Genetic Diversity of Rice (*Oryza sativa* L.) Germplasm from Different Agro-Ecological Zones in Myanmar

November 2015

WUNNA

Genetic Diversity of Rice (*Oryza sativa* L.) Germplasm from Different Agro-Ecological Zones in Myanmar

A Dissertation Submitted to

the Graduate School of Life and Environmental Sciences,

the University of Tsukuba

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy in Agricultural Science

(Doctoral Program in Biosphere Science and Technology)

WUNNA

LIST OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

Abstract

Rice (*Oryza sativa* L.) is the one of vital cereal species on earth, providing the dominant staple food for Asian countries including Myanmar. Myanmar has the long history of rice production which was influence by culture, religion and belief and national economy. Moreover, she has a high genetic diversity of rice, along with diverse eco-geography and agricultural practices. Major Rice production depends on lowland and upland cultivation systems, which covered 8 million ha in 2009 (http://faostat.fao.org/). Upland landrace cultivars are adapted to less reliable environmental conditions. The diversity of lowland landrace cultivars, which are adapted to more controllable conditions, depends on farmers' economic, social and cultural choices. Varietal diversity, which ensures stability of crop production, is interested by Myanmar farmers. Nowadays, landraces are rapidly being lost due to drastic development such as land-use, agriculture practice impacts and government's rice production policy. And current existence of landraces diversity and its distribution, become interesting information for conservation and utilization. This study is an attempt to delineate the genetic diversity of Myanmar rice landrace gene pool and to conduct the comparison assessment of rice landrace diversity among upland and lowland ecosystem for conservation measures. Genetic diversity of Myanmar rice landraces and the comparison of landrace diversity among upland and lowland ecosystems, were assessed through 65 SSR markers. The variation of three agronomic traits; heading date, eating and cooking quality and rice blast resistant were also determined among upland and lowland populations.

Considerable high genetic diversity existed in the Myanmar rice landrace accessions over upland and lowland ecosystems. SSR profiles placed all accessions into two main clusters, I and II which are thought to correspond to the conventional groups, Indica and Japonica. Moreover, four genetic distinct groups; Japonica-type upland group (A-1), Japonica-type lowland group (A-2),

Indica-typed lowland and upland mixture group (B-1) and Indica-typed upland group (B-2) were identified again by clustering of same genetic background individuals with model-based method. With assuming germplasm exchanges among regions, independent clustering pattern of landraces from different origins in the UPGMA analysis and revealing of high molecular variance among accessions within populations in AMOVA, pointed to human pressure upon the dissemination of seed among populations. But there was significant genetic differentiation among upland and lowland populations indicated by higher F_{ST} (0.31) than the threshold for significant population differentiation. Main evidence is interspecific variation and distinct genetic background among upland and lowland populations. Landraces of three distinct groups from upland ecosystem are highly genetically diverse, widely overlapped and adapted among three mountainous agro-ecological zones. Although, there was relatively high genetic diversity in every region, north-eastern population had higher potential rate of genetic diversity among regional populations, especially, majority of the accessions has two genetic backgrounds (A-1 and B-1). Lowest diversity rate was observed in lowland landrace population from Southern plain which have Japonica-type (A-2) genetic background. This research elucidated upon genetic diversity and intraspecific variation of landraces among different ecosystems and agro-ecological regions for supporting the sustainable utilization and conservation.

Among upland and lowland ecosystems, characterization of landraces base on agronomic traits such as, heading date, eating and cooking quality, and rice blast resistant, is important in determining the regional landrace distribution and diversity under considering of adaptability of local environment and human preferences. Detection of trait variation among landraces as well as among regions is helpful to design strategies for landrace germplasm on-farm management, *ex-situ* conservation and improvement for sustainable regional rice production. Significant differences of

agronomic traits variation were indicated among regional populations with Tukey-Kramer HSD test. Among two agro-ecotype populations, variation of seed size, cooking and eating quality and resistant reactions upon the rice blast within mountainous upland landraces, are intriguing genetic diversity to assume the population for conservation. With the stable variation of intermediate amylose content, high candidate resistance to rice blast character and wide range of heading variation, lowland landrace population can be recognized as breeding resources for quality improved breeding purposes and searching the R gene for rice blast pathogen control. More clear variation of these agronomic traits was observed among upland and lowland populations. And upland population was more variable than lowland group except heading date trait. Diverse agro-ecology, seasonal and regional climate, cultivation systems and social diversity, may attribute the genetic variability among upland and lowland populations.

Generally, landrace diversity was found as high potential frequency in upland ecosystem with effect of harsh environment and diverse human tribes. Current population of lowland landrace from Southern plain region showed low genetic diversity under the forcing of improved cultivars introductions. Intraspecific SSR variation, variation of three agronomic traits and diversity indexes of regional population, were distinctly different among upland and lowland landrace pool. But, higher molecular variances within regional populations under significant variation of agronomic traits among regional populations are preferable broad genetic landrace pool for utilization and further research purposes. With this study, I identified and pointed out the potentially low or high diverse landrace population among two ecosystems and conservation measures are also discussed to be helpful while devising similar studies in future.

Chapter I

General introduction

Myanmar is known as rice- based agriculture country and large fraction of national economy rely on the surplus rice exporting. From historical time to now, she has long history of rice production. Moreover, culture, religion and belief, politic and national economy were influenced by rice and rice production. Therefore, civilization of Myanmar was established base on rice and rice cultivation. Much more relying on the rice and rice production, rice becomes the culture and the national symbol of Myanmar.

Myanmar is the most extensive center of ecological and crop genetic diversity. It constitutes part of the center of genetic diversity of cultivated rice (*Oryza sativa* L.), an area that includes East Nepal, Bhutan, Assam, Myanmar, Laos and northern Thailand (Chang, 1976). Diverse topography from East to West, heterogonous water regime from South to North and, country-wide diversity of tribes and its culture and resilient agriculture system are evidence factors in shaping the Myanmar rice germplasms diversity. The high potential of rice germplasm diversity directly affect to economy of Myanmar. For sustainable rice production in Myanmar, these germplasms are needed to conduct the collection, conservation, and research and utilization procedure systematically.

1.1 Introduction of Myanmar

1.1.1 Location

Myanmar is a large country (with a land area of 676,577 square kilometers) and geographically locate between $9^0 58'$ to $28^0 31'$ N and $92^0 9'$ to $101^0 10'$ E. Geographic location of Myanmar is land bridge position between South and South-east Asia to link the Mekong sub region and India sub-continent. It is closely attached on the northwest by India and Bangladesh, on the North and East by China, Laos and Thailand and 2,800-kilometer (km) coastline along the

Western side to Southern with the Bay of Bengal. According to topographic and climatic data, there are seven agro-ecological zones, such as Northern Mountain Region (NMR), Western Hilly Region (WHR), Eastern Mountain Region (EMR), Central Dry Zone (CDZ), Southern Coastal Strip Region (SCSR), Western Coastal Strip Region (WCSR) and Southern Plain and Delta Area (SPD) of Ayeyarwaddy and Sittaung rivers (Ye Goung *et al.*,1978) (Figure 1).

1.1.2 Demography and ethnic diversity

The population is estimated to be approximately 60 million and growing at about 1.5% annually. Due to census data, population density is 89 per square kilometer. Although, urban population is 18 million, rural population is 42 million and its seventy-five percent contribute the agri-sector as farm labor. Myanmar unites with 8 major human tribes consisting of so many sub-tribes as 135, each of which has their own languages and dialects. There are 12 sub-tribes in the Kachin, 9 in the Kayah , 12 in the Kayin, 51 in the Chin, 9 in the Bamar, 7 in the Rakhine and 34 in the Shan (Min Naing, 2000). High number of tribe groups overwhelms mountainous and hilly regions, especially Western hilly region and Eastern plateau region. But, the population density in the Western and Northern mountainous regions and the Eastern plateau is low. Majority of their living was based on agriculture.

1.1.3 Indigenous PGR of Myanmar

Myanmar is one of the largest crop genetic diversity reservoirs in south-east Asia, providing a rich habitat for tropical and subtropical vegetables, fruit and cereal crops (Thein Tun and Mya Than,1995). Myanmar is well known as the primary center of plant genetic resources diversity for rice and secondary for other crops. High levels of diversity were found in rice, maize, sorghum, millets, pigeon pea, cowpea, sesame, groundnut, banana, mango, citrus, okra, tomato, chilies, pepper, ginger, cambric bamboos and many medicinal plants (San San Yi *et* *al.*,2007; Hirano *et al.*, 2011). Besides agriculture diversity resources, the huge forest reserve provides an important component of biodiversity, ecological preservation, renewable bio-resource and environmental sustainability within south- east Asia.

1.1.4 Role of Agriculture sector

Myanmar's economy and food security totally rely on agriculture sector. Agricultural production takes place about 12.4 million hectares, or 20% of total land area; about 68 million ha (MOAI, 2013). These cultivable areas spread among seven agro-ecological zones and there is regional specific crop production. Rice, which covers about two-thirds of the total area under cultivation, is the most important crop in Myanmar, requiring large investments, large manpower, and earning the large return from the land. The second principle crop is beans and pulses, which have recently become major export crops and are grown on about 4.2 million ha. Oilseeds crop (especially in the Central Dry Zone) is third, grown on 3.3 million ha; production is insufficient to meet national demand and about 200,000 tons of palm oil is imported annually. Currently, oil-palm plantations are founded at southern coral Thaninthayi division to catch the domestic need. The last one is vegetables and chili, grown on about 0.8 million ha, principally in highland areas. Other crops, including maize, cotton, rubber, sugarcane, and tropical fruit crops are also important crop for agriculture sector.

1.1.5 Rice growing conditions

Rice is grown in the different climatic conditions and various topography of the country. And it can be grown throughout the year because Myanmar possess different sorts of rice verities which have prominent adaptability to the wide range of growing conditions. In Myanma Agri-sector, there are two main types of rice cultivation systems; "La" cultivation (Wet; lowland) and "Ya or Taung Ya" cultivation (Dry; upland). "Ya" cultivation is not bounded, but land preparing and seeding conduct under dry condition. Farmers from central dry-zone area and mountainous area use the subsistence “Ya” cultivation system. “La” cultivation system is usually found in plain rain-fed and irrigated area. Geographically, major rice cultivation is Ayeyarwadde delta region, which has 55% of the total rice sown areas, and then follow the coastal strips (14%), Central dry zone (13%) and the mountain area (18%) (Ministry of Agriculture and Irrigation, 2013). In Southern plains region, percolation is important for rain-fed lowland and choosing of varieties is also vital for regional adaptability. The introduction of modern high yielding varieties in lowland area was initiated in the year 1967-68 (Khin Win, 1991) and it take 70 % of total lowland area currently.

1.2 Myanmar rice production

Myanmar’s first priority crop is rice because of its crucial role in food security, as well as its attribute to the economic viability, political stability, and social prosperity of the country (Khin Win, 1991). Presently, domestic rice consumption is rising gradually with population rate. With the politic transformation during 2011 to 2015, country’s recent re-engagement with the international community has set the stage for renewed and reinvigorated attention to develop the agricultural export market and regain the world rice market share. That is also a good remedy for reducing the poverty and boost-broadening economic growth of Myanmar.

Rice is sown in Myanmar as monsoon crop in the rainy season. But, summer rice has been cultivated wherever irrigation is available since 1992-93 cropping season (Ye Tint Tun, 2006). Current national average rice yields are around 3.2 to 3.4 ton per hector. In accordance with national planning target, cultivation of rice is being implemented, aiming to reach the total area of 7.3 million ha, comprising 5.7 million ha under monsoon rice and 1.6 million ha under summer rice. Yield per unit area is also targeted to reach about 5.2 ton per hector and nation surplus rate is 7% (Ministry of Agriculture and Irrigation, 2013). But there are many limiting factors to catch that aim.

1.2.1 Rice cultivation systems

Traditional rice cultivation methods in Myanmar are divided into three categories: dry land (Ya), upland (Taung Ya) and lowland (La) cultivation. “Ya” cultivation is also called as dry-land and can be found in central dry zone. Although, onion, sesame and bean and pea are main production of “Ya” cultivation, a few area grow dry-land rice with scarce rainfall. But, irrigated rice is grown where irrigation system exists, mostly in Mandalay and Saging dry zone area. Cultivation of rice on the mountainous and hill side condition is “Taun Ya” (upland), naturally well drain soils without surface water accumulation, normally sloppy topography. Northern Mountain Region (NMR), Western Mountain Region (WMR), and Eastern Mountain Region (EMR) are Taun Ya cultivated area. “Ya” and “Taun Ya” rice landraces are one form of natural agriculture resources because of its adapting to harsh environmental conditions through more resilient agricultural systems (Khin Win and Nyi Nyi, 1979). In present day, both “Ya” and “Taung Ya” are generally called “Ya”.

Lowland (La) cultivation is major method for rain-fed plains region and irrigation facilitated area. Rain-fed lowland (the largest ecosystem) and deep-water rice are found in the delta region and coastal strip of Rakhine State. Nearly 60% of the delta region, including the Ayeyarwaddy, Bago, and Yangon region of Lower Myanmar, is major rice productive lowland rain-fed areas. Lowland rice cultivation start with tillage operation in nursery plot and plow field with pre-monsoon rain, follow hollowing, transplanting, inter-cultivating, some cultural practices and end at winter season by harvesting, threshing and winnowing. All cultural practice follows traditional methods using simple implements, such as hoe, knife and fork. Commercialized farming and some farmers use machinery from land-preparation to harvesting but poor farmers conduct farming with intensive family labor and cattle for farm-preparation.

“Taun Ya” upland cultivation, generally practiced at wooded hillsides is typical slash and burn methods and use for subsistence rice production. Upland cultivation on the mountainous area is temporary farming system (cultivation in one place depend on variable time interval) and farm cleaning with the help of fire, alternating of growing and fallowing period and growing crops with tree (Agro-forestry) are characteristics of Myanmar Taung Ya. Main theme of rice cultivation in upland mountainous area is culture value and regional self-sufficiency. For rice cultivation, farm cleaning start with fire burning at the end of raining season, final weeding and farm cleaning before raining season and sowing seed with rain by using the seeder stick are procedure of traditional upland cultivation method (Khin Win and Nyi Nyi, 1979). But culture practice and fallowing period are different among regions or tribes.

1.2.2 Social factors and rice production

Although Myanmar is rice-surplus country, rice cultivation was originated from subsistence agriculture. Therefore, traditional methods using simple implements and farmer indigenous knowledge are widely adopted in Myanmar rice cultivation and production since colonial time (FAO, 1996). And local cultivars and traditional landraces are special seed sources among local farmers for the self-sufficiency rice production as well as the commercial production. All tribe farmers and rural farmers take attention upon landrace diversity and conservation because landrace have regional preferable characters or culture values. Therefore, rice is life for Myanmar people, being interwoven with the social and culture, social and economic fabric of the people’s lives. All norms regarding with rice cultivation base on socio-economic and socio-culture rather than market oriented agriculture.

1.2.3 Government policy and rice production

With huge cultivable areas and a great labor source in the rice industry, all local governments emphasize and give attention to develop the rice production. Along the Myanmar rice production history, policy and implementation for rice industry development differ from government to government depend on the market economy and evolution of efficient technology (Ministry of Agriculture and Irrigation, 2015). But settings of all policy base on the stable and sustainable rice production. Nowadays, innovation and motivation of modern technology, which contribute to increase the rice production, need urgently to fill the rapid increasing of domestic consumption as well as foreign demand. However, rice production is continuous challenge for Agriculture Department and Agri-sector.

1.2.4 Problem and barriers of rice cultivation in Myanmar:

Major constrains and barriers of rice production in Myanmar are unstable and uneven policies, financial status and climate changes, and poor technology innovation and motivation at farmer level.

1.2.4.1 Dry-zone area:

In dry zone area, low yield is major problem for regional rice self-sufficiency. Maximum production rate decrease nine folds than the delta rice productive area (FAO, 2005). Unfavorable environmental conditions in traditional cultivation system are possible evidence for yield lost. Vital role to get regional expected target yield depend on the right combination of short duration varieties and regional reliable cropping pattern. To avoid the drought period and adjust the sowing time for winter crop, farmers choose the short growth varieties. Different productivity levels among farmers depend on the successive cares; irrigation and application of additional inputs. Conducting in several steps of crop care management relies on farmer's financial ability but majority of farmers cannot do. Unfortunately, the poor economic situation of farmers in "dry-land" area is fundamental barrier for "dry-land" rice production. Currently, all dryland area becomes major production area of pea and bean, and rice farming is rarely found in irrigated area. Dryland landraces or local cultivars are disappeared gradually in that area and substitution of irrigated improved cultivars are increased promptly.

1.2.4.2 Mountainous and hilly region

In mountainous area, shifting cultivation system is major livelihood for poor tribe-farmers. These farmers conduct the slush and burn shifting cultivation for upland rice and other crops (maize, poppy, sesame and pulse). Soil fertility exhaustion, increasing weedy problem, disease outbreak and low temperature at seedling and reproductive stages affect the upland (Taun Ya) rice productivity. “Taun Ya” cultivated area is naturally sloppy and favors the rice plant to adapt the aerobic condition during their lifespan (FAO, 1984). However, locally adapted landraces and traditional cultivars are important for regional rice production stability and sustainability.

At altitude about 750m to 1500m or more, the sustainability of rice-based cropping pattern is difficult not only for local farmer to practice but also for extension workers to support the technical advices. Besides, poor in awareness, innovation and motivation, and finance within local communities is one kind of social-economic harsh condition for sustainable upland rice production. The sustainable development of “Taung Ya” rice cultivation totally depends on systematic cropping system based on agriculture conservation such as, no tillage, marching of crop residues, systematic integrated pest and disease management and soil fertility management through the crop rotations.

1.2.4.2 Lowland plains area

Lowland area is major rice production realm. But problematic agriculture practices such as low rate and uncorrected way of applying manure and chemical fertilizers, poor seed quality and poor weeding and water management; appear to be the most serious limitations in rice production. Particularly, low yield of lowland is based on application of very low amount of fertilizer (Tin Aye Aye Naing *et al.*, 2008). Using of high yielding cultivars with low input intensities orient to low yield is occasionally found in resource poor rural farmers' farm. In irrigated lowland area, there is decrease in the overall average rate of fertilizer application, an increase in the prevalence of rice-legume cropping systems, and only localized insect pest or disease problems. But, rice yields are found to be higher than rain-fed area, likely the results of more suitable weather conditions and better irrigation.

In seed-source acceptation, most of farmers keep their own seeds from their own harvest or from neighboring farms and use as seed source rather than purchasing seed as recommended from the Myanmar Agriculture Service (MAS). As a result, a considerable amount of varietal degeneration is found. Occasionally, using the poor quality or deteriorated seed is serious problem in rice cultivation and production. Nowadays, the seed production sector and seed company stand as one role to supply quality seeds with affordable prices to farmers throughout the country. Moreover, extension workers train the farmer how to produce the registered seed for local cultivars (Ministry of Agriculture and Irrigation, 2013).

Constraints to rice production in Myanmar vary regionally but tightly link to the management practices among farmers and socioeconomic conditions of the farmers. Above all of these barriers not only distract to Myanmar rice production processes but also threat to Myanmar rice germplasm diversity.

1.3 Genetic variations of natural resources in rice cultivation

Asia cultivated rice (*Oryza sativa*. L) is normally classified into two subspecies; *indica* and *japonica*. Indica-type landraces predominates in Myanmar as 81 percentage of total landraces (Khush *et al.*,2003) and Japonica-type landraces specially influence in Eastern plateau region (Ohm Mar Saw, 2007). However, landrace diversity and its dissemination depends on the adaptation to local agro-ecology, socio-economic and environmental harsh conditions; biotic and abiotic stress. There are many rice varieties in Myanmar. A wide range of vernacular names for each variety does not always mean to represent genetically diversified cultivars because of linguistic differences among different ethnic groups (Watanabe *et al.*,2007). If the farmer-named varieties are not genetically distinct, farmer taxonomies and nomenclature which are typically localized and culturally determined would not affect in identification and qualification of diversity in agricultural ecosystem (Sadiki *et al.*, 2007). But consistency in naming and distinguishing landraces is essential in evaluation of diversity and socio-economic studies.

Myanmar farmers classify rice with some standards; growth duration, water regime and seed size (Table-1). Actually, agro-morphological trait is important at farmer community because farmers identify or distinguish varieties by using these traits' class. Some of these traits are preferred or valued by the farmer; that is, the farmer chooses to plant a particular variety because certain of its distinguishing characteristics are desirable. Grain shape and size has long been used as a convenient criterion among Myanmar farmers (Irie *et al.*, 2004). Moreover, seed size is important in production processes and milling processes. Size, shape and weight of rice grains of Myanmar landraces were diverse countrywide as well as locally.

Day length and temperature are crucial roles to determine heading time of rice plant, which affects the regional and the seasonal adaptation of rice landraces. Moreover, wide range of photoperiod sensitivity among landraces is important for adaptability crop at different ecological environments. Less sensitive photoperiod cultivars get a popular role in irrigated dry-zone areas because they are widely adaptable and suitable in cultivating for year around. On the other hand, photoperiod sensitive cultivars are also adapted to rice growing areas with an unpredictable water regime (Mackill, 1986). Ye Tint Tun *et al.*, (2005) reported that high sensitive landraces to photoperiod predominate countrywide in Myanmar but low sensitive landraces are exclusively distributed in the mountainous regions. In the low to intermediate rain-fed lowland areas, medium to medium-late maturing-type, photoperiod sensitive or insensitive-type varieties are usually overwhelming, while, the traditional medium-late to late maturing varieties are dominant in large areas of the intermediate to semi-deep rain-fed lowland. In deep water areas, the traditional tall pant-type with photoperiod sensitive are predominant. Mainly, traditional rice are monsoon rice and grown only once in a year. Early maturing landraces are more adaptable to high altitude mountainous regions (Ye Tint Tun, 2006). Almost all of dry-land and lowland farmers decide the crop rotation pattern depend on theirs landraces' maturity type. Dryland farmers use some landraces that have trait of early maturity or short growth duration to escape the drought period. Therefore, that trait is dryland farmer preference trait and prevails among the irrigated lowland, dry-land and upland area. This sort of differentiation among zones is considered to be closely associated with cultivated ecotypes, the prevailing cropping system and the natural conditions of each rice-growing area (Irie *et al.*, 2003). However, diversity of heading time is vital among rice varieties for adaptation to various rice growing environments and rice growing seasons.

Amylose content of grain starch and type of endosperm are one attraction for human selection. High ethnical complexity to diversity dietary habits and arts of rice cooking are main factor to create a great diversity of rice landraces in Myanmar. Pa Pa Aung (2003) reported that Myanmar local rice cultivars have a wealth of genetic diversity for seed storage protein and endosperm starch characters. Very low amylose landraces are frequently found in the North-East mountainous zone. Opaque and waxy endosperm type are dispersal in the mountainous region related with diverse cooking and eating habit and processing technique traditionally transmitted by various ethnic groups settled in mountainous regions and affected by cultural influences of surrounding areas, especially the so-called waxy rice zone expending round Thailand, Laos and Cambodia. Above these traits such as grain shape, heading date, amylose content, are important traits within landraces in determining the genetic diversity related with adaptable to natural environments and various rice-growing conditions.

In dry-land and hillside region, water shortage is prime limiting factor for the semi-aquatic-type rice species. Natural selection turn to crop adaptability events is prominent in landrace gene pool of harsh environment conditions. Detecting of sequence analysis base on the *OsLEA27* (dehydrin gene; drought tolerance) and drought adaptable traits among upland and dryland rice accessions, significant positive selection was observed among landraces from Northern mountainous region (Wunna *et al.,*2015). The mountainous ecosystem is a natural selector for upland landraces, which remain under continuous selections pressure on slope areas that are unable to retain water. Moreover, the cold and humid upland ecosystem condition favors the blast epidemics and rice landraces which thrive in blast problematic area is huge divergence resources of R-gene pool as well as source to resolve blast disease problem.

Genetic variations and dissemination of Myanmar rice landrace cultivars in rice cultivation depends on the adaptation to local agro-ecology, socio-economic and environmental harsh conditions; biotic and abiotic stress. This tremendous diversity of Myanmar rice gene pool is considered to be precious and irreplaceable property.

1.4 Value of rice landrace cultivars

Myanmar is a union of 8 main tribes consisting of so many sub-tribes and each of which has their own languages and dialects. High numbers of tribe-groups are dispersal among mountains and hilly regions. Rice consumption is more in the mountainous rural area than in the urban. All ethnic groups cultivate landraces that have both symbolic and social values and they handle huge gene pool to choose with economically, socially, and culturally purposes (Gupta *et al.,* 1996). Socio-economic factors defined by Brush (1995) such as land fragmentation, marginal growing environments, imperfect market conditions, cultural identity and preference on-farm diversity, and agro-ecology and agro-ecosystem, may vary among ethnic groups and their settlement areas. The Northern part of Myanmar survey results, every household occupy the high rice landrace resources for their socio-cultural and religious values (Watanabe *et al.,* 2007, Kawase *et al.,* 2011, Yamamoto *et al.,* 2011). In addition, landrace resource-richness in each household, which maintain higher varietal diversity, show their stronger livelihood status in the harsh environmental conditions and complex social communities. Therefore, landrace diversity is totally related with ethnic diversity and appreciation of tribe-group upon landrace richness. Major tribe, Burma, settles and distributes alone the lowland plain regions and their cultural norm, social status, religious value and economic sense are nearly similar. And richness of lowland landraces resource in plain directly related with farmers interesting upon productivity rather than culture and religious facts. In plain region, therefore, landrace diversity is illustrated by irrigation facilities, farmer's choice and market demands.

With the possessing of social and cultural values, tremendous diversity of Myanmar rice landraces is considered to be breeding resources. Landraces or traditional cultivars, which are grown in subsistence agriculture, are diverse and carry genetic variability in their populations. With combination of natural and deliberated selection, frequent or sporadic recombination, and mutation and adaptable characters to particular environment are established in landrace pool. Consequently unique combination of useful genes is thought to be piled in the genome of landraces. They are the main source of genetic material for breeding materials (San San Yi *et al.*, 2007). To efficient use of these valuable germplasm, we must know the genetic diversity and structure of the existing germplasm.

1.5 Assessment of rice landrace diversity

Genetic diversity is a ubiquitous property of all species in nature. Therefore, systematic evaluation, characterization and classification of plant genetic diversity are important and that information can be successfully applied in genetic resource management (Karp, 2002) for long-term sustainability. Especially, traditional cultivars or landrace diversity are important reservoirs of useful genes and can be exploited to broaden genetic base and enrich the important favorable agronomic trait. Study of genetic diversity is the process by which variation among individuals or groups of individual or population is analyzed by a specific method or combination of methods (Mohammadi and Prasanna, 2003). For assessing the genetic diversity and classification, data sets consisting with measurements of several methods (molecular, morphological and agronomic characters) upon target population are necessary.

In general, extent and exist of genetic diversity within a collection sample or conservative area are needed information. Pre-breeding work or evaluation of germplasm within regional or on farm area is also important for accessions selection for use and guiding the management of germplasms. Breeders also need available information of germplasm variability for effective breeding (Loresto *et al.,* 2000). Luck of characterization and evaluation has undoubted limited to extend the wide use of the rich genetic diversity available and the enhancement of germplasm (Weidong *et al.,* 2000). Therefore, assessment of genetic diversity and genetic variation is important research subject.

1.5.1 Assessment base on molecular markers

Studying the genetic diversity and classification, morphological characterization is labor intensive, and subject to environmental fluctuations and soil condition. For these reasons, attention has been directed to laboratory based methods, such as arrays for storage proteins, isozymes and molecular characterization of crop (Cooke, 1984). Assessment of genetic diversity, population structure and relationship among plant germplasm collections, molecular marker is a powerful tool and has been applied to cultivated rice (*Oryza sativa* L.) (Garris *et al.,* 2005). Microsatellite or simple sequence repeat (SSR) markers are particularly powerful and have become the versatile molecular markers not only for germplasm diversity studies but also for exploration of targeted gene. Because, they are abundant and dispersed throughout the entire genome and have high information contents (Rice *et al.,* 2006), codominant inheritance, reproducibility, and locus specificity (Ellis and Burke, 2007).

1.5.2 Assessment base on Agronomic traits

Agronomic traits are important because these are used by farmers to identify or distinguish varieties. Some of these traits are preferred or valued by the farmer because some distinguishing characteristics are desirable. Furthermost, farmer may identify a named variety of rice by its agro-ecological habit and region of origin, value it for its cooking quality, and select for higher-yielding plants to increase the yield potential of the variety. Assessment of genetic diversity using agronomic trait is the traditional method of measuring diversity. Simpson and Withers (1986) stated that fundamental importance in the management of plant genetic resources is the measurement and characterization on the variation they present. Especially, morphological traits are primary essential characters (Ikeda, 1999). Using morphological and agronomic descriptors in genetic diversity studies is still worthwhile and necessary (Sounigo *et al.,*1996), but natural and artificial selection impact upon the genetic diversity related with some traits , such as adapted to agro-ecological habitat, cooking quality and resistant to stress and so on. Genetic variation of agronomic character among individuals is subject to environment variation; which interact with crop’s genetically heritable characters.

1.6 Current circumstance of landrace cultivars from upland and lowland area

Acceptable rate of diverse landrace or local cultivars pools are observed both lowland and upland ecosystems (Khin Soe *et al.,*2002). Lowland ecosystem of Southern delta region and upland ecosystem of Western, Northern and Eastern hilly and mountainous region are prosperous area for rice landrace pool. Changes of meteorological factors (Figure 1.2), diverse ethnic factors and heterogeneous topography among these four regions favor the landrace diversity. Ability of a species or a population to adapt the changing environments is outcomes of vast genetic gene pool. Local cultivars which are adapted to specific environment, are valuable source for farmer to cope harsh environmental constrain. Nowadays, current landrace cultivars populations face thread of endangerment with several factors. That precious and irreplaceable resource urgently needs to conserve before they disappear.

1.6.1 Factors influencing upon on-farm landrace diversity

Most of Myanmar farmers use their own seed from year to year. Once well-adapted named varieties are available to farmers, the seed can be readily multiplied and distributed through farmer-to-farmer channel. Especially in farmer's seed, temporal variability and spatial heterogeneity among landraces can be found and it depend on the landrace's local adaptability, and farmer selection impact and their cultural practices. Foremost, on-farm seed source is important and popular for indigenous selection for regional adaptable. For survival of landraces, market-based incentives are important to encourage farmers to grow landraces as economy interesting. Therefore, a complex combination of environmental, socio-cultural and economic factors influence upon farmer manageable on-farm varietal diversity.

Rice growing farmer in mountainous and hilly region have better possession of diverse landraces and maintain their richness or evenness of landrace diversity due to their assets of cultivating diverse land types, dispersed plots that are located further away from market centers (Watanabe *et al.*, 2007) and coexistence of diverse tribes. Mountainous and hilly regions farmers know the benefit of diversity richness and appreciate upon it as cultural, social and religious norm. In irrigated lowland and dry-land area, local farmer groups link to extension service, therefore, government's regional self-sufficiency policy and incentive of irrigation facility are negatively influenced on landrace diversity. Due to overwhelmed introducing of modern varieties among lowland rain-fed and irrigated area, promising local landraces are needed to conserve at *in situ* and *ex site* level.

Involving public sectors and government extension service in evaluation of promising landraces among farmers/ communities/ ethnic groups followed by gene bank procedures in government side and contribution of on-farm conservation knowledge in farmer community would enhance the survival chances of landraces at on-farm and fulfill regional food security (Rana *et al.,*2007). The continuous maintenance of landrace *in situ* is an essential component of sustainable agricultural development (FAO, 1998).

1.6.2 Destroyed circumstances upon sustainable landrace diversity

All farmers assume that the choice of varieties and choosing the quality seed are important components before growing. Adaptable to growing environment, eating/cooking preferences of the consumers, market preference /price and cost of seed are facts thought by farmers to choice the variety. Most farmers sow seed from their own harvest or neighboring farm where there is without caring of varieties selection norms (Tin Aye Aye Naing *et al.,*2008). As a result, a considerable amount of varietal degeneration becomes the problem for facial harvesting season as well as for next growing season's seed source.

Nowadays, awareness and interesting of farmers upon the modern varieties increase promptly within two decades. Seed quality, improving yield and government supporting are forces for changing of farmer attitudes. Actually, a substantial number of high yielding rice varieties (HYV) have been developed and released for rain-fed lowland and irrigated ecologies since 1960 (Khin Win, 1991). Recently lowland landraces face with erosion at on-farm and they arrive to gene bank as quiescence condition for conservation.

In 2011 to 2015, government is taking aggressive measures to disseminate Good

Agricultural Practices (GAP) through the extension services, adoption of good-quality high-yielding varieties (including hybrids) in favorable ecosystems, transformation of conventional farming into mechanized production system, and conversion intensify rice farming and achieve higher production by input-use efficiency (Ministry of Agriculture and Irrigation, 2015). They aim to enhance local rice production by substituting with high yield improved varieties in local landrace varieties. Therefore, landrace and local cultivars; their role and survival in the Agri-sector arrive under the threat of endangerment level.

1.7 Problem addressed

Among upland and lowland ecosystems, there are tremendous factors which affect upon the landrace diversity. Major rice landrace cultivar pool is from resilient agriculture of upland and lowland ecosystems. Whatsoever, the approaching of intensive cultivation technology in these two subsistence agriculture systems and several factors, which addressed above, distract the existence of landrace diversity and structure (Figure 1.3). Leading to threaten upon the rice landrace diversity and structure, conservation measures should be identified and taken.

1.8 Goals and purposes

Study about the interaction of the genetic diversity and agronomic variation of landrace genepool under the affecting of natural and artificial factors are also needed for planning the landrace conservation and utilization effectively. Therefore, evaluation of landrace population structures and assessment of their genetic diversity and agronomic trait variation among agro-ecological zones or upland and lowland ecosystems are prime implementations and necessary measures in setting of rice landrace conservation strategies (Figure 1.4).

1.9 Hypothesis

1. Assessment of genetic diversity and structure of landrace populations and wide range of agronomic trait among different agro-ecological zones is critical for sustainable conservation and utilization. In Myanmar rice ecosystem, there are tremendous contrast factors among upland and lowland cultivation systems which affect upon the landrace diversity. With high ethnic diversity, proliferation of culture and heterogeneous topography, diversity of upland population are expected to be higher than lowland population.
2. Before setting the conservation and utilization plans, knowing about the population differentiation and genetic partition among upland and lowland populations is important because they have different adaptable and survival profiles under different regional ecology. And, we need to do representative strategies for each group depends on the evident profile of each group. Among our emphasized upland and lowland populations, there may be different genetic background and subspecies differentiation rather than agronomic characterization among groups.

1.10 Objectives

Two major objectives set for this study are

1. The characterization and diversity assessment of Myanmar rice landraces pools
2. Comparative assessment of landrace cultivars diversity among upland and lowland landrace groups (Figure 1.5)

The approaches used to achieve these two objectives are;

1. Genetic diversity of rice cultivars in Myanmar based on chromosome components.
2. Genetic variation of agronomic traits

Table 1.1. Rice classification based on grain type, growth duration, water regime and amylose content in Myanmar.

Classification	**Category**	**Criteria**	
		Length (mm)	length/ width
Type of grain	(A)Emata type	9.4<	3.30<
	(B)Latywezin type	9.4-9.8	2.8-3.3
	(C) Ngasein type	7.75-9	2.4-2.8
	(D) Meedon type	7.35-8.6	2.0-2.4
	(E) Byat type	<9	2.25-3
Growth duration		DTH	Date(Harvest)
	Short-duration rice (Kauk-yin),	< 150	October
	Medium-duration rice (Kauk-latt)	150-170	November
	Late-duration rice(Kauk kyi)	170<	December
Water regime	Irrigated (Lowland)		
	Rain fed (Lowland)		
	Dryland (Central Dry zone – depend on scattered rainfall)		
	Upland (sleep slope mountainous region- depend on rainfall)		
Stickiness and texture		Level of sticky	Amylose content
	Kauk Hnyin	Most sticky	0-5.1%
	Kauk Sei	< most sticky	2.6-17.1%
	Kauk Chaw	> Least sticky	17.2-24.4%
	Kauk Kyan	Least sticky	24.5-30.2%

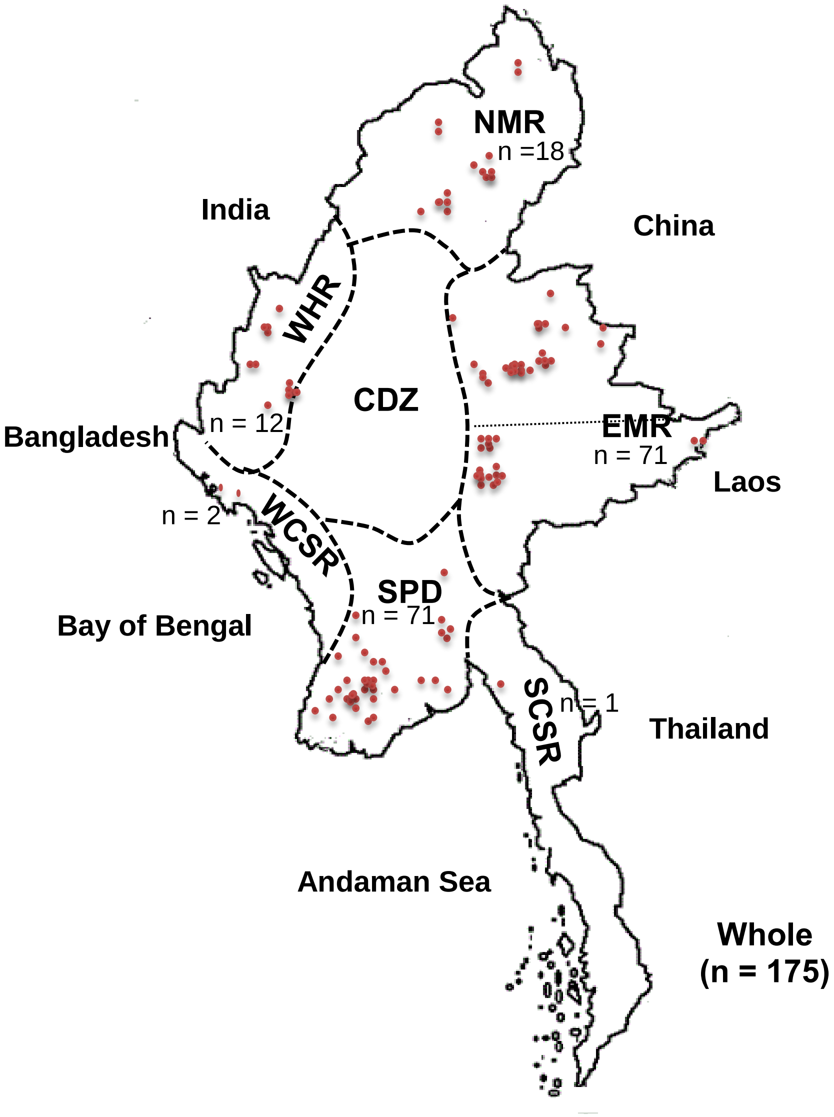


Figure 1.1. Map of Myanmar showing seven geographical regions, as follows: Western Hilly (WH), Northern Mountain (NM), Eastern Mountain (EM), Southern Plain and Delta Area (SPD), Central Dry Zone (CDZ), Western Coastal Strip (WCS), and Southern Coastal Strip (SCS). The red spots indicate the accessions' collection sites.

