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Research Article

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Diversity Analysis, Population Structure and Association Mapping for Grain Quality and Yield Traits in Lowland Rice of Nagaland, India Using SSR Markers

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Abstract

North-east India is known for the hot spot of rice species in the world. Nagaland alone harbor great diversity of rice and possessed immense potential of rice production. In this study, 81 genotypes across the state including 2 commercial varieties were evaluated for diversity studies, a total of 102 alleles, 13% variation among the population, 87% variation among the individuals, 0% variation within the individual, PIC value of 40 SSR markers from 0.23 to 0.99 were observed. The whole accessions were divided into two main group through STRUCTURE and an unweighted neighbour-joining cluster analysis. In PCoA, the total variation of the first three axes of differentiation was 17.37%. A total of 59 association between traits and markers was observed, among these, association between RM209, RM6836, RM247, RM440, RM3575 and grain dimension; RM5709 and RM3331 with flowering and grain yield have been reported. The observed genetic diversity, population structure and association between traits and markers can be useful in further improvement of rice grain quality and yield using the collected germplasm. This will aid in breeding superior variety that can benefit the state as well as the region in increase production of quality rice.

Keywords: Association Mapping, Genetic Diversity, Grain Quality, Yield and SSR Markers

1. Introduction

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 [1]. It is globally grown in an area of around 154 million hectares annually with a total production of 509.2 million tons [2]. 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 [3]. The constant growth of global population and improvement in economy has led to rise in demand for better quality rice which is equipped with high productivity [4,5]. 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.

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 plant 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 lowland, upland and deep-water conditions. It has been estimated that at least 10,000 indigenous cultivars are grown in this region [6]. 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. But Nagaland state is one of the states in north east India that have surplus production of rice with a potential to give rise to an improved variety from available local landraces [7].

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, test, aroma and also marketability, which are not present in many cases in the improved varieties [8]. 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 [9]. 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 variety, thorough identification and characterization of each genotype at phenotypic and genotypic level is required.

An 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 [4,10,11]. 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 [12,13]. Hundreds of valuable genes have been identified that relate to yield and quality in rice [14-17]. Grain weight related genes such as BG3, OsPL3, OsSBN, OsBT1, TGW6 and OsSPL18 have been identified. Furthermore, genes such as NOG1, OsSPX1 and DEP1 were related to grains number per panicles [14,18-25]. Panicle number related genes such as OsIAGLU and DEP1 were also identified. Main component for eating quality; Amylose content is also related to gene *Wx* [18,22].

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 [22]. 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 [22]. Morphological markers were earlier utilised for the analysis of genetic stock, but these markers are not reliable to generate the correct picture of genetic makeup of plant as they are limited in numbers, highly influenced by environment and subjected to epistasis and pleiotropic effect [26-28]. Therefore, new technologies needed to be developed to accelerate the breeding process through more advanced phenotyping and genotyping methods. For this purpose 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 different 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 [29,30]. 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. Association analysis, or linkage disequilibrium mapping, is a notable strategy used 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. 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-pollinating species which is expected to have high linkage

disequilibrium thereby requiring fewer markers, 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.

2. Materials and Methods

In this study 81 rice genotypes, low landraces collected from various districts of Nagaland with improved varieties viz. RCM09 and Ranjit from ICAR, NEH region, Nagaland were analysed in randomized block design with three replications during *Kharif* 2022. Phenotyping of important yield attributing traits and various traits for grain quality was done 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. 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. The total genomic DNA from each accession was extracted following a Della porta DNA extraction method from 100- pooled individuals per accession to ensure better presentation of the accession than a single plant [31,32]. The quality and quantity of extracted genomic DNA was estimated based on agarose gel electrophoresis and spectral analysis. A photograph of the gel band was digitally recorded under UV light in a gel documentation system so as to enable estimation of each sample by LABWARE system. 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 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. 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.

