0.6538	0.1324	0.7060	0.7054	0.6762
RM131	9.0000	4.4825	0.3595	0.6842	0.1154	0.7775	0.7769	0.7442
RM135	10.0000	5.7514	0.2722	0.5736	0.1859	0.8268	0.8261	0.8004
RM153	3.0000	1.0849	0.9659	0.6319	0.1456	0.0783	0.0783	0.0640
RM190	4.0000	2.2061	0.6222	0.8063	0.0600	0.5471	0.5467	0.5172
RM125	3.0000	1.5892	0.7571	0.8690	0.0377	0.3711	0.3708	0.3066
RM72	7.0000	4.6448	0.3111	0.8448	0.0459	0.7853	0.7847	0.7531
RM171	5.0000	3.5446	0.4143	0.8802	0.0340	0.7184	0.7179	0.6456
RM287	3.0000	1.8270	0.6635	0.9418	0.0155	0.4530	0.4527	0.4077
RM117	4.0000	2.6843	0.6929	0.6261	0.1493	0.6280	0.6275	0.4258
Mean	5.6363	3.3379	0.5301	0.7599	0.0878	0.6100	0.6095	0.5517
SD	2.5009	1.6038	0.2234	0.1275	0.0587	0.2322	0.2320	0.2304

na = observed number of alleles, ne = effective number of alleles, MAF = major allele frequency, Fst = F-statistics, Nm = gene flow estimated from Fst = 0.25(1 – Fst)/Fst, H_E = expected heterozygosity computed using Levene (1949), Nei = Nei's (1973) gene diversity, PIC = polymorphism information content.

4.4 Population-wise diversity

Population-wise diversity indices (Table 4) showed that the number of alleles per population ranged from 23 to 13 with an average of 16.8 for all populations. The highest number of alleles were amplified by two varieties, Biruchuk and Baimasa and the least number was found in 7 varieties, viz., Dumte, Zongam, Fazupui, Fangsin, Buhban sen, Buhban zam, Malcheng. of Biruchuk was found to have the highest expected heterozygosity (or gene diversity) at 0.3382, followed by Baimasa (0.3346) then Kawnglawng (0.3013), while the least value was found in Buhban zam (0.0158), followed by Zongam (0.0281) and Bahipui (0.0298). The average expected

heterozygosity of indigenous cultivars (0.1389) was lower than that of improved varieties (0.1638) in the present study.

Table 5: Population-wise diversity indices.

Sl. No.	Name	na	na (A)	Polymorphic loci	Polymorphim %	H_E
1.	Kungtawi sen	15	1.25	3	25	0.1259
2.	Vaiphei	19	1.58	4	33.33	0.1890
3.	Kawnglawng	22	1.83	7	58.33	0.3013
4.	Biruchuk	23	1.92	7	58.33	0.3382
5.	Tuikuk buh	15	1.25	2	16.67	0.0443
6.	Fare	19	1.58	4	33.33	0.1956
7.	Kawnglawng tial	19	1.58	5	41.67	0.1987
8.	Kawnglawng var	17	1.42	5	41.67	0.1382
9.	Buhban Langakthou	19	1.58	5	41.67	0.2101
10.	Buhbial	15	1.25	2	16.67	0.0456
11	Fazai	21	1.75	6	50	0.1811
12.	Laithangnu	19	1.5	5	41.67	0.1838
13.	Tai sanghar	18	1.5	5	41.67	0.2105
14.	Tai te	14	1.17	2	16.67	0.0557
15.	Zawngin buh	15	1.25	3	25	0.1167
16.	Baimasa	23	1.92	8	66.67	0.3346
17.	Bialte	19	1.58	7	58.33	0.2487
18.	Buhban hmui	17	1.42	5	41.67	0.1570
19.	Buhngat	18	1.5	6	50	0.2092
20	Fazai ban	15	1.25	3	25	0.1228
21.	San	20	1.67	4	33.33	0.1689
22.	Idaw	18	1.5	5	41.67	0.2053
23.	Mangbuh	21	1.75	6	50	0.2500
24.	Buhpui	19	1.58	6	50	0.2329
25.	Naga	16	1.33	4	33.33	0.1167
26.	Fazu	18	1.5	6	50	0.1991
27.	Phodum	17	1.42	5	41.67	0.1912
28.	Vaibuh	17	1.42	5	41.67	0.1259
29.	Varsiama	16	1.33	4	33.33	0.1061
30.	Dengchungnunga	15	1.25	3	25	0.0895
31.	Dumte	13	1.08	1	8.33	0.0368
32.	Mawitawi	16	1.33	4	33.33	0.1316
33.	Zongam	13	1.08	1	8.33	0.0281
34.	Fazupui	13	1.08	1	8.33	0.0439
35.	Fangsin	13	1.08	1	8.33	0.0437

36.	Tai buhpui	17	1.42	5	41.67	0.1245
37.	Kungrei	14	1.17	2	16.67	0.0702
38.	Tailuaia hmui	18	1.5	5	41.67	0.1754
39.	Tailuaia hmui lo	17	1.42	4	33.33	0.1579
40.	Buhban sen	13	1.08	1	8.33	0.0329
41.	Buhban zam	13	1.08	1	8.33	0.0158
42.	Zaitlai	14	1.67	2	16.67	0.0557
43.	Khawzawl buh	16	1.33	3	25	0.1035
44.	Bahipui	14	1.17	1	8.33	0.0298
45.	Pawnbuh	14	1.17	2	16.67	0.0439
46.	Malcheng	13	1.08	1	8.33	0.0438
47.	Tai bial	17	1.42	3	25	0.0899
48.	BPL	18	1.5	4	33.33	0.1403
49.	Pana	14	1.17	2	16.67	0.0316
50.	Robula	19	1.58	5	41.67	0.1965
51.	Zamzathanga	18	1.5	5	41.67	0.1943
52.	Roenga	15	1.25	3	25	0.1298
53.	Pi Hui buh	15	1.25	3	25	0.1070
54.	BM71	17	1.42	4	33.33	0.1825
55.	IR71033-121-15B	17	1.42	4	33.33	0.1846
56.	MO1	16	1.33	3	25	0.1386
57.	PTB33	17	1.42	4	33.33	0.1829
58.	TN1	19	1.58	5	41.67	0.2123
59.	CAUR1	16	1.33	3	25	0.1123
60.	Gomati	16	1.33	3	25	0.1386
61.	RCM9	16	1.33	4	33.33	0.1474
62.	RCM10	19	1.58	6	50	0.2127
63.	RCM13	17	1.42	3	25	0.1263

na = number of alleles, na (A) = average number of alleles per locus, H_E = Expected heterozygosity.

4.5 Analysis of molecular variance

Analysis of molecular variance (AMOVA) showed that 74% of total variation was due to among-population differentiation while the remaining 26% was due to within individual differentiation (Figure 10). Among the populations, most variation within population was found in Biruchuk (38.550 %), which was followed by Baimasa (38.150 %), Kawnglawng (34.400 %) and Mangbuh (28.500 %) while

the least variations within population was found in Buhban zam (1.800 %) and Bahipui (3.400 %).

Table 6: Sum of squares of variation within the population.

Sl. No.	Population	SSWP
1.	Kungtawi sen	14.350
2.	Vaiphei	21.550
3.	Kawnglawng	34.400
4.	Biruchuk	38.550
5.	Tuikuk buh	5.050
6.	Fare	22.300
7.	Kawnglawng tial	22.650
8.	Kawnglawng var	15.700
9.	Buhban Langakthou	23.950
10.	Buhbial	5.200
11	Fazai	20.650
12.	Laithangnu	20.950
13.	Tai sanghar	24.000
14.	Tai te	6.350
15.	Zawngin buh	13.300
16.	Baimasa	38.150
17.	Bialte	28.350
18.	Buhban hmui	17.900
19.	Buhngat	23.850
20	Fazai ban	14.000
21.	San	19.250
22.	Idaw	23.400
23.	Mangbuh	28.500
24.	Buhpui	26.550
25.	Naga	13.300
26.	Fazu	22.700
27.	Phodum	21.800
28.	Vaibuh	14.350
29.	Varsiama	12.100
30.	Dengchungnunga	10.200
31.	Dumte	4.200
32.	Mawitawi	15.000
33.	Zongam	3.200
34.	Fazupui	5.000
35.	Fangsin	5.000
36.	Tai buhpui	14.200

37.	Kungrei	8.000
38.	Tailuaia hmui	18.200
39.	Tailuaia hmui lo	18.000
40.	Buhban sen	3.750
41.	Buhban zam	1.800
42.	Zaitlai	6.350
43.	Khawzawl buh	11.800
44.	Bahipui	3.400
45.	Pawnbuh	5.000
46.	Malcheng	5.000
47.	Tai bial	10.250
48.	BPL	16.000
49.	Pana	3.600
50.	Robula	22.400
51.	Zamzathanga	22.150
52.	Roenga	14.800
53.	Pi Hui buh	12.200
54.	BM71	20.800
55.	IR71033-121-15B	21.050
56.	MO1	15.800
57.	PTB33	20.850
58.	TN1	24.200
59.	CAUR1	12.800
60.	Gomati	15.800
61.	RCM9	16.800
62.	RCM10	24.250
63.	RCM13	14.400

SSWP = The sum of squared differences within populations.

Table 7: Analysis of molecular variance (AMOVA).

Source	df	SS	MS	%
Among Populations	62	3204.965	51.693	74
Within Individuals	630	564.000	0.895	26

df = degree of freedom, SS = sum of square, MS = means of square, % = percentage variation.

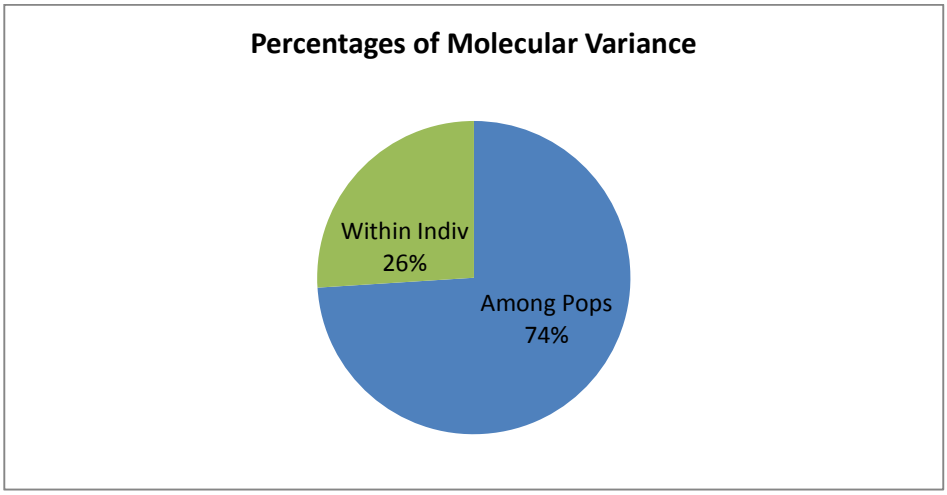

Figure 10: Analysis of molecular variance (AMOVA) showing the distribution of genetic diversity among populations (74%) and within individuals (26%);

4.6 Principal Coordinates Analysis

Principal coordinates analysis (PCoA) analysis using Nei's genetic distance showed a grouping of the studied populations into three distinct groups (Figure 11) which were further supported by the STRUCTURE results and UPGMA dendrogram. The first three principal coordinates explained 48.76 % of the total variation. Coordinate 1 extracted 22.44 % of the total variation, coordinate 2 extracted 17.25 % of the total variation and coordinate 3 explained 9.08 %. Group I was represented by *Japonica* check-varieties and 11 indigenous varieties, Group II was represented by *Indica* varieties and 12 indigenous varieties, while Group III comprised of 30 indigenous varieties.

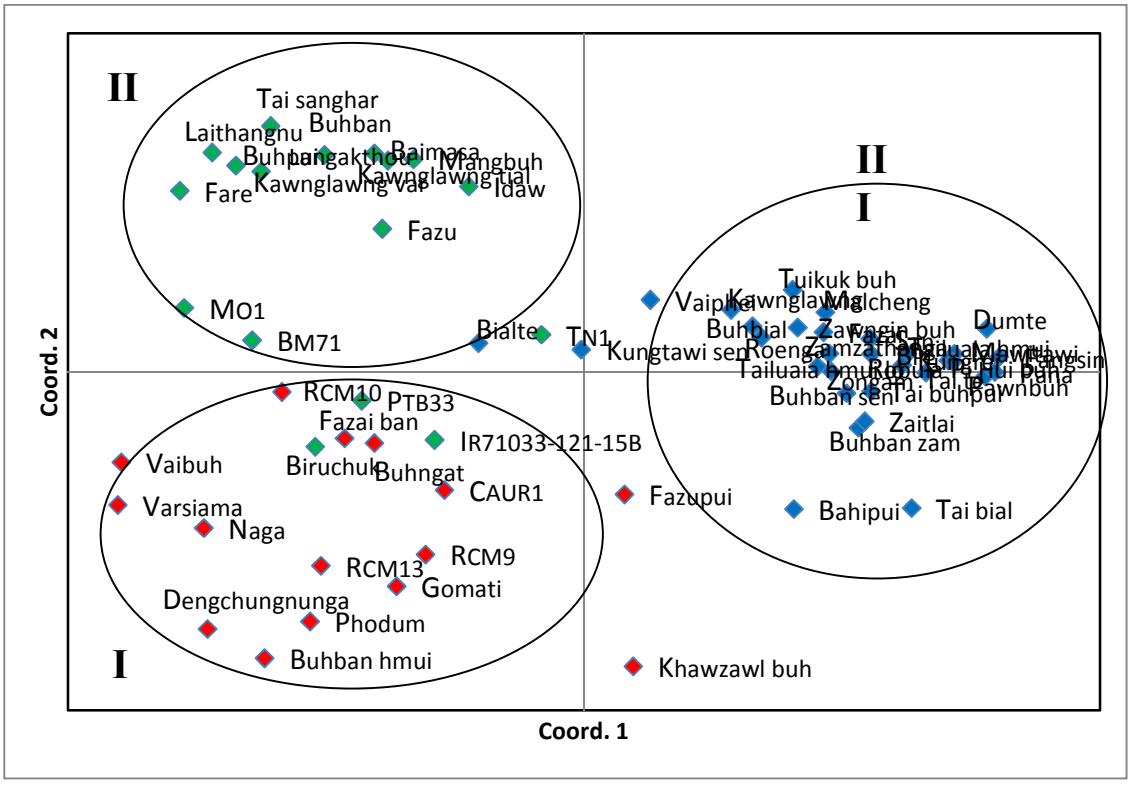

Figure 11: Principal coordinates analysis (PCoA) based on Nei's genetic diversity. Different colours indicated different groups.

4.7 Genetic Similarity

Pairwise population matrix of Nei's genetic similarity (Appendix 1) performed using GenALEX indicated that Pawnbuh and Pana were mostly related among the studied populations with the similarity index of 0.994, which was followed by Fangsin and Pana, Fangsin and Pawnbuh and Malcheng and Roenga with similarity indices of 0.977, 0.974 and 0.964 respectively. The most diverse cultivars were Buhban hmui and Mangbuh with a genetic similarity index of 0.175, which was followed by Buhban hmui and Idaw, Buhban hmui and Dumte and

Buhban langakthou and Buhban hmui with the similarity indices of 0.180, 0.193 and 0.189 respectively.

4.8 Population structure analysis

Bayesian model-based method, STRUCTURE supported the structuring of the populations into three clusters with ΔK at K=3 (Figure 12). The UPGMA tree (Figure 15) based on Nei's genetic distance also clustered the populations into three clusters. Cluster I was represented by *Japonica* check varieties and 11 indigenous varieties- Fazupui, Vaibuh, Varsiama, Fazai ban, Bialte, Buhngat, Naga, Khawzawl buh, Phodum, Buhban hmui, and Dengchungnunga. Cluster II was represented by *Indica* check varieties and 12 indigenous varieties- Biruchuk, Fazu, Kawnglawng tial, Buhpui, Idaw, Mangbuh, Fare, Buhban langakthou, Kawnglawng var, Laithangnu, Tai sanghar, and Baimasa. Cluster III comprised of 30 indigenous varieties viz., Pawnbuh, Pana, Fangsin, Robula, Tai buhpui, Dumte, Mawitawi, Buhban zam, BPL, Zamzathanga, Tai bial, Malcheng, Roenga, Pi Hui buh, Tai te, Kungrei, Tailuaia hmui, Zongam, Buhban sen, Zaitlai, Tailuaia hmui lo, Bahipui, Vaiphei, Tuikuk buh, Kungtawi sen, Kawnglawng, Buhbial, Fazai and San. Bar plot obtained from sort by Q option of STRUCTURE differentiate the studied populations into three groups indicated by different colours (Figure 13 & 14), this clustering was totally similar to UPGMA tree obtained from MEGA. The average distance between individuals ranged from 0.3328 (in cluster II), 0.3984 (in cluster III) and 0.5359 (in cluster I). Allele-frequency divergence among populations ranged from 0.1093 (Group I and II), 0.2168 (Group II and III) and 0.2266 (Group I and III).

The mean Fst value of cluster I was 0.2540 and that of cluster II and cluster III were 0.5424 and 0.4860 respectively, while the mean alpha value was 0.0744.

Table 8: Allele-frequency divergence among populations (Net nucleotide distance), computed using point estimates of P.

	Group 1	**Group 2**	**Group 3**
Group 1	-		
Group 2	0.1903	-	
Group 3	0.2266	0.2168	-

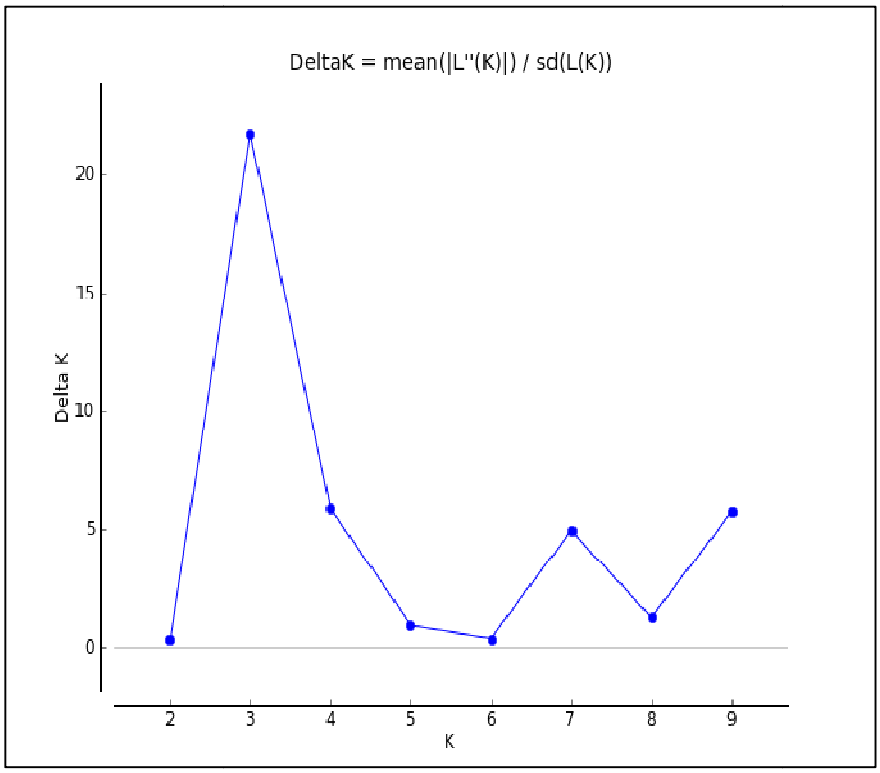

Figure 12: Relationship between K and ΔK showing highest ΔK at K=3.