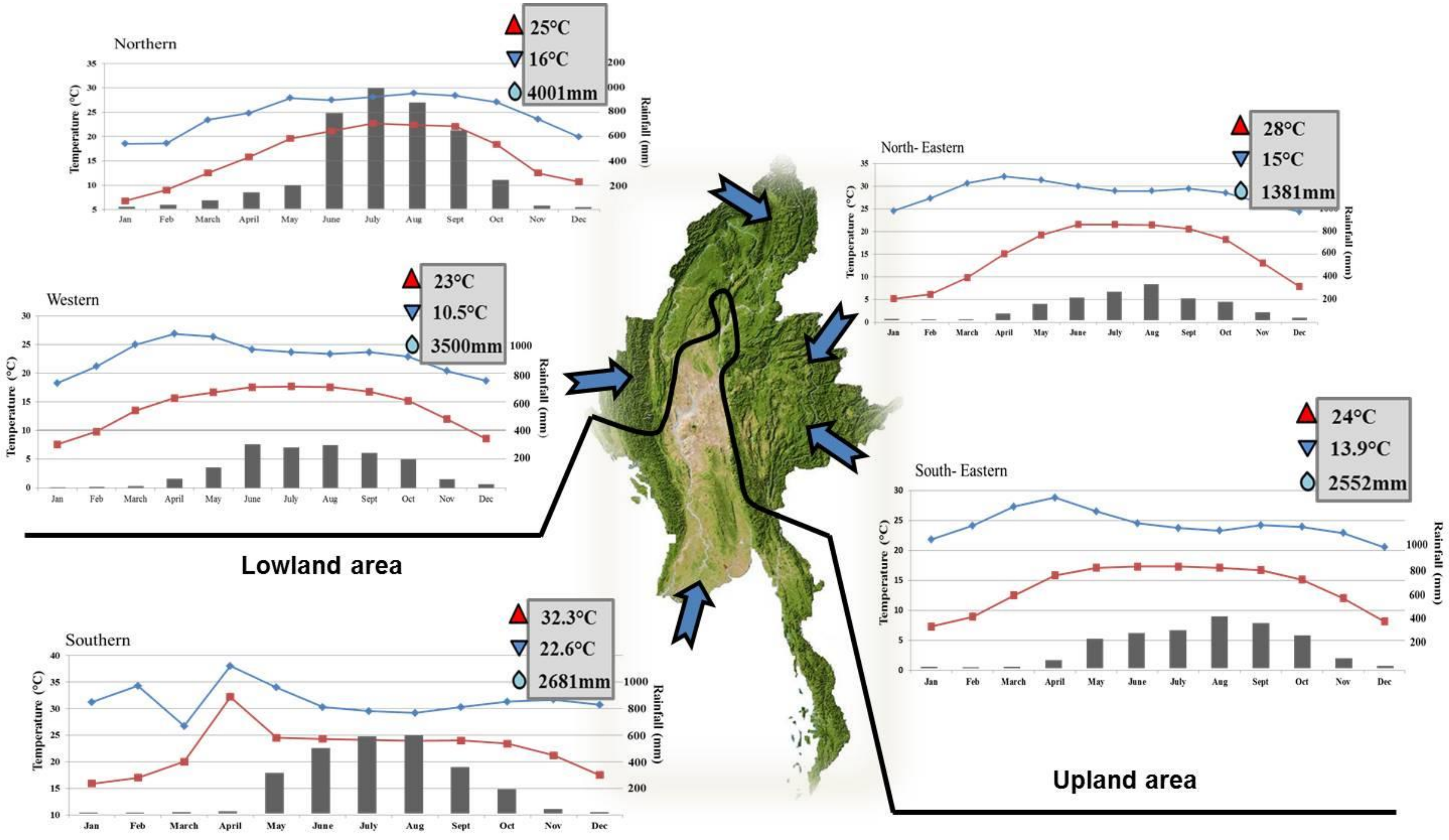


Figure 1.2. Map of Myanmar showing dominant area of upland and lowland ecosystem among seven geographical regions and changes of meteorological factors.

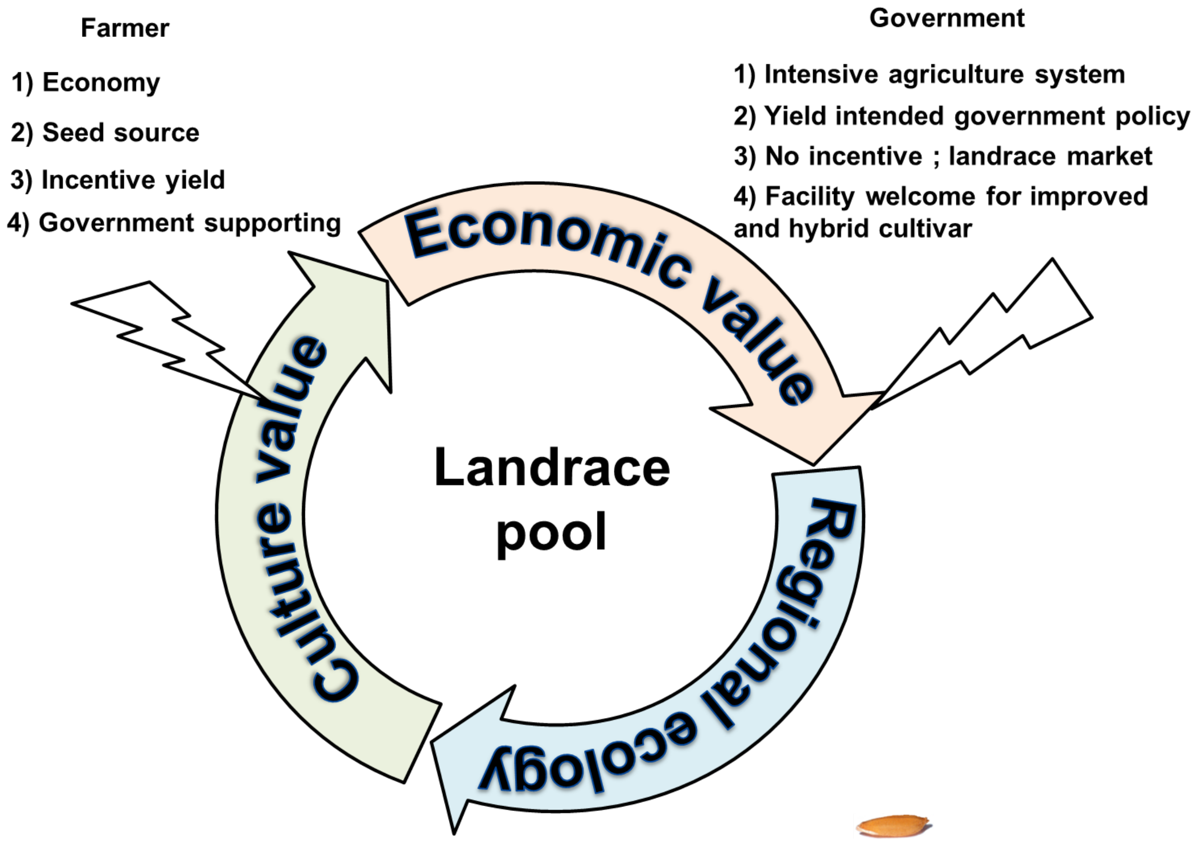


Figure 1.3. Current condition of rice gene pool under the threat of endangerment due to several factors.

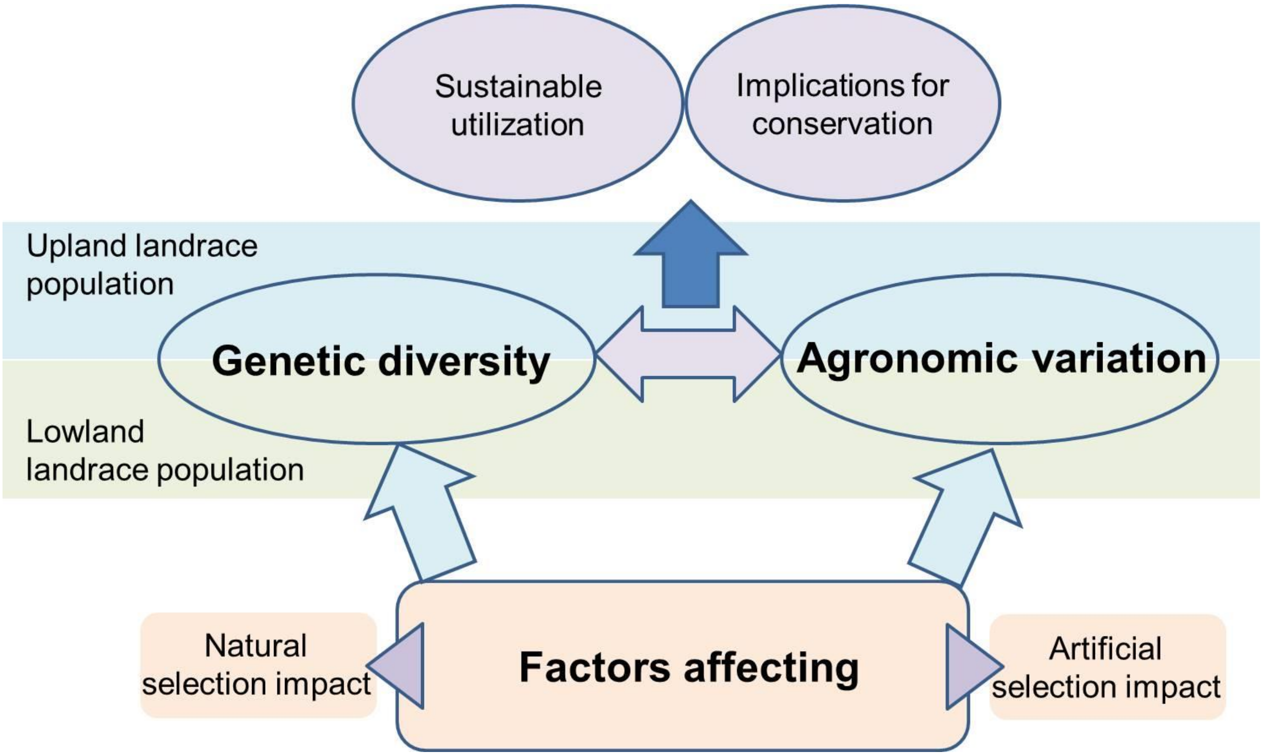


Figure 1.4. Schematic representation of whole study showing the interaction of the genetic diversity and agronomic variation of landrace genepool under the affecting of natural and artificial factors that needs to be evaluate prior to plan sustainable utilization and conservation measures of upland and lowland landraces.

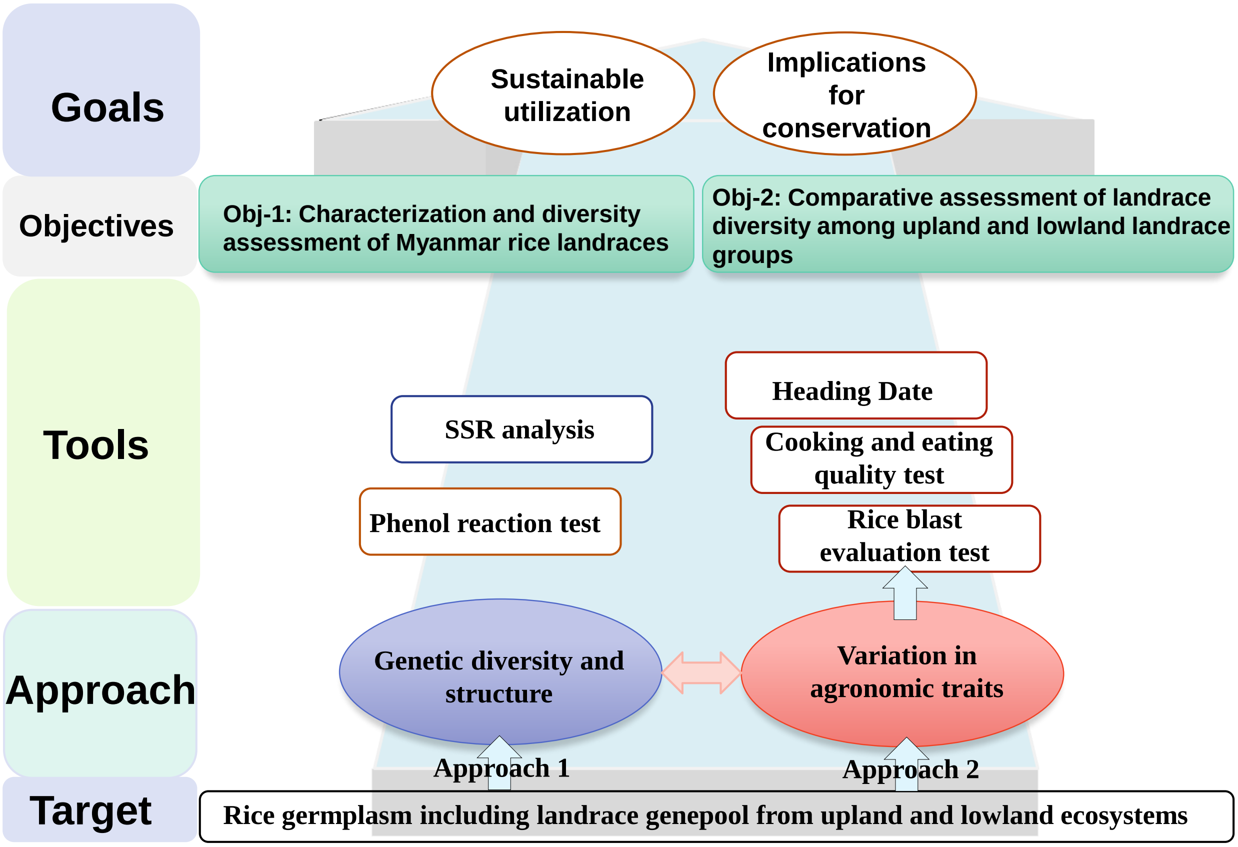


Figure 1.5. General schematic diagram represents correlation of goals and objectives, and approaches.

Chapter 2

Genetic diversity of rice cultivars in Myanmar based on chromosome components

2.1 Introduction

Globally, rice is the most important crop in terms of its contribution to human diets and value of production. It is a tremendously variable species with worldwide distribution. Phylogeographical and archeological evidences suggest that the Asian cultivated rice has a polyphyletic origin (Aggrwal, 2003) and divided into two sub-species, *O.sativa* ssp *indica* was domesticated in Southeast of South Asia while *O.sativa* ssp *japonica* was domesticated in southern China (Huang *et al.,* 2012). Situation of Myanmar among these two rice diversity hot-spots and its geographic position seem like key position of rice genetic resource. Genetic diversity of rice varieties is expected to be high in Myanmar (Khin Myo Myint *et al.,*2012).

From colonial times to present time, economy and food security of Myanmar had been monopolized by rice production with two main types of rice cultivation systems; "La" cultivation (Lowland) and "Ya" cultivation (Upland), which covered eight million hectares (http://faostat.fao.org, 2009). Upland (Ya) rices are one form of natural agriculture resource because of its adapting to harsh environmental conditions through more resilient agricultural systems (Ye Tint Tun, 2006). But lowland (La) rice diversity depend on farmers' economic, social and cultural choices (Khin Win, 1991). Cultivar variation is also essential for rice farmers assuring crop production stability with relevant adaptation, cropping systems and culinary quality. However, landrace diversity is important, especially, for subsistent rice grower or ethnic farmer to do choice for local adaptable and regional specific preference. Their seed selection practices and management create the genetic structure of rice cultivars (Ohm Mar Saw, 2007). Nowadays, traditional cultivars are rapidly losing due to land-use and agriculture practice impacts. Therefore, landraces structure and diversity is interesting information for conservation and utilization.

Studies of Myanmar rice landraces using Isozyme for classifying (Khush *et al.,* 2003 and Nakagahra and Hayashi,1977), molecular markers for comparative studies between on-farm population and seed-bank collection (Yamanaka *et al.,* 2011) and grain morphological characters for comprehensive interpretation of Myanmar landrace diversity (Ye Tint Tun, 2006) were conducted and documented as high diversity profile of Myanmar rice cultivars and suggested for effective conservation. Actually, rice genetic resource conservation and evaluation is essential to guarantee germplasm source for further breeding purposes. Better understanding of genetic diversity, structure in each agro-ecotype population and partition within upland and lowland ecosystem is one unexplored issue for Myanmar Agri-sector's rice improvement program.

This study evaluate the genetic variation and structure of rice cultivars from different Agro-ecological zones by using SSR markers and phenol reaction. Outcomes of this study will provide not only an image of the relationship between agro-ecological region and genetic variation but also scope for illustrations of conservation and sustainable utilization of rice landraces in Myanmar.

2.2 Materials and methods

2.2.1 Plant materials

A total of 175 rice accessions from upland and lowland in Myanmar were analyzed (Table Appendix 1). The Ministry of Agriculture and Irrigation's Seed Bank Section in Yezin, Myanmar, provided 101 upland accessions (93 landraces and 8 improved cultivars) from three upland regions, Western, Northern and Eastern. The Plant Biotechnology Centre (PBC), Yangon, Myanmar, provided 74 lowland accessions (60 landraces and 14 improved cultivars) from the delta and Southern lowland plains regions. All accessions were grown three generations for cultivar purification at PBC.

2.2.2 Phenol reaction detecting

The phenol reaction of rice accessions were investigated by standard method for varietal purity testing by Walls (1965). Two replicates of 5 seeds for each accession were soaked in distilled water for 18 hours. The seeds were placed in polyethylene bags which was pinched and embedded in 2% phenol solution and kept at room temperature (28°C). After 24 hours, the seeds were examined for staining. Depend on the non-staining or staining level, we designated as negative reaction (-) and positive reaction (+),(++) and (+++).

2.2.3 Genomic characterization

All 175 accessions and the two control cultivars; Japonica-type cultivar ('Nipponbare'), and Indica-type cultivar ('Kasalath') were genotyped with 65 SSR markers distributed on 12 chromosomes. Thus markers were selected from a public database (http://www.gramene.org/) and used for analysis of Japanese cultivars by Tanaka and Fukuta (2014). DNA was extracted from one plant in each accession using the method of Monna *et al.* (2002), with a slight modification. In brief, 1 cm of rice leaf was ground in 100 µl NaOH in a Mixer Mill MM200 (Retsch) and then mixed well with 400 µl Tris·HCl, pH 8.0. After centrifugation (10min at 10,000 rpm), the supernatant was diluted 1:20 with sterile water and used as the DNA template for PCR. PCR amplification was performed in 10- µl PCR mixture containing 1 µl sterile H_2O, a total of 1.5 µl forwards primer (µM) and reverse primer (µM), 7.5 µl of 2 × Quick Taq HS DyeMix (Toyobo Co., Ltd.), and 5 µl DNA template.

PCR amplifications were carried out as one initial denaturation step at 94 °C for 2 min, followed by 40 cycles of denaturation step at 94 °C for 30 s, primer annealing at 55 °C for 30s , and primer extension at 72 °C for 1 min. PCR products were separated by gel electrophoresis in 3% agarose containing ethidium bromide, under TBE buffer, at 150 V. Bands were documented with a gel documentation system (Bio-Rad, USA) and scored against a 100-bp DNA ladder by using the Quality One software (Bio-Rad). The extracted data were exported as allele sizes and formatted for further statistical analysis.

2.2.4 Data analysis

Genotypes of each accession were represented by size of amplified bands at 65 SSR loci. Gene diversity, number of alleles per locus and expected heterozygosity were calculated for each locus. The genetic variation of each locus was also measure in terms of Polymorphic Information Index (PIC) using the Power Marker v. 3.0 software (Liu and Muse, 2005). PIC shows the utility and general information of the informativeness of each SSR as a tool.

A phylogenetic tree was constructed using the Mega v.6.0 software. And genetic structure was examined on the SSR marker using the assignment method implemented in the STRUCTURE software version 2.3.3(Pritchard *et al.,* 2000). Partition of individuals into number of clusters (K) based on the 65 SSR data. Probabilities of (K) were estimated using a Markov-chain Monte Carlo method, in which the chains were run with a burn-in of 10 000 iterations with a length of 100 000, in an admixture model with correlated allele frequencies. After performing the ten runs of Structure by setting the number of populations (K) from 1 to 10, the real K value was determined using the method proposed by Evanno *et al.* (2005), based on the changes in the log probability of data between successive K values. The membership probabilities (Q) calculated from STRUCTURE ≥ 0.75 were used to assign rice accession to clusters. Possible genetic exchange among groups of rice accessions can be detected in rice accessions with membership probabilities (Q) < 0.75 for all clusters. The Structure Harvester v 6.0 (Earl and von Holdt, 2011) was used to determine the final population.

In order to detect the partition among individuals within the same populations as well as among different populations, analysis of molecular variance (AMOVA) was performed using the ARLEQUIN 3.11 software (Excoffier *et al*.,2005). By using the GenAlEx v. 6.5 software (Peakall and Smouse, 2006), all data were then standardized and subjected to multivariate analysis using principle-coordinates analysis (PCoA), based on matrix of Nei's genetic distance (Nei *et al.*,1983), and F statistic (F*st*), based on allelic distance matrix, to indicate the degree of population differentiation were calculated.

2.3 Results

2.3.1 SSR diversity in Myanmar rice landraces

A total of 65 microsatellite primers were used and 414 bands were amplified among 175 rice accessions. The number of alleles per locus detected by microsatellite primers varied from 2 to 15 with an average of 6.4 alleles per locus. The highest polymorphism was observed at RM 7000 on chromosome 3 which showed 15 alleles. Based on the level of polymorphism detected by individual primers, three most informative primers (RM7000, RM8137 and RM276) were identified with polymorphic bands 15, 12 and 11, respectively. But, RM6313 belong to chromosomes (5) showed lowest alleles number (2) among 65 SSR markers (Table 2.6).

As a measure of the informativeness of the microsatellites, the average polymorphic information content (PIC) value was found 0.82 per locus with the range of 0.519 (RM291) to 0.919 (RM276) (Table 2.6). The large range of PIC values for the respective accessions provides greater confidence for the assessment of genetic diversity and relationships.

2.3.2 Genetic diversity among sub-populations

The distribution of accessions among agro-ecological zones revealed seven sub-populations The highest gene diversity and PIC value were found in the control group and the North-eastern population (0.82, 0.80), and the lowest in the lowland landrace population (0.73, 0.68) (Table 2.1). All SSR markers were polymorphic within the Northern, North-eastern, South-eastern and lowland cultivar populations and the control group, but monomorphic in the Western, lowland landrace and upland cultivar populations. The Western and upland cultivar populations accounted for only 6.9% and 4.6% of all 175 accessions, but their genetic diversity indexes ranked third and fourth. Comparison between landrace cultivars populations and improved cultivar population within each ecosystem, lowland improved population showed higher diversity indexes than lowland landrace population at Southern plain region, whereas, all upland populations except Northern population were higher genetic diversity than upland improved population (Table 2.1)

Genetic diversity of upland accessions showed slightly higher than the lowland accessions (Table 2.1). AMOVA and *F* statists results apportioned (31.12% of total variance and degree of differentiation within a population among groups of demes Fct = 0.34, P = 0.001) of the total molecular variation to the difference between upland and lowland groups, (10.24 % of total variance) to differences among the seven populations, and (58.08 % of total variance) to differences within populations (Table 2.2). Higher molecular variance (58%) was observed among landraces within populations than among populations and groups. F_{ST} also indicated significant differences between upland and lowland populations, with values ranging from 0.043 (western vs northern) to 0.219 (northern vs lowland landraces) (Table 2.5).

2.3.3 PCoA analysis, Clustering and detecting of phenol reaction

The two distinct groups were distinguishable by cluster analyses, UPGMA and PCoA on the basis of genetic distance among the 175 accessions. In the dendrogram drawn by UPGMA analysis (Figure 2.1 & 2.2), two main clusters were observed and tentatively designated as cluster I and cluster II. The accessions in cluster I included those from upland landraces and lowland improved cultivars with Indica control Kasalth. Furthermore, the cluster I could be subdivided into 3 sub-groups: group-Ia, group-Ib and group-Ic which overlapped in the PCoA scatter diagram (Figure 2.2). Groups Ia was distributed thoroughly among four upland regions, especially, South-eastern, Western and Northern regions were main distributed areas. In group Ib, almost all of the lowland improved cultivars were grouped with three lowland landrace cultivars; Nga Pya Gyi, Nat Pyi Hmwe and Nga Kywe. Indica-type control, Kasalth was separated from Ia and Ib groups, and designated as Ic (Figure 2.1).

Cluster II composed with lowland landraces from Southern plain and upland landrace and improved cultivars from North-eastern with Japonica control Nipponbare. Cluster II contained two sub-clusters, IIa, lowland landraces group and IIb, North-eastern upland landrace and improved cultivars group, and accessions of which were also distributed separately in the PCoA scatter diagram. The results of the two dimensional principal coordinate analysis agreed with forming of two main clusters base on the UPGMA cluster analysis (Figures 2.1 & 2.2).

In detecting the interspecific differentiation among and within the two main clusters, landrace accessions in Group I and Group II were associated with phenol color reaction. Almost all of upland landrace accessions and lowland improved cultivars from group I showed a positive color reaction, whereas, lowland landraces, and some upland landraces and improved cultivars in group II showed the negative color reaction (Figure 2.1 & 2.2). But a positive color reaction was found at twelve accessions from lowland landraces from IIa. Prominent of different phenol reactions among accessions, these two main clusters were thought to fit to two sub-species types, mainly Indica and Japonica.

2.3.4 Genetic structure analysis

The Bayesian-based clustering method gave the highest log-likelihood score when the number of populations (K) was 4. When K = 3, cluster I divided into two sub-populations, B-1 and B-2 (data not shown). At K = 4, cluster II divided into upland (A-1) and lowland (A-2) accession groups. Base on phenol detecting and UPGMA clustering result, these four groups were identified as Japonica-type upland group (A-1), Japonica-type lowland group (A-2), Indica-typed lowland and upland mixture group (B-1) and Indica-type upland group (B-2) (Figure 2.3). Of the 175 accessions, 167 (95.4%) had greater than 0.750 of membership probability (Q value) and they shared >0.750 membership with one of the four groups, and were thus classified as members of that group (Appendix Table 2). The remaining eight accessions each had a membership probability of <0.750 in any group and designated as admixture-type. These admixture accessions were found in the western (2), upland cultivar (1), lowland landrace (2) and lowland cultivar (3) populations (Table 2.3). Among these eight accessions of admixture-type, the range of Q value was from 0.38 to 0.71.

The Japonica and Indica-type groups were also clearly separated in the UPGMA tree but distribution of accessions was independent of their original agro-ecological zones (Figure 2.1). So we designated the representative regional group on the basis of the major accessions from the respective populations. STRUCTURE analysis showed that 53% of the accessions in A-1 came from the North-eastern population, 98% of those in A-2 came from the lowland landrace population, and 62% of those in B-2 came from the South-eastern population. In B-1genetic distinct group, which had the highest diversity indexes, 30% and 70% of accessions came from lowland and upland groups (Figure 2.4 and Table 2.3). Majority of lowland improved cultivar clustered in B-1 (Tables 2.3). A-1, the Japonica-type upland group, had the second highest diversity indexes, followed by A-2 and B-1 (Table 2.1). The population structure result when K = 4 showed similar results to the PCoA (Figure 2,2). Among the four main groups, A-2 versus B-2 had the highest F_{ST} value, and B-1 versus B-2 had the lowest F_{ST} value (Table 2.4).

2.3.5 Relationship between UPGMA cluster groups and genetic distinct groups

The cluster analysis of five regional populations using with UPGMA method divided into four groups; Ia, Ib, IIa and IIb. In model-base clustering analysis, the bar plots of all 175 individuals were drawn to estimate the distinct groups using an optimal values of K=4 as the number of clusters. Between two clustering methods, there was some variation between distance base and model-base clustering.. Clustering variation of accessions between UPGMA and model-base analysis was Indica-type group accessions. Upland accessions from Ia (UPGMA) included 50% of each B-1 and B-2 genetic distinct groups. All of lowland improved-type from Ib (UPGMA) were B-1 genetic background. Clustering of Japonica-type accessions was not different between distance-based UPGMA analysis and model-base STRUCTURE analysis (Table 2.4). These results were confirmed by principle coordinate analysis, in which the first coordinate account for 45.38% of the variation and second coordinate accounts for 16.64% of the variation. Distribution pattern of accessions in PCoA and model-base genetic distinct groups showed consistency in grouping.

2.4 Discussions

2.4.1 Genetic diversity within and among regional populations

According to the records, the 175 rice accessions utilized in this study were collected from five geographical regions of Myanmar; the southern lowland areas and four upland mountainous regions. Different rice growing environments, diverse rice consumption behaviors and complex environmental conditions among upland and lowland ecosystems were associated with landrace diversity differences among and within the agro-ecological zone. The assessment of genetic diversity among agro-ecological zones is an essential component in germplasm characterization and conservation.

The present study revealed the considerable high genetic diversity, with mean number of alleles (6.2) and an overall Nei's gene diversity of (0.846) among the 175 rice accessions and that value was higher than the former study of country-wide gene bank collection set (0.809) and similar country-wide on-farm population set (0.826; Yamanaka *et al.,* 2011). Therefore, current studied sample reflected a trend of diversity status of rice landraces at farmer's field. Among upland and lowland populations, there was clear differentiation of genetic variation such as different level of gene diversity and expected heterozygosity, and genetic diversity indexes. Evidently, the cultivation systems are considerably different between these two ecosystems.

Genetic diversity assessment among regional populations, there was considerable high genetic diversity in every regional population. The North-eastern population showed highest gene diversity (0.82) and PIC value (0.80) among the five regional populations and then Western, Northern, South-eastern and Southern plain followed respectively. In contract, Northern upland population showed lower genetic diversity than not only other upland landrace-type regional populations but also upland improved-type population. But, the lowest diversity indexes were observed in lowland landrace-type population from Southern plain. Comparison between upland and lowland populations, almost all of upland accessions are from the Western, Northern and Eastern parts of Myanmar, which are close to the rice diversity hot-spot of Assam, India (Choudhury *et al.,* 2014), and the region of japonica domestication in Yunnan, China (Huang *et al.,* 2012). This proximity dominates the regional diversity of upland landraces. Moreover, Nakagahra *et al,* (1977) pointed out the remarkable of diverse accessions from Northern and North-eastern part of Myanmar and estimate the center of genetic diversity of the cultivated rice. Additionally, the low diversity of landrace from Southern region is related to the extensive interesting of improved cultivars. Lowland area is rice productive area and almost all of farmer attitude shift to improved cultivars.

2.4.2 Differentiation of upland and lowland landrace groups base on SSR markers and phenol reaction

According to DNA polymorphism analysis and phenol detecting results, phylogenetic tree revealed that Myanmar rice landrace population was clearly differentiated into two main clusters; I and II. In positive color reaction dominant main cluster, I, piling of upland accessions from South-eastern in Ia, and clustering of upland accessions from Northern, Western and Eastern in Ib were observed. Independently clustering of upland accessions in group Ib indicate that genetic diversity in each upland region is correlated with ecological variability, environmental heterogeneity and human impact. In all studied accessions, seventy-three percent of upland landraces were grouped in cluster I and thought to be Indica-type landraces which have a broad zone of adaptation across an altitudinal cline, and their distribution is not strongly influenced by temperature (Xiong *et al.,* 2010; Ghaley *et al.,* 2012). This adaptation among the Myanmar landraces will prove useful for introducing or improving rice for particular regions. All of lowland improved cultivars were grouped with three lowland landrace cultivars; Nga Pya Gyi, Nat Pyi Hmwe and Nga Kywe and one improved upland cultivar; Yar 9, as Ib in cluster-I.

Cluster II was organized by two sub-groups, IIa (lowland landraces from Southern region) and IIb (upland landrace from North-eastern and upland improved cultivars) from contrast agro-ecological regions. Although cluster II was thought to correspond to the conventional varietal Japonica-type group, blending of accessions where positive phenol reaction accessions in the major accessions group of negative phenol reaction was observed in sub-group IIa. According to some reports, the Southern lowland areas of Myanmar is known as one of the natural habitats for both *O. nivara* and *O. rufipogon* (Vaughan 1994, Than Sein *et al.*,2004). Therefore, possible reasons are intraspecific or interspecific crossings within landraces and among landraces and wild under the influence of ecological and density-dependent genetic neighborhoods.

2.4.3 Molecular variance and fixation index among and within regional populations

Within upland ecosystem, *Fst* placed the Northern, Western and South-eastern groups genetically close (*Fst* = 0.043 to 0.046). The low values of *Fst* suggest human-mediated gene flow. Dispersal of upland landraces in those regions is based on adaptability to the upland ecosystem and to social factors of local tribes (Rana *et al.,* 2007). In the Eastern plateau region, a strong contrast between the genetic diversity of the North-eastern (Highest gene diversity) and South-eastern (Lowest gene diversity in upland group) was observed. Moreover, South-eastern population genetically closed to Northern and Western rather than North-eastern. But significant value of *Fst* among populations within upland ecosystem was lower than significant threshold level of population differentiation (*Fst* = 0.25 ; Hartl and Clark, 1997).

And AMOVA analysis indicated that 58% of total variation was due to difference within regional populations (Table 2.2). It is also supported by cluster analysis where clustering pattern of landrace accessions from different regions, even in the same region, tend to reflect variability at molecular level. Therefore, the relative closeness of some landraces from different regions clustered together in a dendrogram can possibly be explained by the fact that there is germplasm cross introductions among regions with attribution of ethnicity, traditional farming systems and consumption behavior within and among the regional populations.

However, clear genetic differentiation between upland and lowland groups was demonstrated by fixation index (F_{ST}). Value of F_{ST} among upland and lowland group (F_{ST}= 0.31) was higher than the threshold for significant population differentiation (Tables 2.2 & 2.5). Distinct phenol reaction, distribution of landrace diversity and different rice growing environments in each ecosystem may be responsible for clear identifying of diversity differences among upland and lowland groups.

2.4.4 Distribution of structure groups among different agro-ecological zones

The determination of population structure is usually based on geographical origin or phenotype (Petit *et al.,*2001; Evanno *et al.,*2005; Gwag, 2008). Here, the rice genotypes were clustered into four distinct groups reflecting the ecotype and geography that showed possible cues to detect the selection impacts. A-1, the upland Japonica-type group, had greater genetic diversity than A-2, the lowland Japonica-type group. The high genetic diversity in the upland rice ecosystem is considered a function of its ecological and evolutionary history (Hamrick and Godt, 1996) and an adaptation to the diverse conditions of the mountainous regions. In contrast, the lowland landraces are adapted to reliable conditions. In the South-eastern region, the (B-2) Indica-type upland group predominated (Figure 2.4), and had lower diversity than B-1, the Indica-type upland and lowland mixture group. This lower diversity shows that A-2 landraces in the Southern plain are affected by selection to adapt the regional environmental and socioeconomic factors. The B-1 improved cultivars are disseminated by farmers in the lowlands of the Southern plains. Among four genetic distinct group, three distinct group; A-1 and B-1 &2 overlap among upland populations. But, almost all of lowland landrace's genetic background was A-2. Among ecosystems, genetic background of population may be one genetic partition.

2.4.5 Comparative landrace diversity assessment among upland and lowland population

Overall, clear distribution of landrace structure and diversity among upland and lowland ecosystems was detected with diversity indexes, population differentiation, clustering and phenol color reaction. With high genetic diversity indexes and predominant of positive color reaction among populations, piling of accessions from three genetic backgrounds; A1, B1 and B2, are character of landrace pool from upland ecosystem. But, lowland ecosystem occupies accessions with negative phenol color reaction and A-2 genetic background. Whatsoever, distinct level of high genetic diversity was observed in landrace pool from upland ecosystem.

Moreover, there was higher genetic viability within populations and lower genetic variation among populations within ecosystems and among ecosystems, which is generally the case of normal larger populations. There was lesser vital number of individuals in each population and lower genetic variation was found among populations. Because there is no boundary for seed dissemination from one region to another. Thus, regional socio-economic, socio-culture base on ethnic diversity need to be considered for regional landrace diversity. To trace the human impacted landrace diversity, variation of regional adaptable and human preference agronomic trait were detected in Chapter III.

2.5 Conclusion

In this study, high genetic diversity was detected in each group and regional population. SSR profiles placed all landraces into two main clusters, I and II which are thought to correspond to the conventional groups, Indica and Japonica. With assuming of human pressure, high genetic diversity was detected in upland regional populations. Upland landrace populations which possess three genetic distinct groups were highly genetically diverse, widely overlapped and adapted among three mountainous agro-ecological zones. Especially, North-eastern population was high rate of genetic diversity among regional populations. Although, there was higher genetic variation among landraces within populations, clear genetic differentiation between upland and lowland agro-ecotype groups was clarified with intraspecific differentiation (phenol reaction) and diversity indexes of regional landrace population. Overall, this research highlights upon landrace diversity, intraspecific variation and landraces adaptable among different ecosystems and agro-ecological regions for supporting the sustainable utilization and conservation.

Table 2.1. Summary of SSR markers polymorphisms within seven sub-populations by region and model-based genetic group.

Population	No. of accessions	No. alleles	Gene diversity	Expected heterozygosity	Percentage polymorphic	PIC
Upland						
Landrace-type						
Northern	18	8	0.773	0.551	100.0	0.74
Western	12	7	0.776	0.548	98.0	0.74
South-eastern	33	9	0.769	0.537	100.0	0.73
North-eastern	30	10	0.820	0.642	100.0	0.80
Sum	93	11	0.800	0.640	100.0	0.80
(North-eastern) Improved-type	8	6	0.773	0.552	98.0	0.74
Total	101	12	0.830	0.661	100.0	0.81
Lowland						
(Southern) landraces-type	60	8	0.728	0.455	97.0	0.68
(Southern) Improved-type	14	8	0.794	0.587	100.0	0.76
Total	74	11	0.777	0.553	100.0	0.74
Control	10	8	0.820	0.642	100.0	0.80
Ground total	185	13	0.846	0.564	99.0	0.83
Model-based genetic distinct group						
A-1	34	8	0.787	0.577	97.0	0.75
A-2	55	6	0.700	0.408	78.0	0.64
B-1	54	10	0.807	0.614	97.0	0.77
B-2	32	5	0.663	0.327	78.0	0.59
Total	175	13	0.841	0.482	100.0	0.82

PIC = polymorphic information content. Total of 65 SSR markers selected from a public database (http://www.gramene.org/) were used. Identification and classification of these markers were carried out by Tanaka and Fukuta (2014).

Table 2.2. Analysis of molecular variance (AMOVA) based on 65 SSR loci of upland and lowland rice groups.

Source	d.f.	SS	Variance components	percentage of variation	*F*	*P*- value
Among groups	1	960.6	5.2	31.12	*Fct* = 0.31	0.001
Among populations within groups	5	740.2	1.9	10.24	*Fsc*= 0.19	0.001
Within populations	343	4409.5	12.5	58.64	*Fst* =0.16	0.001
Total	349	6110.3	19.6			

d.f., degrees of freedom; SS, sum of squares. Degree of differentiation within a population among groups of demes (F_{ct}), within groups among demes (F_{sc}), and within a population among demes (F_{st})

Table 2.3. Geographic distributions of rice accessions classified into four genetic distinct groups.

	Upland					Lowland		
Genetic distinct groups	Landrace-type				Improved-type	Landrace-type	Improved-type	Total
	Northern	Western	North-eastern	South-eastern	North-eastern	Southern	Southern	
A -1	2	1	18	4	7(1)	0	2(2)	34
A -2	0	0	0	0	0	55(1)	0	55
B -1	10	8(2)	9	9	1	5(1)	12(1)	54
B -2	6	3	3	20	0	0	0	32
Total	18	12	30	33	8	60	14	**175**

Number in the blank mean number of admixture accessions in that group

Table 2.4. Relationship between cluster groups of UPGMA and genetic distinct groups.

Cluster group	A-1	A-2	B-1	B-2	No. of accessions (%)
Ia	0	0	37	32	69(39)
Ib	0	0	16	0	16(9)
Ic *	0	0	1	0	1(0.5)
IIa	3	55	1	0	59(33.5)
IIb *	30	0	0	2	32(18)
Total	33 (18)	55(31)	55(31)	34(19)	177(100)

* Indica-type control, Kasalath from Ic was categorized into B-1 and Japonica-type control, Nippobare from IIb was categorized into A-1 genetic distinct group.

Table 2.5. Pairwise F_{ST} values within sub-populations, model-based groups and ecotypes.

