GENETIC VARIATION AND ASSOCIATION MAPPING FOR GRAIN QUALITY, YIELD AND YIELD ATTRIBUTING TRAITS IN LOWLAND RICE OF NAGALAND

Thesis

submitted to

NAGALAND UNIVERSITY

in partial fulfillment of requirements for the Degree of

Doctor of Philosophy (Agriculture)

in

Genetics and Plant Breeding

By

Lalrinchhani Chhangte

Admn. No. Ph-289/19 Regn. No. Ph.D./GPB/00342

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School of Agricultural Sciences and Rural Development,
Nagaland University, Medziphema Campus – 797106
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DECLARATION

I, **Lalrinchhani Chhangte** hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis had not been submitted by me for any research degree in any other university/institute.

This is being submitted to Nagaland University for the degree of Doctor of Philosophy in **Genetics and Plant Breeding**.

Date:

Place: **Medziphema** **(LALRINCHHANI CHHANGTE)**

……..

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CERTIFICATE – I

This is to certify that the thesis entitled "***Genetic variation and association mapping for grain quality, yield and yield attributing traits in lowland rice of Nagaland"*** submitted to Nagaland University in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in **Genetics and Plant Breeding** is the record of research work carried out by Miss **Lalrinchhani Chhangte,** Registration No. **Ph.D./GPB/00342** under my personal supervision and guidance.

The result of the investigation reported in the thesis have not been submitted for any other degree or diploma. The assistance of all kinds received by the student has been duly acknowledged.

Date :
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CERTIFICATE – II

VIVA VOCE ON THESIS OF DOCTOR OF PHILOSOPHY IN GENETICS AND PLANT BREEDINGS

This is to certify that the thesis entitled "**Genetic variation and association mapping for grain quality, yield and yield attributing traits in lowland rice of Nagaland**" submitted by Miss. Lalrinchhani Chhangte Admission No. Ph-289/19, Registration No. Ph.D./GPB/00342 to the NAGALAND UNIVERSITY in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Genetics and Plant Breeding has been examined by the Advisory Board and External examiner on …….....

The performance of the student has been found **Satisfactory/Unsatisfactory.**

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1. Prof. Kigwe Seyie (Supervisor)	.....
2. …… (External examiner)	.....
3. Dr. Harendra Verma	.....
(Co-supervisor)	.....
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Date: **(Lalrinchhani Chhangte)**

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LIST OF ABBREVIATIONS

%	=	Percentage
<	=	Less than
>	=	More than
AC	=	Amylose Content
AMOVA	=	Analysis of Molecular Variance
ANOVA	=	Analysis of Variance
ASV	=	Alkali Spreading Value
Av.	=	Average
C.D	=	Critical Difference
cM	=	Centi Morgan
Cm	=	Centimetre
DF	=	Days to Flowering
Df	=	Degree of Freedom
DGL	=	Decorticated Grain Length
DGW	=	Decorticated Grain Width
DM	=	Days to Maturity
DNA	=	Deoxyribonucleic Acid
DRR	=	Director of Research Centre
DUS	=	Distinctiveness, Uniformity and Stability
ECV	=	Environmental Coefficient of Variation
et al.	=	Et alia (and others)
Even.	=	Evening

FGPP	=	Filled Grains per panicle
GA	=	Genetic Advance
GC	=	Gel Consistency
GCV	=	Genotypic Coefficient of Variation
GEL	=	Grain Elongation Ratio
GL	=	Grain Length
GLM	=	General Linear Method
GT	=	Gelatinization Temperature
GV	=	Genotypic Variance
GW	=	Grain Width
GW	=	Grains Weight
GY	=	Grain Yield
h^2bs	=	Heritability in Broad Sense
ICAR	=	Indian Council of Agricultural Research
i.e	=	Id est (That is)
K	=	Kinship matrix
KOH	=	Potassium Hydroxide
m	=	Meter
MAS	=	Maker Assisted Selection
Max.	=	Maximum
mg	=	Milligram
Min.	=	Minimum
MLM	=	Mixed Linear Method

Mm	=	Millimeter
Mor.	=	Morning
N	=	Normality
NaOH	=	Sodium Hydroxide
NEH	=	North East Hilly
nm	=	Nanometre
no.	=	Number
PCoA	=	Principal Coordinate Analysis
PCR	=	Polymerase Chain Reaction
PCV	=	Phenotypic Coefficient of Variation
PH	=	Plant Height
PIC	=	Polymorphism Information Content
PL	=	Panicle Length
PPP	=	Panicles per Plant
PV	=	Phenotypic Variance
Q	=	Population Structure
QTL	=	Quantitative Trait Loci
RBD	=	Randomized Block Design
RC	=	Research Centre
RCM	=	Research Centre Maniphou
RH	=	Relative Humidity
RM	=	Rice Microsatellite
rpm	=	Repetition per Minute

Sl.no.	=	Serial Number
SSR	=	Simple Sequence Repeats
TASSEL	=	Trait Analysis by Association, Evolution and Linkage
TBE	=	Tris-borate-EDTA
TE	=	Tris-EDTA (Ethylenediamine tetra acetic acid)
UFGPP	=	Unfilled Grains per panicle
ul	=	Microliter
UPGMA	=	Unweighted Pair Group Method with Arithmetic Mean
UPOV	=	The International Union for the Protection of New Varieties of Plants

ABSRTACT

An experiment entitled **'Genetic variation and Association mapping for Grain Quality, Yield and Yield Attributing traits in Lowland rice of Nagaland'** was undertaken at the research farm and Central laboratory of ICAR, Medziphema, Nagaland Centre during *Kharif* 2020 and 2021. A total of 81 genotypes including two check varieties Ranjit and RCM09 was laid down in randomised block design with three replications. Important physical and chemical quality and yield traits were recorded by following DUS guidelines. The whole accessions were genotyped using 40 SSR markers covering all the 12 chromosomes for the identification of important traits and marker association. Analysis of variance revealed significant variation among the genotypes. Genetic parameters studied revealed influence of environment in the development of the characters under study. The pattern of high heritability coupled with high genetic advance was observed in important traits such as grain elongation ratio, no. of unfilled grains per panicle, amylose content, yield per plant, decorticated grain width, gel consistency and no. of panicles per plant. These traits can be improved with simple selection or progeny selection. A significant positive correlation with grain yield was observed in component traits such as no. of filled grains per panicle (0.64,0.60), days to 50% flowering (0.41, 0.40), no. of panicles per plant (0.37, 0.35) and days to maturity (0.37, 0.36). High direct effects of component traits on grain yield were recorded in no. of filled grains per panicle (0.73,0.62), no. of panicles per plant (0.53,0.50) and 1000 grains weight (0.35,0.28). Six clusters were generated using UPGMA in which cluster VI comprises maximum genotypes (73), followed by cluster V (3), cluster IV (2), cluster III, II and I with one genotype each. Principal component analysis revealed that PC1 and PC2 accounted for18.34% and 15.56% to total variation respectively. The PIC value of 40 SSR markers range from 0.23 for marker RM53 to 0.99 for RM1256 with average value of 0.64. A total of 102 alleles were detected with an average 2.55 alleles per locus. AMOVA revealed the presence of 13% variation among the population, 87% variation among the individuals and 0% variation within the individual. Two subpopulations with 10 admixtures were observed from STRUCTURE analysis. An unweighted neighbour-joining cluster

analysis separated the whole accessions into two main group with admixtures distributed in each cluster. Cluster I comprise of 48 genotype and cluster II with 33. From PCoA, the total variation of the first three axes of differentiation was 17.37%. Association with SSR markers were observed between quality traits such as decorticated grain width, decorticated grain length, grain width, grain length, gel consistency, grain elongation ratio and gelatinization temperature and some important yield traits such as filled grain per panicle, plant height, panicle length, 1000 seed weight, panicles per plant, days to maturity, days to 50% flowering unfilled grains per panicle and grain yield per plant. The observed genetic variations among the genotypes under study and association between traits and markers can be an important tool in further development of an improved grain quality of rice with higher yield.

Keywords: Association mapping, Genetic variation, Grain quality, SSR markers, Yield.

CHAPTER I

INTRODUCTION

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major food crops that is consumed by more than half of the world population. It is globally grown in an area of around 154 million hectares annually with a total production of 509.2 million tons (World Food Situation, 2020). India stands as the second largest producer of rice in the world next to China, around 44.5-million-hectare area is under rice cultivation with production of 116.42 million tonnes (Agricultural Statistics at a Glance, 2019). The constant growth in global population and conversion to rice as a staple food from native foods such as roots and tubers in some countries has led to an urgent need of boosting rice production at the rate of approximately 30% to meet the increasing global demands (Mohanty *et al*., 2010; Ray *et al*., 2013; Seck *et al*., 2013). The standard of living is also improving simultaneously with increase in population and this has led to the demand of superior quality of rice as the consumers are willing to purchase at higher price. This opens a gateway in global market and become a priority issue in most of the rice producing countries across the world and thus challenges plant breeders in developing a variety having a desirable grain quality for the consumers as well as high yielding for the farmers. Particularly in rice breeding, the most important qualities desired by breeders have been high yielding, premium grain and eating quality along with resistance to major biotic and abiotic stresses. Improvement of grain yield is either difficult or not suitable through increase in arable land due to competition between food and energy, as a result of these, plant breeders seek alternative method to improve grain yield by selecting high yielding cultivars or improving grain yield by either selection or hybridization. However, simultaneous improvement of grain yield and grain quality is difficult since there is conflict between these two important traits as much emphasis on either of them causes into poor results of the other trait (Khan *et al*., 2015).

These agriculturally important traits such as yield and quality are controlled by polygenes and multifactorial factors that greatly depends on genetic x environmental (G x E) interactions, this made them a complex trait which are depending on additive effect of component traits. The complexity of grain yield has made direct selection base on this trait ineffective. Since grain yield alone is the ultimate contribution of other components such as plant height, panicle length, no. of filled grains per panicle and 1000 grains weight, the identification of these component traits and simultaneous improvement of their genotype is an important strategy in yield improvement program. For the success of any crop improvement program the knowledge of genetic variability present in a given crop species is of paramount importance and further analysis of association between each component trait and their path coefficient analysis helps in selecting the most suitable strategy for improvement of grain yield. The study of the association of yield with yield components is important for fixing up the character, which plays an ultimate role in influencing the yield (Rajeswari and Nadarajan, 2004). When it comes to grain quality in rice, the diverse preference in different culture broadens the aspect of rice grain quality but its characteristics are mainly defined by grain size and shape, cooking quality and eating characteristics, nutritional quality and milling properties. But the most relevant quality properties are appearance and cooking quality which is directly related to amylose content, gel consistency and gelatinization temperature (He *et al*., 1999 and Fan *et al*., 2005). Grain quality is an important character to study not only because of its contribution to yield, but also because of its influence in rice marketing and trade (Kumar *et al*., 2010). Since appearance quality has a direct influence on marketing it ultimately influences the success of commercial varieties. Grain quality traits are controlled by major and minor quantitative trait loci (QTLs), implying that the genetic mechanisms underlying quality traits are complex. QTL analyses have identified several markers linked to grain quality (Fan *et al*., 2005; Lestari *et al*., 2009; Shao *et al*., 2010; Rani *et al*., 2011 and

Tabkhar *et al*., 2012). But validation of those markers is essential to add value to those markers in a diverse set of germplasm before using them in marker aided breeding programme. Such information is very limited in rice particularly for quality traits. It is important to know the information on the number and location of genes in chromosomes that influence the expression of trait. Their relative contribution to expression of trait, possible pleiotropic effects or epistatic interaction among the loci and their sensitivity to variations in environments are very important for the utilization of these loci for crop improvement.

Apart from being one of the largest producers of rice in the world, India is also a home to diverse genetic resources of rice such as landraces, primitive cultivars and wild rice. These diverse genetic resources which are ultimately useful in breeding program can be found in north east region of India which is often considered as one of the hot pockets of rice genetic resources in the world. Considerable number of diverse germplasms of rice are found in this region most of which are landraces cultivated by local farmers as this suit to their taste, provide food security and adapted to local environmental condition. These landraces are grown in both lowland, upland and on deep-water conditions. It has been estimated that at least 10,000 indigenous cultivars are grown in this region (Hore, 2005). Rice plays an important role in the culture not only as the most important staple food but in rituals and ceremony. Even though rice occupies 80% of total cultivated area of North east region, most of the states are not self-sufficient in rice production. However, Nagaland state is one among the four states in north east India viz: Arunachal Pradesh, Manipur, Nagaland and Tripura having surplus production of rice (Roy *et al*, 2015) with a potential to give rise to an improved variety from available local landraces. The farmers of this region are persistent in growing local landraces with low yield over the improved high yielding varieties as this attributed to their adaptability to environmental conditions, resistance to pests, diseases, grain quality, taste,

aroma and also marketability, which are not present in many of the improved varieties (Dey, 2009). So, improvement of these locally available landraces can be beneficial for the state as well as for the region surrounding the state. These rice varieties are broadly categorised as glutinous, brown and aromatic, and most of them are grown under *jhum* or shifting cultivation system practised by different Naga tribes in the state (Sharma, 2017). These landraces carry appreciable genetic information on their genome that can be exploited for developing new varieties with desirable characteristics for grain quality. Several ethic groups inhabiting at different altitudes and climatic situations has unknowingly practiced selection from olden days which contributed to some extent towards the diversity of rice crop in the region. For proper utilization of these genetic resources particularly for development of superior quality of rice and high yielding variety, thorough identification and characterization at phenotypic and genotypic level is required.

Morphological markers were earlier utilised for the analysis of genetic stock, but these markers are limited in number and are highly influenced by environment and are subjected to epistasis and pleiotropic effect, hence, they are not reliable to generate the correct picture of genetic makeup of plant (Higgins, 1984; Xiao *et al*., 1996; Ovesna *et al*., 2002). Therefore, new technologies need to be developed to accelerate the breeding process through more advanced phenotyping and genotyping methods. Molecular marker technologies can assist conventional breeding efforts and are valuable tools for the analysis of genetic relatedness, identification and selection of desirable genotypes for crosses as well as for germplasm conservation in gene banks. However, DNA markers portray genome sequence composition, thus, enabling to detect differences in the genetic information carried by the diverse individuals. Among many molecular techniques, microsatellite DNA markers (SSR markers) consisting of AT repeats were found to be highly polymorphic, co-dominant, abundant and well distributed throughout the rice genome and could distinguish even closely

related cultivars (Akagi *et al*., 1997; Temnykh *et al*., 2001). SSRs are particularly useful for studying the population structure and demographic history of domesticated species such as rice and are extensively used to genotype rice germplasm collections (Agrama *et al*., 2007). Association analysis, or linkage disequilibrium mapping has been a notable strategy for identifying genes controlling important traits. This approach identifies quantitative trait loci (QTLs) by examining the marker trait association that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse germplasm and locate valuable genes in a genome (Zhu *et al*., 2008). Association mapping doesn't need the development of mapping population; this method utilizes natural variation and requires lesser marker per chromosome without the loss of genetic resolution for marker assisted selection (MAS), furthermore, rice is self-pollinated species which is expected to have high linkage disequilibrium thereby requiring fewer markers. (Borba *et al*., 2010), it is supposed to have a great potential in evaluation and characterization of a wide range of alleles. This method offers an opportunity for increasing the exploitation of germplasm accessions in the search for advantageous allele combinations.

With the above information and consideration for the need to breed rice with better quality and higher yield the following **objectives** were formulated for the present investigation.

1. To study genetic variation for grain quality and yield attributing traits in lowland rice landraces of Nagaland.
2. To study association for grain quality traits and yield attributing traits in lowland rice.
3. To identify molecular marker associated with grain quality traits in rice.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Rice, apart from being a staple food for more than half of the world's population is a suitable model plant for genomic research because of its small genomic size. Rice has 21 wild and 2 cultivated species giving a rich genetic diversity in the form of thousands of land races and progenitor species (Kumar *et al*., 2012). Landraces are valuable genetic resources as they contain huge genetic variability which can be used to complement and widen the gene pool of various advanced genotypes (Kobayashi *et al*., 2006). Evaluation and characterization of existing land races of rice is important due to increasing needs of varietal improvement (Yawen *et al*., 2003).

Evaluation of germplasm at phenotypic and genotypic level provide fundamental knowledge of the genetic behaviour of each genotype through which we can figure out the carrier of our gene of interest. Analysis of genetic parameters, association analysis, diversity and trait-marker association studies can provide tremendously useful information that can be employed in genetic improvement of our germplasm collection. However, before setting up any investigation thorough understanding of economic importance and phylogeny of experimental materials, feasibility of the methodology, expected results, records on different set of accession, possible errors and its causes will help the breeder in preparing suitable strategy. Therefore, scrutinizing previous works on the related field of experiment conducted by various experts is a pre-requisite that can ensure the success of our experiment.

2.1 Genetic variability analysis.

The genetic improvement of any breeding population largely depends upon the amount of genetic variability present. Grain yield in rice is known to be a complex trait which is controlled by various yields components such as no.

of grains per panicle, no. of panicles per plant, panicle length etc. In order to understand the genetic variability of yield attributing traits, relationship among them and their relation with yield are pre-requisite to execute any breeding programme (Tiwari *et al.*, 2019). Therefore, the information obtained from different genetic parameters such as genotypic covariance (GCV), phenotypic covariance (PCV), heritability and correlation of morphological traits with grain yield could be useful in formulating successful rice breeding programme and for genetic improvement of grain yield and its quality.

Since grain yield is a complex quantitative trait which is affected by two or several genes and furthermore affected by environmental factor, a magnitude of GCV, PCV and their difference have an impact on selection of trait for improvement. Small difference between GCV and PCV indicate less influence of environment on the trait and a high estimate for GCV and PCV also indicates the possibility of genetic improvement through direct selection. Since estimates value of GCV and PCV alone are not helpful in determining the heritable portion of variation, estimation of heritability is crucial. Heritability value reflects the portion of genetic variability that is transmitted from parents to offspring. It acts as a predictive instrument in expressing the reliability of phenotypic value. Therefore, high heritability helps in effective selection for a particular trait. Genetic advance is a useful indicator of the progress that can be expected as a result of exercising selection on the pertinent population. Genetic advance is the difference between mean of the main population and mean of the selected population. Heritability in conjunction with genetic advance would give a more reliable index of selection value (Johnson *et al*., 1955).

Estimates of GCV and PCV reflect average inter-accession differences that are useful to understand variability presence among the germplasm accessions. Karim *et al.* (2007) reported low GCV for days to maturity in 40 aromatic rice genotypes, while a high estimate of GCV was reported for 1000 grain weights, grain yield per hill and no. of filled grains per panicle. High

heritability with high genetic advance was also reported for 1000 grains weight and no. of filled grains per panicle. Whereas days to maturity was reported to show high heritability with low genetic advance. Mustafa and Yassir Elsheikh (2007) study 14 African rice genotypes and observed wide range of variability for most of the traits such as no. of filled grains per panicle, no. of panicles per plant, no. of panicles per plant and no. of tillers per plant etc., indicating genotypes exhibit difference in genetic potential for these characters, they recorded high GCV value on grain yield, no. of unfilled grains per panicle, no. of grains per panicle and no. of unfilled grains per panicle, whereas, low coefficient of variation was observed for plant height, days to 50% flowering, days to maturity, panicle length and 1000 grains weight. Chakraborty and Chakraborty (2010) reported small difference between GCV and PCV for 50% flowering in Assam rice, they also reported wide difference between GCV and PCV for character such as plant height, panicle length and yield per plant. They also reported high heritability associated with high genetic advance for grain yield per plant, this association reflected that this character is predominantly governed by additive genes action. A low heritability coupled with low genetic advance was also reported in character days to 50% flowering and this indicated that this character is governed by non-additive gene action (dominance and epistasis). A small difference between GCV and PCV in general, high values of GCV and PCV for alkali spreading were reported by Rathi *et al*. (2010) in upland rice of Assam. Selvaraj *et al*. (2011) evaluated 21 genotypes along with 64 hybrids; they reported considerable variation for all the traits, a slightly greater value of PCV in comparing to GCV was reported revealing a small influence of environment on the development of such traits. Parikh *et al*. (2012) reported high heritability for plant height in 71 aromatic rice germplasm from IGKV, Raipur. They also reported high genetic advance for grain yield per plant. Babu *et al*. (2012) evaluated hybrid rice for quantitative traits and reported high GCV and PCV for no. of filled grains per panicle and low value for amylose content and

moderate value for gel consistency. Less difference between GCV and PCV was also reported in this character. A high heritability coupled with high genetic advance was also reported for no. of filled grains per panicle indicating simple selection could be effective for improving no. of filled grains per panicle. Seyoum *et al*. (2012) report on high estimates for GCV and PCV important traits such as days to 50% flowering, plan height, grains per panicle, spikelets per panicle, 1000 grains weight and grain yield per plant. High heritability indicates high heritable portion of variation, such heritability was found in plant height, days to 50% flowering, 1000 grains weight, days to maturity and panicle length. Low, medium and high estimates of GCV and PCV was also reported by Veni *et al*. (2013) where panicle length exhibited low value. Their overall PCV was slightly higher than GCV. They recommend selection based on traits such as 1000 grains weight and grain yield itself as they have high value for heritability, genetic advance as per cent of mean and genetic coefficient of variation. Islam *et al*. (2015) reported relatively high GCV and PCV for 50% with overall higher PCV than GCV for different yield contributing traits which indicate that they are interacted with the environment to some extent. They also reported high heritability for grain width, days to maturity and no. of filled grains per panicle which indicates high heritable portion of variation. High to medium estimates of heritability and genetic advance were also obtained for grains per panicle, days to 50% flowering and days to maturity which indicates the role of additive gene action and a good scope of selection using their phenotypic performance. Rukmini *et al*. (2016) also reported high significance differences among 27 genotypes of rice for yield components, and a less magnitude differences between GCV and PCV. High estimates of GCV and PCV for yield per plant and filled seeds per panicle were also reported. But low (< 10%) estimates for character such as kernel elongation ratio, kernel length and kernel width were reported indicating selection for these traits would offer very little scope for genetic improvement of the genotypes. Nandini *et al*. (2017) also reported low,

medium and high value of GCV and PCV for both yield attributes and quality traits. High heritability with even high genetic advance was also reported in days to 50% flowering, grain length and grain breadth length ratio. This combination is a good criterion for effective selection. Days to 50% flowering, days to maturity, plant height and panicle length was reported to have medium GCV and PCV whereas, traits such as no. of panicles per plant, 1000 grains weight, grain length breadth ratio and grain yield were reported to have high GCV and PCV (Kumar *et al*. 2018). Sanghamitra *et al*. (2018) evaluated 11 pigmented rice genotypes to estimate genetic parameter, they observed highly significant variation for most of the traits including amylose content. The observed PCV and GCV suggest that there was little effect of environment on the development of their traits under study. Akter *et al*. (2019) observed a slightly higher GCV than PCV for traits such as days to maturity, plant height, and grains per panicle, panicle length, thousand grain weight and yield in ton per hectare in hybrid rice. This is an indication that genetic factor plays greater role on the expression of these traits. Akshay *et al*. (2022) evaluated 44 rice genotypes to estimate variability and genetic parameters for yield, yield traits, quality and nutritional traits and observed close value between GCV and PCV suggesting little effect of environment on the development of characters. The traits plant height, number of productive tillers per plant, panicle length, number of grains per panicle, 1000 grain weight, grain yield per plant, head rice recovery percentage, grain length, grain width, length/breadth ratio, protein content, iron and zinc content all showed moderate to high variability, high heritability coupled with high genetic advance as per cent of mean revealing the role of additive gene effect and simple selection procedures may be effective for improving these traits. Sadhana *et al*. (2022) reported high GCV and PCV for no. of filled grains per panicle and 1000 grains weight, they also reported high heritability coupled with high genetic advance as percentage of mean in important traits such as total no. of grains per panicle, no. of filled grains per panicle, 1000 grains weight, grain yield per plant,

length of kernel and kernel length/breadth ratio. Faysal *et al*. (2022) found low to medium GCV and PCV on important traits such as days to 50% flowering, panicle length, plant height, no. of effective tillers, 1000 grains weight and grain yield per plant. They also reported high heritability with medium genetic advance as percentage of mean in many important traits except panicle length. Although high heritability suggests high component of heritable portion of variation that can be exploited by breeders in the selection of superior genotypes (Ali *et al*., 2002). On the basis of phenotypic performance, heritability estimates along with genetic advance will be more useful in predicting the effect for selecting the best individual. Presence of high heritability coupled with high to medium genetic advance indicates the presence of additive gene action that can ensure the effectiveness of selection. Whereas, in presence of moderate to low heritability coupled with low genetic advance indicate the existence of non-additive gene action, in such situation heterosis breeding maybe useful (Thakur and Pandey, 2020).

