Annals of Plant and Soil Research 27(1): 32-40 (2025)
https://doi.org/10.47815/apsr.2025.10433

Genetic variability and diversity using SSR marker in indigenous winter rice germplasm of Assam

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Received: January, 2025; Revised accepted: Febuary, 2025

ABSTRACT

The study of nature and magnitude of genetic diversity is crucial for selection of appropriate genotypes in a crop improvement programme. This paper reports an assessment of genetic diversity in a set of 100 indigenous winter rice genotypes of Assam based on morphological, biochemical traits and molecular markers. The traits, viz., biological yield, filled grains per panicle, effective tillers per plant along with amylose and protein content were found promising for obtaining further genetic gain on selection from the set of genotypes under study. Molecular diversity analysis revealed wide diversity in the germplasm with respect to 25 polymorphic simple sequence repeat (SSR) markers with mean polymorphic information content (PIC) of 0.53, Shannon information index ranging from 0.4-1.157 and expected heterozygosity with an average value of 0.52. Based on these parameters SSR markers RM 1375, RM 2615, RM 3866. RM6378, RM10864, RM314, RM21, RM31, RM152, RM211, 5M335, RM444, 1357, RM916, RM3331, RM190 and RM 555 could be deemed highly informative to study genetic diversity at molecular level in the set of rice germplasm. Three major clusters (I, II, and III), each divided into two minor clusters were seen in the UPGMA-based dendrogram derived from the binary data inferred from the germplasms' DNA profiles based on the dissimilarity coefficient of 0.291 to 0.7838. The largest value of dissimilarity coefficient or the maximum Jaccard distance was 0.7934 between Hindubor and Sapor Aijung, followed by Hindubor and Machuri (0.7934). These findings on the phenotypic data and marker information will be helpful to formulate hybridization plans for parental selection of rice germplasm.

Key Words: Winter rice; Genetic variation, Genetic diversity; SSR markers and Clustering

INTRODUCTION

The North-East Indian state of Assam is the home of a large array of rice germplasm representing various seasonal groups and quality classes suiting to various ethnic uses. In fact, 'Rice is the life' for the people of North-East India. It provides the food, nutritional and livelihood security to the people of the region and occupies more than 90 *per cent* of the cultivated area. The group of winter rice varieties, locally known as Sali comprises of various quality groups which is characteristically different from the winter rice (Aman) germplasm of mainland India. The winter rice varieties of Assam may be classified as normal Sali, Bora (waxy or glutinous), Chokuwa, Komal or Boka (semi-waxy or semi glutinous) and Joha (the small grained scented types) rice (Ghosh, 2018). This variety, considered as special gifts from nature, has been cultivated by farmers since times immemorial to meet various ethnic needs. Grown in Assam for its exquisite flavour and subtle scent, the small grained Joha is similar to expensive Basmati rice in terms of explicit aroma and fetches premium market price. The Bora varieties are characterised by high amylopectin content making it sticky on cooking (Das *et al.,* 2018). The traditional Chokuwa group, is semi-glutinous and has an intermediate amylose content of 11–20 *per cent*. They are widely used as instant rice and does not require prior cooking (Kalia and Hazarika, 2022). Despite unique diversity in rice types in the region, the productivity of rice has always been lower than the national average. Therefore, it is utmost important to comprehend the genetic variability and diversity not only for the yield and yield attributes but also for quality features especially those that were grown for their quality and are still valued by farmers for meeting local demands (Sarma *et al.,* 2022). Apart from the phenotypic traits representing yield and quality, it is equally important to study the diversity pattern based on molecular markers to arrive at precise understanding of genetic diversity. Out of

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different molecular markers available, SSR markers have been widely used for plant genotyping because of their high polymorphism levels, broad distribution across the majority of plant genomes, and most importantly, lack of sensitivity to biotic or abiotic stress. Keeping the above in view, an attempt was made to elucidate the genetic variability and diversity with respect to morphological and quality traits and 30 SSR markers in a set of one hundred indigenous winter rice cultivars of Assam.

