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RESEARCH ARTICLE

Unveiling genetic diversity, fingerprinting, phenotypic and molecular characterization of rice (*Oryza sativa* L.) germplasm from Northeast India using SRAP and TRAP markers

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Abstract

Northeast India, a part of the Indo-Burma biodiversity hotspot, harbours the richest genetic diversity reservoir for agricultural crops. The region is not only a centre of origin of rice but also a critical area where conservation of genetic diversity in crops is required. The analysis of 197 landraces/germplasm using phenotypic, genotypic and combined genotypic/phenotypic distances revealed ample genetic variation in the collections. Multivariate analysis for phenotypic variability indicated that 11 out of 13 phenotypic traits assessed were useful in discriminating the genotypes. Cluster analysis based on phenotypic data distinguished three clusters, while a corresponding analysis with SRAP and TRAP markers indicated four groups. Also, the combined analysis for the phenotypic and genotypic data provided four distinct clusters, revealing valuable information about the diversity among economically important agronomic traits. The present study also partitions the genotypes into distinct heterotic groups, thereby making it possible for parental selection and hybridization to maximize genetic diversity in the rice breeding program. Moreover, two combinations of SRAP and SRAP5 (ME01 and EM10) and SRAP6 (ME01 and EM07) with four TRAP combinations, TRAP1 (Auxr1 and FT14), TRAP2 (Auxr1 and T03), TRAP3 (Auxr1 and FT14) and TRAP5 (Auxr1 and T13) with high informative PIC score, greater than 0.70 effectively discriminated the current collections/genotypes for a robust fingerprint system.

Keywords: Rice, diversity, SRAP, TRAP, principal component analysis, polymerase chain reaction, PIC.

Introduction

The Northeastern region of India, comprising Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura, is home to about 220 diverse ethnic groups and is part of the Indo-Burma biodiversity hotspot (Vanlalsanga et al. 2019). It harbours exceptional biodiversity and is a major reservoir of genetic diversity for agricultural crops, including rice. Rice is the main crop, covering 72% of cultivated land, and is grown in various conditions using at least 10,000 indigenous varieties. Due to local taste preferences, high-yielding varieties (HYVs) from other regions are less popular, prompting the development of locally adapted HYVs with low amylose content, such as the RC Maniphou series in Manipur, which upholds the local palatability with enhanced crop yield and performance. Many farmers continue to grow traditional landraces suited to local climates, valued for their unique grain qualities, nutritional and medicinal properties, and adaptability (Verma et al. 2024. These landraces offer a wealth of untapped genetic traits important for crop improvement.

The development of several new bio-technological techniques has emerged in plant breeding, offering innovative ways for designing effective breeding programs. Molecular markers are widely used to study genetic diversity and fingerprint various crop species (Yang et al. 2022). Different types of molecular markers, such as RAPDs, AFLP, ISSRs, SSRs, and EST-SSRs, have been developed and used to assess genetic diversity and fingerprint at the DNA level. These multi-locus marker systems do not require a priori sequence information and also produce numerous amplicons (Culley and Wolfe 2001; Koopman et al. 2008). However, they are typically used for investigating more shallow taxonomic levels of variation and sometimes show inconsistencies in data replication and involve numerous time-demanding steps (Robarts and Wolfe 2014).

A more recently developed dominant marker, Sequence-Related Amplified Polymorphism (SRAP), originally developed for gene tagging in *Brassica oleracea* L., offers a simple, inexpensive, and effective way to produce genome-wide fragments with high reproducibility and versatility (Li

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and Quiros 2001). It specifically amplifies coding regions of the genome with ambiguous primers targeting GC-rich exons (forward primers) and AT-rich promoters, introns and spacers (reverse primers). Several reports suggest SRAP markers at comparable levels of variation to AFLP markers but with substantially less technical effort and cost (Lou et al. 2010; Levi and Thomas 2007). Target region amplification polymorphism (TRAP) markers are another type of multi-locus markers that have been successfully used to study genetic diversity in a variety of crop species, including sweet sorghum, castor bean, sugarcane, mango, sunflower, wheat, and lettuce (Khidr et al. 2020; Simões et al. 2017; Luo et al. 2015; Menzo et al. 2013) and also offer simplicity, high yield, and reproducibility. It involves a fixed primer designed from a known partial sequence of a candidate gene (EST sequence data), combined with an arbitrary primer having an AT- or CG-rich core sequence (Hu et al. 2003).

Reports have shown that TRAP and SRAP markers were applied combinatorial manner to efficiently explore genetic variability and facilitate selection in crop breeding programs (Mirajkar et al. 2017; El-Shahed et al. 2017; da Silva et al. 2016). The success of any crop improvement program depends on the magnitude of genetic variability in the base population or materials. Hence, assessment of genetic diversity and identification of genotypes becomes a key step in a breeding program. There is no extensive report on dissecting the genetic diversity and fingerprinting of rice germplasm of Northeast India using TRAP and SRAP molecular markers. As these multi-locus marker systems target the open reading frames (ORFs) with high repeatability. A better understanding of the extent of genetic variability is essential for translating genetic diversity into a breeding program. Therefore, the current study was carried out to investigate the genetic variation at morphological and molecular levels and fingerprint the local landraces and other germplasm of Northeast India, which would serve as a huge genetic resource for rice improvement in the region as well as the whole country.