Population	Western	Northern	South-eastern	North-eastern	Lowland landraces	Upland cultivars	Lowland cultivars
Western	0						
Northern	0.043**	0					
South-eastern	0.046**	0.046**	0				
North-eastern	0.100**	0.098*	0.097**	0			
Lowland landraces	0.213**	0.219**	0.208**	0.130**	0		
Upland cultivars	0.159*	0.157*	0.157*	0.098	0.154**	0	
Lowland cultivars	0.115**	0.114**	0.124**	0.113**	0.181**	0.119**	0

	B-1	B-2	A-1	A-2
B-1	0			
B-2	0.227*	0		
A-1	0.243**	0.433**	0	
A-2	0.342**	0.522**	0.237**	0

	Lowland
Upland	0.311**

* and ** F_{ST} significant at either $P \leq 0.05$ and $P \leq 0.01$, respectively

Tale 2.6. Diversity statistic at 65 SSR loci among 175 rice accessions.

Locus	Chromosome	Sample size	Major. Allele. Frequency	No. allele	No. effective allele	Expected Heterozygosity	PIC
4RM1	1	175	0.12	10	6.0	0.83	0.91
RM495	1	174	0.23	6	3.4	0.71	0.84
RM3604	1	175	0.24	5	3.1	0.67	0.82
RM259	1	175	0.18	4	3.7	0.73	0.85
RM6840	1	175	0.16	5	3.9	0.74	0.86
RM8111	1	175	0.28	5	2.4	0.58	0.76
RM8137	1	175	0.12	12	6.0	0.83	0.91
RM262	2	175	0.19	4	3.2	0.68	0.82
RM1367	2	175	0.25	7	2.9	0.66	0.81
RM3847	2	175	0.17	9	4.9	0.80	0.89
RM406	2	175	0.44	6	1.3	0.22	0.53
RM324	2	175	0.23	5	3.2	0.69	0.83
RM3865	2	175	0.22	5	3.5	0.71	0.84
RM6378	2	175	0.21	10	3.8	0.73	0.85
RM234	2	175	0.19	6	4.0	0.75	0.86
RM1347	2	175	0.19	6	3.6	0.72	0.84
RM240	2	175	0.16	6	4.6	0.78	0.88
RM8208	3	175	0.20	5	3.1	0.68	0.82
RM8203	3	175	0.21	5	2.9	0.65	0.80
RM168	3	175	0.16	7	4.9	0.79	0.89
RM7389	3	175	0.29	7	2.6	0.62	0.79
RM6959	3	175	0.16	9	5.2	0.81	0.90
RM7000	3	175	0.16	15	4.8	0.79	0.89
RM5586	4	175	0.23	5	2.9	0.66	0.81
RM3317	4	175	0.28	4	2.2	0.55	0.74
RM3367	4	175	0.15	8	4.6	0.78	0.88
RM3836	4	175	0.18	7	4.1	0.76	0.87
RM8213	4	175	0.18	7	4.3	0.77	0.87
RM3524	4	175	0.18	8	4.3	0.77	0.87
RM3476	5	175	0.29	7	2.4	0.58	0.76
RM3790	5	175	0.24	5	3.0	0.67	0.81
RM6313	5	175	0.44	2	1.3	0.20	0.52
RM413	5	175	0.17	8	4.4	0.77	0.88
RM267	5	175	0.16	6	4.5	0.78	0.88
RM405	5	175	0.19	4	3.2	0.69	0.83
RM1089	5	175	0.39	6	1.7	0.40	0.65
RM3663	5	175	0.41	6	1.5	0.33	0.61
RM276	6	175	0.11	11	6.6	0.85	0.92

RM162	6	175	0.23	4	2.9	0.66	0.81
RM3138	6	175	0.13	8	4.7	0.79	0.89
RM510	6	175	0.13	6	4.7	0.79	0.88
RM508	6	175	0.13	7	5.2	0.81	0.90
RM11	7	175	0.18	5	3.6	0.72	0.85
RM1134	7	175	0.32	3	2.0	0.51	0.71
RM1235	8	175	0.15	5	4.2	0.76	0.87
RM3153	8	175	0.28	8	2.6	0.62	0.78
RM408	8	175	0.23	4	2.9	0.65	0.80
RM3395	8	175	0.16	6	4.4	0.77	0.88
RM152	8	175	0.20	5	3.8	0.74	0.86
RM284	8	175	0.21	4	3.2	0.68	0.82
RM7356	8	175	0.19	8	4.3	0.77	0.87
RM6948	8	175	0.27	5	2.3	0.57	0.75
RM3164	9	175	0.15	8	5.1	0.80	0.89
RM7048	9	175	0.18	6	4.6	0.78	0.88
RM171	10	175	0.31	3	2.2	0.55	0.74
RM8201	10	175	0.21	4	3.2	0.69	0.83
RM258	10	175	0.21	8	4.1	0.76	0.87
RM271	10	175	0.14	7	5.0	0.80	0.89
RM21	11	174	0.20	5	3.4	0.71	0.84
RM552	11	175	0.28	10	3.0	0.66	0.81
RM5704	11	172	0.17	8	4.3	0.77	0.88
RM7376	12	175	0.26	5	2.5	0.61	0.78
RM247	12	175	0.21	9	4.3	0.77	0.87
RM17	12	175	0.35	4	1.9	0.48	0.70
RM7619	12	173	0.28	6	2.3	0.57	0.76
Mean			0.22	6.4	3.6	0.68	0.82
SE				0.3	0.1	0.02	

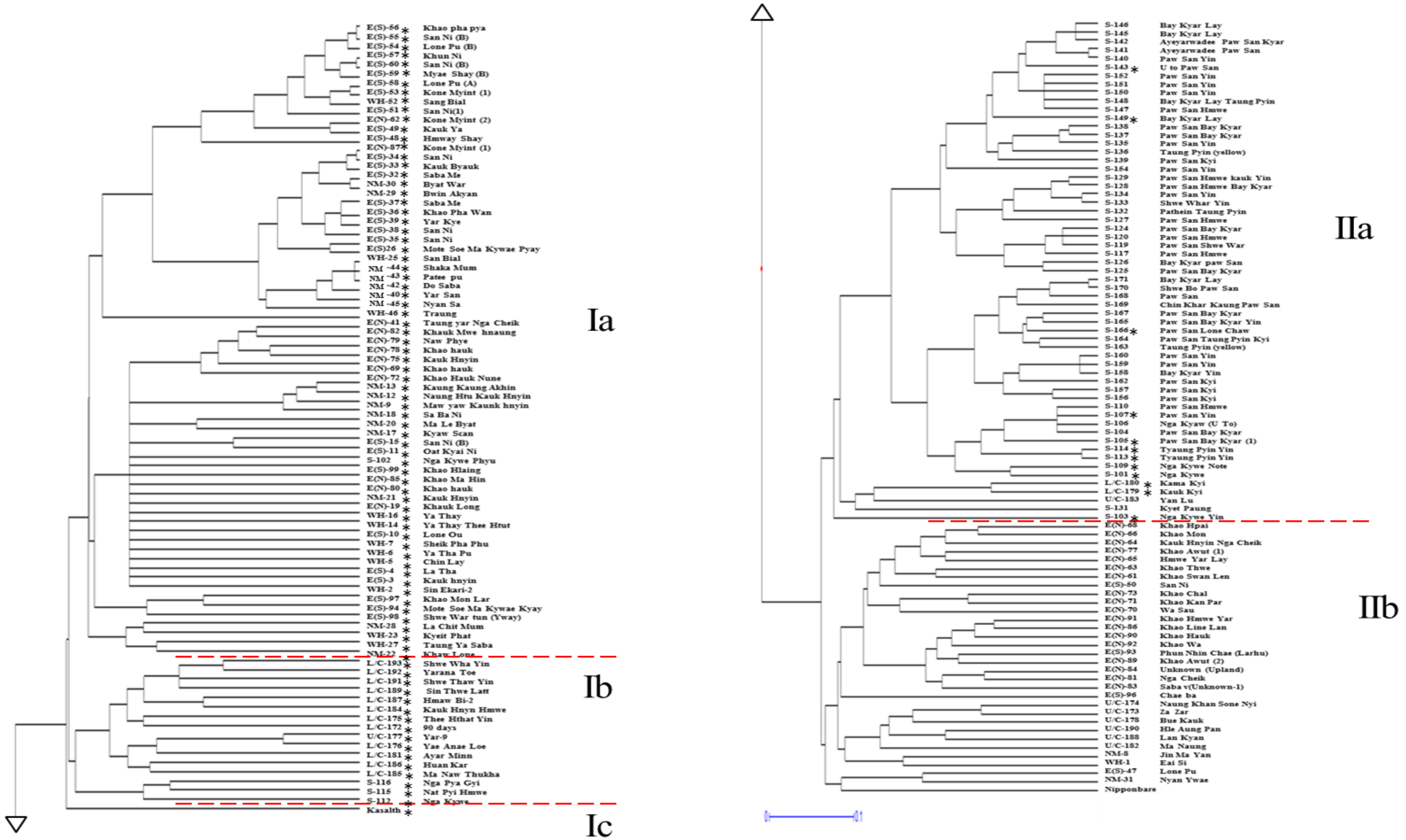


Figure 2.1. Dendrogram indicating relationships among 175 rice accessions based on UPGMA cluster analysis of SSR. Phenol positive reaction in each accession was shown by asterisk.

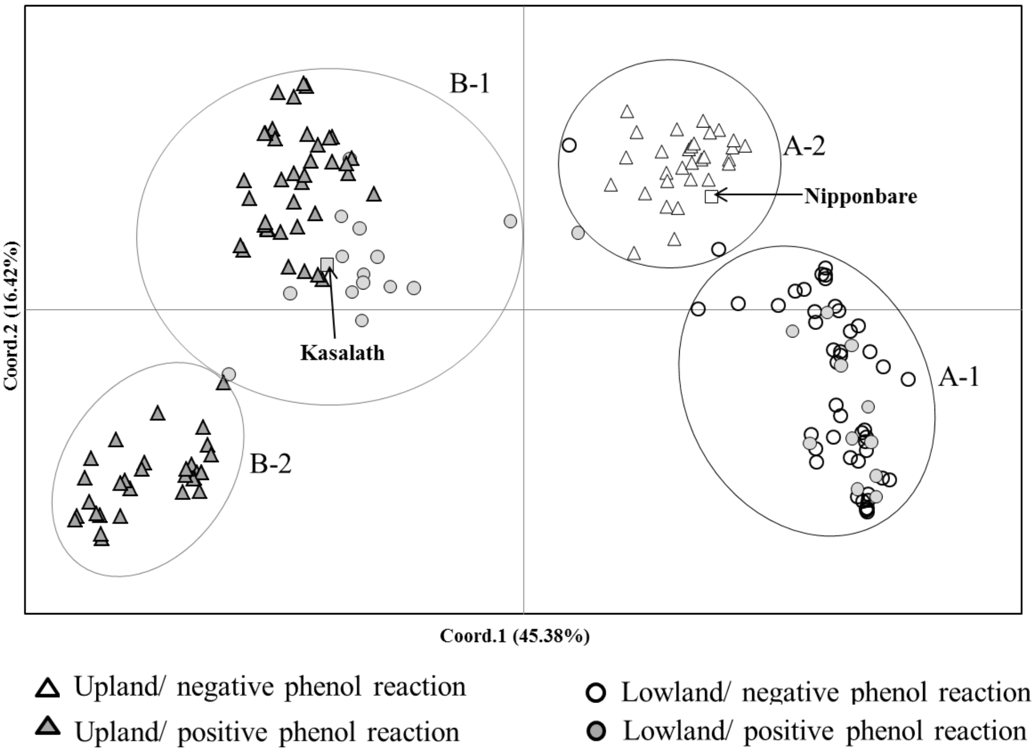


Figure 2.2. Graphic representation of principle coordinate analysis of upland and lowland landrace rice varieties generated through the SSR analysis of 175 rice accessions. Contribution of coordinate1 and coordinate 2 were 45.38% and 16.42% respectively. Dotted circle indicate the genetic distinct groups. Information of cultivated ecotype and phenol color reaction were mention with circle and triangle with black and white color.

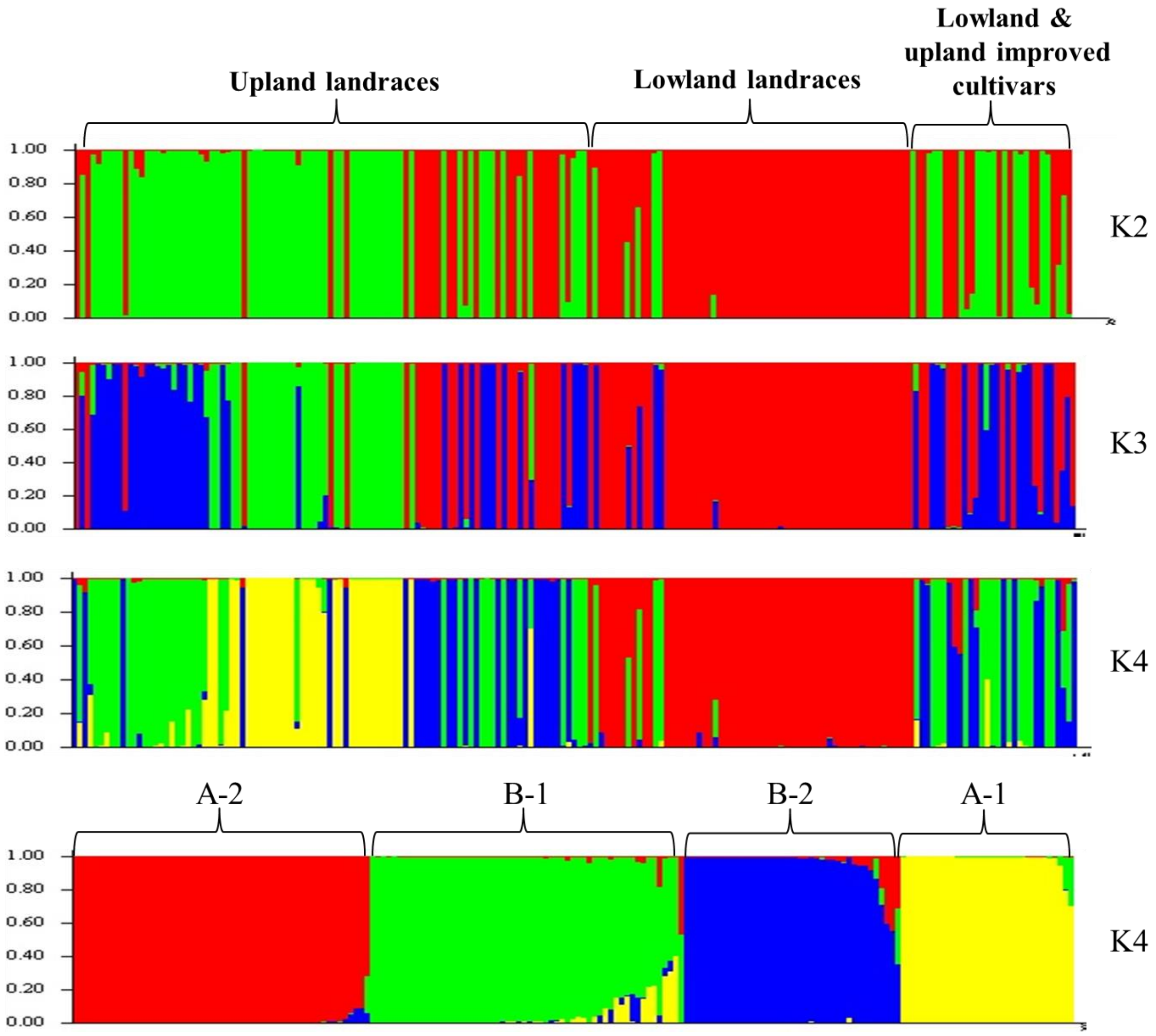


Figure 2.3. Bar plot of the STRUCTURE analysis with different numbers of ancestry groups (K) that ranges from 2 to 4. A-1 and A-2 groups were corresponding to the Japonica-type ancestry groups and B-1 and B-2 were corresponding to the Indica-type ancestry groups.

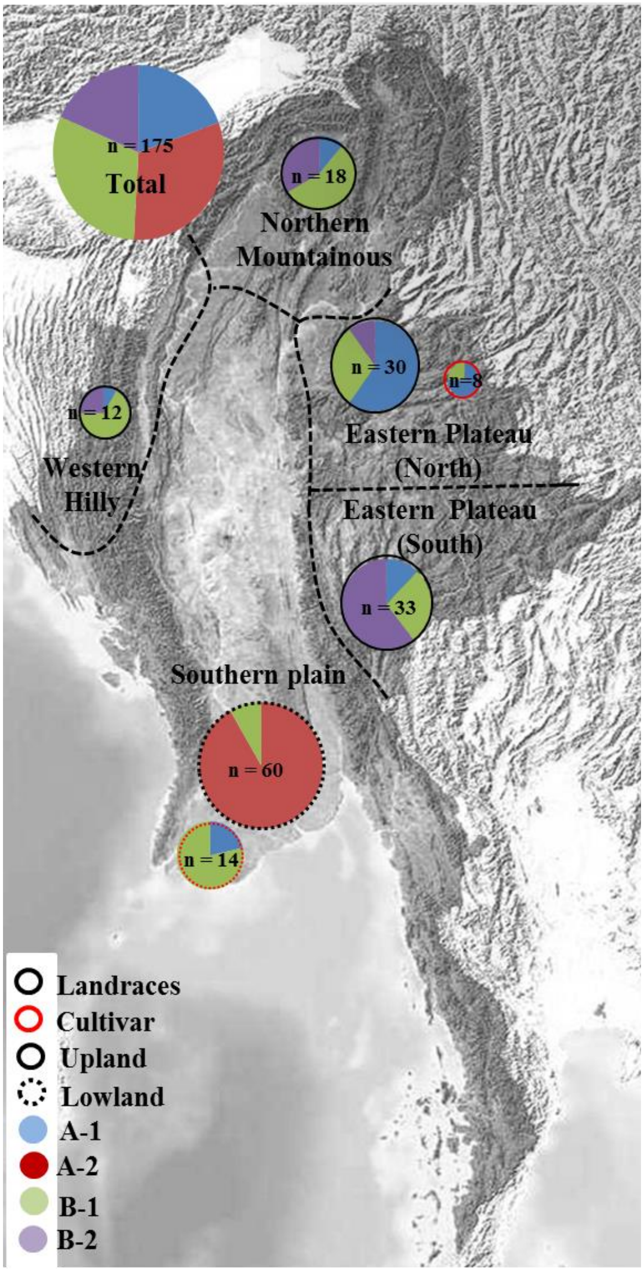


Figure 2.4. Genetic information of structure group of Myanmar landrace germplasms. Circle size corresponds to number of samples. Circle outlines indicate landrace types, as indicated in the key.

Chapter 3

Genetic variation of agronomic trait among Myanmar rice germplasms

3.1 Introduction

Rice is the main cereal crop and grown in various ecosystems of Myanmar. Actually, Myanmar rice cultivation was based on subsistence agriculture and farmers select varieties suited to a wide range of environment. Diversity of rice landraces, its genetic variation and distribution provides information regarding with its adaptation to the environmental changes and human attitude over time, to make a way for sustainable conservation and utilization of landraces (Anon 1985).

Information related with characterization and classification of germplasm support the management of germplasm and increases the efficient use of germplasm. Although molecular characterization is more efficient (Ni *et al.*, 2002), effective assessment of genetic variation, characterization and classification have been done on the basis of the agronomic traits and characters which are still used by many researchers for the breeding purposes.

Myanmar traditional landraces are still under cultivation by resource poor farmers who practice subsistence farming (Yamanaka *et al.*, 2011) and outcome is low yield. Although output is less productive, these landraces have wide adaptation to local or harsh conditions and they are assumed as a harbor of great genetic potential for rice improvement. Farmers interest the diversity of local landraces; result from association of complex environmental conditions and diverse farming systems, to choose their interested agronomic traits such as growth duration, resistant to biotic and abiotic stress and good quality. In previous chapter, there was considerable high in genetic diversity in each upland and lowland regional group. Besides knowing the genetic diversity, understanding the magnitude of agronomic trait variability in the population is necessary before exploiting a population for trait improvement. Therefore, agronomic trait variation among upland and lowland groups and different genetic background of landrace accessions is fundamental for genetic improvement and important for conservation plan.

Three studies of different agronomic traits among upland and lowland groups were conducted to determine the level of variations among regional populations within each ecosystem and understand which trait variations do partition between upland and lowland landrace groups. The specific objective is to study the variation pattern base on three agronomic traits, and association of trait variation patterns and different genetic backgrounds or Agro-eco types.

3.2 Heading date variation in Myanmar rice germplasms

3.2.1 Introduction

Rice plant reaches reproductive stage under the short day condition but reaching to reproductive stage or day to heading varies largely among rice cultivars. Fundamentally, heading time of rice plant is important trait for adaptability because regional and seasonal day length and temperature play crucial roles to define heading date (HD). Therefore, HD is a critical causal factor of distribution and regional adaptability of plant (Gao, 2014) and thus, is an important breeding objective (Ye Tint Tun, 2006). The differentiation of heading properties is closely related with types of rice and farming ecosystems in different ecological environments. In Myanmar, heterogeneous natural environments and various agro-ecosystems create the tremendous not only the diversity of rice landraces but also the variation of heading date.

The heading of cultivated rice varieties are evaluated and classified by farmer and selects varieties and adjust with local environment for sustainable production. Myanmar rice growers classified maturity of rice cultivar based on HD into three groups; early (Kauk-yin), medium (Kauk-latt) and late (Kauk-kyi), and their HDs are less than 150 days, 150-170days and more than 170 days, respectively. Moreover, Farmer categorized the rice varieties again base on growing time, such as 'pre-monsoon rice' (sown in March and harvest in July), 'monsoon rice' (sown in June-July and harvest in Oct.-Nov.), 'late monsoon rice' (sown in Aug. and harvest in Jan.) and 'winter rice'(sown in Nov.-Dec. and harvest in March) (Khin Win, 1991). Early maturity-type is an essential characteristic of pre-monsoon, late-monsoon and winter rice for multiple cropping in tropical and subtropical regions.

Myanmar rice cultivation comprises mainly with wet cultivation (Lowland) and dry cultivation (Upland).The topography and ecological environments are distinctly diverse.

Critical affecting factors upon HD such as temperature and day-length are different among two ecosystems. In high latitude (28°25’ N) of Northern part upland area of Myanmar, the yearly change of day-length ranges from 10:22 to 13:55 hrs. But 11:36 to 12:40 hrs range of day-length is observed in the southern part of the low latitude (15°16’ N). As country-wide observation, the long day condition is in March through September and the short day condition in October through February.

In observing the varieties adaption and distribution base on HD, knowing about the environmental fitness and survival changes of plant under control of extrinsic stimuli (e.g. day-length and temperature) is important. In this study, we report the heading date variation of upland and lowland rice landraces under two growing environments, Yangon (16° 47′ N) and Ishigaki (24° 34′ N). These results will be helpful in elucidating the variation pattern of HD base on the environmental conditions of different growing sites, and association of HD pattern and different growing environments or genetic backgrounds or Agro-eco types.

3.2.2 Materials and Methods

At Yangon (16°47’N), the ordinary rice growing season (monsoon) starts in June-July and ends in November-December. Day-length range of Yangon is 11:36 to 12:40 hrs. The same collection of 175 accessions of upland and lowland landraces studied in molecular analysis were grown in the ordinary rice cultivating season in 2010 at Yangon, Myanmar to investigate their day to heading. Another growing environment; Ishigaki (24° 34’ N), same collection accessions were grown at February, 2014. Range of day-length in Ishigaki is 10:30 to 13:35 hrs. To know the variation of HDs within and among the regional populations, the Tukey-Kramer HSD test was conducted using R version 3.2.1.

3.2.3. Results

3.2.3.1 Observing DH among two growing environments, Ishigaki and Yangon

A large variation of heading date was observed among Myanmar rice landraces. HD variation data of Myanmar landraces sown in 12 July, 2010 was investigated and the range of heading time was from 17th September to 15th December. The days to heading varied from 62 to 175 days, and three peaks around 75-80, 110, and 170 days were found in the distribution of accessions at Yangon. Majority of upland landraces reached heading between second week of October (HD: 90-days) and second week of November (HD: 120-days). Forty-two upland landraces reached heading before second week of October and their HDs were less than 90 days and classified as very early. In the contrast, two landraces headed after 11th November (HD: over 150-days) and very late.

Investigation of HD variation in Ishigaki, Japan, was observed among 175 rice accessions which were sown in 24 February, 2014 and heading time was from 4th April to 21st October. The days to heading varied from 47 to 260 days, and three peaks around 115, 155-170, and 245-250 days were also observed in the distribution. The variation of HD at Ishigaki was wider than at Yangon (Figure 3.2.1). Fifty percent of accession’s HD extended to over 150 days and 34% of accessions’ HD varied between 90 to 150 days. In two growing environments, HD of accessions at Ishigaki became later than those of Yangon (Figure 3.2.1). Lowland landraces were evident in prolonged DH at Ishigaki.

3.2.3.2 Variation of heading date within each regional group at two environments; Yangon and Ishigaki

Among two growing environments, the wide variation of HDs was found in the 74 accessions cultivating at Southern plain region, and these variation was from 63 to 158 days at Yangon and from 133 to 260 days at Ishigaki. There were three peaks at 80, 110-125 and

155 days in the distribution at Yangon, and two peaks at 140-185 and 245-260 days at Ishigaki.

In Yangon growing environment, the range of HD between 90 to 120 days was observed in upland accessions from four regions; Northern, Western, South-eastern and North-eastern. And there was one peak between 90- 125 days on each upland region. Upland populations from North and South-eastern had one peak at 80-95 days and 95-110 days respectively, and they had same HD distribution pattern at Ishigaki. Wide variation of HDs with two peaks at 95-10 and 230-245 days in Western population, and early HD peak 50-65 and 95-110 days in Northern population were prominent among five regions at Ishigaki rice growing environment (Figure 3.2.1).

3.2.3.3 Relationship of days to heading date among genetic distinct groups at two growing environments; Yangon and Ishgaki

In clustering of 175 Myanmar rice accessions, model based approach using 65 SSR markers reveled four distinct groups: A-1.-2; corresponded to Japonica-type and B-1,-2 corresponded to Indica-type. These four distinct groups were characterized based on the relationships of HD between at two growing environments, Yangon and Ishigaki (Figure 3.2.2).

Group B-2 was composed with early heading accessions and the variation was limited from 98 to 109 days at Yangon and from 87 to 138 days at Ishigaki. A-1 was also the early heading accessions group in compare with the other two groups A-2 and B-1, except for one lowland accession, GMJ-179 which was quite late heading in both growing environments. Day to heading varied from 65 to 175 days at Yangon and from 47 to 234 days at Ishigaki, and these variations were wider than those of B-2. B-1 showed the widest variations from 62 to 132 days at Yangon and from 50 to 241 days at Ishigaki. A-2 showed the quite unique distribution including four different types for HD, middle heading in both sites, late at Yangon and middle heading at Ishigaki, from early to middle at Yangon and late heading at Ishigaki, and late heading at both sites (Figure 3.2.2).

Although, upland adapted early maturity-type landraces were cluster in A-1, B1 and B2, lowland landrace were grouped in A-2. Scatter distribution pattern of landrace from four groups, accessions with B-1&2 genetic back-ground had a wide variation of heading at Ishigaki condition, while accessions with A-1&2 showed high range of heading date diversity at Yangon. Relationship of HDs between two sites; Yangon and Ishgaki in each cluster group, wide variation of heading date was found within A-1 and B-1 group. Early heading date with low variation among two investigation sites was B-2 ; India type upland group. HD of A-2 lowland Japonica-type group, was late and we observed three groups in scatter diagram; 1) late and low variable in HD, 2) late HD in Ishigaki and high variable in Yangon, and 3) low variable in Yangon and high variable in Ishigaki (Figure 3.2.3).

In Yangon environment, the highest coefficient of variation (25%) for HD was investigated in A-1 ranging between 65 and 175 days and followed by A-2(21.5%), B-1 (18.8%) and B-2 (2%) respectively. In observing the Ishigaki's HD variation among four groups, the highest CV for HD was found in B-1(45%), followed by A-1(38%), A-2 (18%) and B-2 (8%) (Table 3.2.1). Comparison between two growing environments, extremely prolong heading was found in A-2 genetic background accessions at Ishigaki environment, but dispersion rate in the variation within this group was lower than B-1 and A-2. Highest accessions dispersion in the variation was B-1 and range of HD was from 50 to 238 days. Second highest was A-2 (Table 3.2.1)

3.2.3.4 Distribution of HD phenotype among upland and lowland areas

Generally, all upland accessions were early maturity type in Myanmar growing environments even if they have difference genetic background. But, lowland accessions were more variation in HD and late-heading type was dominant. In Japonica-type background accessions; (A-1) early heading accessions were found in North-eastern upland population and (A-2) late-heading accessions were observed in lowland population. Even though, B-1 and B-2 Indica-type upland accessions from mountainous regions and lowland improved cultivars from Southern part are generally early heading type. Comparing the heading date between Ishigaki and Yangon sites, majority of accessions were late in heading date at Ishigaki. However, nine accessions from Northern, North-eastern and South-eastern showed early HD in Ishigaki than Yangon.

3.2.3.5 Characteristics of HD variation among regional populations and cluster groups

Heading date was investigated among regional landrace populations and cluster groups at two environments; Yangon, Myanmar and Ishigaki, Japan. The significant difference among genetic distinct groups and populations at two growing environments were identified by the Tukey-kramer HSD test.

A wide variation HD was observed among regional populations and groups at Yangon site, with the average of (102 days) ranging from (62 days) to (175 days). The average HD of North-eastern (75 days) was significantly earlier than those of the other populations, while 121 days in the Southern significantly shown as late flowering than those in Northern (94 days), South-eastern (96 days) and Upland improved cultivars (92 days)(Table3.2.1). Southern lowland landrace-type group and lowland improved-type group showed wide range of HDs within them, whereas, the lowest variation was observed in South-eastern population (Figure 3.2.1). Among the groups, average HD of A-1 (82 days) was significantly earlier than A-2 (125 days), B-1(91 days) and B-2 (101 days). Significant late HD was observed in B-2 (124 days). Majority of upland landrace’s HDs were earlier than 100 days, especially, North-eastern upland landrace's HDs were very early (less than 90 days). There was significant difference of HDs variation between North-eastern vs South-eastern in upland populations and upland population vs Southern lowland population (Figure 3.2.4(a)). Observation of HD in Japonica-type A-1 and A-2 groups, variation of lowland landrace group (A-2) was significant different with North-eastern upland group (A-1). But there was no significant variation among B-1and B-2 groups (Figure 3.2.4(b) & Table 3.2.1). Upland accessions with A-1, B-1 and B-2 genetic background were early HD type.

Large variation and late HDs was observed at Ishgaki site, with the average of heading date (156 days) ranging from 47 days to 260 days. Furthermore, Southern lowland population showed late in average of heading among seven populations. Except Southern population, all upland populations and lowland cultivar groups was no significant differentiation at Ishigaki. The average HD of group A-2 (206 days) was highest among four groups and was significantly higher than those of groups A-1 (116 days), B-1 (156 days), and B-2 (110 days). Although, there was no significant differentiation of HDs variation between A-1 and B-2, within Indica-type groups or Japonica-type groups and among A-2 and B-1 had significant differentiation (Figure 3.2.4 (a & b) & Table 3.2.1).

The considerably high variation among the accessions was found in B-1 at Ishgaki and A-2 at Yangon. If we extend, accessions with Indica-type genetic background had a wide variation of HD at Ishigaki growing environment due to lowland improved cultivars, while Japonica-type accessions showed high range of HD diversity at Yangon growing environment due to photoperiod sensitive local cultivars or landrace group.

3.2.4 Discussions

Generally speaking, rice is a short day plant and reaches maturity under the short day condition but the photoperiod sensitivity varies largely among rice cultivars (Vergara and

Chang, 1985). HD is critical for the regional and the seasonal adaptability of the cultivar, which can be divided into basic vegetative phase (BVP) and photoperiod sensitive phase (PSP). The length of BVP is controlled by temperature but the length of PSP is determined by the day-length during growing period. Vergara and Chang (1976) suggested that various combinations between BVP and PS make HD of rice cultivars exhibit abundant diversity.

HDs of Myanmar rice landraces widely ranged from 65 to 175 days. Result of HD investigation at two growing environments, early maturity type with low photoperiod was observed in North-eastern group. Low photoperiod sensitive cultivars are thought to be widely adaptable because they have little difference in growth duration when planted at different times of the year or at varying latitudes (Vergara and Chang, 1985). In lowland population, very late heading varieties were dominant in observing two growing environments and possible facts are due to high sensitivity to day length and temperature during their PSP and BVP. Early maturing landraces predominated in mountainous and hilly zones of upland ecosystem, while late maturing landraces influenced in Southern plain with early HD improved lowland cultivars. Due to co-existent of high and low sensitive cultivars, the considerably variation of day HD within lowland population was found in Yangon growing environment.

Among Myanmar agro-ecotypes, upland rice is usually sown in May after fire burning at April in the upland farming area and at June or July in the terrace farming area. Monthly average temperatures change from 14.1 to 22.9°C in March through October. Occasionally the temperature goes down to 4°C during November to February (Central Statistical Oragnization , 2001). Consequently early- maturity landraces seem to be welcomed in upland mountainous zone to escape cold damage in the flowering stage. In Myanmar, rice is grown throughout a year in the rain-fed condition and, however, a water regime is the most important factor for rice cultivation. In lowland rice, the photoperiod sensitivity is crucially important in order to adjust their growth periods to seasonal changes of water level.

Regarding with Japonica-type (A-1&2 genetic backgrounds) and Indica-type (B-1&2 genetic backgrounds) distribution among upland and lowland areas, cause of late HD in Japonica-type lowland accessions are due to PSP response rather than BVP because lowland temperature is favorable growing degree-day for vegetative growth. Possible fact in prolonging of heading date among accessions of upland Indica-type at Ishigaki is complex unfavorable temperature and day-length. In investigation of coefficient of variation within Indica-type and Japonica-type groups at two localities, Indica-type groups showed more dispersion in the variation at Ishigaki, while, Japonica-type groups reveled high variation in CV at Yangon. Therefore, Indica or Japonica-type which have geographic specific as well as temperature sensitive was very important in considering the regional adapted HD.

Distinctive geographical cline of HD was recognized between North-East mountainous zones and Southern plain zones (Ye Tint Tun *et al.,* 2005). Rice cultivars are thought to have been selected to adapt the local agro-ecological factors such as temperature, day-length, rainfall or water regime, organic and inorganic stresses etc. Generally, north to south topography declination and vertical distribution of percolation and temperature are reasonable facts for Myanmar landrace's heading date diversity. Varieties adapt to each agro-ecological zone are limited but rice varieties are gradually insensitive to day length via long-period natural selection and artificial domestication for high latitude cold region (Zhen-ling *et al.,* 2011).

DHs (or heading time) of rice landraces are determined by BVP and PSP and closely associated with local adaptability. Majority of landraces collected throughout Myanmar are sensitive to photoperiod and they have response to slightly changes of day length. When they were grown in long-day condition at Japan, most of them became extremely late and couldn't reach heading within the ordinary rice growing season.

Less sensitive cultivars to photoperiod have got popular in irrigated areas of the tropics because they are widely adaptable and suitable for cultivating in whole year round. In upland landrace accessions in this study, low variation within early HD range was observed and that is adaptable characteristic of HD trait for regional and seasonal environmental conditions. In the lowland landrace population, wide range of heading variation was observed to skip the unfavorable factors affecting upon HDs. Consequently the diversity pattern of HD among Myanmar rice landraces is illustrated not only by the variations of natural factors such as day length and temperature, but also by various artificial factors like farming system, cropping pattern, cultivating season and so on.

Table. 3.2.1. Descriptive statistics for day to heading among Myanmar rice accessions.

Location		Group	Minimum	Maximum	Mean	SD	CV%
Myanmar	Yangon	A-1	65	175	82.1	20.6	25.0
		A-2	63	158	125.3	27.0	21.5
		B-1	62	128	91.1	17.1	18.8
		B-2	98	109	101.5	2.0	2.0
Japan	Ishigaki	A-1	47	188	116.3	44.5	38.3
		A-2	133	260	206.2	37.4	18.1
		B-1	50	238	156.4	70.6	45.1
		B-2	87	117	110.4	9.1	8.3
Myanmar	Yangon	Western Hilly	73	116	101.3	12.1	11.9
		Northern Mountainous	75	132	94.1	15.6	16.6
		Eastern Plateau (North)	62	102	75.6	10.9	14.4
		Eastern Plateau (South)	71	109	96.8	9.9	10.3
		Upland (IM)	80	120	92.5	13.6	14.7
		Southern Plain	63	158	121.1	29.5	24.4
		Lowland (IM)	80	175	105.6	24.7	23.4
Japan	Ishigaki	Western Hilly	81	239	170.2	68.6	40.3
		Northern Mountainous	50	229	121.2	57.1	47.1
		Eastern Plateau (North)	47	216	110.3	46.6	42.3
		Eastern Plateau (South)	52	234	133.1	51.8	38.9
		Upland (IM)	84	171	114.5	33.5	29.3
		Southern plain	133	260	208.5	36.5	17.5
		Lowland (IM)	81	234	137.6	53.1	38.6

(IM) : Improved cultivar

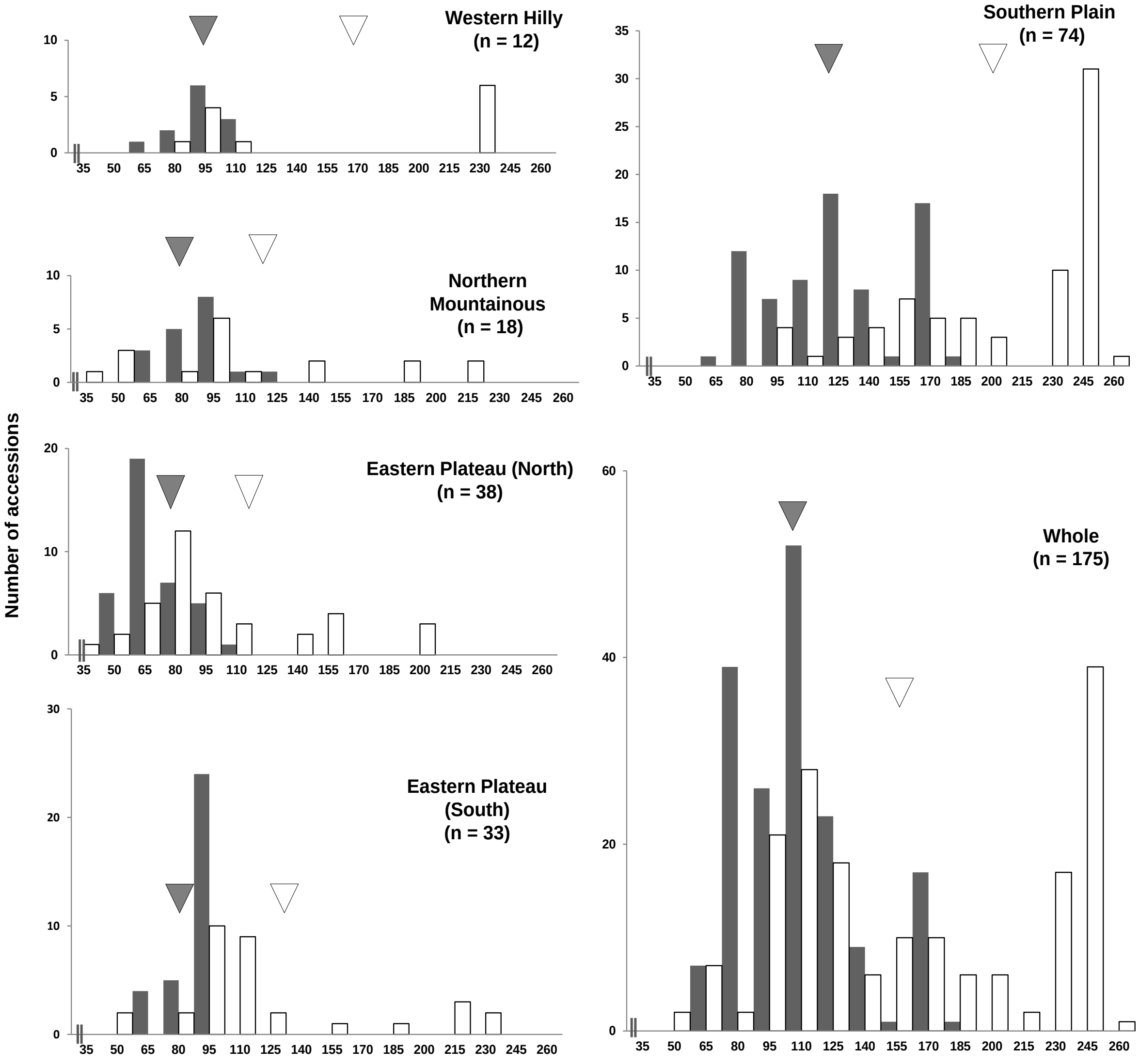


Figure 3.2.1. Variation of days to heading in Myanmar rice accession in each region. Black and white triangles indicate the average of days to heading date at Yangon, Myanmar and Ishigaki, Japan, respectively.