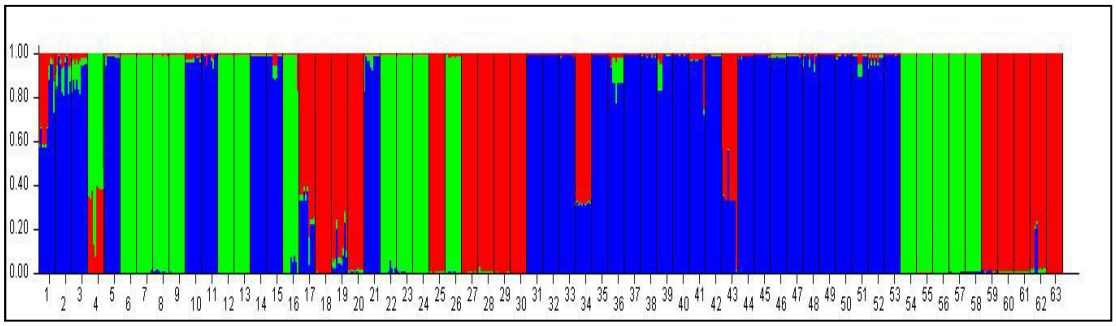

Figure 13: Population structure of indigenous rice varieties of Mizoram.

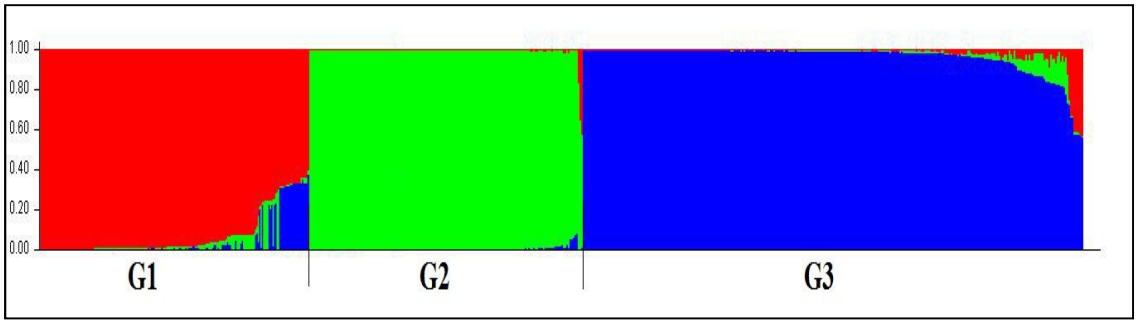

Figure 14: Population structure of rice varieties of Mizoram obtained by sort by Q option from STRUCTURE.

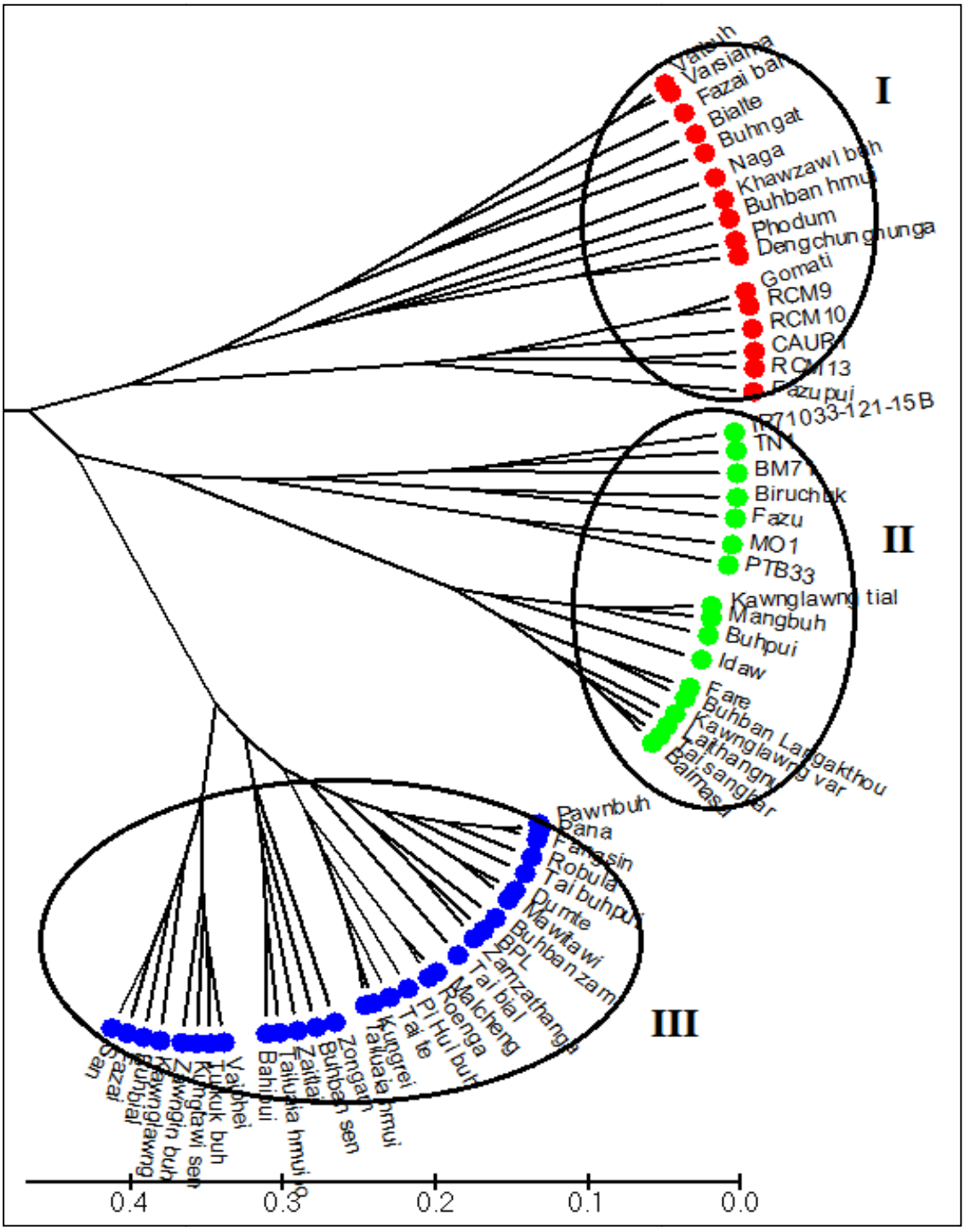

Figure 15: A UPGMA tree of rice varieties of Mizoram showing three clusters.

Chapter 5

Discussion

In the present study, genetic diversity of indigenous rice varieties of Mizoram were estimated based on grain quality traits, biochemical and molecular markers. Kawnglawng cultivar was found to have the highest seed length among the studied cultivars. A similar value was also reported earlier on Indian rice varieties (Pachauri et al. 2013; Vanlalsanga and Singh 2019). According to ICAR, rice varieties can be categorized into short bold, short slender, medium slender, long bold, long slender, basmati type and extra long slender based on grain length and length/width ratio. In the current investigation, 34 cultivars can be categorized into long bold, while 29 cultivars were found to be the extra long slender type. It has been suggested that grains of slender category posses higher market values than the bold ones (Verma et al. 2012). Nearly 50% of the Mizoram rice varieties used in the study were the slender type and could have good market values. However, the average L/W ratio was slightly lesser than the previous study (Pachauri et al. 2013).

Protein profile showed a number of polypeptide bands ranging from 5 to 10 in the studied varieties. The average Jaccard's similarity coefficient of 0.7064 indicated that genetic similarity existed respect to seed storage protein. However, the current investigation result showed more number of clusters than reported earlier on

rice of Uttarakhand State, India and International Rice Molecular Breeding Programme (Jugran et al. 2010; Dhawale et al. 2015). It has been opined that higher cluster number is a result of high variation among the cultivars (Tahir 2014). Other studies have also shown variations in numbers and positions of bands while the seed storage protein profiles of intra-species were very similar (Wei-dong et al. 2006; Vithyashini and Wickramasinghe, 2015). This may be the reason why Ladizinsky and Hymowitz (1979) have earlier proposed seed storage protein banding pattern as a unique and powerful tool for evolutionary and diversity studies. Also, the UPGMA tree constructed based on Jaccard's similarity coefficient showed three clusters similar to an earlier report by Vanlalsanga and Singh (2019).

Assessment of genetic diversity of indigenous rice varieties of Mizoram state was done using twelve polymorphic SSR markers, each mapped to a chromosome of the rice genome. A total of 63 alleles were generated by these markers. The total number of alleles per locus ranged from 1 to 10. In order to get a good result and reduce error, in the genetic diversity study, each locus should exhibit more than 4 alleles (Barker 1994). In the present study, 4 loci (RM153, RM125, RM278, and RM287) generated less than 4 alleles per locus, however, the average alleles per locus were found to be more than 4. The mean expected heterozygosity for polymorphic loci found in the present study was a high value of heterozygosity index and it was higher than the previous study of rice germplasm of Assam (Rathi et al. 2014) and Tripura (Anupam et al. 2017), similar to Mizoram accessions (Vanlalsanga and Singh 2019) but lower than indigenous glutinous rice landraces of Assam (Rathi and Sharma 2012) and rice cultivars of Arunachal Pradesh (Berilus et

al. 2013). It also showed similarity to a previous study of aromatic and quality rice landraces from NE India (Roy et al. 2015).

The PIC value determines the usefulness of the markers for genetic diversity analysis (Elston 2005). It was observed that the mean PIC value in this study was higher than previously reported in NE rice cultivars (Choudhury et al. 2014), Indian rice germplasm (Yadav et al. 2013), Indian and exotic rice (Babu et al. 2014), rice landraces of Bangladesh (Wang et al. 2013), rice accessions from Pakistan (Shah et al. 2013) and rice varieties of Iran (Vazirzanjani et al. 2017) indicating the SSR markers used in this study are good markers for rice genetic diversity analysis. Also, PIC value observed was comparable to a previous study on rice of Mizoram (Vanlalsanga and Singh 2019). Following the parameters set by Babu et al. (2014) about most polymorphic loci at PIC value (≥0.70), expected heterozygosity (≥0.71), polymorphic alleles (≥6); RM1, RM154, RM131, RM135, and RM72 were identified as the most polymorphic loci among the markers used. Based on PIC value, there were seven highly informative markers (PIC>0.50), viz., RM1, RM154, RM131, RM135, RM190, RM72 and RM171, three informative markers (PIC between 0.25 and 0.50), RM125, and RM287 and RM117, and two slightly informative markers (PIC<0.25), RM153 and RM278 (Botstein et al. 1980). The lower the PIC value, less is it's informative in distinguishing the genotypes (Goswami et al. 2017). The genetic diversity of wild rice (*Oryza nivara*), the progenitor of *O. sativa* reported earlier by Juneja et al. (2006) possessed higher genetic diversity than that of the rice cultivars in the present study supported the fact

that wild rice populations are thought to possess higher genetic diversity than cultivated rice varieties (Sun et al. 2001).

The ΔK peak (at K=3) indicated that the studied rice varieties could be grouped into three clusters. This result showed strong agreement with Seed Storage Protein-based phylogenetic tree, PCoA analysis, and the UPGMA tree. Most of the rice varieties in Cluster I was from one village ie. NE Khawdungsei, indicating the possible role of biogeography in cluster analysis. Roy at al. (2015) has also demonstrated a similar result on aromatic and quality rice of NE India. This phenomenon may be due to increased gene flow within intra-species in geographically closer populations. On the other hand, some rice populations from geographic distant areas were also grouped together in the same clusters. A similar finding was also reported on rice using SSR markers by previous workers (Dhama et al. 2018). This clearly suggests that parental selection for breeding programmes should be based on genetic diversity, not only on geographically distant cultivars. Human activities such as farming practices, cultivar preferences, etc. also affect genetic diversity and levels of gene flow (Roy et al. 2015). Evanno et al. (2005) have suggested an alpha (the degree of admixture) value close to zero indicates the individuals are from the a single population or another, and an alpha value greater than 1 implies most of the individuals are admixed. In my study, the mean value of alpha was found to be 0.0744 indicating all the populations were different. Wright (1978) has proposed that Fst values of 0 to 0.05 indicates very little genetic differentiation, 0.05 to 0.15 a moderate, 0.15 to 0.25 a good and more than 0.25 a very high level of genetic differentiation among the populations. In my study, based

on the average Fst value, a great genetic differentiation existed among the three clusters. From the Nei's genetic similarity indices, it was clear that the three varieties, Pawnbuh, Pana and Fangsin were very closely related to each other. The two phylogenetic trees, seed storage protein tree (T1) and SSR tree (T2) were more or less comparable. Four varieties in cluster I of T1 were group together in cluster II of T2. Twenty-two varieties out of twenty-eight in cluster II of T1 were grouped together in cluster III of T2. Cluster III of T1 possessed twenty-six varieties, which were separated into two clusters in T2, twelve varieties grouped together in cluster I and eleven varieties in cluster II.

In Mizoram and some areas of NE India, rice has been cultivated in shifting or jhum lands which only depend on Monsoon rain. Due to limited water resource, they might be thought to possess variable traits so as to survive on a long spell of rainless weather, and other desirable traits also. Other important traits include dark color and aroma in *Chakhao* rice of Manipur, resistance against blast, resistance to gall midge, deep water tolerance in *Baon* of Assam, drought resistance in *Hmawrhang* of Mizoram, etc (Hore 2005; Singh et al. 2006; Mahender et al. 2012). The genetic diversity of some indigenous rice cultivars was lower than that of agronomically improved varieties in the present study. This suggests necessory action on conservation of these landraces. The use of genetic variability in breeding programs is a key factor for crop improvement (Babu et al. 2014). More investigation, utilization, management and conservation of landraces of rice are needed due to the preference of high-yielding varieties. A large number of farmers practice shifting or jhum cultivation in Mizoram. These farmers prefer

local/indigenous varieties, not only rice but also other crops. This preference may be due to the ability of the local cultivars to grow in local microclimate, resistance to drought, heavy rain and salinity, etc. and also easily accessible and lack of lowland farming areas. Though shifting cultivation practice may cause environmental issues, yet it also serves as conservation field for indigenous crops. It has been pointed out that, the upland rice growing regions of India represent a valuable centre for the conservation of indigenous rice (Gayacharan et al. 2015). Introduction of HYVs also resulted in the loss of a large number of indigenous cultivars as well as narrowing of their genetic diversity. It is clear from the above points that both the introduction of HYV and stopping of shifting cultivation result in the loss of diversity of indigenous cultivars. Also, rice landraces are very good sources for future rice improvement programmes since they possess considerable genetic diversity and genetically variable traits, and are also considered as an intermediate stage between wild rice and cultivated rice varieties (Choudhury et al. 2013; Li et al. 2014). So, management, utilization, and conservation of these landraces need to be handled carefully. Understanding on genetic diversity of rice landraces is of prime importance in conservation, utilization, and management since it forms the basis for effective plant breeding strategies and also the genetic resources is required for increasing the germplasm resources for future improvement programmes (Rao and Hodgkin, 2002; Sohrabi et al. 2012).

The major cause of loss of crop genetic resources can be expressed in two ways, first, rapidly releasing and adoption of high yielding varieties and abandonment of indigenous crop varieties and secondly, changing of local farming

practices to modern agricultural systems (Altieri and Merrick 1987). The release of HYVs in India by the Green Revolution in the 1960s can only be grown in irrigated lowland farms, at the same period, the indigenous rice varieties suited to upland and deep-water farms started disappearing. From that period, it is believed that hundreds of indigenous rice cultivars are abandoned due to the preference of HYVs (Sebby 2010). This led to the loss of many important germplasm resources. While releasing high yielding varieties, many rice growing countries starts collecting and conserving their landraces of rice, but most of these old landraces are conserved and available in certain gene banks only, not in their original habitat (Rabbani et al. 2008). It is understood that landraces of rice are not very demanding, only because of their low production but they are specific to adapt to local farming practices like land preparation, seeding, weeding and harvesting and they do not require high soil fertility (Harlan 1975). Generally, farmers are not interested in conserving landraces for the future, in other words, landraces varieties are not grown for their conservation purposes. Farmers' main aim is only the production. But it can be said that the landraces grown in the fields for production also get automatically conserved (Rijal et al. 1998). On-farm conservation in their natural habitats (*in situ conservation*), continuous cultivation and management of indigenous crop cultivars by farmers in agro-ecosystems is very important (Zhu et al. 2003).

Landraces are geographically or ecologically distinct populations so are genetically diverged within and among them. They also represent a unique and critically important source of genetic diversity. The major contributions of indigenous varieties for breeding programmes include their genetically diverse

nature, and their ability to adapt to biotic and abiotic stresses such as drought, salinity, blasts, high temperatures, pests, etc (Dwivedi et al. 2016). Now, genetically uniform HYVs have dominated the genetically diverged and less productive indigenous landraces. So, the faith of these landraces is now threatened due to the rapid spread of these HYVs (Medhabati et al. 2013). To add to the worry, less investigation on the genetic diversity of these landraces at the molecular level has been undertaken thus far, especially from Mizoram State. However, it is worth mentioning that the success of breeding lies in the evolution of local cultivars that have been co-adapted to the local microclimate through natural selection. It is necessary to know that the landraces of rice are the basic source of future rice improvement programmes (Harlan 1975). So, the present study will enrich the knowledge on the genetic diversity of these races and will be useful for designing various breeding and conservation strategies.

Conclusion

The main objective of the assessment of genetic diversity is the identification, quantification, and comparison of genetic differentiation among loci, individuals, populations and species (Bird et al. 2011) and utilization of diverse populations for crop improvement programmes and conservation of germplasm and their genetic diversity.