2.2 Correlation coefficient and path coefficient analysis.

A better understanding of characters and their interactions among themselves can assist in designing a precise plant breeding programme. The directions and magnitude of association between yield and its component characters determine the efficiency of selection for increasing yield. Character association provides information on the nature and extent of association between pairs of metric traits for improvements. Among character association studies corelation coefficient measures the degree to which characters vary or measure the intensity of association within and between them. Improvement in yield as a quantitative trait often requires the improvement of a secondary trait that is positively correlated with yield (Smith *et al*., 1978). Knowledge of correlation is important in identifying important parameters in any selection programme. Genotypic and phenotypic correlation coefficients tell us the association between and among two or more characters. A significant association suggests

that such characters could be improved simultaneously; however, such an improvement depends on phenotypic correlation, additive genetic variance and heritability (Ojo *et al*., 2006). However, correlation coefficient gives only the relation between two variables. Whereas, path co-efficient analysis allows separation of direct and indirect effects through other attributes by partitioning the correlation (Wright, 1921). Path analysis is that, it permits the partitioning of the correlation coefficient into its components, one component being the path coefficient that measures the direct effect of a predictor variable upon its response variable; the second component being the indirect effect(s) of a predictor variable on the response variable through another predictor variable (Dewey and Lu, 1959). On the analysis of F4 and F5 generation form a cross between Zhenshan and IR50, Rajeswari and Nadarajan (2004) reported days to 50% flowering, no. of productive tillers per plant, panicle length, no. of grains per panicle and 100 grain weight as dependent attributes on which the selection would be focus for improving the grain yield. Mustafa and Yassir Elsheikh (2007) suggested improvement of rice yield through selecting rice plants for higher no. of filled grains per panicle, no. of panicles per plant and 1000 grains weight as these characters showed positive correlation with grain yield. The path analysis revealed that number of filled grains per panicle had direct positive contribution to the grain yield per hectare and positive indirect effect on grain yield per hectare through days to 50% maturity and number of grains per panicle; while number of filled grains per particle had negative indirect effect on grain yield per hectare through number of tillers per plant and number of panicles respectively. The relative contribution of characters towards variability and results of correlation and path coefficient indicated the importance of number of grains per panicle, number of filled grain per panicle and number of panicles. Genotypes having these characters would offer a good possibility for the improvement of rice through conventional selection. In grain quality studies, Kumar *et al.* (2010) suggested selection through character such as paddy length

and brown rice length since these characters show positive interrelation among them. Rathi *et al*. (2010) observed higher genotypic correlation coefficient than the phenotypic correlation coefficient in quality traits of 100 upland/ahu rice of Assam, India indicating strong inherent associations among the characteristics studied. Akinwale *et al*. (2011) reported significant positive correlation between grain yield with the number of tillers per plant, panicle weight and number of grains per panicle indicating the possibilities of improvement of grain yield through improving these characters. Kiani and Nematzadeh, (2012) studied 54 rice genotypes selected from F2 populations to study association between grain yield and yield components. From the correlation studies they reported that panicles per plant and filled grains per panicle were significantly correlated to grain yield. Moreover, they revealed high direct effect of these two characters on grain yield. The highest indirect effect was contributed by panicle length through filled grains per panicle. Dhurai *et al*. (2014) investigates 32 rice genotypes to understand the association among 14 contributing traits for yield and quality and their direct and indirect effects on grain yield. They reported higher genotypic correlation coefficient than phenotypic correlation coefficient. Grain yield was reported to have significant association with number of grains per panicle and days to maturity. Kernel elongation ratio, kernel length, kernel length and breadth ratio, kernel breadth, days to maturity, panicle length and plant heigh were reported to have direct effect on grain yield. So, selection based on these characters could help in simultaneous improvement and quality traits. Liu *et al*. (2014) observed negative correlation between amylose content, water absorption and expansion rate with eating qualities, yet gel consistency, alkali spreading values, solid content of rice-water and fat content were positively correlated with eating qualities. Among them, eating quality had an obvious correlation with amylose content and gel consistency, but no significant correlation with protein content. Ratna *et al*. (2015) studied correlation and path coefficients analyses among fourteen morphological characters in six advanced

lines of Basmati rice and one commercial check namely BRRI Dhan 29. In general, genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients suggesting that the environmental influence reduces the relationship between yield and yield contributing characters of rice. Correlation coefficient analysis showed significant positive correlation between plant height and panicle length at genotypic level. Number of filled spikelets/panicle showed significant positive correlation with yield at both genotypic and phenotypic levels but significant negative correlation was observed between plant height and yield. Number of effective tillers per plant had negative significant correlation with panicle length and with number of unfilled spikelets per panicle at genotypic level. Number of ineffective tillers per plant had significant negative correlation with 1000-seed weight at both genotypic and phenotypic levels. Path coefficient analysis revealed highest positive direct effect of number of filled spikelets/panicle on grain yield but plant height and number of unfilled spikelets per panicle had negative direct effect on grain. Abdala *et al*. (2016) conducted correlation and path analysis on eleven F1 generation obtained from crossing Aromatic and Non-aromatic rice parental line from landraces, a high positive correlation between brown rice length and paddy grain width; paddy grain length and brown grain length; brown rice shape and grain rice length were reported. A negative correlation between brown rice shape and brown rice length was also reported. Positive but low direct effect of paddy grain length on brown grain length was also reported, high and positive direct effect of brown rice width and brown grain shape on brown grain length was also observed. These results thus revealed the importance of grain quality on rice grain improvement. Srijan *et al*. (2016) studied 51 genotypes of rice which include 15 parental lines with 38 cross combinations, among the characters studied they recommend no. of filled grains per panicle, panicle length, no. of productive tillers per plant, days to 50% flowering and plant height for selection parameters since they exhibit high significant positive correlation with grain

yield per plant, form their path analysis these characters also show high positive direct effect on grain yield. So, these characters can be used as a selection criterion for any rice breeding programme for improving grain yield. Dhakal *et al*. (2017) reported positive correlation between grain yield and elongation ratio, however they also reported negative association between grain yield and amylose content. Number of grains per panicle and 1000 grains weight were reported to have positive direct effect on grain yield. The grain quality characters elongation ratio, length and breadth ratio were reported to have positive direct effect on grain yield per plant. Sowmya and Venkatesan (2017) studied correlation and path analysis on 80 genotypes of rice, they reported higher magnitude of genotypic correlation coefficient than phenotypic correlation coefficient, this indicated masking or modifying effect of environment. Days to first flowering was reported to show strong significant negative effect on grain yield per plant. This inferred the days to first flower and grain yield per plant can be used as a selection criterion for the improvement of grain yield per plant. Maximum direct effect on grain yield per plant was exhibited by number of panicles per plant. Hence this trait should be taken into account for developing maximum threshold yield for obtaining new rice varieties or hybrid. Girma *et al*. (2018) reported significant and positive phenotypic correlation results between yield and most yield traits except plant height and panicle length. Saghamitra *et al*. (2018) reported negative association between the agronomic and quality traits such as grain fertility and head rice recovery. Chhangte and Devi (2019) reported high positive association between grain yield and no. of grains per plant, no. of panicles per plant, 1000 grains weight, panicle length and plant height showed. Maximum direct effect on grain yield was also observed from no. of grains yield per plant followed by no. of panicles per plant, panicle length, plant height and 1000 grains weight. Kiani and Nematzadeh (2019) also performed the association between grain yield and yield components in fifty-four selected rice genotypes at F2 populations. The results showed that traits, the panicles per plant

and filled grains per panicle correlated significantly with grain yield, while grain yield was negatively associated with non-filled grains per panicle. Path coefficient analysis revealed that grain yield was associated with panicles per plant and filled grains per panicle with the direct effects of 0.691 and 0.568, respectively. The greatest indirect effect belonged to panicle length through filled grains per panicle. Information obtained in this study revealed that traits, the panicles per plant and filled grains per panicle, could be used as selection criteria for grain yield improvement at segregating populations of rice. Tiwari *et al*. (2019) studied rainfed early lowland rice and observed positive and highly significant correlations both in genotypic and phenotypic levels between days to heading and days to maturity, days to heading and grain yield, and days to maturity and grain yield. They also reported negative and highly significant genetic correlation was observed between plant height and 1000-grain weight. These results indicated that days to heading, days to maturity, grain yield, 1000-grain weight demonstrating higher heritability and remarkable genetic advance could be considered the most appropriate traits for improvement and selection of trait to achieve stable and high yielding early rice genotypes under rainfed environments. The degree of correlation among the characters is an important factor especially in economic and complex character as yield. Steel and Torrie (1984) stated that correlations are measures of the intensity of association between traits. The selection for one trait results in progress for all characters that are positively correlated and retrogress for traits that are negatively correlated.

2.3 Genetic diversity analysis and marker-trait association

Importance of landraces can never be denied in agriculture system, because improvement in existing variety depends upon desirable genes which are possibly present in landraces and wild varieties only (Holden *et al*., 1993). Landraces offer a valuable gene pool for future breeding program (Richharia, 1979; Patra, 2000). Assessing the diversity of rice genetic resources involves

identifying phenotypes, analysing biochemistry, and evaluating DNA diversity (Second, 1982; McNally *et al*., 2009). Agro-morphological characterization of germplasm variety is fundamental in order to provide information for plant breeding programs (Lin, 1991). Our information about them is incomplete and is therefore urgent to collect and conserve these landraces of rice (Sinha and Mishra, 2013). Presently more than 90% of rice cultivation is being done using high yielding variety only. Obviously, landraces are disappearing fast (Holden *et al*., 1993; During, 1990; Matson *et al*., 1997). In present era of overpopulation *Ex-Situ* conservation is the best strategy to conserve these landraces (Lipton and Longhurt, 1989) because marginal and poor farmers who are the main keepers of traditional variety of rice are more interested in high production but not in genetic diversity. Several workers reported the use of agro-morphological markers in the characterization of rice diversity. Yawen *et al*. (2003) studied the genetic diversity of 5285 accessions of land races of rice in China and found considerable morphological variation among accession. Patra and Dhua (2003) reported agro-morphological diversity in upland rice of Jeypore tract. Chakravorty *et al*. (2013) also reported multivariate diversity of 51 landraces of rice of West Bengal based upon 18 agro-morphological traits. Sinha and Mishra (2013) conducted research to determine the agro-morphic characterization and relationship between 20 landraces of rice cultivars of Bankura District of West Bengal. They characterized 20 qualitative and 13 quantitative morphological characters with 82 agro-morphic descriptors. They observed polymorphism for most traits except coleoptiles colour, present of leaf collar, shape of ligule and present of secondary branching in panicle. For Cluster analysis of qualitative traits, the cultivars were grouped into five clusters based on similarity coefficient of Jaccard. Pearson correlation matrix, Principal Component Analysis (Pearson –n type), the un-weighted variable pair group method of the average linkage cluster analysis (UPGMA-Person) were used to analysed quantitative data. This analysis enabled assessment of major characters of landraces variety which have

a great impact to the diversity of landraces. Using UPGMA four cluster groups were obtained from 13 quantitative agro-morphological characters. The first three principal components explained about 79.05% of the total variation among the 13 characters. The results of PCA suggested that characters such as leaf length and width ratio, plant height, grain width, decorticated grain width, 50% flowering and maturity time were the principal discriminatory characteristics of landraces of rice variety. Germplasm consist of these characters are better choice for hybridization program. This study indicated that morphological traits were useful for preliminary evaluation for crop improvement program and can be used for assessing genetic diversity among morphologically distinguishable rice landraces. However, agro-morphological markers and biochemical markers are often influenced by environment, therefore, the used of molecular markers has provided better and reliable data. Molecular marker technologies can assist conventional breeding efforts and are valuable tools for the analysis of genetic relatedness and the identification and selection of desirable genotypes for crosses as well as for germplasm conservation in gene banks. Among many molecular techniques, simple sequence repeat (SSR) markers (microsatellites) are co-dominant, hypervariable, abundant and well distributed throughout the rice genome (Temnykh *et al*., 2001). Abundance of microsatellite markers is now available through the published high-density linkage map (McCouch *et al*., 2002; IRGSP, 2005) or public database. Hossain *et al*. (2007) used thirty microsatellite molecular markers across 21 rice genotypes for characterization and discrimination and reported 4.53 alleles per locus. The polymorphism information content (PIC) values ranged from 0.30 to 0.84 in all 30 loci. RM223 was found the best marker for the identification of 21 genotypes as revealed by PIC values. A two-dimensional principal coordinate analysis (PCoA) with 21 genotypes showed that the genotypes Supper Basmoti, Basmati370, BasmatiD, Keora, Chinisakkora, Thakurbhog, Benaful, Kolgochi, Buchi, Awnedtapl and Kalijira-11 were found far away from centroid of the cluster than rest of the

genotypes which placed around the centroid. Being grouped into distant clusters, Supper Basmoti, Thakurbhog, Keora, and Benaful could be utilized as potential parents for the improvement of fine grain aromatic rice varieties. The microsatellite marker based molecular fingerprinting could serve as a sound basis in the identification of genetically distant accessions as well as in the duplicate sorting of the morphologically close accessions. Genetic diversity underlies the improvement of crops by plant breeding. Land races of rice (*Oryza sativa* L.) can contain some valuable alleles not common in modern germplasm. Pervaiz *et al*. (2010) measure diversity using thirty-five microsatellite markers and seventy-five genotypes. They detected a total of 142 alleles among the markers with 32 polymorphic SSR loci. The number of alleles identified by each marker ranged from 2 to 13 with a mean of 4.4. Polymorphism information content ranged from 0.124 to 0.836, with an average of 0.569. At nine microsatellite loci, basmati-type landraces amplified more different alleles than those in the coarse-type. DNA markers RM70 and RM72 divided the rice landraces on the basis of days to flowering. A dendrogram based on total microsatellite polymorphism grouped 75 genotypes into four major clusters at 0.40 similarity coefficient, differentiating tall, late maturing and slender aromatic types from the short, early and bold non-aromatic ones. It inferred that Pakistani landraces have diverse genetic bases and can be utilized in future breeding programs. The DNA markers developed will assist in genotype identification, purity testing and plant variety protection. Kumar *et al*. (2012) analysed the genetic diversity of 64 rice genotypes using 20 SSR primers on chromosome number 7-12. The banding pattern was recorded in the form of 0-1 data sheet which was analysed using unweighted pair group method with arithmetic mean (UPGMA) based on Jaccard's similarity coefficient. The results revealed that out of twenty, eight primers showed distinct polymorphism, indicating the robust nature of microsatellites in revealing polymorphism. The cluster analysis showed higher level of genetic variation among the genotypes.

Similarity coefficients ranged from 0.40 to 0.96. The dendrogram revealed 8 major distinct clusters. Higher range of similarity values for related genotypes using simple sequence repeats (SSR) provides greater confidence for the assessment of genetic diversity and relationships. The polymorphism information content (PIC) value for the SSR loci ranged from 0.36 to 0.98. Higher PIC values were associated with higher level of polymorphism. The information obtained from the DNA fingerprinting studies helps to distinctly identify and characterize the various genotypes. Such information can be used in background selections during backcross breeding programs. Das *et al*. (2013) calculate the genetic distances among the accessions of 83 landraces collected from north eastern states along with 8 check accessions (total 91 accessions) using 23 previously mapped SSR markers and examined the population structure among the accessions using model-based clustering approach. A total of 182 alleles were identified which included 51 rare and 27 null alleles. They reported average PIC value of 0.7467/marker. The non-aromatic landraces from West Bengal were most diverse with 154 alleles and an average PIC value of 0.8005/marker, followed by the aromatic landraces from West Bengal with 118 alleles and an average PIC value of 0.6524/marker, while the landraces from North East ranked third with 113 alleles and an average PIC value of 0.5745/marker. In the dendrogram distinct clusters consisting of predominantly aromatic landraces and predominantly North East Indian landraces were observed. The non-aromatic landraces from West Bengal were interspersed within these two clusters. The accessions were moderately structured, showing four sub-populations (A-D) with an Fst value of 0.398, 0.364, 0.206 and 0.281, respectively. The assigned clustering of accessions was well in agreement in both distance-based and model-based approaches. Park *et al*. (2019) studied genetic diversity within germplasm collections by using simple sequence repeat (SSR) markers and association mapping techniques in coloured rice germplasm collection containing 376 black-purple rice samples and 172 red pericarp

samples. A total of 409 polymorphic amplified fragments obtained using the 16 SSR markers were reported. The reported number of alleles per locus ranged from 11 to 47, with an average of 25.6. The average PIC value was 0.913, ranging from 0.855 to 0.964. Four hundred and nine SSR loci were used to calculate Jaccard's distance coefficients, using the unweighted pair-group method with arithmetic mean cluster analysis. These accessions were separated into several distinctive groups corresponding to their morphology. The results provided valuable information for the coloured rice breeding program and showed the importance of protecting germplasm resources and the molecular markers that can be derived from them.

From the commercial point of view, DNA fingerprinting is a useful tool for varietal protection to prove ownership or derivation of plant lines. Moreover, the analysis of genetic diversity and relationship between or within different species, populations and individuals is a prerequisite towards effective utilization and protection of plant genetic resources (Weising *et al*., 1995). In the late 1980s quantitative trait loci (QTL) mapping methods for identification of genes or QTL controlling quantitative traits was developed which has become an important landmark achievement in plant genetics research (Doerge, 2002; Semagn *et al*., 2010). Due to its limitation such as small resolution in detecting QTLs or long duration for detection as establishment of biparental population is required association mapping method become the alternative method for detection of QTL or for finding marker trait association of major crops. Since linkage analysis required development of mapping population, association mapping doesn't require such population but instead uses diverse populations, or individuals with contrasting geographical origin (Lipka *et al*., 2015). So, this strategy reduced time and other resources since phenotypic data are available for the populations that are used for analysis and controlled crosses are not required for mapping population. Hence, the populations utilize in association mapping have undergone many generations of recombination since domestication and

therefore in general, only markers that are physically located close to the trait of interest will be detected as significant (Verdeprado *et al*., 2018). Grain yield is known to be a complex character contributed by various other traits which lead to difficulties in the identification of genes controlling this quantitative trait. Aluko *et al*. (2004) identify and characterize quantitative trait loci (QTLs) among 312 doubled haploid lines derived from the BC3F1 of an interspecific cross of O. sativa × O. glaberrima, they evaluated grains quality such as amylose content, grain length, grain width and grain shape using 100 polymorphic microsatellite markers. They detected one major QTL, amy6 on chromosome 5 with two additional QTLs on chromosomes 3; 8-amy3 and amy8, respectively. The QTL gl3 for the grain length was detected in chromosome 3 which was also the same region in other populations for grain length. No statistical difference for grain width was reported in this study whereas Tan *et al*. (2000) detected a major QTL for grain width on chromosome 5 and a minor locus on chromosome 6 in both F2, 3 and RIL populations of rice. Ge *et al*. (2005) analysed recombinant inbred population from a cross between two indica cultivars, Zhenshan 97 and Minghui 63 for quantitative trait loci (QTL) by using a linkage map based on 221 molecular marker loci covering a total of 1796 cM. They identified three QTLs for cooked rice grain elongation on chromosome 2, 6 and 11. Another six QTLs was identified for cooked width expansion on chromosome 1-3, 6,9 and 11. A range of studies has been done on rice in relation with association mapping which focus on association with important agronomic and morphological traits. SSRs markers were reported to be used initially in association mapping of rice, Agrama *et al*. (2007) identified a total of 25 marker-trait associations with 21 different SSR markers. RM85 on chromosome 3 was associated with kernel width and 1000 kernel weight, RM122 on chromosome 5 was associated with kernel length and 1000 kernel weight, RM459 also on chromosome 5 was associated with kernel length and 1000 kernel weight, and RM228 on chromosome 10 was associated with grain yield and length width

ratio. Seventeen of the 25 associations were in regions where QTL associated with the given trait had previously been identified. Of the 25 marker-trait associations, seven were identified as explaining 20% or more of the total variation for GY (RM261, RM228), KL (RM284), LWR (RM7, RM228), and TKW (RM440, RM122). Only RM284 associated with kernel length was not in the region of a previously identified QTL for the associated trait. Amaravathi *et al*. (2008) identified one QTL for grain length and cooked kernel elongation ratio on chromosomes 1 and 11 respectively. Mixed linear model (MLM) technique was used to detect SSR marker loci associated with three agronomic traits, i.e., panicle length, plant height, and heading date on chromosome 7 from a diverse Chinese rice germplasm by Wen *et al*. (2009). Eight markers displayed meaningful association with four different parameters as panicle number, amylose content, head-milled rice, and yield (Borba *et al*., 2010). Rathi *et al*., (2014) detected significant marker association for amylose (RM282 on chromosome 3) and grain length ratio (RM142 on chromosome 4). A total 242 rice accessions were examined to find out the association of yield parameters and grain-quality traits with 86 SSR markers by using mixed linear model. Zhang *et al*. (2014) mined elite genes within rice landraces and detected various association on different panel. They used 12 agronomic traits for association studies with 274 SSR markers. They reported 76 significant (P, 0.05) trait-marker associations using mixed linear model (MLM) within Panel 1 in two years, among which 32% were identical with previously mapped QTLs. A total of seven aforementioned trait-marker associations were verified within Panel 2 and 3 when using a general linear model (GLM) and 55 SSR markers of the 76 significant trait-marker associations. However, no significant trait-marker association was reported to be identical within three panels when using the MLM model. several desirable alleles of the loci which showed significant trait-marker associations were identified. The research provided important information for further mining these elite genes within rice landraces and using them for rice

breeding. Association between different markers with grain quality such as amylose content, gel consistency, gelatinization temperature, grain length and width, decorticated grain length were also reported in indigenous rice (Verma *et al*., 2015). Fei-fei *et al*. (2016) investigated 416 rice accessions with 143 markers including 100 SSR markers and 43 gene-tagged markers to help achieve higher yield, phenotype variations of heading date (HD), plant architecture and grain shape through association mapping. The SSR markers, RM267, RM340 and RM346, were linked to at least two traits. Feng *et al*. (2016) utilised 469 indica accessions collected from 20 countries for genome wide association mapping panel. They analysed four grain quality such as grain length, grain width, thousand grain weight and grain length-width ratio. They observed a total of 27 significant loci for four grain traits under study. Out of 27 loci, GS3 and qSW5 was reported to show a strong effect on grain length and width. For grain length trait, 8 major loci were identified on chromosome 2, 3, 4,5, 8 and 12, 4 major loci were identified for grain width on chromosomes 1, 2, 4 and 5. Another 5 loci were associated with length width ratio on chromosomes 2, 3, 4, 5 and 10. For the trait thousand grain weight 10 major loci was identified on chromosome 1, 3, 4, 5, 7 and 11. Asante (2017) described grain quality as a complex trait which comprises milling, appearance such as grain size and chalkiness, eating properties which is influence by starch properties; amylose content, gelatinization temperature, gel consistency as well as nutritional quality. Functional markers, especially those targeting mutations in the BADH2, waxy, *alk* and GS3 genes, are reported to be highly associated with aroma, AAC/RVA, GT and grain size, respectively; and thus, effective for marker-assisted breeding. Rice texture is mostly controlled by the allele at the waxy locus (Wx) on chromosome 6 (Mackill and Khush, 2018). Suman *et al.* (2020) reported a total of 37 significant marker-trait association in a set of 24 genotypes for 17 grain quality characters. Eight markers a (RM246, RM11, RM241, RM16427, RM421, RM3, RM234 and RM257) showed association with more than one

character suggesting their utility for the selection for grain quality characters which can be deployed in the rice crop improvement programmes. Donde *et al*. (2020) did evaluation on 60 genotypes comprising New Plant Types (NPTs), indica rice, tropical and temperate japonica rice for yield and its component traits. They utilised 85 SSR markers to identify QTLs associated with grain yield and its related traits. Sixty-six (66) markers were recorded to be polymorphic. Their association analysis using GLM and MLM models led to identification of association between 30 and 10 SSR markers with 70 and 16 QTLs respectively. Yield attributing traits such as tiller number, panicle length, and flag leaf length, flag leaf width, total no. of grains, 1000 grains weight, fertile grains, seed length-breadth ratio, plant height, days to 50% flowering and grain yield per plant were reported to be associated with 30 novel QTLs that linked with 16 SSRs. Yang *et al*. (2021) studied marker-trait association on 57 Shanlam upland rice accession with 48 SSR markers, they detected 25 25 SSR markers with agronomic traits such as plant height, effective no. of panicles per plant, panicle length, total grain number, filled grain number, seed setting rate and 1000 grains weight. Of these associations, RM208 explained 42.62% of total variations in plant height of Shanlan upland rice, 8 markers were significantly associated with 2 traits, 3 markers with 3 traits, and 3 markers with 4 traits. In particular, RM493 was significantly associated with 6 traits. Therefore, these validated markers and new markers from previous research will be useful to improve rice grain yield, plant architecture and grain quality when performing marker-assisted selection of proper alleles.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation entitled "Genetic variation and association mapping of grain quality, yield and yield attributing traits in lowland rice of Nagaland" was conducted in ICAR-Research Complex for NEH region's research farm and Central Laboratory, Medziphema, Nagaland situated at 98º and 96º East longitude and 26º 6´ and 27º 4´ latitude North of the Equator. The field experiment was carried out for two *kharif* seasons of 2020 and 2021.

Recorded meteorological data such as temperature, rainfall, relative humidity and average sunshine hour for the period of experiments extending from June to December, 2020 and 2021 were collected from ICAR Research Centre for NEH region, Medziphema, Nagaland and are presented in **Table 3.1**.

3.1 Experimental materials

The experimental material for the present investigation comprised of 81 rice genotypes, low landraces collected from various districts of Nagaland including two improved varieties viz. RCM09 and Ranjit from ICAR, NEH region, Nagaland are presented on **Table 3.2**. Each and every germplasm were subjected to phenotyping for various traits for grain quality such as gain length (mm), grain width (mm), decorticated grain length (mm), decorticated grain width (mm), grain shape. Grain elongation ratio, amylose content (%), gel consistency (mm), gelatinization temperature (ºC) was also estimated using standard protocol. Yield attributing traits such as plant height (cm), panicle length (cm), no. of panicles per plant, no. of filled grains per panicle, no. of unfilled grains per panicle, 1000 grains weight (g), days to 50% flowering, days to maturity, grains yield per plant (g) were also recorded. The phenotyping panel was followed by genotyping using 36 SSR markers in order to study the level of genetic diversity among the germplasm and marker-trait association.

Table 3.1 Meteorological data during the period of field experiment i.e., June to December 2020 and 2021.

Months	Av. Temp (°C)			Av. RH (%)			Total rainfall (mm)	Av. Sunshine hours
	Max.	Min.	Av.	Mor.	Even.	Av.		
Jun. 2020	32.4	23.8	28.1	92	72	82	266.2	3.9
Jun. 2021	33.1	24.3	28.7	93	69	81	117.4	3.4
Jul. 2020	32.4	24.5	28.5	94	74	84	199.9	2.6
Jul. 2021	33.3	24.8	29.1	92	72	82	272.2	3.9
Aug. 2020	33.7	25.0	29.4	93	70	81.5	80.3	4.4
Aug. 2021	32.6	24.6	28.6	93	72	83	138.8	3.2
Sept. 2020	32.5	24.3	28.4	95	73	84	157.6	4.8
Sept. 2021	33.1	23.8	28.5	94	68	81	116.2	5.9
Oct. 2020	31.2	23.0	27.1	95	74	84.5	175.7	5.2
Oct. 2021	32.1	22.1	27.1	95	68	82	130	6.4
Nov. 2020	27.9	15.6	21.8	97	59	78	35.2	6.7
Nov. 2021	28.5	14.8	21.7	96	51	74	0.0	7.9
Dec. 2020	24.5	9.8	17.2	97	52	74.5	0.0	7.0
Dec. 2021	25.1	11.3	18.2	95	51	73	16.4	6.3

Table 3.2 List of genotypes under study

Sl. No.	Name of genotypes	Source	Sl. No.	Name of genotypes	Source
1	Pluchama Ngoba	Kohima	36	Tsomone	Tuensang
2	Tenyizhu Ngoba	Kohima	37	Aodong Tsonyak	Tuensang
3	Kemenya (Rüjoi)	Kohima	38	Tu-Tso	Tuensang
4	White Mekrilha	Kohima	39	China-Tsone	Tuensang
5	Mekrilha	Kohima	40	Betguti	Mokokchung
6	Bokadzii	Phek	41	Nuno Tsuk	Mokokchung
7	Japan Rice	Phek	42	Aspa	Zunheboto
8	Thevure	Phek	43	Abor	Kohima
9	Kerebe	Phek	44	MehÜrÜ	Kohima
10	Förie	Phek	45	Mekrilha	Kohima
11	Otokewerü	Phek	46	Tsorenyu	Kohima
12	Makre Tanye	Phek	47	ThevÜrÜ	Kohima
13	Kumure	Phek	48	Ngoba Kerieu	Kohima
14	Tsive	Phek	49	Ngoba Kenou	Kohima
15	Daha	Phek	50	Kemenya I	Kohima
16	Kutsanie	Phek	51	Kemenya II	Kohima
17	Poramunyo	Phek	52	Ngoba (Short)	Kohima
18	Nechorei	Phek	53	Mekrilha II	Kohima
19	Pochury	Phek	54	Runlonya	Kohima
24	Vamuzo	Phek	55	Tengo	Kohima
25	Thuzolha	Phek	56	TevÜrÜ	Kohima
26	Kemene	Phek	57	Kemenga (Pointed)	Kohima
28	Kuthunü	Phek	58	Tevu-Rupru	Kohima
29	Thujure	Phek	59	Kheyahi	Kohima
30	Tsokang	Tuensang	60	Lhasari	Kohima
31	Tochokoi	Tuensang	61	Makilha II	Kohima
32	Ani	Tuensang	62	Kemese-U	SocÜno
33	Chokla Tsou	Tuensang	63	Kemenya	SocÜno
34	Ashay	Tuensang	64	N. Special Bobla	SocÜno
35	Longka	Tuensang	65	Lihati	SocÜno

Table 3.2 List of genotypes under study

Sl.No.	Name of genotypes	Source	Sl.No.	Name of genotypes	Source
66	N.Special	SocÜno	74	Neingutsure	Phek
67	Yeihpho	SocÜno	75	Pelhirie	Phek
68	K.Special I	SocÜno	76	Kofie	Phek
69	Ario Special	RÜziephe	77	Manaba	Phek
70	K. Special II	RÜziephe	78	Tanyomezu	Phek
71	Asukhomi I	Zunheboto	79	Taghaho	Phek
72	Asukhomi II	Zunheboto	80	RCM09	ICAR
73	Egiru	Phek	81	Ranjit	ICAR

3.2 Experimental methods

3.2.1 Layout of the field experiment

The field experimental materials were laid down in Randomised Block Design (RBD) consisting of three replications. Each replication consists of 81 genotypes which were allotted randomly. The required essential techniques for the success of growth of rice were followed throughout the field preparation. The standard quantity of fertilizers and manures were applied for optimum growth of the plants. The details of the experimental design are given below:

Experimental Design	:	Randomised Block Design (RBD)
No. of replication	:	3 (three)
No. of genotypes	:	81 (eighty-one)
Gross plot size	:	70 X 16 m^2
Net plot size	:	51 X 9 m^2
Spacing	:	30 X 30 cm^2
Season	:	Kharif 2020 and 2021

3.2.2 Recording of field observations

In the present investigation, by using simple random sampling method five representative plants were selected from the middle rows of each plot for recording phenotypic data using Protection of New Plant genotypes (UPOV) guidelines (UPOV, 1985) and National guidelines (DRR, 2007), for DUS (Distinctiveness, Uniformity and Stability) test (Rani *et al*., 2006). The phenotypic data were collected from the following parameters for the present trial.