2.1. Data Analysis

The mean value of each trait of the recorded field experiment data was tabulated and subjected to analysis of variance by following [33]. Population structure was studied using STRUCUTURE software [34]. 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 which was implemented in the software STRUCTURE [34,35]. For analysis of the dataset parameters of the population admixture model and correlated frequency of alleles was considered. The genetic distance among the K structure clusters was computed by 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. 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 $p \leq 0.05$.

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 (Rüjoi)	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	Förie	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	Otokewerü	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
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
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
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
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
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
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
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
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
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 1: Mean Performance of 81 Rice Genotypes for Yield, Yield Attributes and Quality Traits

Df-Days to 50% flowering; DM-Days to maturity; PH-Plant height; PL-Panicle length; NPPP- Number of panicles per plant; FGPP-Filled grains per panicles; UFGPP-Unfilled grains per panicle; TW-Thousand grains weight; GL-Grain length; GW-Grain width; DGL-Decorticated grain length; DGW-Decorticated grain width; GEL- Gel elongation ratio; AC-Amylose content; GC- Gel consistency; GT- Gelatinisation temperature; GY-Grain Yield; cm-centimeter; mm-millimeter; g-gram;%-percentage.

3. Results And Discussion

Analysis of variance (Table 2) revealed significant difference among the treatments. Important traits such as days to 50% flowering (DF), days to maturity (DM), plant height (PH), panicle length (PL), no. of panicles per plant (PPP), no. of filled grains per plant (FGPP), no. of unfilled grains per plant (UFGPP), 1000 grains weight (GW), grain length (GL), grain width (GW), decorticated grain length (DGL), decorticated grain width (DGW), grain elongation ratio (GEL), amylose content (AC), gel consistency (GC), gelatinization temperature (GT) and grain yield (GY) per plant were showing significant variation at 1% level of significant.

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

Table 2: Analysis of Variance for Yield, Yield Attributing and Quality Traits

3.1. SSR Polymorphism Among the Accession

The PIC value ranges from 0.23 for marker RM53 to 0.99 for RM1256 with average 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 [36]. The highest frequency allele was detected from RM53 with lowest frequency from RM524 giving a mean value of 0.52. reported higher average PIC value by using 16 SSR markers in a collection of different coloured rice of Korea, the range of PIC value was also reported to be 0.85 to 0.96 [37]. The higher value of PIC could be due to the use of more diverse set of germplasm or due to highly polymorphic SSR markers. Reported average PIC value of 0.507 by using 65 SSR markers in a collection of 114 rice genotypes of NE India [4]. reported average PIC more than 0.5 in a collection of landraces, local selections and improved varieties, whereas, reported lower average PIC value of 0.34 indicating lesser diversity in their accession [38,39]. Wide range of PIC value, 0.30 to 0.84; 0.123 to 0.836 and 0.36 to 0.98 was also reported [40-42]. 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.

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 3: Polymorphism Information Observed Among 81 Genotypes Based on SSR Markers. Note: Major Allele is Described as the Allele with the Highest Frequency

3.2. Genetic Diversity of the Population at Molecular Level Using SSR Markers

A total of 102 alleles with average of 2.55 alleles per locus were detected using 40 SSR markers. 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 using 36 HvSSR, in this accession 3.11 alleles per locus was recorded [43]. More number of alleles were reported by various researchers, Reported a total of 147 alleles in 144 rice genotypes of NE India characterized by using 65 SSR polymorphic SSR markers [4]. 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 [38]. reported 86 alleles in a set of 124 diverse rice genotypes with 32 SSR markers. 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 [44]. Lesser alleles, 52 total alleles with average of 2.7 alleles per marker was reported [45]. 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 [39].

The highest gene diversity (He) 0.71 was shown by RM517 followed by RM444 (0.69) with average value of 0.48. also reported a gene diversity value of 0.48 in Bangladesh rice [46]. reported lower value of gene diversity of 0.33, reported high value of gene diversity (0.91) among Bangladesh local rice cultivars, reported 0.52 average gene diversity in Asian rice involving indica and japonica rice, reported average gene diversity 0.69 in modern rice and local landraces of rice in Bangladesh [4,43,44,47,48]. The diversity panel with global accessions were observed to have a gene diversity of 0.45 to 0.70 (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 [49]. Therefore, the diversity that exist in 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 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.