Rice is the most important crop and is a staple food in Mizoram. It supplies nutritional needs much more than any other crops or other food items to the people of the State just like other rice-consuming countries. But the production can withstand only 25-30% of the total rice needed (Lallianthanga et al. 2013; Sati and Rinawma 2014). Of course, improved varieties are needed to increase rice production to meet the demand, but at the same time, these indigenous rice varieties are needed to be protected from being disappeared and conserved for their valuable diversity which can be utilized in crop improvement programmes.

Indigenous rice varieties play a very important role in rice improvement programme as they are known to possess high genetic diversity. But by changing the cropping system from shifting/jhuming cultivation to terrace/settled/lowland cultivation and by rapidly releasing high yielding varieties, the number, as well as

their genetic diversity, become decreasing. Farmers in many villages of Mizoram have stopped the cultivation of rice due to settled farming and other jobs from about five to ten years back. This leads to the loss of many indigenous crop cultivars especially rice. After 10 years, it is not clear that the indigenous rice varieties of Mizoram used in the present study will remain available to plant and study. Loss of many indigenous cultivars due to the rapid increase of HYVs is happening in various states of India (Choudhury at al. 2013) and other rice-growing countries (Zhu et al. 2003; Dwivedi et al.2016). So, it is necessary to take action on the conservation of these indigenous cultivars, in their natural ecosystem (on-farm) as well as in the gene banks. It is necessary to avoid being abandoned. Farmers' role takes a very important place in maintaining or abandoning these landraces (Bellon 1997).

The results obtained in my present study suggested that grain quality trait, seed storage protein, and SSR/microsatellite markers used can be powerful tools to study genetic diversity among individuals within a population as well as between populations in a species. They are effective and promising markers for detecting genetic variation. Previous researchers reported that the effectiveness of SSR markers in genetic diversity analysis of rice (Sivaranjani et al. 2010; Babu et al. 2014; Shamin et al. 2016) and other crops also (Park et al. 2009).

Pawnbuh and Pana showed the highest genetic similarity while Buhban hmui and Mangbuh showed the highest genetic distance among all the populations studied. The information on genetic distance and similarity in this study could avoid the

chance of using genetically similar or identical genotypes and will also be helpful to select genetically diverse parents for further breeding programmes.

The results obtained in the present study indicated that there is a considerable level of genetic diversity among rice populations of Mizoram. The high level of genetic diversity observed among the indigenous rice of the state such as Biruchuk, Baimasa, Kawnglawng, etc as well as genetically diverse varieties like Buhban hmui and Mangbuh, Buhban hmui and Idaw can be useful as germplasm resources for future rice breeding or improvement programmes. On the other hand, some cultivars such as Buhban zam, Zongam, Bahipui, etc. showed very low levels of genetic diversity, calling for necessary conservation strategies. The present study will improve the data on grain quality traits and genetic diversity of rice landraces of Mizoram. Further investigations are sought on agronomy, qualitative and quantitative traits in order for selecting important parental lines for effective breeding programmes of the indigenous rice of Mizoram.

Appendix: Pairwise population matrix of Nei's genetic similarity.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1	1.000																
2	0.880	1.000															
3	0.799	0.757	1.000														
4	0.515	0.453	0.480	1.000													
5	0.821	0.891	0.869	0.354	1.000												
6	0.328	0.430	0.414	0.425	0.347	1.000											
7	0.503	0.629	0.577	0.418	0.523	0.784	1.000										
8	0.471	0.522	0.469	0.468	0.483	0.796	0.645	1.000									
9	0.408	0.517	0.503	0.437	0.419	0.905	0.877	0.762	1.000								
10	0.576	0.640	0.794	0.398	0.649	0.350	0.536	0.372	0.460	1.000							
11	0.661	0.799	0.787	0.375	0.779	0.371	0.575	0.460	0.476	0.884	1.000						
12	0.519	0.527	0.597	0.446	0.521	0.792	0.741	0.864	0.862	0.439	0.433	1.000					
13	0.474	0.515	0.509	0.368	0.513	0.729	0.702	0.855	0.793	0.357	0.417	0.903	1.000				
14	0.627	0.679	0.775	0.343	0.811	0.294	0.385	0.466	0.378	0.630	0.761	0.424	0.440	1.000			
15	0.819	0.851	0.752	0.455	0.796	0.312	0.513	0.542	0.406	0.651	0.787	0.458	0.469	0.805	1.000		
16	0.481	0.528	0.564	0.433	0.508	0.755	0.754	0.861	0.828	0.456	0.496	0.895	0.939	0.477	0.507	1.000	
17	0.521	0.644	0.577	0.389	0.561	0.585	0.561	0.449	0.494	0.522	0.565	0.398	0.357	0.431	0.523	0.403	1.000
18	0.341	0.373	0.250	0.515	0.245	0.290	0.201	0.305	0.198	0.217	0.267	0.211	0.199	0.209	0.319	0.217	0.575
19	0.496	0.511	0.401	0.299	0.422	0.511	0.506	0.366	0.406	0.425	0.485	0.347	0.351	0.273	0.380	0.331	0.682
20	0.387	0.485	0.428	0.365	0.428	0.560	0.468	0.500	0.468	0.444	0.469	0.489	0.481	0.419	0.390	0.459	0.543
21	0.670	0.774	0.802	0.384	0.803	0.375	0.582	0.390	0.468	0.800	0.908	0.371	0.384	0.796	0.799	0.467	0.608
22	0.426	0.508	0.478	0.435	0.458	0.700	0.768	0.605	0.742	0.361	0.436	0.648	0.737	0.434	0.426	0.751	0.433
23	0.468	0.592	0.530	0.398	0.497	0.716	0.908	0.658	0.783	0.536	0.578	0.703	0.762	0.397	0.523	0.808	0.527
24	0.517	0.592	0.555	0.424	0.528	0.681	0.840	0.663	0.751	0.583	0.514	0.766	0.760	0.340	0.466	0.771	0.497
25	0.375	0.346	0.343	0.418	0.289	0.393	0.385	0.359	0.413	0.327	0.360	0.404	0.363	0.263	0.294	0.361	0.388

26	0.541	0.531	0.536	0.722	0.462	0.566	0.588	0.598	0.637	0.422	0.401	0.616	0.617	0.414	0.486	0.612	0.423
27	0.481	0.475	0.363	0.649	0.374	0.240	0.240	0.313	0.239	0.240	0.328	0.289	0.268	0.348	0.398	0.260	0.381
28	0.468	0.472	0.447	0.483	0.424	0.522	0.455	0.411	0.448	0.472	0.381	0.498	0.415	0.333	0.364	0.380	0.651
29	0.442	0.420	0.414	0.480	0.383	0.514	0.395	0.401	0.438	0.398	0.335	0.486	0.405	0.384	0.317	0.374	0.599
30	0.455	0.484	0.336	0.503	0.359	0.278	0.287	0.324	0.286	0.267	0.360	0.335	0.312	0.358	0.380	0.316	0.399
31	0.547	0.688	0.587	0.340	0.667	0.385	0.476	0.372	0.469	0.529	0.690	0.327	0.341	0.745	0.720	0.450	0.480
32	0.544	0.666	0.635	0.422	0.664	0.385	0.480	0.392	0.473	0.565	0.681	0.343	0.359	0.778	0.742	0.477	0.483
33	0.513	0.536	0.648	0.354	0.522	0.332	0.409	0.450	0.395	0.682	0.692	0.465	0.428	0.590	0.609	0.546	0.372
34	0.528	0.475	0.571	0.496	0.487	0.325	0.430	0.363	0.414	0.531	0.561	0.384	0.347	0.569	0.560	0.428	0.273
35	0.490	0.616	0.680	0.341	0.670	0.387	0.477	0.348	0.471	0.614	0.692	0.328	0.343	0.794	0.679	0.423	0.541
36	0.405	0.476	0.538	0.437	0.535	0.413	0.488	0.345	0.483	0.472	0.544	0.347	0.361	0.647	0.541	0.435	0.399
37	0.633	0.675	0.815	0.440	0.809	0.303	0.397	0.456	0.389	0.649	0.761	0.439	0.445	0.896	0.788	0.492	0.434
38	0.604	0.676	0.772	0.456	0.779	0.339	0.437	0.490	0.430	0.654	0.751	0.458	0.475	0.896	0.810	0.506	0.432
39	0.629	0.624	0.755	0.489	0.628	0.411	0.517	0.454	0.501	0.627	0.678	0.461	0.427	0.687	0.681	0.582	0.463
40	0.592	0.584	0.686	0.421	0.619	0.291	0.381	0.461	0.374	0.537	0.672	0.419	0.435	0.733	0.730	0.513	0.368
41	0.528	0.533	0.739	0.388	0.609	0.326	0.427	0.351	0.412	0.678	0.678	0.380	0.345	0.682	0.588	0.463	0.442
42	0.484	0.566	0.575	0.339	0.509	0.388	0.480	0.324	0.474	0.534	0.669	0.330	0.345	0.638	0.602	0.466	0.477
43	0.318	0.357	0.389	0.428	0.334	0.284	0.233	0.276	0.232	0.476	0.553	0.213	0.218	0.514	0.404	0.295	0.355
44	0.496	0.471	0.581	0.558	0.441	0.323	0.427	0.250	0.411	0.519	0.554	0.288	0.250	0.530	0.511	0.415	0.369
45	0.481	0.602	0.653	0.341	0.644	0.396	0.468	0.328	0.463	0.583	0.666	0.323	0.337	0.752	0.654	0.425	0.539
46	0.519	0.634	0.598	0.305	0.639	0.377	0.477	0.524	0.471	0.627	0.716	0.515	0.533	0.735	0.736	0.526	0.356
47	0.421	0.526	0.579	0.531	0.563	0.290	0.386	0.239	0.379	0.572	0.639	0.243	0.253	0.669	0.561	0.320	0.414
48	0.464	0.608	0.688	0.369	0.591	0.427	0.548	0.401	0.533	0.778	0.801	0.419	0.379	0.670	0.600	0.495	0.539
49	0.496	0.612	0.687	0.347	0.674	0.375	0.465	0.330	0.459	0.606	0.688	0.335	0.350	0.782	0.668	0.425	0.526
50	0.576	0.652	0.794	0.454	0.706	0.445	0.514	0.432	0.502	0.725	0.789	0.399	0.373	0.808	0.699	0.493	0.579
51	0.511	0.617	0.703	0.373	0.631	0.471	0.579	0.469	0.571	0.694	0.758	0.494	0.447	0.755	0.661	0.526	0.485
52	0.505	0.624	0.597	0.362	0.632	0.434	0.461	0.569	0.459	0.619	0.709	0.519	0.533	0.732	0.731	0.526	0.405
53	0.474	0.647	0.642	0.463	0.694	0.294	0.395	0.396	0.388	0.710	0.791	0.339	0.354	0.782	0.685	0.407	0.428
54	0.471	0.463	0.395	0.626	0.332	0.472	0.458	0.518	0.571	0.459	0.421	0.552	0.507	0.283	0.389	0.548	0.252
55	0.614	0.519	0.552	0.649	0.455	0.356	0.433	0.351	0.439	0.413	0.401	0.415	0.351	0.424	0.506	0.403	0.336

56	0.446	0.479	0.476	0.502	0.352	0.592	0.511	0.638	0.617	0.448	0.413	0.630	0.522	0.322	0.455	0.613	0.471
57	0.506	0.487	0.552	0.526	0.399	0.469	0.475	0.498	0.484	0.492	0.458	0.491	0.388	0.419	0.524	0.506	0.439
58	0.639	0.638	0.658	0.542	0.548	0.496	0.481	0.518	0.593	0.591	0.586	0.563	0.508	0.556	0.648	0.565	0.465
59	0.465	0.412	0.541	0.510	0.395	0.365	0.456	0.313	0.469	0.546	0.498	0.442	0.360	0.516	0.434	0.410	0.294
60	0.408	0.343	0.468	0.483	0.365	0.354	0.354	0.323	0.351	0.377	0.387	0.348	0.302	0.518	0.412	0.347	0.292
61	0.459	0.345	0.465	0.382	0.367	0.351	0.354	0.330	0.347	0.372	0.388	0.344	0.304	0.524	0.417	0.348	0.288
62	0.350	0.401	0.480	0.367	0.356	0.551	0.578	0.558	0.584	0.505	0.476	0.603	0.516	0.465	0.425	0.599	0.402
63	0.331	0.309	0.393	0.439	0.290	0.332	0.353	0.342	0.345	0.413	0.395	0.354	0.303	0.427	0.346	0.354	0.254

	18	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**
18	1.000																
19	0.539	1.000															
20	0.558	0.626	1.000														
21	0.234	0.427	0.479	1.000													
22	0.180	0.397	0.458	0.530	1.000												
23	0.175	0.495	0.473	0.598	0.830	1.000											
24	0.202	0.458	0.572	0.521	0.698	0.900	1.000										
25	0.551	0.571	0.606	0.342	0.345	0.345	0.389	1.000									
26	0.326	0.244	0.408	0.452	0.679	0.585	0.640	0.409	1.000								
27	0.607	0.376	0.544	0.362	0.290	0.243	0.282	0.579	0.477	1.000							
28	0.540	0.618	0.702	0.409	0.372	0.452	0.601	0.508	0.379	0.511	1.000						
29	0.555	0.597	0.694	0.355	0.376	0.389	0.516	0.555	0.382	0.529	0.960	1.000					
30	0.668	0.415	0.612	0.339	0.267	0.286	0.326	0.652	0.323	0.821	0.557	0.622	1.000				
31	0.193	0.327	0.361	0.764	0.525	0.481	0.356	0.261	0.410	0.281	0.285	0.291	0.266	1.000			
32	0.244	0.327	0.398	0.772	0.551	0.487	0.369	0.321	0.500	0.364	0.299	0.305	0.279	0.946	1.000		
33	0.325	0.434	0.467	0.544	0.348	0.416	0.373	0.309	0.334	0.280	0.288	0.285	0.327	0.645	0.650	1.000	
34	0.360	0.347	0.362	0.588	0.432	0.434	0.386	0.442	0.336	0.409	0.449	0.480	0.454	0.563	0.592	0.500	1.000

35	0.240	0.364	0.408	0.799	0.527	0.483	0.357	0.278	0.411	0.329	0.286	0.292	0.298	0.867	0.878	0.630	0.543
36	0.302	0.369	0.359	0.655	0.544	0.499	0.375	0.259	0.426	0.365	0.321	0.327	0.354	0.750	0.732	0.561	0.562
37	0.313	0.304	0.441	0.797	0.476	0.408	0.352	0.370	0.512	0.353	0.290	0.296	0.296	0.738	0.802	0.647	0.573
38	0.296	0.338	0.458	0.780	0.510	0.450	0.390	0.401	0.541	0.388	0.330	0.334	0.293	0.764	0.857	0.642	0.605
39	0.346	0.377	0.482	0.698	0.533	0.493	0.425	0.396	0.529	0.380	0.303	0.310	0.323	0.764	0.841	0.806	0.637
40	0.307	0.298	0.486	0.699	0.467	0.393	0.336	0.440	0.503	0.456	0.284	0.295	0.354	0.685	0.806	0.669	0.562
41	0.378	0.323	0.449	0.724	0.423	0.424	0.382	0.385	0.409	0.325	0.282	0.314	0.413	0.675	0.673	0.740	0.678
42	0.332	0.438	0.566	0.730	0.523	0.485	0.359	0.388	0.407	0.438	0.302	0.327	0.452	0.806	0.817	0.742	0.516
43	0.614	0.514	0.607	0.496	0.259	0.272	0.219	0.513	0.223	0.544	0.489	0.565	0.707	0.431	0.466	0.598	0.583
44	0.421	0.397	0.394	0.600	0.459	0.421	0.307	0.363	0.390	0.463	0.360	0.370	0.424	0.671	0.737	0.677	0.691
45	0.295	0.409	0.462	0.771	0.522	0.478	0.352	0.293	0.405	0.329	0.295	0.301	0.329	0.870	0.861	0.685	0.513
46	0.217	0.442	0.453	0.606	0.455	0.487	0.434	0.370	0.369	0.268	0.363	0.359	0.276	0.737	0.728	0.759	0.565
47	0.444	0.421	0.394	0.679	0.435	0.383	0.265	0.395	0.399	0.465	0.360	0.361	0.419	0.661	0.737	0.534	0.585
48	0.315	0.429	0.490	0.752	0.459	0.533	0.443	0.334	0.368	0.307	0.330	0.318	0.353	0.738	0.722	0.808	0.539
49	0.292	0.391	0.454	0.790	0.518	0.471	0.353	0.301	0.418	0.332	0.284	0.290	0.327	0.844	0.843	0.669	0.519
50	0.303	0.386	0.472	0.854	0.561	0.525	0.418	0.343	0.483	0.368	0.340	0.343	0.310	0.818	0.858	0.682	0.647
51	0.310	0.428	0.533	0.729	0.488	0.537	0.441	0.352	0.396	0.327	0.344	0.360	0.388	0.719	0.723	0.762	0.563
52	0.306	0.502	0.530	0.595	0.476	0.489	0.434	0.434	0.407	0.305	0.417	0.413	0.288	0.689	0.723	0.727	0.555
53	0.252	0.323	0.389	0.761	0.403	0.404	0.349	0.323	0.511	0.384	0.284	0.283	0.276	0.761	0.799	0.642	0.512
54	0.338	0.278	0.225	0.315	0.385	0.408	0.447	0.253	0.586	0.436	0.361	0.358	0.412	0.280	0.304	0.399	0.421
55	0.363	0.247	0.272	0.451	0.408	0.359	0.392	0.285	0.550	0.482	0.421	0.426	0.456	0.420	0.441	0.353	0.656
56	0.349	0.238	0.399	0.366	0.357	0.452	0.491	0.460	0.496	0.445	0.376	0.363	0.486	0.319	0.335	0.408	0.320
57	0.395	0.227	0.365	0.458	0.415	0.451	0.459	0.501	0.532	0.446	0.335	0.363	0.544	0.415	0.436	0.451	0.604
58	0.313	0.298	0.421	0.595	0.436	0.434	0.446	0.334	0.555	0.389	0.385	0.386	0.392	0.551	0.578	0.529	0.514
59	0.292	0.375	0.469	0.467	0.373	0.420	0.428	0.407	0.362	0.409	0.567	0.609	0.492	0.422	0.443	0.474	0.693
60	0.393	0.365	0.438	0.435	0.389	0.358	0.333	0.566	0.292	0.573	0.529	0.608	0.664	0.410	0.449	0.363	0.749
61	0.316	0.457	0.440	0.436	0.391	0.360	0.331	0.648	0.284	0.492	0.444	0.551	0.582	0.412	0.456	0.364	0.675
62	0.326	0.332	0.544	0.429	0.483	0.589	0.586	0.610	0.418	0.410	0.490	0.543	0.577	0.399	0.416	0.492	0.569
63	0.313	0.382	0.482	0.349	0.312	0.371	0.352	0.480	0.217	0.525	0.502	0.549	0.613	0.317	0.356	0.405	0.631