Materials and methods

Plant material and the phenotypic traits recorded

A total of 197 rice landraces/germplasm collection comprised 5 accessions from the states of Arunachal Pradesh, 114 from Manipur, 9 from Meghalaya and 66 from Nagaland of Northeast India. This germplasm is maintained at the ICAR Research Complex for Northeastern Hill Region (ICAR RC NEHR), Manipur Centre, Lamphelpat, Imphal, Manipur, India. The material was evaluated at the Lamphelpat Research Farm, ICAR RC NEHR, Manipur Centre, Imphal (Supplementary Table S1) planted in rows during the *kharif* 2023. Thirteen agronomic traits namely, plant height (cm), no. of panicles per plant, panicle length (cm), 1000 seeds weight (g), decorticated grain length (mm), decorticated grain width (mm), grain length (mm), grain width (mm), stem thickness (mm), blade length (mm), and blade width (mm), days to 50% flowering and days to 80% maturity were recorded.

DNA isolation

Genomic DNA was isolated from the leaves of 3-week-old seedlings after transplanting by using the standard CTAB procedure (Murray and Thompson 1980). Integrity of DNA samples was checked on 0.8% agarose gel and quantified with absorbance ratio A260/280 using Genova Nano Spectrophotometer (Decibel). Samples were diluted to make up a final concentration of ~20 ng/µL with sterile molecular-grade water and stored at 4°C.

Genotyping with SRAP and TRAP markers

The information on both SRAPs used in the study was obtained from previous studies of genetic diversity and fingerprinting in crop plants (da Silva et al. 2016; Ferriol et al. 2003; Li and Quiros 2001). TRAP polymerase chain reaction (PCR) was performed using the arbitrary markers of SRAP and the fixed markers of SSR or expressed sequence tag (EST)-SSR markers. The 16 combinations of both SRAP and TRAP markers with their sequences of forward and reverse primers are presented in Table 1. The final reaction volume was made up to 15 µL using EmeraldAmp® GT PCR Master Mix.

For SRAP markers, polymerase chain reaction (PCR) - mediated amplification was programmed as follows: 94°C for 2 minutes, followed by five cycles of 94°C for 30 seconds, 35°C for 30 seconds, and 72°C for 45 seconds, then an additional 35 cycles of 94°C for 30 seconds, annealing temperature of each marker combination for 30 seconds, and 72°C for 45 seconds. For TRAP markers, the PCR reactions were standardized according to the protocol described by Hu and Vick (2003), with modifications for the current species. The final reaction volume was 15 µL using EmeraldAmp® GT PCR Master Mix. The amplification program consisted of a denaturation step at 94°C for 2 minutes and five cycles of 94°C for 45 seconds, 35°C for 45 seconds, and 72°C for 1 minute. These steps were followed by 35 cycles of 94°C for 45 seconds, annealing temperature of each marker combination for 45 seconds, and 72°C for 1-minute with a final extension at 72°C for 7 minutes. The PCR products were separated in 2% VWR agarose gel and visualized using a gel documentation system (Gel Doc XR System, Bio-Rad).

Data analysis

The phenotypic data were normalized (min-max scaling) with data points ranging from 0 to 1 in Microsoft Excel 2016. For molecular markers, the amplicons of each marker were scored using BioVision software and the obtained molecular data were coded into a binary matrix for the presence (1) or absence (0) of amplified fragments. Expected Hetrerozygosity (H) of each marker was calculated using Nei's genetic diversity measure with formula $H = n\ (n-1) \times (1-\sum P^2 i)$, where *Pi* is the frequency of alleles i^{th} allele in the population, *n* is the number of alleles and $\sum Pi^2$ is sum of the squares of the allele frequencies. The polymorphism information content (PIC) for each locus was calculated using the formula $PIC = 1 - \sum P^2 i$, where *Pi* is the frequency of genotype *I*. D^2 analysis was carried out to assess the genetic variability among the phenotypic traits and to estimate hierarchical clustering of Ward using molecular data.

The individual Dissimilarity matrices and dendrograms of SRAP, TRAP molecular markers and combined analysis used the 'NbClust' package in R Software with Ward.D2 method, with the optimal number of clusters predicted using the Silhouette method.

A Mantel test was performed using the 'vegan' package in R with 9999 permutations for estimation of significance. And also the similarity or correlation coefficients, Cophenetic and Bakers gamma of the two derived dendrograms were assessed using the 'dendextend' R package. The analysis was performed in R software.

Results

Diversity analysis based on phenotypic traits

Principal component analysis was performed to predict the most discriminatory traits among the 13 phenotypes. The optimal number of principal components was predicted to be eight, explaining a total of 87% phenotypic variation (Supplementary Fig. 1). Principal component 1 accounted for 20.4% of total variation with main contributions (>10%) from four traits viz., plant height, days to 50% flowering, number of panicles per plant, decorticated grain width and the second principal component of 16.6% total variance, with major contributions deriving from 5 traits namely, plant height, blade width, days to 50% flowering, days to 80 % maturity, decorticated grain length and grain width (Supplementary Table S2). Based on the contribution to the most informative principal components, variables like plant height, blade length, blade width, days to 50% flowering, number of panicle per plant, days to 80% maturity, 1000 seed weight, decorticated grain length, decorticated grain width, grain length, grain width were found to be relevant in discriminating the germplasm (Fig. 1). Pearson correlation analysis revealed a higher and positive correlation between blade length and panicle length with the plant height; blade length with panicle length, 1000 seed weight with decorticated grain width. And a high negative correlation could be found between plant height with days to 50% flowering and days to 80% maturity, blade length with number of panicles per plant, decorticated grain width with decorticated grain (Supplementary Fig. 2).