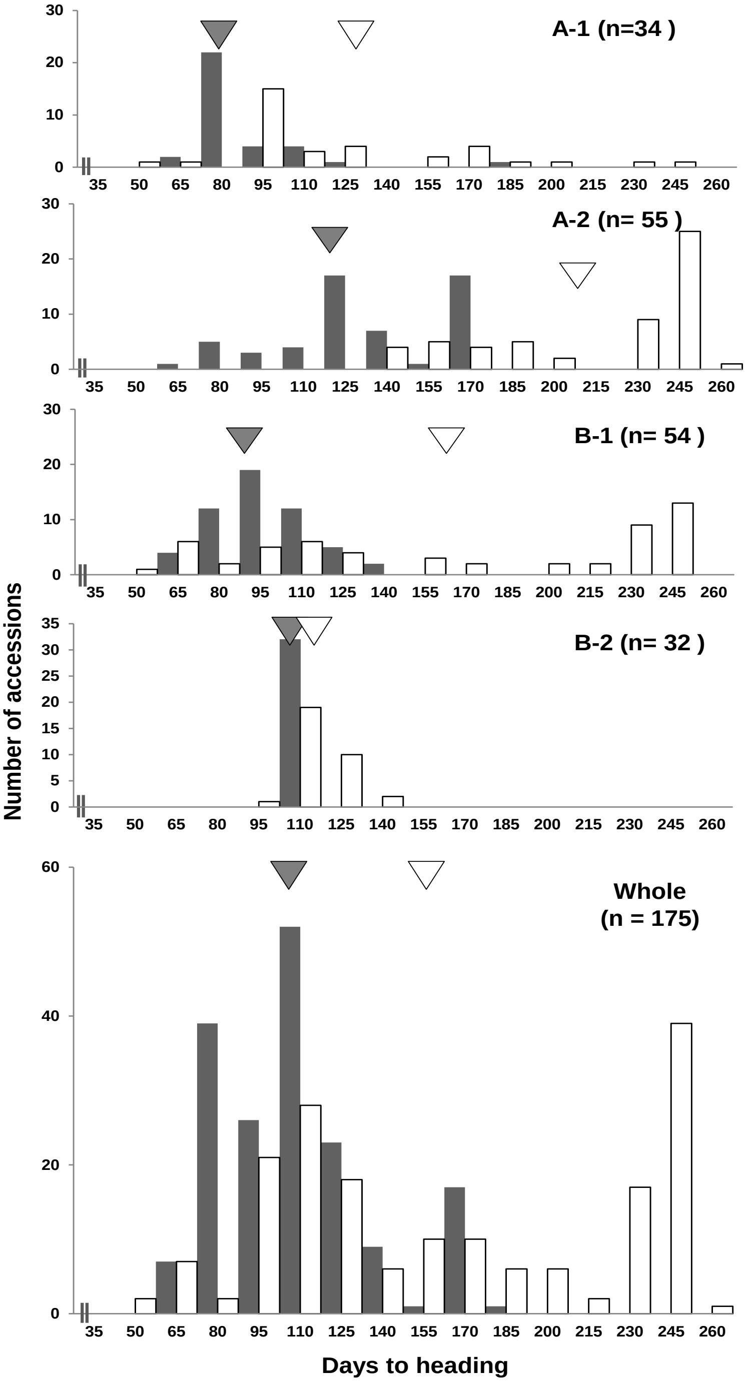


Figure 3.2.2. Variation of days to heading in Myanmar rice germplasms in each distinct group. Black and white triangles indicate the average of days to heading at Yangon, Myanmar and Ishigaki, Japan, respectively.

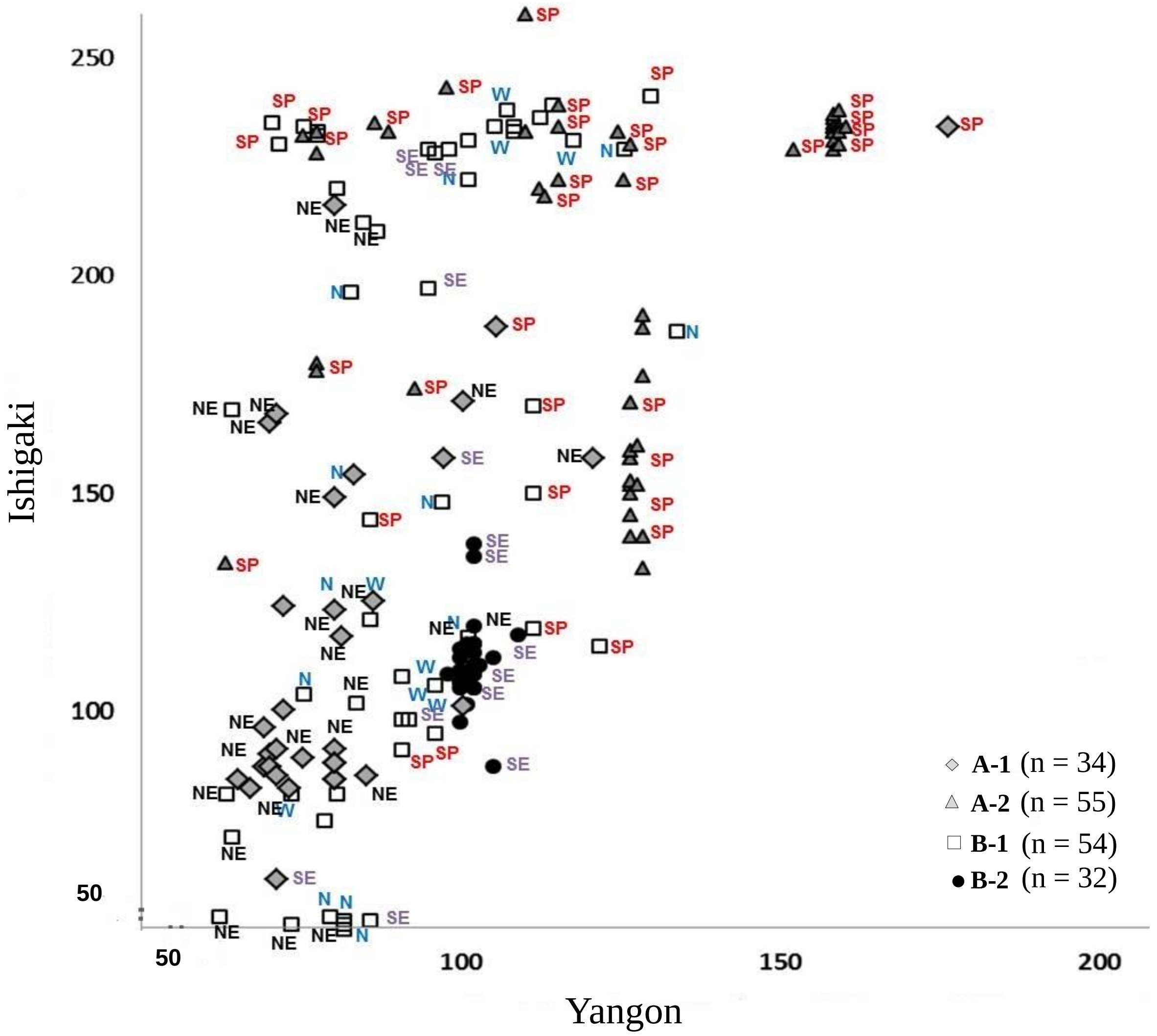


Figure. 3.2.3. Scatter distribution of four groups of days to heading at two growing environments; Ishigaki and Yangon.

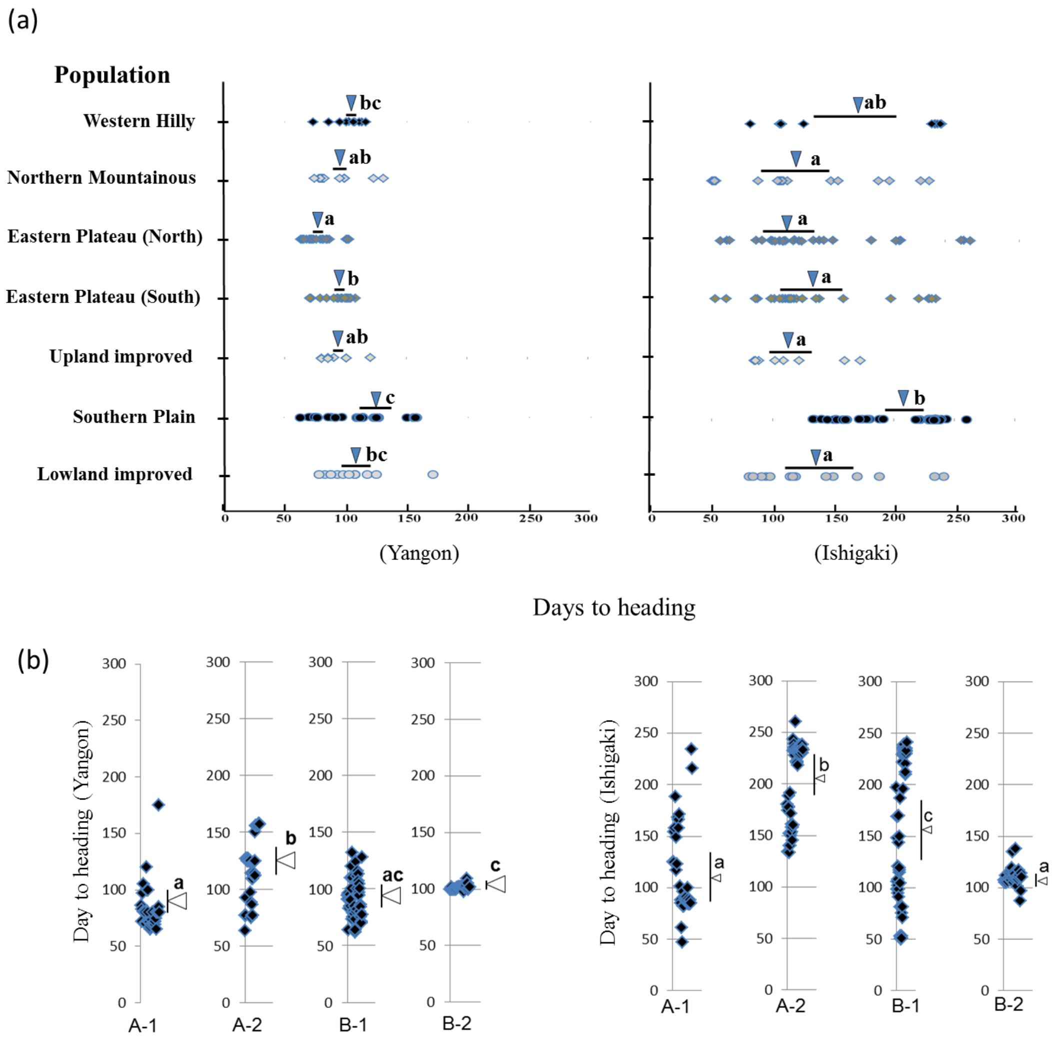


Figure 3.2.4. Characteristics of heading date of rice accessions (a) among seven populations and (b) among four cluster groups from two growing environments; Yangon and Ishigaki. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

3.3 Cooking and eating quality and seed shape variation in Myanmar rice germplasms

3.3.1 Introduction

The eating and cooking qualities (ECQ) of rice grains and grain shape are the major determinants of consumer preference and, consequently, the economic value of a specific rice variety. ECQ is mainly influenced by the physicochemical properties of starch, which constituent in milled rice differs widely among varieties (Juliano, 1979). Amylose content of the rice starch is principal factor in volume expansion, texture tenderness, and gloss scores of cooked rice regardless of water / rice ratio used during cooking (Juliano and Boulter, 1976). Another two physiochemical traits related with cooking and eating quality is Gelatinization Temperature; determine the time required to cook milled rice (Beachell and Halick, 1957) and Gel Consistency; measures the tendency of the cooked rice to harden on cooling.

In addition, Myanmar rice germplasms are greatly varied in grain morphological and physiological characters. Beale (1915) categorized five groups of Myanmar rice as Emata (A), Letywezin (B), Ngasein (C), Meedon (D), and Byat (E) based on the grain length / width ratio of rice (Table 1.1). The Emata and Letywezin groups have relatively long, thin grains whereas the Ngasein, Meedon and Byat groups are classified as bold, short-medium grain types. Emata, Letywezin and Ngasein are hard and translucent, and the Meedon and Byat groups are softer and more opaque. This classification is still used widely among Myanmar farmers for milling processes and marketing.

Though rice grain class preferences vary across regions among farmers, some cooking and eating quality preferences are widely shared in Myanmar. Because eating habits and preferences of Myanmar people not only vary from region to region but also between rural and urban areas (Pa Pa Aung, 2004). Moreover, rice is possible to be grown all year round in various environments and agro-eco conditions. The regional distribution of rice landrace with different cooking and eating quality, and grain size has not yet been characterized among and within agro-eco types and agro-ecological zones. In this section of this chapter, we assess the genetic diversity of upland and lowland landraces with regards to eating and cooking quality and seed size for the effective utilization of rice germplasm.

3.3.2 Materials and methods

To determine the CEQ and seed size among upland and lowland accessions, we measured grain length, grain width, and length-to-width ratio of 50 randomly selected seeds; measured the amylose content of fresh seed samples by the method of Cruz and Khush (2000); measured alkali digestibility for Gelatinization temperature by method of Little *et al*.(1958); and measured Gel-consistency by method of Cruz and Khush (2000).

3.3.2.1 Amylose content

Twenty whole-grain rice of all rice varieties were dehusked and polished. After that these were ground using beam shaker. 100 mg of rice powder was put into a 100 ml volumetric flask and 1 ml of 95% ethanol and 9 ml of 1N sodium hydroxide were also added. These flasks were boiled on boiling water bath to gelatinize the starch. After 1 hr cooling in room temperature, volume make up was done with distilled water (100ml) and shake well and stand for 20 mins. 5ml of this starch solution was added into a 100 ml volumetric flask and treated with 1 ml of 1N acetic acid and 2 ml of iodine solution (0.2 g Iodine and 2.0g Potassium Iodide in 100 ml of aqueous solution). Volumes make up were done using distilled water, then shake well and stand for 20 mins. Absorbance of the solutions was measured at 620 nm using spectrophotometer (Cruz and Khush, 2000).

3.3.2.2 Gel consistency

Ten whole-grain rice of all rice varieties were dehusked and polished. After that these were ground using beam shaker. 100 mg of powder was weighed in duplicate into the culture tubes (13 x 100 mm). 0.2 ml of 95% ethyl alcohol containing 0.025% thymol blue and 2.0 ml of 0.2 N KOH was added. Contents were mixed using a vortex. Tubes were covered with glass marbles and cooked in boiling water bath for 8 min (make sure tube contents reach 2/3 height of tube). Then tubes were cooled down at room temperature for 5 mins and transferred to an ice-water bath for 20 min. After that tubes were laid on graphing paper. Total length of gel was measured in mm from the bottom of the tube to the gel front (Cruz and Khush, 2000). The Gel consistency value (gel length) was classified as hard gel (5-40mm), medium gel (41-60mm) and soft gel (61-100mm).

3.3.2.3 Gelatinization temperature

Two sets of six whole milled kernels without cracks were selected and placed in petri dish. 10ml of 1.7% (0.3035 N) potassium hydroxide (KOH) solution was added. These petridishes were placed at 30°C in an oven for 23 hrs. Starch endosperm was rated visually based on a 9-point numerical spreading scale. Standard check varieties of high, intermediate and low gelatinization types of rice were used as control.

To know the variation of seed shape and eating and cooking quality traits within and among the regional populations, the Tukey-Kramer HSD test was conducted using R version 3.2.1.

3.3.3 Results

3.3.3.1 Diversity of amylose content of endosperm starch

Total of 175 accessions analyzed were classified into four apparent amylose groups: very low (6~12%), low (12~18%), intermediate (18~24%) and high (>24%). Studied sample of upland and lowland groups showed considerable high diversity in amylose content. The frequency distribution of landraces with different contents of amylose showed two peaks ranging from 9 to 35% (Figure 3.3.1).These two peaks appear at low amylose content class (12-18%) and Intermediate class (18-24%). In grouping of landrace cultivars among the amylose content class, very low (2, 1%), low (54, 31%), intermediate (76, 43%) and high type (43, 25%) were observed among 175 accessions of landraces (Appendix table 2). Majority of upland accessions were distributed in low and high amylose content, while, lowland accessions were dominant in intermediate type.

3.3.3.2 Regional variation of amylose content

Wide range of AC distribution among low, intermediate and high level was observed in upland populations, whereas, low variation of AC, especially in intermediate type, was investigated in lowland population. Among regional populations, the average AC of North-eastern (16%) was significantly lower than those of the other populations, while (26% and 25%) in Western and South-eastern population significantly higher than those in other populations. Southern lowland landrace group showed intermediate level of AC range within population, but no significant differentiation between upland and lowland improved groups was observed. Wide variation of AC was investigated in Northern population and South-eastern population (Figure 3.3.2) (Table 3.7.1).

3.3.3.3 Variation of amylose content among four genetic distinct groups

Among Indica-type; A-1 and A-2, and Japonica-type; B-1 and B-2, genetic distinct groups, average AC of A-1 (17%) and B-1(19%) were lower than A-2 (22%) and B-2 (28%). Almost all of lowland landraces clustered in A-2 were intermediate class, meanwhile, upland accessions from B-2 showed high AC class. Wide variation of AC was investigated in B-1 ranging between 9% and 34% (Figure 3.3.2) (Table 3.7.1).

3.3.3.4 Distribution of Gel consistency (GC) and Gelatinization temperature (GT) among agro-ecological zones and genetic distinct groups

Although Myanmar rice cultivars possessed the hard to soft gel consistency value ranged from 30 to 100mm, majority were medium and soft gel consistency properties of starch. In grouping of landrace cultivars among GC class, hard (4, 2%), intermediate (31, 18%), and soft (140, 80%) classes were observed among 175 accessions. The significant variations of GC were observed in regional populations, but no variation among four distinct groups was investigated. Generally, Myanmar rice landraces was governed with soft GC trait (Figure 3.3.5).

Time required for cooking is determined by gelatinization temperature(GT) and classified by low (55° to 74°C), intermediate (70° to 74°C) and high (>75° C). The alkali digestion, as an index for GT was performed and detected GT among Myanmar rice landrace germplasms. Low alkali digestibility ranging scores from 1 to 3 showed no dispersion to a little dispersion, while intermediate alkali digestibility ranging scores from 4 to 6 showed moderate dispersion. High alkali digestibility ranging scores from 7 to 9 showed almost complete dispersion. Wide distribution pattern of alkali digestibility scores was found in Myanmar landrace rice cultivars. Forty-six percent of cultivars showed alkali spreading score 1 to 3; 18% of cultivars possessed score 4 to 6 and 35% of cultivars had score 7 to 9 (Figure 3.3.3). These results indicated that Myanmar local rice cultivars are very diverse for the gelatinization or alkali digestibility properties. In addition, more than 40% of the studied cultivars had alkali digestibility scores of 1 to 3, suggesting that most of the Myanmar local rice cultivars are resistant to alkali. Observation of GT among regional populations, average of alkali scores in South-eastern population was significant lower than southern landraces population and northern population (Figure 3.3.4).

3.3.3.5 Genetic diversity of grain shape character in upland and lowland landrace germplasms and its geographical distribution

With the wide range of length-breath ratio from 1.7 to 4.7, tremendous variation of grain size was observed among Myanmar germplasms. Majority of upland accessions were dominant in slender-type or long and slender type. In North-eastern upland population, considerable high frequency rate of short-medium type was observed. Wide variation pattern of seed size was investigated in Northern, South-eastern and lowland improved populations. Prominent distribution of short-bold type was observed specially in Southern lowland landrace population. Among regional populations, significant changes of seed size were observed, especially South-eastern population vs Southern and South-eastern vs North-eastern (Figure 3.3.6). Within four genetic distinct groups, significant length-breath ratio variation was investigated between Japonica-type groups, A-1 & 2, and Indica-type groups, B-1 &2, but A-1 and B-1 showed the high variation of L/B ratio within groups.

3.3.4 Discussion

Grain quality depends on features such as Amylose Content (AC), Gelatinization Temperature (GT), Gel Consistency (GC) and Length/Width ratio, etc. Currently, grain quality has become the primary consideration of rice customers and breeding programs. For medicinal, ceremonial, or special production purposes, cultivars with different grain qualities are also required. In determining the grain quality, eating and cooking quality is important genetic basic norm and mainly affected by three physicochemical properties: amylose content (AC), gel consistency (GC), and gelatinization temperature (GT). Amylose content (AC) is recognized as one of the most important determinants of eating and cooking quality of rice and its synthesis is controlled by Waxy (Wx), encode starch granule-bound starch synthase (GBSS) (Hirano and Sano, 1991) and situate at chromosome 6 as a major QTL cluster (Hsu *et al.,* 2014). Moreover, involvement of complex starch biosynthesis system compose with four classes of enzymes: ADP-glucose pyrophosphorylase (AGP), starch synthase (SS), starch branching enzyme (SBE), and starch debranching enzyme (DBE) (Tian *et al.,* 2009). Each enzyme plays a distinct role (Ball *et al.,* 1996) but their interaction depends on the genetic background (*indica/japonica*) and environment. But variation of ECQ among germplasms and regions largely rely on the preference of Human and their utility.

Rao *et al.* (1952) and Juliano (1979) reported that the ratio of amylose and amylopectin in the rice grain influences many of the cooking and eating characteristics of milled rice. Amylose is almost absent from the waxy (glutinous) rice, and low amylose cooked rice is moist and sticky. Intermediate-type is moist and tender, and do not become hard after cooling. High amylose type show high volume expansion and a high degree of flakiness after cooling. In my studied accessions, a few samples were determined as very low amylose content especially from Southern region. Majority of upland landrace accessions were included in both low AC and high AC class, but intermediate AC class was few. Low AC type distribute especially Northern, North-eastern and South-eastern where are close to China, Laos and Thailand. Among 175 accessions, 54 accessions were Low AC type and 80% of them were collected from upland area. Therefore, stickiness texture of cooked rice is preference of hill man. As the same way, high AC type (25% of total) was also dispersal in upland regions for their regional preference and some utilities. In major rice productive lowland area, intermediate AC type was predominant due to market and customer preference. Among four clusters, accessions from B-1 &2 with Indica-type genetic background were dominant in high AC type rather than low AC. Takeda and Hizukuri 1987 also mentioned that Indica-type cultivars have more apparent amylose contents than Japonica-type rices. Actually, AC is recognized as one of the most important determinants of the eating and cooking quality but eating quality still differs among varieties with a similar AC, which can be determined by difference in the endosperm types, and amylose and amylopectin ratio (Farias and DeLaCruz, 1995).

Gelatinization temperature is the range of temperature wherein a physical properties of starch change (Cruz and Khush, 2000). Taking time for cook is determined by the gelatinization temperature of starch. The gelatinization temperature of rice varieties is classified as low (55-69°C), intermediate (70-74°C) and high (> 74°C). The alkali digestion score indicate the GT and alkali spreading among materials tested range from 1 to 9, and 46%, 18% and 35% of studied samples showed high GT, Intermediate GT and low GT. Although, high frequency of accessions was found at high and Low GT, wide range distribution of GT was observed among Myanmar landraces (Figure 3.3.3). The alkali digestibility behavior of rice endosperm starch granule depends on amylopectin side chain (Pa Pa Aung *et al.*, 2003). Possessing all variations for alkali digestibility can be suggested that upland and lowland landrace area in Myanmar are important for maintaining the genetic resources of amylopectin properties. Another observation of bimodal distribution of GT at high and low level among Myanmar landrace cultivars may be related with environment condition because temperature during ripening effect on the GT. During grain development, high ambient temperature causes the higher GT result, while, higher AC and lower GT result can come out under low temperature (Klush *et al.*,1979, Beachell and Stansel, 1963).

Gel consistency is also another good index of cooked rice texture. Varieties having the same amylose content may be differentiated in eating quality of cooked rice by the gel consistency test (Cagampang *et al.*, 1973). In total of 175 rice landrace accessions, the gel consistency value ranged from 30 to 100mm and majority were soft GC. Soft viscosity properties in Myanmar local rice cultivars ensure to utilize for the rice grain quality improvement because materials with soft GC is the most important objective of rice-quality breeding program (Klush *et al.*,1979). Wide variation for gel length was observed in the cultivars from Northern, Western and South –eastern populations (Table 3.7.1).

A broad genetic base in grain shape showed among Myanmar local rice cultivars, suggesting that large rice-crop diversity exists in Myanmar. Mountainous rice cultivars with its grain shape diversity play a prominent role in Myanmar and are still maintained and used by ethnic farmers because of their adaptability, and endemic characters preferred by the farmers(Khin Than Nwe and Tin Tin Myat, 2000). In lowland plain region, farmers rely and interest on market demand and productivity, uniform seed shape is preferred by market and reliable to milling processes.

Base on the grain shape and quality evaluation, upland landraces with high diverse in ECQ and seed shape were observed as high genetic diversity pool. Actually variation of cooking, eating, and processing qualities among rice is not solely a varietal characteristic but also depends on the crop production environment, harvesting, processing and handling systems. Therefore, high range of genetic diversity within and among populations depends on the complex interaction of crop adaptable, environmental attributions upon cultivation and human affecting upon crop such as selection and cultural practice.

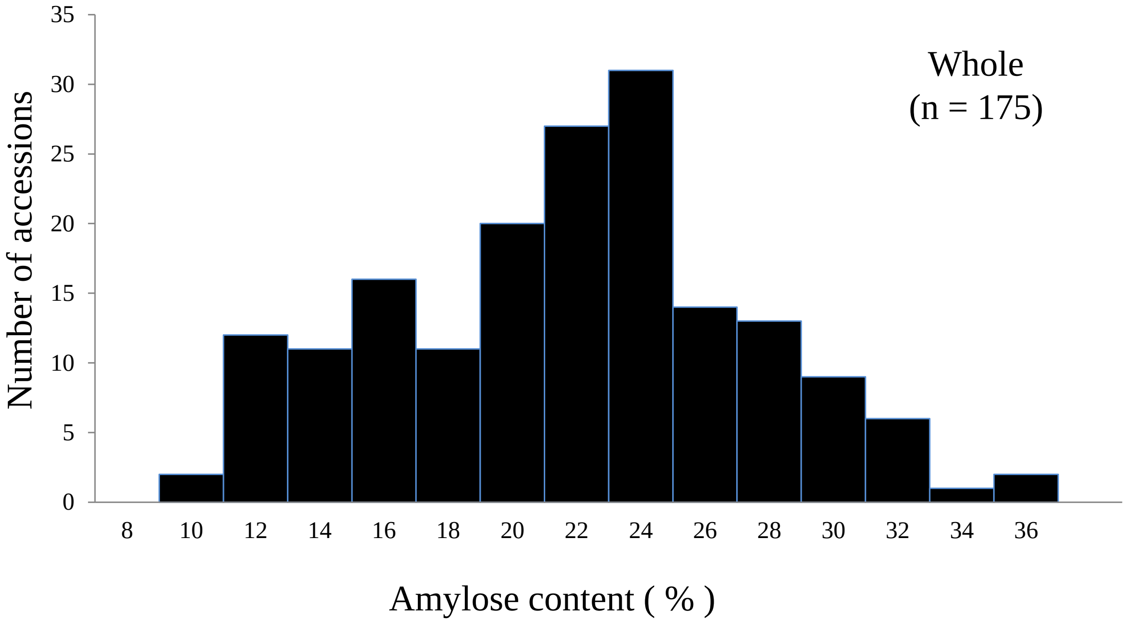


Figure 3.3.1. Distribution of amylose content in Myanmar rice accessions.

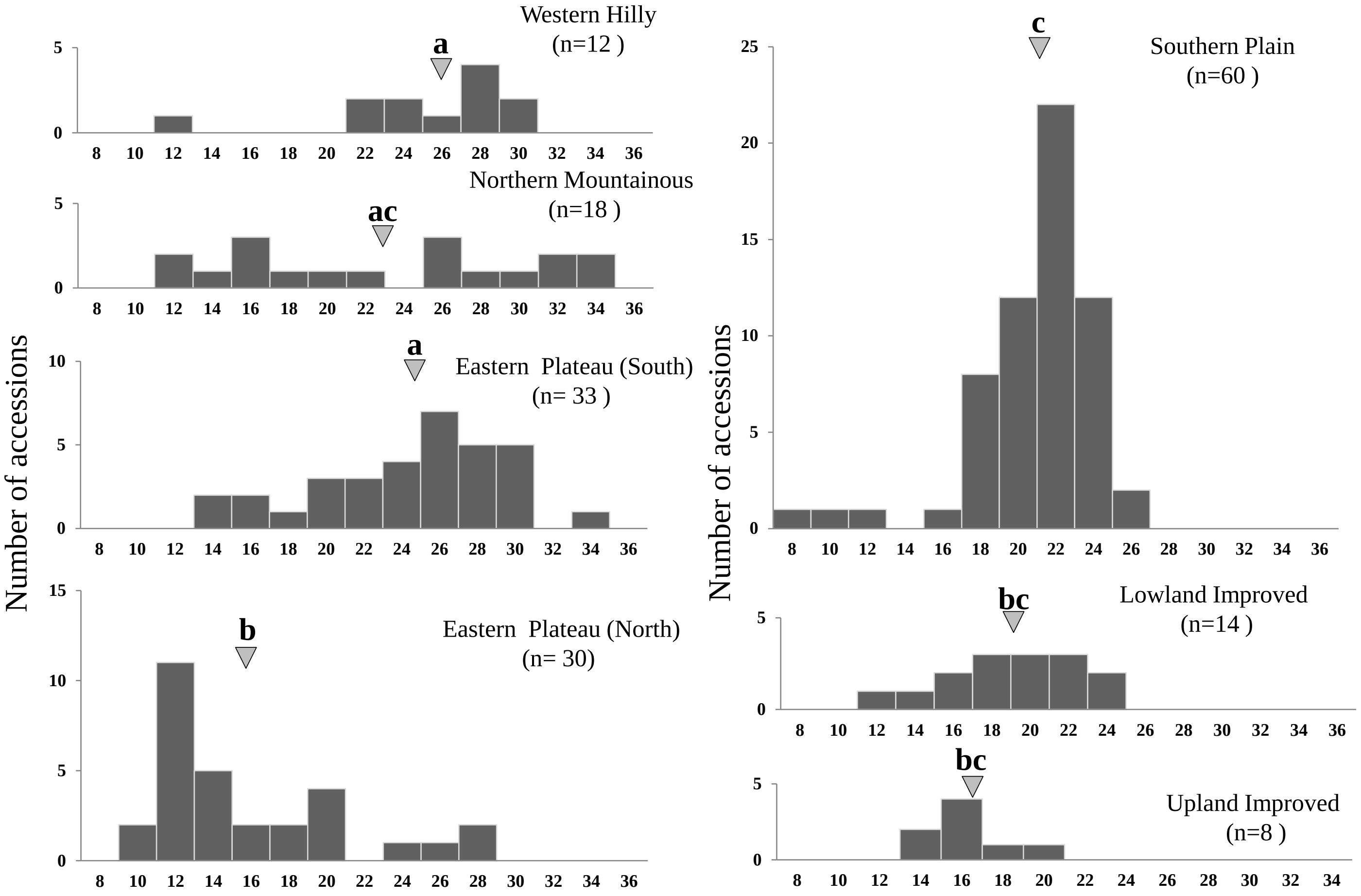


Figure 3.3.2. Characteristics of amylose content of rice accessions among seven populations. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

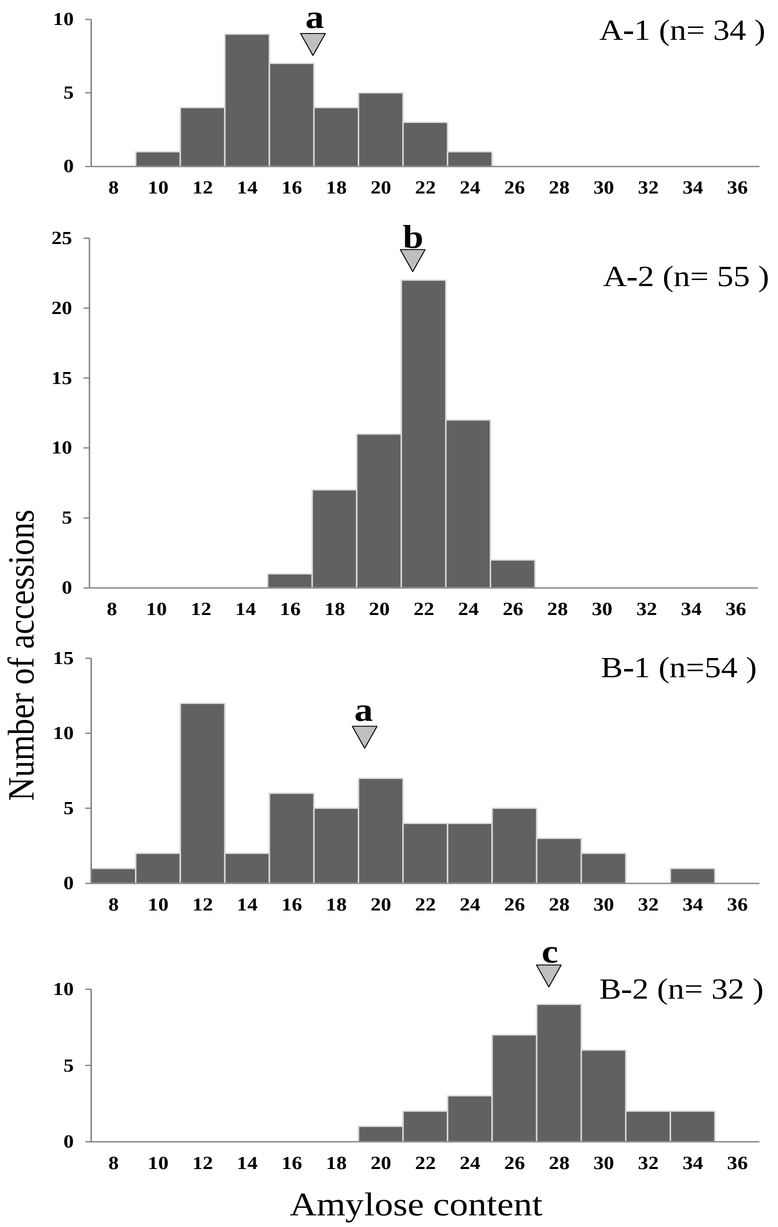


Figure 3.3.3. Characteristics of amylose content of rice accessions among four cluster groups. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

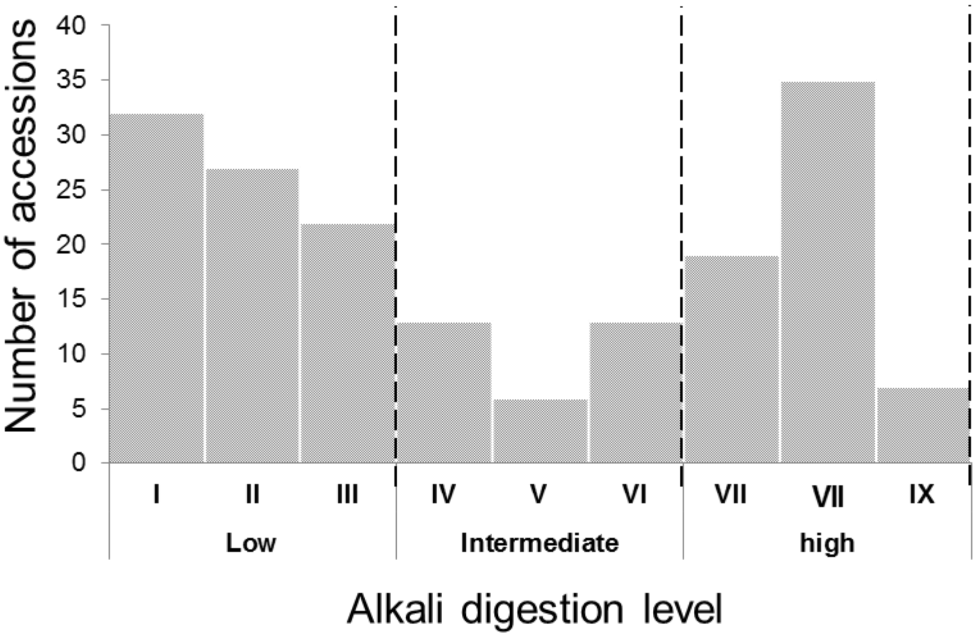


Figure 3.3.4. Distribution of alkali digestibility in Myanmar upland and lowland landrace cultivars. Alkali digestibility levels were evaluated into nine classes from I to IX by visual scoring based on Little et al., 1958. A total of 91 accessions were classified into I. II and Iii scores, 22 accessions into IV, V and VI, and 62 accessions into VII, VIII and IX.