	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
35	1.000																
36	0.870	1.000															
37	0.793	0.680	1.000														
38	0.823	0.678	0.963	1.000													
39	0.720	0.611	0.801	0.773	1.000												
40	0.638	0.487	0.802	0.825	0.845	1.000											
41	0.806	0.724	0.739	0.693	0.770	0.589	1.000										
42	0.792	0.716	0.647	0.642	0.820	0.774	0.729	1.000									
43	0.466	0.544	0.463	0.463	0.505	0.535	0.584	0.668	1.000								
44	0.656	0.720	0.648	0.609	0.867	0.670	0.681	0.782	0.632	1.000							
45	0.974	0.894	0.784	0.782	0.748	0.614	0.823	0.844	0.522	0.708	1.000						
46	0.717	0.598	0.714	0.818	0.609	0.649	0.592	0.590	0.457	0.440	0.687	1.000					
47	0.790	0.832	0.782	0.781	0.661	0.583	0.637	0.648	0.621	0.800	0.797	0.576	1.000				
48	0.864	0.755	0.713	0.707	0.740	0.555	0.857	0.763	0.537	0.681	0.869	0.713	0.733	1.000			
49	0.977	0.893	0.823	0.814	0.740	0.619	0.835	0.816	0.508	0.693	0.994	0.691	0.819	0.874	1.000		
50	0.920	0.773	0.868	0.856	0.849	0.730	0.857	0.771	0.498	0.755	0.891	0.665	0.786	0.882	0.903	1.000	
51	0.813	0.718	0.771	0.760	0.753	0.613	0.795	0.751	0.553	0.676	0.831	0.724	0.730	0.906	0.842	0.835	1.000
52	0.668	0.548	0.755	0.855	0.627	0.687	0.538	0.551	0.501	0.456	0.644	0.964	0.612	0.660	0.651	0.672	0.697
53	0.804	0.665	0.803	0.866	0.624	0.625	0.682	0.601	0.480	0.498	0.763	0.799	0.734	0.784	0.784	0.793	0.735
54	0.281	0.434	0.294	0.328	0.351	0.279	0.324	0.292	0.386	0.418	0.272	0.375	0.426	0.384	0.279	0.320	0.335
55	0.422	0.508	0.437	0.428	0.502	0.420	0.464	0.415	0.377	0.559	0.412	0.328	0.480	0.384	0.419	0.473	0.386
56	0.320	0.257	0.342	0.340	0.448	0.318	0.409	0.322	0.235	0.318	0.320	0.320	0.234	0.442	0.318	0.416	0.406
57	0.417	0.356	0.441	0.431	0.536	0.414	0.594	0.414	0.383	0.414	0.417	0.361	0.302	0.470	0.414	0.524	0.452
58	0.553	0.419	0.570	0.580	0.628	0.550	0.589	0.551	0.355	0.501	0.543	0.514	0.444	0.571	0.549	0.615	0.581
59	0.423	0.460	0.447	0.475	0.531	0.421	0.502	0.456	0.542	0.608	0.414	0.495	0.516	0.502	0.421	0.508	0.566
60	0.429	0.457	0.426	0.466	0.478	0.436	0.536	0.436	0.640	0.553	0.411	0.429	0.471	0.378	0.409	0.497	0.446
61	0.436	0.390	0.428	0.473	0.482	0.445	0.539	0.439	0.558	0.474	0.413	0.436	0.390	0.381	0.411	0.503	0.448

62	0.407	0.358	0.406	0.445	0.498	0.390	0.569	0.423	0.472	0.388	0.400	0.503	0.294	0.527	0.396	0.503	0.550
63	0.340	0.360	0.340	0.391	0.429	0.350	0.416	0.351	0.543	0.505	0.318	0.431	0.423	0.415	0.316	0.433	0.472

	52	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**
52	1.000											
53	0.789	1.000										
54	0.352	0.416	1.000									
55	0.304	0.358	0.722	1.000								
56	0.334	0.330	0.549	0.477	1.000							
57	0.377	0.384	0.455	0.643	0.759	1.000						
58	0.498	0.531	0.675	0.731	0.658	0.611	1.000					
59	0.480	0.422	0.553	0.665	0.393	0.431	0.643	1.000				
60	0.449	0.360	0.374	0.547	0.437	0.622	0.470	0.693	1.000			
61	0.456	0.365	0.282	0.455	0.434	0.620	0.468	0.604	0.921	1.000		
62	0.516	0.410	0.382	0.362	0.673	0.751	0.509	0.632	0.730	0.732	1.000	
63	0.451	0.342	0.425	0.492	0.460	0.503	0.483	0.860	0.803	0.726	0.751	1.000

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4.	M.Sc.	First – 67.39%	Botany	2013	MZU

Ph.D. Registration No. and Date : MZU/Ph.D/713 of 22.05.2015

Department : Botany

Title of Research : Study of genetic diversity of selected indigenous rice varieties of Mizoram

Supervisor : Dr. Y Tunginba Singh

PUBLICATIONS

1. Vanlalsanga, Singh YT (2019) Genetic diversity and population structure in upland rice (*Oryza sativa* L.) of Mizoram, North East India as revealed by morphological, biochemical and molecular markers. *Biochemical Genetics* 57(3):421–442.

2. Vanlalsanga, Singh YT (2019) Salt tolerance profiles in indigenous rice (Oryza sativa L.) varieties of Mizoram, India based on physiological and salt-link microsatellite markers. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences* 21(1):220–224.

PAPERS PRESENTED

1. 'Determination of salt tolerance in some upland rice (*Oryza sativa* L.) varieties of Mizoram, India.' Mizoram Science Congress held at Mizoram University during 13th – 14th October 2016.

2. 'Genetic Structure and Diversity of Indigenous Rice (*Oryza sativa*) varieties of Mizoram, NE India.' National Seminar on Biodiversity, Conservation and Utilization of Natural resources with reference to Northeast India (BCUNRNEI) organized by Department of Botany, Mizoram University, Aizawl held on 30th - 31st March 2017.

3. 'Population structure in the indigenous rice of Manipur, NE India.' National Seminar on Biodiversity, Conservation and Utilization of Natural resources with reference to Northeast India (BCUNRNEI) organized by Department of Botany, Mizoram University, Aizawl held on 30th - 31st March 2017.

SEMINAR AND WORKSHOP ATTENDED

1. 'Mizoram Science Congress' held at Mizoram University during 13th-14th October 2016 organized by: MISTIC, MSS, MAS, STAM, MMS, GSM & BIOCONE.

2. 'National Seminar on Biodiversity, Conservation and Utilization of Natural resources with reference to Northeast India (BCUNRNEI)' during 30th - 31st March 2017organized by Department of Botany, Mizoram University.

3. 'Statistical and Computing Methods for Life-Science Data Analysis' during 5th - 10th March, 2018 organized by Department of Botany, Mizoram University and Indian Statistical Institute, Biological Anthropology Unit, Kolkata at Department of Botany, Mizoram University.

PARTICULARS OF THE CANDIDATE

NAME OF CANDIDATE		: VANLALSANGA
DEGREE		: DOCTOR OF PHILOSOPHY
DEPARTMENT		: BOTANY
TITLE OF THESIS		: STUDY OF GENETIC DIVERSITY OF SELECTED INDIGENOUS RICE VARIETIES OF MIZORAM
DATE OF ADMISSION		: 19.08.2014
APPROVAL OF RESEARCH PROPOSAL		:
1.	BOS	: 12.05.2015
2.	SCHOOL BOARD	: 22.05.2015
	REGISTRATION NO. & DATE	: MZU/Ph.D./713 of 22.05.2015
	EXTENTION (IF ANY)	:

(Prof. S.K. MEHTA)

Head of Department

Photo plate: 15-day old seedlings of collected rice varieties of Mizoram.

1. Kungtawi sen

2. Vaiphei

3. Kawnglawng

4. Biruchuk

5. Tuikuk buh

6. Fare

7. Kawnglawng tial

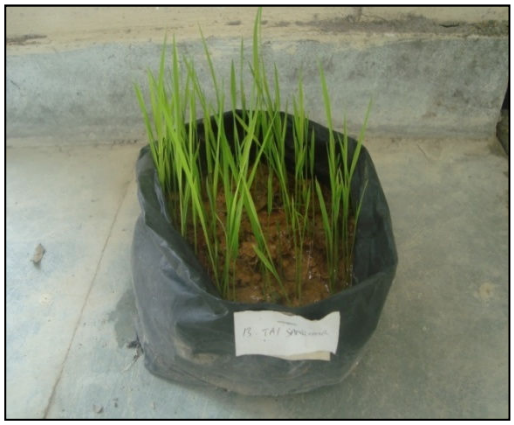
8. Kawnglawng var

9. Buhban langakthou

10. Buhbial

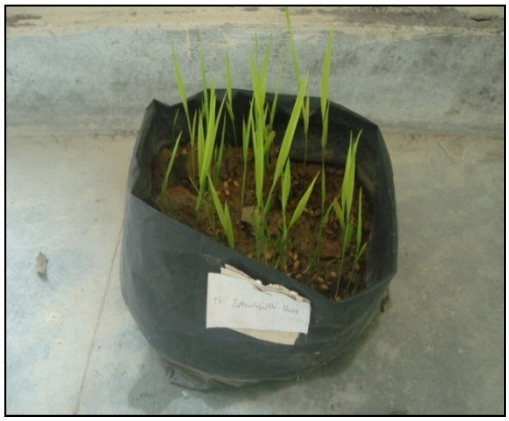
11. Fazai

12. Laithangnu



13. Tai sanghar

14. Tai te



15. Zawngin buh

16. Baimasa

17. Bialte

18. Buhban hmui

19. Buhngat

20. Fazai ban

21. San

22. Idaw

23. Mangbuh

24. Buhpui

25. Naga

26. Fazu

27. Phodum

28. Vaibuh

29. Varsiama

30. Dengchungnunga

31. Dumte

32. Mawitawi

33. Zongam

34. Fazupui

35. Fangsin

36. Tai buhpui

37. Kungrei

38. Tailuaia hmui

39. Tailuaia hmui lo

40. Buhban sen

41. Buhban zam

42. Zaitlai

43. Khawzawl buh

44. Bahipui

45. Pawnbuh

46. Malcheng

47. Tai bial

48. BPL

49. Pana

50. Robula

51. Zamzathanga

52. Roenga

53. Pi Hui buh

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ORIGINAL ARTICLE

Genetic Diversity and Population Structure in Upland Rice (*Oryza sativa* L.) of Mizoram, North East India as Revealed by Morphological, Biochemical and Molecular Markers

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Abstract

Upland rice landraces from different villages of Mizoram, Northeast India were analyzed for seed morphology, amylose content, aromatic characteristic, seed storage protein profiling and genetic diversity. Results revealed variation in grain length, width, weight and shape. Protein profiling showed polypeptide bands ranging from 7 to 10 with similarity coefficient from 0.556 to 1.000 in the studied populations. Population genetic analysis using simple sequence repeats markers revealed a total of 63 alleles with a high level of gene diversity at 0.6468. High values of Fst and PIC estimates were found at 0.7239 and 0.5984 respectively. The Biruchuk population was found to be the most genetically diverse cultivar and least gene diversity was found in Tuikuk buh. The UPGMA trees based on seed morphology, seed storage protein profiling and simple sequence repeats diversity showed the grouping of rice cultivars into three clusters which were further supported by model-based STRUCTURE analysis. This finding is the first-hand report in upland rice of the state and can be useful for selecting suitable rice lines for prebreeding and germplasm conservation of indigenous hill rice cultivars of Mizoram.

Keywords Conservation · Genetic diversity · Microsatellite marker · Northeast India · Seed protein · Upland rice

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crop for Asia which has most diversified and adopted to a wide range of geographical, ecological and climatic regions (Yadav et al. 2013). The nutritional value of rice crops is determined by

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its types and quantities of metabolites content, which in turn is strongly influenced by environmental and genetic factors (Lemaux 2008).

Local farmers in many regions of the world favor landraces due to better adaptations to microclimate, therefore these varieties are cultivated though long duration as compared to the modern rice varieties (Parzies et al. 2004; Pusadee et al. 2009; Roy et al. 2016). India is very rich with regard to the genetic diversity of rice germplasm that includes indigenous rice varieties, wild rice species, natural hybrids between the cultivar and wild relatives, and in addition the germplasm resources generated from strong and robust breeding programs adopted by the Indian agricultural research system (Rai 1999). It has been documented that West Bengal and North Eastern (NE) States of India consisting Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura, are home to a large number of indigenous rice varieties and detailed examination of these land races with regard to morphology and genetics is very essential (Das et al. 2013; Choudhury et al. 2013).

The NE India, being the region of origin and one of the zones of domestication of rice, represents a valuable center for the conservation of genetic diversity of rice. It is well noted that rice landraces of this region possess unique traits which can be used for future crop improvement programs. However, information on the molecular characterization of this germplasm is very limited (Choudhury et al. 2014). In addition, it is known that genetic diversity plays a vital role in providing traits responsible for survival and adaptation of a species (Rao and Hodgkin 2002), detailed information and identification of superior varieties becomes essential. Some of the useful qualities identified in these landraces include unique adaptive traits for cold tolerance, flooding and salt tolerance etc. Once these traits are identified, accessed and catalogd, large number of these germplasm could be easily used as a source of genetically important traits for rice improvement programs (Pusadee et al. 2009; Roy et al. 2016).

The Indian state of Mizoram lies within the International boundary of Myanmar in the East and Bangladesh in the South West, and State boundaries of Manipur in the North East, Assam in the North and Tripura in the North West. It lies between 21°56′N to 24°31′N Latitude and 92°16′E to 93°26′E Longitude in an area span of 21,087 km^2. Rice, in Mizo language is called '*buh*' and is the main crop for people of Mizoram. The indigenous rice landraces or hill rice of the state are grown in upland areas such as *jhumland* and shifting cultivation sites where farmers directly seed the rice in these traditional farming areas.

The present study was undertaken to investigate seed morphological characters, amylose content, aromatic characteristic, seed protein profiles and genetic diversity using microsatellite or simple sequence repeats (SSR) markers of hill rice of Mizoram, to gain a better understanding on the diversity of indigenous rice cultivars and in turn device conservation strategies and facilitate their effective use for future breeding programs.

Materials and Methods

Collection and Planting

Seeds of indigenous rice cultivars were collected from local farmers from different villages of Mizoram (Fig. 1). *Indica* and *Japonica* varieties were kind gifts from ICAR Kolasib, ABF Hyderabad, and ICGEB New Delhi. A total of 30 indigenous, 5 *indica* and 5 *japonica* cultivars were analyzed in the current study. Seeds were planted on poly pots and grown at Department of Botany, Mizoram University (Table 1).

Estimation of Seed Morphology

Grain quality traits viz. grain length (mm), grain width (mm), 1000-grain weight (g) and grain length/width ratio were measured and recorded from all test entries. A total of five seeds per cultivar in triplicates were used for investigation. Grain quality data were used to construct a dendrogram for genotype diversity with the help of statistical computer software NTSYSpc 2.21 (Rohlf 2009).

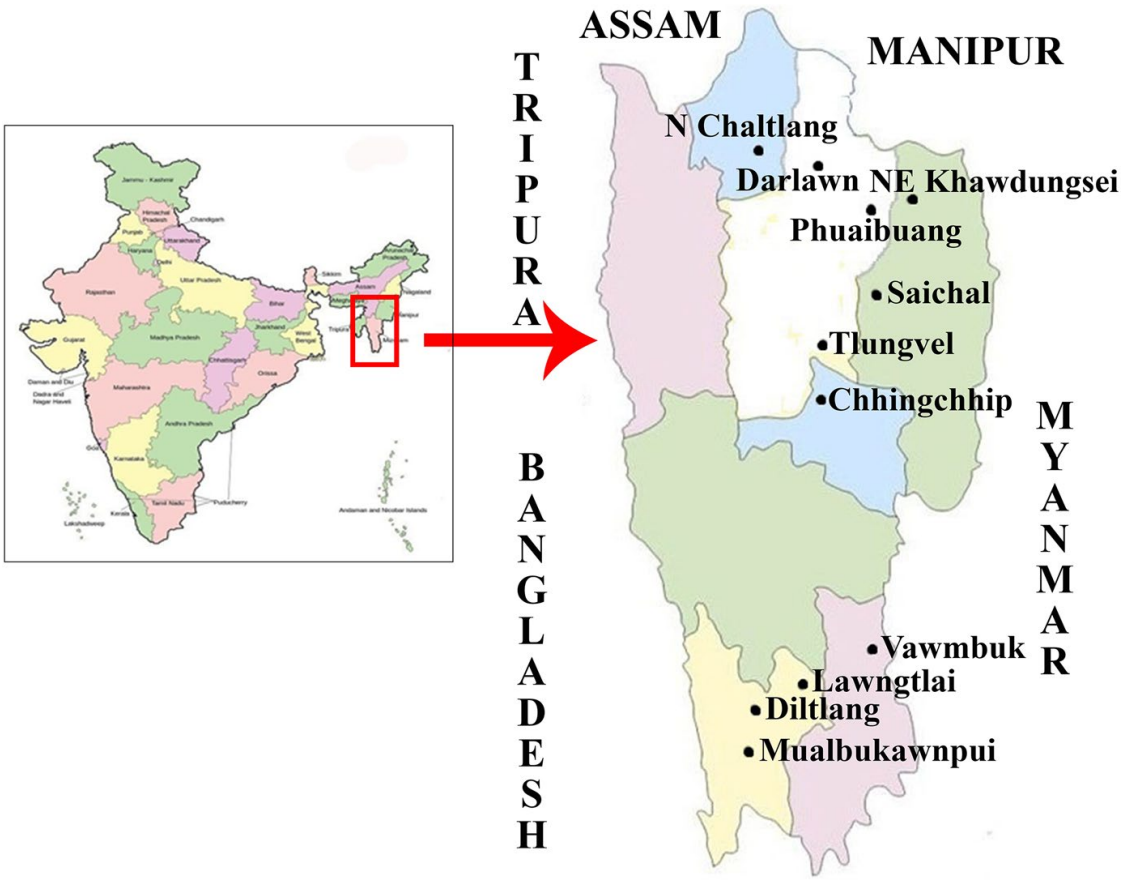

Fig. 1 Political map of India (inset) and Mizoram showing collection sites

Table 1 Details of rice cultivars used in the study

S. no.	Cultivar name	Place of collection	Location	Elevation (ft)	District	Type
1.	Kungtawi sen	Lawngtlai	22°31′42.40″ N 92°53′33.48″ E	2377	Lawngtlai	Landrace
2.	Vaiphei	Lawngtlai	22°31′42.40″ N 92°53′33.48″ E	2377	Lawngtlai	Landrace
3.	Kawnglawng	Diltlang South	22°29′33.48″ N 92°43′32.64″ E	2321	Lawngtlai	Landrace
4.	Biruchuk	Lawngtlai	22°31′42.40″ N 92°53′33.48″ E	2377	Lawngtlai	Landrace
5.	Tuikuk buh	Lawngtlai	22°31′42.40″ N 92°53′33.48″ E	2377	Lawngtlai	Landrace
6.	Fare	Diltlang South	22°29′33.48″ N 92°43′32.64″ E	2321	Lawngtlai	Landrace
7.	Kawnglawng tial	Mualbukawnpui	22°20′00.78″ N 92°42′43.41″ E	1601	Lawngtlai	Landrace
8.	Kawnglawng var	Mualbukawnpui	22°20′00.78″ N 92°42′43.41″ E	1601	Lawngtlai	Landrace
9.	Buhban Langak-thou	Vawmbuk	22°35′52.75″ N 93°04′35.06″ E	4195	Siaha	Landrace
10.	Buhbial	Vawmbuk	22°35′52.75″ N 93°04′35.06″ E	4195	Siaha	Landrace
11	Fazai	Vawmbuk	22°35′52.75″ N 93°04′35.06″ E	4195	Siaha	Landrace
12.	Laithangnu	Darlawn	24°00′51.63″ N 92°55′28.06″ E	3591	Aizawl	Landrace
13.	Tai sanghar	Darlawn	24°00′51.63″ N 92°55′28.06″ E	3591	Aizawl	Landrace
14.	Tai te	Darlawn	24°00′51.63″ N 92°55′28.06″ E	3591	Aizawl	Landrace
15.	Zawngin buh	Darlawn	24°00′51.63″ N 92°55′28.06″ E	3591	Aizawl	Landrace
16.	Baimasa	Phuaibuang	23°55′35.59″ N 93°07′17.46″ E	4571	Aizawl	Landrace
17.	Bialte	NE Khawdungsei	23°58′30.53″ N 93°12′51.77″ E	3802	Champhai	Landrace
18.	Buhban hmui	NE Khawdungsei	23°58′30.53″ N 93°12′51.77″ E	3802	Champhai	Landrace
19.	Buhngat	NE Khawdungsei	23°58′30.53″ N 93°12′51.77″ E	3802	Champhai	Landrace
20	Fazai ban	NE Khawdungsei	23°58′30.53″ N 93°12′51.77″ E	3802	Champhai	Landrace
21.	San	Saichal	23°43′07.92″ N 93°04′05.75″ E	3649	Champhai	Landrace
22.	Idaw	Tlungvel	23°36′17.20″ N 92°51′14.29″ E	3780	Aizawl	Landrace
23.	Mangbuh	Chhingchhip	23°28′15.32″ N 92°51′23.27″ E	3526	Serchhip	Landrace