MATERIALS AND METHOD

The material for the present investigation comprised of a set of 100 Sali (Winter rice) rice cultivars of Assam including Joha, Bora, Chokuwa, Bao and Hill rice varieties. They were collected from farmers' field of various districts of Assam and maintained at the Biotech Hub, BN College of Agriculture, Assam (Table 1). The experiment was conducted in the Kharif season of 2021 and 2022 at the experimental field of Biswanath College of Agriculture, Biswanath Chariali, represented by the latitude of 26°15′N, 27°45′ N, the longitude of 92°42′E, 95°30′E and altitude of 104 m MSL and was laid out in a randomized block design followed by three replications. Recommended package of practice was followed to raise the experimental crop. Observations on 17 quantitative traits were recorded as per the standard evaluation system of International Rice Research Institute. The observations on all the traits except for days to 50 *per cent* flowering and maturity were recorded based on five randomly taken plants per plot. Days to flowering and maturity were recorded on plot basis. The volume expansion was calculated with the method by Sidhu *et al.* (1975). Amylose and amylopectin estimation was done following the protocol of Juliano (Juliano, 1971). Lowry method (Lowry, 1954) was used for protein estimation. The data obtained were subjected to analysis of variance (ANOVA) and to derive the genetic parameters of variation.

Table 1: List of germplasms under study

Sl. No.	Germplasm	Sl. No.	Germplasm	Sl. No.	Germplasm	Sl. No.	Germplasm
1	Baismuthi	26	Malbhoog	51	Nania	76	Gandhari
2	Chapor Aijung	27	Betguti	52	Gitesh	77	Inglong a lahi
3	Kola Joha	28	Xorujahingya	53	Dubai sanga	78	Sangamohini
4	Joy Bangla	29	Ronga Sali	54	Thupi Sali	79	Gethu
5	Inglongkiri	30	Manipuri Joha	55	Parimal	80	BorJahingya
6	Sok Soi soi	31	Luit	56	Bokul	81	Sorufolia Sali
7	Maibee	32	Mala	57	Bokul Joha	82	Kurmi Sali
8	Haccha	33	Panindra	58	Til Bora	83	Ampakhi
9	Dehangi	34	Bishnuprasad	59	Bioi Sali	84	Bhanumati
10	Hindubor	35	Bora	60	Siyal Sali	85	Senduri Sali
11	Karbi Dhan	36	Kumoldhan	61	Mes 9	86	Moni Sali
12	Sok Jongthi	37	Agni Sali	62	Mamolsinga	87	Bhaboli Joha
13	Sok Votung	38	Bhogali	63	Bejel Bao	88	Mout Bora
14	Maizubiron	39	Prasadbhog	64	Nol Bora	89	Sok naka
15	Mashuri	40	Bahadur	65	Kona Musori	90	Piolee
16	Hakky	41	Prafulla	66	Komal 101	91	Tora Sali
17	Ranjit	42	Moinagiri	67	Swarna Joha	92	Hurupi bao
18	Solpuna	43	Kanaklata	68	Bao dhan	93	Lau Dubi
19	Sulsuli Bao	44	Vandana	69	Rongali Bao	94	Keteki Joha
20	Boga Joha	45	KajoliChokua	70	Kunkuni Joha	95	Badol Sali
21	Basanta Sali	46	Abor sali	71	Joldubi	96	Phul Pakhori
22	Bijoy Sali	47	Tulaipanju	72	Amona bao	97	Kutkuti Sali
23	Maguri	48	TTB404	73	Ekora Sali	98	Buruli bao
24	Gomidhan	49	Bokul Bora	74	TTB 103	99	Kati neuli
25	Swagmoni	50	Gopinath	75	Basudev	100	Nepali Sokua

Molecular Assay

For the DNA extraction, fresh leaf tissue from 20 days rice seedling (2g) was collected. Total genomic DNA extraction was carried out by cetyl-trimethyl-ammonium bromide (CTAB) as described previously by Doyle *et al.* (1987). The DNA pellet was dissolves in 100 µL molecular graded water and stored at 4ºC for further analysis. The overall genomic DNA concentration, quality and optical density were measured at a wave length of 260/280 nm by Nano Drop for all the samples. The final PCR was setup for 20 µLvolume of 1x Taqbuffer (Emarald Amp GT PCR Master Mix) for 10 µL, Template DNA 1 µL, forward and reverse primer 0.5 µL each, and molecular graded water 8 µL. A total of 30 SSR marker taken from International Rice Research Institute under the Generation Challenging Program (http://gramene.org/markers/microsat/50_ssr.html) were used to study molecular diversity amongst the 100 genotypes under investigation. The PCR amplifications were performed in a 96-well Veriti thermal cycler (Applied Biosystems, USA). The PCR temperature cycling conditions was set up at Initial denaturation at 95°C for five minutes; Denaturation at 95°C for 30 seconds; Annealing for 32 seconds at 55°C and Elongation for 45 seconds at 72°C; Denaturation, annealing, and elongation were repeated 35 times; The final cycle was followed by five minutes extension at 72°C. The amplified PCR product was separated using 2 % agarose gel stained with ethidium bromide and visualized using Gel image reader. The band size was recorded against a 100 bp ladder. For diversity analysis, the binary data sheet was generated using PCR amplification data. Only clear and unambiguous SSR markers were scored. All the genotypes were scored for the presence (score '1') and absence (score '0') of the SSR bands.