1. Days to 50% flowering

   The no. of days from the date of sowing to the day of initiation of flowers in 50% of plants from each plot was recorded.

2. Days to maturity

   The no. of days from the date of sowing to the day of grain maturation (80% maturity) was recorded from each plot.

3. Plant height (cm)

   The height of five randomly selected plants were measured form the base of the stem just above the ground to the tip of the spikelet.

4. Panicle length (cm)

   The length of five randomly selected plants from each accession were measured from the neck to the tip of the panicle.

5. No. of panicles per plant

   Five hills were selected from each plot and the no. of panicles in each hill were counted to record no. of panicles per plant.

6. No. of filled grains per panicle

   The no. of filled grains were counted after removing chaffy grains from each panicle of five randomly selected plants.

7. No. of unfilled grains per panicle

   The no. of unfilled grains were counted from each panicle of five randomly selected plants.

8. 1000 grains weight (g)

   Weight of one thousand randomly chosen filled grains were recorded on gram from each accession.

9. Grains yield per plant (g)

   Total weight of grains per plant from each accession were recorded to express grains yield per plant in gram.

3.2.3 Determination of quality parameters

3.2.3.1 Determination of physical quality parameters

For determination of physical quality parameters, measurements were recorded as per international union for Protection of New Plant genotypes (UPOV) guidelines (UPOV, 1985) and National guidelines (DRR, 2007), for DUS (Distinctiveness, Uniformity and Stability) test (Rani *et al*., 2006).

1 Grain length (mm)

The length of random ten unhusked grains from sampled grains were measured by using dial calliper in terms of millimetre and the average of the measurements was taken to determine the length of the grain.

2 Grain width (mm)

The width of random ten unhusked grains from sampled grains were measured by using calliper in terms of millimetre and the average were taken to determine the width of the grain.

3 Decorticated grain length (mm)

Decorticated grain length was measured in millimetre from ten random fully dried hulled grains from sampled grains using dial calliper. The average of the measurements was taken to express the length of decorticated grain.

4 Decorticated grain width (mm)

Decorticated grain width was measured in millimetre from ten random fully dried hulled grains from sampled grains using dial calliper. The average of the measurements was taken to express the width of decorticated grain.

5 Grain shape

Grain shape of the accessions were computed from the ratio of decorticated grain length to decorticated grain width which were measured in millimetre by using dial calliper. The shape of grain was determined by using the following standards.

Grain shape as referred in Rice research in India: ICAR publication, 1985 (Ramaiah, 1969):

Kernel length (mm)	Length/breadth ratio	Grain shape
<6.0	>3.0	Short slender
<6.0	<2.5	Short bold
<6.0	>2.5-3.0	Medium slender
>6.0	>3.0	Long slender
>6.0	<3.0	Long bold
>6.61	>3.0	Basmati type
>7.5	>3.0	Extra long

6 Grain elongation ratio

The ratio of length of cooked rice to uncooked rice was computed from ten randomly selected grains from the sampled grains to express grain elongation ratio.

3.2.3.2 Determination of biochemical properties

For determination of certain rice grain quality i.e., biochemical parameters, healthy grain samples were collected from each accession. Dehusked rice grain

were grounded into powder using mortar and pestle and used for different estimation.

1 Determination of Amylose content

Amylose content in each accession was determined by following the method described by Juliano (1971). The method has been described below.

A moisture free powdered rice grain (100 mg) was taken in a capped volumetric flask of 100 ml. One millilitre of 95% alcohol was added followed by gently pouring of nine millilitres of 1N NaOH at the side of the volumetric flask. The volumetric flask was kept in a boiling water bath for 15 minutes. After cooling in room temperature, the volume of the content was made up to 100 millilitres with distilled water. Then five millilitre of above dispersion was pipette out and transferred into another 100-millilitre volumetric flask followed by addition of one millilitre acetic acid and two millilitre iodine (0.2%) solution and the final volume was made to 100 millilitres with distilled water.

The solution was well mixed and allowed to stand for 20 minutes in a dark place. The intensity of colour developed was recorded by using spectrophotometer at 630 nm against reagent blank. Transmittance was recorded and standard graph was prepared from standard amylose solution.

A standard amylose solution was also prepared as well as a blank reagent solution. One millilitre of a standard solution was taken treated using the same procedure.

Amylose content in the sample was calculated by following the relationship given below.

$$Amylose\ content = \frac{R}{A} X\ \frac{a}{r} X \frac{1}{5} X100$$

Where, R = 630 nm reading for the sample

A = 630 reading for standard amylose solution

a = Amount of standard amylose taken

r = Amount of sample taken

The estimation was done in triplicate and their mean was recorded as gram of amylose per 100 g in moisture free sample.

Based on the amylose content percentage the accession was grouped as follows (Juliano 1979).

Category	**Amylose content (%)**
Waxy	1-2
Very low amylose	2-9
Low	10-20
Intermediate	20-25
High	25-30

2 Determination of Gel consistency

Gel consistency in each accession was determined by the method of Cagampang *et al*. (1973).

A 100 mg powdered rice grain sample of each accession was taken in a culture tube (13 x 100 mm). A 0.2 ml of 95% ethanol which contain 0.025% thymol was used to wet the samples. The ethanol will prevent clumping of the rice powder during alkali gelatinization, while thymol blue imparts colour to the alkali paste to make the gel front easier to read. The culture tubes were then shacked followed by immediately adding 2.0 ml of 0.2N KOH and the mixture was dispersed. The culture tubes were then covered with a lid and placed in a vigorously boiling water bath for 8 minutes. Then the culture tubes were then removed from the water bath and left at room temperature for 5 minutes and then cooled in ice water for 15 minutes. The tubes were then laid horizontally over a ruled paper graduated in millimetres.

The length of the gel consistency of the samples were classified as given below (Cagampang *et al*. 1973).

Category	Gel consistency (mm)
Soft	61-100
Medium	41-60
Medium hard	36-40
Hard	26-35

3 Determination of Gelatinization temperature

Gelatinization temperature (GT) of each sample was assessed indirectly as the alkali spreading value (ASV) of hulled kernels as described by Little *et al*. (1958). Thirty whole grains were immersed in a petri plate, 1.7% KOH in such a way that every grain in the plate were not in contact with each other. The plates were then incubated at room temperature for 24 hours. The ASV was then determined by visual scoring of the appearance of the grains and disintegration on a 1-7 linear scale, as given below:

1 = grains not affected

2 = grains swollen

3 = grains swollen, collar incomplete or narrow

4 = grains swollen, collar complete and wide

5 = grains split or segmented, collar complete and wide

6 = grains dispersed, merging with collar

7 = grain completely dispersed and intermingled.

Gelatinization temperature is inversely proportional to Alkali Spreading Value (ASV) therefore, a higher value of ASV will give a low gelatinization

temperature. A scoring of ASV value 1.00 -2.99 was taken as high gelatinization temperature of more than 70^{o}C, a value between 3.00-4.99 as intermediate gelatinization temperature of between 70 to 74^{o}C. Whereas, ASV scoring value between 5.00-7.00 was taken as low gelatinization temperature of 55 to 68^{o}C.

3.2.4 DNA analysis

3.2.4.1 DNA isolation and purification

The total genomic DNA from each accession in the present investigation was extracted following a Della porta DNA extraction method (Della porta *et al*., 1983). DNA was extracted from 100- pooled individuals per accession (Virk *et al*., 1995a) to ensure better presentation of the accession than a single plant.

Young leaf samples were taken and washed with distilled water, the dried tips were removed and kept in room temperature for surface dry. After drying the leaves were chopped and crushed into fine powder using liquid nitrogen in mortar and pestle. The fine powder was quickly transferred into 1.5 ml eppendorf tube followed by adding 1.0 ml of extraction buffer. It was then mixed well and incubated in hot water bath at 65^{o}C for 1 hour with occasional mixing by shaking the tubes side wise and invertedly. After incubation, the supernatant was taken out in another 1.5 ml eppendorf tube and equal volume of chloroform: isoamyl alcohol (24:1) was added and the content were mixed well. The samples were then centrifuged in a bench top centrifuge at 10,000 rpm for 15 minutes. The supernatant was carefully removed and transferred in a new eppendorf tube. After adding equal volume of chilled DNA diluent, the samples were kept for 15 hours at 4^{o}C. Samples were spun at 1000 rpm for 10 minutes in order to pellet down the DNA. The DNA pellet was then washed with 70% ethanol by centrifuge at 300 rpm for 3-4 minutes. After centrifuge, the pellet was air dried and dissolved in 50 ul 1 x TE buffer.

3.2.4.2 DNA quality evaluation and quantification

Quality and quantity of extracted genomic DNA was estimated based on agarose gel electrophoresis and spectral analysis. The isolated genomic DNA of

each accession was subjected to agarose gel electrophoresis as described below, to judge their integrity.

Prior to assembling the gel casting plate, each member was thoroughly cleaned with distilled water and subsequently with 70% ethanol. Agarose gel of 0.8% was prepared by melting 0.8g of agarose in 100ml 1xTAE by heating, 10 ul of ethidium bromide was added and cooled down to 50^{o}C. The mixture was then poured into a levelled pre-set casting tray fitted with cleaned comb. After solidification, the comb was carefully separated from the gel followed by the surround and then the casting tray was detached from the casting plate. The casting tray with the gel was mounted on a submarine electrophoresis chamber, 4 ul of DNA of each sample were mixed with 2ul of 6X loading buffer. The DNA samples were loaded in each well of the gel, the gel tank was then covered with the lid and electrode was connected to the power supply unit keeping the positive terminal away from the wells. The power supply was put on and the gel was run at 50 V (5 volts/cm). The power supply was stopped when the bromophenol blue dye reached 2/3rd of the gel length.

A photograph of the gel was digitally recorded under UV light in a gel documentation system so as to enable estimation of each sample by LABWARE system.

3.2.4.3 SSR analysis

For analysing the genetic variability to grain quality in the given accessions, 40 SSR markers were used (**Table 3.3**). The amplification condition was based on the procedure of Panaud *et al*. (1996).

3.2.4.4 PCR and DNA amplification

A reaction mixture consisting of 5 ul Takarn Emerald Amp ® €T PCR master mix, 0.5 ul forward primer, 0.5 ul reverse primer, 1 ul extracted DNA sample and 3 ul H_2O.

The reagents were mixed thoroughly and then placed in a Thermal cycler, Applied Biosystems 9700 (Perkin Elmer Applied Biosystems, Foster City, CA) using the following cycling protocol for amplifications.

Step 1 (Initial denaturation)	94^{o}C for 3 min
Step 2 (Denaturation)	94^{o}C for 45 sec
Step 3 (Annealing)	58^{o}C for 30 sec
Step 4 (Extension)	72^{o}C for 30 sec
Step 5 (Final annealing)	72^{o}C for 10 min
Step 6 (Storage)	4^{o}C to hold the sample

Note: The step 2,3 and 4 were repeated for 35 times.

3.2.4.5 Gel electrophoresis and photography

For separation of PCR products with SSR markers, 2.5% agarose gel was used. The reagents used for preparation of 2.5% are given in Appendix. Agarose gel of 2.5% strength was prepared by melting 5 gram of agarose in total volume of 200 ml 1x TBE by heating, 10 ul of ethidium bromide (10mg/ml) was added, the solution was cooled down to 50^{o}C. Th solution was then poured into pre-cleaned casting tray. After solidification, comb was carefully removed and then the casting tray was mounted in a gel tank containing 1000 ml of 1xTBE buffer. To each PCR tube containing the amplified products, 2ul of 6x loading buffer was added, mixed well and then a total of 10 ul of each PCR product was loaded into the wells of the agarose gel. Gel was run after adjusting the voltage till bromophenol blue reached the end of the gel. The photograph of the gel was digitally documented in Gel Documentation System.

3.2.4.6 Scoring of DNA data

By using DNA ladder of known molecular weight, the PCR products obtained for each primer from SSR analysis were scored per the format of different software utilised in the present investigation while rejecting faint bands and bands with smeared background. Only intense bands were scored by designating as '1' if the product was present in a certain genotype, and '0' if the product was absent to study genetic diversity and markers polymorphism. The base pairs size for each SSR product were also recorded to study population structure and marker-trait association. Accession failing to amplify a product was assigned null allele at that locus.

3.3. Statistical analysis

3.3.1 Analysis of variance

The mean value of each trait of the recorded field experiment data was tabulated and subjected to analysis of variance by following Gomez and Gomez (1987).

Standard error of mean difference and Critical difference were also calculated to find out the significance of mean difference between genotypes.

$$S.\ Ed = \sqrt{2EMS/r}$$

Where,

EMS = error mean sum of squares,

r = number of replications.

C.D = S. Ed x t at error degree of freedom at 5 percent

3.3.2 Analysis of genetic parameter

1 Genotypic variance (σ_g^2)

Genotypic variance was computed according to Burton and Devane (1953) as given below:

$$\sigma_g^2 = \frac{MSg - MSe}{r}$$

Where, MSg = Mean square due to genotype

MSe = Mean square due to error

R = number of replications

2 Phenotypic variance (σ_p^2)

Phenotypic variance was also computed by following the formula given hereunder:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

Where,

σ_e^2 = error variance which is nothing but error mean square

3 Genotypic co-efficient of variation (GCV), Phenotypic co-efficient of variation (PCV) and Environmental co-efficient of variation (ECV)

Co-efficient of variation for genotype, phenotype and environment were calculated using the following formula given by Burton and Dvane (1953).

$$\text{GCV} = \frac{\sqrt{\text{genotypic variance } (\sigma_g^2)}}{\bar{X}} \times 100$$

$$\text{PCV} = \frac{\sqrt{\text{phenotypic variance } (\sigma_p^2)}}{\bar{X}} \times 100$$

$$ECV = \frac{\sqrt{environmental\ \ variance\ (\sigma_e^2)}}{\bar{X}} \times 100$$

Where,

$\bar{X}$ = general mean of the character under study

4 Heritability in broad sense (h^2)

Heritability in broad sense is the ratio of genotypic variance to the phenotypic variance expressed in percentage. It was calculated by the formula given by Johnson, Robinson and Comstock (1956).

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

5 Expected genetic advance (GA)

Expected genetic advance for each character was calculated by using the formula given by Allard, 1960.

$$GA = \ k.\sigma_p.h^2$$

Where,

K = Selection differential at 5% selection intensity, the value of which is 2.06

σ_p = Phenotypic standard deviation

h^2 = Heritability in broad sense

Genetic advance as per cent of population = $\frac{\text{Genetic advance}}{\text{X}}$ x 100

Where,

X = Mean of base population

6 Correlation studies

The genotypic and phenotypic correlation coefficients between two characters X and Y under studies were calculated. For this data was subjected to the analysis of covariance and the respective correlation coefficients were computed using the following formula:

a) Genotypic correlation coefficient

$$r_{gxy} = \frac{\sigma_{gxy}}{\sqrt{\sigma_{gx}^2} \times \sqrt{\sigma_{gy}^2}}$$

Where,

r_{gxy} = genotypic correlation between x and y

σ^2_{gx} = genotypic variance of x

σ^2_{gy} = genotypic variance of y

σ_{gxy} = genotypic covariance of x and y

$$\sigma_{gxy} = \frac{MSP_g - MSP_e}{r}$$

Where,

MSP_g = Genotypic mean sum of product

MSP_e = Error mean sum of product

R = Number of replications

b) Phenotypic correlation coefficient

$$r_{pxy} = \frac{\sigma_{pxy}}{\sqrt{\sigma^2_{px}} \times \sqrt{\sigma^2_{py}}}$$

Where,

r_{pxy} = Phenotypic correlation between x and y

σ^2_{px} and σ^2_{py} = Phenotypic variances of x and y respectively

σ_{pxy} = Phenotypic covariances between x and y

$\sigma_{pxy} = \sigma_{gxy} + \sigma_{pxy}$

C. Path coefficient analysis

By *Wright*, 1921

The path coefficient analysis measures the direct and indirect contribution of various independent characters on a development character. The path diagram may be explained in terms of cause-and-effect relationship of different variables.

The direct and indirect effect will be rates as follows by Lenka and Mishra (1937).

0.00-0.09 --------- Negligible

0.10-o.19 --------- Low

0.20-0.29 --------- Moderate

0.30-1.00 ---------- High

3.3.3 Genotypic data analysis

From the generated binary data. Polymorphic Information Content (PIC) (Nei, 1973) and D (Discrimination power) scores (Kraakman *et al*., 2004) of each marker were calculated using the following formula:

$$PIC_j = 1 - \sum_{i=1}^{I} p_i^2$$

Where, *Pi* is the frequency of the *i*th allele of a given *j*th SSR locus across all genotypes.

For calculating D for each SSR marker the following formula was used.

$$D_j = 1 - \sum_{i=1}^{I} p_i \frac{(Np_i - 1)}{N-1}$$

Where, N is the total number of accessions and *Pi* is the frequency of the *i*th allele at a given *j*th SSR locus.

For calculating genetic similarity (GS*j*) between a pair of genotypes, Jaccards's Index (Ingvarsson, 2010) was used and cluster analysis, by means of a

dendogram was conducted on the GS*j* estimates using UPGMA and Jaccards's index procedure in Darwin software.

3.3.4 Study of population structure

Population structure was studied using STRUCUTURE software (Pritchard *et al*., 2000). For analysing the population structure and kinship, number of randomly inherited polymorphic SSR markers was selected from a set of markers. The Q matrix was constructed by following the model-based approach as described by Pritchard *et al* (2000) and Falush *et al.* (2003) which was implemented in the software structure (Pritchard *et al.* 2000). For analysis of the data, set parameters of the population admixture model and correlated frequency of alleles was considered. The genetic distance among the K structure clusters was computed applying the neighbour- joining algorithm to the matrix of allele frequency divergence among clusters in GenALex ver. 6.5. The kinship matrix was generated for MLM study using Tassel 5.2.86 package.

3.3.5 Linkage disequilibrium analysis

Several statistics have been proposed for LD, and these measurements largely differ in how they are affected by marginal allele frequencies and small sample sizes (Hedrick, 1987). Both *D′* (Lewontin, 1964) and r^2 (Hill and Robertson, 1968) have been widely used to quantify LD. For two biallelic loci, *D′* and r^2 have the following formula:

$$D' = \frac{|D|}{D_{\max}}$$

$$\text{where} \quad D_{\max} = \min(p_A p_b, p_a p_B) \text{ if } D > 0;$$

$$D_{\max} = \min(p_A p_B, p_a p_b) \text{ if } D < 0$$

$$r^2 = \frac{D^2}{p_A p_a p_B p_b}$$

3.3.6 Study of marker-trait association to identify genomic regions for economically important traits in rice

Both genotypic and phenotypic data along with Q and K used to identify markers linked with target traits. Marker-traits association was studied applying GLM and MLM in TASSEL software. Single factor analysis of variance (SFA) was Implemented without Q & K, general linear model (GLM) with Q (individuals' membership in the population) (Bradbury *et al*., 2007), mixed linear model (MLM) with K (genetic relatedness) and MLM with K + Q (Yu et *al*., 2006). Markers-trait association was considered significant at $p \leq 0.05$.

Plate 3.1 Uprooting of Seedlings

Plate 3.2 Seedling transplantation

Plate 3.3 View of experimental field

Plate 3.4 Panicle length of some genotypes

Plate 3.5 Determination of Amylose Content

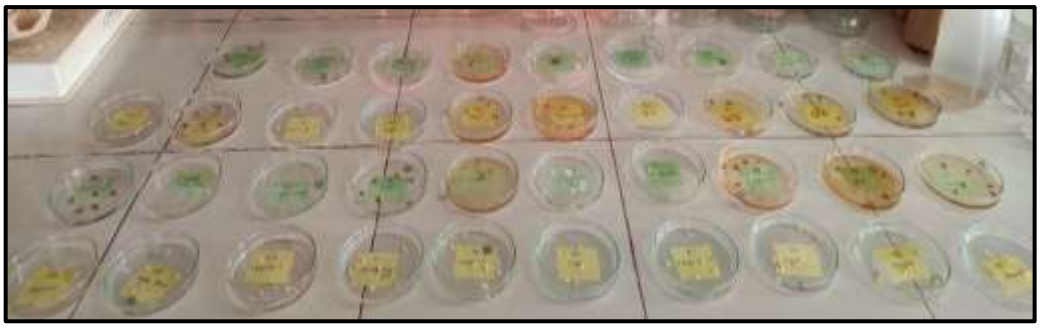

Plate 3.6 Determination of Gelatinization Temperature

Plate 3.7 Determination of Gel Consistency

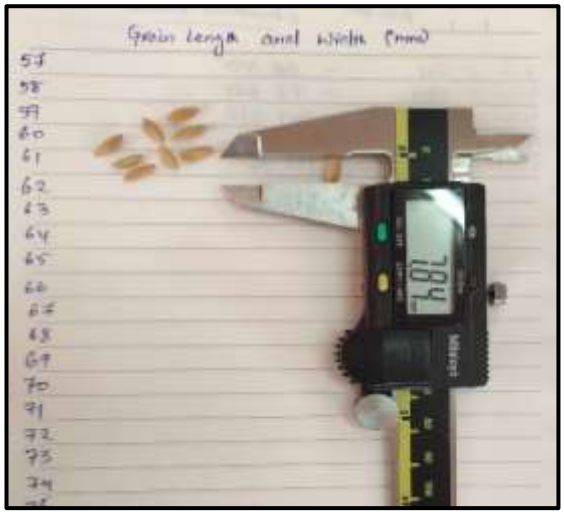

Plate 3.8 Determination of Grain Shape

Plate 3.9 Determination of Grain elongation ratio

Plate 3. 10 Extraction, Amplification and Documentation of DNA

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Rice is one of the most important staple crops and consumed by half of the world population. It provides an important dietary requirement and serves a 21% source of calories in human (Zhao *et al*., 2020). The constant growth of global population and improvement in economy of life has led to rise in demand for better quality rice which is equipped with high productivity (Verma *et al*., 2019; Cheng *et al*., 2021). Therefore, an increase in grain yield is needed to ensure food security. Since, agricultural lands are limited and clearing of untouched forest for the purpose of increase in production is not the ideal strategy to meet increase in demand of rice because of its ultimate impact on global climate as well as reduction in wildlife diversity. The role of geneticist and plant breeders has become crucial in creating an ultimate crop variety that serve various nutritional needs of mankind without disturbing the flow of ecological balance. The knowledge of genetic variation, relatedness, genetic diversity and molecular breeding thus helped the breeder in identifying useful alleles that has never been tag before and become an important tool in developing a better variety that serve the necessities of mankind for survival and for higher standard of living.

A thorough understanding on the nature and magnitude of genetic variation in order to establish a genetic relationship among the individuals and to identify and preserve the promising genotype for systematic breeding, study of genetic diversity is crucial for evaluating and comprehending the genotypes (Tomar *et al*., 2021; Roy and Shil 2020; Verma *et al*., 2019). For rice, yield is a complex character which is controlled by three main quantitative traits; panicle number, grains number per panicle and grain weight. These traits are regulated by a number of genes and some genes are highly pleiotropic (Zhao *et al*., 2011; Qian *et al*., 2016;). Hundreds of valuable genes have been identified that relate to yield and quality in rice (Liu *et al*., 2018; Yano *et al*., 2016; Huang *et al*., 2010; Huang *et al*., 2012). Grain weight related genes such as BG3 (Xio *et al*., 2021; Xia *et*

al., 2019; Yin *et al*., 2020), OsPL3 (Xio *et al*., 2021; Sun *et al*., 2020), OsSBN (Xio *et al*., 2021), OsBT1 (Song *et al*., 2020), TGW6 (Ishimaru *et al*., 2013) and OsSPL18 (Yuan *et al*., 2019) have been identified. Furthermore, genes such as NOG1 (Xiao *et al*., 2021; Huo *et al*., 2017), OsSPX1 (Xiao *et al*., 2021; Zhang *et al*., 2016) and DEP1 (Liu *et al*., 2018; Huang *et al*., 2009) were related to grains number per panicles. Panicle number related genes such as OsIAGLU (He *et al*., 2020) and DEP1 were also identified. Main component for eating quality; Amylose content is also related to gene *Wx* (Xiao *et al*., 2021). For past 30 years, these identified genes for yield and quality traits have played a crucial role in increasing grain yield and developing excellent taste characteristics in rice (Xiao *et al*., 2021). In molecular design breeding, it is very difficult to pyramid multiple target traits because of the uncertainty of the genetic structure of the existing breeding population and the lack of high throughput genotyping for genomic selection. However, illustrating superior alleles of yield and quality trait and drawing a whole genome linkage in a particular breeding population guide in balancing multiple traits based on genome-based strategy and perform breeding in a rapid, high throughput and with more precision (Xiao *et al*., 2021).

Therefore, the present study was set up with the following three objectives: (1) To study genetic variation for grain quality and yield attributing traits in lowland rice landraces of Nagaland. (2) To study association for grain quality traits and yield attributing traits in lowland rice. (3) To identify molecular marker associated with grain quality traits in rice.

4.1 Classification of genotypes based on variation in grain shape

The grain shape of each genotype was determined from kernel length and kernel length breadth ratio. Polymorphism in grain shape was observed in the present rice accession with varied frequency giving ample scope for selection on the basis of grain shape. Different genotypes with their respective grain shape are given in **Table 4.1**. Maximum genotypes, 53 genotypes had long bold type

grain shape giving 64.20% frequency to the total variation, another 14 genotypes were having short bold type grain shape contributing 17.28% frequency. Basmati type grain shape was also observed in 8 genotypes contributing 9.88% frequency. Extra-long grain shape was found in three genotypes with 2.47% frequency. One genotype each for long slender and short slender type of grain shape was also observed contributing minimum frequency of 1.23% to the total variation in grain shape.

Grain shape has effects on both yield and quality, since rice are eaten as a whole grain, its shape and size become an important quality factor that even determine the market value of rice. Extra-long and medium slender type of grain shape is mostly preferred by consumers in north India and rice growers (Singh *et al*., 2006). Roy *et al*. (2021) also observed medium slender, short medium and short slender type of grain shape in Assam rice. Grain shape such as short bold, short medium and long slender type were also reported by Mathure *et al*. (2011) in 88 aromatic rice cultivars of Maharashtra. Amudha and Thiyagarajan (2008) observed medium, bold and slender type of grain shape in landraces of Nilgiris, Tamil Nadu. Sahu *et al.* (2017) reported short slender and short bold grain shape in 215 indigenous rice landraces of Chhattisgarh and Mohan *et al*. (2021) reported short slender type grain shape as fine grain in a high yielding variety of Tamil Nadu. A collection of short bold, long slender, medium slender and medium short were also reported by Islam *et al*. (2016) in aromatic and fine rice germplasm of Bangladesh. Large grain type and slender type grain were also reported by Xiongsiyee and Prom-U-Thai (2016) in landraces cultivars of Lao, Thailand. In most of Asian countries, medium or long grain class have a good fetch at commercial scale, long- and slender- grained Basmati cultivars of India and Basmati serve a premium price in the global market. On the other hand, countries such as Japan and Sri Lanka have much preference for short- and bold grain class. Even though Indian consumer normally prefers dry flaky (non-sticky) rice the preference itself is varied within the country (Roy *et al.*,2021).