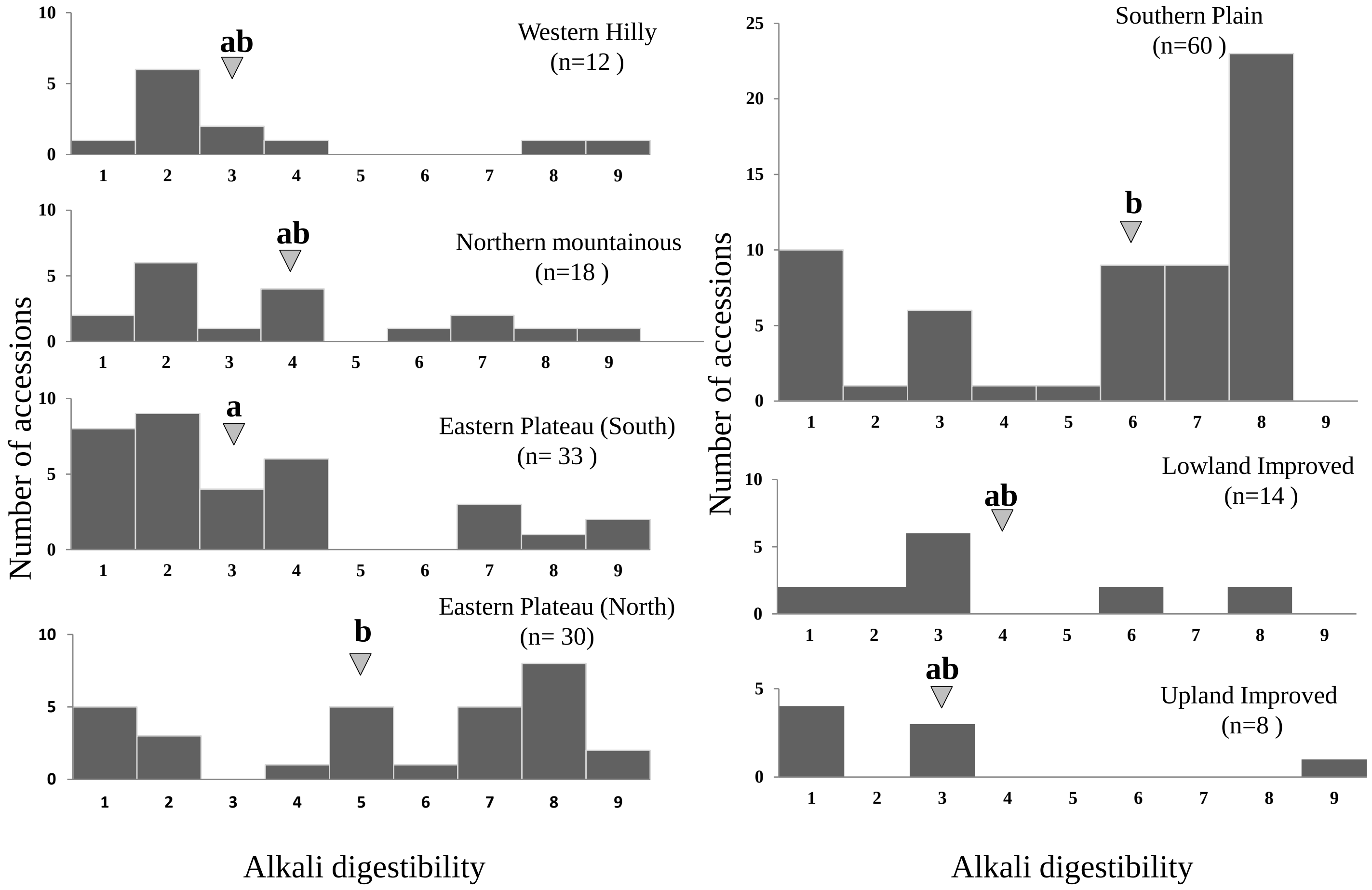


Figure 3.3.5. Characteristics of alkali digestion of rice accessions among seven populations. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

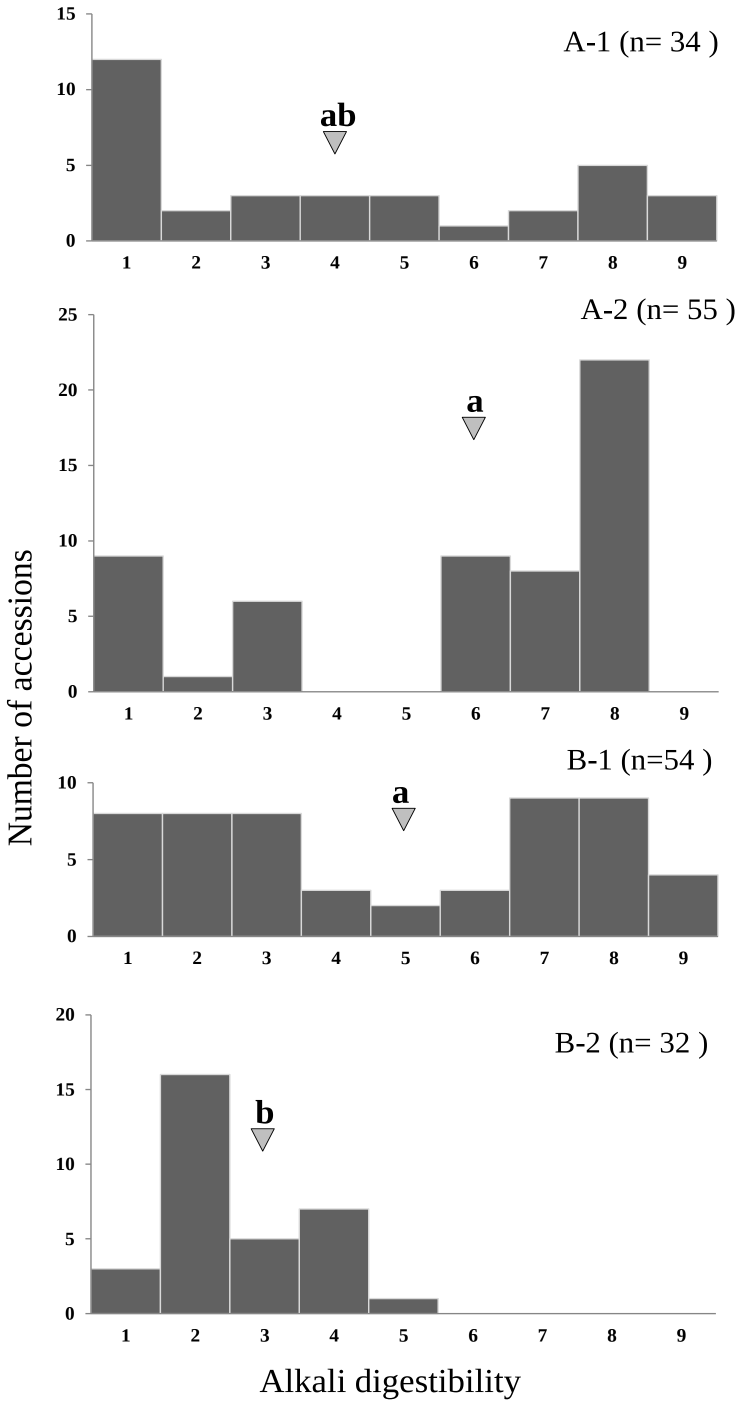


Figure 3.3.6. Characteristics of alkali digestion of rice accessions among four cluster groups. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

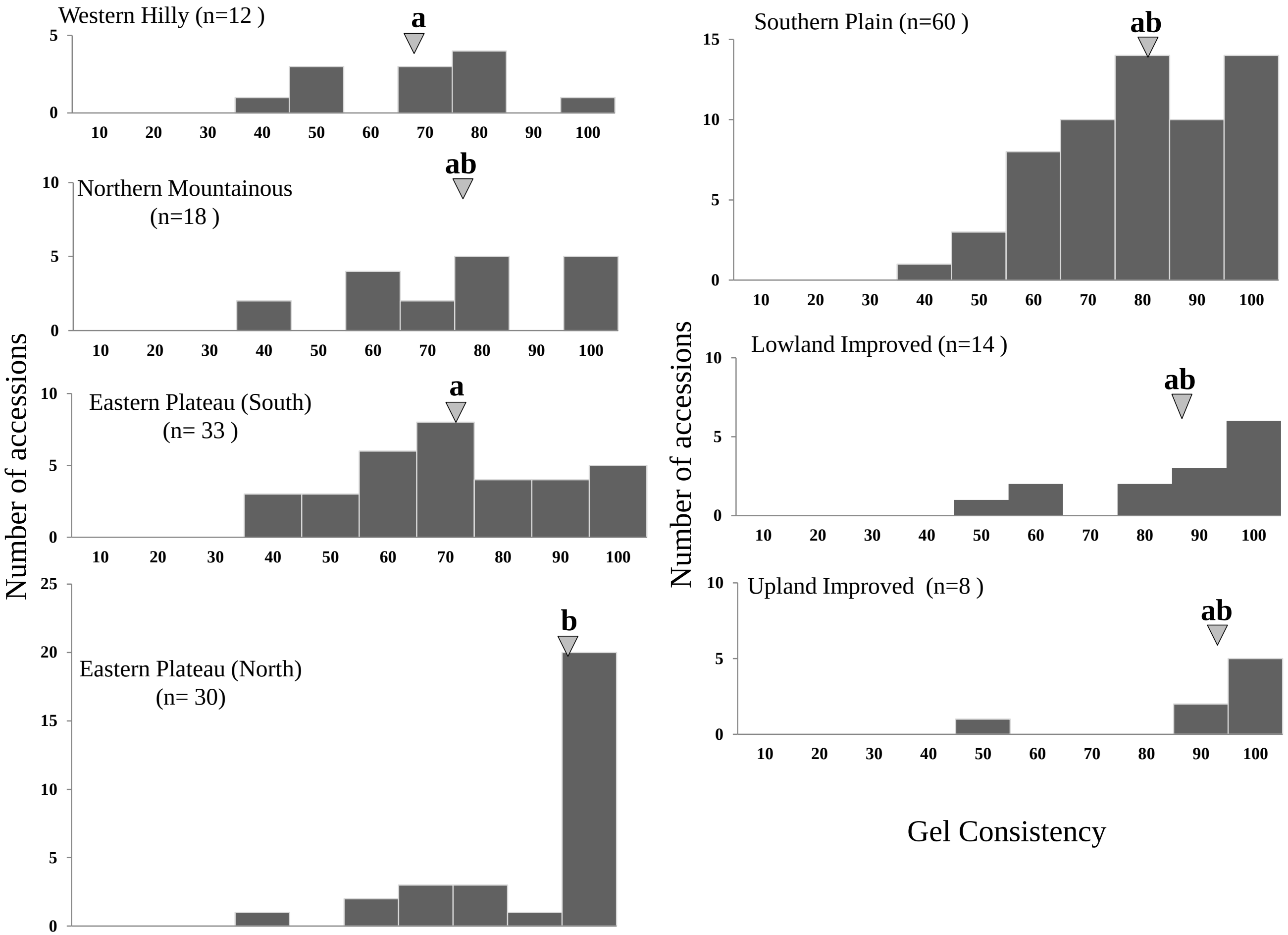


Figure 3.3.7. Characteristics of gel consistency of rice accessions among seven populations. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

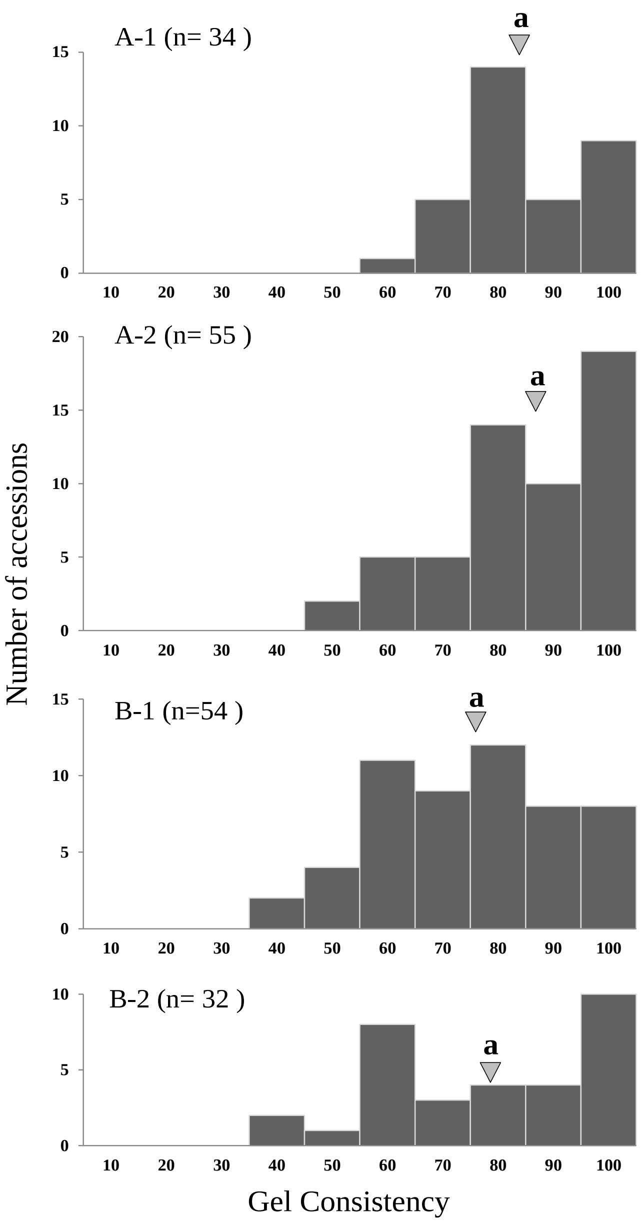


Figure 3.3.8. Characteristics of gel consistency of rice accessions among four cluster groups. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

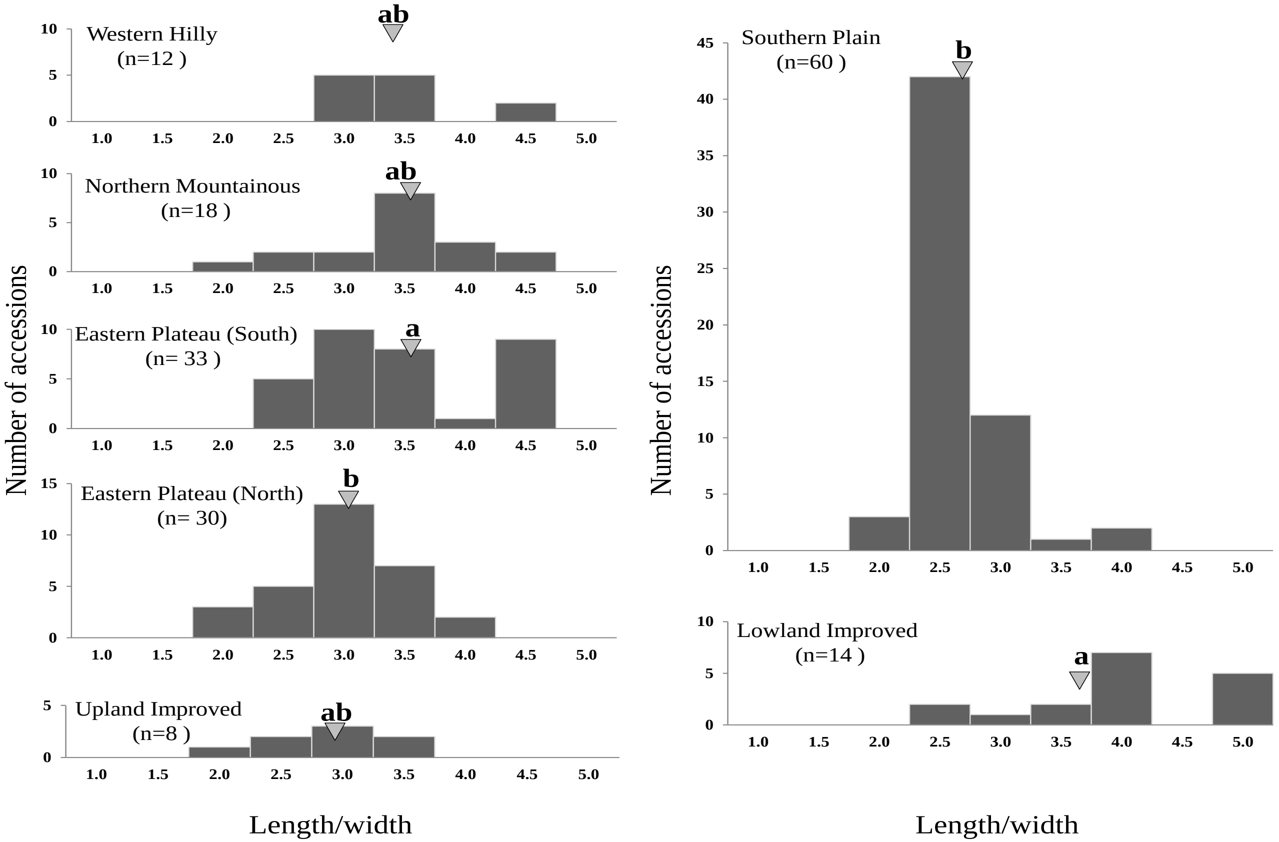


Figure 3.3.9. Characteristics of seed size of rice accessions among seven populations. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

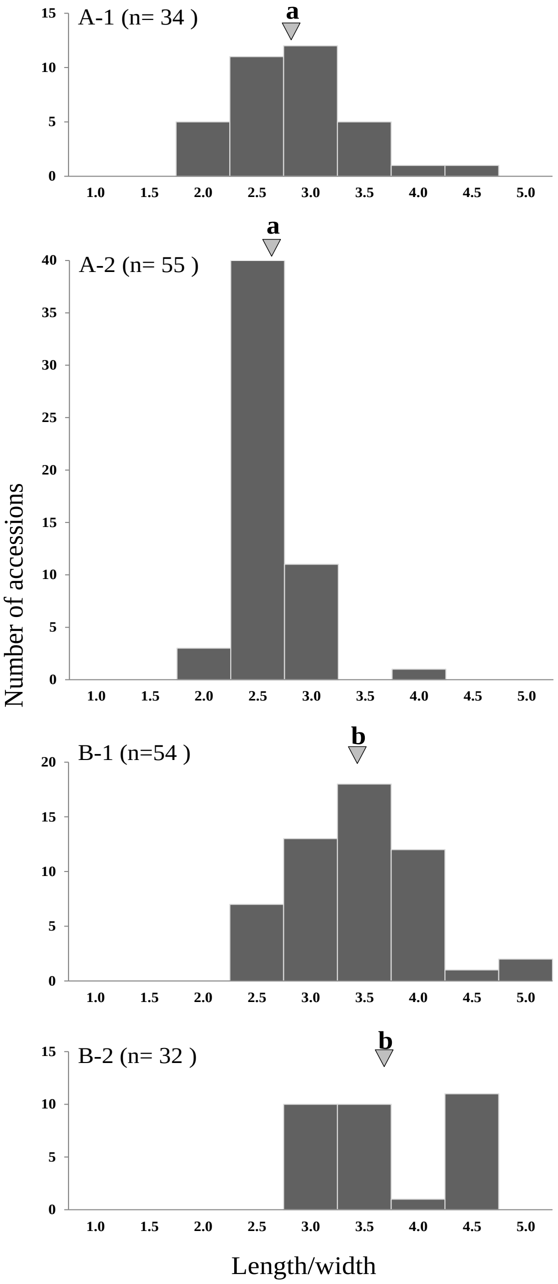


Figure 3.3.10. Characteristics of seed size of rice accessions among four cluster groups. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

3.4 Evaluation of resistant to blast fungus (*Pyricularia oryzae* Cavara) among Myanmar rice germplasm

3.4.1 Introduction

Rice blast caused by fungus *Pyricularia oryzae* Cavara, one of the most severe diseases in different ecosystems of rice growing area, was first discovered by Sasaki (1922). Nowadays, blast problem is still solving problem due to the extent of variation and genetic instability of *Pyricularia oryzae*. According to the gene to gene model, locally adapted rice landrace in disease favorable environment have co-evolved with the blast pathogen leading to accumulation of variants of resistant gene in each landrace (Thakur *et al.,* 2013). However, locally adapted landraces could be potential genetic resource with respect to rice blast resistant. Generally, growing the resistant varieties in rice blast problematic area is an effective and economically feasible method to control rice blast disease (Wang *et al.,* 1994) but blast disease is constant problem for breeding researcher. To skip the blast problem via breeding objectives, exploration of resistant gene in rice landraces or cultivars is an important task.

In host-patho system, there are two types of disease resistance, complete resistance (CR) and partial resistance (PR). CR in many plant; host–pathogen relationships is hypersensitive, racespecific, and governed by major R genes with gene-for-gene system. In contrast, PR is quantitative, presumably non-racespecific, and controlled by polygenes (Koch and Parlevliet, 1991; Li *et al.,* 2007). There are 85 R genes for blast resistance has been identified (Ballini *et al.,* 2008). For mining of new R and partial resistant genes, attention upon the regional adapted landraces or traditional cultivars is right approach. Therefore, exploration of the use of rice genetic diversity is important for improving blast resistance and for pathology study.

However, Myanmar is a primary center of rice, tremendous diversity of resistance to *Pyricularia oryzae* Cavara may exist. There was unrecorded information regarding with blast problem and seldom outbreak but endemic problem occur never. Although identification of resistant donors is essential in the breeding program for rice blast resistance, information on the identification of Myanmar rice germplasm resistant to *Pyricularia oryzae* is still lack. Objectives of this study are to assess the genetic diversity in blast resistance and to identify potential blast resistance source within diverse Myanmar landraces and outcomes will help the breeding programs in the use of genetic resources for blast resistant.

3.4.2 Materials and methods

3.4.2.1 Plant material

A total of 163 rice landraces (90 upland; 85 landrace and 5 improved cultivars, and 73 lowland; 59 landraces and 14 improved cultivars) among original 175 accessions were used to investigate the genetic variation of resistance to blast disease. Twelve accessions were discarded from experiment set due to lack of enough seed number.

3.4.2.2 Pathogenicity seedling test

In evaluation of blast resistant, nine Japanese standard blast isolates were used to assess resistance / susceptibility to blast disease. Six seeds of each accession were sown in plastic trays (40cm X 28cm X 5cm X 64 vertical holes) and grown to the 4th to 5th –leaf stage under day temperature at 27°C and night temperature at 22°C with 75% humidity. As susceptible check varieties, LTH and US2 varieties were also grown in each tray. Spore concentration was standardized to 1x 105 /ml and 50ml of the suspension was sprayed on each tray 3 weeks after sown. After seven days, necrosis and lesions on rice leaves were recorded according to the infection scale of 0 to 5 derived from Hayashi *et al.* (2009). Infection rating of 0,1 and 2 , were classified as resistant and ratings 3, 4 and 5 were designated as susceptible.

3.4.2.3 Classification of varieties

Base on the reaction pattern upon the nine Japanese standard blast isolates, rice accessions were classified using Ward's hierarchical clustering method with the computer program JMP 7.7.2 (SAS Institute, Inc., NC, USA). To know the variation of rice blast infection degree of rice accessions within and among the regional populations, the Tukey-Kramer HSD test was conducted using R version 3.2.1.

3.4.3 Result

3.4.3.1 Clustering of rice landraces group based blast resistant phenotype

To assess resistance / susceptibility to blast disease, 163 accessions were inoculated with nine Japanese standard blast isolates and observed as resistant germplasm pool. With reaction patterns upon the nine Japanese standard blast isolates, studied samples were classified into three cluster groups, I, II and III. There was unclear in identifying the cluster base on the reaction of specific isolates, especially in cluster I and II. To grain clear cluster identification, average degree of reaction upon nine blast isolates was observed among clusters (Table 3.4.1) (Figure 3.4.1).

In cluster I, contained forty-three rice germplasms, the average frequency of resistant reactions to all isolates was 96% and susceptible reaction to five isolates; BN 1-1-1, IW-85-04, Kyu 93-229, Ina 93-3 and TH69-8, was 3%. The average degree of infection from nine blast isolates was 0.5 for all accessions and degree of infection range was from 0.4 to 0.7 among four genetic distinct groups. There were 111 rice germplasms in cluster II and 97 %

of average frequency of reaction exhibited resistant to all isolates. But 1.7% of average reaction frequency showed the susceptible at three strains; BN 1-1-1, Kyu 93-229 and INA 93-3. Majority of lowland accessions (81%) were grouped in this cluster. Thus, this cluster was most resistant group among three clusters. Nine accessions belonged to cluster III and susceptible reaction frequency was found among eight isolates; 1804-4, Mu-95, 446-2a, BN 1-1-1, IW-85-04, Kyu 93-229, Ina 93-3 and TH 69-8. Average degree of infection from nine isolates for nine accessions was 1.9. And this cluster was lowest resistant level among three groups (Table 3.4.1 & 3.4.4).

A total of nine differential isolates were involved in this study, reactions to five distinct isolates; 1804-4, Mu-95, 446-2a, IW-85-04 and TH 69-8, were considered for clustering. The susceptible accessions were detected mostly with two distinct isolates; BN 1-1-1 and Kyu 93-229 but all accessions were resistant to Kyu 9439013 isolate. Average degree of infection from nine isolates for all studied sample was 0.4 (Table 3.4.1). In evaluation of 163 landraces, 82% of accessions showed resistance upon all isolates and less than 20% of accessions showed susceptible from one to six isolates. Resistant accessions which showed zero score level to all isolates were found fifty-three percent in lowland group and four percent in upland group.

By detail observation among and within groups, there may be harbored some resistant genes or resistant genetic mechanisms among upland and lowland genetic background of Myanmar landrace pool. However, Myanmar rice accessions were differentiated as three groups based on their resistant ability upon Japanese isolated strains.

3.4.3.2 Distribution of blast resistant phenotype groups among different agro-ecological groups

Distribution of rice accession groups classified according to reaction pattern of blast resistant was observed among five geographic regions, namely Western hilly (WH), Northern Mountainous (NM), North-eastern Plateau (EP), South-eastern Plateau (EP) and Southern Plain. Among these five regions, there are four upland regions and one lowland region.

The frequency of the 163 rice accessions in clusters I, II and III were 26%, 69% and 5% respectively, and dissimilar frequency distribution of varietal groups were observed among five regions. In Southern plain region, frequency of accessions among blast resistant phenotype clusters I, II and III were 15%, 81% and 4% respectively (Figure 3.4.2) and group II was dominant in lowland population. In upland, frequency of three varietal clusters were 36%, 58% and 6%, respectively, and number of upland accessions in cluster I was larger than lowland (Table 3.4.3).

The two resistant clusters; I and II, were distributed among five regions, but lowest resistant cluster; III, was distributed four regions only. Comparison among five regions, landrace accessions from lowland Southern plain region were markedly resistant because of it infection degree of resistant to nine blast isolates. And frequency of accessions which showed susceptible reactions in lowland population (10%) was less than upland (23%). In detecting of significant differences between regional populations by using with the Tukey-Kramer HSD test, Northern population and Southern population showed significant different in average degree of infection (Figure 3.4.3).

3.4.3.3 Relationship between genetic distinct groups and blast resistant phenotype groups

The collection of 163 upland and lowland cultivars, which were classified into four groups; A1, A2, B1 and B2 with SSR polymorphic profile, was inoculated with nine Japanese standard blast isolates to assess resistance / susceptibility to blast disease. Within four genetic groups, B1 showed more frequency of susceptible reactions among six Japanese fungus isolates than A1, A2 and B2. Although, there were three avirulent isolates among accessions of A1, A2 and B1, all accessions from B2 group was resistant to seven isolates (Appendix table 3). Reaction pattern of Indica-type groups (B1 and B2) upon three isolates; Mu-95, IW-85-04 and Kyu 9439013 and Japonica-type group (A1 and A2) upon two isolates; 1804-4 and Kyu 9439013 were clear resistant reaction and these isolates were avirulent isolates for each group. Susceptible reaction and fluctuation of resistant pattern at BN 1-1-1 and Kyu 93-229 were observed among four groups.

When we observed the relationship among four genetic distinct groups and three blast resistant phenotype clusters, uneven distribution of four genotype groups among three blast resistant phenotype clusters was found. Among three clusters, Indica-type distributed among three cluster as I; 40%, II; 53% and III; 7%, whereas, Japonica-type also scattered among three cluster as I; 12%, II; 84% and III; 4% (Figure 3.4.4).

In observing blast resistant phenotype cluster I, B-1 and B-2 groups' accessions (76% of cluster I accessions) were predominant than A-1 and A-2 groups. In Cluster II, lowland (A-2) was major populated group (49 acc.; 48%), and others; A-1, B-1and B-2 were also clustered with 20 acc.; 18%, 25 acc.; 22% and 17 acc.; 15%, respectively. Except the upland (B-2), other three genetic distinct groups were distributed among three blast resistant phenotype groups (Table 3.4.4).

Relationship between genotyping groups and blast resistant phenotyping cluster, Indica-type landraces were dominant in Cluster I and Japonica-type landrace took major group in cluster II. That result gave hint that genetic background (*indica*/ *japonica* and upland /lowland) of accessions for each cluster was more fundamental in evaluation of rice blast resistant.

3.4.4 Discussions

This study reports result of an integrated genotype-phenotype analysis assessing genetic diversity of rice blast resistant among Myanmar rice landraces. The analysis of the 65 set SSR loci revealed four genetic groups among upland and lowland germplasms. And we assessed the blast resistant level of 163 rice accessions with nine Japanese standard blast isolates and classified as three clusters. Round about 80% of all accessions showed resistant to all blast isolates. Actually these resistant accessions originate from different geographical area and cluster to different subgroups in the dendrogram, they may have different genetic background for resistant (van dar Linden *et al.,* 2004)

Among the two agro-ecotypes; upland and lowland, most accessions from lowland were found to be resistant to all Japanese standard fungal strains. Majority was lowland elite landraces from (A-2) and included in blast resistant cluster II. Lowland improved cultivars are B1 group and their breeding purpose orient to yield rather than biotic stress. But, they showed resistant reaction upon Japanese fungus isolates. Actually, Southern lowland plain is tropical humid area where have favorable conditions for disease infection and outbreak except summer period. And, there was unrecorded information regarding with blast problem and seldom outbreak but endemic problem occured never. Observation of lowest average infection degree among lowland landrace pool, they may have resistant gene or genetic mechanisms to pathogen (Smilde *et al.,* 2005).

Studied upland population showed wide variation of resistant reaction and their sensitive isolates were Kyu 93-229 and BN1-1-1. In more detail observation, B-1 and B-2 upland accessions showed susceptible reactions upon these two isolates than A-1 upland group. Both susceptible and resistant genotypes upon Japanese isolates were observed in Indica-type upland group. The coexistence of resistant and susceptible genotype might have ecological meaning, possibly in preventing the pathogen breaking the resistant too quickly.

In this study, nine blast isolates with different pathogenic reactions were chosen to inoculate 163 rice landraces. The reactions to the nine blast isolates were shown to be less diverse resistant pattern than expected, that is, around 80% of the total tested landraces were resistant to all blast isolates and less than 20% of them showed susceptible to seven isolates. This evaluation of Myanmar rice landraces are based on temperate Standard differential blast isolates (SDBI). Actually, evaluation of co-evolutional diversity between landraces and blast isolates is one important circumstance to consider being control the outbreak (Song *et al.,* 2003). In order to examine pathogenic differentiation of blast in Myanmar and to establish a differential system to distinguish blast isolates, the development of an efficient screening technique for resistance to blast is needed to identify the promising breeding material with stable resistant to rice blast isolates prevailing in Myanmar.

Table 3.4.1. Variation of resistant in each cluster group classified by reaction patterns to standard differential blast isolates and mean value of infection degree in each isolate.

Resistant subgroups (Groups based on reaction to blast isolates)	Score	No. of rice accessions										
		Standard differential blast isolates in Japan									Mean	
		1804-4	Mu-95	446-2a	BN 1-1-1	IW-85-04	Kyu 9439013	Kyu 93-229	Ina 93-3	TH 69-8	Mean	(%)
I (n = 43)	0	24	13	39	26	36	31	6	27	24	25.1	(58.4)
	1	17	24	4	11	6	12	15	12	14	12.8	(29.7)
	2	2	6	0	2	0	0	16	2	4	3.6	(8.3)
	3	0	0	0	3	1	0	3	1	1	1.0	(2.3)
	4	0	0	0	1	0	0	1	0	0	0.2	(0.5)
	5	0	0	0	0	0	0	1	0	0	0.1	(0.2)
Infection degree (Mean)		0.3	0.7	0.1	0.5	0.4	0.2	1.3	0.5	0.6	0.5	
II (n = 111)	0	96	106	102	79	103	110	64	87	108	95.0	(85.6)
	1	15	5	8	16	7	1	28	20	3	11.4	(10.3)
	2	0	0	1	5	0	0	12	2	0	2.2	(2.0)
	3	0	0	0	5	0	0	5	1	0	1.2	(1.1)
	4	0	0	0	3	0	0	1	0	0	0.4	(0.4)
	5	0	0	0	2	0	0	0	0	0	0.2	(0.2)
Infection degree (Mean)		0.2	0.1	0.1	0.6	0.1	0.1	0.9	0.3	0.1	0.3	
III (n= 9)	0	2	2	0	1	3	0	0	1	1	1.1	(12.3)
	1	4	5	2	2	3	4	1	5	5	3.4	(38.2)
	2	2	1	3	2	1	5	2	1	1	2.0	(22.2)
	3	1	1	2	2	2	0	2	0	1	1.2	(13.6)
	4	0	0	1	2	0	0	2	1	1	0.8	(8.6)
	5	0	0	1	0	0	0	2	1	0	0.4	(4.9)
Infection degree (Mean)		1.2	1.5	2.0	2.6	1.9	1.2	3.3	2.3	1.5	1.9	
(Ground mean)		0.5	0.7	0.6	1.1	0.7	0.4	1.7	0.9	0.6	0.4	

Infection degree of each blast isolate was calculated in each cluster group and whole accessions (Total scores/No. of accession in each group).

Table 3.4.2 Variation of resistant in each distinct genetic group.

Cluster group classified based on the polymorphism data of SSR markers	Score	No. of rice accession										
		Standard differential blast isolates in Japan									Mean (%)	
		1804-4	Mu-95	446-2a	BN 1-1-1	IW-85-04	Kyu 9439013	Kyu 93-229	Ina 93-3	TH 69-8	Mean	(%)
A-1 (n = 28)	0	20	20	23	17	19	24	13	15	22	19.2	(68.6)
	1	8	7	4	10	8	4	13	12	5	7.9	(28.2)
	2	0	1	0	0	0	0	0	1	0	1.0	(3.6)
	3	0	0	1	1	1	0	0	0	1	1.0	(3.6)
	4	0	0	0	0	0	0	0	0	0	-	-
	5	0	0	0	0	0	0	1	0	0	1.0	(3.6)
Infection degree (Mean)		0.2	0.4	0.3	0.4	0.4	0.2	0.7	0.5	0.3	0.4	
A-2 (n = 54)	0	52	52	51	42	48	52	47	50	50	49.3	(91.4)
	1	1	1	2	4	1	2	4	1	3	2.1	(3.9)
	2	1	0	1	1	0	0	2	0	0	1.3	(2.3)
	3	0	1	0	3	2	0	0	1	1	1.6	(3)
	4	0	0	0	1	0	0	0	0	0	1.0	(1.8)
	5	0	0	0	1	0	0	0	1	0	1.0	(1.8)
Infection degree (Mean)		0.1	0.1	0.1	0.5	0.1	0.1	0.2	0.2	0.1	0.2	
B-1 (n = 49)	0	28	33	36	25	43	35	9	19	32	28.9	(58.9)
	1	17	14	7	10	5	9	17	23	11	12.6	(25.6)
	2	3	2	3	4	1	5	15	4	5	4.7	(9.5)
	3	1	0	1	4	0	0	2	1	0	1.8	(3.7)
	4	0	0	1	5	0	0	4	1	1	2.4	(4.9)
	5	0	0	1	1	0	0	2	0	0	1.3	(2.7)
Infection degree (Mean)		0.5	0.5	0.5	1.1	0.1	0.4	1.6	0.7	0.5	0.6	
B-2 (n = 32)	0	22	16	31	20	30	30	1	31	29	23.3	(72.9)
	1	10	12	1	5	2	2	10	1	3	5.1	(16)
	2	0	4	0	4	0	0	15	0	0	7.7	(24)
	3	0	0	0	2	0	0	6	0	0	4.0	(12.5)
	4	0	0	0	0	0	0	0	0	0	-	-
	5	0	0	0	0	0	0	0	0	0	-	-
Infection degree (Mean)		0.4	0.6	0.0	0.6	0.1	0.1	1.7	0.1	0.1	0.4	
	Ground mean	0.3	0.4	0.2	0.7	0.2	0.2	1.1	0.4	0.3	0.4	

Infection degree of each blast isolate was calculated in each cluster group and whole accessions (Total scores/Number of accessions in each group).

Table 3.4.3. Relationships between cluster groups of resistant and geographical regions collected of rice accessions.

Geographical region	No. of accessions (%)			
	Cluster groups based on the reaction patterns to standard differential blast isolates			
	I	II	III	Total
Upland				
Western Hilly	5	7	0	12
Northern Mountainous	6	8	3	17
Eastern Plateau (North)	9	20	2	31
Eastern Plateau (South)	12	17	1	30
Total	32 (36%)	52 (58%)	6 (6%)	90 (100%)
Lowland				
Southern Plain	11(15%)	59 (81%)	3 (4%)	73 (100%)
Total	43 (26%)	111(69%)	9(5%)	163(100%)

(Majority of improved upland and lowland accessions were discarded from experiment set due to lack of enough seed number.)

Table 3.4.4. Relationships of cluster groups between reaction to blast isolates and DNA polymorphism of rice accessions.

DNA polymorphism of SSR markers		No. of accessions (%) Cluster groups Reaction patterns to standard differential blast isolates I	II	III	Total
A	1	6	20	2	28
	2	4	49	1	54
	Sum	10 (6)	69 (42)	3 (2)	82 (50)
B	1	18	25	6	49
	2	15	17	0	32
	Sum	33(20)	49 (30)	6 (4)	81(50)
Total		43(26)	111(68)	9(6)	163(100)

Table 3.4.5. Variation of blast resistant in each cluster group.

Groups	No. of rice accession									
	Standard differential blast isolates in Japan									
	1804-4	Mu-95	446-2a	BN 1-1-1	IW-85-04	Kyu 9439013	Kyu 93-229	Ina 93-3	TH 69-8	Mean
Resistant subgroups (Groups based on reaction to blast isolates)										
I (n = 43)	0.3	0.7	0.1	0.5	0.4	0.2	1.3	0.5	0.6	0.5
II (n = 111)	0.2	0.1	0.1	0.6	0.1	0.1	0.9	0.3	0.1	0.3
III (n= 9)	1.2	1.5	2.0	2.6	1.9	1.2	3.3	2.3	1.5	1.9
Ecotype group										
Upland (n=90)	0.4	0.7	0.3	0.8	0.2	0.2	1.5	0.4	0.3	0.5
Lowland (n= 73)	0.2	0.4	0.1	0.6	0.1	0.1	0.4	0.3	0.2	0.3
Regional										
Western (n =12)	0.3	0.5	0.1	1.9	0.1	0.2	1.8	0.6	0.4	0.6
Northern (n = 17)	0.7	0.4	0.7	1.1	0.2	0.6	2.5	0.6	0.6	0.8
North-eastern (n = 31)	0.3	0.3	0.3	0.3	0.2	0.2	0.9	0.4	0.3	0.4
South- eastern (n = 30)	0.4	0.5	0.2	0.6	0.2	0.0	1.6	0.3	0.2	0.4
Southern Plain (n = 73)	0.2	0.3	0.1	0.6	0.1	0.1	0.4	0.3	0.2	0.3
Genetic distinct group										
A-1 (n = 28)	0.2	0.4	0.3	0.4	0.4	0.2	0.7	0.5	0.3	0.4
A-2 (n = 54)	0.1	0.1	0.1	0.5	0.1	0.1	0.2	0.2	0.1	0.2
B-1 (n = 32)	0.4	0.6	0.0	0.6	0.1	0.1	1.7	0.1	0.1	0.4
B-2 (n = 49)	0.5	0.5	0.5	1.1	0.1	0.4	1.6	0.7	0.5	0.6

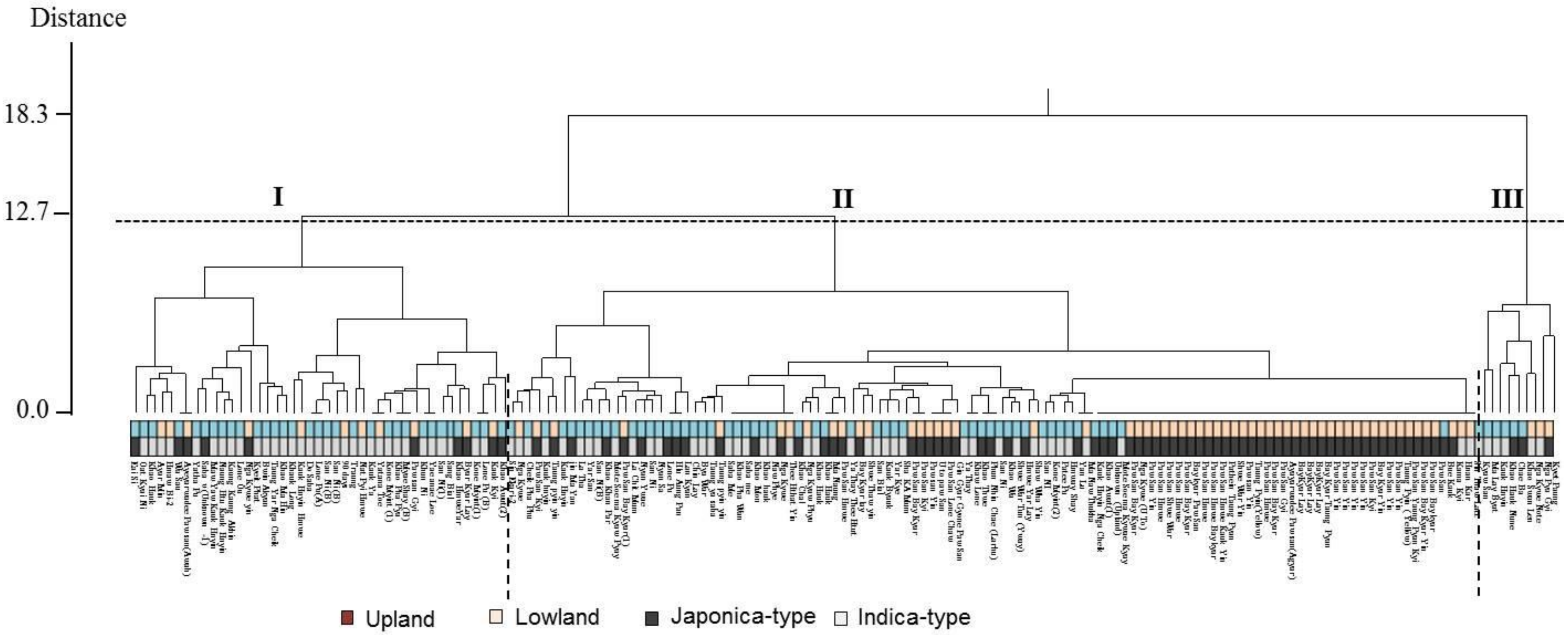


Figure 3.4.1. Classification of rice accessions in Myanmar based on the reactions to standard differential blast isolates. Cluster analysis was performed based on reaction data of 9 blast isolates in Japan and by Ward's hierarchical clustering method. A total of 163 rice accessions were classified into three groups; I, II and III. Color bars represent accessions' agro-ecotype and subtype.