Statistical analysis

The pooled data for morphological traits over two years (2021 and 2022) were subjected to analysis of variance (ANOVA) following Panse and Sukhatme (1967). Phenotypic and genotypic variance and the coefficients of variation were calculated using the formula suggested by Burton (1952). Heritability in broad sense was estimated following Hanson *et al.* (1956) and genetic advance was calculated as suggested by Johanson *et al.* (1955). Polymorphism information content (PIC) for 25 polymorphic SSR marker was calculated, according to the formula of Weir (1996): PIC = 1- ($\sum Pi^2$), where, 'i' is the total number of alleles detected for SSR marker and 'Pi' is the frequency of the i^{th} allele in the set of genotypes investigated. Na (Number of Different Alleles), Ne (Number of Effective alleles), I (Shannon's Information Index), He (Expected heterozygosity) were calculated using GENAlex. Matrix of Jaccard coefficient of dissimilarity was used for cluster analysis using the Unweighted Pair Group method with Arithmetic Averages (UPGMA) and the dendrogram for the 100 rice genotypes was constructed using the software DARwin 6.

RESULTS AND DISCUSSIONS

Study of genetic parameters of variation

The estimates of genetic parameters of variation in the set of 100 indigenous rice cultivars for 17 quantitative traits are presented in Table 2. There was good agreement of the values of genotypic and phenotypic variance, indicating relatively less influence of environment in expression all the traits under study. A measure of variability comparison and an indication of the validity of characteristics for selection are provided by the study of coefficient of variation (Kumar *et al.,* 2015). In this study, highest genotypic coefficient of variation (GCV) along with phenotypic coefficient of variation (PCV) was observed for biological yield followed by grain yield and harvest index. Similar to our study, higher PCV than GCV was observed by Akshay *et al.* (2022) and Das *et al.* (2021). Chouhan *et al.* (2014) reported fertile spikelets per panicle had the highest estimates of PCV and GCV (56.21 and 55.39), followed by total grains per panicle (42.07 and 40.86) and grain yield per plant (28.51 and 27.60), demonstrating the significance of these variables in selection for yield improvement. Highest heritability was observed for days to flowering followed by filled grains per panicle and grain length. More than 80 *per cent* heritability in broad sense was observed for all other traits except for protein content, amylose and amylopectin content, grain

Table 2: Genetic parameters of yield and yield related traits

Characters	Mean	SE	Range	GCV	PCV	h^2 (broad sense) percent	GAM	CV (%)
DF	119.3	1.78	179.1-78.2	14.22	14.3	95.6	74.7	14.23
DM	151.1	4.5	183.5-106.5	9.5	11.6	94.34	49.4	9.325
PL	24.12	0.55	33.8-6.75	11.98	12.575	86.4	23.76	4.275
P H	141.19	6.05	200.3-93.4	17.08	19.255	84	56.83	9.03
ETP	9.23	0.52	13.8-4.46	18.73	22.095	88.54	75.84	12.515
FGP	115.47	2.82	212.52-10.5	28	27.53	95.95	85.5	5.19
GW	2.2	0.07	3.12-1.29.	17.02	17.76	89.43	85.32	5.445
GL	7.46	0.23	9.43-3.16	11.22	12.285	96.5	46.4	6.89
GB	2.99	0.08	8.5-1.4	24.2	24.35	93.94	65	4.24
L:B	2.66	0.11	4.14-1.02	18.36	20.1	87.5	65.9	7.565
BY	103.9	1.86	235.82-9.04	49.3	44.805	72	53.4	3.65
HI	25.57	0.86	51.7-7.9	32.6	34.2	73.9	32.63	7.175
VER	1.8	0.07	1-3.7	11.27	12.58	85.7	20.2	8.55
Amylose	19.3	0.05	1.57-26.9	9.36	9.27	78.9	38.04	5.71
Amylopec	80.7	0.05	73.08-98.42	7.01	7.02	77.9	27.21	4.14
Protein	6.49	0.36	3.4-10.5	12.51	10.74	65	36.5	12.05
GYP	20.99	1.06	51.4-7.8	38.24	39.48	75	31.6	9.895