Hence, this significant variation present in the accession for this particular trait could provide a substantial opportunity for selection of suitable parent for gene contribution in breeding grain shape of our choice or high-quality rice grain through selection.

4.2 Estimation of genetic variance

Estimation of genetic variability is the fundamental step for the success of any plant improvement programme. The presence of adequate variability in traits under study and the knowledge of their system of variation can be helpful in identifying useful genotypes to be used as a donor parent in varietal development for better yield and quality rice.

4.2.1 Mean *per se* performance of 81 rice genotypes against yield and quality traits

Based on the mean *per se* performance given in **Table 4.2** Pelhirie showed minimum days of flowering (88 days) and reach maturity in 122 days, while N. Special showed longest duration to flower (124 days) and reach maturity in 154 days. On average the present study accessions took 101 days to attain 50% flowering and 126 days for maturity. As per national guidelines for DUS criteria in rice (Rani *et al*. 2006), this accession falls under 'medium' (between 121-140 days) to 'late' (141-160 days) category for maturity. However, short duration variety of rice is preferential when it comes to multiple cropping as it offers optimum time for the preparation of field for the next crop (Chen *et al*., 2022). In addition to this, the crop attains maturity before scarcity of water especially in case of rainfed cultivation and required lesser interculture operation (Ohno *et al*., 2018). Besides this, short duration rice cultivars are also reported to have high yield potential (Won *et al*., 2020). The short growth duration rice cultivars are having lower biomass accumulation and higher biomass production, it is believed that this high biomass production is a pre-requisite for high yield and high crop growth (Lang *et al*., 2012; Ibrahim *et al*.,

2013; Wang *et al*., 2017; Xu *et al*., 2018). The accession here in the present investigation can be opted for a donor parent in breeding a rice variety for different growth duration. The above results on duration of crops in the present investigation were in conformity with the reports given by Karthikeyan *et al.* (2010) in 36 rice genotypes of Anamalainagar, Tamil Nadu, under coastal salinity condition; Osundare *et al.* (2017) in 6 upland rice varieties of Nigeria, and Thakur and Pandey, (2020) in local rice germplasm of Himachal Pradesh, India.

After careful evaluation on recordings of plant height, Betguti was found to be shortest among the accession with 100.27 cm height, while Kutsanie was the tallest among the accession with 180.23 cm. The average of plant height for all the genotypes was 144.88 cm. Plant height is an important deciding factor in rice breeding for increasing yield potential. Mostly, the stature of the plant is governed by its genetic make-up, however, the influenced of environmental factors is also reported (Ashrafuzzaman *et al*., 2009). Dwarf or semi-dwarf varieties are found to be more resistant to lodging than their taller variety as lodging is reported to have a positive correlation with plant height (Wu *et al*., 2022). Since lodging is one of the environmental effects that caused reduction in grain yield the shorter genotypes could be an important gene contributor in breeding dwarf or semi-dwarf variety. In addition to this, semi dwarf varies are reported to produce higher grain yields as more resources are allocated to the grain rather than the vegetative tissues (Yang and Hwa, 2008). Even though dwarf phenotypes are beneficial to counteract rice lodging, excess short stature of plant will lead to insufficient growth and ultimately affect the yield potential of rice (Zhang *et al*., 2017). Therefore, it is essential to explore and identify gene that control plant height and exploit them in rice breeding. Hien *et al*. (2007) have reported the possibility of increasing rice yield through reduction of plant height. In relation with plant height, our results are in conformity with the report given by Nishant *et al*. (2017) in submergence tolerance of rice germplasm

which include indigenous landraces, local cultivars, released varieties and breeding lines; Tiwari *et al*. (2019) also reported the similar results on rainfed lowland rice of Nepal; Chakraborty and Chakraborty (2010) in bold grained rice gene pool of Barak valley, Assam; Akshay *et al*. (2022) and Faysal *et al*. (2022) in Aman rice genotypes of Bangladesh.

In the present investigation Kerebe was recorded with longest panicle length (39.93 cm), while Vamuzo showed shortest panicle length (19.91 cm), the average length of panicle for the whole accessions was 29.66 cm. More or less similar range of variation in panicle length to the present investigation was also reported by Akshay *et al.* (2022) and Thakur and Pandey, (2020) in local rice germplasm of Himachal Pradesh. Nishant *et al.* (2017) and Sadhana *et al.* (2022) reported panicle length as short as 17 and 19 cm. Panicle length is also one among yield-related traits. Panicle length with grain number and density, seed setting rate and grain plumpness determines the grain yield per panicle, hence, grain yield potential (Liu *et al*., 2016).

More number of panicles per plant is an ideal trait for increasing yield potential. Makre Tanye showed maximum number of panicles per plant (17.71) comparing to other genotypes, while Lhasalu was recorded with minimum number of panicles per plant (5.85). The whole accession was found to have 9.53 panicles per plant in average. Whereas, Indigenous *Ahu* rice germplasm of Assam has a record of 4 to 8 panicles per plant (Sarma *et al*., 2022), Ahmed *et al*. (2021) also observed a 4 to 16 panicles per plant in Assam rice. Chakraborty and Chakraborty (2010) observed a wide range of variation for this character in bold grained genotypes of Assam, while some genotypes had 3 panicles some genotypes had as many as 20 panicles. Karthikeyen *et al.* (2010) observed an average count of 11.31 panicles or plant in rice genotypes of south India, Nishant *et al.* (2017), Nandini *et al.* (2017) submergence tolerant germplasm of Karnataka, and Sadhana *et al.* (2022) in 36 hybrid rice genotypes also reported the similar findings in relation to the number of panicles per plant.

No. of filled grains per panicle highly determined the yield of grains per plant, if there are numerous unfilled grains the yield of the plant will be lesser irrespective of the length and number of panicles per plant. In the present study, Lhasalu had highest number of filled grains per panicle (211.73) while Yeipho has highest number of unfilled grains per panicle (94.25). Thevuru was recorded with least number of filled grains per panicle (21.97) while Furie was recorded with lowest number of unfilled grains per panicle (7.61). The overall performance of the accessions for fill grains per panicle and unfilled grains per panicle was 75.05 and 36.65 respectively. Mustafa and Yassir Elsheikh, (2007) in 14 rice genotypes of Sudan; Islam *et al*. (2016) in 113 aromatic and fine rice and Naik *et al*. (2020) also reported more or less similar findings in hybrid rice related to filled grains per panicle and unfilled grains per panicle in the present study.

Kemenya was observed with highest 1000 grains weight of 40.20 g while minimum weight of 18.90 g was recorded in Lhasalu for 1000 grains. The overall mean performance of the accession for 1000 grains weight was 28.65g. Selection of genotypes having higher grain density is also an important and effective measure to develop high yielding variety (Shahidullah *et al*., 2017). A wide range of variation for 1000 grains weight can exist in a collection of rice germplasm, earlier reports on diverse rice germplasm such as rice genotypes of Sudan (Mustafa and Yassir Elsheikh, 2007), aromatic and fine rice of Bangladesh (Islam *et al*., 2016), traditional rice varieties (Nandini *et al*., 2017) and hybrid rice (Naik *et al*., 2020) were also observed to be in conformity with our result on 1000 grains weight.

A wide range of variation was observed among the accession for grain yield per plant, Egiru was recorded to give lowest yield per plant (4.48 g) while N. Special Bobla gave highest yield (66.75 g) which was higher than two check varieties i.e., RCM09 (33.33 g) and Ranjit (26.66 g). The average performance of all the genotype for grain yield was 22.79 g. Rukmini *et al*. (2016) and Nishant

et al. (2017) also reported similar results in related to grain yield per plant south India rice. Lakshmi *et al*. (2017) also reported similar findings in advanced generation of aromatic rice and Selvaraj *et al.* (2017) in blast resistant rice.

Among the grain quality traits, longest grain length was found in White Mekrilha (11.17 mm) while Betguti had shortest grain length (6.92 mm). The average performance of the genotypes for this trait was 8.91 mm. Highest grain width was recorded in China-Tsone (4.25 mm) while least grain width was recorded in Lihati (1.75 mm), for this trait, the average performance was 3.12 mm. Islam *et al*. (2016) and Nandini *et al*. (2017) also reported a range value in grain length and grain width in different set of germplasm which is similar to the findings in the present study.

Maximum and minimum decorticated grain length was recorded in White Mekrilha (7.97 mm) and Betguti (4.94 mm) respectively with overall average of 6.46 mm. Poramuanya was recorded with decorticated grain width of 3.38 mm while White Mekrilha was recorded with 1.28 mm decorticated grain width making them highest and lowest value for the particular trait. The mean value of decorticated grain width was 2.61 mm. The above findings for decorticated grain size are in conformity with reports given by Das *et al.* (2007) in 20 promising rice genotypes of CRRI, Cuttack, Lakshmi *et al*. (2017) in advance generation of aromatic rice, Veni *et al*. (2013), Rukmini *et al*. (2016), and Rathan *et al*. (2019).

Highest grain elongation ratio was recorded in Betguti (1.41) while White Mekrilha was recorded to have lowest ratio (0.68) with mean value of 0.81. More or less similar to these findings were also reported by Rathi *et al*. (2010) in upland rice of Assam and Rukmini *et al*. (2016). The elongation ratio is an important cooking quality trait of rice, the higher values of this traits is desirable and much preferred by the consumers (Parikh *et al*., 2012).

Taghaho showed highest amylose content (19.72 %) while Kuthunu was recorded with least amylose content (10.44 %), mean value for this trait was 15.39%. Rathi *et al*. (2010) reported a range of amylose content between 14.00 and 25.00% in upland rice of Assam, Majumder *et al*. (2019) also reported 14.9 to 22.80% content of amylose in 36 rice genotypes, while Singh *et al*. (2022) reported amylose content as low as 8.30% in short grain aromatic rice accessions. Amylose content of rice grain is considered as one of the most important compositional indices for cooking and processing behaviour. Amylose content of 2 to 9% are considered 'very low', while, 10-20% are 'low' and 20-25% are intermediate. Whereas, amylose content of more than 26% in rice is considered 'high' amylose content. Depending on the desired for rice variety the amount of amylose content can be different. Since, genotypes having low amylose content are sticky, moist and tender when cooked, they are favourable for food processing purpose. A very low amylose is found in waxy (glutinous) rice, such rice does not expand on volume, gave glossy appearance, sticky and remain firm after cooked. Since, amylose content of rice is an important determination of rice hardiness and stickiness when cooked, the knowledge on the amount of its quantity on rice genotypes could be an important selection criterion for better and desirable rice varieties.

Highest gel consistency was recorded in Makilha II, Kemeya, Yeipho, K. Special I, K. Special II, Neingutsure, Pelhirie and Kofie (110 cm) on the other hand Tanyomezu was recorded with lowest gel consistency (20 cm). Whereas the mean value for gel consistency was recorded to be 72.69 cm. Based on gel consistency, rice genotypes can be categorised as soft (if length of gel >61mm), medium (if length of gel 41-60mm) and hard (if length of gel <40mm) (Graham, 2002). Soft and medium types are generally preferred (Amudhya and Thiyagarajan, 2008). Naik *et al.* (2020) and Zhang *et al.* (2020) also reported soft, intermediate and hard gel consistency in F1 hybrids and RIL population respectively. Anjum and Hossain (2019) reported soft, medium and hard type of

gel consistency in local rice varieties of Bangladesh. While, Amudhya and Thiyagarajan (2008) observed soft gel consistency in south Indian landraces. Gel consistency measure the tendency of cooked rice to harden on cooling by measuring the cold paste viscosity of cooked milled rice flour. This measurement is an index used in distinguishing cooked rice texture of high amylose content group of genotypes (Chemutai *et al*., 2016). Biallelic variability in the waxy locus could be responsible for the soft and hard gel consistency (Wanchana *et al*., 2003). This physicochemical test is performed in rice improvement programs to ascertain if high amylose genotypes are soft or hard textured when cooked (Tran *et al*., 2011).

Gelatinization temperature (GT) of each sample was assessed indirectly as the alkali spreading value (ASV) of hulled kernels as described by Little *et al*. (1958). Out of 81 genotypes under study, 52 genotypes were observed with high gelatinization temperature. Low gelatinization temperature was observed in 19 genotypes while 11 genotypes were observed with intermediate gelatinization temperature. All three classes of GT were also reported by Singh *et al.* (2012) in 38 rice germplasm accessions; Prasad *et al*. (2021) in south Indian landraces rice germplasm; Kalpana *et al*. (2018) in a mixture of varieties, landraces and breeding lines of rice collection. Whereas, Abd El-Aty *et al.* (2022) observed low gelatinisation temperature in Egyptian rice. Oko *et al.* (2012) also observed low and intermediate type of gelatinization temperature in 15 rice cultivars of Nigeria while, Roy *et al*. (2021) observed intermediate to high gelatinization temperature in Assam rice.

GT is an important characteristic of quality rice as it determines the water uptake and the duration of cooking. Processing of starch-based products are also relying on thermal properties such as GT (Saif *et al*., 2003). Rice varieties with high GT are expected to require more water and longer duration for cooking, it may also reflect the hardiness of rice starch granules (Mady, 1994), during the process of gelatinization in starch granules there is closing of the cracks present

in the endosperm which make it possible to have a consolidated grain (Bauer and Knorr, 2004; Ituen and Ukpakha, 2011). In order to have an optimum consolidation, it is important to know the level of gelatinization temperature required by the particular rice variety which is significantly affected by soaking/steaming combination treatments (Ayamdoo *et al*., 2013a). The intermediate class of GT is reported to be a good quality parameter as it is mostly preferred by consumers (Roy *et al***.,** 2021). However, according to Juliano and Villareal (1993) a low or intermediate GT is required for high quality rice. So, the genotypes in the present study with a record of low to intermediate gelatinization temperature may be a useful material in developing an improved variety with desirable cooking and processing qualities.

Table 4.1 Variation in grain shape of 81 rice genotypes

Grain shape	Genotypes	Count of Genotypes	Frequency (%)
Basmati type (>6.61- >3.0 mm)	Bokadzii, Kumure, Kumugha, Lhasari, Makilha II and Ario Special	6	**9.88**
Extra-long (>7.5 - > 3.0 mm)	RCM09, Tsive and Tanu	3	**2.47**
Long bold (>6.0 - <3.0 mm)	Pluchama Ngoba, Tenyizhu Ngoba, Kemenya (Rüjoi), White Mekrilha, Mekrilha, Thevure, Kerebe, Fürie, Otokewerü, Daha, Kutsanie, Poramunyo, Nechorei, Pochury, Lhasalu, Zazhobe, Manabe, Vamuzo, Thuzolha, Kemene, Kuthunü, Thujure, Tsokang, Ani, Chokla Tsou, Ashay, Longka, Tsomone, Aodong Tsonyak, Ranjit, China-Tsone, Aspa, MehÜrÜ, Tsorenyu, ThevÜrÜ, Ngoba Kerieu, Ngoba Kenou, Ngoba (Short), Tengo, TevÜ-RÜprÜ, Kheyahi, Kemenya, N. Special Bobla, K.Special I, K. Special II, Asukhomi I, Asukhomi II, Neingutsure, Pelhirie, Kofie, Tanyomezu, Taghaho and Abor.	**53**	**64.20**
Long slender (>6.0 > 3.0 mm)	Mekrilha.	**1**	**1.23**
Medium slender (<6.0 – 2.5-3.0 mm)	Mekrilha II, Kemese-U and Yeihpho.	**3**	**3.70**
Short bold (<6.0 - <2.5 mm)	Japan Rice, Makre Tanye, Tochokoi, Tu-Tso, Betguti, Nuno Tsuk, Kemenya I, Kemenya II, Rulonya, TevÜrÜ, Kemenga (Pointed), N.Special, Egiru and Manaba.	**14**	**17.28**
Short slender (<6.0 – >3.0 mm)	Lihati.	**1**	**1.23**

Table 4.2 Mean performance of 81 rice genotypes for yield, yield attributes and quality traits

Sl. No.	Genotypes	DF	DM	PH (cm)	PL (cm)	NPPP	FGPP	UFGPP	TW (g)	GL (mm)	GW (mm)	DGL (mm)	DGW (mm)	GEL	AC (%)	GC (mm)	GT	GY (g)
1	**Pluchama Ngoba**	99	127	120.74	27.73	8.46	92.86	15.12	22.75	8.86	3.05	6.70	2.70	0.72	16.64	74.00	8.86	19.08
2	**Tenyizhu Ngoba**	101	131	121.42	27.20	12.80	28.44	66.33	25.80	9.88	2.83	7.70	1.32	0.68	16.32	38.00	9.88	13.59
3	**Kemenya (Rӧjoi)**	120	151	154.38	20.17	6.34	59.12	34.17	36.75	9.68	4.05	7.24	3.20	0.76	14.20	81.00	9.68	19.48
4	**White Mekrilha**	103	133	146.72	24.07	9.86	113.18	38.07	29.77	11.17	2.87	7.97	1.28	0.68	15.28	81.00	11.17	36.93
5	**Mekrilha**	116	132	123.51	25.39	12.92	102.71	28.89	28.63	9.81	2.88	7.34	1.96	0.78	15.96	35.00	9.82	54.26
6	**Bokadzii**	103	128	115.77	28.74	7.02	135.93	37.83	22.80	9.33	2.96	6.93	1.72	0.73	17.72	87.00	9.34	23.57
7	**Japan Rice**	103	130	151.48	28.20	7.38	63.68	27.07	24.78	8.40	3.33	5.68	2.88	0.71	14.88	94.00	8.40	19.00
8	**Thevure**	98	125	151.21	29.60	7.36	47.82	59.09	26.22	9.31	3.27	7.08	2.40	0.73	19.40	27.00	9.31	13.03
9	**Kerebe**	99	125	150.06	35.93	10.57	81.61	16.36	27.70	8.49	3.70	6.27	2.80	0.74	14.80	83.00	8.49	26.20
10	**Fӧrie**	93	125	155.46	29.67	11.56	63.60	7.61	26.97	8.55	3.23	6.56	2.82	0.82	10.24	83.00	8.56	24.73
11	**Otokewerӧ**	94	123	145.51	27.75	11.65	79.32	14.51	23.40	8.94	2.95	6.87	2.46	0.79	18.00	40.00	8.94	23.00
12	**Makre Tanye**	98	126	175.93	29.41	17.71	38.38	26.45	27.53	8.29	3.22	5.77	2.83	0.81	10.84	110.00	8.29	20.78

Table 4.2 Mean performance of 81 rice genotypes for yield, yield attributes and quality traits

Sl. No	Genotypes	DF	DM	PH (cm)	PL (cm)	NPPP	FGPP	UFGPP	TW (g)	GL (mm)	GW (mm)	DGL (mm)	DGW (mm)	GEL	AC (%)	GC (mm)	GT	GY (g)
13	**Kumure**	97	129	156.40	28.36	12.77	111.28	30.44	26.00	9.94	2.74	7.48	2.43	0.75	17.00	92.00	9.94	44.63
14	**Tsive**	98	124	149.63	29.33	8.76	70.95	60.13	27.85	10.44	3.02	7.52	2.40	0.72	14.00	46.00	10.45	23.26
15	**Daha**	94	125	145.98	31.51	7.92	89.00	23.16	25.55	8.97	2.91	6.78	2.62	0.76	13.84	56.00	8.97	22.31
16	**Kutsanie**	101	127	180.23	35.09	5.00	83.22	25.10	27.80	8.91	3.46	6.17	2.97	0.78	12.92	103.00	8.92	13.79
17	**Poramunyo**	114	132	169.65	29.73	5.54	73.35	19.92	30.51	8.28	4.04	6.20	3.38	0.79	16.04	98.00	8.28	16.70
18	**Nechorei**	99	127	147.47	29.09	7.06	26.29	52.03	24.80	9.46	3.16	7.04	2.70	0.75	11.36	34.00	9.46	5.62
19	**Pochury**	105	140	128.11	29.23	10.62	84.47	51.07	22.75	9.28	2.93	7.43	2.59	0.77	15.00	44.00	9.29	26.61
20	**Lhasalu**	117	147	113.88	27.92	5.85	211.73	24.94	18.90	8.72	2.72	6.34	2.40	0.72	15.08	26.00	8.73	27.13
21	**Zazhobe**	91	125	161.06	30.27	8.84	106.41	28.10	31.09	8.77	3.66	6.27	3.28	0.76	16.64	41.00	8.77	26.64
22	**Manabe**	102	131	147.91	30.19	10.64	94.46	21.66	27.61	9.17	3.72	6.61	3.07	0.83	14.16	94.00	9.17	29.38
23	**Tanu**	111	134	151.72	30.02	6.97	129.73	45.92	27.93	10.07	2.86	7.76	2.45	0.85	19.16	66.00	10.08	34.51
24	**Vamuzo**	109	134	116.98	19.91	6.90	63.65	21.75	37.51	10.12	3.61	7.54	3.10	0.80	17.44	41.00	10.12	21.28

Table 4.2 Mean performance of 81 rice genotypes for yield, yield attributes and quality traits

Sl. No	Genotypes	DF	DM	PH (cm)	PL (cm)	NPPP	FGPP	UFGPP	TW (g)	GL (mm)	GW (mm)	DGL (mm)	DGW (mm)	GEL	AC (%)	GC (mm)	GT	GY (g)
25	**Thuzolha**	119	149	125.37	28.63	6.88	134.44	28.44	24.96	9.23	3.13	6.71	2.73	0.79	18.24	49.00	9.23	26.68
26	**Kemene**	104	134	173.80	31.69	9.97	103.25	48.56	31.60	9.12	4.00	6.67	3.09	0.71	11.92	98.00	9.13	36.62
27	**Kumugha**	99	131	151.54	29.50	11.34	69.02	50.13	25.86	10.38	2.91	7.43	2.36	0.74	15.52	46.00	10.38	23.45
28	**Kuthunü**	99	131	175.60	30.64	8.38	82.76	84.67	26.82	8.40	3.95	6.16	3.26	0.80	10.44	92.00	8.41	35.94
29	**Thujure**	103	129	135.09	35.37	7.25	95.11	82.00	28.78	9.80	3.80	7.01	3.07	0.69	10.68	95.00	9.80	21.52
30	**Tsokang**	95	127	142.54	31.65	8.97	93.50	18.83	24.60	8.52	2.80	6.22	2.36	0.78	17.28	58.00	8.53	22.69
31	**Tochokoi**	100	133	179.04	28.25	11.78	62.28	25.67	24.95	7.70	3.30	5.77	2.77	0.87	12.88	72.00	7.70	22.92
32	**Ani**	104	130	139.10	32.15	11.54	76.38	15.33	29.87	9.22	3.53	7.07	2.81	0.81	12.76	88.00	9.22	27.94
33	**Chokla Tsou**	101	126	134.07	28.90	6.95	85.68	38.67	23.72	8.52	2.77	6.14	2.44	0.72	13.00	48.00	8.52	17.84
34	**Ashay**	111	133	136.26	23.25	5.30	53.92	28.72	39.95	9.71	3.98	6.83	3.29	0.81	10.32	92.00	9.71	14.45
35	**Longka**	98	129	135.30	25.40	14.76	77.20	44.23	25.04	8.37	3.12	6.12	2.64	0.85	15.24	28.00	8.37	27.52
36	**Tsomone**	108	140	145.98	27.21	6.74	57.33	53.67	30.75	9.54	3.72	7.02	2.90	0.78	16.52	98.00	9.55	14.43

Table 4.2 Mean performance of 81 rice genotypes for yield, yield attributes and quality traits

Sl. No	Genotypes	DF	DM	PH (cm)	PL (cm)	NPPP	FGPP	UFGPP	TW (g)	GL (mm)	GW (mm)	DGL (mm)	DGW (mm)	GEL	AC (%)	GC (mm)	GT	GY (g)
37	**Aodong Tsonyak**	102	137	112.68	29.47	7.88	124.32	37.20	23.03	8.67	3.14	6.17	2.80	0.87	15.08	84.00	8.37	27.50
38	**Tu-Tso**	98	120	174.90	29.08	13.74	66.85	39.56	27.17	8.03	3.26	5.97	2.78	0.89	15.00	90.00	9.55	25.38
39	**China-Tsone**	92	127	126.47	31.25	6.81	42.82	84.50	28.91	9.93	4.25	7.13	3.35	0.86	19.00	80.00	8.68	11.53
40	**Betguti**	98	125	100.27	27.95	10.49	76.25	24.70	27.65	6.92	3.46	4.94	2.87	1.41	17.00	75.00	8.04	22.66
41	**Nuno Tsuk**	96	126	116.93	24.41	11.92	99.13	12.40	26.07	8.61	3.27	5.96	3.00	0.75	10.48	24.00	9.94	34.21
42	**Aspa**	120	133	145.92	27.34	12.59	103.77	29.50	27.58	8.16	3.63	6.63	2.93	0.86	14.72	73.00	6.93	38.27
43	**Abor**	89	125	135.88	32.29	5.29	33.22	28.42	28.94	8.90	3.50	6.00	3.10	0.80	14.00	85.00	8.62	8.37
44	**MehÜrÜ**	97	124	119.00	32.18	14.50	84.71	26.96	28.04	8.40	2.90	6.05	2.65	0.83	15.00	85.00	8.16	36.55
45	**Mekrilha**	98	124	153.14	29.78	13.35	60.93	22.50	30.55	9.55	2.70	6.85	2.20	0.80	14.37	77.00	8.90	19.61
46	**Tsorenyu**	98	123	166.74	31.38	14.92	50.53	48.47	24.97	8.65	3.20	6.25	2.75	0.77	17.82	80.00	8.40	14.31
47	**ThevÜrÜ**	93	126	169.59	31.68	12.25	21.97	36.44	27.74	8.50	3.10	6.00	3.05	0.83	18.60	60.00	9.55	9.07
48	**Ngoba Kerieu**	98	124	129.29	35.69	10.93	58.24	40.20	27.73	8.85	3.05	6.55	3.00	0.77	13.77	70.00	8.65	12.50

Table 4.2 Mean performance of 81 rice genotypes for yield, yield attributes and quality traits