The germplasm was categorized into three clusters with taller height, longer panicle length, a large number of panicles per plant, slender grain types and early maturing grouping together in Cluster I. Accessions grouped into Cluster II were also of taller plant height but of stronger culm, medium maturing and bolder seed/grain. The Cluster III consisted of shorter germplasm with late maturing types, smaller leaf blade length and width, and higher effective panicle number per plant (Table 1).

Sequence-related Amplification Polymorphism (SRAP) Marker Profiling

The ten SRAP marker pairs amplified a total of 109 bands from the 197 germplasm. The lowest number of amplicons

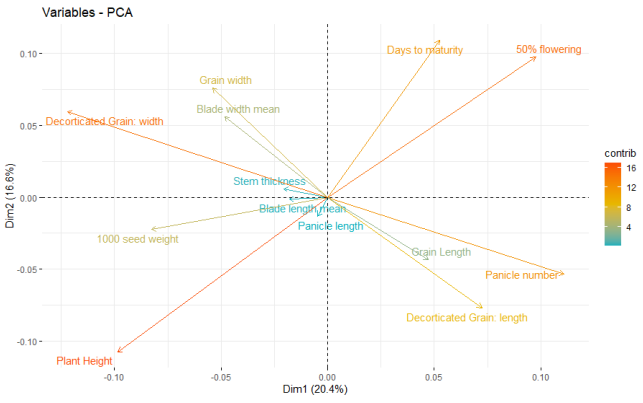

Fig. 1. PCA variables plot predicting the extend of contributions of all the phenotypic traits assessed in the collection of 197 germplasm

Table 1. Phenotypic variation of the 13 traits studied in the three clusters obtained from the hierarchical clustering of Ward

Phenotypic traits	Cluster I		Cluster II		Cluster III	
	Average	Std	Average	Std	Average	Std
Plant height (cm)	141.54	14.10	141.00	22.81	106.53	22.57
Blade length (cm)	55.25	8.57	55.24	11.04	52.56	13.28
Blade width (cm)	1.33	0.24	1.59	0.19	1.37	0.21
Days to 50% flowering	87.35	7.88	85.78	10.01	92.49	9.84
Stem thickness (cm)	0.72	0.11	0.92	0.54	0.69	0.10
Panicle length (cm)	27.14	4.81	25.97	2.47	24.91	2.52
Number of panicles per plant	12.80	4.25	8.59	3.02	12.90	3.37
Days to 80% maturity	127.75	6.14	131.15	12.22	136.76	9.83
1000 seed weight (g)	26.01	2.83	30.10	4.27	25.19	3.29
Decorticated grain length (mm)	6.63	0.44	6.08	0.63	6.55	0.43
Decorticated grain width (mm)	2.19	0.17	2.74	0.27	2.15	0.19
Grain length (mm)	9.28	0.55	8.92	0.51	9.22	0.63
Grain width (mm)	2.60	0.18	2.96	0.27	2.89	0.41

std = Standard deviation

was observed in SRAP4 (ME05 and EM07) and the highest in SRAP8 (ME10 and EM07) with 15 numbers of bands. The PIC scores range from 0.43 to 0.83, with a mean score of 0.61 among the SRAP marker combinations (Table 2). Eight out of 10 primer combinations were highly informative, with PIC scores more than 0.50, with the two highest values in marker combinations SRAP5 (ME01 and EM10) and SRAP6 (ME01 and EM07), with scores 0.80 and 0.83, respectively. The expected heterozygosity, Nei's H, calculated as the proportion of heterozygous individuals in the population, varied from 0.73 to 0.91 with an average of 0.85. However, 80% of the loci or SRAP marker combinations showed heterozygosity variation from 0.84 to 0.91 with an average of 0.87, indicating high genetic diversity among the germplasm.

D^2 was performed on the molecular group data of SRAP markers of the 197 local germplasm; four clusters were predicted (Supplementary Fig. 3). The quality and optimal number of clusters were assessed by Silhouette analysis. The rice accessions were distributed into four clusters with a minimum 4.67 inter-cluster average to centroid distances between Cluster I and Cluster II and a maximum of 6.73 between Cluster III and Cluster IV (Supplementary Table S3). Cluster I was composed of 48% of accession with two subs-clusters and Centroid intra-cluster distances of 3.01. Among the 4 clusters, Cluster IV represented the lowest proportion (11%) with 22 accessions. Mantel's test indicated a correlation value of 0.82.