DF, days to flowering; DM, days to maturity; PL, panicle length; PH, plant height; ET, effective tillers per plant; FG, filled grains per panicle; GW, grain weight; GYP, grain yield per plant; GL, grain length; GB, grain breadth; BY, biological yield; and HI, harvest; VER, volume expansion ratio

yield per plant, harvest index and biological yield. Grain yield is a complex polygenic trait with low heritability governed by contributing yield attribute such as grain size, weight, and number (Ahmed *et al.,* 2021). Hence, indirect selection of these traits will be profitable to obtain a high yield for the crop. Thus, the traits, *viz*., biological yield and filled grains per panicle having high GCV and higher magnitude of heritability could be considered for further genetic improvement through selection. In order to quantify the genetic gain obtained from selection, along with heritability, estimation of genetic advance is also important (Rheneen *et al.,* 2019). Genetic advance as *per cent* of mean was highest for filled grains per panicle (88.27), followed by100- grain weight (84.65) and effective tillers (78.67). Genetic advance in quality trait was seen to be high for amylose (38.04 %) and protein content (36.5 %). Varying results on heritability of amylose content in rice was penned by Nirmaladevi *et al.* (2015), stating a very high heritability and low genetic advance compared to our findings. As per their finding, highest broad sense heritability and genetic advance for amylose content was 93% and 29%, respectively. Also, high percentage of heritability for amylose (90.6) and protein content (95.5) was reported by Mahesh *et al.* (2022). Suggested by Hillerislambers *et al.* (1973), the heritability of amylose and protein content in rice can be affected by a number of factors, including the genetic makeup of the rice and the environment in which it grows, for which diverse results might be obtained by different workers. Thus, the traits, *viz*., biological yield, filled grains per panicle, effective tillers per plant alongwith amylose and protein content were found promising for obtaining of further genetic gain on selection from the set of genotypes under study. The importance of understanding the variability for grain yield and its attributes along with the quality and nutritional parameters were also emphasised by several workers earlier for successful crop improvement program (Parikh *et al.,* 2012, Ahmed *et al.,* 2021 and Verma *et al.,* 2020).

Genetic diversity study using SSR markers

The 25 polymorphic SSR markers out of 30 SSR markers, investigated in the rice cultivars together showed 104 alleles (Table 3). The number of alleles per locus ranged from 1 - 4. The highest number of allele was detected for RM2615, RM336 and RM444 (Fig. 1). The SSR markers, RM211, RM335, RM21, RM10864, and RM3866 exhibited three allelic bands. Two allelic bands were shown by RM480, RM8213, RM186, RM152, RM11, RM1357, RM31, RM314, and RM341. A similar study on 100 upland rice germplasm of Assam using 120 SSR marker generated 1-3 alleles

with an average value of 1.867 per locus (Rathi *et al.,* 2014). The effective number of alleles (Ne) varied from 3.040 in RM 6378 to 1.278 in RM 31, with an average of 2.06. Very high allele numbers 6 to 14 were also reported earlier (Jin *et al.,* 2010).

Table 3: Summary Statistics of genetic diversity parameters among 25 polymorphic SSR markers used in the study