Table 1 (continued)

S. no.	Cultivar name	Place of collection	Location	Elevation (ft)	District	Type
24.	Buhpui	N Chaltlang	24°01′20.21″ N 92°46′18.44″ E	2668	Kolasib	Landrace
25.	Naga	Tlungvel	23°36′17.20″ N 92°51′14.29″ E	3780	Aizawl	Landrace
26.	Fazu	Saichal	23°43′07.92″ N 93°04′05.75″ E	3649	Champhai	Landrace
27.	Phodum	NE Khawdungsei	23°58′30.53″ N 93°12′51.77″ E	3802	Champhai	Landrace
28.	Vaibuh	NE Khawdungsei	23°58′30.53″ N 93°12′51.77″ E	3802	Champhai	Landrace
29.	Varsiama	NE Khawdungsei	23°58′30.53″ N 93°12′51.77″ E	3802	Champhai	Landrace
30.	Dengchungnunga	NE Khawdungsei	23°58′30.53″ N 93°12′51.77″ E	3802	Champhai	Landrace
31.	BM71[a]	ABF, Hyderabad	17°23′06.16″ N 78°29′12.02″ E	–	Hyderabad	Improved
32.	IR71033–121-15B[a]	ABF, Hyderabad	17°23′06.16″ N 78°29′12.02″ E	–	Hyderabad	Improved
33.	MO1[a]	ABF, Hyderabad	17°23′06.16″ N 78°29′12.02″ E	–	Hyderabad	Improved
34.	PTB33[a]	ABF, Hyderabad	17°23′06.16″ N 78°29′12.02″ E	–	Hyderabad	Improved
35.	TN1[a]	ICGEB, New Delhi	28°31′47.21″ N 77°10′05.37″ E	–	New Delhi	Improved
36.	CAUR1[b]	ICAR, Kolasib	24°12′44.00″ N 92°40′32.69″ E	2057	Kolasib	Improved
37.	Gomati[b]	ICAR, Kolasib	24°12′44.00″ N 92°40′32.69″ E	2057	Kolasib	Improved
38.	RCM9[b]	ICAR, Kolasib	24°12′44.00″ N 92°40′32.69″ E	2057	Kolasib	Improved
39.	RCM10[b]	ICAR, Kolasib	24°12′44.00″ N 92°40′32.69″ E	2057	Kolasib	Improved
40.	RCM13[b]	ICAR, Kolasib	24°12′44.00″ N 92°40′32.69″ E	2057	Kolasib	Improved

[a]Represents *Indica* varieties, [b]Represents *Japonica* varieties.

ABF Agri Biotech Foundation, *ICGEB* International Centre for Genetic Engineering and Biotechnology, *ICAR* Indian Council of Agricultural Research

Amylose Content Analysis

Amylose content was measured by following the method described by Juliano (1971). Rice seeds were ground into a fine powder and 100 mg was placed into a 100 ml volumetric flask. Then, 1 ml of 95% ethanol and 9 ml of 1 M sodium hydroxide were added. The contents were boiled for 10 min. After cooling down to room temperature, the volume was made up to 100 ml with distilled water. A 5 ml solution

was taken into a fresh 100 ml volumetric flask, and 1 ml of 1 M acetic acid and 2 ml of 2% I_2KI solutions were added. The final volume was made up to 100 ml with distilled water. The absorbance was measured at 620 nm. Amylose contents of the rice samples were determined in reference to standard curve and expressed on percent basis.

Aroma Test

Aromatic characteristic of rice cultivars were identified by following the method described by Sood and Siddiq (1978). One gram of rice seed powder was taken and placed in petri dishes with5 ml of 1.7% KOH solution. After 30 min, the dishes were opened and smelled. The presence (+) or absence (−) of aroma was scored.

Extraction of Seed Storage Protein and SDS-PAGE

Seed storage protein was estimated by following the protocol suggested by Jugran et al. (2010). Where 500 µl of extraction buffer (0.5 M Tris pH 6.8, 20% glycerol, 10% SDS, 0.1% bromophenol blue and 2-mercaptoethanol) was added to 0.5 g of powdered seed and mixed by vortexing for 2 min. The sample was denatured at 100ºC for 5 min and centrifuged at 7000 rpm for 12 min. The resulting supernatant was used to run on a 10% denaturing SDS-PAGE. Sizes of the bands were estimated with reference to Protein Molecular Weight Marker (Genei, India).

SDS-PAGE Data Analysis

Polypeptide bands were scored as present (1) or absent (0) for all the samples analyzed using Image Lab 5.0 (Bio-Rad Laboratories, USA). Based on these scores, Jaccard's similarity coefficient between rice cultivars was calculated and UPGMA (The unweighted pair group method with an arithmetic mean) tree was constructed using open access online software, DendroUPGMA (https://genomes.urv.cat/UPGMA) (Garcia-Vallve and Puigbo 2002).

Genomic DNA Isolation and PCR Amplification

Genomic DNA of indigenous rice cultivars were isolated as per protocol suggested by Edwards et al. 1991. Where, leaflet from a 15-day-old seedlings after sowing was macerated using micro-pestle in a 2 ml centrifuge tube and 400 µl of extraction buffer (200 mM Tris–HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, 0.5% SDS) was added. The sample was vortex vigorously for 1 min and centrifuged at 13,000 rpm for 5 min. Then, 300 µl of the supernatant was transferred to a fresh tube with equal volume of isopropanol and then centrifuged at 13,000 rpm for 5 min. The resulting pellet was air-dried and dissolved in 100 µl TE (10 mM Tris, 1 mM EDTA) buffer.

Twelve SSR primers (Table 2), which were chosen according to their location on the rice chromosomes, were used to amplify genomic DNA (Panaud et al. 1996; Temnykh et al. 2000). Amplification was performed in an ABI Veriti 96 well

Table 2 Details of SSR primers used in the present study

S. no.	Primer name	Sequences (forward primer/reverse primer)	Chr. no.	Expected size	T_a (ºC)
1.	RM1	Fp: 5′-GCGAAAACACAATGCAAAAA-3' Rp: 5′-GCGTTGGTTGGACCTGAC-3'	1	113	55
2.	RM154	Fp: 5′-ACCCTCTCCGCCTCGCCTCCTC-3' Rp: 5′-CTCCTCCTCCTGCGACCGCTCC-3'	2	183	61
3.	RM131	Fp: 5′-TCCTCCCTCCCTTCGCCCACTG-3' Rp: 5′-CGATGTTCGCCATGGCGTCTCC-3'	4	215	61
4.	RM135	Fp: 5′-CTCTGTCTCCTCCCCCGCGTCG-3' Rp: 5′-TCAGCTTCTGGCCGGCCTCCTC-3'	3	131	55
5.	RM153	Fp: 5′-GCCTCGAGCATCATCATCAG-3' Rp: 5′-ATCAACCTGCACTTGCCTGG-3'	5	201	55
6.	RM190	Fp: 5′-CTTTGTCTATCTCAAGACAC-3' Rp: 5′-TTGCAGATGTTCCTGATG-3'	6	124	55
7.	RM125	Fp: 5′-ATCAGCAGCCATGGCAGCGACC-3' Rp: 5′-AGGGGATCATGTGCCGAAGGCC-3'	7	127	55
8.	RM72	Fp: 5′-CCGGCGATAAAACAATGAG-3' Rp: 5′-GCATCGGTACTAACTAAGGG-3'	8	166	55
9.	RM278	Fp: 5′-GTAGTGAGCCTATCAATAATC-3' Rp: 5′-TCAACTCAGCATCTCTGTCC-3'	9	141	55
10.	RM171	Fp: 5′-CGATCCATTCCTGCTGCTCGCG-3' Rp: 5′-CGCCCCCATGCATGAGAAGACG-3'	10	328	55
11.	RM287	Fp: 5′-TTCCCTGTTAAGAGAGAAATC-3' Rp: 5′-GTGTATTTGGTGAAAGCAAC-3'	11	118	55
12.	RM117	Fp: 5′-CGCCCCCATGCATGAGAAGACG-3' Rp: 5′-CGATCCATTCCTGCTGCTCGCG-3'	12	208	55

Chr. no. chromosome number, T_a annealing temperature

Thermal cycler (ABI, USA) in 25 µl reaction containing 1× PCR buffer, 200 µM dNTP mixture, 3 mM $MgCl_2$, 0.5 U Taq polymerase (Genei, India), 50 ng of each primer and 30 ng template DNA. The amplification conditions were set as, initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing temperature (depending on primer) for 30 s, extension at 72 °C for 1 min followed by final extension at 72 °C for 7 min. The amplified products were electrophoresed on 2.5% agarose gel and visualized by standard ethidium bromide staining (Sambrook et al. 2001) in an AlphaImager Mini (Protein Simple, USA). Sizes of amplified bands were ascertained by comparing with molecular weight marker (Genei, India) using Alpha View software (Protein Simple, USA).

Analysis of SSR Data

The total number of alleles, number of polymorphic loci, expected heterozygosity, Nei's gene diversity, Fst and population-wise diversity index were calculated using genetic analysis package POPGENE 1.31 (Yeh et al. 1999). Population-wise diversity index was calculated using Arlequin 3.5 (Excoffier and Lischer 2010). Analysis

of molecular variance (AMOVA) and principal coordinate analysis (PCoA) were performed in GenAlEx 6.5 (Peakall and Smouse 2012). Major allele frequency (MAF) and polymorphism information content (PIC) were calculated using PowerMarker 3.25 (Liu and Muse 2005) and UPGMA tree based on Nei's genetic distance was constructed using MEGA 6 (Tamura et al. 2013). The possible population structure was detected using a Bayesian model-based STRUCTURE 2.3.4 (Pritchard et al. 2000). The parameter was set at 100,000 burn-in periods and 100,000 Markov Chain Monte Carlo (MCMC) repeats after burn-in. A possible number of clusters (K) was determined by setting $K=1$ to $K=10$ with 10 replicate runs per K value (Evanno et al. 2005). Online program Structure Harvester (Earl and von Holdt 2012) was used to identify final K value.

Results

Seed Morphology, Amylose Content and Aroma Test

Seeds of the studied rice cultivars showed distinguished variation in its grain quality traits (Table 3). The longest grain length was recorded for Kawnglawng (11.4 mm), whereas the shortest grain length for Zawngin buh (7.16 mm) with mean population grain length of 9.32 mm. On the other hand, grain width was ranged from 2.66 mm (Biruchuk) to 3.82 mm (Buhban langakthou) with an average of 3.17 mm. The highest length/width ratio was observed in Biruchuk (3.77), while the least value was recorded in Buhbial (2.08). The average length/width ratio was found to be 2.96. A 1000-grain weight ranged from Dengchungnunga (22.87g) to Kawnglawng (38.84 g) with an average of 28.33 g. Dendrogram constructed based on grain quality traits using NTSYSpc grouped indigenous rice cultivars into three clusters (Fig. 2). Cluster I comprising 9 cultivars, cluster II was represented by 9 populations and cluster III comprising 12 cultivars.

Amylose content of studied rice cultivars ranged from 9.72% (Fazai ban) to 47.31% (Vaibuh) with an average of 25.97% (Table 3). The amylose content of rice can be classified as waxy (0–2%), very low (3–9%), low (10–19%), intermediate (20–24%), high (25–29%) and very high (> 30%) (Juliano 1971). According to this classification, one cultivar (Fazai ban) possessed very low amylose content, 14 cultivars (Vaiphei, Kawnglawng, Tuikuk buh, Kawnglawng tial, Buhban langakthou, Laithangnu, Tai sanghar, Tai te, Buhban hmui, Idaw, Mangbuh, Naga, Fazu and Dengchungnunga) possessed low amylose content. Three cultivars (Kungtawi sen, Buhngat and San) contained intermediate amylose. Kawnglawng var possessed high amylose content and 10 cultivars (Biruchuk, Fare, Buhbial, Fazai, Zawngin buh, Baimasa, Bialte, Buhpui, Phodum, Vaibuh and Varsiama possessed very high amylose content.

Aroma test was performed for indigenous rice cultivars using 1.7% KOH solution. Eleven out of thirty rice cultivars viz., Kungtawi sen, Kawnglawng, Tuikuk buh, Kawnglawng tial, Kawnglawng var, Laithangnu, Tai sanghar, Tai te, Fazu and Phodum possessed aromatic characteristics (Table 3).

Table 3 Seed morphological features of rice cultivars of Mizoram

S. no.	Name	Grain length (mm)	Grain width (mm)	1000-grain weight (g)	L/W ratio	State[a]	Amylose content (%)	Aroma test[b]
1.	Kungtawi sen	10.14	3	23.08	3.38	ELS	20.38	+
2.	Vaiphei	9.3	3.36	30.12	2.77	LB	16.08	−
3.	Kawnglawng	**11.4**	3.36	**38.84**	3.40	ELS	15.17	+
4.	Biruchuk	10.02	*2.66*	26.92	**3.77**	ELS	36.97	−
5.	Tuikuk buh	10	3.16	26.44	3.16	ELS	13.94	+
6.	Fare	9.14	3.04	27.32	3	ELS	34.91	−
7.	Kawnglawng tial	10.22	3.36	33.09	3.04	ELS	13.77	+
8.	Kawnglawng var	9.12	3.62	30	2.52	LB	28.96	+
9.	Buhban Langakthou	10.6	**3.82**	37.23	2.77	LB	17.24	−
10.	Buhbial	7.28	3.5	26.05	*2.08*	LB	40.28	−
11.	Fazai	9.2	3	27.12	3.06	ELS	30.03	−
12.	Laithangnu	9.68	3.38	28.57	2.86	LB	17.57	+
13.	Tai sanghar	10.46	3.02	27.16	3.46	ELS	13.19	+
14.	Tai te	8.92	2.7	23.25	3.3	ELS	18.56	+
15.	Zawngin buh	*7.16*	3.08	24.91	2.32	LB	41.93	−
16.	Baimasa	9	2.98	26.7	3.02	ELS	37.22	−
17.	Bialte	7.72	3.46	25.42	2.23	LB	36.48	−
18.	Buhban hmui	10.92	2.96	31.23	3.69	ELS	10.96	+
19.	Buhngat	9.1	3.12	29.15	2.92	LB	24.25	−
20.	Fazai ban	10.74	3.06	30.88	3.51	ELS	*9.72*	−
21.	San	9.02	3.04	26.98	2.97	LB	24.01	−
22.	Idaw	10.24	2.98	30.04	3.44	ELS	12.94	−
23.	Mangbuh	8.28	3.02	24.61	2.74	LB	17.32	−
24.	Buhpui	8.3	3.06	25.25	2.71	LB	31.11	−
25.	Naga	9.18	3.16	30.42	2.9	LB	11.71	−
26.	Fazu	9.16	3.56	30.89	2.58	LB	10.05	+
27.	Phodum	9.26	3.12	29.17	2.96	LB	30.37	+
28.	Vaibuh	8.08	3.28	25.98	2.46	LB	**47.30**	−
29.	Varsiama	8.9	3.5	30.11	2.54	LB	30.62	−
30.	Dengchungnunga	9.06	2.76	*22.87*	3.28	ELS	19.38	−
	Mean	9.32	3.17	28.33	2.96		23.75	
	SE	1.03	0.27	0.68	0.42		1.98	

[a]As referred in Rice Research in India: ICAR Publication, 1985. *ELS* Extra long slender, *LB* Long bold.
[b] + represents presence of aroma and − represents absence of aroma. For each grain quality trait, highest value cell was indicated in bold and lowest value cell was indicated in italics