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

Table 4: Genetic Diversity of 40 SSR Markers in the 81 Rice Genotypes

3.3. 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 (Figure 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 (Figure 4). 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. also detected 2 sub-populations in 59 rice accessions using SNP's, Reported 3 sub-populations in a collection of NE India rice, reported 4 sub-populations in a collection of 83 landraces rice of NE India, reported 7 sub-populations in a collection of coloured rice, reported 3 sub-populations in a collection of 6984 NE rice accessions, also reported 3 sub-populations in 50 rice genotypes, reported 4 sub-populations in 124 diverse rice genotypes [4,37-39,50-52].

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 [53]. 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 [43]. 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, then we can say that most accessions of populations are admixed [54,55]. The genetic differentiation among populations were considered highly differentiated as per classification given [56]. 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.

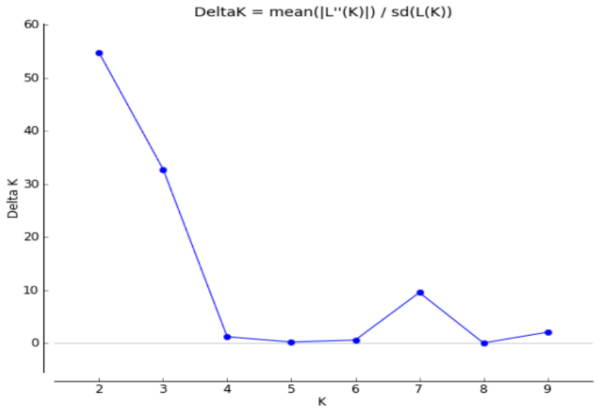

Figure 1: A Plot of Delta K Values from the Structure Analyses of 81 Rice Accessions, Obtained Through Structure Harvester ver. 0.6. Application (Earl and Vonholdt)