Sl No	Locus	Na	Ne	I	He	PIC
1	RM186	2	1.812	0.638	0.446	0.350
2	RM336	4	2.485	0.962	0.577	0.350
3	RM480	2	1.616	0.561	0.376	0.377
4	RM1375	2	1.914	0.668	0.475	0.530
5	RM2615	4	2.955	1.137	0.657	0.540
6	RM3472	2	1.811	0.637	0.445	0.380
7	RM3866	2	1.897	0.713	0.420	0.550
8	RM6378	4	3.040	0.762	0.504	0.550
9	RM8213	2	1.943	0.678	0.485	0.480
10	RM10864	3	2.372	0.949	0.565	0.590
11	RM314	2	1.837	0.640	0.449	0.520
12	RM11	2	1.754	0.609	0.421	0.360
13	RM21	3	2.554	1.001	0.604	0.620
14	RM31	2	1.278	0.297	0.190	0.620
15	RM152	2	1.562	0.498	0.329	0.610
16	RM211	3	2.295	0.892	0.553	0.540
17	RM335	3	2.255	0.892	0.652	0.620
18	RM444	4	2.978	1.153	0.679	0.630
19	RM1357	2	1.714	0.588	0.403	0.530
20	RM225	2	1.390	0.400	0.258	0.340
21	RM8020	2	1.992	0.691	0.498	0.440
22	RM916	2	1.882	0.661	0.469	0.570
23	RM3331	2	1.992	0.691	0.898	0.750
24	RM190	2	1.853	0.721	0.553	0.580
25	RM555	4	2.483	0.862	0.521	0.584

Na: No of Different Alleles; Ne: No of Effective alleles; I: Shannon's Information Index: He: Expected heterozygosity; PIC: Polymorphism Information Content

The number of alleles produced by SSR markers indicates the richness of diversity in a studied germplasm. Hence allele number higher than 2 could be considered significant. The Shannon Information Index (I) used to quantify the richness and evenness of alleles within a population showed high values (>1) for markers RM2615, RM 21 and RM 444, and almost equal to 1, for RM336 and RM 10864. The Shannon information index ranging from 0.4-1.157 showed that for those loci with I>1, the number of alleles were equal to 4, and PIC >0.5. Thus, I estimate show positive correlation with allele number and PIC value, and therefore can be used to quantify diversity of SSR markers. Expected heterozygosity (He), an indicator of genetic differentiation in a population for the studied markers, showed a moderate range of diversity lying mostly above 0.5, the highest being for RM3331(0.89) with a mean value of 0.57. High Shannon's mean value of 0.7, expected heterozygosity with an average of 0.6311 and number of alleles per locus (6.722) was recorded by Valsalanga *et al.* (2019) in a set of 65 germplasm from the North East.

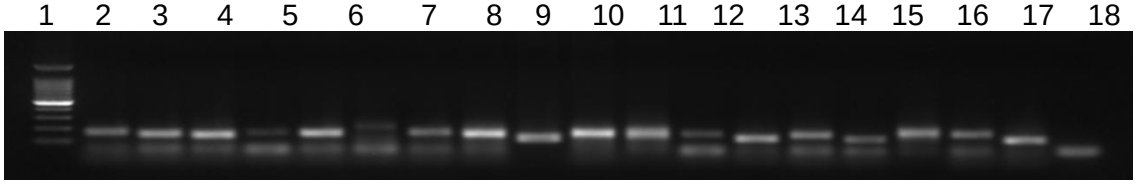

Fig 1: Agarose gel of 2% showing polymorphism across a few rice germplasm under study using SSR marker RM 444

The PIC value gives information about the diversity of the allele and its frequency among landraces. The PIC of 25 SSR markers showed variable polymorphism across the cultivars ranging from 0.35 to 0.75, indicating a good discriminating power of the employed markers. However, SSR markers namely RM 319, RM 402, RM 207, RM 80, and RM 206 were found to be monomorphic in the studied rice cultivars. Assessment of genetic diversity in Phillipines rice germplasm, carried out by Lapitan *et al.,* 2007 reported SSR marker RM 206 and RM 207 to be highly polymorphic with a PIC value of 0.8. In the present study grouping, monomorphism of the same markers might be attributed to the different set of genotypes owing to a different origin than the Phillipines germplasm. Low GD and PIC value with a mean of 0.36 and 0.54 was reported by Ajumaili *et al.* (2018) in Malayasian aromatic rice; GD value of 0.442 and PIC value of 0.404 by Hassan *et al. (*2021) in Turkish rice. In this study, the mean PIC value above 0.5 indicated that the set of markers used in the study were effective to detect the range of variability in the cultivars studied (Saikia *et al.,* 2021 and Pathak *et al.,* 2016). SSR markers have been used widely to study diversity in Assam and North East rice germplasm to facilitate various breeding programs. However, previous reports on molecular diversity analysis in traditional rice genotypes including all the groups of winter rice such as Joha, Bora, Chokuwa was, however, very scanty. Based on the SSR marker analysis, the markers RM 1375, RM 2615, RM 3866, RM6378, RM10864, RM314, RM21, RM31, RM152, RM211, 5M335, RM444, 1357, RM916, RM3331, RM190 and RM 555 ranging PIC value above 0.5, coupled with high Shannon information index and effective number of alleles could be considered highly informative to study genetic diversity at molecular level in the set of rice germplasm. These markers can be immensely valuable in studies related to the indigenous rice germplasm of the state to detect variation, diversity analysis, phylogeny, population structure, gene mapping and association studies.