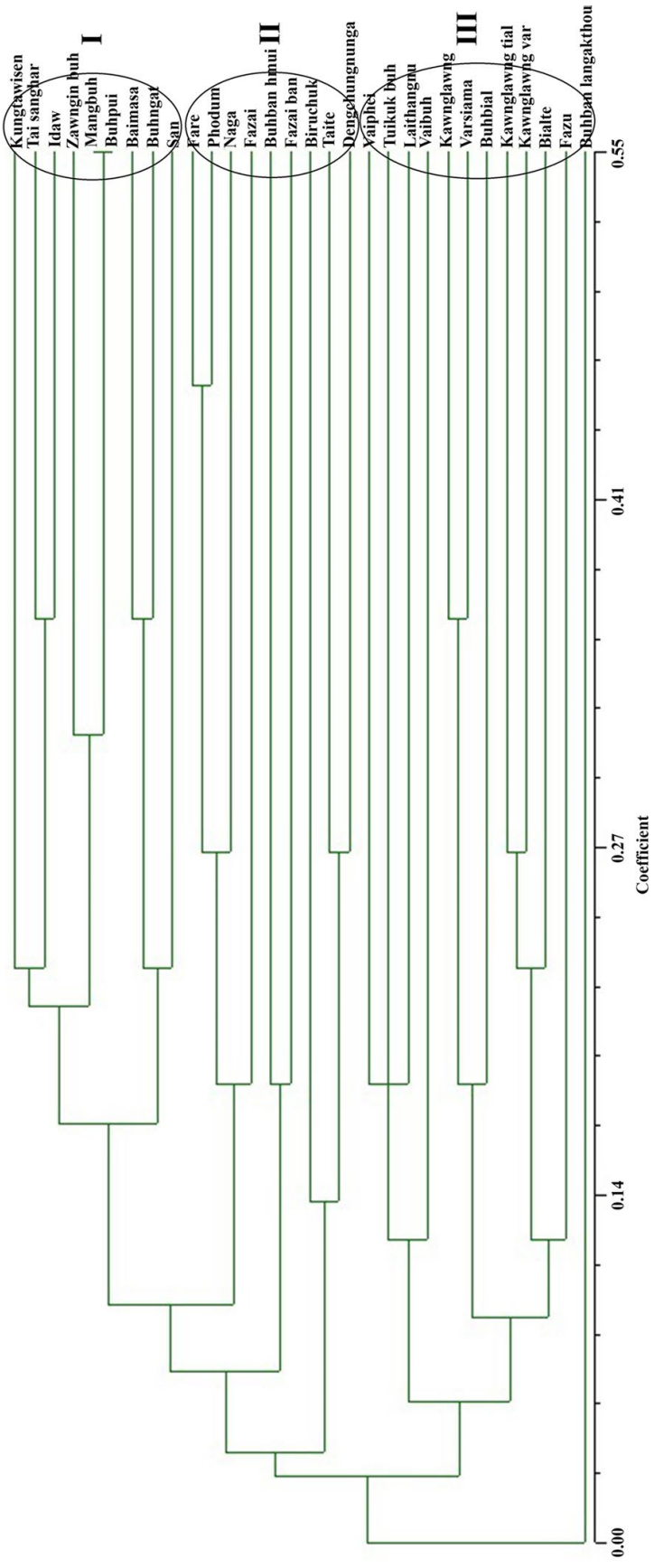

Fig. 2 Dendrogram based on seed morphology of indigenous rice cultivars of Mizoram

Protein Profiling

The SDS-PAGE analysis of seed storage proteins of hill rice cultivars of Mizoram showed little variation among different populations. The number of polypeptide bands per cultivar ranged from 7 to 10. Fourteen cultivars viz., Kawnglawng, Tuikuk buh, Fare, Kawnglawng tial, Buhban Langakthou, Buhbial, Fazai, Laithangnu, Tai sanghar, Zawngin buh, Bialte, Fazai ban, Idaw and Fazu exhibited seven bands, ten cultivars viz., Kungtawi sen, Vaiphei, Kawnglawng var, Tai te, Baimasa, Buhban hmui, San, Buhngat, Vaibuh and Varsiama exhibited 8 bands, five cultivars viz., Buhngat, Mangbuh, Naga, Phodum and Dengchungnunga exhibited 9 bands and only one population, Biruchuk possessed 10 bands. A UPGMA tree constructed based on Jaccard's similarity coefficient using DendroUPGMA showed three clusters. Cluster I was represented by only one cultivar (Biruchuk), cluster II by 9 cultivars and Cluster III comprise of 20 cultivars (Figs. 3, 4).

Analysis of SSR Diversity

Out of 12 simple sequence repeats primers used in the present study, 11 were found to be polymorphic (91.67% polymorphism). A total of 63 bands were detected using these 12 primers and the maximum band (10) was amplified by primer RM135 while the minimum band (1) was generated by RM278 across all the cultivars screened (Table 4). Major allele frequency (MAF) varied from 0.2475 (RM72) to 0.9463 (RM153) with an average of 0.4674 while expected heterozygosity ranged from 0.1210 (RM153) to 0.8320 (RM135) with averages of 0.6468, respectively.

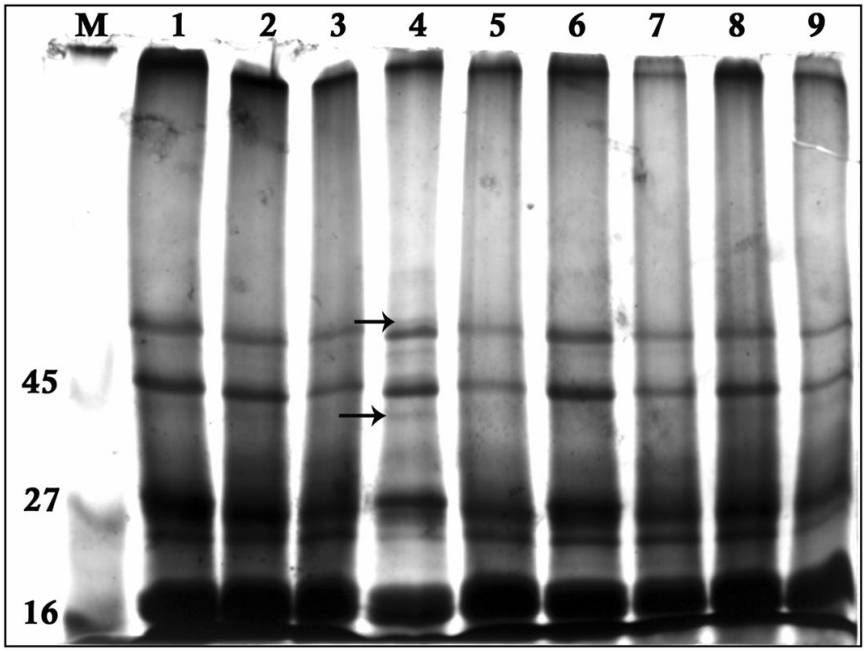

Fig. 3 Protein profile of rice cultivars of Mizoram. (Lanes 1–9 indicated individuals from Kungtawi sen, Vaiphei, Kawnglawng, Biruchuk, Tuikuk buh, Fare, Kawnglawng tial, Kawnglawng var and Buhban langakthou.) Lane M represents protein marker. Arrows indicated polymorphic bands

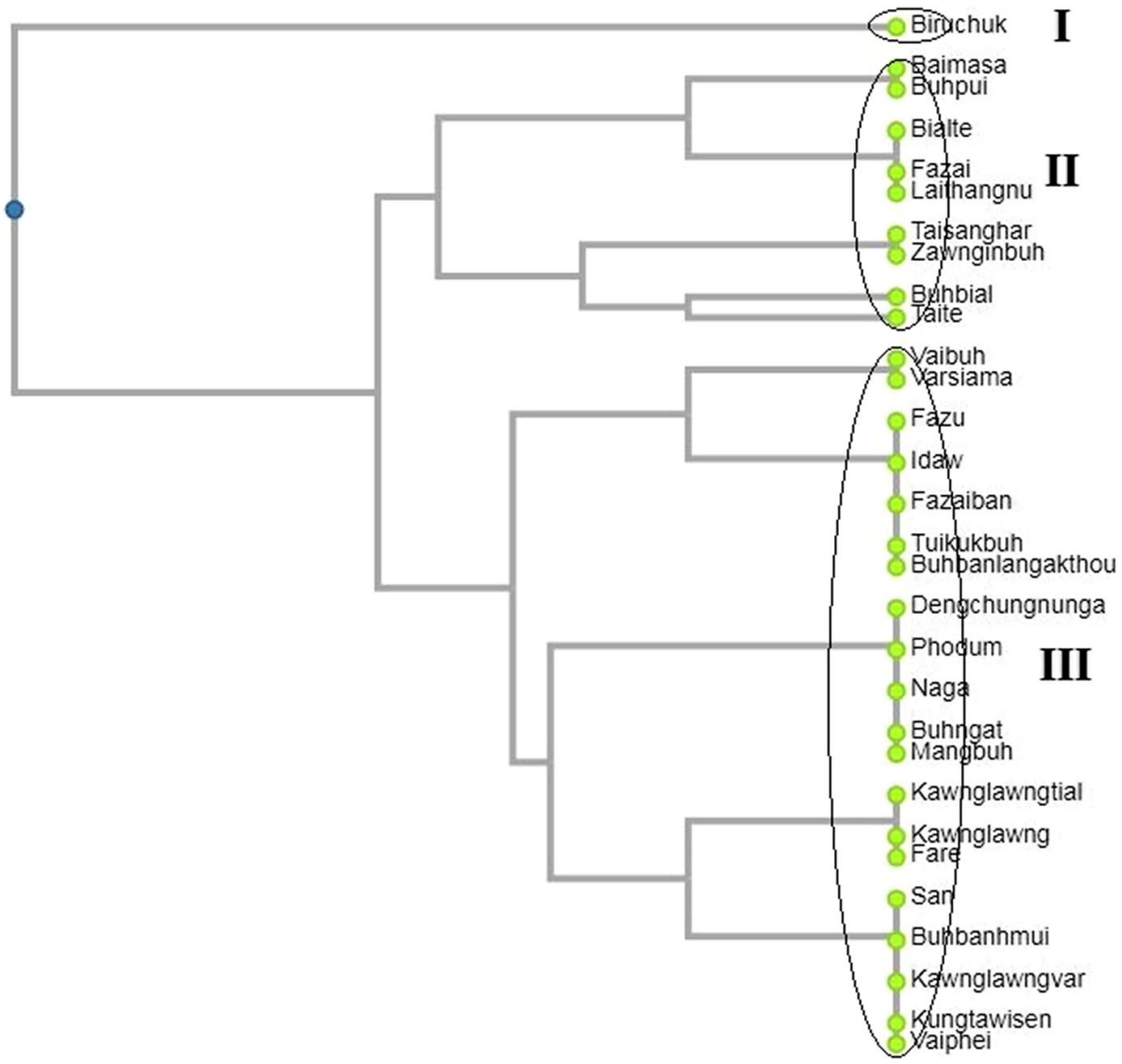

Fig. 4 Dendrogram of indigenous rice cultivars of Mizoram based on seed storage protein

Nei's gene diversity varied from 0.1208 (RM153) to 0.8310 (RM135) with an average of 0.6460. Genetic differentiation (Fst) varied from 0.4400 (RM117) to 0.9344 (RM287) with an average of 0.7239. The polymorphism information content (PIC) value ranged from 0.0973 (RM153) to 0.8134 (RM135) with an average of 0.5984 (Table 5; Fig. 5).

Analysis of Population Structure Based on SSR Data

Model-based grouping method, STRUCTURE, supported genetic structure of the population into three groups with the highest ΔK at $K = 3$ (Fig. 6 and 7). An UPGMA clustering based on genetic distance also grouped the studied cultivars into three clusters. Cluster I represents all *Japonica* check varieties used in the present study and 9 indigenous cultivars—Bialte, Buhngat, Fazai ban, Vaibuh, Varsiama, Buhban hmui, Phodum, Dengchungnunga, and Naga. Cluster II consists of *Indica* varieties and cluster III comprising 21 indigenous cultivars viz., Biruchuk, Kawnglawng, Laithangnu, Fare, Buhban Langakthou, Kawnglawng tial, Tai sanghar, Baimasa,

Table 4 Genetic parameters as revealed by polymorphic SSR primers

Locus	na	ne	MAF	H_E	Nei's	Fst	PIC
RM1	7.0000	4.6881	0.3263	0.7877	0.7867	0.8796	0.7564
RM154	7.0000	3.9412	0.3088	0.7472	0.7463	0.5630	0.7006
RM131	9.0000	4.8522	0.3838	0.7949	0.7939	0.5618	0.7673
RM135	10.0000	5.9157	0.3025	0.8320	0.8310	0.5258	0.8134
RM153	3.0000	1.1374	0.9463	0.1210	0.1208	0.6245	0.0973
RM190	4.0000	3.0936	0.4750	0.6776	0.6768	0.8201	0.6243
RM125	3.0000	1.8891	0.6350	0.4712	0.4707	0.8598	0.3689
RM72	7.0000	5.6286	0.2475	0.8234	0.8223	0.8524	0.7982
RM171	5.0000	3.4649	0.3625	0.7123	0.7114	0.9016	0.6617
RM287	3.0000	2.0420	0.6400	0.5109	0.5103	0.9344	0.4434
RM117	4.0000	2.7467	0.5138	0.6367	0.6359	0.4400	0.5611
Mean	5.6363	3.5817	0.4674	0.6468	0.6460	0.7239	0.5984
SD±	2.7345	1.6753	0.1973	0.2750	0.2747	0.1722	0.2097

na observed number of alleles, *ne* effective number of alleles, *MAF* major allele frequency, H_E expected heterozygosity, *Nei* Nei's gene diversity, *Fst* genetic differentiation, *PIC* polymorphism information content

Mangbuh, Buhpui, Idaw, Fazu, Kungtawi sen, Vaiphei, Tuikuk buh, Kawnglawng var, Taite, Zawngin buh, Buhbial, Fazai and San. G1 in barplot and cluster I in dendrogram were totally similar, G2 cultivars were located in cluster III except *Indica* varieties which formed cluster II, G3 cultivars were present together in cluster III. Analysis of molecular variance (AMOVA) showed that 67% of total variation was due to among-population differentiation and 33% was due to within-individual differentiation. Population-wise diversity indices (Table 4) showed that the expected heterozygosity (or gene diversity) of Biruchuk was highest at 0.3382, followed by Baimasa (0.3346) and Kawnglawng (0.3013) and the least expected heterozygosity was found in Tuikuk buh (0.0443). The average distance between individuals in the same cluster ranged from 0.4019 (cluster III), 0.4549 (cluster II) and 0.4867 (cluster I). The mean Fst value of cluster I was 0.3253 and that of cluster II and cluster III were 0.3357 and 0.4965, respectively. The mean alpha value was found to be 0.0341. Principal coordinates analysis showed three distinct groups among the studied populations which were further confirmed by the STRUCTURE results and UPGMA tree. Coordinate 1 extracted 26.58% of the variation while coordinate 2 extracted 16.46% of the variation (Tables 6, 7; Figs. 8, 9).

Discussion

Grain quality traits of upland rice genotypes of Mizoram were examined and the average grain length and width were found to be 9.32 mm and 3.17 mm, respectively. Seed length of Kawnglawng was the highest among the studied cultivars which is a similar value to an earlier report on Indian rice (Pachauri et al. 2013). As

Table 5 Population-wise diversity indices

S. no.	Name	H_E	No. of polymorphic loci
1.	Kungtawi sen	0.1228	3
2.	Vaiphei	0.1890	4
3.	Kawnglawng	0.3013	7
4.	Biruchuk	0.3382	7
5.	Tuikuk buh	0.0443	2
6.	Fare	0.2114	4
7.	Kawnglawng tial	0.1987	5
8.	Kawnglawng var	0.0803	5
9.	Buhban Langakthou	0.2101	5
10.	Buhbial	0.0539	2
11	Fazai	0.1811	6
12.	Laithangnu	0.1838	5
13.	Tai sanghar	0.2105	5
14.	Tai te	0.0557	2
15.	Zawngin buh	0.1167	3
16.	Baimasa	0.3346	8
17.	Bialte	0.2487	7
18.	Buhban hmui	0.1627	5
19.	Buhngat	0.2092	6
20	Fazai ban	0.1228	3
21.	San	0.1531	4
22.	Idaw	0.2053	5
23.	Mangbuh	0.2500	6
24.	Buhpui	0.2329	6
25.	Naga	0.1167	4
26.	Fazu	0.1991	6
27.	Phodum	0.1754	5
28.	Vaibuh	0.1259	5
29.	Varsiama	0.1430	4
30.	Dengchungnunga	0.0895	3
31.	BM71	0.1825	4
32.	IR71033-121-15B	0.1846	4
33.	MO1	0.1386	3
34.	PTB33	0.1829	4
35.	TN1	0.2123	5
36.	CAUR1	0.1123	3
37.	Gomati	0.1386	3
38.	RCM9	0.1474	4
39.	RCM10	0.2127	6
40.	RCM13	0.1263	3

H_E expected heterozygosity (Nei 1978)

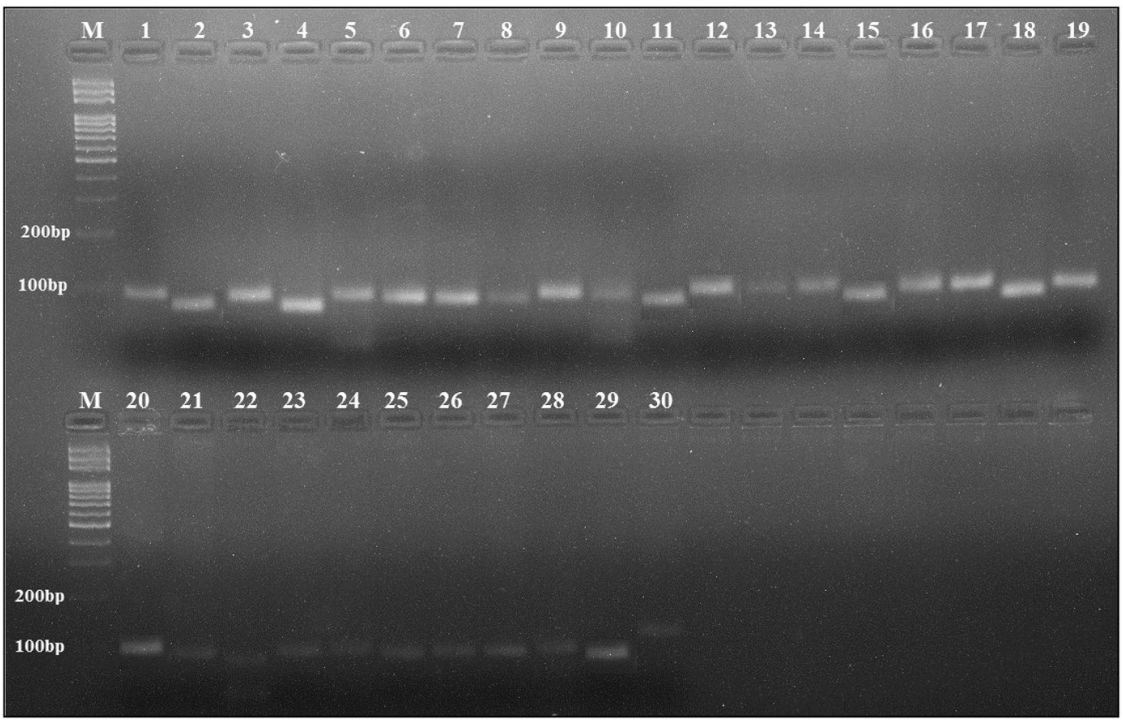

Fig. 5 A 2.5% agarose gel showing banding pattern of Mizoram rice cultivars generated by RM1. M represents 100bp DNA ladder