Target region amplification polymorphism (TRAP) marker profiling

In the case of six combinations of TRAP markers, a total of 72 bands were amplified with a polymorphism of 83%. The loci yielded an average of 12 amplicons. The number of amplicons per combination ranged from 7 TRAP5 (Auxr1 and T13) to 16 TRAP3 (Auxr1 and FT14). The expected heterozygosity varied from 0.66 to 0.92, with an average of 0.87. However, five out of six combinations showed expected heterozygosity of Nei's H score 0.90 or more. The PIC value is also showing the highest discrimination power for each marker combination. The lowest PIC value was 0.53 and was found in TRAP4 (Cystf and T03) and the highest was 0.82 in TRAP2 (Auxr1 and T03), with a mean value of 0.68 (Table 2). The collection is highly diverse and it has high allelic variability as indicated by a high PIC score. In analysis of the dendrogram generated from the TRAP marker data, four main clusters were identified, which were estimated by Ward.D2 method as implemented in the package 'NbClust' with maximum intercluster distance between Cluster II and Cluster IV and minimum between Cluster 1 and Cluster IV with value 5.14 and 4.02, respectively (Supplementary Table 4; Supplementary Fig. 4). Optimal number of clusters was assessed Silhouette method. Mantel's test based on Pearson's product-moment correlation indicated a correlation value of 0.84. In the first cluster, a total of 95 genotypes were grouped into two sub-groups similar to SRAP observations. The Cluster IV consisted of the second-largest group with 37 accessions from different germplasm

Table 2. Details of SRAP and TRAP marker profiling of the rice germplasm (with bold PIC value having more than 0.7)

S. No.	Marker	Sequence (5′-3′)		Tm (°C)	Number of band amplified	Amplicon range (bp)	H (Nei 1987)	PIC
1	SRAP1 (ME04 & EM07)	TGAGTCCAAACCGGACC	GACTGCGTACGAATTCAA	53	12	80-1180	0.9	0.56
2	SRAP2 (ME05 & EM03)	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTGAC	53	7	120-695	0.84	0.43
3	SRAP3 (ME05 & EM10)	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCAG	53	12	140-1200	0.86	0.66
4	SRAP4 (ME05 & EM07)	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCAA	52	5	120-510	0.73	0.56
5	SRAP5 (ME01 & EM10)	TGAGTCCAAACCGGATA	GACTGCGTACGAATTCAG	53	13	130-1180	0.85	0.80
6	SRAP6 (ME01 & EM07)	TGAGTCCAAACCGGATA	GACTGCGTACGAATTCAA	55	8	120-1010	0.78	0.83
7	SRAP7 (ME01 & EM03)	TGAGTCCAAACCGGATA	GACTGCGTACGAATTGAC	51	12	145-1190	0.88	0.46
8	SRAP8 (ME10 & EM07)	TGAGTCCTTTCCGGTCC	GACTGCGTACGAATTCAA	52	15	140-1475	0.91	0.66
9	SRAP9 (ME19 & EM03)	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTGAC	53	12	100-670	0.88	0.54
10	SRAP10 (ME19 & EM10)	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTCAG	55	13	100-1100	0.88	0.62
11	TRAP1 (Auxr1& FT14)	TCATCACCCGCTTGTATG	GTCGTACGTAGAATTCCT	52	10	180-1200	0.9	0.72
12	TRAP2 (Auxr1& T03)	CACAGACCCCGCCTTATAAA	CGTAGCGCGTCAATTATG	53	12	130-1100	0.9	0.82
13	TRAP3 (Auxr1& FT14)	CACAGACCCCGCCTTATAAA	GTCGTACGTAGAATTCCT	55	16	150-1100	0.91	0.78
14	TRAP4 (Cystf & T03)	AGGAGGTGGTCATGGTCCTG	CGTAGCGCGTCAATTATG	52	15	120-1240	0.92	0.53
15	TRAP5 (Auxr1& T13)	TCATCACCCGCTTGTATG	GCGCGATGATAAATTATC	52	7	210-600	0.66	0.71
16	TRAP6 (Cystf & FT14)	AGGAGGTGGTCATGGTCCTG	GTCGTACGTAGAATTCCT	52	12	130-1230	0.91	0.56

types and origins.

Comparison of SRAP and TRAP-derived clustering of germplasm

The comparison of TRAP and SRAP dendrograms showed a consistent level of genetic relationship among genotypes clustering into four groups. 93.6% of genotypes in cluster I are similarly group together in both cases. All the genotype in cluster II and cluster IV of SRAP are grouped in either clusters III or cluster IV of TRAP dendrogram (Fig. 2). The majority of genotypes were grouped persistently in both dendrograms regardless of marker type with higher correlation as predicted by Cophenetic and Bakers gamma correlation co-efficient value of 0.65 and 0.68.

Combined analysis of phenotypic and genotypic data

Genetic diversity assessment using the combined transformed phenotypic and molecular information revealed the presence of four well-defined genetic groups in the current set of materials. Cluster III is composed of the highest number of genotypes, with 91 accessions, constituting 46% of the total accessions and Cluster IV with the minimum number of 29 genotypes (Table 3). Cluster I and Cluster IV showed the highest intercluster distance of 6.92, while Cluster I and Cluster III had with lowest value of 5.10 (Supplementary Table 5).

Principal component analysis had been performed to check the consistency with the clustering event. The first 10 principal components cumulatively explained 51% of the total phenotypic variation. The first 4 components were predicted to explain sufficient variance, with their main contribution (>10%) coming from 7 variables, viz. SRAP 8, SRAP 9, TRAP 1, TRAP 2, TRAP 3, TRAP 4 and TRAP 6 (Supplementary Fig. 5, Supplementary Table 6). The pattern of distribution in factorial analysis of the genotypes in the PCA was highly similar to the finding in hierarchical clustering into 4 groups (Fig. 3).