Sl. No	Genotypes	DF	DM	PH (cm)	PL (cm)	NPPP	FGPP	UFGPP	TW (g)	GL (mm)	GW (mm)	DGL (mm)	DGW (mm)	GEL	AC (%)	GC (mm)	GT	GY (g)
49	**Ngoba Kenou**	100	128	118.44	30.32	11.15	76.38	21.91	25.32	8.75	2.65	6.20	2.25	0.76	18.05	65.00	8.75	23.89
50	**Kemenya I**	106	128	155.57	30.42	12.81	78.69	22.33	31.90	8.85	3.55	5.70	3.00	0.76	19.69	90.00	8.85	35.67
51	**Kemenya II**	108	135	163.08	29.01	14.35	86.33	36.17	26.94	8.15	3.15	5.35	2.60	0.86	18.92	70.00	8.15	29.80
52	**Ngoba (Short)**	100	126	123.04	30.29	7.51	95.88	33.10	23.89	7.80	2.70	6.00	2.15	0.89	13.62	93.00	7.80	21.92
53	**Mekrilha II**	92	129	156.96	33.80	9.27	75.56	21.31	22.30	8.60	2.40	5.85	2.25	0.98	18.55	80.00	8.60	18.68
54	**Rulonya**	98	126	126.21	29.36	5.95	26.97	43.16	29.38	7.40	3.25	4.95	2.80	0.93	18.12	65.00	7.40	6.87
55	**Tengo**	100	127	161.97	28.38	6.91	51.63	17.47	33.16	7.95	3.10	6.40	2.75	0.82	12.40	70.00	7.95	16.00
56	**TevÜrÜ**	89	127	142.88	31.53	8.69	40.15	54.67	32.10	7.75	3.20	5.75	2.70	0.85	19.17	55.00	7.75	17.55
57	**Kemenga (Pointed)**	106	135	167.37	30.21	6.55	62.90	30.72	40.20	8.50	3.75	6.45	3.05	0.84	19.00	65.00	8.50	16.79
58	**TevÜ-RÜprÜ**	97	123	160.08	35.75	6.96	59.23	45.33	33.34	8.95	2.50	6.70	2.25	0.83	18.20	70.00	8.95	15.36
59	**Kheyahi**	101	123	160.48	35.54	7.82	48.61	28.24	29.45	8.60	2.65	6.25	2.35	0.75	17.07	60.00	8.60	14.01
60	**Lhasari**	100	129	160.16	34.00	8.77	33.48	65.44	29.12	9.55	2.40	7.10	2.15	0.79	16.57	30.00	9.55	10.12

Table 4.2 Mean performance of 81 rice genotypes for yield, yield attributes and quality traits

Sl. No.	Genotypes	DF	DM	PH (cm)	PL (cm)	NPPP	FGPP	UFGPP	TW (g)	GL (mm)	GW (mm)	DGL (mm)	DGW (mm)	GEL	AC (%)	GC (mm)	GT	GY (g)
61	**Makilha II**	96	125	140.33	33.68	9.53	34.39	47.71	25.09	8.70	2.50	6.00	2.00	0.77	13.70	110.00	8.70	20.10
62	**Kemese-U**	107	127	177.06	24.31	8.56	69.19	35.00	32.23	8.30	2.50	5.80	2.25	0.79	12.25	30.00	8.30	13.78
63	**Kemenya**	114	129	138.33	27.53	9.87	89.20	19.97	31.12	9.50	3.05	6.05	2.50	0.87	18.02	110.00	9.50	25.23
64	**N. Special Bobla**	125	149	136.69	27.69	11.26	104.53	22.30	32.87	8.75	3.55	6.10	2.70	0.75	14.37	85.00	8.75	66.75
65	**Lihati**	123	144	148.63	25.04	12.98	66.49	31.43	21.98	7.20	1.75	5.70	1.50	0.96	15.47	100.00	7.20	17.93
66	**N.Special**	125	155	137.72	24.80	9.31	92.30	31.52	33.62	8.75	3.35	5.75	2.75	0.89	12.67	85.00	8.75	34.52
67	**Yeihpho**	95	129	124.06	33.64	9.50	74.63	94.25	28.43	7.50	2.80	5.30	2.35	0.82	14.60	110.00	7.50	39.54
68	**K.Special I**	100	131	121.11	31.50	10.27	74.24	51.11	29.78	8.70	2.50	6.10	2.15	0.77	14.82	110.00	8.70	21.39
69	**Ario Special**	96	122	106.92	28.25	9.81	37.36	50.08	30.00	9.55	2.35	7.30	2.15	0.84	16.72	110.00	9.55	8.11
70	**K. Special II**	104	128	136.50	35.81	10.29	90.58	21.33	36.82	10.50	2.70	7.00	2.35	0.80	14.22	85.00	10.50	36.94
71	**Asukhomi I**	100	122	121.69	34.46	9.28	54.74	23.78	32.15	9.05	2.70	6.35	2.35	0.91	15.62	110.00	9.05	14.17
72	**Asukhomi II**	100	130	121.89	29.01	12.11	85.31	16.86	34.95	9.00	2.80	6.45	2.55	0.88	18.97	110.00	9.00	23.04

Table 4.2 Mean performance of 81 rice genotypes for yield, yield attributes and quality traits

Sl. No.	Genotypes	DF	DM	PH (cm)	PL (cm)	NPPP	FGPP	UFGPP	TW (g)	GL (mm)	GW (mm)	DGL (mm)	DGW (mm)	GEL	AC (%)	GC (mm)	GT	GY (g)
73	**Egiru**	96	123	177.46	32.09	10.87	23.51	55.72	26.68	7.62	3.10	5.00	2.85	0.95	17.90	79.00	7.62	4.48
74	**Neingutsure**	103	124	165.14	30.63	10.84	86.09	13.22	32.80	8.39	3.05	6.05	2.70	0.82	10.82	110.00	8.39	25.21
75	**Pelhirie**	88	123	143.29	31.12	9.78	55.14	14.77	32.15	8.43	3.05	6.40	2.70	0.82	17.17	110.00	8.43	18.59
76	**Kofie**	100	128	133.10	24.54	9.39	112.13	76.17	26.33	8.61	2.95	6.15	2.55	0.78	14.45	110.00	8.61	27.72
77	**Manaba**	97	129	169.35	30.21	6.91	83.05	9.22	35.63	10.04	3.35	5.70	2.80	0.78	10.50	85.00	10.04	25.44
78	**Tanyomezu**	99	129	172.17	32.45	6.31	50.76	51.33	31.57	8.67	3.23	7.35	2.80	0.74	18.00	20.00	8.67	11.92
79	**Taghaho**	101	126	155.98	31.67	7.86	66.46	50.15	30.65	8.45	3.23	6.15	2.80	0.81	19.72	22.00	8.45	16.49
80	**RCM09**	101	136	89.98	21.73	9.00	66.06	33.02	27.13	10.41	2.55	7.73	2.43	0.79	9.67	25.00	9.64	33.33
81	**Ranjit**	122	147	95.22	23.62	11.3	62.51	54.00	32.52	19.32	2.69	6.39	2.44	0.74	11.80	38.00	10.90	26.66
	Range	88-124	111-150	100.27-180.22	19.91-35.93	4.99-17.71	21.97-211.72	7.61-94.25	18.90-40.19	6.92-11.17	1.75-4.25	4.64-7.97	1.28-3.38	0.68-1.41	10.44-19.72	20.00-110.00	6.84-11.71	4.48-66.75
	Grand Mean	101	126	144.88	29.66	9.53	75.05	36.39	28.65	8.91	3.12	6.46	2.88	0.81	15.39	72.69	8.91	22.79
	SEd	1.61	0.62	5.05	0.48	0.26	7.40	3.73	0.72	0.09	0.04	0.08	0.04	0.03	0.17	1.09	0.11	1.10
	CD5%	4.48	1.72	14.01	1.33	0.72	20.57	10.37	2.01	0.25	0.12	0.24	0.13	0.10	0.47	3.05	0.33	3.08
	CD1%	5.91	2.27	18.48	1.75	0.95	27.09	13.66	2.65	0.33	0.16	0.32	0.18	0.13	0.63	4.03	0.43	4.05

Table 4.3 Analysis of variance for yield, yield attributing and quality traits

Source of Variation	Treatments	Replication	Error
Df	80	2	160
DF	386.21**	165.60	15.64
DM	325.24**	56.11	2.32
PH (cm)	2313.53**	453.60	153.06
PL (cm)	66.70**	14.71	1.38
No. of PPP	42.73**	0.38	0.40
No. of FGPP	5511.82**	1707.68	328.68
No. of UFGPP	2090.67**	271.59	83.58
1000 GW (g)	103.51**	21.25	3.15
GL (mm)	2.09**	0.05	0.02
GW (mm)	0.65**	0.01	0.01
DGL (mm)	1.4**	0.36	0.02
DGW (mm)	4.76 **	0.07	0.01
GEL	19.51 **	0.01	0.00
AC (%)	84.96 **	0.05	0.08
GC (mm)	2159.88**	2.74	3.58
GT	162.29**	1.37	6.83
GY (g)	671.59 **	118.08	7.37

4.2.2 Analysis of variance

Analysis of variance allow us to see the presence of significant difference among the treatments by following a standard procedure for respective experimental design such as randomized block design. On the perusal of **Table 4.3** it is observed that there is significant variation among the genotypes for yield and quality traits. Important traits such as days to 50% flowering, days to maturity, plant height, panicle length, no. of panicles per plant, no. of filled grains per plant, no. of unfilled grains per plant, 1000 grains weight, grain length, grain width, decorticated grain length, decorticated grain width, grain elongation ratio, amylose content, gel consistency and grain yield per plant were showing significant variation at 1% level of significant. The presence of this variation implies that there is scope for selection of promising genotypes from the present accession for yield and quality traits improvement. Presence of such variation was also reported by Kalpana *et al.* (2018) in a collection of rice varieties, landraces and breeding lines for yield and quality traits, Kausar Ali *et al.* (2018) also reported significant variation for yield and quality traits in parents and F1 genotypes of rice and Roy *et al*. (2021) in a collection of germplasm that include north east cultivars.

4.2.3 Variability parameters for yield and quality traits of 81 rice genotypes

The continuous variation exhibited by quantitative traits include both heritable and non-heritable components, the heritable component is due to the genetic make-up, while the other component is mainly due to unknown environmental effects (Fisher, 1918). Assessment of such variation is difficult through assessing genotypes directly, therefore, assessment of phenotypic expression which is the outcome of genotype and environmental interaction in the existing material is one method. Hence, the study of such phenotypic variability for various yield and quality traits is of great importance.

In the present study, highest genotypic and phenotypic variance was observed in a trait no. of filled grains per panicle (863.85, 918.63). In general, the phenotypic coefficient of variation for each trait was slightly higher than the corresponding genotypic coefficient of variation indicating little influence of environment on the expression of each trait. If a trait expressed coefficient of variation less than 10%, the value is said to be low, while between 10%-20% are called moderate and coefficient of variation more than 20% is said to be high in value (Deshmuk *et al.*, 1986). The GCV and PCV was recorded similar for grain yield per plant and grain elongation ratio indicating little or no effect of environment. Among yield traits, no. of panicle per plant (27.84%,27.97%), no. of filled grains per plant (39.16%,40.38%), no. of unfilled grains per plant (51.24%,51.28%) and grain yield per plant (46.40%,46.40%) were recorded with high genotypic and phenotypic coefficient of variation. Grain quality traits such as gel consistency (37.10%,37.13%), decorticated grain width (44.83%,44.86%), amylose content (57.35%,57.37%), and grain elongation ratio (208.26%,208.26%), were recorded with high genotypic and phenotypic coefficient of variation. Panicle length (11.12%,11.23%), grain width (13.03%,13.10%), plant height (13.09%,13.055%) and 1000 grains weight (14.27%,14.49%) were recorded with moderate genotypic and phenotypic coefficient of variation. Whereas, traits such as, days to maturity (5.78%,5.80%), days to 50% flowering (7.71%,7.87%), grain length (8.86%,8.92%) and decorticated grain length (9.78%,9.87%) were detected to have low coefficient of variation at both the level. These different magnitude of both genotypic and phenotypic coefficient of variation as well as higher value of phenotypic coefficient of variation in comparison to corresponding genotypic coefficient of variation was also reported by Seyoum *et al.* (2012) in grain yield parameters of upland rice of southwest Ethiopia, Nandini *et al*. (2017) in both quality and yield traits of traditional rice varieties collected from southern Karnataka, Akter *et al.* (2019) in yield parameters of promising rice hybrids, Naik *et al.* (2020) in both

quality and yield traits, Faysal *et al*. (2022) in grain yield traits of Aman type of rice in Bangladesh and Sadhana *et al*. (2022) in both yield and quality traits. The difference between PCV and GCV were very low which indicates that the genotypes chosen on the basis of these characters may be suitable in the crossing programme for obtaining good transgressive segregants (Kausar Ali *et al*., 2018).

Even though nature and magnitude of coefficient of variation provides a measure of comparison of variability and provide some indication for selection of traits it does not provide a clear-cut picture on the extent of genetic gain to be expected from selection of such phenotypic traits unless heritable fraction of variation which is known as heritability is not estimated (Burton, 1952). Estimation of heritability in broad sense includes both fixable (additive) and non-fixable (dominant and epistasis) variances. It also provides a good indication about the repeatability of the traits.

In the present investigation high heritability (>60%) were observed for every trait under study (**Table 4.4**) on the basis of classification by Johnson, Robinson and Comstock (1995). Highest heritability value (99.98%) was observed for the trait, grain elongation ratio followed by decorticated grain width (99.86%), amylose content and gel consistency (99.85%). Babu *et al.* (2012), Akter *et al.* (2019), Naik *et al*. (2020), Akshay *et al.* (2022) and Faysal *et al.* (2022) also gave reports of high heritability for such characters under study. Although the presence of high heritability values indicates the effectiveness of selection on the basis of phenotypic performance, it does not show any indication to the amount of genetic progress for selecting the best individuals which is possible by using the estimate of genetic advance (Kalpana *et al*., 2018). In medium heritability (30%-60%), more influence of environment on the trait is expected. Therefore, direct selection for those traits with medium heritability will not be effective. Since heritability does not always indicate genetic gain, heritability coupled with genetic advance is more effective for selection.

Genetic advance indicates the expected progress as the result of selection. It is used to estimate the types of gene action in polygenic traits (Girma *et al*., 2018). According to Johnson (1955) genetic advance of a treatments are classified into three group based on their value. Genetic advance less than 10% are classified as low, while between 10%-20% are classified as medium, more than 20% are classified as high. In the present study, medium and high genetic advance were observed for both yield and quality traits. Maximum traits were found to have high genetic advance in which grain elongation ratio show highest value (428.98%) followed by amylose content (118.13%) and no. of unfilled grains per panicle (101.42%). Four traits viz; decorticated grain length (19.98%), grain length (8.14%), days to 50% flowering (15.55%) and days to maturity (11.875) were recorded to have medium genetic advance. Since broad sense heritability includes both additive and epistatic effects. It will be reliable only when accompanied by high genetic advance, heritability estimates along with genetic advance is more useful than heritability alone in predicting the effectiveness of selection (Johnson *et al*., 1955). So, these character that possessed high genetic advance are also coupled with high heritability and therefore selection based on these traits will be effective.

4.3 Association analysis for yield and quality traits in 81 rice genotypes

4.3.1 Genotypic and phenotypic correlation coefficient

Direct selection of genotypes based on their performance on grain yield alone is not an ideal strategy in crop breeding programme since grain yield is a complex character that depends on several component traits. Measurement of relationship and association between yield and its component characters is an essential activity that can determine the direction of selection and no. of component traits to be considered in improving grain yield. This association between them can be measured using correlation coefficients which present the

degree of relationship between grain yield and its component traits as well as relationship among the component traits.

The coefficient of correlation at genotypic and phenotypic level is presented in **Table 4.5.** A significant positive correlation with grain yield was observed in component traits such as no. of filled grains per panicle (0.64**,0.60**), days to 50% flowering (0.41**, 0.40**), no. of panicles per plant (0.37**, 0.35**) and days to maturity (0.37**, 0.36**). Whereas, plant height, 1000 grains weight and no. of unfilled grains per panicle show negative correlation with grain yield per plant. Among the component traits, days to 50% flowering was recorded to exhibit a significant positive correlation with days to maturity (0.69**,0.69**) and no. of filled grains per panicle (0.42**,0.41**). Days to maturity was also recorded to have significant positive association with no. of filled grains per panicle (0.41**,0.41**).

No significant positive correlation was observed between grain yield per plant and quality parameters. However, among the grain quality traits, grain length shows significant positive correlation with decorticated grain length (0.83**,0.82**) at both genotypic and phenotypic level. While decorticated grain width also shows significant positive correlation with grain width (0.25*,0.25*) and decorticated grain length (0.25*,0.25*).

Important yield attributes were also observed to have a significant positive correlation with grain quality traits. Association between days to maturity with grain width (0.26*,0.26*) and decorticated grain length (0.20*). A significant positive correlation was also observed between 1000 grain weight with grain length (0.26*,0.26*) and grain width (0.34*,0.34*).

In the above overall correlation studies, a highest significant correlation with grain yield was observed in no. of filled grain per panicle (0.64**,0.60**) followed by days to 50% flowering (0.41**,0.40**) while association between grain length and decorticated grain length was recorded to be highest value

(0.83**,0.82**). The pattern of the above relationship was also reported by Dhurai *et al.* (2014), Ratna *et al.* (2015) and Sadhana *et al*. (2022). However, negative relationship between days to 50% flowering and grain yield per plant was also observed by other researchers such as Mustafa and Yassir Elsheik (2007), Chakrabarty and Chakraborty (2010) and Seyoum *et al.* (2012), Faysal *et al*. (2022). Therefore, selection of those traits that have significant positive association with grain yield could be beneficial for varietal improvement.

4.3.2 Path coefficient analysis

Even though the studies of correlation coefficient reflect association between important traits and their impact on grain yield, selection based on correlation component characters may sometimes be misleading due to over and under-estimation. Splitting of correlation coefficient into direct and indirect effects would provide a more meaningful interpretation of such association. Path analysis splits the genotypic correlation coefficient into the measure of direct and indirect effects (Train *et al*., 1992). Therefore, correlation in combination with path coefficient analysis will be an important tool to find out the association and quantify the direct and indirect influence of one character upon another (Dewey and Lu, 1959). Path coefficient analysis provides an exact picture of the relative importance of direct and indirect effects of each of the component character towards yield.

In the present study high direct effects (**Table 4.6**) of component traits on grain yield were recorded in no. of filled grains per panicle (0.73,0.62), no. of panicles per plant (0.53,0.50) and 1000 grains weight (0.35,0.28) indicating that these traits have high contribution on increasing yield. A positive direct effect of days to 50% flowering (0.06,0.10), days to maturity (0.04,0.05), panicle length (0.08,0.06), no. of unfilled grains per panicle (0.12,0.09), grain width (0.04,0.05), decorticated grain width (0.05, 0.03) and gel consistency (0.00,0.01) were negligible. Direct effect of plant height (-0.04, -0.06), grain length (-

0.41,0.01), decorticated grain length (-0.05, -0.07), grain elongation ratio (-0.04, -0.01) and amylose content (-0.09, -0.12) were recorded to be negative at negligible level.

Days to 50% flowering (0.31, 0.25) and days to maturity (0.30, 0.24) were observed to have moderate positive effect on grain yield through no. of filled grain per panicle. Amylose content (0.11, 0.11) shows low indirect effect through no. of panicles per plant. The indirect effect of plant height (-0.20, -0.16) and panicle length (-0.13, -0.10) through no. of filled grains per panicle were recorded to be negative at low to moderate level. In the overall path analysis highest direct effect was observed in no. of filled grains per panicle (0.73, 0.62) while indirect effect of days to 50% flowering (0.31, 0.25) through no. of filled grains per panicle was highest, which suggest that improving these traits will have high positive effect on increasing grain yield. Highest negative direct effect and indirect effect was observed in grain length (-0.41, 0.01) and plant height (-0.20, -0.16) through no. of filled grain per panicle. The above findings are in conformity with the report given by Mustafa and Yassir Elsheikh (2007), Faysal *et al.* (2022) and Sadhana *et al*. (2022).

Table 4.4 Genetic variability of yield, yield attributing and quality traits

Characters	GV	PV	GCV (%)	PCV (%)	ECV (%)	h^2 bs (%)	GA as 5% of mean
DF	61.76	64.36	7.71	7.87	3.88	96.00	15.55
DM	53.82	54.20	5.78	5.80	1.26	99.29	11.87
PH (cm)	360.07	385.50	13.09	13.55	8.53	93.38	26.07
PL (cm)	10.88	11.11	11.12	11.23	3.96	97.92	22.67
PPP	7.05	7.12	27.84	27.97	6.69	99.05	57.08
FGPP	863.85	918.63	39.16	40.38	24.15	94.04	78.22
UFGPP	334.51	348.44	50.24	51.28	25.11	96.00	101.42
TW (g)	16.72	17.25	14.27	14.49	6.19	96.95	28.94
GL (mm)	0.65	0.66	8.86	8.92	1.74	98.73	18.14
GW (mm)	0.18	0.18	13.03	13.10	2.27	99.00	26.72
DGL (mm)	0.43	0.43	9.78	9.87	2.28	98.21	19.98
DGW (mm)	2.53	2.53	44.83	44.86	2.92	99.86	92.28
GEL	6.09	6.10	208.26	208.26	5.10	99.98	428.98
AC (%)	16.02	16.07	57.35	57.37	2.19	99.85	118.13
GC (mm)	648.26	649.24	37.10	37.13	2.50	99.85	76.38
GT	0.66	0.70	9.13	9.42	2.31	93.00	18.22
GY (g)	110.70	111.93	46.40	46.40	11.91	98.90	94.54

Table 4.5 Genotypic (G) and Phenotypic (P) correlation coefficient of grain yield with yield attributes and quality traits

Characters		DF	DM	PH	PL	NPP	NFGPP	NUFGPP	TW	GL	GW	DGL	DGW	GER	AC	GC	GT	YPP
DF	**G**	1	0.69**	-0.08	-0.47	-0.05	0.42**	-0.14	0.14	0.09	0.12	0.09	0.04	-0.16	-0.28	0.04	0.09	0.41**
	P	1	0.69**	-0.08	-0.46	-0.05	0.41**	-0.14	0.15	0.09	0.10	0.10	0.05	-0.16	-0.27	0.04	0.10	0.40**
DM	**G**		1	-0.15	-0.49	-0.07	0.41**	-0.11	0.10	0.18	0.26*	0.19	0.05	-0.06	-0.26	-0.12	0.17	0.37**
	P		1	-0.14	-0.47	-0.07	0.41**	-0.11	0.11	0.19	0.26*	0.20*	0.05	-0.06	-0.26	-0.12	0.18	0.36**
PH	**G**			1	0.19	0.05	-0.28	-0.14	0.18	-0.09	0.18	-0.10	-0.12	0.12	-0.49	-0.02	-0.09	-0.15
	P			1	0.18	0.05	-0.26	-0.01	0.17	-0.12	0.18	-0.10	-0.11	0.12	-0.05	-0.02	-0.08	-0.14
PL	**G**				1	-0.04	-0.18	0.13	-0.10	-0.14	-0.13	-0.17	-0.18	0.16	0.20	0.18	-0.14	-0.19
	P				1	-0.04	-0.17	0.12	-0.09	-0.12	-0.12	-0.15	-0.17	0.16	0.19	0.18	-0.11	-0.18
NPP	**G**					1	-0.06	-0.14	-0.22	-0.18	-0.23	-0.15	-0.10	-0.10	0.21*	0.13	-0.16	0.37**
	P					1	-0.06	-0.14	-0.21	-0.17	-0.22	-0.15	-0.10	-0.09	0.20*	0.12	-0.15	0.35**
NFGPP	**G**						1	-0.22	-0.28	0.13	-0.02	0.10	0.05	0.03	-0.34	-0.01	0.12	0.64**
	P						1	-0.21	-0.25	0.12	-0.01	0.10	0.05	0.03	-0.32	-0.01	0.12	0.60**
NUFGPP	**G**							1	-0.12	0.10	0.03	0.17	0.05	0.00	-0.02	-0.08	0.10	-0.16
	P							1	-0.11	0.10	0.03	0.16	0.05	0.00	-0.02	-0.07	0.10	-0.15
TW	**G**								1	0.26*	0.34*	0.11	-0.11	0.14	0.14	0.15	0.24*	0.00
	P								1	0.26*	0.34*	0.12	-0.11	0.14	0.14	0.15	0.25*	0.00
GL	**G**									1	0.00	0.83**	0.18	-0.02	-0.30	-0.19	0.99**	0.10
	P									1	0.01	0.82**	0.19	-0.02	-0.29	-0.18	0.87**	0.09
GW	**G**										1	-0.03	0.25*	-0.02	-0.46	0.10	0.00	0.10
	P										1	0.00	0.25*	-0.02	-0.45	0.10	0.03	0.10
DGL	**G**											1	0.25*	-0.01	-0.33	-0.13	0.83**	0.04
	P											1	0.25*	-0.01	-0.32	-0.29	0.84**	0.04
DGW	**G**												1	-0.05	-0.32	-0.09	0.19	0.03
	P												1	-0.05	-0.32	-0.13	0.19	0.03
GER	**G**													1	0.07	-0.09	-0.02	-0.03
	P													1	0.07	-0.09	-0.02	-0.03
AC	**G**														1	0.04	-0.16	-0.22
	P														1	0.04	-0.32	-0.22
GC	**G**															1	-0.31	0.13
	P															1	-0.16	0.13*
GT	**G**																-0.15	0.10
	P																1	0.10
YPP	**G**																1	1
	P																	1

Table 4.6 Genotypic (G) and Phenotypic (P) path coefficient analysis of grain yield, yield attributes and quality traits