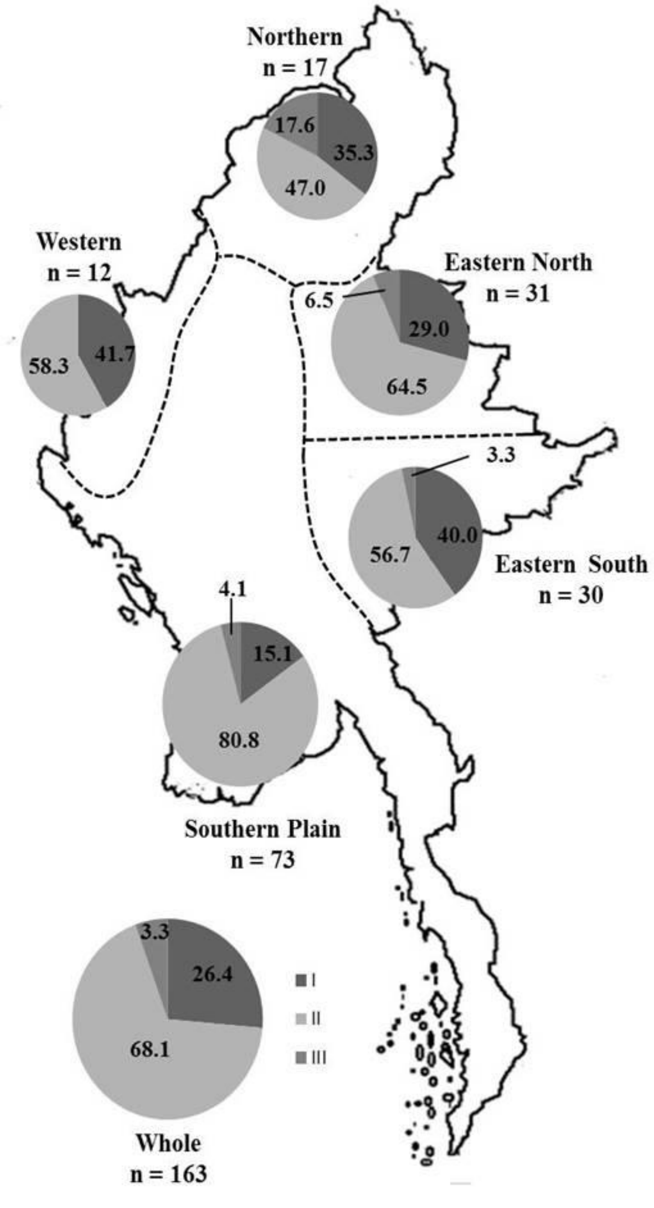


Figure 3.4.2. Geographical distributions of rice accessions classified by reactions to standard differential blast isolates in five agro-ecological zones of Myanmar.

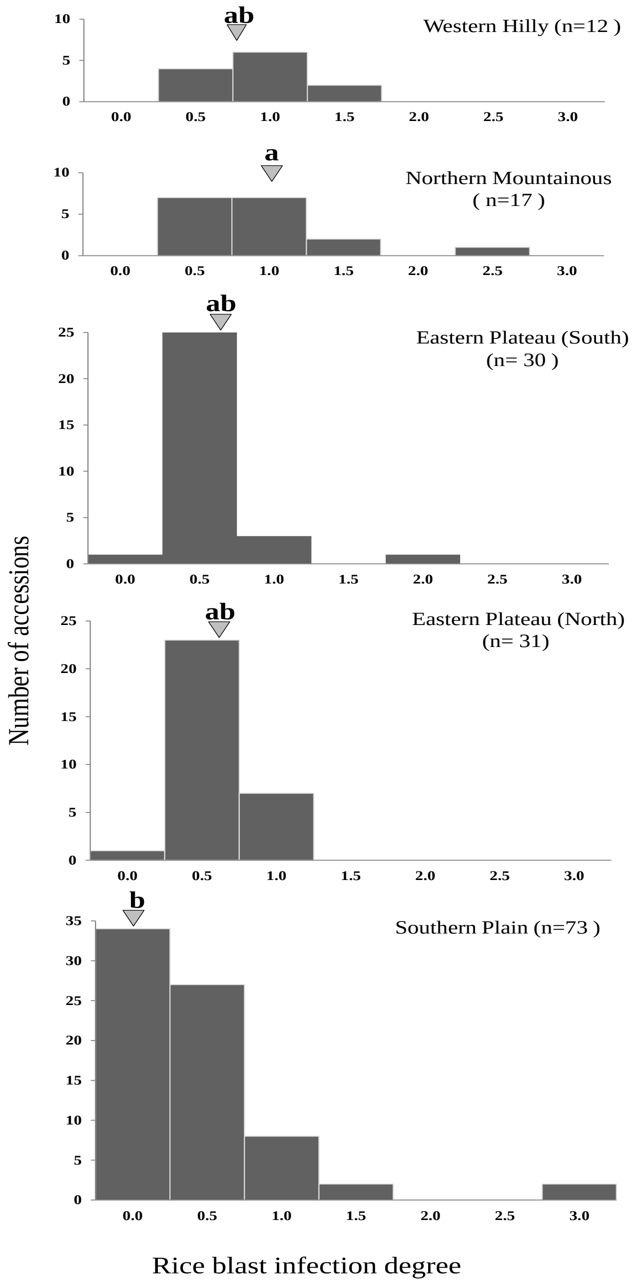


Figure 3.4.3. Characteristics of rice blast infection degree of rice accessions among seven populations. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

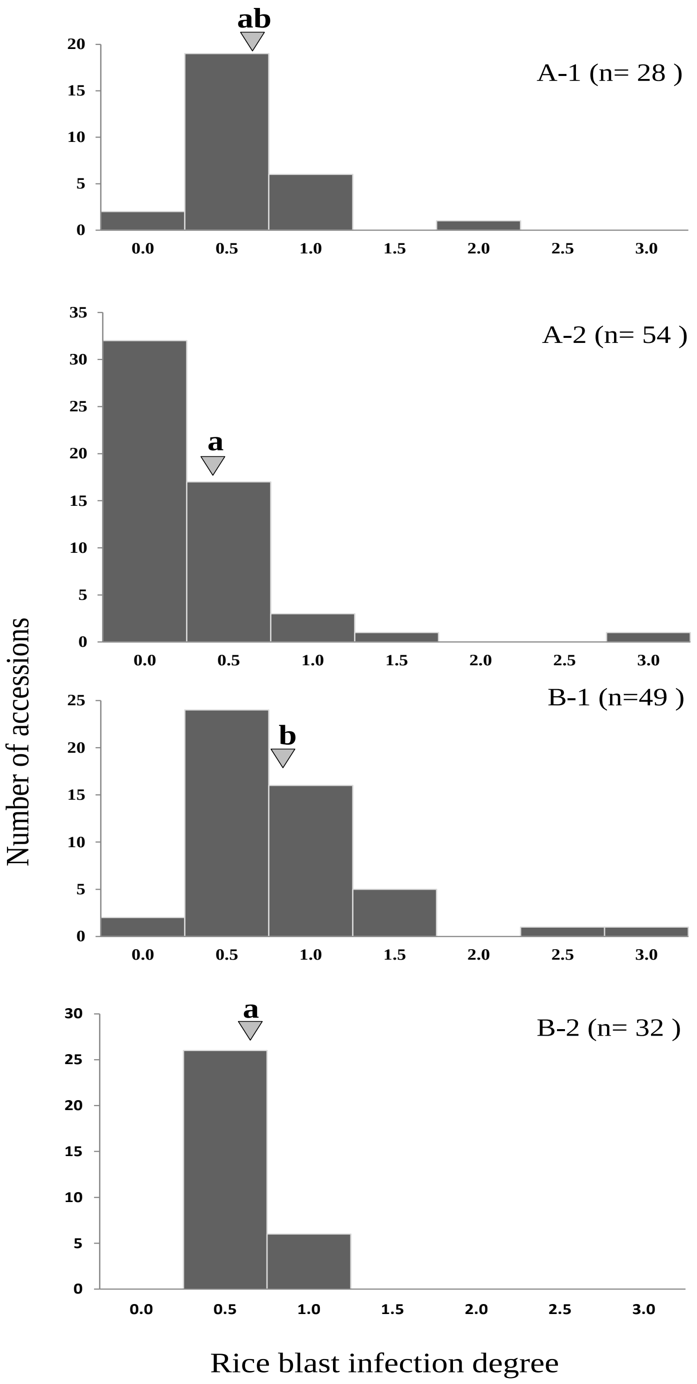


Figure 3.4.4. Characteristics of rice blast infection degree of rice accessions among four cluster groups. Arrowheads indicate the mean value of heading date of each group. Black line bar indicate the standard deviation of each group. Different letters represent significant differences at 5% using the Tukey-Kramer HSD test.

3.5 Comparative assessment of agronomic variation among upland and lowland landrace groups

Among two agro-ecotype populations, significant variation of agronomic trait was investigated among upland and lowland populations. Variation of seed size, cooking and eating quality and resistant reactions upon the rice blast among upland landraces are interesting information for Myanmar rice germplasms conservation and use. With the stable variation of intermediate amylose content, highly resistant to rice blast characters and wide range of heading variation, lowland landrace population can be defined as promising breeding materials for quality improved breeding purposes and searching the R gene for rice blast control.

With observance of high genetic diversity and broad agronomic trait variation, upland landrace pool may serve as a source for future studies, thus leading to regional conservation and regional adapted breeding purpose.

3.6 Summary of three agronomic trait variations

Agronomic traits are important because these are used by farmers to identify or distinguish varieties; these identifying characteristics are often the basis for the names farmers given to varieties. Some of these traits are preferred or valued by the farmer because certain of its distinguishing characteristics are desirable at farmer and regional adaptable level. Farmers select among the plants in the crop population to maintain these desirable characteristics and to increase the prevalence of other valued traits in the population over time. Therefore, evaluation of agronomic traits variation among farmers' handling landrace pools, which was shaped by cultural practices, ecosystems and diverse ethnic groups, may serve as a source for study leading to effective utilization and conservation for regional specific.

In identifying of genetic variation of heading date, cooking and eating quality, seed size and resistant to blast, there was significant variation of agronomic traits among regional populations. Moreover, the clear different variation of agronomic traits was investigated among lowland and upland landrace populations. High variation of agronomic traits was observed all upland regional populations except North-eastern groups. In lowland landrace population, heading date and amylose content traits were highly variated. Agronomic trait variation of accessions from A-1 and B-1 genetic distinct groups was higher than the A-2 and B-2 groups. Among agro-ecotype groups, all agronomic traits in upland group showed more variable than lowland group except heading date trait (Table 3.7.1). Distribution of agronomic variation at regional level depends on human factors and crop adaptable. Moreover, intraspecific variation within and among of population affect the genetic diversity of agronomic traits. At intraspecific level, Indica-type landraces were more variable than Japonica-type. This present study elucidated the variability pattern of rice landrace cultivars on the base of three agronomic traits variation. From this investigation, upland landraces which processes the A-1 and B-1 genetic background showed the wide variation of agronomic trait.

Table 3.7.1. Gene diversity and agronomic trait variability patterns of rice accessions among regional groups, genetic distinct groups, agro-ecotype groups and variety-type groups.

Group	Gene Diversity	Heading Date			Amylose content			Alkali digestibility			Gel consistency			Seed shape			Rice blast reaction degree		
		Mean	STD	CV %	Mean	STD	CV %	Mean	STD	CV %	Mean	STD	CV %	Mean	STD	CV %	Mean	STD	CV %
Regional group																			
Western	0.776	101	12	12	26	5	19	3	3	76	64	18	28	3.2	0.6	18	0.6	0.3	49
Northern	0.773	94	16	17	23	7	32	4	2	64	73	20	28	3.3	0.7	21	0.8	0.5	67
South-eastern	0.769	97	10	10	25	5	21	3	2	74	68	19	27	3.3	0.7	23	0.4	0.3	76
North-eastern	0.831	79	13	17	16	5	28	5	3	61	88	18	20	2.7	0.5	17	0.4	0.3	73
Southern	0.777	118	29	25	21	3	16	5	3	51	77	16	21	2.7	0.6	23	0.3	0.5	195
Genetic distinct group																			
A-1	0.787	82	21	25	17	4	21	4	3	74	80	11	14	2.6	0.6	22	0.4	0.4	100
A-2	0.700	125	27	22	22	2	9	6	3	47	82	14	18	2.4	0.3	11	0.2	0.4	254
B-1	0.807	91	17	19	19	6	33	5	3	58	72	17	24	3.2	0.6	18	0.6	0.5	84
B-2	0.663	102	2	2	28	3	12	3	1	40	75	20	27	3.5	0.6	18	0.4	0.2	38
Agro-ecotype																			
Upland	0.841	90	15	17	22	7	31	4	3	68	76	21	27	3.1	0.7	22	0.5	0.4	77
Lowland	0.777	118	29	25	21	3	16	5	3	51	77	16	21	2.7	0.6	23	0.3	0.5	195
Type																			
Landrace	0.853	102	27	26	22	6	26	5	3	59	76	19	25	2.8	0.6	22	0.4	0.5	118
Improved	0.787	101	22	22	18	3	18	3	2	73	84	17	21	3.2	0.8	25	0.3	0.2	75

SD = Standard deviation, CV= Coefficient of variation

Chapter 4

General discussion, Conclusion and Recommendations

4.1 General discussion

Myanmar has prosperous plant genetic diversity source with a wide range of extensive ecological background. It constitutes part of the center of genetic diversity of cultivated rice (*Oryza sativa* L.). Moreover, ethnic factors, socio-economic, and social-cultural value are also diverse among the different geographic conditions and affect upon the regional landrace diversity. Adaptable of crops among different ecosystems; upland and lowland agro-ecotype is also another important to be consider for regional landrace diversity and structure. Currently, changes of government policy, farmer attitude and land use impacts affect the local landrace diversity. These rice germplasms has to be collected and conserved for future uses before they disappeared. To efficient conservation and use, about genetic diversity and structure of the existing germplasm on each region is important to be known. Furthermore, the genetic variation of agronomic traits within landrace genepool needs to be evaluated prior for planning the sustainable use and conservation measures of landraces. This current study was undertaken with the aim to characterize the genetic diversity as well as the agronomic variation between and among upland and lowland landrace groups, and as no such attempt regarding with farmer handling landrace pools in Myanmar, has been conducted yet.

4.1.1 Genetic diversity among different agro-ecology zones, genetic distinct groups and ecosystems

The strategy of this study is evaluation of landrace rice cultivars from two rice ecosystems for conservation and use under the sustainable production. Evaluating the genetic diversity and genetic structure is proofing survey for planning of conservation measures.

Assessment of genetic diversity within all populations and among upland and lowland ecosystems is main objective and detecting with 65 SSR microsatellite markers and assessing of human interested three agronomic traits; heading date, eating and cooking quality, grain shape and resistant to rice blast are main tools in this study.

The present study of two agro-ecotypes rice landraces revealed the considerable high genetic diversity, with mean number of alleles (6.2) and an overall Nei's gene diversity of (0.846) among the 175 rice accessions collected from the Southern lowland areas and three upland mountainous regions and that value was higher than the country-wide gene bank collection set (0.809) and similar country-wide on farm-population set (0.826; Yamanaka *et al.,* 2011). Among upland and lowland accessions groups, higher gene diversity was observed in upland population (0.82) than lowland (0.78). This high diversity in upland accessions is due to the nature of subsistence rice farming in mountainous regions, the diversity of land types, distance from market centers (Watanabe *et al.,* 2007) and the coexistence of diverse tribes. And farmers in mountainous regions appreciate the benefit of diversity as a cultural, social and religious norm (Kawase *et al.,* 2011; Yamamoto *et al.*, 2011).

In chromosomal component analysis with 65 SSR markers, all rice accessions were classified mainly two groups; I and II, and which showed the positive and negative reactions of phenol, respectively. Thus, cluster groups I and II were expected to correspond with Indica and Japonica-types, respectively. Moreover, four genetic distinct groups were identified again by clustering of same genetic background individual with model-based method. With the result of phenol test and accession's distributed ecosystem, these four groups was designated as A-1(Japonica-type upland), A-2(Japonica-type lowland), B-1(Indica-type lowland and upland mixture) and B-2 (Indica-type upland). Indica-type, B-1 was highest in diversity indexes among four groups, followed by A-1, A-2 and B-2. Higher genetic diversity indexes and 70% of Indica-type influence were prominent among upland populations and within upland ecosystem, while, landrace population from lowland ecosystem was observed as low genetic diversity and high dominant of Japonica-type (80%) group. The distribution of Japonica-type accessions in lowland, and those of Indica-type's one in upland were different from the other tropical countries, such as the Philippines, Laos, Vietnam and so on, and it is quite unique situation in Myanmar. Stressful harsh environment condition upon the rice cultivation (natural impact) and tremendous purposes upon rice growing among high tribe diversity (human impact) are main causes in affecting of upland landrace diversity. That results proof my hypothesis that "diversity of upland population are expected to be higher than lowland population" and "different genetic background and intraspecific differentiation are main partition among upland and lowland".

Among the regional populations, there was relatively high genetic diversity in each regional population. North-eastern population showed highest gene diversity among regional populations and majority of accessions has two genetic backgrounds (A-1 and B-1). Topography of region, closeness Yunnan's diversity hot-sport area and tribe diversity are major effects upon North-eastern upland populations' diversity status. Lowest gene diversity was investigated in Southern lowland populations which had (A-2) Japonica-type genetic background. That is related with regional farmer attitude, market attribute and government policy.

In analysis of (AMOVA), there was high variability within populations than among populations within ecosystems and among ecosystems. Independent clustering pattern of landraces from different regions in the UPGMA supported to AMOVA results and tended to human pressure affect upon genetic variability among regional population level. But there was significant genetic differentiation among upland and lowland populations due to higher F_{ST} (0.31) than the significant threshold level of population differentiation (F_{ST} =0.25; Hartl and Clark, 1997). The low pairwise F_{ST} value range (0.043 to 0.046) among the upland populations was observed, thus, there was lesser vital number of individuals in each population and lower molecular variation was found among populations. Overlapping of three genetic distinct groups; A-1, B-1 and B-2 among upland populations was one possible cause in lowering of population differentiation (F_{ST}) among upland populations. Therefore, there are germplasm cross introduction among populations with human interference.

Above we mentioned regarding with genetic diversity, genetic differentiation and intraspecific variation among ecosystems, we detected the clear genetic differentiation among upland and lowland populations. Main observances were intraspecific variation and distinct genetic background among upland and lowland populations. Indica-type population showed high genetic diversity (highest genetic diversity indexes in B-1) and they had wide range distribution among upland regions. Moreover, population with higher and lower genetic diversity among upland and lowland ecosystem were identified for conservation purposes. Higher genetic viability within regional populations relate with adaptable ability of regional population to response to harsh environment and tend to lesser change of genetic drift (Frankham *et al.,* 2007) and diverse allele under the same human preference trait, and may be select for conservation plan.

4.1.2 Agronomic trait variation among different agro-ecology zones, genetic distinct groups and ecosystems

Landraces and traditional cultivars had been under cultivation by grower with their specific preferences such as, good taste and impressive cooking quality, good response to stress condition and suitability in particular agro-climate regions. Natural and artificial selection among the landrace pools create the landrace genetic diversity and structure; moreover, the genetic differentiation among the populations can emerge due to strong impacts. This present study revealed high agronomic variation and significant differentiation of trait variation among populations, and regional attributed agronomic characters. And this information is necessary for regional crop improvement program.

Myanmar rice grower select seed with their traditional norm and use and conserve it by them for seed source. And dissemination of seed within and among regions is very easy due to extensive agriculture system. Among farmers, seed choices are based on adaptability of crop with regional environment and fitting with customer desire. In this study, we identified the significant variation of seed shape among regional populations and seed shape character could be assumed as regional specific character. Beside the appearance of grain character, farmers are interested upon growth duration, palatability and ability of stress response.

Regional and seasonal adaptable trait, heading date is important and farmer evaluate growth duration base on that character. Depend on the day-length and temperature distribution, Myanmar rice landrace show high diversity of HD. This sort of diversity observed in heading time seem to be caused not only by adaptation to various rice growing conditions like upland and lowland conditions but also by adaptability to rice growing seasons like summer, monsoon and winter. Prevailing of early heading in upland ecosystem and wide range of heading date distribution in lowland ecosystem are distinct regional attributed agronomic characters (Irie *et al.,* 2003). Cold temperature in mountainous regions or drastic changes of water level at lowland area is possible cues to create the HD diversity of rice landraces (Ye Tint Tun, 2006).

In evaluation of grain quality, palatability is important and controlled by amylose content (AC) mainly (Okuno,1978) and others two, Gel-consistency (GC) and Gelatinization temperature (GT). Seed size is additional character in observing the grain quality. Eating habits and preferences of Myanmar people vary not only from region to region but also between rural and urban areas, therefore, wide range of eating and cooking quality traits in

Myanmar rice landraces is undoubted information. Wide variation in AC, GT and GC, and seed size were found in upland area. But accessions from lowland landrace were completed with intermediate-type AC, soft-type GC and intermediate-type GT which are important objective for quality rice breeding program (Khush *et al.*,1979). Medium short and bold seed shape was prominent in lowland. Actually, lowland ecosystem is major productive area and all criteria upon seed selection base on market need. Therefore, filling with standard ECQ in lowland landrace is not interesting.

Investigation of rice blast resistant among germplasms with nine Japanese standard blast isolates, low average infection degree (0.4) was observed among Myanmar rice landraces and could be assumed as resistant gene pool to rice blast. Among upland and lowland ecosystems, highest resistant group is lowland population and upland population showed wide variation of resistant reaction upon nine JSBIs. Upland mountainous and Southern delta region have high chance for disease out-break because temperature and rainfall in that regions favor to be the disease out-break humid environment. But, there is seldom infection problem but no record regarding with outbreak. Therefore, evaluation of upland and lowland landraces base on rice blast and knowing about as a resistant germplasm population is valuable information to use as an important genetic resource in the future rice blast resistant breeding program (Gnanamanickam *et al.*, 1999).

In this study, there was significant variation among regional populations under different ecosystems. More clear variation of agronomic traits was observed among upland and lowland landrace pools. But, upland group was more variable than lowland group except heading date trait. Distribution of agronomic variation level depends on the natural and artificial impacts of each region. As a detailed statement, traditional farming system and local environment associated with diverse conditions including water un-detainable condition on the slopes and hilly region (Wunna *et al.*, 2015) and tribes related culture diversity (Kawase *et al.,* 2011) may attribute the high genetic variability among upland landrace populations.

4.1.3 Influence of selection impact upon landrace diversity

Rice landraces or traditional cultivars grown in subsistence agriculture are diverse. They carry genetic variability in their populations with possible condition of crossing with natural adapted wild-type or off-type or other varieties. Consequently, unique combination of useful genes related with regional adaptable and human preference trait is thought to be accumulated in the genome of a landrace (Ohm Mar Saw, 2007; San San Yi *et al.*,2007). Therefore, landraces diversity is important and useful for breeding purpose.

The high gene diversity demonstrates the broader genetic base of the rice landrace accessions examined. The lower genetic diversity in lowland landrace is due to the fact that this study collection was from customer derived rice productive area, while, the upland population that were from diverse multi-ethnic groups and heterogeneous environment show high genetic diversity. Therefore, the different in diversity indexes between two agro-ecotype groups relate with artifact base on human and nature.

Actually, genetic diversity of landrace population is influence by natural and human selection events. Natural selection through the ecological factors (day-length, temperature, rainfall, etc) and biotic factors (disease resistant) influence upon the regional landrace population (Kauffman *et al.*,1973; Ding *et al.*,2011). As heading date example, varieties adapt to each agro-ecological zone is limited but rice varieties are gradually insensitive to day length via long–period natural selection and artificial domestication for high latitude cold region (Zhen-ling *et al.,* 2011). Observation of days to heading within my studied samples, mountainous upland landrace accessions are early HD-type and show low variation among upland populations. As a human impact, farmers select among the plants in the crop population to maintain some desirable characters and to increase the prevalence of other valued traits in the population over time. Main interesting trait for seed source selection among farmers is seed size and eating quality. Evidently, tribe men from hilly region prefer the sticky and hard texture for their traditional event utility. And, Myanmar people from plain prefer bold-type grain, good elongation on cooking and soft texture after cooked. All of the landraces from lowland population showed low variation in these characters. By observing the low genetic diversity and variation in agronomic traits in lowland landrace population, possible genetic drift among lowland rice accessions can be assumed.

This higher genetic diversity within regional populations under same regional and seasonal adaptable ability or diverse allele under the same human preference traits are preferable broad genetic base landrace population which are thought to be the result of natural selection or artificial selection after introducing to each area(Domestication) (Tenaillon *et al.*,2001).

4.1.4 Implication for conservation and sustainable utilization

As rice based agricultural country, Myanmar, the conservation of rice germplasm is important for economic development and food security. With the great complex ethnicity, diverse geographic create the high rate of rice germplasm diversity which direct affect to the economy of Myanmar. Therefore, appropriate conservation and use of rice diversity is one of the key elements which can greatly contribute to the success of agriculture development and food security in Myanmar.

This current study of genetic diversity and agronomic variation studies of rice landraces show the current status of landrace diversity within and among the agro-ecology zones and ecosystems. In Southern lowland area, low diverse landrace population has been identified based on genetic diversity and agronomic variation studies. Current population of landrace is maintained by Southern plain’s farmers because of their adaptability, and endemic characters preference by the farmers such as eating quality. Regarding the landraces or traditional cultivars in some regions, farmers protected and maintained them systematically even if they were forced to replace these landraces with improved varieties, they would refuse to do so. Thus, current landraces survival and landrace diversity is totally relay on the farmers, and on-farm management for landraces conservation is best option with further evolution of landraces diversity under the traditional farming.

In upland ecosystem, landraces diversity was found as high diversity rate under the harsh environment and diverse tribes. Finding of considerable landrace diversity in upland area, rural farmers' seed source and seed's characters become important information for conservation and utilization. Actually, these upland landraces are under cultivation by hill man for a long time where adaptation and domestication forces facilitated the fitness of these landrace according to local harsh condition. With the regional adapted characters, these landrace become the valuable resource for breeding purposes.

Nowadays, these local landrace genetic resources lead to the problem by threating to their existing diversity, while they are not effectively used yet in our country for developing the new local adapted varieties. Therefore, assessing the current status of diversity and setting of possible ways of implementation in conservation is urgently needed not only in national level, but equally so in regional contexts. With this study, we identified and pointed out the low or high genetic diversity of landrace population among two ecosystems and conservation measures are also discussed to be helpful while revising the similar studies in future.

4.1.5 Approaching to purpose via objectives

This current study was conducted with the aim to evaluate genetic diversity as well as agronomic variation between and among upland and lowland landrace groups. Generally, all regional populations have considerable high in genetic diversity and high potential rate of gene diversity was observed in upland rice landrace pool with effect of harsh environment and diverse tribes. But current population of lowland landraces from Southern plain region showed low genetic diversity. Therefore, upland populations have higher genetic diversity than lowland population. Intraspecific variation, genetic background of accessions and diversity indexes of regional populations were distinctly different among upland and lowland landrace pools than variation of three agronomic traits. The result of this comparative assessment between upland and lowland landrace populations supports my second hypothesis 'intraspecific differentiation and genetic background are main partition among upland and lowland population'. This information is also important for in setting of regional germplasm conservation plan and regional effective breeding program.

With this study, we identified and pointed out current landrace diversity condition among two ecosystems. Although, the existence of upland landrace population is not yet thread and higher genetic diversity still remain, the conservation program is necessary to be started. The lowland landrace has lower gene diversity than upland population but variation pattern of heading date and rice blast resistant showed wider than upland population. Main facts of wide variation in heading date and rice blast resistant at lowland ecosystem depend on cultivation system (rainfed vs irrigated) and cultivation season (summer vs raining).

Higher molecular variances within regional populations under significant variation of agronomic traits among regional populations are observed at both upland and lowland population. The broad genetic basis of the germplasms and wide variability in agronomic trait provide a basis for sustainable utilization and crop improvement. This studied landrace pool is preferable broad genetic landrace pool for utilization and further research purposes.

4.2 Conclusions

1. Investigation of current rice landrace accessions from Myanmar displayed moderate to high genetic diversity in both upland and lowland cultivation systems. These accessions were classified into mainly two groups; I and II by the cluster analysis using the polymorphism data of 65 SSR markers. Thus, cluster groups I and II were expected to correspond with Indica-type and Japonica-type, respectively. Although, there was high genetic diversity in every regional populations, upland agro-ecotype was higher than lowland. Especially, North-eastern upland accessions showed broader base of genetic diversity among five regional populations. On the other hand, southern lowland landrace accessions were lowest in genetic diversity indexes among five regions.

2. Results of model-based method base on the detecting of intraspecific differentiation and accession's collection environments, all accessions were classified as A-1(Japonica-type upland), A-2 (Japonica-type lowland), B-1(Indica-type lowland and upland mixture) and B-2 (Indica-type upland). Higher genetic diversity indexes and 70% of Indica-type influence were prominent among upland populations and within upland ecosystem, while, high dominant of Japonica-type (80%) group was observed lowland ecosystem.

3. Higher genetic viability within populations was observed rather than among populations within ecosystems and among ecosystems at AMOVA analysis. Independent clustering of accessions from different origins in UPGMA analysis and overlapping of genetic distinct groups among populations support the AMOVA analysis to assume that there was germplasm cross introductions among regions with attribution of ethnicity, traditional farming systems, crop adaptable to local environment and consumption behaviors of regional population.

4. Clear genetic differentiation between upland and lowland agro-ecotype groups was clarified with significant value of population differentiation (Fixation index), moreover,

interspecific variation and diversity indexes of regional population among upland and lowland landrace pool were distinctly different. Landraces of three distinct groups from upland ecosystem are highly genetically diverse and widely overlapped and adapted among upland regions (Figure 4.1). Therefore, higher genetic variability within regional populations related with regional adaptable.

5. Although clear distribution of landrace structure and diversity among upland and lowland ecosystems, agronomic variation of heading date, eating and cooking quality, seed size and blast resistant are variated from low, moderate to high depend on the regional. Although, high variation of eating and cooking quality (ECQ) was observed in upland area, especially Northern, Western and South-eastern, broad variation of day to heading trait and blast disease resistant was found in Southern lowland population. But, upland landrace diversity and agronomic trait variation associated with the diverse upland landscape, wide range of geographic and ecological niches, and different cultivation system under diverse tribe were higher than lowland and this information may serve as a source for future studies, leading to regional adapted breeding purposes.

6. The significant variation of agronomic traits among regional populations under different ecosystems depends on the natural and artificial impacts. More clear variation of agronomic traits was observed among upland and lowland landrace populations. These specific trait variations on each region provide a good indication for future crop improvement in that relevant trait. And these landrace become the valuable resource for breeding purpose.

7. Moreover, populations with higher and lower genetic diversity and their specific variation of regional agronomic traits were identified for conservation purposes. In upland ecosystems, higher genetic viability within regional populations related with adaptable ability of regional population to response to harsh environment and tended to lesser change of

genetic drift and diverse allele under the same human preference trait, and may be select for conservation plan. This study represents landrace diversity and distribution among cultivation systems and agro-ecological regions for supporting the sustainable utilization and conservation.

4.3 Recommendations

4.3.1 Conservation plans

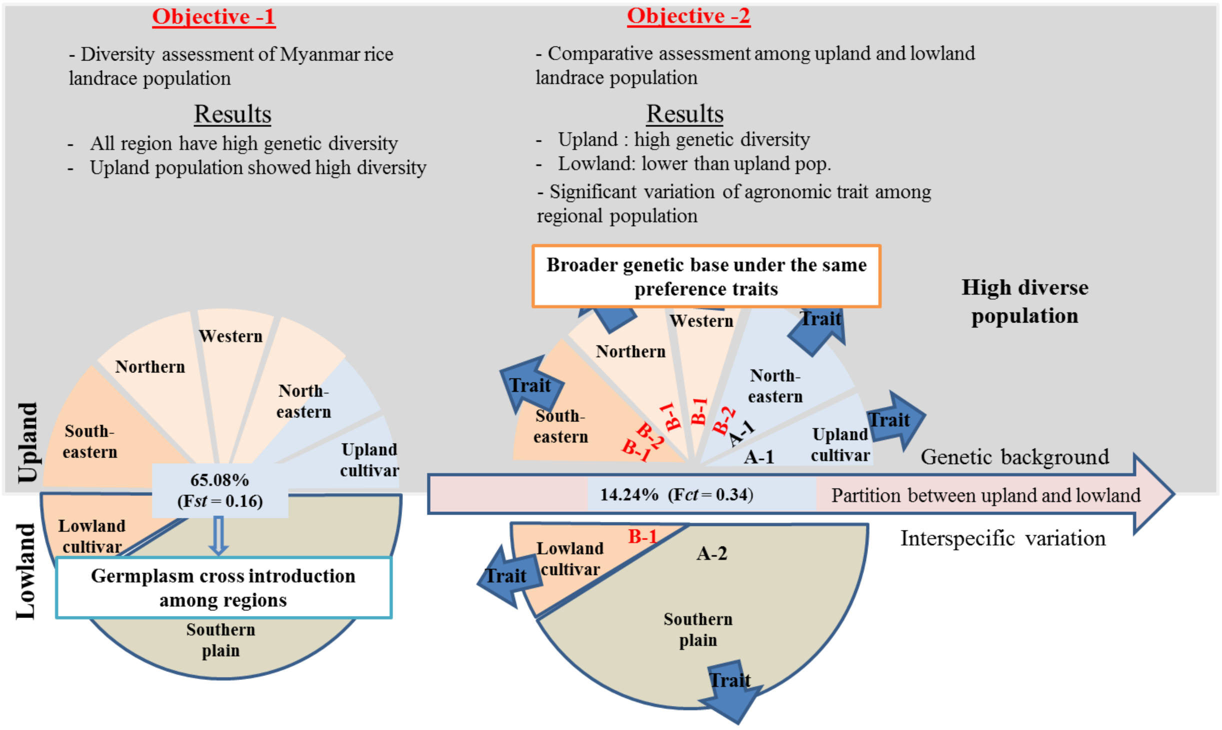
1. Genetic diversity and population structure of landraces from upland and lowland ecosystems were analyzed with two objectives; 1) evaluation of Myanmar rice landraces' genetic diversity and 2) comparative assessment of landrace diversity among upland and lowland ecosystems, for Myanmar landrace sustainability. Genetic diversity of upland rice cultivars showed high diversity rate compare with lowland. And genetic diversity and survival of local cultivars or landraces is totally relying on farmers and their on-farm management. Therefore, innovation and motivation of systematic on-farm conservation procedures among farmers is best option for sustainability of landrace diversity under the traditional farming.
2. In Myanmar, *ex-situ* conservation of rice landrace had already set as national level under the collaboration between MOAI and IRRI, and MOAI and JICA. Regional level of *ex-situ* conservation is also need to support for the regional landrace erosion recovery and to conserve germplasm materials with genetic stability under the same environments (original environment and conservation environment).

3. Lowland landraces population showed lower genetic diversity indexes than upland regional populations. Attention upon the lowland landrace population need for further conservation measures and plan. The elite landrace markets need to promote for supporting

the stability and sustainability of landrace diversity among farmer level.

4.3.2 Sustainable utilization

1. Population with higher variation at molecular level as well as agronomic phenotypic level was found in the upland population to assume the potential pool for research and utilization purposes. Lowland landrace population can also define as promising gene pool for quality improved breeding purposes and searching the resistant gene for rice blast control because stable variation of intermediate amylose content, highly resistant to rice blast character and wide range of heading date variation are main useful traits among landraces in lowland.
2. For promoting the regional landrace market, regional adapted rice improvement plan by introducing of gene from local landrace is more appropriated plan for sustainable utilization rather than the introducing of improved cultivars from abroad.



Objective -1

- Diversity assessment of Myanmar rice landrace population

Results

- All region have high genetic diversity
- Upland population showed high diversity

Objective -2

- Comparative assessment among upland and lowland landrace population

Results

- Upland : high genetic diversity
- Lowland: lower than upland pop.
- Significant variation of agronomic trait among regional population

Figure 4.1 Schematic model of objectives and detailed results of this study.

Summary

Myanmar is Agro-resource richness country due to high potential access of Plant Genetic Resource. It constitutes part of the center of genetic diversity of cultivated rice (*Oryza sativa* L.). Myanmar rice genetic diversity depends on the environmental heterogeneity and various other factors, which are agro-ecological conditions, resilient agriculture systems and ethnological complexity in Myanmar. Moreover, rice production is prominent contribution upon national food security and economy. Major Rice production relies on lowland and upland cultivation systems. Adaptable of crops among different ecosystems; upland and lowland is also another important in considering for regional landrace diversity and structure. Farmers are also interested upon the landrace diversity for their yield stability and sustainability. Currently, changing of government policy and farmer attitudes, and land use impacts affect the local landrace diversity and turn to disappear. These rice germplasm has to be collected and conserved for future uses before they disappeared. To efficient conservation and utilization, we must to know the genetic diversity and structure of the existing germplasm on each region. This current study was conducted with the aim to evaluate genetic diversity as well as agronomic variation between and among upland and lowland landrace groups, and as no such attempt regarding with farmer handling landrace pools in Myanmar has been conducted yet.

In assessing of genetic diversity through DNA based SSR markers, Myanmar rice landraces pool show the considerable broad genetic diversity (gene diversity : 0.840 and expected heterozygosity: He 0.685). In all upland and lowland accessions, two major clusters was identified base on the polymorphism of SSRs and phenol reaction to seed grain. Thus, cluster I and II were expected to correspond with Indica and Japonica-types, respectively. According to Structure analysis, genetic background of all accessions was

categorized into four groups, Japonica-type upland group (A-1), Japonica-type lowland group (A-2), Indica-typed lowland and upland mixture group (B-1) and Indica-typed upland group (B-2). These four genetic groups were distributed among five rice cultivated agro-ecological zones.