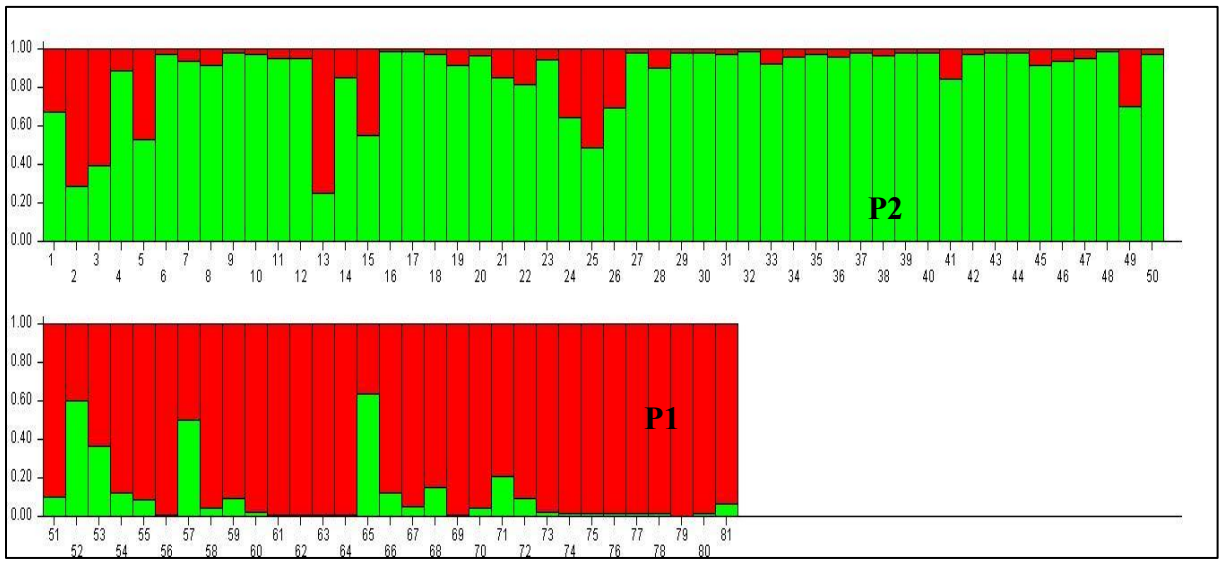

Figure 2: Population Structure of 81 Rice Accession Based on 65 SSR Markers. Note: Numbering of Genotypes Corresponds to the Serial no. and Gemotypes in Table 1; P1-Population 1; P2-Population

Populations	P1	P2
P1	0	38.12
P2	38.12	0

Table 5: Pairwise Population Differentiations (Fst Value) Below Diagonal and Gene Flow (Nm) Values Above Diagonal

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

Table 6: 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)

3.4. Analysis of Molecular Variance

AMOVA 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. Also reported higher proportions of variation among individuals in a collection of NE India rice and also reported the similar results on AMOVA [4,7,43,50,52]. 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 [44]. The Fst inbreeding coefficient within subpopulations relative to the total provides a measure of the genetic differentiation between subpopulations [57]. 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 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 [56]. 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 indicating moderate differentiation between the sub-population.

Source	Df	SS	MS	Est.Var	%Var	F statistics	Value	P (r-and >= 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 7: Analysis of Molecular Variance of 81 Rice Genotypes

Populations	P1	P2
P1	0	0.18
P2	0.18	0

Table 8: Pairwise Population Matrix of Nei Genetic Distance of 81 Rice Genotypes at Sub-Population Levels

3.5. Neighbour-Joining Based Clustering

An unweighted neighbour-joining tree 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.

3.6. Principal Coordinate Analysis

PCoA using SSR markers data 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. also reported a cumulative variation of 15.9% [43]. Whereas, reported 43% cumulative variation in a collection of Nagaland rice [52]. 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 as this help in separation of admixtures from pure genotypes [4]. 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 [58]. 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.

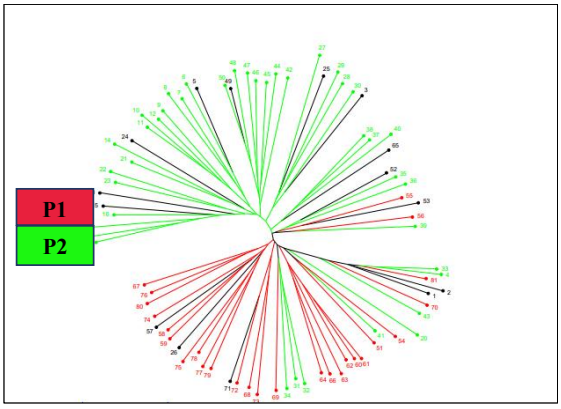

Figure 3: Unrooted Neighbor Joining Tree of 81 Rice Genotypes Using SSR Markers

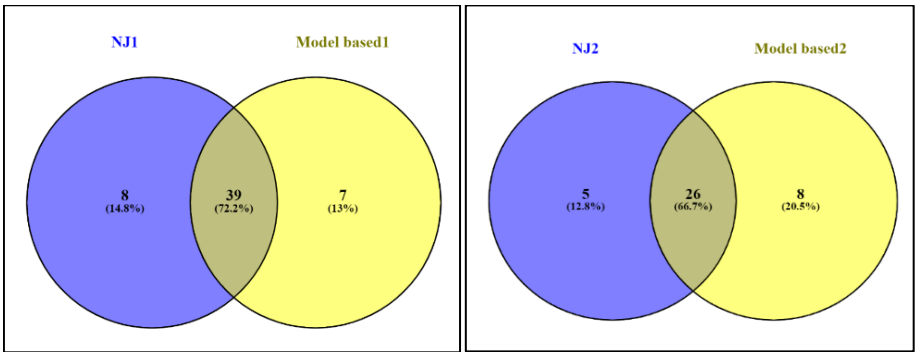

Figure 4: Venn Diagram Showing Co-Linearity Between Neighbour Joining Based Clusters and Model-Based Sub-Populations