Characters		DF	DM	PH	PL	NPP	NFGPP	NUFGPP	TW	GL	GW	DGL	DGW	GER	AC	GC	GT	YPP
DF	**G**	**0.06**	0.03	0.00	-0.03	-0.03	0.31	-0.02	0.05	-0.04	0.01	0.00	0.00	0.01	0.03	0.00	0.00	0.41**
	P	**0.10**	0.03	0.00	-0.03	-0.03	0.25	-0.01	0.04	0.00	0.01	-0.01	0.00	0.00	0.03	0.00	0.01	0.40**
DM	**G**	0.04	**0.04**	0.01	-0.04	-0.04	0.30	-0.01	0.04	-0.08	0.01	-0.01	0.00	0.00	0.02	0.00	0.00	0.37**
	P	0.07	**0.05**	0.01	-0.03	-0.03	0.24	-0.01	0.03	0.00	0.01	-0.01	0.00	0.00	0.03	0.00	0.00	0.36**
PH	**G**	0.00	-0.01	**-0.04**	0.01	0.03	-0.20	0.00	0.06	0.04	0.01	0.01	-0.01	0.00	0.00	0.00	0.00	-0.15
	P	-0.01	-0.01	**-0.06**	0.01	0.02	-0.16	0.00	0.05	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	-0.14
PL	**G**	-0.03	-0.02	0.00	**0.08**	-0.02	-0.13	0.02	-0.03	0.06	-0.01	0.01	-0.01	-0.01	-0.02	0.00	0.01	-0.19
	P	-0.05	-0.02	-0.01	**0.06**	-0.02	-0.10	0.01	-0.02	0.00	-0.01	0.01	-0.01	0.00	-0.02	0.00	0.00	-0.18
NPP	**G**	0.00	0.00	0.00	0.00	**0.53**	-0.05	-0.02	-0.08	0.07	-0.01	0.01	-0.01	0.00	-0.02	0.00	-0.08	0.37**
	P	-0.01	0.00	0.00	0.00	**0.50**	-0.04	-0.01	-0.06	0.00	-0.01	0.01	0.00	0.00	-0.03	0.00	-0.07	0.35**
NFGPP	**G**	0.03	0.02	0.01	-0.01	-0.03	**0.73**	-0.03	-0.10	-0.05	0.00	-0.01	0.00	0.00	0.03	0.00	0.09	0.64**
	P	0.04	0.02	0.02	-0.01	-0.03	**0.62**	-0.02	-0.07	0.00	0.00	-0.01	0.00	0.00	0.04	0.00	0.07	0.60**
NUFGPP	**G**	-0.01	0.00	0.00	0.01	-0.07	-0.16	**0.12**	-0.04	-0.04	0.00	-0.01	0.00	0.00	0.00	0.00	0.01	-0.16
	P	-0.01	0.00	0.00	0.01	-0.07	-0.13	**0.09**	-0.03	0.00	0.00	-0.01	0.00	0.00	0.00	0.00	0.00	-0.15
TW	**G**	0.01	-0.01	-0.01	-0.01	-0.11	-0.20	-0.01	**0.35**	-0.11	0.02	-0.01	-0.01	-0.01	-0.01	0.00	0.08	0.00
	P	0.01	0.00	-0.01	-0.01	-0.10	-0.16	-0.01	**0.28**	0.00	0.02	-0.01	0.00	0.00	-0.02	0.00	0.07	0.00
GL	**G**	0.01	0.01	0.00	-0.01	-0.09	0.09	0.01	0.09	**-0.41**	0.00	-0.04	0.01	0.00	0.03	0.00	-0.41	0.10
	P	0.01	0.01	0.01	-0.01	-0.09	0.08	0.01	0.07	**0.01**	0.00	-0.06	0.01	0.00	0.04	0.00	0.00	0.09
GW	**G**	0.01	0.01	-0.01	-0.01	-0.12	-0.01	0.00	0.12	0.00	**0.04**	0.00	0.01	0.00	0.04	0.00	0.00	0.10
	P	0.01	0.01	-0.01	-0.01	-0.11	-0.01	0.00	0.09	0.00	**0.05**	0.00	0.01	0.00	0.06	0.00	0.00	0.10
DGL	**G**	0.01	0.01	0.00	-0.01	-0.08	0.07	0.02	0.04	-0.34	0.00	**-0.05**	0.01	0.00	0.03	0.00	-0.04	0.04
	P	0.01	0.01	0.01	-0.01	-0.07	0.06	0.01	0.03	0.01	0.00	**-0.07**	0.01	0.00	0.04	0.00	-0.05	0.04
DGW	**G**	0.00	0.00	0.01	-0.01	-0.05	0.04	0.01	-0.04	-0.08	0.01	-0.01	**0.05**	0.00	0.03	0.00	0.00	0.03
	P	0.00	0.00	0.01	-0.01	-0.05	0.03	0.00	-0.03	0.00	0.01	-0.02	**0.03**	0.00	0.04	0.00	0.00	0.03
GER	**G**	-0.01	0.00	-0.01	0.01	-0.05	0.02	0.00	0.05	0.01	0.00	0.00	0.00	**-0.04**	-0.01	0.00	0.00	-0.03
	P	-0.02	0.00	-0.01	0.01	-0.05	0.02	0.00	0.04	0.00	0.00	0.00	0.00	**-0.01**	-0.01	0.00	0.00	-0.03
AC	**G**	-0.02	-0.01	0.00	0.02	0.11	-0.25	0.00	0.05	0.12	-0.02	0.02	-0.02	0.00	**-0.09**	0.00	0.03	-0.22
	P	-0.03	-0.01	0.00	0.01	0.11	-0.20	0.00	0.04	0.00	-0.02	0.02	-0.01	0.00	**-0.12**	0.00	0.03	-0.22
GC	**G**	0.00	-0.01	0.00	0.01	0.07	-0.01	-0.01	0.05	0.08	0.00	0.02	-0.01	0.00	0.00	**0.00**	0.00	0.13
	P	0.00	-0.01	0.00	0.01	0.06	-0.01	-0.01	0.04	0.00	0.00	0.02	0.00	0.00	0.00	**0.01**	0.00	0.13*
GT	**G**	0.00	0.00	0.00	0.01	-0.08	0.09	0.01	0.08	-0.41	0.00	-0.04	0.00	0.00	0.03	0.00	**0.40**	0.10
	P	0.01	0.00	0.00	0.00	-0.07	0.07	0.00	0.07	0.00	0.00	-0.05	0.00	0.00	0.03	-0.00	**0.01**	0.10

4.4 Genetic diversity based on phenotypic traits among the germplasm

Analysis of variance **Table 4.3** revealed significant variation among the germplasm, The UPGMA based dendrogram (**Fig. 4.1**) revealed grouping of 81 genotypes into 6 clusters using grain quality, yield and yield attributes. Based on the cluster division of genotypes cluster I to III contain 1 genotype each, cluster IV had 2 genotypes, while cluster V had three genotypes. The last cluster, cluster VI had maximum genotypes which was 73 genotypes. The only genotype in cluster I, Betguti is the best candidate for developing semi-dwarf variety based on its mean *per se* performance among 81 genotypes but cluster I was characterized by highest amylose content and gel consistency. Vamuzo in cluster II was recorded to have a highest value for 1000 grains weight which is an important selection criterion for high yielding genotype and the cluster was characterized by highest value of 1000 grains weight, grain width and decorticated grain length. Lihati in cluster III had highest record for grain yield per plant, it possessed short bold grain type with high gelatinization temperature, cluster III was characterized by highest value in days to 50% flowering, plant height and grain yield per plant. Cluster IV was characterized by highest value for days to maturity, no. of filled grains per panicle and grain length. Cluster V was recorded with highest value for no. of unfilled grains per panicle, decorticated grain width and grain elongation ratio while, cluster VI was characterized with panicle length. PCA analysis (Fig. 2) revealed that the first principal component accounted for 18.34% to total variation and second principal component accounted for 15.56% to total variation. In conclusion, all the variability analysis revealed that in the present study accession diversity is governed by grain elongation ratio, decorticated grain length, grain length, filled grains per panicle and yield per plant. This study revealed the presence of diversity and genotypes identified with superior in one or two traits from different clusters could be useful in the hybridization programme to produce desirable segregants for the traits under study.

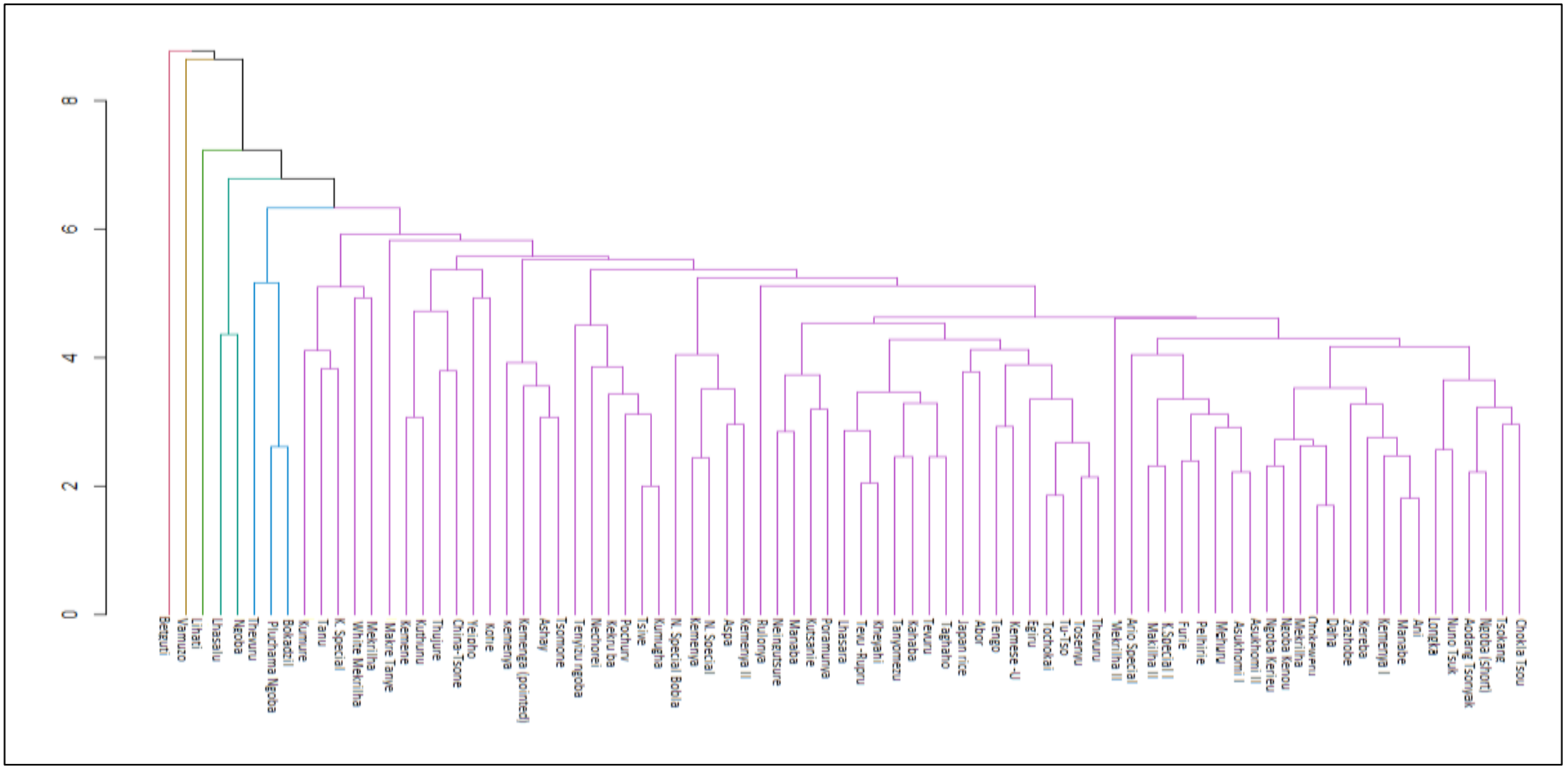

Fig. 4.1 Grouping of genotypes based on the yield, yield related and quality traits using UPGMA.

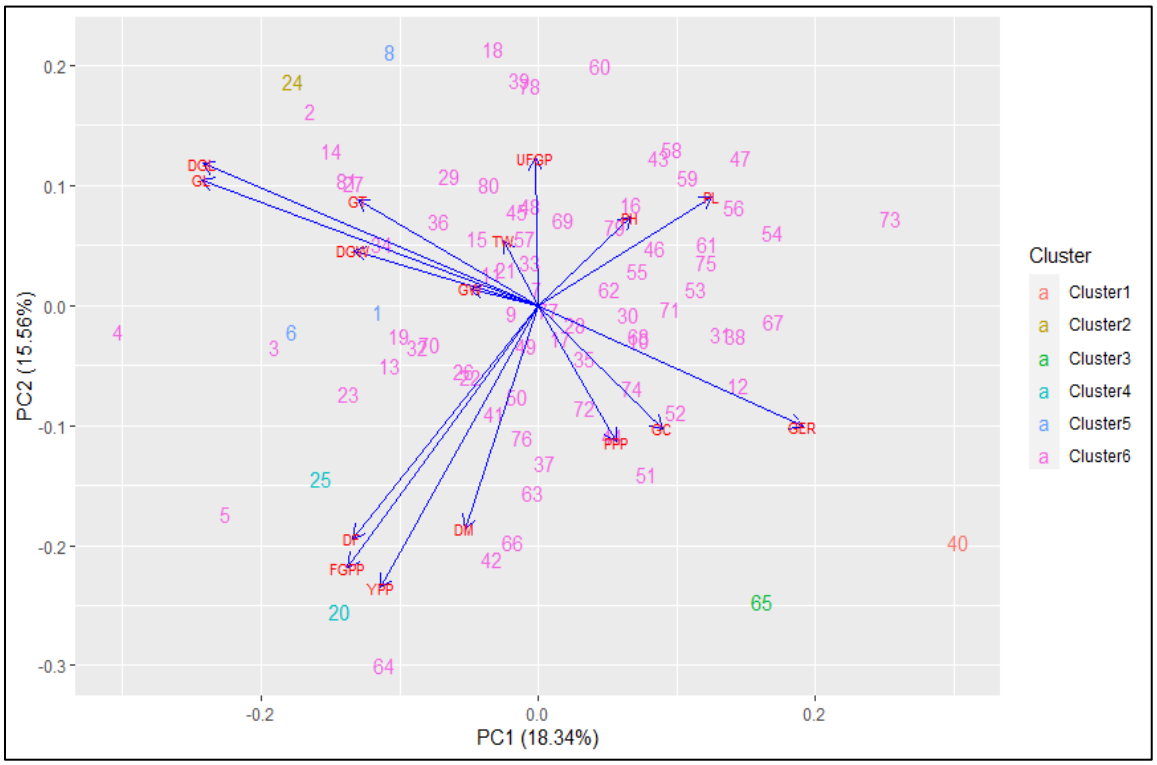

Fig.4.2 Grouping of genotypes based on the two principal components

4.5 Polymorphism of SSR markers among the accession

In the present investigation the PIC value (**Table 4.7)** ranges from 0.23 for marker RM53 to 0.99 for RM1256. The average PIC for the overall markers was 0.64. Marker with high value of PIC gave higher discriminatory power to distinguish genotypes from one another. The PIC value of SSR markers more than 0.5 were considered highly informative while the PIC value of 1 indicate that the marker is highly polymorphic and would have an infinite number of alleles that is more informative (Hildebrand *et al*., 1992). The highest frequency allele was detected from RM53 while lowest frequency was observed from RM524 with a mean value of 0.52. Park *et al*. (2019) reported higher average PIC value by using 16 SSR markers in a collection of different coloured rice of Korea, the range of the PIC value was also reported to be 0.85 to 0.96. The higher value of PIC could be due to the use of more diverse set of germplasm or due to highly polymorphic SSR markers. Verma *et al*. (2019) reported average PIC value of 0.507 by using 65 SSR markers in a collection of 114 rice genotypes of NE India. Khumbar *et al*. (2015) reported average PIC more than 0.5 in a collection of landraces, local selections and improved varieties, whereas, Bollineni *et al*. (2020) reported lower average PIC value of 0.34 indicating the less diversity in their accession. Wide range of PIC value, 0.30 to 0.84 by Hossain *et al*. (2007); 0.123 to 0.836 by Pervaiz *et al*. (2010) and 0.36 to 0.98 by Kumar *et al*. (2012) was also reported. Different range and average PIC value reported could be due to varied polymorphism of SSR markers or range of diversity in different set of germplasm collection.

4.6 Genetic diversity of the population at molecular level using SSR markers

The population level genetic diversity (**Table 4.8**) of the present accession revealed that 102 total alleles were detected with 40 SSR markers, on an average 2.55 alleles were detected per locus. The lowest alleles per locus was

2 which was detected in majority of the markers (21 SSR markers), 16 markers were detected to give 3 alleles per locus while 3 markers; RM3331, RM1866 and RM1132 were detected to have 4 alleles per locus. A total of 112 alleles in a set of 729 Indian rice varieties was reported by Singh *et al*. (2016) by using 36 HvSSR, in this accession 3.11 alleles per locus was recorded. More number of alleles were reported by various researchers, Verma *et al*. (2019) reported a total of 147 alleles in 144 rice genotypes of NE India characterized by using 65 SSR polymorphic SSR markers. Kumbhar *et al*. (2015) used 15 polymorphic SSR markers in a landraces and improved varieties of rice, they detected 189 polymorphic alleles with 2 to 23 range and 9 unique alleles. Bollineni *et al*. (2020) reported 86 alleles in a set of 124 diverse rice genotypes with 32 SSR markers. Nachimuthu *et al*. (2015) also reported 205 total alleles in 192 diverse rice germplasm lines with 61 genomes wide SSR markers with a varied range of 2 to 7 alleles per locus. Lesser alleles, 52 total alleles with average of 2.7 alleles per marker was reported by Rashmi *et al*. (2017). The variation in the number of alleles detected in different studies might be due to the use of varied diverse genetic material and polymorphic markers in each study.

The highest gene diversity (He) 0.71 was shown by RM517 followed by RM444 (0.69) with average value of 0.48. Akter *et al*. (2022) also reported a gene diversity value of 0.48 in Bangladesh rice. Verma *et al*. (2019) and Singh *et al*. (2016) reported lower value of gene diversity of 0.33, Hoque *et al*. (2021) reported high value of gene diversity (0.91) among Bangladesh local rice cultivars, Nachimuthu *et al*. (2015) reported 0.52 average gene diversity in Asian rice involving indica and japonica rice, Shakil *et al*. (2013) reported average gene diversity 0.69 in modern rice and local landraces of rice in Bangladesh. The diversity panel with global accessions were observed to have a gene diversity of 0.45 to 0.70 (Garris *et al*., 2005; Ni *et al*., 2002), these rice accessions in various investigation include different species of rice such as indica, japonica, temperate japonica, wild relatives, pigmented and quality rice and even improved varieties.

Therefore, the diversity that exist our panel represents a considerable proportion of the genetic diversity that possibly present in the major rice-growing Asian countries.

From the value of observed heterozygosity (Ho) which was 0.00 for all the locus, the whole accession was assumed to be pure and homozygous for SSR markers used in the present study owing to rice being self-pollinated crop. On the perusal of **Table 4.8** the observed heterozygosity (0.00) was much lower than the total expected heterozygosity (0.72) which was supported by low gene flow (Nm) for most of the loci even though some markers such as RM19696, RM1100 and RM22 were observed with high value of gene flow with lower difference between observed heterozygosity and expected heterozygosity. However, as rice is self-pollinated crop, the value of gene flow (Nm) is expected to be lower than 1, so, the higher value of gene flow for some markers in the present study could be due to the inclusion of sister line or, landraces with different name, or some extent of natural cross-pollination with adjacent crop.

Table 4.7 Polymorphism information observed among 81 genotypes based on SSR markers. Note: Major allele is described as the allele with the highest frequency.

Sl. No	Primer	Chromo. no.	Annealing Temp.	Allele no	Size range (bp)	Highest frequency		PIC
						Size (bp)	Frequency (%)	
1	**RM53**	8	55	3	180-200	200	0.88	0.23
2	**RM240**	2	55	4	120-200	150	0.59	0.64
3	**RM1256**	3	55	3	110-150	150	0.79	0.99
4	**RM1352**	3	55	5	210-230	230	0.72	0.45
5	**RM15078**	3	55	4	200-300	280	0.19	0.96
6	**RM15429**	3	55	5	590-600	600	0.80	0.35
7	**RM22**	3	55	5	180-190	190	0.83	0.31
8	**RM15448**	3	55	5	150-170	170	0.84	0.29
9	**RM7563**	4	50	4	120-150	150	0.88	0.23
10	**RM1100**	4	50	4	120-150	120	0.51	0.62
11	**RM5709**	4	55	5	120-300	150	0.33	0.81
12	**RM440**	5	55	6	180-220	200	0.43	0.80
13	**RM19696**	6	55	6	400-450	450	0.80	0.36
14	**RM6836**	6	55	6	230-260	230	0.77	0.41
15	**RM19974**	6	55	5	120-150	140	0.17	0.97
16	**RM527**	6	55	5	210-250	230	0.47	0.76
17	**RM5344**	7	50	4	280-320	280	0.56	0.64
18	**RM1132**	7	55	6	100-250	150	0.52	0.62
19	**RM234**	2	55	5	120-300	150	0.80	0.35
20	**RM1896**	9	55	5	100-180	120	0.42	0.79
21	**RM24181**	9	55	6	100-120	100	0.44	0.68
22	**RM524**	9	55	4	190-200	190	0.10	0.40
23	**RM245**	9	55	3	150-170	170	0.47	0.74
24	**RM444**	11	55	4	180-300	180	0.36	0.82
25	**RM524**	9	55	4	190-200	200	0.58	0.50
26	**RM216**	10	55	3	120-180	150	0.22	0.93
27	**RM209**	11	55	5	150-180	160	0.30	0.91
28	**RM313**	12	55	2	100-130	130	0.14	0.98
29	**RM3331**	12	50	4	100-180	100	0.59	0.60
30	**RM270**	12	55	4	180-210	210	0.43	0.94
31	**RM247**	12	55	4	120-180	120	0.53	0.60
32	**RM28302**	3	55	5	100-130	130	0.46	0.72
33	**RM28519**	12	55	4	130-150	130	0.43	0.75
34	**RM349**	4	55	3	660-670	660	0.41	0.74
35	**RM3894**	3	55	3	180-210	190	0.47	0.75
36	**RM517**	3	55	4	250-300	250	0.37	0.77
37	**RM13600**	2	55	4	120-160	150	0.48	0.72
38	**RM60**	3	55	4	150-180	180	0.85	0.27
39	**RM22**	3	55	3	190-200	190	0.44	0.66
40	**RM232**	3	55	4	130-180	150	0.65	0.55

Table 4.8 Genetic diversity of 40 SSR markers in the 81 rice genotypes.

Sl.No	Locus	Na	Ne	Ht	He	Ho	Fis	Fit	Fst	Nm
1	**RM53**	2.50	1.26	0.21	0.20	0.00	1.00	1.00	0.01	34.72
2	**RM240**	3.50	1.67	0.55	0.39	0.00	1.00	1.00	0.28	0.65
3	**RM1256**	2.50	1.21	0.17	0.17	0.00	1.00	1.00	0.01	23.69
4	**RM1352**	3.00	1.71	0.42	0.42	0.00	1.00	1.00	0.00	518.07
5	**RM15078**	3.50	1.93	0.45	0.38	0.00	1.00	1.00	0.16	1.33
6	**RM15429**	3.00	1.50	0.33	0.32	0.00	1.00	1.00	0.04	6.74
7	**RM22**	3.00	1.47	0.32	0.31	0.00	1.00	1.00	0.02	14.87
8	**RM15448**	2.50	1.43	0.29	0.27	0.00	1.00	1.00	0.06	3.83
9	**RM7563**	2.50	1.34	0.25	0.24	0.00	1.00	1.00	0.05	5.18
10	**RM1100**	3.00	2.54	0.61	0.61	0.00	1.00	1.00	0.01	29.56
11	**RM5709**	3.50	2.63	0.67	0.60	0.00	1.00	1.00	0.10	2.26
12	**RM440**	4.00	2.90	0.70	0.65	0.00	1.00	1.00	0.06	3.72
13	**RM19696**	2.00	1.46	0.31	0.31	0.00	1.00	1.00	0.00	223.09
14	**RM6836**	2.50	1.66	0.40	0.39	0.00	1.00	1.00	0.01	22.11
15	**RM19974**	4.00	1.53	0.34	0.33	0.00	1.00	1.00	0.03	7.69
16	**RM527**	4.00	2.98	0.67	0.66	0.00	1.00	1.00	0.01	26.31
17	**RM5344**	3.00	2.23	0.58	0.55	0.00	1.00	1.00	0.05	4.96
18	**RM1132**	4.00	2.68	0.67	0.60	0.00	1.00	1.00	0.09	2.43
19	**RM234**	4.00	2.04	0.52	0.49	0.00	1.00	1.00	0.05	4.83
20	**RM1896**	5.00	3.31	0.71	0.70	0.00	1.00	1.00	0.03	9.61
21	**RM24181**	2.50	1.87	0.63	0.44	0.00	1.00	1.00	0.30	0.59
22	**RM524**	3.00	1.71	0.41	0.41	0.00	1.00	1.00	0.01	29.98
23	**RM245**	3.00	2.49	0.65	0.59	0.00	1.00	1.00	0.09	2.43
24	**RM444**	4.00	3.24	0.70	0.69	0.00	1.00	1.00	0.02	15.97
25	**RM524**	2.50	1.11	0.51	0.10	0.00	1.00	1.00	0.81	0.06
26	**RM216**	4.00	2.50	0.62	0.59	0.00	1.00	1.00	0.06	4.10
27	**RM209**	4.00	3.30	0.70	0.70	0.00	1.00	1.00	0.01	39.12
28	**RM313**	2.50	1.57	0.33	0.29	0.00	1.00	1.00	0.12	1.83
29	**RM3331**	5.00	2.78	0.66	0.64	0.00	1.00	1.00	0.03	7.12
30	**RM270**	3.50	2.62	0.67	0.60	0.00	1.00	1.00	0.09	2.49
31	**RM247**	3.50	2.63	0.63	0.62	0.00	1.00	1.00	0.01	20.44
32	**RM28302**	3.00	1.55	0.55	0.34	0.00	1.00	1.00	0.39	0.39
33	**RM28519**	3.00	2.56	0.66	0.61	0.00	1.00	1.00	0.08	2.98
34	**RM349**	3.50	2.87	0.66	0.65	0.00	1.00	1.00	0.02	15.31
35	**RM3894**	3.50	2.68	0.64	0.63	0.00	1.00	1.00	0.02	13.08
36	**RM517**	4.50	3.44	0.72	0.71	0.00	1.00	1.00	0.01	18.08
37	**RM13600**	4.00	3.01	0.67	0.66	0.00	1.00	1.00	0.02	12.19
38	**RM60**	2.50	1.39	0.27	0.26	0.00	1.00	1.00	0.05	5.27
39	**RM22**	3.00	2.53	0.63	0.60	0.00	1.00	1.00	0.04	6.39
40	**RM232**	4.00	2.07	0.52	0.52	0.00	1.00	1.00	0.00	79.70

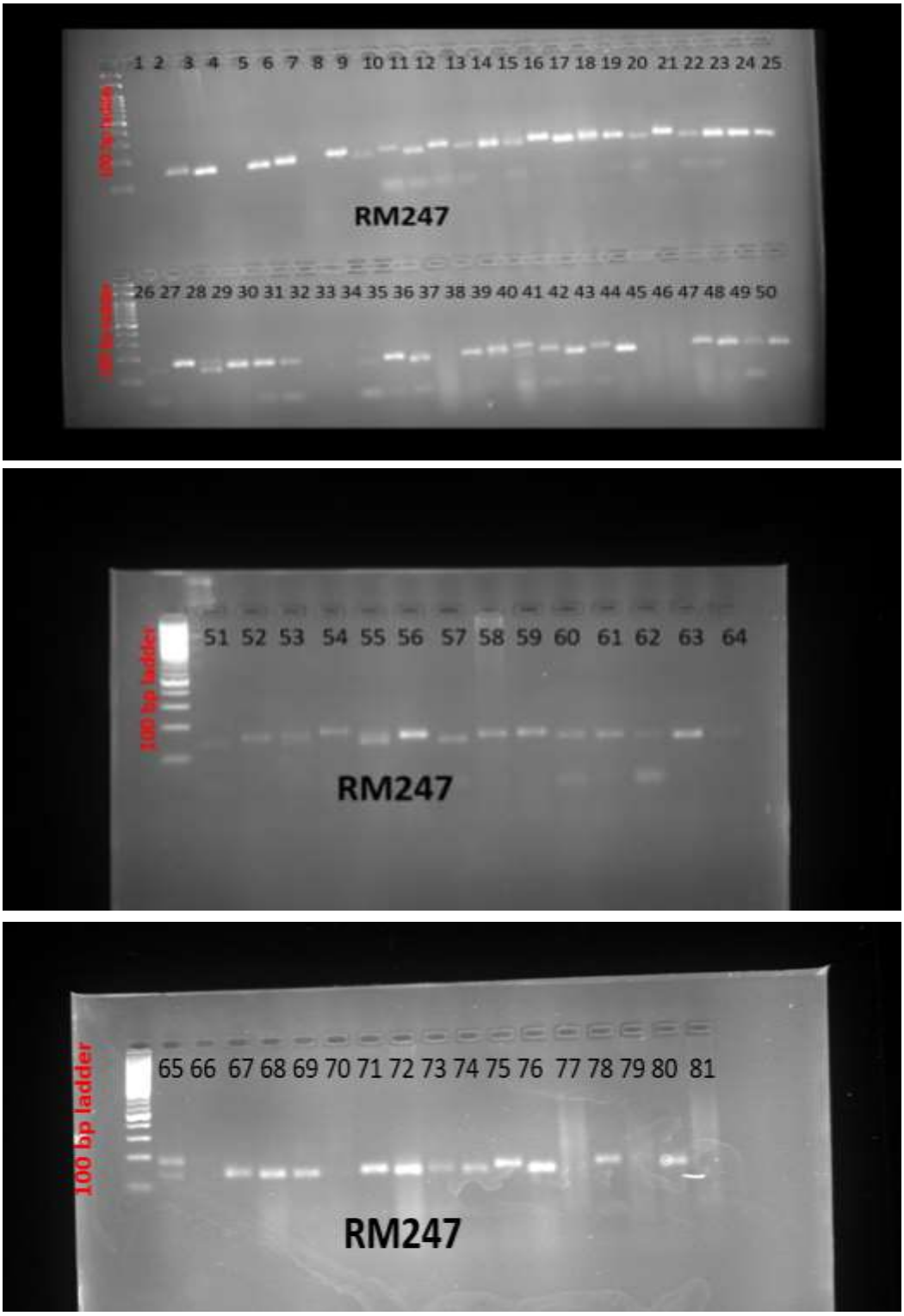

Plate 4.1 Amplification of RM247 in the genotypes; Numbering corresponds to the genotypes no. in the list of genotypes Table 3.2