Discussion

The multivariate analysis of the 13 agronomic/phenotypic traits indicated a substantial diversity among 197 germplasm lines. Eleven out of 13 traits namely, plant height, blade length, blade width, days to 50% flowering, panicle number, days to 80% maturity, 1000 seed weight, decorticated grain length, decorticated grain width, grain length and width had majorly contributed to the principal components and could be used to efficiently assess diversity in the current collections. Several reports have projected the importance of phenotypic traits in unravelling the diversity and characterizing it to relevant, defined germplasm accessions with desirable traits for breeding purposes (Umamaheswar et al. 2024).

Genetic diversity of the germplasm was also assessed using 10 combinations of SRAP and 6 combinations of TRAP molecular markers. The TRAP marker combinations (4 out of 6) exhibited more polymorphism than the SRAP combinations (2 out of 10) in the rice germplasm. Similar

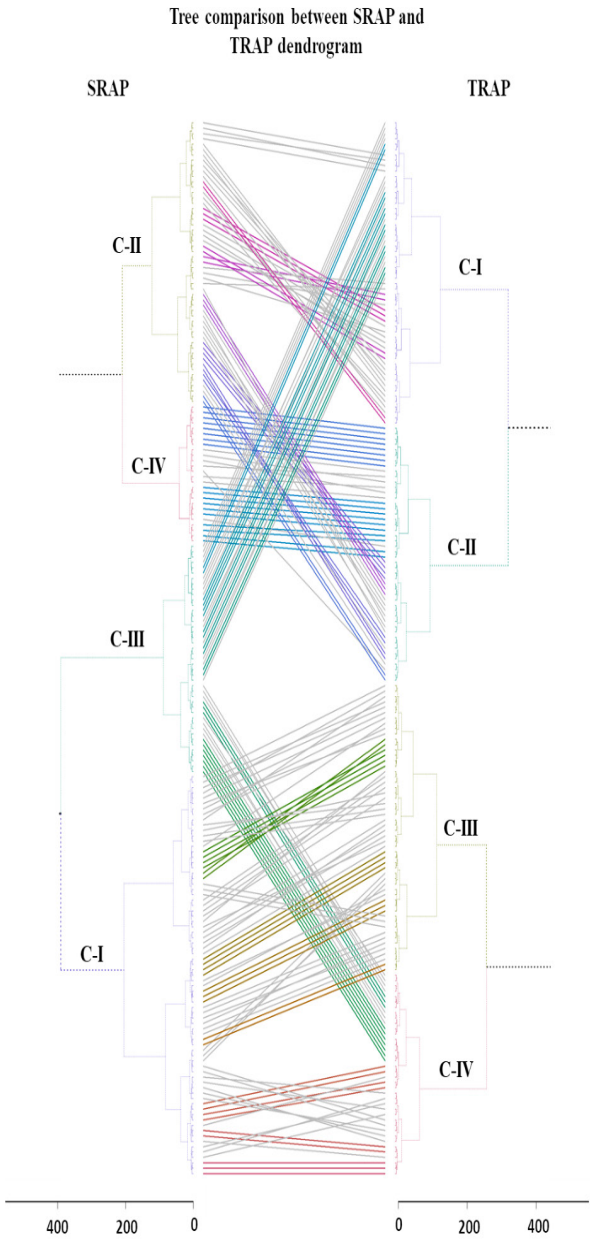

Fig. 2. Comparison of hierarchical cluster dendrograms derived from SRAP and TRAP. The grey lines in between the two dendrograms represent mismatched accessions, while the other colour lines are accessions in a similar grouping/clustering with higher correlation as predicted by Cophenetic and Bakers gamma correlation co-efficient value of 0.65 and 0.68

findings of TRAP markers to be more polymorphic than SRAP were reported in other crops as well, in wheat, sugarcane, *Paullinia cupana,* etc. (da Silva et al. 2016; Devarumath et al. 2013; Barakat et al. 2013). About 68% of the germplasm was found to be similarly grouped in both SRAP and TRAP clusters. The differences observed between the results are partially attributed to the features of the markers that SRAP amplifies preferentially intragenic fragments for polymorphism detection (Li and Quiros 2001), whereas TRAP gathers information associated with expressed regions in the genome that are more conserved (Hu and Vick 2003). SRAP and TRAP markers have been successfully used to analyse genetic diversity in Asian rice (Dai et al. 2012), alfa alfa (Vandemark et al. 2006), sesame (Zhang et al. 2010), banana (Youssef et al. 2011), grape (Guo et al. 2012), etc. The combination SRAP5 (ME01 and EM10), SRAP6 (ME01 and EM07), TRAP1 (Auxr1 and FT14), TRAP2 (Auxr1 and T03), TRAP3 (Auxr1 and FT14) and TRAP5 (Auxr1 and T13) with the PIC score greater than 0.7 is highly informative and indicative of a discriminatory potential of the markers to distinguish the genotypes or individuals and its utilization in a robust fingerprint system of the current germplasm collection (Kaur et al. 2011; Laursen et al. 2011).