Among five geographic regions, North-eastern upland population showed high gene diversity, while, Southern lowland landrace group was lowest gene diversity among regional populations. But upland landrace accessions independently clustered among two main clusters with no source or origin specific distribution. AMOVA also revealed the high variation within populations rather than among populations and ecosystems. Therefore, we can assume germplasm cross introduction among regions under attribution of ethnicity, traditional farming systems and consumption behavior. Clear population differentiation among upland and lowland populations was observed with significant F_{ST} value ($F_{ST} = 0.31$). Intraspecific variation, regional populations' genetic background and genetic diversity indexes level are main partition among upland and lowland populations. Distinct level of high genetic diversity was observed in landrace pool from upland ecosystem because upland landraces are highly genetically diverse, and widely overlapped and adapted among three mountainous agro-ecological zones. Overall, this part of study highlights upon landrace diversity and intraspecific variation among different ecosystems and agro-ecological regions for supporting the sustainable utilization and conservation.

Characterization of three agronomic traits; heading date, eating and cooking quality, and rice blast resistant among landraces is important to determine the regional landrace variation and distribution based on their adaptability of local environment and human preferences. For germplasm management and sustainable regional rice production, detecting of trait variation among landraces as well as among and within regions is vital survey. In observing of heading date (HD), related with regional and seasonal adaptable, significant differentiation was investigated among upland and lowland ecosystems, defined as early HD dominant area and wide range of HD distributed area. Diversity pattern of HD among Myanmar rice landraces was created not only by the variation of natural factors such as day-length and temperature, but also by various artificial factors, farming systems, cropping patterns, cultivation seasons and so on.

Seed appearance quality and palatability of variety are related with landraces dissemination or diversity. Variation of seed size, amylose content, alkali digestible and gel consistency among upland landraces are interesting because these traits variation were significant among upland agro-ecological zones where complex ethnicity, culture events, utility and preference are main option. Actually, variation of cooking, eating and processing qualities among rice is not solely a varietal characteristic but also depends on the growing environment, harvesting, processing and handling systems.

Among Myanmar rice germplasms, low average infection degree of blast fungus (0.4) was observed when we evaluated with nine Japanese isolates (JSBI). Therefore, Myanmar rice landraces can be assumed as resistant gene pool to rice blast. Among two agro-ecotype groups, most accessions from lowland were found to be resistant to all nine Japanese isolates, while upland populations showed the wide variation of resistance reactions upon nine JSBI. Evaluation of Myanmar germplasms base on rice blast resistant evaluation and knowing about the resistant germplasm population is valuable information in formulating the rice blast resistant breeding program.

Generally, landrace diversity was observed as high rate in upland rice landraces with effect of harsh environment and diverse tribes. Current condition of lowland landraces from Southern plain region showed low genetic diversity under forcing of improved cultivars introductions. Intraspecific variation, variation of three agronomic traits and diversity indexes of regional populations were distinctly different among upland and lowland landrace pools. But, higher molecular variances within regional populations under significant variation of agronomic traits among regional populations are preferable broad genetic gene pool for utilization and further research purposes. With this study, we identified and pointed out the potentially low or high diverse landrace population among agroecology-zones and conservation measures are also discussed to be helpful while revising similar studies in future.

LIST OF PUBLICATIONS/ POSTERS

Publications

1. Wunna, Gilini SA, Kawase M, Ohsawa R, and Watanabe KN (2015) Tracking selection signatures based on variation in OsLEA27 within Myanmar landraces of upland and dryland rice. Am J Plant Sci 6: 1937-1950

2. Gilani SA, Kikuchi A, Shimazaki T, Noladhi W, Wunna and Watanabe KN (2015) Molecular genetic diversity of curcuminoid genes in Curcuma amada: Curcuminoid variation, consideration on species boundary and polyploidy. Biochem Systs Ecol 61:186-195.

3. Yamamoto S, Wunna, Rafique T, Arizaga MV, Fukui K, Gutierrez EJC, Martinez CRC, Watanabe K and Niino T (2015) The Aluminum Cryo-plate Increases Efficiency of Cryopreservation Protocols for Potato Shoot Tips. Am J Potato Res 92(2).

4. Niino T, Wunna, Watanabe K, Nohara N, Rafique T, Yamamoto S, Fukui K, Arizaga MV, Martinez CRC, Matsumoto T and Engelmann F (2014) Cryopreservation of mat rush lateral buds by air dehydration using aluminum cryo-plate. Plant Biotech 31(3):281-287.

5. Kawase M, Wunna and Watanabe K (2011) Second Field Survey Collecting Traditionally Grown Crops in Northern Areas of Myanmar, 2009, 植物遺伝資源探索導入調査報告書27:83-93.

6. Yamamoto S, Moe Kyaw Aung, Watanabe K, Wunna and Kawase M (2011) Third Field Survey Collecting Traditionally Grown Crops in Northern Areas of Myanmar, 2011. 植物遺伝資源探索導入調査報告書27:95-109.

Academic meeting presentation

1. Wunna, Fukuta Y, Ohsawa R, Aung PP and Watanabe K (2015) Genetic diversity of Myanmar rice landraces from different agro-ecological zones investigated with SSR markers and agronomic traits.127th Meeting of Japanese Society of Breeding (JBS), Tamagawa University, Machida, Tokyo, Japan, March-2015. (Poster)

ACKNOWLEDGEMENTS

This thesis would not have been possible without the support and guidance of many people. Firstly, I would like to acknowledge and extend my sincere gratitude and profound appreciation to the following persons who have made the completion of this thesis possible:

- Professor Dr. Kazuo N. Watanabe, Faculty of Life and Environmental Sciences, University of Tsukuba
- Professor Dr. Ryo Ohsawa, Faculty of Life and Environmental Sciences, university of Tsukuba
- Professor Dr. Yoshimichi Fukuta, Japan International Research Center For Agricultural Science (JIRCAS), Japan
- Professor Dr. Sachio Maruyama, Faculty of Life and Environmental Sciences, university of Tsukuba
- All Watanabe lab members

Finally, I will extend my great thanks to Ministry of Education, Culture, Sports, Science and technology of Japan for giving a chance to study abroad in Japan by supporting scholarship.

REFERENCES

Agrawal PK (2003) Molecular approaches in evolutionary studies and speciation in genus *Oryza*—present status. In: Monograph on Genus *Oryza*, Jata A. Nanda, S.D. Sharma (Eds.). Science Publishers, Inc. USA. pp. 139–170

Anon (1995) Manual of operations and procedures of the International rice genebank. International Rice Research Institute, Los Banos, Phillipines pp. 56-72

Ball S, Guan H-P, James M, Myers A, Keeling P, Mouille G, Buleon A, Colonna P and Preiss J (1996) From glycogen to amylopectin: a model explaining the biogenesis of the plant starch granule. *Cell* 86: 349–352

Ballini E, Morel JB, Droc G, Price A, Courtois B, Notteghem JL and Tharreau D (2008) A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Molecular Plant-Microbe Interactions* 21: 859–868

Beachell JM and Halick JV (1957) Breeding for improved milling processing and cooking characteristics of rice. *International Rice Commission Newsletter*, Los Banos 6(2):1-7

Beachell JM and Stansel JW (1963) Selection rice for specifying cooking characteristics in a breeding program. *International Rice Commission Newsletter* (Special issue) pp. 25-34

Beale RA (1915) Note on the classification of rice of Lower Burma. *Poona Agriculture Collage Magazine* 7: 31-90

Brush SB (1995) In situ conservation of landraces in centers of crop diversity. *Crop Science*

35: 346–354

Cagampang GB, Perez CM and Juliano BO (1973) A gel consistency test for eating quality of rice. *Journal of the Science of Food and Agriculture* 24: 1589-1594

Central Statistical Organization (2001) Statistical year book, Myanmar, pp. 3 – 9

Chang TT (1976) The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. *Euphytica* 25: 425-441

Choudhury DR, Singh N, Singh AK, Kumar S, Srinivasan K, Tyagi RK, Ahmad A, Singh NK and Singh R (2014) Analysis of genetic diversity and population structure of rice germplasm from North-Eastern region of India and development of a core germplasm set. *PLoS ONE* 9:11

Cooke RJ (1984) The characterization and identification of crop cultivars by electrophoresis. *Electrophoresis* 5: 59-72

Cruz DN and Khush GS (2000) Rice grain quality evaluation procedures. In Aromatic rices, (Eds. R.K. Singh, U.S. Singh and G.S. Khush), pp.15-28, Publisher, Oxford and IBH publishing Co.Pvt. Ltd., New Delhi, Calcutta

Ding Z, Wang C, Chan S and Yu S (2011) Diversity and selective sweep in *OsAMT*1;1 genomic region of rice. *BMC Evolutionary Biology* 11: 61

Earl DA and von Hoidt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. http://taylor0.biology ucla.edu/structureHarvester/

Ellis JR and Burke JM (2007) EST-SSRs as a resource for population genetic analyses.

Heredity 99: 125–132

Evanno G, Regnaut S and Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* 14: 2611-2620

Excoffier L, Laval G and Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1:47-50

FAO (1984) Food balance sheets, 1979-81 average, Rome, FAO.

FAO (1996) Myanmar: Country report to the FAO international technical conference on plant genetic resources. Leipzig

FAO (1998) The State of the world's Plant Genetic Resources for Food and Agriculture. FAO (Rome) pp. 3

FAO (2005) Agriculture Atlas of the Union of Myanmar. FAO (Rome) pp.44

FAO data base [online] http://faostat.fao.org,(Assessed July, 2015)

Farias FJC and DeLaCruz NM (1995) Cooking and eating characteristics in upland and irrigated rice varieties. Pesq agropec bras, Brasília 30(1): 115-120

Frankham R, Ballou JD and Briscoe DA (2007) A Primer of Conservation Genetics. Cambridge University Press, UK

Gao H, Jin M, Zheng XM, Chen J, Yuan D, Xin Y, Wang M, Huang D, Zhang Z, Zhou K, Sheng P, Ma J, Ma W, Deng H, Jiang L, Liu S, Wang H, Wu C, Yuan L and Wan J (2014) Days to heading 7, a major quantitative locus determining photoperiod sensitivity and regional adaptation in rice. *Proceedings of the National Academy of*

Sciences 111: 16337–16342

Garris, JA, Tai TH, Coburn J, Kresovich S and McCouch SR (2005) Genetic structure and diversity in Oryza sativa L. *Genetics* 169: 1631- 1638

Ghaley BB, Christiansen JL and Andersen SB (2012) Genetic diversity in blast resistance of Bhutan rice landraces. *Euphytica* 184: 119–130

Gnanamanickam SS, Brindha Priyadarisini V, Narayanan NN, Preeti Vasudevan and Kavitha S (1999) An overview of bacterial blight disease of rice and strategies for itsmanagement. *Current Science* 77:1435-1443

GRAMENE data base [online] http://www.gramene.org/ (Assessed July 5015)

Gupta SR, Upadhyay MP and Katsumoto T (1996) Status of Plant Genetic Resources in Nepal. Paper presented on the 19th Summer Crops Workshop, Regional Agricultural Research Station (RARS). Parwanipur (Nepal) Feb. 27-29, 1996

Gwag JG (2008) Molecular diversity assessment and population structure analysis in mungbean, *Vigna radiata* (L.) Wilczek. Ph.D. thesis, Graduate School of Seoul National University 125p.

Hamrick JL and Godt MJ (1996) Conservation genetics of endangered plant species. In: Avise JC, Hamrick JL (Eds) Conservation Genetics: Case Histories from Nature. Chapman & Hall, New York, pp. 281–304

Hartl DL and Clark AG (1997) Principles of population genetics 3rd Ed. Sunderland, Massachusetts: Sinauer Associates, Inc

Hayashi N, Kobayashi N, VeraCruz MCM and Fukuda Y (2009) Protocols for the sampling of diseased specimens and evaluation of blast disease in rice. JIRCAS working report No.63. Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, pp. 17-33

Hirano HY and Sano Y (1991) Molecular characterization of the waxy locus of rice (*Oryza sativa*). *Plant Cell Physiology* 32: 989-997

Hirano R, Ishii H, Than Htun Oo, Gilani1 SA, Kikuchi1 A and Watanabe KN (2011) Propagation management methods have altered the genetic variability of two traditional mango varieties in Myanmar, as revealed by SSR. *Plant Genetic Resources Characterization and Utilization* 9(3): 404–410

Hsu YC, Tseng MC, Wu YP, Lin MY, Wei FJ, Hwu KK, Hsing YI and Lin YR (2014) Genetic factors responsible for eating and cooking qualities of rice grains in a recombinant inbred population of an inter-subspecific cross. *Molecular Breeding* 34 (2): 655-673

Huang X , Kurata N, Wei X, Wang ZX, Wang A, Zhao Q, Zhao Y, Liu K, Lu H, Li W, Guo Y, Lu Y, Zhou C, Fan D, Weng Q, Zhu C, Huang T, Zhang L, Wang Y, Feng L, Furuumi H, Kubo T, Miyabayashi T, Yuan X, Xu Q, Dong G, Zhan Q, Li C, Fujiyama A, Toyoda A, Lu T, Feng Q, Qian Q, Li J and Han B (2012) A map of rice genome variation reveals the origin of cultivated rice. *Nature* 490: 497–501

Ikeda R (1999) Descriptors for rice. In: (Eds. Nagemina T and Takeda H), Proceeding of the joint international workshop, NIAR, Tsukuba, Japan 1: 1-7

Irie K, Khin Aye, Nagamine T, Fujimaki H and Kikichi F (2003) Varietal variations of heading time among rice landraces in Myanmar. *Japanese Journal of Tropical Agriculture* 47:198–205

Irie K, Khin Aye, Yi Yi Myint, L. Nang Kha, Ye Tint Tun, Nagamine T, Fujimaki H and Kikuchi F (2004) Regional variations of grain characters observed in rice landraces of Myanmar. *Japanese Journal of Tropical Agriculture* 48:101-110

ISNB [online] available at http://www.bioversityinternational.org (Assessed July 2015)

Juliano BO (1979) The chemical basis of rice grain quality. In: Proceedings of the Workshop on Chemical Aspects of Rice Grain Quality. Manila (Philippines): International Rice Research Institute pp. 69-90

Juliano BO and Boulter D (1976) Extraction and composition of rice endosperm glutelin. *Phytochemistry* 15: 1601-1606

Karp A(2002) The new genetic era: Will it help us in managing genetic diversity? In: (Eds. Engels JMM, Rao VR, Brown AHD, Jacson MT) Managing plant genetic diversity. CABI Publishing, UK pp. 43-56

Kawase M, Wunna and Watanabe K (2011) Second Field Survey Collecting Traditionally Grown Crops in Northern Areas of Myanmar, 2009. *Annual Report on Exploration and Introduction of Plant Genetic Resources* 27: 83-93

Khin Myo Myint, Courtois B, Risterucci AM, Frouin J, Soe K, Thet KM, Vanavichit A and Glaszmann JC (2012) Specific patterns of genetic diversity among aromatic rice varieties in Myanmar. *Rice* 5: 20.

Khin Soe, Tin Soe, Toe Aung, and Fujimura T (2002) Study on the genetic variation among rice varieties (*Oryza sativa* L.) using microsatellite DNA polymorphisms. Myanmar. Agricultural Sciences, proceeding of the annual research conference; Myanmar, pp. 132- 139

Khin Than Nwe and Tin Tin Myant (2000) Aspects of Quality of Rice Improvement in Myanmar. Myanmar Agriculture Journal 2000, pp. 24-34

Khin Win (1991) A Century of Rice Improvement in Burma. Sourcebook produced by IRRI, Philippines pp. 5–15

Khin Win and Nyi Nyi (1979) Rainfed farming systems and practices in relation to agroecological zones. Agricultural Corporation, Rangoon, Burma pp. 9-14

Khush GS, Brar D, Virk PS, Tang SX, Malik SS, Busto GA, Lee YT, McNally R, Trinh LN, Jiang Y and Shat MAM (2003) Classifying rice germplasm by isozyme polymorphism and origin of cultivated rice. In: IRRI Discussion pp. 46- 279

Klush GS, Paule CM and Cruz NM (1979) Rice grain quality evaluation and improvement at IRRI. In: Workshop on Chemical Aspects of Rice Grain Quality, Los Banos . Proceeding . Los Banos: International Rice Research Institute pp. 21-31

Koch M and Parlevliet JE (1991) Residual effects of the Xa-4 resistance gene in three rice cultivars when exposed to a virulent isolate of *Xanthomonas campestris* pv. *oryzae*. *Euphytica* 53:187-193

Li Y, Wang L, Jing JX, Li ZQ, Lin F, Huang LF and Pan QH (2007) The Pikm gene, conferring stable resistance to isolates of *Magnaporthe oryzae*, was finely mapped in a crossover-cold region on rice chromosome 11. *Molecular Breeding* 20: 179–188

Little RR, Hilder GB and Dawson EH (1958) Differential effect of dilute alkali on 25 varieties of milled white rice. *Cereal Chemistry*, 35:111-126

Liu K and Muse SV (2005) PowerMarker: integrated analysis environment for genetic marker data. *Bioinformatics* 31(9): 2128–2129

Loresto GC, Guevarra E and Jackson MT (2000) Use of conserved rice germplasm, In: Plant genetic resource newsletter (124) pp 51-56. International Plant genetic Resources Institute (IPGRI), Rome, Italy

Mickill DJ (1986) Varietal improvement for rainfed lowland rice in Southeast Asia: Results of a survey. In progress in rainfed lowland rice. International Rice Research Institute, Manilla, Philippines pp. 115-144

Min Naing (2000) Books of National ethnic groups of Myanmar. Chotaythan Sarpay (01692), Yangon Press, pp. 1-15

Ministry of Agriculture and Irrigation (2013) Myanmar Agriculture in Brief. Naypyidaw, Myanmar pp. 15-16

Ministry of Agriculture and Irrigation (2015) Myanmar Rice Sector Development Strategy. Naypyidaw, Myanmar pp. 5-9

Mohammadi SA and Prasanna BM (2003) Analysis of genetic diversity in crop plants-Salient statistical tools and considerations. *Crop Science* (43) 4:1235-1248

Monna L, Kitazawa N, Yoshino R, Suzuki J, Masuda H, Maehara Y, Tanji M, Sato M, Nasu S and Minobe Y (2002) Positional cloning of rice semidwarfing gene, *sd-1*: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis. *DNA Research* 9(1): 11-17

Nakagahra M and Hayashi KI (1977) Origin of cultivated rice as detected by isozyme variations. *Japan Agricultural Research Quarterly* 11:1-5

Nei M, Tajima F and Tatena Y (1983) Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution* 19:153-170

Ni J, Colowit PM and Mackill DJ (2002) Evaluation of Genetic Diversity in Rice Subspecies Using Microsatellite Markers. *Crop Science* 42: 601-607

Ohm Mar Saw (2007) Genetic diversity of Myanmar rice cultivar. PhD thesis. Kyushu University, Japan, 110 p.

Okuno K (1978) Gene dosage effect of waxy alleles on amylose content in endosperm starch of rice. *Japanese Journal of Genetic* 53: 219-222

Pa Pa Aung (2004) Evaluation of genetic diversity on seed storage protein and endosperm starch properties in Myanmar Rice Germplasm for rice grain quality improvement. PhD thesis. Kyushu university, Japan,119 p.

Pa Pa Aung, Nishi A, Kumamaru T and Satoh H (2003) The amylopectin chains length distribution of opaque endosperm type in Myanmar local rice cultivar. *Rice Genetic Newsletter* 20 :16

Peakall R and Smouse PE (2006) GenAlEx 6: Genetic Analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6 (1): 288–295

Petit E, Balloux F and Goudet J (2001) Sex-biased dispersal in a migratory bat; a characterization using sex-specific demographic parameters. *Evolution* 55: 635–640

Pritchard JK, Stephens M and Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959

Rana RB, Garforth C, Sthapit B and Jarvis D (2007) Influence of socio-economic and cultural factors in rice varietal diversity management on-farm in Nepal. *Agriculture and Human Values* 24: 461-471

Rao SB, Vasudeva AR and Subrahmanya RA (1952) The amylose and amylopectin contents of rice and their influence on the cooking qualities of cereal. *Proceeding Indian Academic Science Section* (B) 36:70

Rice N, Giovanni C, Shepherd M, Bundock P, Bradbury L, Pacey-miller T, Furtado A and Henry R (2006) DNA banks and their role in facilitating the application of genomics to plant germplasm. *Plant Genetic Resource* 4(1): 64-70

Sadiki M, Jarvis DI, Rijal D, Bajracharya J, Hue NN, Camacho-Villa TC, Burgos-May LA, Sawadogo M, Balma D, Lope D, Arias L, Mar I, Karamura D, Williams D, Chavez-Servia JL, Sthapit B and Rao VR (2007) Variety names: an entry point to crop genetic diversity and distribution in agroecosystems. In Managing biodiversity in agricultural ecosystems (Eds D. I. Jarvis, C. Padoch & H. D. Cooper), New York, NY: Columbia University Press pp. 34–76

San San Yi, Jatoi SA, Fujimura T, Yamanaka S, Watanabe J and Watanabe KN (2007) Potential loss of unique genetic diversity in tomato landraces by genetic colonization of modern cultivars at a non-center of origin. *Plant Breeding* 127(2):189-196

Sasaki R (1922) Existence of strains in rice blast fungus. *Japanese Journal of Plant Protection* 9:631–44

Simpson MJA and Withers LA (1986) Characterization of plant genetic resources using electrophoresis: a guide to the literature. International Board for Plant genetic Resources, Rome

Smilde WD, Brigneti G, Jagger L, Perkins S and Jones JDG (2005), *Solanum mochiquense* chromosome IX carries a novel late blight resistance gene *Rpi-moc1* . *Theoretical and Applied Genetics* 110: 252-258

Song J, Bradeen JM, Naess SK, Raasch JA, Wielgus SM, Haberlach GT, Liu J, Kuang H, Austin-Phillips S, Buell CR, Helgeson JP and Jiang J (2003) Gene RB cloned from Solanum bulbocastanum confers broad spectrum resistance to potato late blight. *Proceedings of the National Academy of Sciences* 100: 9128-9133

Sounigo O, Christopher Y and Umaharan R (1996) Genetic diversity assessment of *Theobroma cacao* L. using isoenzymee and RAPD analyses. In: *CRU, Annual Report 1996* Port of Spain, Trinidad and Tobago pp. 35–51

Takeda Y and Hizukuri S (1987). Structure of rice amylopectins with low and high affinities for iodine. *Carbohydrate Research* 168: 79-88

Tanaka AK and Fukuta Y (2014) Genetic variation in resistance to blast disease (*Pyricularia oryzae* Cavara) in Japanese rice (*Oryza sativa* L.), as determined using a differential system. *Breeding Science* 64: 1-10

Tenaillon MI, Sawkins MC, Long AD, Gaut RL, Doebley JF and Gaut BS (2001) Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. mays L.). *Proceedings of the National Academy of Sciences* 98: 9161-9166

Thakur S, Gupta YK, Singh PK, Rathour R, Variar M, Prashanthi SK, Singh AK, Singh UD, Chand D, Rana JC, Singh NK and Sharma TR (2013) Molecular diversity in rice blast resistance gene *Pi-ta* makes it highly effective against dynamic population of *Magnaporthe oryzae*. *Functional Integrated Genomics* 13; 309–322

Than Sein, Tin Maw Oo, Min San Thein, Soe Pe, Irie K and Ito M (2004) Study on wild rice species in Myanmar. In “Rice Research Activities in Myanmar” Published in commemoration of International Year of Rice 2004. Myanmar Academy of Agricultural, Forestry, Livestock and Fishery Sciences pp. 30-47

Thein Tun and Mya Than (1995) Myanmar: Country report to the FAO international conference on plant genetic resources. Yangon, pp. 1-25

Tian Z, Qianb Q, Liuc Q, Yanb M, Liua X, Yanc C, Liua G, Gaob Z, Tang S, Zeng D, Wanga Y, Yu J, Gu M and Li J (2009) Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. *Proceedings of the National Academy of Sciences* 106 (51): 21760-21765

Tin Aye Aye Naing, Kingsbury AJ, Buerkert A and Finckh MR (2008). A Survey of Myanmar rice production and constraints. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* 109(2): 151-168

van der Linden CG, Wouters DC, Mihalka V, Kochieva EZ, Smulders MJ and Vosman B (2004) Efficient targeting of plant disease resistance loci using NBS profiling. *Theoretical and Applied Genetics* 109: 384-393

Vaughan DA (1994) The Wild Relatives of Rice: A Genetic Resources Handbook, International Rice Research Institute, Manila, Philippines

Vergara BS and Chang TT (1976) The flowering response of the rice plant to photoperiod, a review of literature. *International Rice Research Institute Technology Bulletin* 8: 75

Vergara BS and Chang TT (1985) The flowering response of the rice plant to photoperiod, a review of literature, 4th edn. International Rice Research Institute pp.1-12

Walls FW(1965) A standard phenol method for testing wheat for varietal purity. Hand Book of Seed Testing , AOSA, Contribution No. 28

Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC and Nelson RJ (1994) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics* 136(4):1421-1434

Watanabe KN, Tun YT and Kawase M (2007) Field survey and collection of traditionally grown crops in Northern area of Myanmar, 2006. *Annual Report on Exploration and Introduction of Plant Genetic Resources* 23: 161–175

Weidong G, Fang J, Zheng D, Li Y, Lu X, Rao RV, Hodgkin T and Zongwen Z (2000) Utilization of germplasm conserved in Chinese national genebanks- a survey. In: *Plant Genetic Resource Newsletter*,123: 1-8, IPGRI, Rome, Italy

Wunna, Gilini SA, Kawase M, Ohsawa R, and Watanabe KN (2015) Tracking selection signatures based on variation in *OsLEA27* within Myanmar landraces of upland and dryland rice. *American Journal of Plant Science* 6: 1937-1950

Xiong Z, Zhang S, Wang Y, Ford-Lloyd BV, Tu M, Jin X, Wu Y, Yan H, Yang X, Lui P and Lu B (2010) Differentiation and distribution of indica and japonica rice varieties along the altitude gradients in Yunnan Province of China as revealed by InDel molecular markers. *Genetic Resource and Crop Evolution* 57: 891–902

Yamamoto S, Aung MK, Watanabe KN, Wunna and Kawase M (2011) Third Field Survey Collecting Traditionally Grown Crops in Northern Areas of Myanmar, 2011. *Annual Report on Exploration and Introduction of Plant Genetic Resources* 27: 95–109

Yamanaka S, Jatoi SA, Yi SS, Kothari SL, Htut T and Watanabe KN (2011) Genetic diversity of Myanmar rice and their implementation on management methods. *African Journal of Biotechnology* 10(8):1290–1298

Ye Goung, Khin Win and Win Htin (1978) Rice soils of Burma. Pages 57-71 in Soils and rice. International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Ye Thit Tun, Irie K, Nagamine T, Maw JB, Kikuchi F and Fujimaki H (2005) Genetic diversity of Myanmar rice landraces. In “Rice is life: scientific perspectives for the 21st century”, Proceedings of the World Rice Research Conference, Tokyo and Tsukuba (Japan) pp. 56–60

Ye Tint Tun (2006) Characterization of Myanmar rice landraces based on analyses of genetic diversity of various traits. PhD thesis. Tokyo University of Agriculture, Japan, 248p.

Zhen-ling Z, Xiang-jin W, Jiang L, Kai L, Da-yong X, Hu-qu Z and Jian-min W (2011) Genetic analysis of heading date of Japonica rice cultivars in south west China. *Rice science* 18(4): 287-296

Appendix Table 1 List of Myanmar rice landraces and its ecological origin.

No.	Code no.	Cultivar name	Collection	Agro-ecological area *	Ecotype *	Type of cultivar*
			State/ Region			
1	GMJ1	Eai Si	Chin State	WH	Upland	Landrace
2	GMJ2	Sin Ekari-2	Chin State	WH	Upland	Landrace
3	GMJ3	Kauk hnyin	Shan State(S)	EP(South)	Upland	Landrace
4	GMJ4	La Tha	Shan State(S)	EP(South)	Upland	Landrace
5	GMJ5	Chin Lay	Magway Region	WH	Upland	Landrace
6	GMJ6	Ya Tha Pu	Magway Region	WH	Upland	Landrace
7	GMJ7	Sheik Pha Phu	Magway Region	WH	Upland	Landrace
8	GMJ8	Jin Ma Yan	Kachin State	EP(South)	Upland	Landrace
9	GMJ9	Maw yaw Kaunk hnyin	Kachin State	NM	Upland	Landrace
10	GMJ10	Lone Ou	Shan State(S)	EP(South)	Upland	Landrace
11	GMJ11	Oat Kyai Ni	Shan State(S)	EP(South)	Upland	Landrace
12	GMJ12	Naung Htu Kauk Hnyin	Kachin State	NM	Upland	Landrace
13	GMJ13	Kaung Kaung Akhin	Kachin State	NM	Upland	Landrace
14	GMJ14	Ya Thay Thee Htut	Magway Region	WH	Upland	Landrace
15	GMJ15	San Ni (B)	Shan State(S)	EP(South)	Upland	Landrace
16	GMJ16	Ya Thay	Magway Region	WH	Upland	Landrace
17	GMJ17	Kyaw Scan	Kachin State	NM	Upland	Landrace
18	GMJ18	Sa Ba Ni	Kachin State	NM	Upland	Landrace
19	GMJ19	Khauk Long	Shan State(N)		Upland	Landrace
20	GMJ20	Ma Le Byat	Sagaing Region	NM	Upland	Landrace
21	GMJ21	Kauk Hnyin	Kachin State	NM	Upland	Landrace
22	GMJ22	Khaw Lone	Kachin State	NM	Upland	Landrace
23	GMJ23	Kyeit Phat	Chin State	WH	Upland	Landrace
24	GMJ25	San Bial	Chin State	WH	Upland	Landrace

No.	Code no.	Cultivar name	Collection	Agro-ecological area *	Ecotype *	Type of cultivar*
			State/ Region			
25	GMJ26	Mote Soe Ma Kywae Pyay	Shan State(S)	EP(South)	Upland	Landrace
26	GMJ27	Taung Ya Saba	Magway Region	WH	Upland	Landrace
27	GMJ28	La Chit Mum	Kachin State	NM	Upland	Landrace
28	GMJ29	Bwin Akyan	Kachin State	NM	Upland	Landrace
29	GMJ30	Byat War	Kachin State	NM	Upland	Landrace
30	GMJ31	Nyan Ywae	Kachin State	NM	Upland	Landrace
31	GMJ32	Saba Me	Shan State(S)	EP(South)	Upland	Landrace
32	GMJ33	Kauk Byauk	Shan State(S)	EP(South)	Upland	Landrace
33	GMJ34	San Ni	Shan State(S)	EP(South)	Upland	Landrace
34	GMJ35	San Ni	Shan State(S)	EP(South)	Upland	Landrace
35	GMJ36	Khao Pha Wan	Shan State(S)	EP(South)	Upland	Landrace
36	GMJ37	Saba Me	Shan State(S)	EP(South)	Upland	Landrace
37	GMJ38	San Ni	Shan State(S)	EP(South)	Upland	Landrace
38	GMJ39	Yar Kye	Shan State(S)	EP(South)	Upland	Landrace
39	GMJ40	Yar San	Mandalay Region	EP(North)	Upland	Landrace
40	GMJ41	Taung yar Nga Cheik	Kachin State	EP(North)	Upland	Landrace
41	GMJ42	Do Saba	Kachin State	EP(North)	Upland	Landrace
42	GMJ43	Patee pu	Kachin State	EP(North)	Upland	Landrace
43	GMJ44	Shaka Mum	Kachin State	EP(North)	Upland	Landrace
44	GMJ45	Nyan Sa	Kachin State	EP(North)	Upland	Landrace
45	GMJ46	Traung	Chin State	WH	Upland	Landrace
46	GMJ47	Lone Pu	Shan State(S)	EP(South)	Upland	Landrace
47	GMJ48	Hmway Shay	Shan State(S)	EP(South)	Upland	Landrace
48	GMJ49	Kauk Ya	Shan State(S)	EP(South)	Upland	Landrace
49	GMJ50	San Ni	Shan State(S)	EP(South)	Upland	Landrace

No.	Code no.	Cultivar name	Collection	Agro-ecological area *	Ecotype *	Type of cultivar*
			State/ Region			
50	GMJ51	San Ni(1)	Shan State(S)	EP(South)	Upland	Landrace
51	GMJ52	Sang Bial	Chin State	WH	Upland	Landrace
52	GMJ53	Kone Myint (1)	Shan State (S)	EP(South)	Upland	Landrace
53	GMJ54	Lone Pu (B)	Shan State (S)	EP(South)	Upland	Landrace
54	GMJ55	San Ni (B)	Shan State (S)	EP(South)	Upland	Landrace
55	GMJ56	Khao pha pya	Shan State (S)	EP(South)	Upland	Landrace
56	GMJ57	Khun Ni	Shan State (S)	EP(South)	Upland	Landrace
57	GMJ58	Lone Pu (A)	Shan State (S)	EP(South)	Upland	Landrace
58	GMJ59	Myae Shay (B)	Shan State (S)	EP(South)	Upland	Landrace
59	GMJ60	San Ni (B)	Shan State (S)	EP(South)	Upland	Landrace
60	GMJ61	Khao Swan Len	Shan State (N)	EP(North)	Upland	Landrace
61	GMJ62	Kone Myint (2)	Shan State (N)	EP(North)	Upland	Landrace
62	GMJ63	Khao Thwe	Shan State (N)	EP(North)	Upland	Landrace
63	GMJ64	Kauk Hnyin Nga Cheik	Shan State (N)	EP(North)	Upland	Landrace
64	GMJ65	Hmwe Yar Lay	Shan State (N)	EP(North)	Upland	Landrace
65	GMJ66	Khao Mon	Shan State (N)	EP(North)	Upland	Landrace
66	GMJ68	Khao Hpai	Shan State (N)	EP(North)	Upland	Landrace
67	GMJ69	Khao hauk	Shan State (N)	EP(North)	Upland	Landrace
68	GMJ70	Wa Sau	Shan State (N)	EP(North)	Upland	Landrace
69	GMJ71	Khao Kan Par	Shan State (N)	EP(North)	Upland	Landrace
70	GMJ72	Khao Hauk Nune	Shan State (N)	EP(North)	Upland	Landrace
71	GMJ73	Khao Chal	Shan State (N)	EP(North)	Upland	Landrace
72	GMJ75	Kauk Hnyin	Shan State (N)	EP(North)	Upland	Landrace
73	GMJ77	Khao Awut (1)	Shan State (N)	EP(North)	Upland	Landrace
74	GMJ78	Khao hauk	Shan State (N)	EP(North)	Upland	Landrace

No.	Code no.	Cultivar name	Collection	Agro-ecological area *	Ecotype *	Type of cultivar*
			State/ Region			
75	GMJ79	Naw Phye	Shan State (N)	EP(North)	Upland	Landrace
76	GMJ80	Khao hauk	Shan State (N)	EP(North)	Upland	Landrace
77	GMJ81	Nga Cheik	Shan State (N)	EP(North)	Upland	Landrace
78	GMJ82	Khauk Mwe hnaung	Shan State (N)	EP(North)	Upland	Landrace
79	GMJ83	Saba v(Unknown-1)	Shan State (N)	EP(North)	Upland	Landrace
80	GMJ84	Unknown (Upland)	Mandalay Region	EP(North)	Upland	Landrace
81	GMJ85	Khao Ma Hin	Shan State (N)	EP(North)	Upland	Landrace
82	GMJ86	Khao Line Lan	Shan State (N)	EP(North)	Upland	Landrace
83	GMJ87	Kone Myint (1)	Shan State (N)	EP(North)	Upland	Landrace
84	GMJ89	Khao Awut (2)	Shan State (N)	EP(North)	Upland	Landrace
85	GMJ90	Khao Hauk	Shan State (N)	EP(North)	Upland	Landrace
86	GMJ91	Khao Hmwe Yar	Shan State (N)	EP(North)	Upland	Landrace
87	GMJ92	Khao Wa	Shan State (N)	EP(North)	Upland	Landrace
88	GMJ93	Phun Nhin Chae (Larhu)	Shan State (S)	EP(South)	Upland	Landrace
89	GMJ94	Mote Soe Ma Kywae Kyay	Shan State (S)	EP(South)	Upland	Landrace
90	GMJ96	Chae ba	Shan State (S)	EP(South)	Upland	Landrace
91	GMJ97	Khao Mon Lar	Shan State (S)	EP(South)	Upland	Landrace
92	GMJ98	Shwe War tun (Yway)	Shan State (S)	EP(South)	Upland	Landrace
93	GMJ99	Khao Hlaing	Shan State (S)	EP(South)	Upland	Landrace
94	GMJ101	Nga Kywe	Mon State	SP	Lowland	Landrace
95	GMJ102	Nga Kywe Phyu	Ayeyarwady Division	SP	Lowland	Landrace
96	GMJ103	Nga Kywe Yin	Ayeyarwady Division	SP	Lowland	Landrace
97	GMJ104	Paw San Bay Kyar	Ayeyarwady Division	SP	Lowland	Landrace
98	GMJ105	Paw San Bay Kyar (1)	Ayeyarwady Division	SP	Lowland	Landrace
99	GMJ106	Nga Kyaw (U To)	Ayeyarwady Division	SP	Lowland	Landrace

No.	Code no.	Cultivar name	Collection	Agro-ecological area *	Ecotype *	Type of cultivar*
			State/ Region			
100	GMJ107	Paw San Yin	Ayeyarwady Division	SP	Lowland	Landrace
101	GMJ109	Nga Kywe Note	Bago Division	SP	Lowland	Landrace
102	GMJ110	Paw San Hmwe	Ayeyarwady Division	SP	Lowland	Landrace
103	GMJ112	Nga Kywe	Bago Division	SP	Lowland	Landrace
104	GMJ113	Tyaung Pyin Yin	Ayeyarwady Division	SP	Lowland	Landrace
105	GMJ114	Tyaung Pyin Yin	Bago Division	SP	Lowland	Landrace
106	GMJ115	Nat Pyi Hmwe	Bago Division	SP	Lowland	Landrace
107	GMJ116	Nga Pya Gyi	Unknown		Lowland	Landrace
108	GMJ117	Paw San Hmwe	Unknown		Lowland	Landrace
109	GMJ119	Paw San Shwe War	Ayeyarwady Division	SP	Lowland	Landrace
110	GMJ120	Paw San Hmwe	Ayeyarwady Division	SP	Lowland	Landrace
111	GMJ124	Paw San Bay Kyar	Unknown		Lowland	Landrace
112	GMJ125	Paw San Bay Kyar	Unknown		Lowland	Landrace
113	GMJ126	Bay Kyar paw San	Yangon Division	SP	Lowland	Landrace
114	GMJ127	Paw San Hmwe	Unknown		Lowland	Landrace
115	GMJ128	Paw San Hmwe Bay Kyar	Unknown		Lowland	Landrace
116	GMJ129	Paw San Hmwe kauk Yin	Unknown		Lowland	Landrace
117	GMJ131	Kyet Paung	Yangon Division	SP	Lowland	Landrace
118	GMJ132	Pathein Taung Pyin	Ayeyarwadee Division	SP	Lowland	Landrace
119	GMJ133	Shwe Whar Yin	Ayeyarwadee Division	SP	Lowland	Landrace
120	GMJ134	Paw San Yin	Ayeyarwadee Division	SP	Lowland	Landrace
121	GMJ135	Paw San Yin	Ayeyarwadee Division	SP	Lowland	Landrace
122	GMJ136	Taung Pyin (yellow)	Ayeyarwadee Division	SP	Lowland	Landrace
123	GMJ137	Paw San Bay Kyar	Ayeyarwadee Division	SP	Lowland	Landrace
124	GMJ138	Paw San Bay Kyar	Ayeyarwadee Division	SP	Lowland	Landrace