Genotype grouping or clustering to identify varied parents from a population in a hybridization program is very useful to produce progenies with the highest genetic variability (Barrett *et al.,* 1998). The UPGMA based dendrogram obtained from the binary data deduced from DNA profiles of the germplasms based on dissimilarity coefficient of 0.291 to 0.7838, revealed three broad clusters each subdivided into two minor clusters (**Fig 2**). Similar clustering pattern was reported in agronomically identified as *aus/ahu* cultivars of Northeast India (Verma *et al.,* 2019); 8 exotic and 7 local short grain rice genotypes from Pakistan (Mehmood *et al.,* 2021); 24 aromatic rice genotypes from Indian Institute of Rice Research, Hyderabad (Kumar *et al.,* 2020). Cluster III had the highest entry with 44 entries, followed by cluster II with 27 entries and cluster I with 29 entries (Table 4).

Table 4: Cluster wise distribution of the 100winter rice germplasm based on dendrogram generated by UPGMA cluster analysis of the 25 SSR markers amplicons using Jaccard dissimilarity index

Cluster	Sub cluster	Name of the germplasms
I	IA	Bejel Bao, Mamolsanga, Gandhari, Rongali Bao, Nol Bora, Gethu, BorJahingya, Ranjit, Sulsuli bao, Sok Votung, Boga Joha, Komal 101, Kajoli Chokuwa, Mes 9, Bokul Joha, Buruli Bao, Keteki Joha, Xoru Jahingya, Manipuri Joha, Kola Joha.
	IB	Piolee, Sok Naka, Kanaklata, Prafulla, Bijoy Sali, Joldubi, Mout Bora, Moinagiri, Vandana.
II	IIA	Lao Dubi, Hurupi bao, Parimol, Gitesh,Siyal Sali, Nania, Dehangi, Haccha, Bahadur, Sok soi soi, Inglongkiri, Agnisali, Prasadbhog, Luit, Nepali chokuwa, Kona musori, Hakky, Maizubiron, Mashuri, Panindra, Inglong a lahi,
	IIB	Badol Sali, Bishnuprasad, Betguti, Maibee, Gomidhan
III	IIIA	Bioi Sali, Duboisanga, Tulai Panju, Abor Sali, Kutkuti Sali, TTB404, Hindubor, Joy Bangla, Ekora Sali, Tora Sali, Maguri, Senduri Sali, Karbidhan, Kumoldhan, Mala, Phul pakhori, Bao dhan, Basudev, Bokul, Senduri Sali, Bhanumati, Baismuthi.
	IIIB	Gopinath,Solpuna, Bokul Bora, Kati neuli, Aampakhi, Swarna Joha, Sorufolia Sali, Thupisali, Bhaboli Joha, Til Bora, Amona Bao,Bhogali,Kunkuni Joha, Ronga sali, Kurmi sali, TTB103, Bora, Malbhoog Bora, Swagmoni, Sangamohini, Basanta Sali.