Low correlation or poor consistency have been observed between the genotypic and phenotypic distance matrices and clusters generated in this study, with the cophenetic correlation value of 0.008 (Ikkurti et al. 2022; Andrade et al. 2017). The low association observed could be because of the smaller number of molecular markers used, variation in phenotypic traits as a result of a few mutations, and therefore not in accordance with the overall genetic distances. It may also be that the variation detected by molecular markers is often of the non-adaptive type and hence not liable to natural and/or artificial selection, unlike phenotypic traits where the portions of the genome associated with phenotypic expression are subject to selection under environmental influence (Agre et. al. 2019; Alves et. al. 2013; Collard et. al. 2005). Therefore, analysis had been conducted by combining phenotypic traits with genotypic data, suggested and put forward by several researchers as the best way and more accurate assessment of genetic diversity among the germplasm. The ward.D2 hierarchical clustering distance matrix of the phenotypic and molecular marker data resulted in four distinct clusters. The higher distance observed in the combined dissimilarity matrix than the genotypic and phenotypic dissimilarity matrices and cluster distances (Table 5) indicates the importance of joint analysis in genetic diversity study and corroborates with other findings (Huang et al. 2022; Scossa et al. 2021). The present study also showed that a combination of factor analysis and diversity analysis can give a better understanding of the diversity of a set of accessions. The results further established that there is a high level of diversity in the rice accessions of North East India.

Our study using genotypic, phenotypic, and combined distances revealed substantial genetic diversity in rice collections from Northeast India, a key region for rice origin and genetic conservation. This diversity, identified via SRAP and TRAP markers, enables effective DNA fingerprinting of our collection and grouping into four genetic groups

Table 3. Germplasm lines of each cluster as in the dendrogram generated by the combined analysis of phenotypic and genotypic data, based on the hierarchical clustering method

S. No	Cluster	Genotypes
1	Cluster I	1, 2, 7, 10, 14, 15, 19, 24, 31, 32, 36, 38, 45, 46, 47, 69, 90, 109, 110, 111, 114, 129, 134, 141, 147, 148, 154, 168, 171, 172, 179, 181, 189, 191, 192, 197
2	Cluster II	3, 9, 11, 18, 26, 30, 44, 49, 50, 52, 54, 55, 58, 67, 76, 84, 89, 94, 96, 97, 101, 103, 104, 105, 116, 117, 121, 124, 126, 151, 152, 155, 161, 165, 167, 183, 184, 185, 187, 195, 196
3	Cluster III	4, 5, 6, 8, 12, 13, 16, 17, 20, 21, 22, 23, 25, 29, 33, 34, 35, 37, 39, 40, 41, 42, 48, 53, 64, 65, 66, 68, 70, 71, 72, 73, 74, 75, 80, 81, 82, 85, 99, 100, 106, 107, 108, 118, 120, 123, 125, 127, 128, 130, 131, 132, 133, 135, 136, 137, 138, 139, 140, 142, 143, 144, 145, 146, 149, 150, 153, 156, 157, 158, 159, 160, 162, 163, 164, 166, 169, 170, 173, 174, 175, 176, 177, 178, 180, 182, 186, 188, 190, 193, 194
4	Cluster IV	27, 28, 43, 51, 56, 57, 59, 60, 61, 62, 63, 77, 78, 79, 83, 86, 87, 88, 91, 92, 93, 95, 98, 102, 112, 113, 115, 119, 122