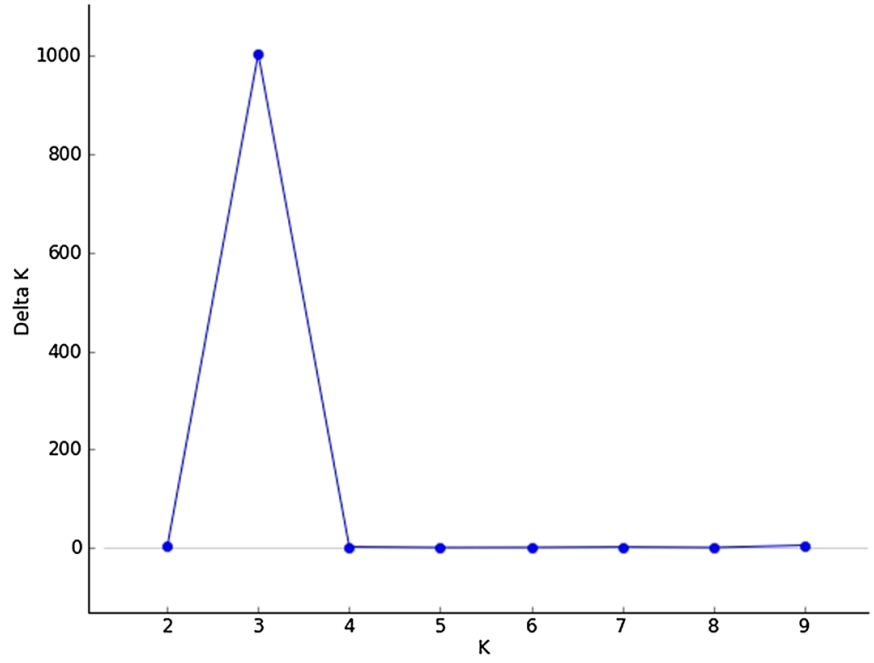

Fig. 6 Relationship between ΔK and K showing a true K for the three groups ($K=3$)

referred in rice research in India, ICAR publication, rice cultivars can be categorized into short slender, short bold, medium slender, long slender, long bold, basmati type and extra long slender based on grain length and length/width ratio. In the present study, 16 cultivars can be categorized into long bold and 14 cultivars were extra

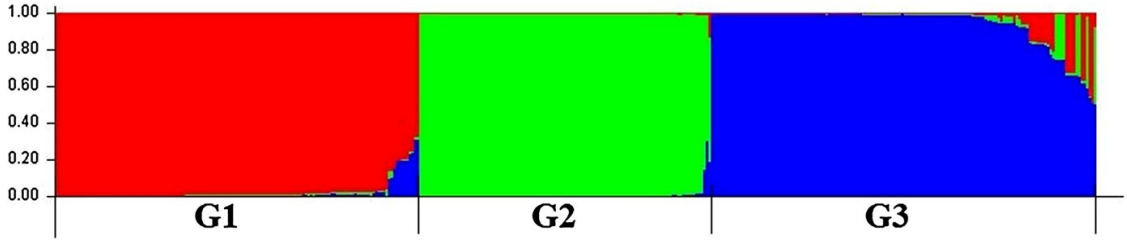

Fig. 7 Population structure of indigenous rice cultivars of Mizoram obtained from STRUCTURE. G1, G2, G3 = Group 1, group 2, group 3. Different groups were represented by different colors

long slender type. It is likely that slender grains have higher market values than the bold ones (Verma et al. 2012) and nearly 50% of the Mizoram rice cultivars fall in slender type. The average L/W ratio was slightly smaller as compared to the previous study (Pachauri et al. 2013). Amylose content is one of the most important grain quality trait (Avaro et al. 2009). Rice cultivars containing low amylose are soft and sticky when cooked, while rice cultivars with high amylose content are dry and less tender when cooked and become hard after cooling (Jain et al. 2012). Rice cultivars with low amylose content found in the present study are also locally known as sticky rice. Aroma characteristic is common in low amylose content cultivars in the present study.

SDS-PAGE analysis showed that the protein bands ranged from 7 to 10 among rice cultivars. Dendrogram constructed based on Jaccard's similarity coefficient using DendroUPGMA showed that more than half of the studied cultivars (66.6%) were grouped into the same cluster which may be indicative of genetic relatedness with respect to seed storage protein profile. While the number of clusters was more compared to earlier studies on rice of Uttarakhand State, India (Jugran et al. 2010) and rice accessions from International Rice Molecular Breeding Programme (Dhawale et al. 2015), Tahir (2014) has indicated that number of clusters might be due to high variation among the cultivars. Although the seed storage protein profiles of intra-species are very similar, the variations in numbers and positions of bands were also reported (Wei-dong et al. 2006; Vithyashini and Wickramasinghe 2015). The similarity banding pattern of seed storage protein made it a unique and powerful tool in evolutionary and diversity studies (Ladizinsky and Hymowitz 1979). Buhban langakthou showed distinct variation among the studied cultivars based on seed quality traits, while Biruchuk showed distinct variation from other cultivars based on protein profile.

Twelve SSR markers, each mapped to a chromosome of the rice genome, detected 63 alleles. The number of alleles per locus ranged from 1 to 10. According to Barker (1994), each locus should exhibit more than four alleles to reduce the standard error and to get a good result in the genetic diversity study. In our study, four loci viz., RM153, RM125, RM278, and RM287 generated less than four alleles but the average alleles per locus were found to be more than four. The mean expected heterozygosity for polymorphic loci found in the present study was a high value of heterozygosity index and showed similarity to a previous study (Roy et al. 2015). PIC determines the usefulness of the markers for linkage analysis (Elston 2005). The

Table 6 Grouping pattern of studied genotypes between barplot obtained from Structure and UPGMA tree constructed using Mega

S. no.	Name	Groups in barplot	Clusters in UPGMA
1.	Bialte	1	I
2.	Buhban hmui	1	I
3.	Buhngat	1	I
4.	Fazai ban	1	I
5.	Naga	1	I
6.	Phodum	1	I
7.	Vaibuh	1	I
8.	Varsiama	1	I
9.	Dengchungnunga	1	I
10.	CAUR1	1	I
11	Gomati	1	I
12.	RCM9	1	I
13.	RCM10	1	I
14.	RCM13	1	I
15.	BM71	2	II
16.	IR71033-121-15B	2	II
17.	MO1	2	II
18.	PTB33	2	II
19.	TN1	2	II
20	Kungtawi sen	2	III
21.	Vaiphei	2	III
22.	Tuikuk buh	2	III
23.	Kawnglawng var	2	III
24.	Buhbial	2	III
25.	Fazai	2	III
26.	Tai te	2	III
27.	Zawngin buh	2	III
28.	San	2	III
29.	Kawnglawng	3	III
30.	Biruchuk	3	III
31.	Fare	3	III
32.	Kawnglawng tial	3	III
33.	Buhban Langakthou	3	III
34.	Laithangnu	3	III
35.	Tai sanghar	3	III
36.	Baimasa	3	III
37.	Idaw	3	III
38.	Mangbuh	3	III
39.	Buhpui	3	III
40.	Fazu	3	III

Table 7 Analysis of molecular variance

Source	*df*	SS	MS	%
Among populations	39	2075.527	53.222	67
Within individuals	400	520.000	1.300	33

df degree of freedom, *SS* sum of square, *MS* means of square, % percentage variation

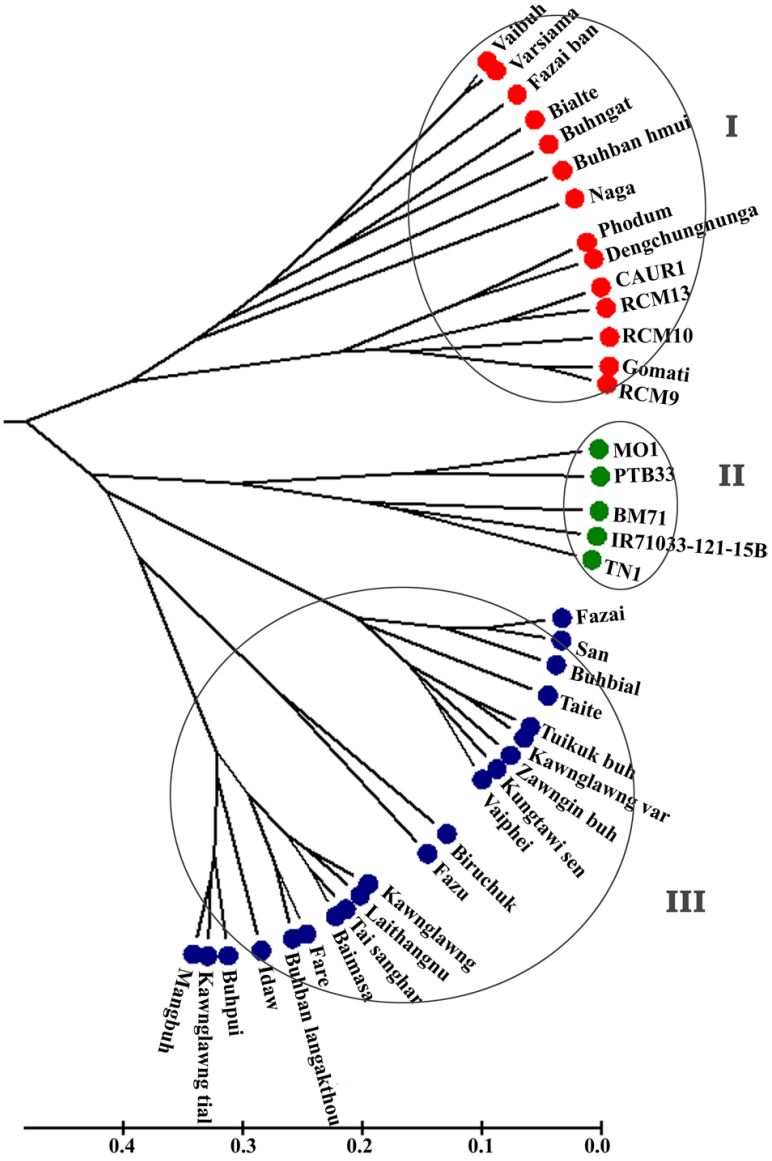

Fig. 8 A UPGMA tree of rice cultivars of Mizoram

mean PIC value in this study was higher than previously reported in NE rice cultivars, indicated that the SSR markers used in the current study were good enough for studying genetic diversity. Considering the parameters (Babu et al. 2014) of PIC value (≥ 0.70), expected heterozygosity (≥ 0.71), polymorphic alleles (≥ 6), RM1, RM154, RM131, RM135, and RM72 were found to be the most polymorphic loci among the studied markers.

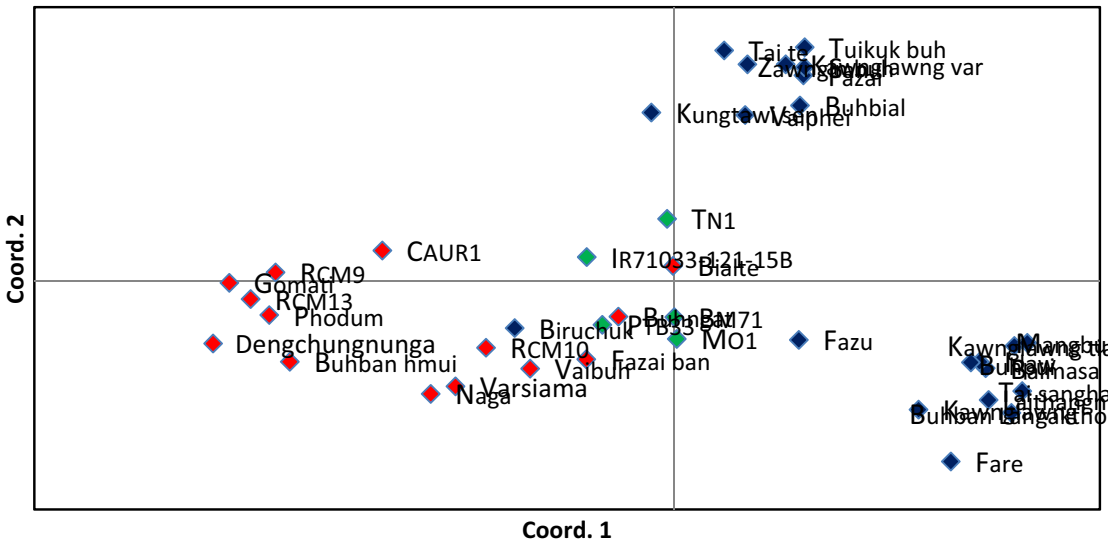

Fig. 9 Principal coordinate analysis (PCoA) of 40 rice cultivars based on Nei's genetic diversity. Group 1, 2 and 3 were indicated by red, green and blue colors, respectively

The ΔK peak at $K=3$ indicated that all the studied rice cultivars could be divided into three clusters. This result was strongly in agreement with principal coordinates analysis obtained and UPGMA tree. Cluster I represents *Japonica* test cultivars and cluster II represents *Indica* group while cluster III cultivars could be admixed or interchanged between *Japonica* and *Indica*. Choudhury et al. (2013) have also found out that Kawnglawng cultivar was interchanged between *Indica* and *Japonica*. Nine cultivars which are present together in cluster III in dendrogram formed a group with *Indica* varieties in bar plot, represented by same color. Another twelve cultivars in cluster III formed one group different from *Indica* and *Japonica* group in bar plot which may be indicated that these cultivars are admixed. Indigenous rice cultivars of Cluster I were from one village which indicated that clustering in the present study is more or less affected by geographic condition. This geographic clustering was also reported in aromatic and quality rice of North East India (Roy et al. 2015). This may be due to the chance of gene flow within intra-species is higher in geographically close cultivars than far distance. Genetic diversity and levels of gene flow were also influenced by human activities, farming practices, cultivar preferences, etc (Roy et al. 2015). According to Evanno et al. (2005), an alpha value close to zero implies most of the individuals are from one population or another, and an alpha value greater than 1 indicated that most individuals are admixed. The mean value of alpha (the degree of admixture) was 0.0341. According to Wright (1978), Fst values from 0 to 0.05 indicate little genetic differentiation, 0.05 to 0.15 indicate moderate, 0.15 to 0.25 indicate great and more than 0.25 indicate very great genetic differentiation. In this study, the average Fst value of three clusters indicated the existence of great genetic differentiation among clusters. Comparing the three phylogenetic trees (Seed tree = T1, Protein tree = T2, SSR tree = T3) in the present study, mention may be made that seed morphology had the least effect on the genetic relationship since the distribution of rice cultivars in T1 was dissimilar to the other T2 and T3. This could be due to less number of morphometric parameters employed in the current study.

However, T2 and T3 were comparable. For instance, cluster II of T2 possessed nine cultivars which were also present together in a cluster in T3 except one cultivar (Bialte). Similarly, Cluster I of T3 possessed nine indigenous cultivars which were also grouped together in Clusters III of T2. It is cleared from these results that the studies of genetic diversity based on seed storage protein and SSR markers had more or less kinship.

Many farmers of Mizoram practice shifting cultivation or jhuming cultivation and prefer indigenous cultivars, not only rice but also other crops, due to their ability to grow in different local conditions, drought to heavy rain, salinity, etc, easily available and lack of lowland for farming practices. Though shifting cultivation causes environmental issues, the practice serves as a conservation field for indigenous crop cultivars. Previous researchers also pointed out that the upland regions of India represent a valuable center for the conservation of the diversity of indigenous rice varieties (Gayacharan et al. 2015). Landraces of rice may contain considerable genetic diversity and genetically variable traits, thus are good sources for future rice improvement because they are thought to be an intermediate stage between wild rice species and cultivated rice (Choudhury et al. 2013; Li et al. 2014). Knowledge on genetic diversity of these landraces of rice is essential for conservation, utilization and management because it is the basis of the plant breeding (Rao and Hodgkin 2002; Sohrabi et al. 2012).

The high genetic diversity observed in the indigenous rice samples such as Biruchuk, Baimasa, Kawnglawng, etc. may be useful as a resource for future rice improvement or breeding program. Some cultivars exhibited a low level of genetic diversity (Table 4) suggesting necessity action with respect to conservation strategies. The present study is a preliminary report on genetic diversity and grain quality traits of landraces of rice of Mizoram. Although it will be helpful for conservation and utilization, further investigations on agronomy, qualitative and quantitative traits are needed to be undergone to select valuable parental lines for successful future breeding programs.

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SALT TOLERANCE PROFILES IN INDIGENOUS RICE (*ORYZA SATIVA* L.) VARIETIES OF MIZORAM, INDIA BASED ON PHYSIOLOGICAL AND SALT-LINKED MICROSATELLITE MARKERS

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Key words: *Rice, Indigenous, NaCl, Tolerance, STI, Microsatellite, Mizoram*

Abstract – The salt tolerance of six indigenous rice varieties of Mizoram and one susceptible variety during germination and early seedling growth was investigated using different NaCl concentrations, and their genetic relationship was assessed using three salt-linked microsatellite markers – RM223, RM493 and RM3412. Germination percentage, shoot and root length, total dry weight and salt tolerance index (STI) were evaluated. Shoot and root length, dry weight and STI decreased as the concentration of NaCl increased. Different concentrations of NaCl had no effect on the germination of the studied varieties. Idaw showed highest level of tolerance, at highest salt concentration, among the studied varieties. Dendrogram based on salt-linked microsatellite markers distinguished salt tolerant varieties similar to that of physiological study.

INTRODUCTION

Rice (*Oryza sativa* L) is one of the most important staple crops of the world. It is one of the major food crops in the world, but is also considered as extremely salt-sensitive (Maas and Hoffman, 1977). Salinity is one of the most common soil problems in rice-growing countries (Senadhira, 1987; Dhar *et al*., 2012). The area affected by salinity in the world covers about 400 million hectares (Flowers *et al*., 1977), of which 54 million hectares are found in South and South East Asia (Akbar and Ponnamperuma, 1982; Thach *et al*., 1999). Salinity causes unfavorable hydrological and ecological situation that restrict the normal crop production throughout the year (Haque, 2006).