No.	Code no.	Cultivar name	Collection	Agro-ecological area *	Ecotype *	Type of cultivar*
			State/ Region			
125	GMJ139	Paw San Kyi	Ayeyarwadee Division	SP	Lowland	Landrace
126	GMJ140	Paw San Yin	Ayeyarwadee Division	SP	Lowland	Landrace
127	GMJ141	Ayeyarwadee Paw San	Ayeyarwadee Division	SP	Lowland	Landrace
128	GMJ142	Ayeyarwadee Paw San Kyar	Ayeyarwadee Division	SP	Lowland	Landrace
129	GMJ143	U to Paw San	Ayeyarwadee Division	SP	Lowland	Landrace
130	GMJ145	Bay Kyar Lay	Ayeyarwadee Division	SP	Lowland	Landrace
131	GMJ146	Bay Kyar Lay	Ayeyarwadee Division	SP	Lowland	Landrace
132	GMJ147	Paw San Hmwe	Ayeyarwadee Division	SP	Lowland	Landrace
133	GMJ148	Bay Kyar Lay Taung Pyin	Ayeyarwadee Division	SP	Lowland	Landrace
134	GMJ149	Bay Kyar Lay	Ayeyarwadee Division	SP	Lowland	Landrace
135	GMJ150	Paw San Yin	Ayeyarwadee Division	SP	Lowland	Landrace
136	GMJ151	Paw San Yin	Ayeyarwadee Division	SP	Lowland	Landrace
137	GMJ152	Paw San Yin	Ayeyarwadee Division	SP	Lowland	Landrace
138	GMJ154	Paw San Yin	Ayeyarwadee Division	SP	Lowland	Landrace
139	GMJ156	Paw San Kyi	Ayeyarwadee Division	SP	Lowland	Landrace
140	GMJ157	Paw San Kyi	Ayeyarwadee Division	SP	Lowland	Landrace
141	GMJ158	Bay Kyar Yin	Ayeyarwadee Division	SP	Lowland	Landrace
142	GMJ159	Paw San Yin	Ayeyarwadee Division	SP	Lowland	Landrace
143	GMJ160	Paw San Yin	Ayeyarwadee Division	SP	Lowland	Landrace
144	GMJ162	Paw San Kyi	Ayeyarwadee Division	SP	Lowland	Landrace
145	GMJ163	Taung Pyin (yellow)	Ayeyarwadee Division	SP	Lowland	Landrace
146	GMJ164	Paw San Taung Pyin Kyi	Ayeyarwadee Division	SP	Lowland	Landrace
147	GMJ165	Paw San Bay Kyar Yin	Ayeyarwadee Division	SP	Lowland	Landrace
148	GMJ166	Paw San Lone Chaw	Ayeyarwadee Division	SP	Lowland	Landrace
149	GMJ167	Paw San Bay Kyar	Ayeyarwadee Division	SP	Lowland	Landrace

No.	Code no.	Cultivar name	Collection	Agro-ecological area *	Ecotype *	Type of cultivar*
			State/ Region			
150	GMJ168	Paw San	Ayeyarwadee Division	SP	Lowland	Landrace
151	GMJ169	Chin Khar Kaung Paw San	Ayeyarwadee Division	SP	Lowland	Landrace
152	GMJ170	Shwe Bo Paw San	Unknown	SP	Lowland	Landrace
153	GMJ171	Bay Kyar Lay	Ayeyarwadee Division	SP	Lowland	Landrace
154	GMJ172	90 days		SP	Lowland	Improved
155	GMJ173	Za Zar		EP(North)	Upland	Improved
156	GMJ174	Naung Khan Sone Nyi		EP(North)	Upland	Improved
157	GMJ175	Thee Hthat Yin		SP	Lowland	Improved
158	GMJ176	Yae Anae Loe		SP	Lowland	Improved
159	GMJ177	Yar-9			Upland	Improved
160	GMJ178	Bue Kauk		EP(North)	Upland	Improved
161	GMJ179	Kauk Kyi		SP	Lowland	Improved
162	GMJ180	Kama Kyi		SP	Lowland	Improved
163	GMJ181	Ayar Minn		SP	Lowland	Improved
164	GMJ182	Ma Naung		EP(North)	Upland	Improved
165	GMJ183	Yan Lu		EP(North)	Upland	Improved
166	GMJ184	Kauk Hnyn Hmwe		SP	Lowland	Improved
167	GMJ185	Ma Naw Thukha		SP	Lowland	Improved
168	GMJ186	Huan Kar		SP	Lowland	Improved
169	GMJ187	Hmaw Bi-2		SP	Lowland	Improved
170	GMJ188	Lan Kyan		EP(North)	Upland	Improved
171	GMJ189	Sin Thwe Latt		SP	Lowland	Improved
172	GMJ190	Hle Aung Pan		EP(North)	Upland	Improved
173	GMJ191	Shwe Thaw Yin		SP	Lowland	Improved
174	GMJ192	Yarana Toe		SP	Lowland	Improved

No.	Code no.	Cultivar name	Collection	Agro-ecological area *	Ecotype *	Type of cultivar*
			State/ Region			
175	GMJ193	Shwe Wha Yin		SP	Lowland	Improved

Appendix table 2. Agronomic trait variation, phenol reaction and genetic background of 175 Myanmar rice germplasm

Code no.	Cultivar name	Sub-type	UPGMA Cluster	Phenol test		DTH (Days)		Amylose content	GT	Gel consistancy	Length/ Width
				+	-	Yangon	Ishigaki				
GMJ1	Eai Si	A-1	IIb		-	86	47	23.34	4	75	2.95
GMJ2	Sin Ekari-2	B-1	Ia	+		95	50	25.90	1	68	3.20
GMJ3	Kauk hnyin	B-1	Ia	++		85	51	31.25	1	98	2.49
GMJ4	La Tha	B-1	Ia	++		104	51	17.82	7	54	2.79
GMJ5	Chin Lay	B-1	Ia	++		111	52	27.60	2	90	3.23
GMJ6	Ya Tha Pu	B-1	Ia	++		106	52	25.17	2	71	2.70
GMJ7	Sheik Pha Phu	B-1	Ia	+++		113	53	23.13	9	80	2.88
GMJ8	Jin Ma Yan	A-1	IIb		-	83	53	23.41	1	71	1.72
GMJ9	Maw yaw Kaunk hnyin	B-1	Ia	++		79	61	13.43	7	80	3.31
GMJ10	Lone Ou	B-1	Ia	+		97	71	13.63	7	82	2.84
GMJ11	Oat Kyai Ni	B-1	Ia	++		94	75	25.07	1	90	2.65
GMJ12	Naung Htu Kauk Hnyin	B-1	Ia	++		81	81	13.27	6	70	3.37
GMJ13	Kaung Kaung Akhin	B-1	Ia	+		81	81	16.07	4	80	3.34
GMJ14	Ya Thay Thee Htut	B-1	Ia	+		107	81	29.34	2	95	2.66
GMJ15	San Ni (B)	B-1	Ia	++		95	82	25.59	1	65	2.85
GMJ16	Ya Thay	B-1	Ia	+		107	82	27.86	2	73	2.73
GMJ17	Kyaw Scan	B-1	Ia	+		124	84	25.61	2	75	3.33
GMJ18	Sa Ba Ni	B-1	Ia	+		81	84	16.22	7	75	3.24
GMJ19	Khauk Long	B-1	Ia	+		83	84	13.22	8	50	2.18
GMJ20	Ma Le Byat	B-1	Ia	++		132	85	19.97	9	99	3.33
GMJ21	Kauk Hnyin	B-1	Ia	+++		100	85	15.03	3	60	3.85
GMJ22	Khaw Lone	B-1	Ia	+		96	87	26.47	8	98	2.35
GMJ23	Kyeit Phat	B-1	Ia	++		116	87	12.60	8	45	3.33
GMJ25	San Bial	B-2	Ia	+		100	87	29.13	2	99	4.33

Code no.	Cultivar name	Sub-type	UPGMA Cluster	Phenol test		DTH (Days)		Amylose content	GT	Gel consistancy	Length/ Width
				+	-	Yangon	Ishigaki				
GMJ26	MoteSoeMa Kywae Pyay	B-2	Ia	+		100	88	28.28	2	65	4.15
GMJ27	Taung Ya Saba	B-1	Ia	+		73	88	30.45	3	48	4.46
GMJ28	La Chit Mum	B-1	Ia	++		82	89	17.70	4	75	3.74
GMJ29	Bwin Akyan	B-2	Ia	+		100	91	34.87	2	78	4.26
GMJ30	Byat War	B-2	Ia	+		100	91	29.93	4	99	4.02
GMJ31	Nyan Ywae	A-1	IIb		-	80	91	17.20	4	78	4.38
GMJ32	Saba Me	B-2	Ia	+		101	90	28.66	4	60	4.23
GMJ33	Kauk Byauk	B-2	Ia	+		101	95	34.58	4	51	4.39
GMJ34	San Ni	B-2	Ia	+		101	96	27.60	4	53	4.38
GMJ35	San Ni	B-2	Ia	+		102	97	26.77	4	99	4.34
GMJ36	Khao Pha Wan	B-2	Ia	+		102	98	26.77	2	85	4.08
GMJ37	Saba Me	B-2	Ia	+		102	98	29.58	2	60	4.27
GMJ38	San Ni	B-2	Ia	+		102	100	31.29	2	75	4.52
GMJ39	Yar Kye	B-2	Ia	+		102	101	27.54	2	99	4.39
GMJ40	Yar San	B-2	Ia	+		102	101	28.97	1	55	2.89
GMJ41	Taung yar Nga Cheik	B-1	Ia	++		75	102	34.32	1	79	2.39
GMJ42	Do Saba	B-2	Ia	+		100	104	27.57	2	60	3.13
GMJ43	Patee pu	B-2	Ia	+		100	105	32.04	2	98	3.16
GMJ44	Shaka Mum	B-2	Ia	+		100	105	32.12	2	98	3.01
GMJ45	Nyan Sa	B-2	Ia	+		100	105	27.23	2	99	2.85
GMJ46	Traung	B-2	Ia	+		100	106	31.21	3	99	3.23
GMJ47	Lone Pu	B-2	IIb	+		71	106	25.14	1	98	2.12
GMJ48	Hmway Shay	B-2	Ia	+		102	107	29.15	3	56	3.07
GMJ49	Kauk Ya	B-2	Ia	+		102	107	29.95	1	58	2.99
GMJ50	San Ni	B-1	IIb	+		72	107	21.37	2	89	2.11

Code no.	Cultivar name	Sub-type	UPGMA Cluster	Phenol test		DTH (Days)		Amylose content	GT	Gel consistancy	Length/ Width
				+	-	Yangon	Ishigaki				
GMJ51	San Ni(1)	B-2	Ia	+		102	107	26.45	4	94	3.04
GMJ52	Sang Bial	B-2	Ia	+		102	108	23.58	2	67	3.10
GMJ53	Kone Myint (1)	B-2	Ia	+		102	108	26.81	2	68	3.08
GMJ54	Lone Pu (B)	B-2	Ia	+		98	108	21.37	1	49	2.99
GMJ55	San Ni (B)	B-2	Ia	+		101	108	23.79	2	80	3.05
GMJ56	Khao pha pya	B-2	Ia	+		102	108	30.97	2	87	2.97
GMJ57	Khun Ni	B-2	Ia	+		103	108	25.56	3	73	2.92
GMJ58	Lone Pu (A)	B-2	Ia	+		109	109	21.81	3	97	3.12
GMJ59	Myae Shay (B)	B-2	Ia	+		105	110	23.63	4	36	3.09
GMJ60	San Ni (B)	B-2	IIb	+		105	110	23.19	3	90	3.08
GMJ61	Khao Swan Len	A-1	IIb		-	65	110	14.96	8	67	3.18
GMJ62	Kone Myint (2)	B-2	Ia	+		100	112	27.64	5	84	2.89
GMJ63	Khao Thwe	A-1	IIb		-	81	112	14.78	8	76	2.83
GMJ64	Kauk Hnyin Nga Cheik	A-1	IIb	+		67	113	12.34	4	78	2.74
GMJ65	Hmwe Yar Lay	A-1	IIb		-	69	114	19.94	1	56	2.09
GMJ66	Khao Mon	A-1	IIb		-	73	114	15.12	8	78	2.56
GMJ68	Khao Hpai	A-1	IIb		-	80	115	15.46	7	96	2.65
GMJ69	Khao hauk	B-1	IIb	++		78	115	11.06	8	60	2.59
GMJ70	Wa Sau	A-1	IIb		-	80	115	11.40	6	81	2.68
GMJ71	Khao Kan Par	A-1	IIb		-	80	115	15.30	8	78	2.84
GMJ72	Khao Hauk Nune	B-1	Ia	++		63	117	12.26	8	65	2.99
GMJ73	Khao Chal	A-1	IIb		-	70	117	20.22	1	80	2.03
GMJ75	Kauk Hnyin	B-1	Ia	++		62	117	11.58	8	60	3.19
GMJ77	Khao Awut (1)	A-1	IIb		-	70	119	16.19	2	72	1.96
GMJ78	Khao hauk	B-1	Ia	++		73	119	12.13	7	78	3.15

Code no.	Cultivar name	Sub-type	UPGMA Cluster	Phenol test		DTH (Days)		Amylose content	GT	Gel consistancy	Length/ Width
				+	-	Yangon	Ishigaki				
GMJ79	Naw Phye	B-1	Ia	++		64	121	11.92	5	65	3.05
GMJ80	Khao hauk	B-1	Ia	+		86	123	12.28	7	57	3.58
GMJ81	Nga Cheik	A-1	IIb		-	80	124	16.43	5	76	3.05
GMJ82	Khauk Mwe hnaung	B-1	Ia	++		84	125	11.75	7	59	3.21
GMJ83	Saba v(Unknown-1)	A-1	IIb		-	71	133	18.90	1	71	2.28
GMJ84	Unknown (Upland)	A-1	IIb		-	69	134	12.26	5	67	3.79
GMJ85	Khao Ma Hin	B-1	Ia	++		64	135	24.85	2	90	2.43
GMJ86	Khao Line Lan	A-1	IIb		-	72	138	12.76	5	67	2.18
GMJ87	Kone Myint (1)	B-2	Ia	+		101	140	26.08	2	35	2.81
GMJ89	Khao Awut (2)	A-1	IIb		-	71	140	20.56	1	68	2.02
GMJ90	Khao Hauk	A-1	IIb		-	75	144	13.01	7	93	2.57
GMJ91	Khao Hmwe Yar	A-1	IIb		-	70	145	17.50	9	80	3.16
GMJ92	Khao Wa	A-1	IIb		-	65	148	19.83	9	95	3.04
GMJ93	PhunNhin Chae (Larhu)	A-1	IIb		-	71	149	21.91	1	98	2.13
GMJ94	MotesoemaKywaeKyay	B-1	Ia	++		100	150	27.26	1	61	2.64
GMJ96	Chae ba	A-1	IIb		-	97	150	13.57	8	91	2.35
GMJ97	Khao Mon Lar	B-1	Ia	+		94	152	16.07	9	75	3.71
GMJ98	Shwe War tun (Yway)	B-1	Ia	+		91	152	19.69	9	84	3.13
GMJ99	Khao Hlaing	B-1	Ia	++		80	153	15.99	7	97	3.39
GMJ101	Nga Kywe	A-2	IIa	++		97	154	18.97	2	76	1.97
GMJ102	Nga Kywe Phyu	B-1	Ia	+++		75	158	19.46	4	38	2.47
GMJ103	Nga Kywe Yin	A-2	IIa	++		75	158	17.45	1	62	2.21
GMJ104	Paw San Bay Kyar	A-2	IIa		-	127	158	18.30	1	78	2.64
GMJ105	Paw San Bay Kyar (1)	A-2	IIa	++		114	158	18.30	1	55	2.03
GMJ106	Nga Kyaw (U To)	A-2	IIa		-	88	160	18.66	3	58	2.31

Code no.	Cultivar name	Sub-type	UPGMA Cluster	Phenol test		DTH (Days)		Amylose content	GT	Gel consistancy	Length/ Width
				+	-	Yangon	Ishigaki				
GMJ107	Paw San Yin	A-2	IIa		-	127	161	20.62	1	49	3.70
GMJ109	Nga Kywe Note	B-1	IIa	+++		77	166	21.34	1	55	2.52
GMJ110	Paw San Hmwe	A-2	IIa		-	86	168	19.53	3	98	2.25
GMJ112	Nga Kywe	B-1	Ib	+++		71	169	11.54	5	65	3.06
GMJ113	Tyaung Pyin Yin	A-2	IIa	++		77	170	18.88	3	74	2.06
GMJ114	Tyaung Pyin Yin	A-2	IIa	++		77	171	20.13	1	54	1.94
GMJ115	Nat Pyi Hmwe	B-1	Ib	+++		70	171	10.07	8	54	3.67
GMJ116	Nga Pya Gyi	B-1	Ib	+++		77	174	9.49	7	65	2.94
GMJ117	Paw San Hmwe	A-2	IIa		-	109	177	23.37	8	49	2.66
GMJ119	Paw San Shwe War	A-2	IIa		-	127	178	26.07	1	89	2.54
GMJ120	Paw San Hmwe	A-2	IIa		-	63	180	20.57	7	58	2.22
GMJ124	Paw San Bay Kyar	A-2	IIa		-	92	187	21.53	3	99	2.20
GMJ125	Paw San Bay Kyar	A-2	IIa		-	77	188	19.48	1	52	2.47
GMJ126	Bay Kyar paw San	A-2	IIa		-	109	188	22.40	1	98	2.53
GMJ127	Paw San Hmwe	A-2	IIa		-	109	191	20.35	1	89	2.56
GMJ128	Pawsan HmweBay Kyar	A-2	IIa		-	127	196	19.43	3	80	2.36
GMJ129	PawsanHmwe kauk Yin	A-2	IIa		-	77	197	19.53	3	89	2.30
GMJ131	Kyet Paung	A-2	IIa		-	150	210	20.76	8	96	2.76
GMJ132	Pathein Taung Pyin	A-2	IIa		-	123	212	20.02	8	70	2.17
GMJ133	Shwe Whar Yin	A-2	IIa		-	126	216	21.3	8	91	2.47
GMJ134	Paw San Yin	A-2	IIa		-	125	218	21.6	7	94	2.43
GMJ135	Paw San Yin	A-2	IIa		-	127	220	20.4	8	97	2.34
GMJ136	Taung Pyin (yellow)	A-2	IIa		-	156	220	24.28	6	97	2.17
GMJ137	Paw San Bay Kyar	A-2	IIa		-	156	222	24.13	8	98	2.38
GMJ138	Paw San Bay Kyar	A-2	IIa		-	156	222	22.56	8	95	2.45

Code no.	Cultivar name	Sub-type	UPGMA Cluster	Phenol test		DTH (Days)		Amylose content	GT	Gel consistancy	Length/ Width
				+	-	Yangon	Ishigaki				
GMJ139	Paw San Kyi	A-2	IIa		-	156	222	23.91	6	93	2.75
GMJ140	Paw San Yin	A-2	IIa		-	156	228	22.94	8	91	2.31
GMJ141	Ayeyarwadee Paw San	A-2	IIa		-	156	228	24.36	8	95	2.41
GMJ142	Ayeyarwadepawsan kyar	A-2	IIa		-	156	229	24.62	8	96	2.61
GMJ143	U to Paw San	A-2	IIa	++		156	229	24.44	8	84	2.76
GMJ145	Bay Kyar Lay	A-2	IIa		-	125	229	23.28	8	80	2.55
GMJ146	Bay Kyar Lay	A-2	IIa		-	125	229	23.52	8	88	2.52
GMJ147	Paw San Hmwe	A-2	IIa		-	114	229	23.66	8	80	2.53
GMJ148	BayKyar Lay Taung Pyin	A-2	IIa		-	114	230	22.42	8	88	2.68
GMJ149	Bay Kyar Lay	A-2	IIa	+++		124	230	22.67	7	89	2.57
GMJ150	Paw San Yin	A-2	IIa		-	111	230	20.89	8	88	2.43
GMJ151	Paw San Yin	A-2	IIa		-	125	231	23.84	7	91	2.29
GMJ152	Paw San Yin	A-2	IIa		-	125	231	23.25	8	70	2.52
GMJ154	Paw San Yin	A-2	IIa		-	156	231	22.02	6	74	2.30
GMJ156	Paw San Kyi	A-2	IIa		-	156	232	24.49	6	97	2.41
GMJ157	Paw San Kyi	A-2	IIa		-	156	232	22.77	7	99	2.39
GMJ158	Bay Kyar Yin	A-2	IIa		-	126	233	22.45	7	73	2.48
GMJ159	Paw San Yin	A-2	IIa		-	125	233	22.81	7	86	2.37
GMJ160	Paw San Yin	A-2	IIa		-	112	233	22.77	6	89	2.39
GMJ162	Paw San Kyi	A-2	IIa		-	157	233	23.17	7	80	2.70
GMJ163	Taung Pyin (yellow)	A-2	IIa		-	125	233	22.14	8	74	2.44
GMJ164	Paw San Taung Pyin Kyi	A-2	IIa		-	158	233	22.35	8	77	2.30
GMJ165	Paw San Bay Kyar Yin	A-2	IIa		-	125	233	22	6	78	2.48
GMJ166	Paw San Lone Chaw	A-2	IIa	+++		157	233	24.49	6	74	2.44
GMJ167	Paw San Bay Kyar	A-2	IIa		-	157	233	26.66	6	96	2.26

Code no.	Cultivar name	Sub-type	UPGMA Cluster	Phenol test		DTH (Days)		Amylose content	GT	Gel consistancy	Length/ Width
				+	-	Yangon	Ishigaki				
GMJ168	Paw San	A-2	IIa		-	157	233	24.4	6	93	2.27
GMJ169	ChinKharKaung Paw San	A-2	IIa		-	157	233	22.5	8	70	2.20
GMJ170	Shwe Bo Paw San	A-2	IIa		-	125	233	23.17	8	76	2.39
GMJ171	Bay Kyar Lay	A-2	IIa		-	125	234	23.4	8	67	2.65
GMJ172	90 days	B-1	Ib	+++		85	234	20.69	8	86	4.00
GMJ173	Za Zar	A-1	IIa		-	100	234	13.95	1	92	3.00
GMJ174	Naung Khan Sone Nyi	A-1	IIa		-	120	234	16.27	1	98	3.39
GMJ175	Thee Hthat Yin	B-1	Ib	+		90	234	22.31	2	87	3.63
GMJ176	Yae Anae Loe	B-1	Ib	+++		90	234	20.71	3	87	2.80
GMJ177	Yar-9	B-1	Ib	+++		85	234	18.20	3	46	2.86
GMJ178	Bue Kauk	A-1	IIa		-	80	234	16.32	3	93	2.29
GMJ179	Kauk Kyi	A-1	IIa		-	174	234	19.40	3	72	2.67
GMJ180	Kama Kyi	A-1	IIa		-	105	234	18.52	3	82	2.28
GMJ181	Ayar Minn	B-1	Ib	+		110	235	21.03	8	31	3.91
GMJ182	Ma Naung	A-1	IIa		-	80	235	14.03	1	83	2.14
GMJ183	Yan Lu	A-1	IIa		-	80	235	16.15	1	75	2.28
GMJ184	Kauk Hnyn Hmwe	B-1	Ib	+++		120	236	11.87	6	99	3.64
GMJ185	Ma Naw Thukha	B-1	Ib	+++		100	236	22.41	2	60	3.16
GMJ186	Huan Kar	B-1	Ib	+++		128	237	24.99	1	93	3.50
GMJ187	Hmaw Bi-2	B-1	Ib	+++		110	238	17.60	6	76	4.65
GMJ188	Lan Kyan	A-1	IIa		-	85	238	17.19	1	86	1.74
GMJ189	Sin Thwe Latt	B-1	Ib	+++		110	239	16.89	3	95	4.81
GMJ190	Hle Aung Pan	A-1	IIa		-	100	239	14.23	9	67	3.18
GMJ191	Shwe Thaw Yin	B-1	Ib	+		80	241	22.09	3	56	3.91
GMJ192	Yarana Toe	B-1	Ib	+		95	243	21.47	3	98	3.63

Code no.	Cultivar name	Sub-type	UPGMA Cluster	Phenol test		DTH (Days)		Amylose content	GT	Gel consistancy (mm)	Length/ Width
				+	-	Yangon	Ishigaki				
GMJ193	Shwe Wha Yin	B-1	Ib	+		90	260	15.81	3	70	3.75

Appendix Table 3. Resistant reaction pattern of Myanmar rice germplasm upon nine Standard differential blast isolates in Japan.

Entry No.	**Name**	**Blast isolates**								
		1804-4	**Mu-95**	**446-2a**	**BN 1-1-1**	**IW-85-04**	**Kyu 9439013**	**Kyu 93-229**	**Ina 93-3**	**TH 69-8**
GMJ-1	Eai Si	0	0	0	0	1	2	0	0	1
GMJ-2	Sin Ekari-2	0	0	1	0	0	0	1	1	0
GMJ-3	Kauk hnyin	1	0	2	1	0	0	4	1	0
GMJ-4	La Tha	1	1	0	0	0	0	2	0	0
GMJ-5	Chin Lay	0	1	0	2	0	0	1	0	0
GMJ-6	Ya Tha Pu	1	1	0	3	0	0	2	1	1
GMJ-7	Sheik Pha Phu	0	1	1	4	0	0	2	1	0
GMJ-8	Jin Ma Yan	1	1	1	1	0	1	1	1	0
GMJ-9	Maw yaw Kaunk hnyin	1	1	0	1	0	0	1	1	2
GMJ-10	Lone Ou	2	0	0	4	0	0	2	0	1
GMJ-11	Oat Kyai Ni	0	1	0	0	0	1	2	1	1
GMJ-12	Naung Htu Kauk Hnyin	1	1	0	2	0	0	2	1	2
GMJ-13	Kaung Kaung Akhin	1	1	0	1	0	0	2	1	2
GMJ-14	Ya Thay Thee Htut	0	1	0	5	0	0	2	2	0
GMJ-15	San Ni (B)	1	0	0	0	0	0	0	0	0
GMJ-16	Ya Thay	0	0	0	0	0	0	2	1	0
GMJ-17	Kyaw Scan	1	1	5	3	2	2	5	1	1
GMJ-19	Khauk Long	1	2	1	0	0	0	3	0	1
GMJ-20	Ma Le Byat	1	1	2	2	0	2	4	2	1
GMJ-21	Kauk Hnyin	2	1	4	1	0	2	4	1	0
GMJ-22	Khaw Lone	0	0	0	0	0	0	2	1	0
GMJ-23	Kyeit Phat	1	1	0	2	0	1	4	1	2
GMJ-25	San Bial	0	0	0	3	0	0	2	0	0
GMJ-26	Mote Soe Ma Kywae Pyay	1	0	0	2	0	0	1	0	0
GMJ-27	Taung Ya Saba	0	0	0	4	0	0	1	0	0
GMJ-28	La Chit Mum	1	0	0	1	0	0	2	0	0
GMJ-29	Bwin Akyan	1	0	0	2	0	1	2	0	1
GMJ-30	Byat War	0	0	0	2	0	0	1	0	0
GMJ-31	Nyan Ywae	1	0	0	1	0	0	-	0	0
GMJ-32	Saba Me	0	0	0	0	0	0	1	0	0
GMJ-33	Kauk Byauk	0	0	0	1	0	0	2	0	0
GMJ-34	San Ni	1	0	0	1	0	0	3	0	0
GMJ-35	San Ni	0	0	0	0	0	0	2	0	0
GMJ-36	Khao Pha Wan	0	0	0	0	0	0	1	0	0
GMJ-37	Saba Me	0	0	0	0	0	0	1	0	0

GMJ-38	San Ni	0	1	0	2	0	0	1	1	0
GMJ-39	Yar Kye	0	0	0	-	0	0	2	0	0
GMJ-40	Yar San	1	1	0	0	0	0	3	0	0
GMJ-41	Taung yar Nga Cheik	1	1	0	0	0	1	5	1	1
GMJ-42	Do Saba	1	1	0	0	0	0	2	0	0
GMJ-43	Patee pu	0	1	0	0	0	0	3	0	0
GMJ-44	Shaka Mum	0	0	0	3	0	0	3	0	0
GMJ-45	Nyan Sa	1	0	0	1	1	0	2	0	0
GMJ-46	Traung	1	2	0	0	0	1	3	0	0
GMJ-47	Lone Pu	1	0	0	1	0	0	1	1	0
GMJ-48	Hmway Shay	0	0	0	0	0	0	3	0	0
GMJ-49	Kauk Ya	0	1	0	0	0	0	1	0	0
GMJ-51	San Ni(1)	0	2	0	0	0	0	2	0	1
GMJ-52	Sang Bial	0	1	0	1	0	0	2	0	1
GMJ-53	Kone Myint (1)	0	2	0	0	0	0	2	0	0
GMJ-54	Lone Pu (B)	0	2	0	0	0	0	2	0	0
GMJ-55	San Ni (B)	1	2	0	0	0	0	2	0	0
GMJ-56	Khao pha pya	0	1	0	0	0	0	2	0	0
GMJ-57	Khun Ni	0	1	0	1	0	0	1	0	0
GMJ-58	Lone Pu (A)	1	1	0	0	0	0	2	0	0
GMJ-59	Myae Shay (B)	0	1	0	0	0	0	2	0	0
GMJ-60	San Ni (B)	1	1	1	0	1	0	1	0	0
GMJ-61	Khao Swan Len	1	1	1	1	1	1	1	1	3
GMJ-62	Kone Myint (2)	0	1	0	0	0	0	2	0	0
GMJ-63	Khao Thwe	0	0	0	0	0	0	0	1	0
GMJ-64	Kauk Hnyin Nga Cheik	0	0	0	0	0	0	0	0	0
GMJ-65	Hmwe Yar Lay	0	0	0	1	0	0	1	1	0
GMJ-66	Khao Mon	0	0	0	0	0	0	1	0	0
GMJ-69	Khao hauk	0	0	0	0	0	0	1	0	0
GMJ-70	Wa Sau	0	0	0	0	0	1	0	0	0
GMJ-71	Khao Kan Par	1	0	0	0	0	0	0	1	0
GMJ-72	Khao Hauk Nune	0	0	2	0	1	1	2	1	1
GMJ-73	Khao Chal	0	0	0	0	1	0	1	0	0
GMJ-75	Kauk Hnyin	0	0	1	0	0	0	1	1	1
GMJ-77	Khao Awut (1)	0	0	0	0	0	0	0	0	0
GMJ-78	Khao hauk	0	0	0	0	1	0	1	1	0
GMJ-79	Naw Phye	0	0	0	0	0	0	1	0	0
GMJ-80	Khao hauk	0	1	0	1	0	1	1	1	1
GMJ-83	Saba v(Unknown-1)	1	1	0	1	1	0	1	1	1
GMJ-84	Unknown (Upland)	0	0	0	0	0	0	0	0	0
GMJ-85	Khao Ma Hin	1	1	0	3	0	1	0	1	1
GMJ-87	Kone Myint (1)	0	1	0	0	0	0	1	0	0
GMJ-89	Khao Awut (2)	0	2	1	1	1	0	1	0	0

GMJ-90	Khao Hauk	0	0	0	0	1	0	0	0	0
GMJ-91	Khao Hmwe Yar	0	1	0	0	1	0	1	1	1
GMJ-92	Khao Wa	0	0	0	0	0	0	1	1	0
GMJ-93	Phun Nhin Chae (Larhu)	0	0	0	0	0	0	0	1	0
GMJ-94	Mote Soe Ma Kywae Kyay	0	0	0	0	0	0	0	0	0
GMJ-96	Chae ba	0	1	3	3	3	1	5	0	1
GMJ-98	Shwe War tun (Yway)	0	1	0	0	0	0	1	1	0
GMJ-101	Nga Kywe	0	0	0	0	0	0	1	0	0
GMJ-102	Nga Kywe Phyu	0	1	0	0	1	0	1	0	0
GMJ-103	Nga Kywe Yin	0	0	0	0	3	0	3	0	3
GMJ-104	Paw San Bay Kyar	0	0	0	0	0	0	0	0	0
GMJ-105	Paw San Bay Kyar (1)	1	1	0	1	0	0	1	0	0
GMJ-106	Nga Kyaw (U To)	0	0	0	0	0	0	0	0	0
GMJ-107	Paw San Yin	0	0	0	0	0	0	0	0	0
GMJ-109	Nga Kywe Note	1	2	1	2	1	2	2	1	2
GMJ-110	Paw San Hmwe	0	0	0	0	0	0	0	0	0
GMJ-112	Nga Kywe	0	1	1	0	0	0	1	1	0
GMJ-113	Tyaung Pyin Yin	0	0	0	2	1	0	1	0	0
GMJ-114	Tyaung Pyin Yin	0	1	1	3	0	0	1	1	1
GMJ-115	Nat Pyi Hmwe	1	2	0	3	0	1	1	1	0
GMJ-116	Nga Pya Gyi	3	2	3	4	0	2	3	4	4
GMJ-117	Paw San Hmwe	0	0	0	0	-	0	0	0	0
GMJ-119	Paw San Shwe War	0	0	0	0	0	0	0	0	0
GMJ-120	Paw San Hmwe	0	0	0	0	0	0	0	0	0
GMJ-124	Paw San Bay Kyar	0	0	0	0	0	0	0	0	0
GMJ-125	Paw San Bay Kyar	0	0	0	3	0	0	0	0	0
GMJ-126	Bay Kyar paw San	0	0	0	0	0	0	0	0	0
GMJ-127	Paw San Hmwe	0	0	0	0	0	0	0	0	0
GMJ-128	Paw San Hmwe Bay Kyar	0	0	0	0	0	0	0	0	0
GMJ-129	Paw San Hmwe kauk Yin	0	0	0	0	0	0	0	0	0
GMJ-131	Kyet Paung	2	3	2	4	3	1	3	5	1
GMJ-132	Pathein Taung Pyin	0	0	0	0	0	0	0	0	0
GMJ-133	Shwe Whar Yin	0	0	0	0	0	0	0	0	0
GMJ-134	Paw San Yin	0	0	0	1	0	0	0	0	0
GMJ-135	Paw San Yin	0	0	0	0	0	0	0	0	0
GMJ-136	Taung Pyin (yellow)	0	0	0	0	0	0	0	0	0
GMJ-137	Paw San Bay Kyar	0	0	0	0	0	0	0	0	0
GMJ-138	Paw San Bay Kyar	0	0	0	0	0	0	0	0	0
GMJ-139	Paw San Kyi	0	1	0	0	0	0	-	-	0
GMJ-140	Paw San Yin	0	0	0	0	0	0	0	0	0
GMJ-141	Ayeyarwadee Paw San	0	0	0	0	0	1	0	0	0
GMJ-142	Ayeyarwadee Paw San Kyar	0	0	0	0	0	0	0	0	0
GMJ-143	U to Paw San	0	0	0	1	0	0	0	0	0

GMJ-145	Bay Kyar Lay	0	0	0	0	0	0	0	0	0
GMJ-146	Bay Kyar Lay	0	0	0	0	0	0	0	0	0
GMJ-147	Paw San Hmwe	0	0	0	0	0	0	0	0	0
GMJ-148	Bay Kyar Lay Taung Pyin	0	0	0	0	0	0	0	0	0
GMJ-149	Bay Kyar Lay	0	0	0	5	0	0	0	5	0
GMJ-150	Paw San Yin	0	0	0	0	0	0	0	0	0
GMJ-151	Paw San Yin	0	0	0	0	0	0	0	0	0
GMJ-152	Paw San Yin	0	0	0	0	0	0	0	0	0
GMJ-154	Paw San Yin	0	0	0	0	0	0	0	0	0
GMJ-156	Paw San Kyi	0	0	0	0	0	0	0	0	0
GMJ-157	Paw San Kyi	0	0	0	3	0	0	0	0	0
GMJ-158	Bay Kyar Yin	0	0	0	0	0	0	0	0	0
GMJ-159	Paw San Yin	0	0	0	0	0	0	0	0	0
GMJ-160	Paw San Yin	0	0	0	0	0	0	0	0	0
GMJ-162	Paw San Kyi	0	0	1	0	0	0	0	0	0
GMJ-163	Taung Pyin (yellow)	0	0	0	0	0	0	0	0	0
GMJ-164	Paw San Taung Pyin Kyi	0	0	0	0	0	0	0	0	0
GMJ-165	Paw San Bay Kyar Yin	0	0	0	0	0	0	0	0	0
GMJ-166	Paw San Lone Chaw	0	0	0	1	0	0	0	0	0
GMJ-167	Paw San Bay Kyar	0	0	0	0	0	0	0	0	0
GMJ-168	Paw San	0	0	0	0	0	0	0	0	0
GMJ-169	Chin Khar Kaung Paw San	0	1	0	1	0	0	0	0	0
GMJ-171	Bay Kyar Lay	0	1	0	0	0	0	0	0	1
GMJ-172	90 days	1	1	0	0	1	0	0	0	0
GMJ-175	Thee Hthat Yin	0	0	0	0	0	0	1	0	0
GMJ-176	Yae Anae Loe	0	1	0	1	0	0	1	0	0
GMJ-178	Yar-9	0	0	0	0	0	0	0	0	0
GMJ-179	Kauk Kyi	0	3	0	1	0	0	1	2	0
GMJ-180	Kama Kyi	0	0	0	0	0	0	0	0	0
GMJ-181	Ayar Minn	0	1	0	1	0	1	2	3	0
GMJ-182	Ma Naung	0	0	0	0	1	0	0	0	0
GMJ-183	Yan Lu	0	1	0	0	0	0	0	0	0
GMJ-184	Kauk Hnyn Hmwe	2	2	1	0	0	0	1	0	0
GMJ-185	Ma Naw Thukha	0	1	0	0	0	0	0	0	0
GMJ-186	Huan Kar	0	0	0	0	0	0	0	0	0
GMJ-187	Hmaw Bi-2	0	1	0	0	0	1	2	2	0
GMJ-188	Lan Kyan	1	0	1	1	0	0	1	1	0
GMJ-189	Sin Thwe Latt	0	0	0	0	0	0	0	0	0
GMJ-190	Hle Aung Pan	1	0	0	1	0	0	1	1	0
GMJ-191	Shwe Thaw Yin	0	0	0	4	0	0	0	-	0
GMJ-192	Yarana Toe	0	1	0	0	0	0	1	0	0
GMJ-193	Shwe Wha Yin	0	0	0	1	0	0	0	1	0

*In rice blast evaluation, NIAS method of evaluation was used.

Rice blast disease assessment

1. Infection type on individual differential lines Susceptibility of the test plants is assessed by examining the leaves for blast symptoms which appear on the latest expanding leaf. Five to seven days after inoculation, infection type is rated as the following:

0 = No evidence of infection

1 = Brown specks smaller than 0.5mm in diameter, No sporulation. Uniform or scattered brown specks, no sporulation

2 = Brown specks about 0.5-1.00mm in diameter, no sporulation. Small lesions with distinct tan centers surrounded by a darker brown margin approximately 1mm in diameter, No sporulation.

3 = Roundish to elliptical lesion about 1-3 mm in diameter with gray center surrounded by brown margins, lesions capable of sporulation. Small eyespot lesions less than one and a half times the interval between thin veins or less than 1.5mm in diameter surrounded by dark brown, lesions capable of sporulation.

4 = Typical spindle shaped blast lesion capable of sporulation, 3 mm or longer with necrotic gray centers and water soaked brown margins little or no coalescence of lesion. Intermediate size eyespot lesions less than twice the interval between thin veins or less than 2 mm in diameter.

5 = Lesions as in 4 but about half of one or two leaf blade killed by coalescence of lesion. Large eyespot lesions more than twice the interval between thin veins or more than 2 mm in diameter.

1) Infection type 0 to 2 is resistance reaction, and infection type 3 to 5 is susceptible reaction basically.

2) When we inoculated it into the rice plant which the most upper leaf extremely slightly emergence, the lesion developed on a tip of the leaf becomes sensitive, and there is a case more than infection type 3, but does not determine as the susceptible lesion.

Also record the presence or absence of sporulation on lesions after incubation for 24 h at 27°C. .