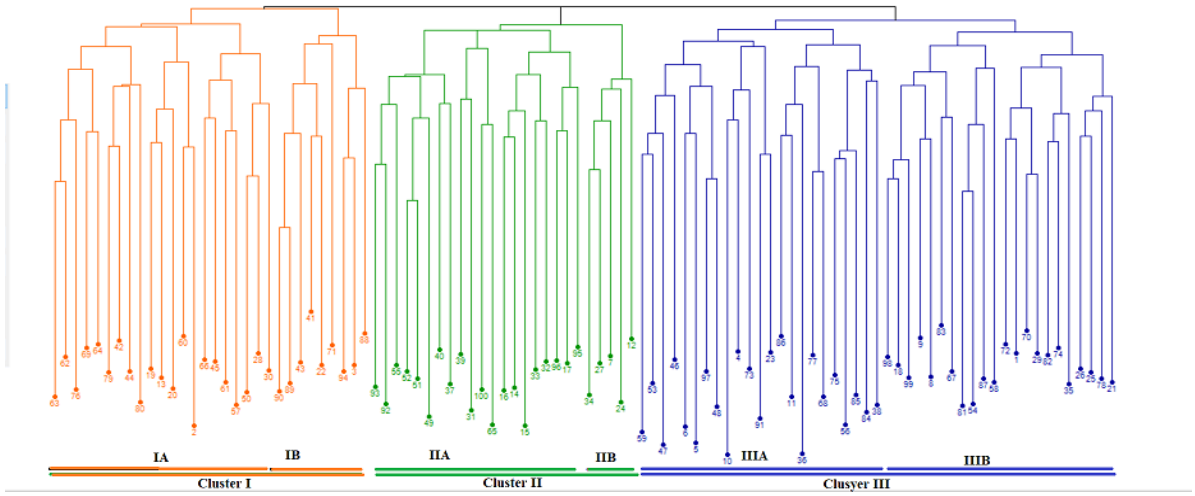

Fig 2: Hierarchical vertical representation of three clusters formed out of 100 cultivars constructed as per the distance-based approach using Jaccard dissimilarity coefficient in a scale of 0.29 -0.79

The Joha varieties were placed under the sub group IA, the sticky Bora varieties were placed under sub group IIIA, the Bao varieties under sub group IA, and the Hill and the normal Sali varieties were seen to be distributed amongst the clusters. Thus, it appeared that based on the 25 polymorphic markers, the traditional quality groups could very well be distinguished from other varieties. No distinct sub group was obtained for the normal Sali and Hill rice indicated the marker were relatively uniformly distributed amongst them and could not isolate the varieties into a distinct group. The sub group division of the varieties showed a considerable range of diversity among the studied varieties. It is presumed that the varieties having same genetic relationships tends to group together due to common ancestors or closely related genetic background (Ferdous *et al.,* 2018, Mohumud *et al.,* 2021; and Akhter *et al.,* 2021). For a number of cases, however it was observed that varieties belonging to different groups *viz* Joha, Bao, Bora, Hill, and normal Sali were included together in the same cluster. This might be due to the similar allelic diversity with respect to the markers under study irrespective of sub group of varieties. It could be inferred that trait specific markers, if known, could clearly distinguish different quality groups under Sali rice. The dissimilarity coefficient, used to study the dendrogram and the relatedness between the genotypes, ranged from 0.291 to 0.7938. The genotypes close to 0.29 showed less dissimilarity, while the genotypes close to 0.7938 showed high dissimilarity. The largest value of dissimilarity coefficient or the maximum Jaccard distance was 0.7934 between Hindubor and Sapor Aijung, followed by Hindubor and Machuri (0.7934), Machuri and Kumoldhan (0.7926), and Bokul Bora and Bor Jahingya (0.7923). The lowest value of dissimilarity was found between Piolee and Sok Naka (0.291), and Ronga Sali and Agni Sali (0.307). A high dissimilarity between the genotypes indicates their usefulness as parents in the hybridization programs. Similar findings were reported by Kiranmayee *et al.* (2022), while studying 100 maintainer lines of rice hybrids using 80 SSR markers. Saikia *et al.* (2020) reported similarity coefficient of 0.72 to 0.20 in 15 germplasms of Joha using 34 SSR markers.

The present study elucidated the worth of a set of 100 indigenous winter rice genotypes of Assam in terms of genetic diversity. The traits, *viz*., biological yield, filled grains per panicle, effective tillers per plant along with amylose and protein content were found promising for obtaining further genetic gain on selection from the set of genotypes under study. Hence, direct selection and improvement of these traits will prove fruitful in further crop improvement programs. The study of allelic diversity using SSR markers, followed by cluster analysis have well established that the indigenous rice cultivars studied are genetically rich and diverse. Considering the ample diversity with respect to phenotypic traits and genotyping by molecular markers, it could be inferred that the group of germplasm is potential for further crop improvement

programme. More precise phenotyping and genotyping with a greater number of markers will further generate more useful information for their exploitation for genetic improvement. Identification of molecular markers, particularly associated with the quality attributes, appears to be key in improving the indigenous quality rice through marker assisted selection.

ACKNOWLEDGMENT

Authors are thankful to Advanced Level Biotech Hub and Department of Plant Breeding and Genetics, B N College of Agriculture, Biswanath Chariali, 784176, Assam Agricultural University, Jorhat, 785013, Assam, India

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