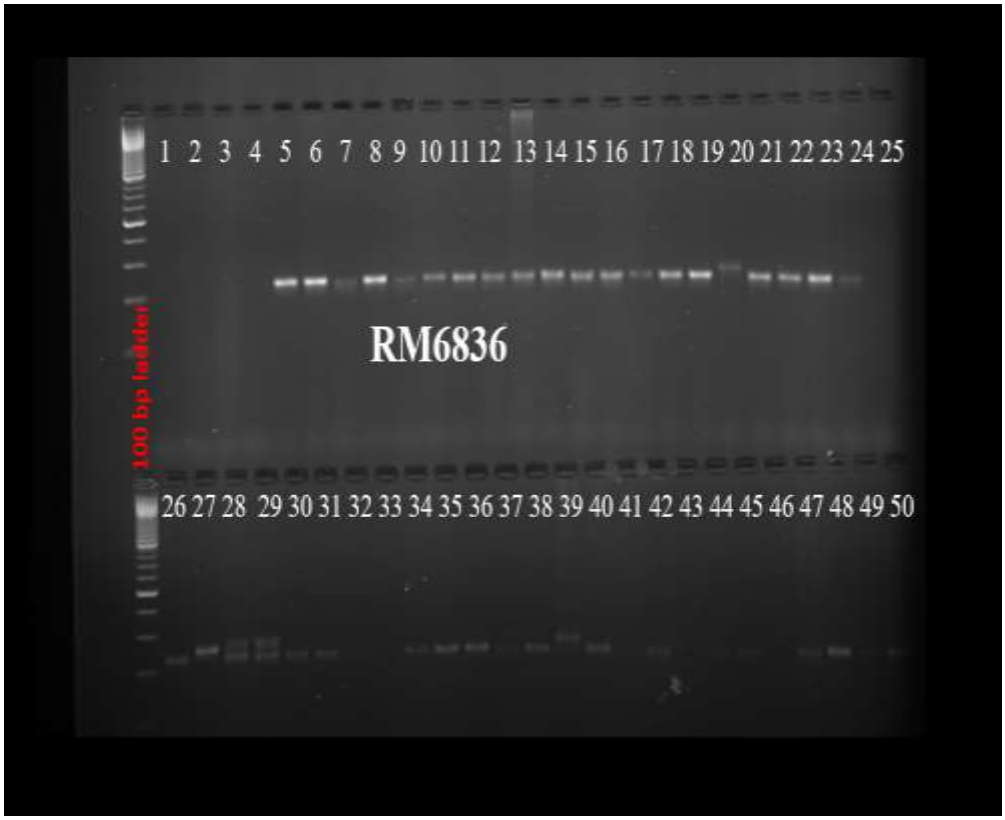

Plate 4.2 Amplification of RM6836 in the genotypes; Numbering corresponds to the genotypes no. in the list of genotypes Table 3.2

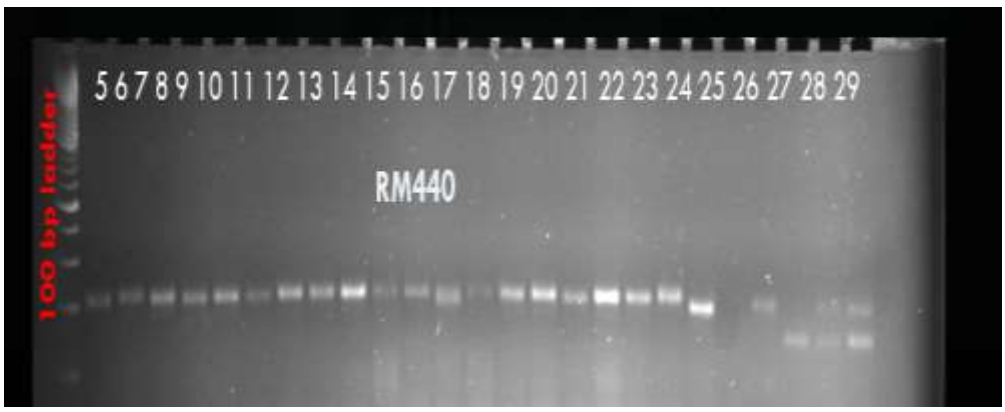

Plate 4.3 Amplification of RM440 in the genotypes; Numbering corresponds to the genotypes no. in the list of genotypes Table 3.2

4.7 Genetic relationship among the genotypes

Population structure analysis was conducted using Bayesian model-based STRUCTURE v 2.3.4, with admixture and K value at 1 to 10 with 9 iterations using 40 polymorphic markers. The length of burning period was set at 50000 with no. of MCMC at 100000. Structure harvester 0.6 results revealed highest ΔK for the model parameter K=2 (Fig. 4.2), so the true number of subpopulations was considered to be two indicating the whole population of rice accessions can be divided into two sub-population i.e., P1 and P2 (Fig. 4.3). Genotypes were classified as pure and admixture based on their probability value. Genotypes with probability value >80% were considered as pure for their respective subpopulation while the remaining genotypes with value <80% were considered as admixtures. Out of 81 genotypes, P1 consist 26 pure and 7 admixtures landraces while P2 consist 40 pure with 8 admixtures. Best performing genotypes such as N. Special Bobla for yield per plant and Taghaho for amylose content were found in P1, while P2 consist of best performing genotypes such as Lhasalu for no. of filled grains per plant, Makre Tanye for no. of panicles per plant, Kerebe for panicle length, Betguti for grain elongation ratio, China-Tsone for grain width and White Mekrilha for grain length. Among admixtures Pluchama Ngoba was recorded as a best performing genotype for grain width while Kemenga (pointed) was recorded with highest 1000 grains weight. Mogga *et al*. (2018) also detected 2 sub-populations in 59 rice accessions using SNP's, Verma *et al*. (2019) reported 3 sub-populations in a collection of NE India rice, Das *et al*. (2013) reported 4 sub-populations in a collection of 83 landraces rice of NE India, Park *et al*. (2019) reported 7 sub-populations in a collection of coloured rice, Choudhury *et al*. (2014) reported 3 sub-populations in a collection of 6984 NE rice accessions, Kumbhar *et al*. (2015) also reported 3 sub-populations in 50 rice genotypes, Bollineni *et al*. (2020) reported 4 sub-populations in 124 diverse rice genotypes. These differences in number of sub-populations might be attributed to the use of different marker system or a

different set of germplasm. On comparison, STRUCTURE software offers better results on population structure analysis using model-based analysis than frequentist approach of clustering since model-based clustering rely on Bayesian methods, in which certain parameters like correlated allele frequencies no-prior population information were used. Fst value for the two sub-population calculated from the STRUCTURE software were 0.20 and 0.26 with an average value of 0.23. An Fst value greater than 0.15 can be considered as significant in differentiating populations (Frankham *et al*., 2002). The mean value of alpha was estimated to be 0.15, this small value reveals that only few genotypes in the present study were admixed (Singh *et al*., 2016). If the alpha value approach zero, the, we can say that the most individuals in the study are from different populations, while if the alpha value approach 1 (Li *et al*., 2014), then we can say that most accessions of populations are admixed (Ostrowski *et al*., 2006). The genetic differentiation among populations were considered highly differentiated as per classification given by Wright (1978). Pairwise Fst value between the two sub populations (Table 4.10), P1 and P2 was 38.12 indicating significant differentiation between the two populations. Fst is a measure of population differentiation due to genetic structure.

The mean no. of alleles (Na), no. of effective alleles (Ne), observed heterozygosity (Ho), gene diversity (He), unbiased expected heterozygosity (uHe) and fixation index (F) were used to determine genetic diversity at the sub population level (Table 4.11), the value of Na (3.25, 3.42), Ne (2.13, 2.23), I (0.82, 0.88), Ho (00, 00), He (0.46, 0.49), uHe (0.47, 0.50) and F (1, 1) were found to be comparable between the two sub populations.

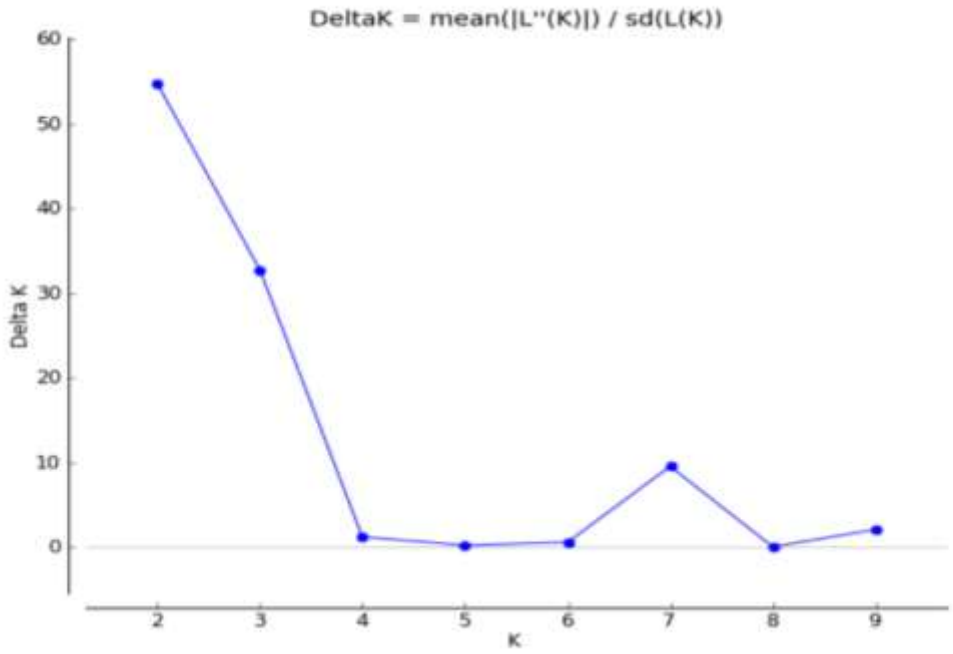

Fig. 4.3 A plot of delta K values from the Structure analyses of 81 rice accessions, obtained through Structure harvester ver. 0.6. Application (Earl and Vonholdt).

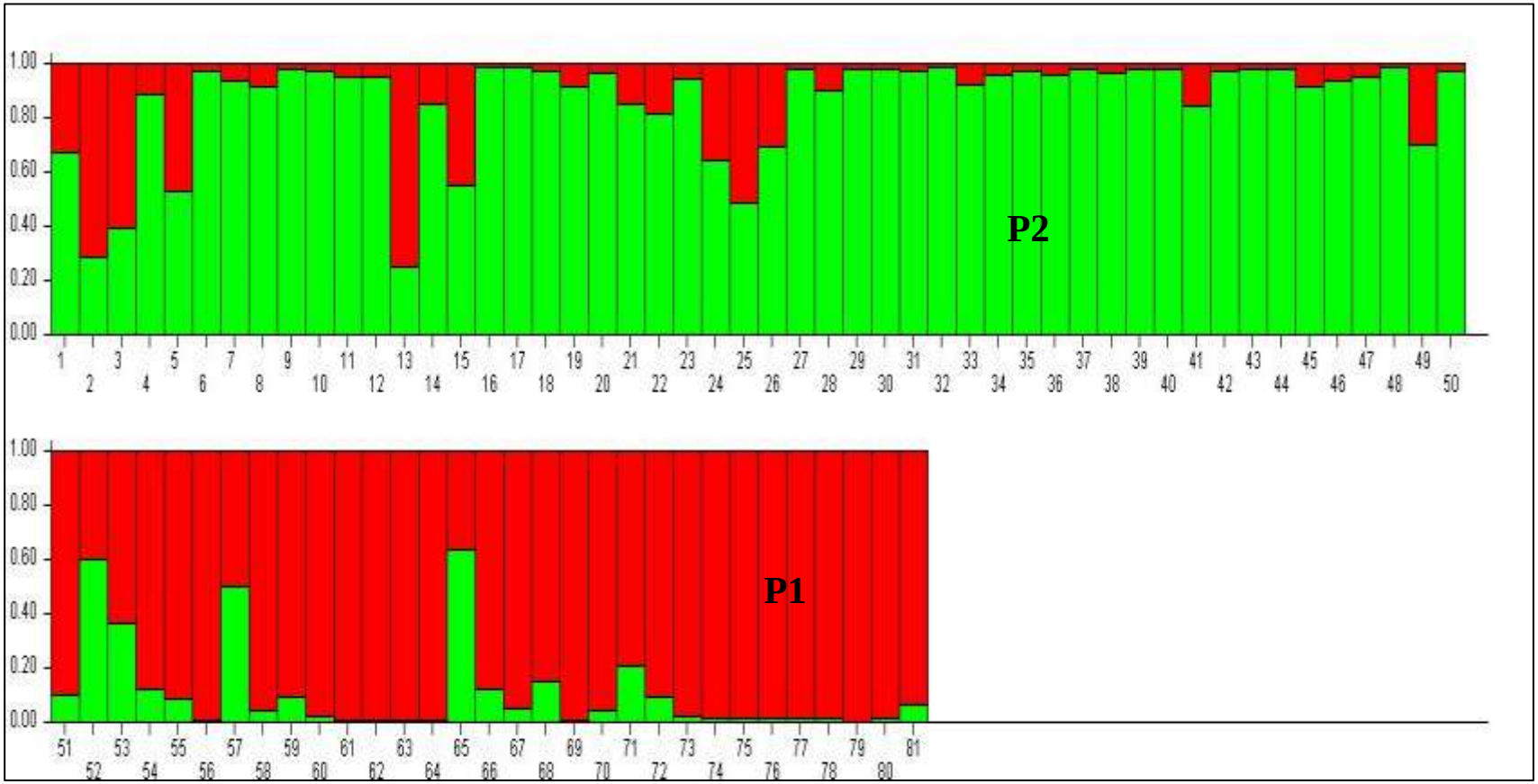

Fig. 4.4 Population structure of 81 rice accession based on 65 SSR markers. Note: Numbering of genotypes corresponds to the serial no. in 'List of genotypes under study' Table 3.2.

Table 4.9. Pairwise population differentiations (Fst value) below diagonal and gene flow (Nm) values above diagonal.

Populations	P1	P2
P1	0	38.12
P2	38.12	0

Table 4.10 Genetic diversity statistics of 81 rice genotypes at sub-population level. No. of different alleles (Na); No. of effective alleles or allelic richness (Ne); shanon information indiex (I), observed heterozygosity (Ho), genetic diversity (He), unbiased expected heterozygosity (uHe) and fixative index (F).

Pop.	Na	Ne±SE	I±SE	Ho±SE	He±Se	uHe±SE	F
P1	3.25±	2.13±0.12	0.82±0.05	0.00±0.00	0.46±0.03	0.47±0.03	1.00±0.00
P2	3.42	2.23±0.11	0.88±0.05	0.00±0.00	0.49±0.03	0.50±0.03	1.00±0.00

4.8 Analysis of molecular variance

AMOVA (Table 4.11) revealed the presence of 13% variation among the population, 87% variation among the individuals and 0% variation within the individual. The variation among the individuals was more than variation among the population. This can be due to the collection of 81 genotypes in the present study from various parts of Nagaland. A 0% variation was observed within individual which indicate that genotypes were highly pure and maintained without any mixture. Verma *et al*. (2019) also reported higher proportions of variation among individuals in a collection of NE India rice, Mogga *et al*. (2018), Singh *et al*. (2016), Roy *et al*. (2015) and Choudhury *et al*. (2014) also reported the similar results on AMOVA. The observed Wright's F statistic Fst was 0.13, Fis was 1.00 and Fit was also 1.00. A very high Fit value indicated lack of heterozygosity most likely due to the inbreeding nature of rice which is self-pollinated (Nachimuthu *et al*., 2015). The Fst inbreeding coefficient within subpopulations relative to the total provides a measure of the genetic differentiation between subpopulations (Ochoa and Storey, 2016). The determination of Fst using structure analysis for the subpopulations of the present study was 0.23 which indicated moderate differentiation between subpopulation as per Wright (1978) classification, Fst value range between 0.15 to 0.25 indicates a moderate differentiation, a value greater than 0.25 explain a very high differentiation between sub-populations, while if Fst is 0.05 or less, differentiation is negligible. This AMOVA calculated from model-based analysis was reported to be reliable and consistent to provide detail information on the genetic constitution of the population. Nei genetic distance of 81 rice genotypes at sub population was recorded to be 0.18 (Table 4.13) indicating moderate differentiation between the sub-population.

Table 4.11 Analysis of molecular variance of 81 rice genotypes

Source	Df	SS	MS	Est.Var	%Var	F statistics	Value	P(rand >= data)
Among Pops	1	135.90	135.90	1.48	13%	Fst	0.13	0.001
Among Indivuals	79	1565.18	19.81	9.90	87%	Fis	1.00	0.001
Within Indivdual	81	0.00	0.00	0.00	0%	Fit	1.00	0.001
Total	161	1701.08		11.39	100%			

Table 4.12 Pairwise population matrix of Nei genetic distance of 81 rice genotypes at sub-population levels.

Populations	P1	P2
P1	0	0.18
P2	0.18	0

4.9 Neighbour-joining based clustering

An unweighted neighbour-joining tree (**Fig 4.4**) based on the alleles that were detected by 40 SSR markers displayed the genetic relationships among the 81 genotypes, cluster analysis using this method separated the whole accessions into two main group with admixtures distributed in each cluster. Cluster I comprise of 48 genotype and cluster II with 33. Using Venn diagram from Venny 2.1, the unweighted neighbour-joining clustering and model-based genetic relationship clustering were compared, cluster 1 generated using unweight neighbour-joining was detected to have 72.2% similarity with sub-population 1 generated through model-based analysis, cluster II had 66.7% similarity with sub-population 2. This pattern support that grouping of genotypes based on hierarchical cluster and model-based approach was more than 72 % similar.

4.10 Principal Coordinate analysis

PCoA using SSR markers data (**Fig 4.5**) determines the genetic relatedness among the accession. The total variation of the first three axes of differentiation was 17.37%. The first two coordinate explained 7.86% and 5.49% variation respectively. Singh *et al*. (2016) also reported a cumulative variation of 15.9%. Whereas, Choudhury *et al*. (2014) reported 43% cumulative variation in a collection of Nagaland rice. Observed differences in a grouping of accessions could be due to the differences in methodology of grouping, model-based analysis grouping is based on the Bayesian model approach whereas, unweighted neighbour-joining clustering is distance-based approach in which admixture genotypes were not included. Since the detail information about the genetic constitution is included in model-based approach it is considered to be more informative (Verma *et al*., 2019) as this help in separation of admixtures from pure genotypes.

Grouping or clustering genotypes help in identifying diverse genotypes which can be useful in hybridization programme that result in creating segregating progenies with high genetic variability for further selection (Barret *et al*., 1998). The present study revealed two sub-populations carrying 15 admixtures with majority variation among individuals. Existence of broad genetic base was also observed based on our studies in gene and allele diversity. Therefore, based on the results of this study the selection for genotypes from different population that perform well with yield and quality traits could help in developing transgressive segregants for rice varieties with improved quality and yield traits. The detection of high level of genetic diversity among the accessions at genotypic and phenotypic level in the present investigation could be due to the fact that the landraces of these NE rice were cultivated for long period of time in a diversified ecological niche that finally result in the evolution of new alleles that help each genotype to adapt well in their respective growth habitat.

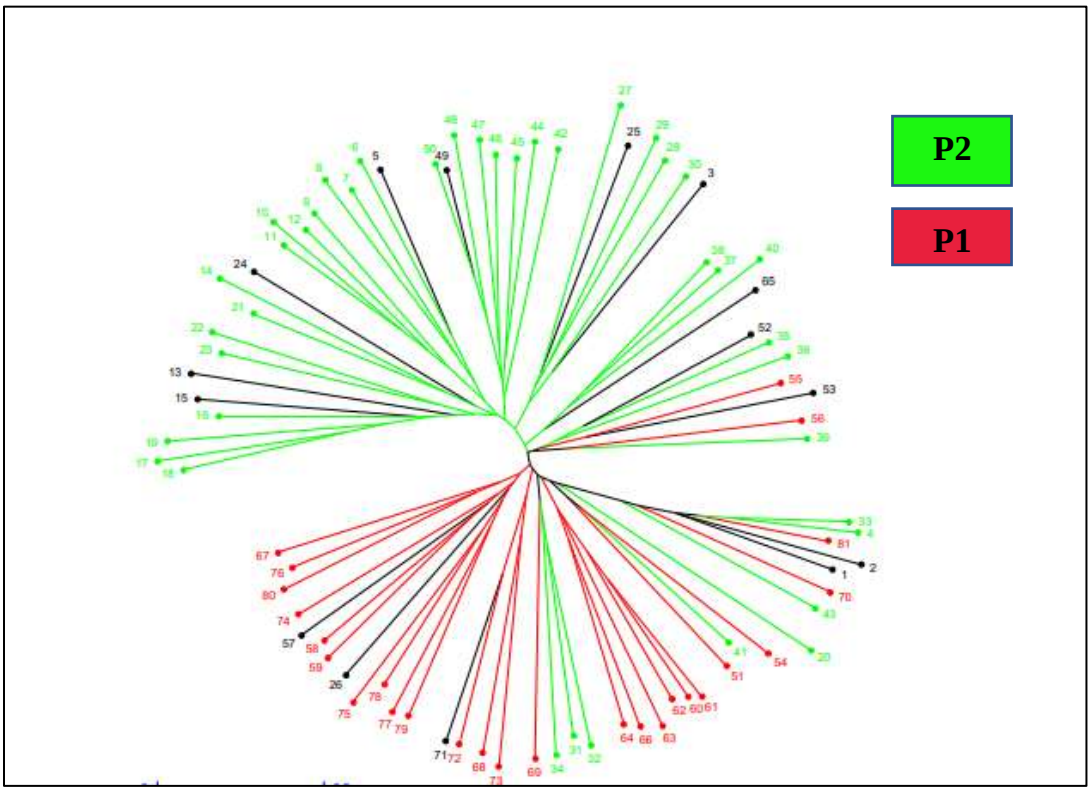

Fig. 4.5 Unrooted neighbor joining tree of 81 rice genotypes using SSR markers.

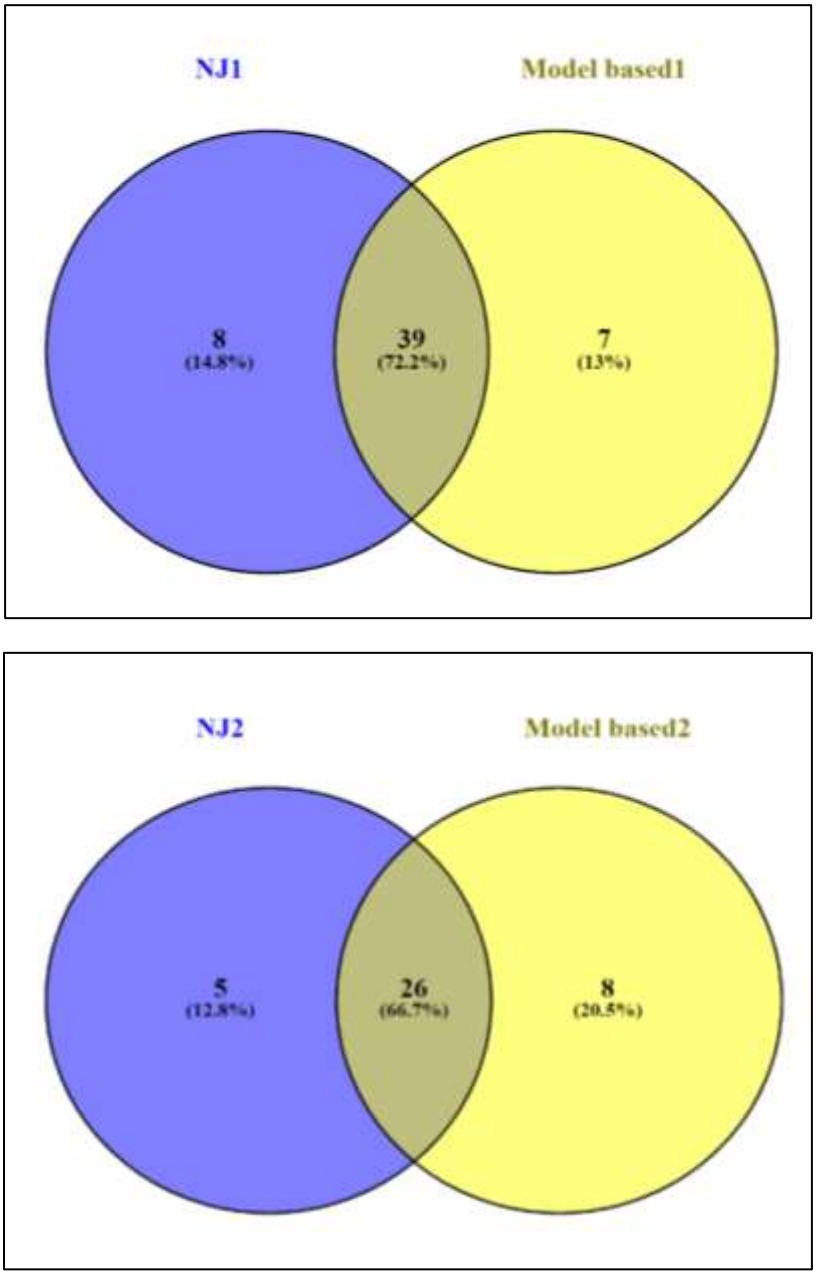

Fig. 4.6 Venn diagram showing co-linearity between neighbour joining based clusters and model-based sub-populations.