Salt tolerance is defined as the ability of the plant to complete the growth cycle and survive under salinity (Bagci *et al*., 2003). Zeng *et al*., (2002) opined that salinity problems in crop production would become worst as human population is increasing and decreasing better quality water resources would force the growers to use poor quality water for irrigation.

Saline soils are enriched with salts that include sodium chloride (NaCl), sodium sulfate (Na_2SO_4), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$), and sodium chloride (NaCl) is a major salt contaminant in most saline soils (Cha-um *et al*., 2010). The effects of Na+ ions are well established because this ion can cause damage to plant cells by both ionic and osmotic effects, which lead to growth retardation, low productivity and eventually cell death (Hasegawa *et al*., 2000).

Mizoram is one of the north eastern states of India. The area covers about 21,087 km^2 and lies between 21°56′N to 24°31′N Latitude and 92°16′E to 93°26′E Longitude. Rice is the largest crop grown in Mizoram. Indigenous crop varieties are mostly grown in upland regions, so that these regions of the State represent valuable conservation center for genetic variability of indigenous varieties, not only rice, but also other crops.

In the present study, the salt tolerance levels of six rice varieties of Mizoram were evaluated using different NaCl concentrations and salt-linked microsatellite markers. A susceptible variety, TN1 was also included for comparative analysis.

MATERIALS AND METHODS

Plant materials

Six indigenous rice varieties, namely, Tailuaia hmui, Zaitlai, Tai buhpui, Fangsin, Fazupui and Idaw were collected from different villages of Mizoram. TN1

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was a kind gift from Dr. Suresh Nair, ICGEB, New Delhi. Table 1 shows the names and places of collection of rice varieties.

Salt tolerance at seedling stage

Seeds were surface sterilized with 10% NaClO for 15 minutes and treated with varying NaCl concentrations (0 mM, 30 mM, 60 mM, 90 mM, 120 mM and 150m mM) and then allowed to germinate. Ten day old seedlings were measured for germination percentage. Shoot and root lengths were calculated three weeks after treatment. Then, shoots and roots were dried at 70^0C for 45 minutes and their dry weights measured. Salt tolerance Index (STI) was calculated as total plant dry weight obtained from different salt treatments compared to total plant dry weight obtained from control (Bagci *et al.,* 2003).

STI = (TDW at Sx / TDW at S1) x 100

Where, STI = salt tolerance index, TDW = total dry weight, Sx = different salt treatments, S1 = control treatment. All experiments were performed in replicates.

Molecular screening using salt-linked SSR markers

Genomic DNA was isolated following the protocol described by Edwards *et al.,* (1991). Three simple sequence repeats (SSRs) primers linked to salt tolerance (Lang *et al.,* 2001; Karmakar *et al.,* 2012; Ali *et al.,* 2014) were used for amplification of genomic DNA. The details of markers are obtained from GRAMENE (http://www.gramene.org). Amplification was performed in ABI Veriti 96 well Thermal cycler (ABI, USA) in 10 µL reaction containing 1X PCR buffer, 100µM dNTP mixture, 3mM $MgCl_2$, 0.1U Taq polymerase (Genie, India), 50ng of each primer and 50ng template DNA. The amplification conditions were set as, initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute followed by final extension at 72°C for 7 minutes. The amplified products were electrophoresed on 2.5 % agarose gel and visualized by standard ethidium bromide staining (Sambrook *et al.,* 2001).

Alleles were scored using Alpha View software (Alpha Imager, Protein Simple, USA). The unweighted pair group method with an arithmetic mean (UPGMA) dendrogram was constructed using MEGA 6 (Tamura *et al.,* 2013) based on Nei's genetic distance.

RESULTS

Germination percentage

All seeds of studied varieties show 100% germination percentage for all salt treatments.

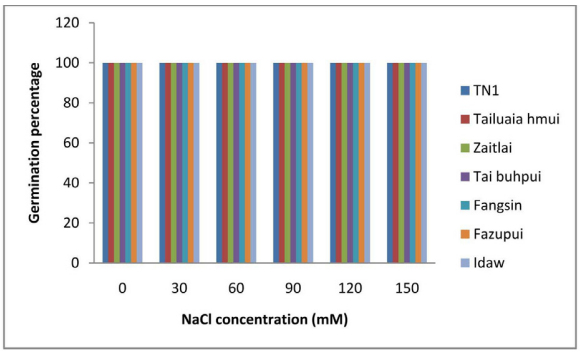

Fig. 1. Effects of NaCl salinity on germination percentage.

Shoot and root length

The shoot and root lengths decreased as the concentration of NaCl increased (Figure 2 and 3). The shoot length of Fazupui at 30 mM was longer than control treatment. Similarly, the shoot lengths of Fangsin and Fazupui at 150 mM were longer than at 120 mM. Tai buhpui also produced the shoot length longer at 90 mM than 60 mM. Idaw produced the longest root among varieties for all salt treatments except with 150 mM treatment.

Table 1. Rice varieties used in the present study.

Sl. No.	Variety	Place of collection	District
1.	TN1	ICGEB	New Delhi
2.	Tailuaia hmui	Khawruhlian	Aizawl, Mizoram
3.	Zaitlai	Mimbung	Champhai, Mizoram
4.	Tai buhpui	Rawpuichhip	Mamit, Mizoram
5.	Fangsin	Zobawk	Lunglei, Mizoram
6.	Fazupui	Zobawk	Lunglei, Mizoram
7.	Idaw	Zobawk	Lunglei, Mizoram

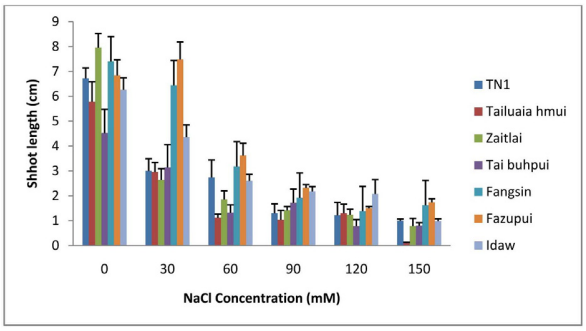

Fig. 2. Effects of NaCl salinity on shoot length.

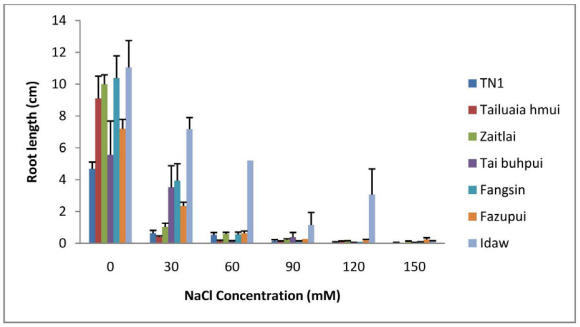

Fig. 3. Effects of NaCl salinity on root length.

Dry weight

Similar to the shoot and root length, total dry weight also decreased as the NaCl concentration increased with the exception of Fazupui where dry weight was exactly the same at 30 mM as that of control treatment, then decreased with increasing salt concentration.

Salt tolerance index: The salt tolerance index (Figure 5 and table 2) varied between 100% (Fazupui) to 46.86% (Zaitlai) at 30 mM of NaCl, 52.99% (Idaw) to 13.14% (Tai buhpui) at 60 mM of NaCl, 37.64% (Idaw) to 7.52% (Fangsin) at 90 mM of NaCl, 33.57% (Idaw) to 4.78% (Tai buhpui) at 120 mM of NaCl and 13.18% (Idaw) to 1.31% (Tailuaia hmui) at 150 mM of NaCl. The ranges of salt tolerance indices among concentrations were very wide ranging from 100% at 30 mM of NaCl to 1.31% at 150 mM of NaCl. The highest salt tolerance indices were recorded for Fazupui, Fangsin and Idaw (100%, 93.2% and 90.64%) treated with 30 mM. The lowest indices were at Tailuaia hmui, TN1 and Tai buhpui treated with 150 mM. STI of Idaw was highest (13.18%) at 150 mM followed by Zaitlai (12.52%).

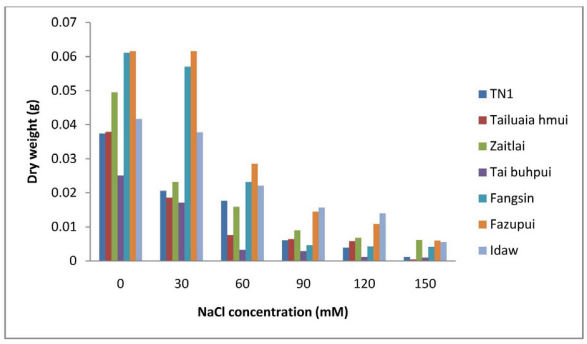

Fig. 4. Effects of NaCl salinity on total dry weight.

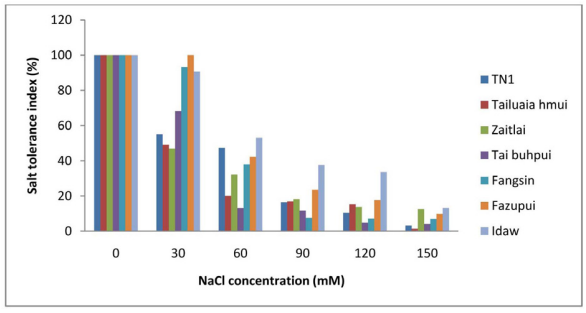

Fig. 5. Effects of NaCl salinity on salt tolerance index.

Molecular Screening using SSR markers

The allele profiles of three SSR markers, viz., RM223, RM492 and RM3412 were used to construct a dendrogram based on Nei's genetic distance. The dendrogram grouped the studied varieties into two

Table 2. SSR primers used in the present study.

Sl No	Primer name	Sequences (Forward primer/Reverse primer)	Chr. no.	Anneal. temp.
1.	RM223	Fp – 5′-GAGTGAGCTTGGGCTGAAAC-3′		
		Rp – 5′-GAAAGGCAAGTCTTGGCACTG-3′	8	55
2.	RM493	Fp – 5′-TAGCTCCAACAGGATCGACC-3′		
		Rp – 5′-GTACGTAAACGCGGAAGGTG-3′	1	55
3.	RM3412	Fp – 5′-AAAGCAGGTTTTCCTCCTCC-3′		
		Rp – 5′-CCCATGTGCAATGTGCTCTC-3′	1	55

Chr. no. = Chromosome number, Anneal. temp. = Annealing temperature.

clusters. Cluster I comprises four varieties including susceptible variety TN1, and Tai buhpui, Fangsin and Tailuaia hmui. Cluster II comprises three varieties Zaitlai, Fazupui and Idaw which were the most three tolerant varieties in the present study.

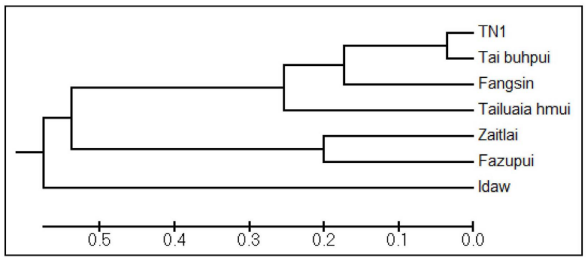

Fig. 6. Dendrogram of seven rice varieties based on Nei's genetic distance.

DISCUSSION

In this investigation, the germination percentage results clearly show that NaCl did not decreased seed germination even though its concentration increased. It is clear that the growth of plant decreased as the salt concentration increased after germination. It has been reported that increasing salinity concentration at germination or seedling stage cause osmotic effect which lead to decreasing the rate of water uptake and/or specific ion toxicity and as a result, may reduce germination percent or growth of the plant (Huang and Redmann 1995; Ashraf 2002; Abbas *et al.,* 2013). The shoot and root length decreased as the concentration of NaCl increased in the present study confirmed these reports. High salt tolerance at germination stage but great sensitivity at early seedling stage was observed, which proved that salinity is fatal for early seedling growth stage. Similar case was also reported by Alam (2001).

Low concentration of salt (30 mM) had no or very little effect on the growth of Fazupui, Fangsin and Idaw. Especially in case of Fazupui, the shoot length was higher in 30 mM of NaCl than in control treatment. Shoot and root lengths were not always related to shoot and root weight and salt tolerance indices. The shoot lengths of Fangsin and Fazupui at 150 mM were longer than at 120 mM. Tai buhpui also produced the shoot length longer at 90 mM than 60 mM. But the dry weight and STI of Fangsin and Fazupui were higher at 120 mM than at 150 mM. Similarly, the dry weight and STI of Tai buhpui were higher at 60 mM treatment that at 90 mM treatment. For this reason, Bagci *et al.,* (2003) advised that dry weight should be the primary selection criterion. The growth of shoot and root of plant was strongly inhibited by high concentrations of NaCl as compared to lower concentrations. Similar results were reported by Bagci *et al.,* (2003) and Abbas *et al.* (2013).

Idaw, Zaitlai and Fazupui show highest percentage of salt tolerance index (Table 3) at 150 mM of NaCl in the studied varieties. At 30 mM, which was the lowest concentration used in the present study, all the varieties show more than 46% salt tolerance index with average of 71.85%, and the highest percent was observed in Fazupui (100%) followed by Fangsin (93.2%) and Idaw (90.64%). Salt concentration of 60mM showed decreased in STI of less than 50%, which mean that more than 50% of the individuals cannot withstand salt stress of 60mM or above. Clustering using salt-linked molecular markers revealed similar results of tolerance of the studied varieties as that of NaCl stress on early germination growth. It clearly distinguished susceptible varieties and tolerant varieties.

The present study investigates the first hand report on salt tolerance on plant growth at early seedling stage and at molecular level of upland rice of Mizoram. Further investigations are needed to be

Table 3. Salt tolerance index (STI) values of 7 rice varieties of Mizoram.

Sl. No.	Varieties	STI percent on different NaCl concentrations (mM)					
		0	30	60	90	120	150
1.	TN1	100	55.08	47.32	16.31	10.42	3.20
2.	Tailuaia hmui	100	49.07	20.05	16.88	15.30	1.31
3.	Zaitlai	100	46.86	32.12	18.18	13.73	12.52
4.	Tai buhpui	100	68.12	13.14	11.55	4.78	3.98
5.	Fangsin	100	93.2	37.97	7.52	7.03	6.87
6.	Fazupui	100	100	42.26	23.53	17.69	9.74
7.	Idaw	100	90.64	52.99	37.64	33.57	13.18
Mean	100	71.85	35.12	18.80	14.64	7.25	

undergone in order to select salt tolerant landraces for successful future breeding programs.

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ABSTRACT

STUDY OF GENETIC DIVERSITY OF SELECTED INDIGENOUS RICE VARIETIES OF MIZORAM

BY

VANLALSANGA

DEPARTMENT OF BOTANY

SUBMITTED

IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN

BOTANY OF MIZORAM UNIVERSITY, AIZAWL

ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important crop and staple food for more than half of the World's human population. So, rice production needs to be increased to meet the ever-increasing demand. Landraces or indigenous rice varieties are primary sources of germplasm for rice improvement programmes, since they contain considerable level of genetic diversity and the knowledge on genetic diversity of such cultivars is very important for development of improved varieties. Microsatellite markers are the widely preferred markers to analyze genetic diversity within and between populations among many molecular markers systems.

In the present study, indigenous rice varieties of Mizoram, Northeast India were analyzed for seed morphology, seed storage protein profiling and genetic diversity. Seeds of indigenous rice varieties were collected from local farmers from different villages of all the eight districts of Mizoram. A total of 63 rice varieties including 53 local varieties, five *Indica,* and five *Japonica* were used in the current study. Twelve simple sequence repeat (SSR)/microsatellite primers RM1, RM154, RM131, RM135, RM153, RM190, RM72, RM125, RM278, RM171, RM287 and RM117 were chosen and used in the present study to amplify genomic DNA.

Results revealed variation in grain length, width and weight. The longest grain length was observed in Kawnglawng (11.4 mm) while the shortest grain length was recorded in Tai bial (6.88 mm). And grain width ranged from 2.21 mm (BM71) to 3.82 mm (Buhban langakthou). A 1000-grain weight ranged from Gomati (18.61g) to Buban sen (46.8g). Protein profiling showed polypeptide bands ranging from 5 to 10 with average similarity coefficient of 0.7064 indicated that genetic similarity existed respect

to seed storage protein in the studied cultivars. A dendrogram (UPGMA) constructed based on seed protein profiling showed three clear clusters. Cluster I consists of 9 cultivars, Cluster II 28 cultivars and Cluster III comprises of 26 cultivars. Genetic diversity analysis using SSR markers revealed a total of 63 alleles ranging from 1 (in RM278) to 10 (in RM135) among the studied rice varieties. A high level of gene diversity at 0.6100 was also observed. High values of Fst (genetic differentiation) and PIC (Polymorphic Information Content) estimates were found at 0.7599 and 0.5517 respectively. This high value of PIC indicated that the SSR markers used in this study are good markers for rice genetic diversity analysis. Among the markers used, RM1, RM154, RM131, RM135, and RM72 were identified as the most polymorphic loci markers. The Biruchuk cultivar was found to be the most genetically diverse cultivar with expected heterozygosity index of 0.3382 and least gene diversity was found in Buhban zam with expected heterozygosity index of 0.0158. Analysis of molecular variance (AMOVA) showed that 74% of total variation was due to among-population differentiation while the remaining 26% was due to within individual differentiation and most variation within population was found in Biruchuk. Principal coordinates analysis (PCoA) analysis using Nei's genetic distance showed a grouping of the studied populations into three distinct groups, the first three principal coordinates explained 48.76 % of the total variation. Coordinate 1 extracted 22.44 % of the total variation, coordinate 2 extracted 17.25 % of the total variation and coordinate 3 explained 9.08 %. Among the population studied, Pawnbuh and Pana were mostly related with the similarity index of 0.994 while the most diverse cultivars were Buhban hmui and Mangbuh with a genetic similarity index of 0.175. The UPGMA trees based on

microsatellite diversity showed the grouping of rice cultivars into three clusters which were further supported by Bayesian model-based STRUCTURE analysis. Cluster I was represented by *Japonica* check-varieties and 11 indigenous varieties, Cluster II was represented by *Indica* check-varieties and 12 indigenous varieties, while Cluster III comprised of 30 indigenous varieties. Comparing the two phylogenetic trees, seed storage protein tree (T1) and SSR tree (T2) were more or less kinship.

The considerable level of genetic diversity among rice varieties of Mizoram was observed in the present study. The populations possessing high genetic diversity such as Biruchuk, Kawnglawng, Baimasa, etc. and genetically diverse varieties like Buhban hmui and Mangbuh, etc. can be utilized in rice breeding programmes. Some cultivars exhibited a low level of genetic diversity suggesting necessity action with respect to conservation strategies. The present finding can be useful for selecting suitable rice lines for breeding programmes and germplasm conservation of indigenous rice varieties of Mizoram.

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