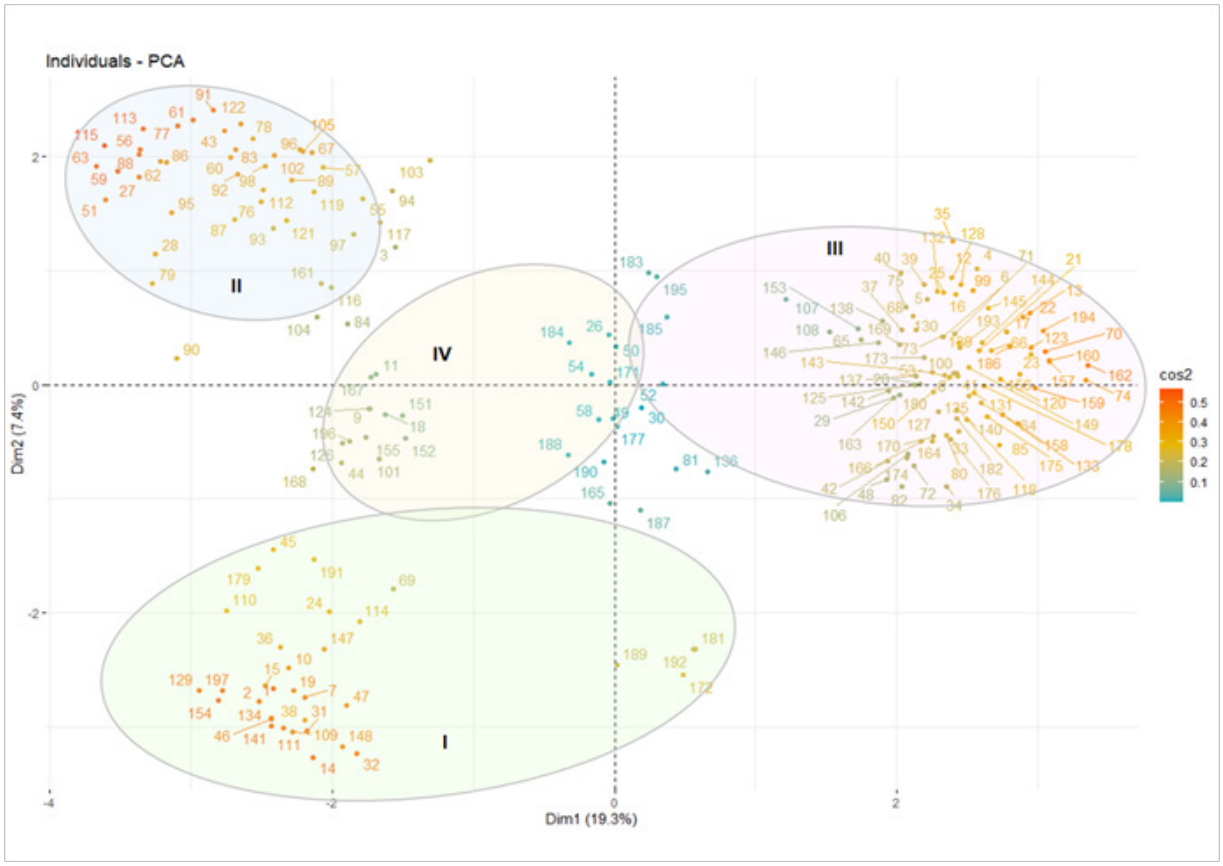

Fig. 3. Principal component analysis with 13 phenotypic traits and molecular data of SRAP and TRAP markers.

for informed parental selection and maximizing genetic variation in rice breeding programs.

Supplementary material

Supplementary Tables S1 to S6 and Supplementary Figures 1 to 5 are provided, which can be accessed at www.isgpb.org

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Authors' contribution

Conceptualization of research (IMS, KS); Designing of the experiments (KS, IMS); Contribution of experimental materials (KS, IMS); Execution of field/lab experiments and data collection (KS, SGS, TRD, CCM, AGD, AK); Analysis of data and interpretation (KS, NU, ELD, HV); Preparation of the manuscript (KS, IMS, RL)

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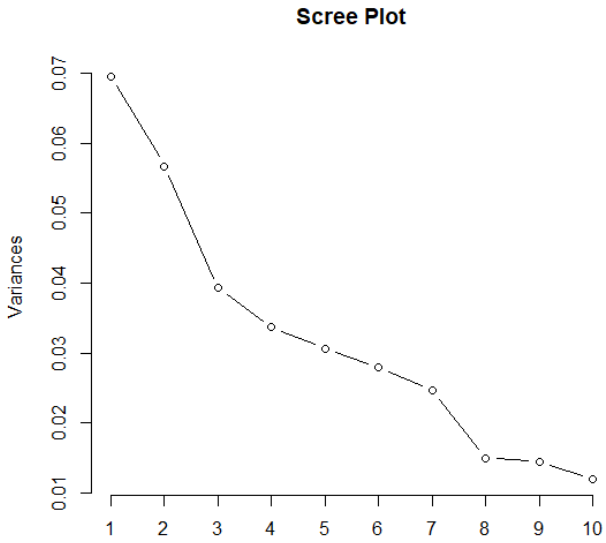
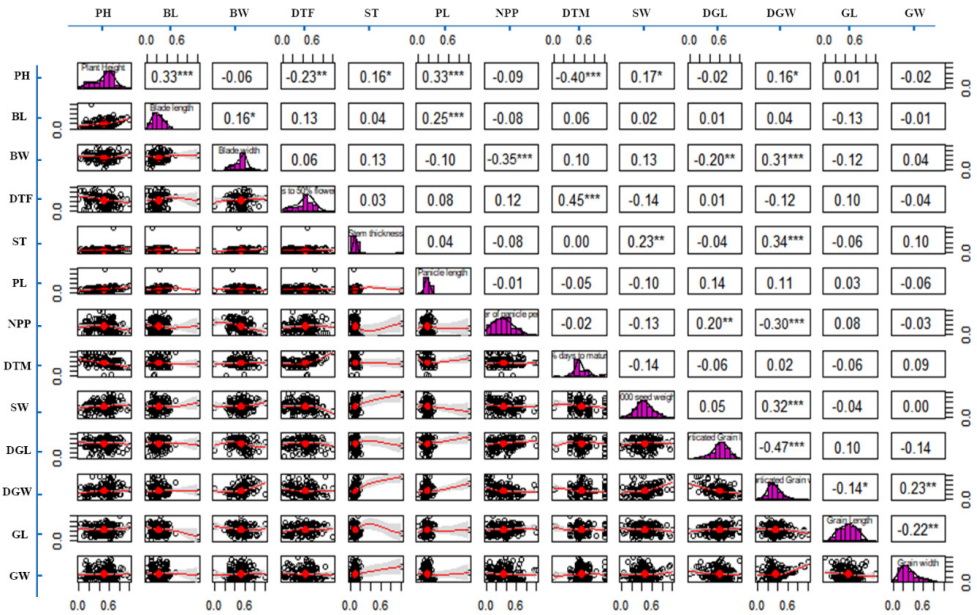