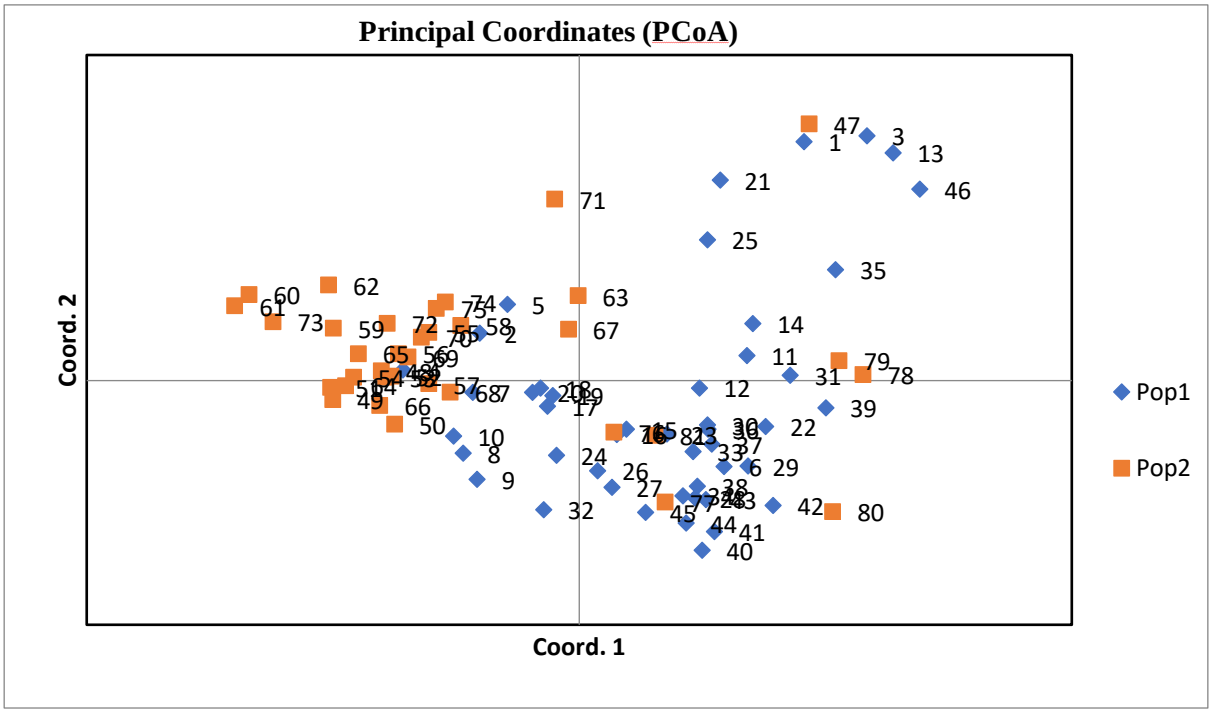

Fig. 4.7 Principal Coordinate Analysis of 81 rice genotypes using SSR markers. Note: Numbering of genotypes corresponds to the serial No. in 'list of genotypes'.

4.11 Association mapping analysis

Association analysis, or linkage disequilibrium is one of the strategies that have been used for identifying genes that control important traits. It has been successfully used in detecting genes that control human disease (Borba *et al*., 2010). However, this strategy is also being used to identify important genes in various plant species such as rice, wheat, cotton and even in horticultural crops (Borba *et al*., 2010; Shi *et al*., 2017; Igbal and Rahman, 2017; Font *et al*., 2019). The classical method of identifying genomic regions through QTL mapping has been found to possessed some limitations such as limited segregating alleles per locus in a segregating population that result in less genetic diversity in a given population, low number of traits per cross as it is difficult to identify parent materials with contrasting genotypes and phenotypes for all those of traits of interest (Buntjer *et al*., 2005) and the large segregating population required for high resolution mapping that is required for marker assisted selection is possibly difficult for some species (Skot *et al*., 2005). On the other hand, association mapping comes with a principle of detecting association between marker and trait of interest through linkage disequilibrium (Zondervan and Cardon, 2004). The mapping population which is usually germplasm population are partitioned into various classes on the basis of variability of traits. The correlative statistical analysis between this phenotypic data and genotypic data based on marker per locus of the individual in the mapping population forms the basis of association mapping. The presence of co-segregation between marker and trait of interest will notify the association between them which is further used in finalising the results of association mapping or QTL identification. Association mapping took advantage of all the meiotic and recombinant events that may occur in the evaluated population and high linkage disequilibrium is expected to present in rice as it is a self-pollinated crop (Patron *et al*, 2002) with this high degree of linkage disequilibrium, even lesser markers per locus will not result in lower resolution for MAS (Rostoks *et al*, 2006). Furthermore, association mapping

does not necessarily require developing a mapping population, as the sampling of non-related individuals as in germplasm accessions offer a great opportunity in finding the advantageous allele combination (Abdurakhmonov and Abdukarimov, 2008). In a panel of highly diverge individuals coupled with random mating, only polymorphisms with a tight linkage to a locus with a desirable phenotypic effect are likely to be significantly associated with a concerned trait (Remington *et al*., 2001). In addition, association analysis can be benefitted by including data collected form several years of experimental analysis with genotypes of breeding programs with the additional possibility of analysing several traits of interest simultaneously (Borba *et al*., 2010).

The association between 40 SSR markers and grain quality traits as well as yield attributing traits were analysed with TASSEL software version 5.2.86 by following General Linear Model (GLM) and Mixed Linear Model (MLM). Association analysis (**Table 4.13**) identified 59 association between traits and markers at $P<0.05$ threshold level of significance with percentage of phenotypic variation (R^2) ranging from 7.44 by RM313 to 37.68 by RM5709. Out of 40 SSR markers 4 markers; RM440, RM349, RM5344 and RM22 were associated with grain breadth, out of these 4 markers RM440 located at chromosome no. 5 offer highest phenotypic variation (34.54%) and lowest phenotypic variation (10.42%) by RM349 located at chromosome no. 4, another six association were detected between grain length and RM232, RM209, RM247, RM6836, RM1100 and RM60, in these association RM232 and RM60 both located at chromosome no. 3 gave highest (25.69%) and lowest (12.24%) phenotypic variation respectively.

For decorticated grain width, 10 association were detected with markers such as RM209, RM240, RM28302, RM524, RM22, RM15429, RM28519, RM232, RM19974 and RM53 out of which RM209 at chromosome 11 gave highest variation (22.02%) on the other hand, RM53 at chromosome 8 gave lowest variation (8.01%). Six markers namely RM209 which gave highest

variation (22.02%), RM232, RM15448, RM19974, RM6836 and RM60 located at chromosome 3 giving lowest variation (11.85%) were detected to have association with decorticated grain length.

RM15078 which is located at chromosome no. 3 gave highest variation (35.81%) while RM28519 at chromosome 12 gave lowest variation (12.75%) among 5 markers in association with grain elongation ratio. Other markers were RM6836, RM15429 and RM524.

For gelatinization temperature markers RM6836, RM15078 and RM1132 were detected to have tight association out of which RM15078 at chromosome 3 gave highest variation (27.42%). Among those markers that have association with quality traits, RM232 and RM209 have association with multiple grain quality traits such as grain length, decorticated grain breadth and decorticated grain length. RM6836 was also detected to have association with multiple traits such as grain length, decorticated grain length, gelatinization temperature and grain elongation ratio.

Apart from grain quality traits, SSR markers were also detected to have association with yield attributing traits. Two markers, RM7563 and RM440 have association with panicle length, RM440 at chromosome 5 gave higher variation (21.18%), another two markers RM15448 which gave higher variation (14.44%) and RM313 were also detected to have association with no. of panicles per plant. RM24181, RM53 and RM19696 were also recorded to have association with no. of filled grains per panicle, among those markers that have association with no. of filled grains per panicle. RM24181 at chromosome 8 was responsible for the highest variation (16.06%) while RM53 was for lowest variation (8.54%). Days to maturity have association with two markers; RM19974 and RM5709 in which RM5709 at chromosome 4 was for higher variation (22.88%), while 7 marker-trait association were detected between RM5709 which gave highest variation (37.68%), RM247, RM3894, RM245, RM3331, RM440 and RM22 that gave

lowest variation (11.11%) with days to 50% flowering. These two traits were found to have associated with common marker; RM5709. Two markers each were detected to have association with 1000 grains weight and grain yield per plant. Between RM440 and RM1256 that associated with 1000 grains weight, RM440 was responsible for higher variation (30.79%) for the concerned trait. RM24181 and RM5709 were associated with grain yield per plant in which RM5709 at chromosome 4 gave higher variation (26.55%). Among two markers that have association with unfilled grains per panicle, RM440 gave higher variation percentage (19.01) while the other marker being RM13600. RM232, RM209 and RM245 were also detected to have association with plant height in which RM209 at chromosome 11 gave highest variation (14.78%).

Grain shape plays an important role in rice breeding as it determines yield and market values (Kato *et al*. 2011; Wang *et al*. 2012). Understanding the genetic mechanism controlling the grain shape has become crucial for molecular biologists and plant breeders as it leap breeding program in many folds yet providing accurate outcome. Aluko *et al*. (2004) detected the association between RM209 with grain width in interspecific cross between *Oryza sativa* and *Oryza glabberima*. Guo *et al*. (2009) identified two genes for grain weight GW3 and GW6 in which RM6836 is linked to GW3 in a population of *indica* and *japonica* derivatives, according to this report, GW3 could control the long large grain trait that is reported to be identical with GS3, which is a major QTL for grain length and weight, as well as a minor QTL for grain width and thickness in rice (Fan *et al*., 2006). Dai *et al*. (2016) two QTL; qGW2n and qGW6 in which qGW6 is flanked by RM6836 and RM527 on chromosome 6. Govindaraj *et al*. (2005) also reported the significant association between RM247 with grain breadth and cooked grain breadth in F2 individuals of a cross between Basmati and non-basmati.

Major QTLs that control grain weight have been detected between RM166 and RM344 on chromosome no. 10 (Bian *et al*. 2013). Wang *et al*.

(2014) also reported digenic epitasis effecting grain length and thousand grain weight on chromosome no. 5 flanked by RM440 and RM3575. RM5709 was reported to be associated with several traits including days to 50% flowering and grain yield in a collection of new plant type of rice, *indica*, tropical and temperate *japonica* (Donde *et al*., 2020). Sharma *et al*. (2017) detected consistent QTL for days to flowering on chromosome no. 12 flanked by RM519 and RM3331in upland rice cultivars of Assam.

These identified marker trait association in the present study supported by earlier findings can be immediately used in MAS whereas, those marker trait association which were not reported yet by other researchers need further validation and confirmed in other population even in bi-parental mapping population and could be used in improvement of a particular trait in any rice variety through marker assisted breeding. Moreover, it is ideal to have more marker to allow optimum coverage across the genome for more precise location of gene and marker validation

Table 4.13 Association analysis between SSR markers and grain quality and yield traits.

Sl.No.	Trait	Marker	Chromo. No.	P value	MarkerR2
1	GB	**RM440**	5	9.18E-06	0.345427
2	GB	**RM349**	4	0.026772	0.104207
3	GB	**RM5344**	7	0.0288	0.15734
4	GB	**RM22**	3	0.038957	0.132175
5	GL	**RM232**	3	1.92E-04	0.256928
6	GL	**RM209**	11	0.005988	0.219003
7	GL	**RM247**	12	0.014476	0.1542
8	GL	**RM6836**	6	0.024348	0.198634
9	GL	**RM1100**	4	0.025448	0.159404
10	GL	**RM60**	3	0.046192	0.122433
11	DGW	**RM209**	11	8.12E-07	0.356296
12	DGW	**RM240**	2	0.01013	0.178657
13	DGW	**RM28302**	12	0.011158	0.193045
14	DGW	**RM524**	9	0.011165	0.140407
15	DGW	**RM22**	3	0.013041	0.15521
16	DGW	**RM15429**	3	0.022434	0.17537

Sl.No.	Trait	Marker	Chromo. No.	P value	MarkerR2
17	DGW	**RM28519**	12	0.027554	0.119373
18	DGW	**RM232**	3	0.030026	0.117309
19	DGW	**RM19974**	6	0.038333	0.145159
20	DGW	**RM53**	8	6.61E-02	0.080123
21	DGL	**RM209**	11	0.002945	0.220294
22	DGL	**RM232**	3	0.011788	0.148125
23	DGL	**RM15448**	3	0.01694	0.158546
24	DGL	**RM19974**	6	0.025091	0.166282
25	DGL	**RM60**	3	0.038338	0.118534
26	DGL	**RM6836**	6	0.045672	0.166211
27	DM	**RM19974**	6	0.032666	0.14468
28	DM	**RM5709**	4	0.036081	0.228836
29	FL	**RM5709**	4	7.02E-04	0.376862
30	FL	**RM247**	12	0.013305	0.154073
31	FL	**RM3894**	3	0.016573	0.126339
32	FL	**RM245**	9	0.020319	0.121221
33	FL	**RM22**	3	0.030125	0.111183
34	FL	**RM3331**	12	0.030292	0.189102
35	FL	**RM440**	5	0.049953	0.173536
36	FGPP	**RM24181**	9	0.007385	0.160651
37	FGPP	**RM53**	8	0.044228	0.085462
38	FGPP	**RM19696**	6	0.047761	0.13281
39	PL	**RM7563**	4	0.004078	0.16173
40	PL	**RM440**	5	0.004818	0.211869
41	PPP	**RM15448**	3	0.028231	0.144479
42	PPP	**RM313**	12	0.042677	0.07441
43	GT	**RM6836**	6	1.23E-04	0.269607
44	GT	**RM15078**	3	5.22E-04	0.274199
45	GT	**RM1132**	7	0.025652	0.24267
46	TW	**RM440**	5	1.24E-05	0.307982
47	TW	**RM1256**	3	0.023986	0.096636
48	YP	**RM24181**	9	0.004837	0.188602
49	YP	**RM5709**	4	0.022093	0.265581
50	GEL	**RM15078**	3	2.19E-04	0.35811
51	GEL	**RM6836**	6	0.001449	0.275722
52	GEL	**RM15429**	3	0.024505	0.199013
53	GEL	**RM524**	9	0.037363	0.1288
54	GEL	**RM28519**	12	0.039064	0.127546
55	UFGPP	**RM13600**	2	0.01389	0.156719
56	UFGPP	**RM440**	5	0.014525	0.190137
57	PH	**RM232**	3	0.036402	0.117544
58	PH	**RM209**	11	0.043677	0.147826
59	PH	**RM245**	9	0.046145	0.092465

CHAPTER V

SUMMARY AND CONCLUSION

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Rice plays a crucial role in everyday life of half of the people in the world as a staple food and supply most of dietary needs, its demand around the world is increasing exponentially with increase in global population. With this increase in demand, consumers started to seek better quality of rice that suit their taste and needs. This rise in demand of high yielding quality rice has become an opportunity in improving the economy of the nation through global market as India is one of the largest producers of rice in the world. Plant breeders play an important role in seeking an alternative measure through identifying ideal genotypes among our landraces that carry valuable genes for yield and quality traits as increasing in yield of rice through other method such as increasing cultivable land is not in the option as it can affect global climate and other forms of life that inhabit our untouched forest. At the same time, improvement of yield and quality traits is not an easy task as focusing on either of them can have a negative impact on the other trait. Therefore, identification, documentation and evaluation of our landraces available in Nagaland at both genotypic and phenotypic level become a preliminary step in obtaining an ideal genotype with ideal strategy for improving rice yield and quality simultaneously. Since, conventional breeding alone takes longer duration, the development of molecular markers technology increases the speed of breeding and at the same time it is more accurate and predictable. Study of genetic parameters, characters association and identification of traits and markers association in our collection of landraces for grain yield and quality traits will be of immense help in identifying better genotypes and markers that can be of use in marker assisted breeding.

The present study was conducted in research farm and central laboratory of ICAR, NEH region, Nagaland center, Medziphema during *Kharif*, 2020 and 2021. The experimental materials consist of 81 lowland rice landraces from

different regions of Nagaland, two check varieties; RCM09 and Ranjit were also included. The field experiment was laid down in randomized block design with three replications and standard interculture operation were followed to ensure the success of crop growth. The whole accessions were genotyped with 40 polymorphic SSR markers in order to study the association between traits and markers. Eight important yield attributing traits were collected by following DUS guidelines, four physical grain quality parameters were recorded as per standard procedure.

Grain shape was determined from decorticated grain length and decorticated grain length and width ratio. The grain shape of each accession was classified according to ICAR publication, 1985. Maximum genotypes (53) were having long bold grain shape, 14 genotypes were short bold type, 6 genotypes had Basmati type grain shape, extra-long and medium slender grain shape were observed in 3 genotypes each, long slender and short slender type grain shape were also found in 1 genotype each.

Biochemical grain quality parameters such as amylose content, gel consistency and gelatinization temperature were also estimated including grain elongation ratio in central laboratory by following standard protocol. Maximum genotypes (51) were detected with high gelatinization temperature, while 19 genotypes had low gelatinization temperature and 11 genotypes were recorded with intermediate gelatinization temperature.

Based on the mean *per se* performance of each genotype against yield and quality traits under study, Pelhirie was recorded to have shortest duration to attain 50% flowering and maturity, whereas, N. Special was taking the longest duration to reach flowering and maturity. However, the whole accessions fall into a category of ‘medium’ and ‘late’ duration as per DUS guidelines.

The shortest plant height was observed in Belguti and this particular genotype can be recommended for contributing gene for developing dwarf

segregants in hybridization works. On the other hand, Kutasnie was tallest among the germplasm.

One of the most important yield traits, long panicle length was observed maximum for Kerebe while, Vamuzo was recorded with shortest panicle length.

Makre Tanye was recorded with maximum no. of panicles per plant comparing to other genotypes. But, Lhasalu was recorded with least no. of panicles per plant.

Lhasalu was recorded with highest number of filled grains per panicle and Yeipho had highest no. of unfilled grains per panicle.

Maximum 1000 grains weight was observed in Kemenya while Lhasalu gave minimum weight among the accessions.

A wide range of variation was observed among the accessions in grain yield per panicle, N. Special Bobla gave highest yield per plant which is higher in comparison to the two check varieties.

Among the quality traits, longest grain and decorticated grain length were recorded in White Mekrilha while China-Tsone was recorded with highest gain width. Poramunya was found to have highest decorticated grain width. Egiru yield lowest grain per plant from 81 genotypes.

From the estimation of grain elongation ratio, Betguti possessed highest record, the elongation ratio is an important cooking quality of rice. Taghaho was recorded with highest amylose content among 81 genotypes. Numerous genotypes such as Makilha II, Kemenya, Yeipho, K. Special I, K. Special II, Neingutsure, Pelhirie amd Kofie were recorded to have a gel consistency of 110 cm.

Form the analysis of variance presence of significant variation was observed among the yield and quality traits. The amount of this much variation implies that there is scope for selection.

The estimates of genetic parameter helped in understanding the gene actions of traits and ensure reliability of selection against recommended traits.

The estimation of genetic parameter in the present study revealed that highest genotypic and phenotypic variance was observed in no. of filled grains per panicle, in general, the phenotypic coefficient of variation of each trait was slightly higher than their corresponding genotypic coefficient of variation indicating little effect of environment on the development of these character except grain yield per plant and grain elongation ratio where both values were same.

High value of both GCV and PCV was observed in no. of panicle per plant, no. of filled grains per panicle, no. of unfilled grains per panicle, grain yield per plant, gel consistency, decorticated grain width, amylose content and grain elongation ratio.

Moderate GCV and PCV was also observed in panicle length, plant height and 1000 grains weight. Traits such as days to maturity, days to 50% flowering, grain length and decorticated grain length were having low GCV and PCV.

High heritability was observed for all the trait, since high heritability coupled with high genetic advance is ideal for selection, important traits such as grain elongation ratio, amylose content and no. of unfilled grain per panicle were observed to have high value for both heritability and genetic advance, Hence, selection based on grain elongation ratio and amylose content might be effective in improving such traits.

High value of GCV, PCV, heritability and genetic advance was observed in grain elongation ratio, amylose content and no. of unfilled grains per panicle. Therefore, this inferred the presence of additive gene action which is effective against selection.

Since direct selection of genotypes based on their performance alone in yield is not an ideal strategy in crop breeding programme since grain yield is a complex character, the measurement of relationship and association between yield and its component traits can determine the direct of selection. In this study, a higher phenotypic correlation coefficient was observed on comparison to their corresponding genotypic coefficient of variation.

Significant positive correlation was observed between yield and no. of filled grains per panicle, days to 50% flowering, no. of panicles per plant and days to maturity.

Among the component traits, days to 505 flowering showed significant positive correlation with days to maturity and no. of filled grains per panicle, also, days to maturity exhibited significant positive correlation with no. of filled grains per panicle.

No significant association was observed between grain yield and quality traits.

However, among the quality traits, significant associations were observed, grain length and decorticated grain length; grain width and decorticated grain width showed significant positive correlation either at both genotypic and phenotypic level or at phenotypic level alone.

Yield attributes such as days to maturity, plant height, panicle length, no. of panicles per plant, 1000 grains weight were observed to show significant positive correlation with grain quality parameters.

Path coefficient analysis revealed high direct effects of no. of filled grains per plant, no. of panicles per plant and 1000 grains weight on grain yield.

Direct effects of plant height, grain length, decorticated grain length, grain elongation ratio and amylose content were observed to be negative at negligible level.

Therefore, selection traits that have high direct effects towards grain yield might ensure the success of breeding of high yielding genotypes.

Genetic diversity and population structure analysis

The UPGMA based dendrogram grouped 81 genotypes into six clusters, cluster I to cluster III contain one genotype each. Cluster I was characterized by highest amylose content and gel consistency. Cluster II was characterized by 1000 grains weight, grain width and decorticated grain length. Cluster III was characterized by highest value in days to 50% flowering, plant height and grain yield per plant.

Whereas, cluster IV was observed with two genotypes and characterized by highest value for days to maturity, no. of filled grains per panicle and grain length.

Cluster V consisted three genotypes and was recorded with highest value for no. of unfilled grain per panicle, decorticated grain width and grain elongation ratio.

The last cluster, cluster VI carried 73 genotypes and it was characterized by panicle length.

PCA analysis revealed that the first principal component accounted for 18.34% to total variation and second principal component accounted for 15.56% to total variation.

A maximum diversity in 81 rice genotypes was governed by 1000 grains weight, grain width, decorticated grain width, grain length and decorticated grain length.

The study based on this categorization revealed presence of diversity among the accessions and genotypes with superior traits from different clusters could be used in hybridization programme to developed a desirable segregants having high genetic variations.

Analysis of genetic diversity of 40 SSR markers, 81 rice genotypes, analysis of molecular variance and principal coordinate analysis was done using GenALex 6.502.

Among 40 SSR markers, average PIC value was 0.64, the PIC value ranges from 0.23 to 0.64. Markers with higher PIC value are reported to have high discriminatory power to distinguish genotypes from one another. The PIC value of 0.5 is highly informative but PIC value of 1 is highly polymorphic and would have an infinite number of alleles that is more informative (Hilderband *et al*., 1992).

The population level of genetic diversity revealed a total of 202 alleles with average of 2.55 alleles per locus. The highest gene diversity was detected for RM517.

From the observed value of heterozygosity (Ho), the whole accessions were assumed to be pure and homozygous for SSR markers owing to the self-pollinated behavior of rice.

Gene flow value more than 1 was observed for some markers which might be due to the inclusion of sister line or, landraces with different name or some extent of natural cross pollination from the adjacent crop.

From the population structure analysis using Bayesian model-based STRUCTURE v. 2.3.4, the whole accessions were divided into two sub-populations. Sub-population 1 was observed with 26 pure and 7 admixture landraces. While, sub-population 2 was observed with 40 pure and 8 admixtures.

Pairwise Fst value between the two sub-populations was 38.12 indicating significant differentiation between them. The mean no. of alleles (Na), no. of effective alleles (Ne), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe), gene diversity (He) and fixation index (F) were used to determine the genetic diversity at the sub-population level and these values were detected to be comparable between the two sub-populations.

From the analysis of molecular variance, it was detected that variation among the individuals (78%) was higher than variation among the population (13%), this could be due to the collection of 81 genotypes from various parts of Nagaland. A 0% variation within the individual indicated that genotypes were highly pure and maintained without any mixture. Nei genetic distance between the two sub-populations was 0.18 indicating moderate differentiation between the two populations.

An unweighted neighbour-joining tree generated by using alleles of 40 SSR markers displayed the genotypic relationships among the 81 genotypes, cluster analysis using this method separated the whole accessions into two main group with admixtures distributed in each cluster. Cluster I comprised 48 genotypes and cluster II comprised 33 genotypes.

Venn diagram between model-based sub-populations and neighbour-joining cluster detected 72.2% similarity between sub-population 1 and cluster I generated through unweighted neighbour-joining approach. Whereas, 66.7% similarity was observed between sub-population 2 and cluster II of unweighted neighbour-joining. This pattern support that grouping of genotypes based on hierarchical cluster and mode-based approach was more than 72% similar.

Principal coordinate analysis using SSR markers data determines genetic relatedness among the accession. The first two axes explained 7.86% and 5.49% variation respectively. The cumulative variation of the axes was 17.37%.

Grouping or clustering of genotypes help in identifying diverse genotypes which can be useful in hybridization programme as diverse parents in crossing resulted in segregating progenies with high genetic variability for further selection (Barret *et al*., 1998).

Marker trait association analysis

Association of 40 polymorphic markers with yield and quality traits was analyzed on Tassel software version 5.2.86 by following GLM and MLM method.

Through GLM 59 association between markers and traits were detected at $P<0.05$ threshold level of significance with percentage of phenotypic variation (R^2) ranging from 7.44 by RM313 to 37.68 by RM5709. However, association between markers and traits were not detected through MLM.

Out of 40 SSR markers, 4 markers; RM440, RM349, RM5344 and RM5709 were associated with grain breadth.

Grain length was detected to have association with 6 markers such as RM232, RM209, RM247, RM6836, RM1100 and RM60.

Ten markers; RM209, RM240, RM28302, RM524, RM22, RM15429, RM28519, RM232, RM19974 and RM53 were detected to have association with decorticated grain width.

Polymorphic markers such as RM232, RM15448, RM19974, RM6836 and RM60 were also detected to have association with decorticated grain length.

Five associations were observed between grain elongation ratio and RM15078, RM28519, RM6836, RM15429 and RM524.

Gelatinization temperature was detected to have association with markers RM6836, RM15078 and RM1132.

RM232 and RM209 were observed to have association with multiple grain quality traits such as grain length, decorticated grain breadth and decorticated grain length.

RM6836 was also detected to have association with multiple traits such as grain length, decorticated grain length, gelatinization temperature and grain elongation ratio.

Apart from grain quality, association between yield traits and SSR markers were also detected through GLM. Two markers, RM7563 and RM440 were associated with panicle length.

RM24181, RM58 and RM1969 were also recorded to have association with no. of filled grains per panicle.

Days to maturity was detected to have association with two markers such as RM19974 and RM5709.

A total of 7 markers were detected to have association with days to 50% flowering, those markers were RM5709, RM247, RM3894, RM245, RM3331, RM440 and RM22.

RM440 and RM1256 were associated with 1000 grains weight. Grain yield per plant was also associated with two markers; RM24181 and RM5709.

Conclusion

The results of the present investigation revealed the existence of genetic variation among the genotypes in their performance toward yield and quality traits which guarantee the effectiveness of selection. In addition, the study on character association for grain and quality traits helped in identifying the direct of selection.

Character that has high value on variability parameters, significant positive correlation with grain yield and high direct effects towards grain yield will ensure the success of selection for improvement of such character. Therefore, selection on the basis of grain elongation ratio, amylose content and no. of unfilled grains per panicle is recommended based on their performance towards GCV, PCV, heritability and genetic advance.

To improve grain yield per plant, selection on the basis of no. filled grains per panicle, days to 50% flowering, no. of panicles per plant and days to maturity

is recommended since they have significant positive correlation with grain yield per plant.

However, selection based on no. of filled grains per plant, no. of panicles per plant and 1000 grains weight will ensure yield improvement as they have high direct effects on grain yield per plant.

In order to have transgressive segregant progenies with high genetic variability, selection of genotypes from diverse population that perform well for the concerned traits for parent material will help in developing such progenies. Therefore, studies on genetic diversity of the population are crucial before implementing any hybridization works.

Since molecular marker technology has been assisted conventional breeding in various ways, the marker trait association detected in the present study which were previously identified in other investigations will be helpful in future marker assisted breeding programme. Association of markers such as, RM209, RM6836, RM247, RM440, RM3575 with grain dimension; RM5709 and RM3331 with flowering and grain yield were reported previously, therefore, these markers can be used directly in markers assisted selection. whereas, the rest of the association need further validation.

On the other hand, utilization of more polymorphic markers that has more coverage across the genome for more accurate location of gene and validation of our novel marker trait associations in other set of germplasm such as bi-parental mating population will be recommended to provide more satisfying results.

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