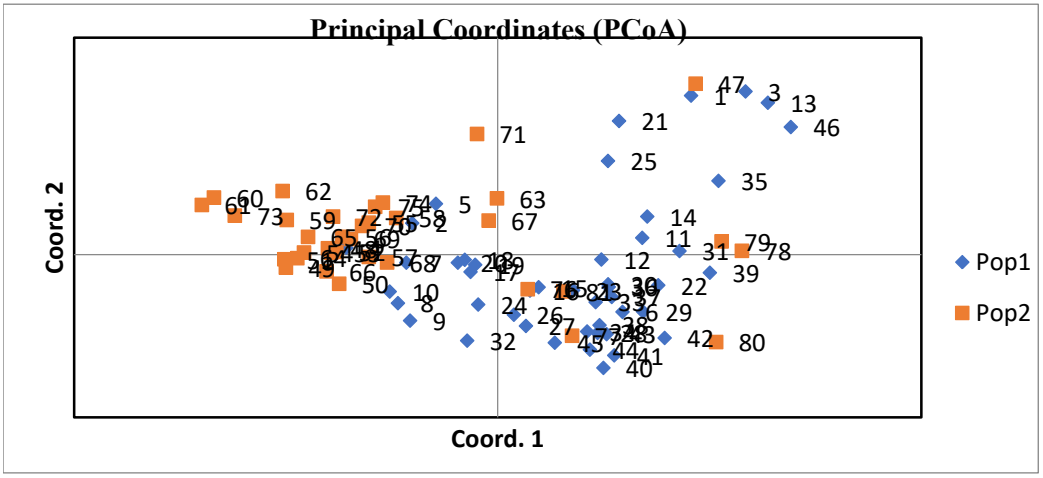

Figure 5: Principal Coordinate Analysis of 81 Rice Genotypes Using SSR Markers. Note: Numbering of Genotypes Corresponds to the Serial No. and Genotypes in Table no. 1

3.7. 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 [59]. 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 [59-62]. 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 and the large segregating population required for high resolution mapping that is required for marker assisted selection is possibly difficult for some species [63,64]. On the other hand, association mapping comes with a principle of detecting association between marker and trait of interest through linkage disequilibrium [65]. 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 with this high degree of linkage disequilibrium, even lesser markers per locus will not result in lower resolution for MAS [66,67]. 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 [68]. 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 [69]. 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 [59].

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 9**) 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 [70,71]. 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. detected the association between RM209 with grain width in interspecific cross between *Oryza sativa* and *Oryza glabberima* [72]. 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 [73,74]. two QTL; qGW2n and qGW6 in which qGW6 is flanked by RM6836 and RM527 on chromosome 6 [75]. 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 [76]. Major QTLs that control grain weight have been detected between RM166 and RM344 on chromosome no. 10 [77]. also reported digenic epitasis effecting grain length and thousand grain weight on chromosome no. 5 flanked by RM440 and RM3575 [78]. 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* [9,79]. 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. The existence of genetic variation among the genotypes in their performance toward yield and quality traits which guarantee the effectiveness of selection. 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.

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
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

Table 9: Association Analysis Between SSR Markers and Grain Quality and Yield Traits

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Author Contributions

Conceptualization and Methodology-H.V.; Resources- H.V. and L.C.; Formal analysis, Investigation and Data curation- L.C.; Writing- original draft- L.C.; Writing- Review and editing-H.V. and H.C.; Visualization-B.V., H.N.L., H.L, L., J.L., K.K.L and L. All authors have read and approved the final manuscript.

Declarations

Conflict of interest

The authors declare no conflict of interest

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