Supplementary Fig. 1. Scree plot predicting eight number of principal components to be retained in PCA analysis of 197 germplasm for the 13 phenotypic traits



(Plant height = PH, Blade length=BL, Blade width=BW, Days to 50% flowering=DTF, Stem thickness=ST, Panicle length=PL, No. of panicles per plant=NPP, Days to 80 % maturity=DTM,1000 seed weight=SW, Decorticated grain length=DGL, Decorticated grain width=DGW, Grain length=GL and Grain width=GW)

Supplementary Fig. 2. Pearson correlation of the 13 studied phenotypic traits (Correlation is significant at *p < 0.01, **p < 0.01, ***p < 0.001)

Supplementary Table S2. Variables contribution on each Principal components (Values in bold indicate the most relevant traits with more than 10% variation of the components)

Phenotypic Traits	*Dim.1*	*Dim.2*	*Dim.3*	*Dim.4*	*Dim.5*	*Dim.6*	*Dim.7*	*Dim.8*
Plant Height (cm)	13.94	**20.40**	0.50	**35.68**	6.76	1.23	0.27	1.25
Days to 50% flowering	**13.73**	**16.69**	**10.04**	**32.93**	0.40	0.12	0.18	0.60
Days to 80 % maturity	3.96	**20.99**	0.75	0.88	0.72	0.97	0.00	20.87
No. of panicles per plant	**17.70**	5.04	**19.44**	**10.21**	3.50	**19.41**	**14.17**	5.03
Panicle length (cm)	0.03	0.31	0.15	2.56	0.63	0.38	0.05	0.62
1000 seed weight (g)	9.79	0.87	3.02	0.63	**67.81**	1.04	0.16	0.90
Decorticated Grain length (mm)	7.53	**10.40**	0.03	0.00	**16.99**	**27.27**	7.26	0.27
Decorticated Grain width (mm)	**21.47**	6.25	0.18	1.43	0.85	**12.97**	1.00	0.88
Grain Length (mm)	3.22	3.30	**11.39**	0.00	0.19	**28.07**	**46.72**	0.97
Grain width (mm)	4.20	**10.12**	**48.99**	3.91	0.50	0.55	**28.83**	1.10
Stem thickness (mm)	0.61	0.06	0.03	0.73	0.70	0.07	0.00	0.00
Blade length (mm)	0.47	0.00	0.97	**10.71**	0.79	6.63	1.00	2.42
Blade width (mm)	3.36	5.55	4.50					

Supplementary Table S3. Intercluster and Centroid intracluster distances predicted based on SRAP molecular data

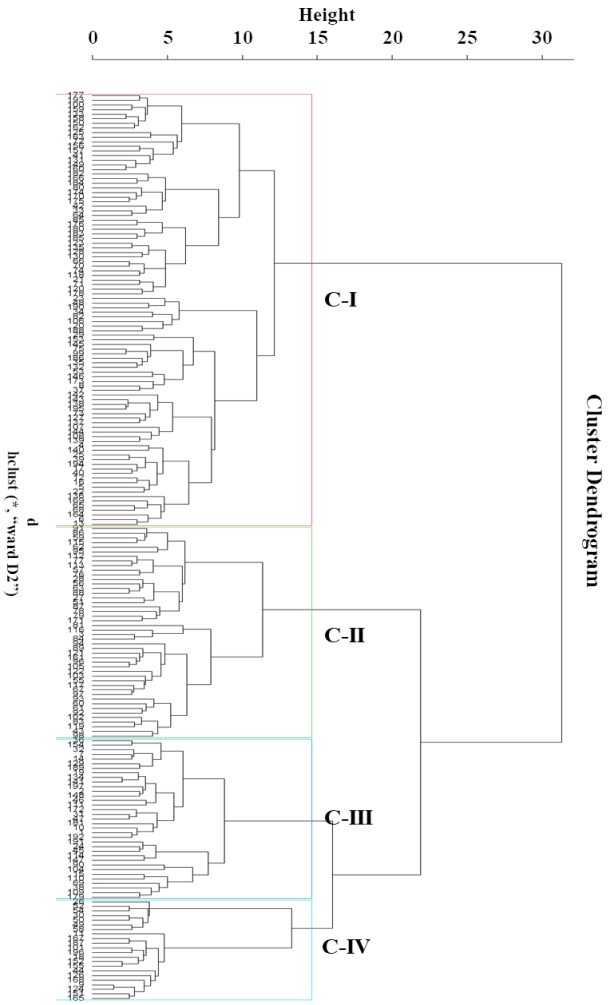
Cluster distance (SRAP)	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	**2.97**	-	-	-
Cluster II	4.67	**3.03**	-	-
Cluster III	5.42	5.01	**3.14**	-
Cluster IV	6.05	6.24	6.73	3.01

Supplementary Table S4, Inter-cluster and Centroid intra-cluster distances as predicted based on TRAP molecular data

Cluster distance (TRAP)	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	**3.11**	-	-	-
Cluster II	4.48	**3.02**	-	-
Cluster III	4.88	5.04	**2.87**	-
Cluster IV	4.02	5.14	4.85	**2.99**

Supplementary Table S5. Intercluster and Centroid intracluster distances predicted based on combined phenotypic and genotypic data of 197 germplasm

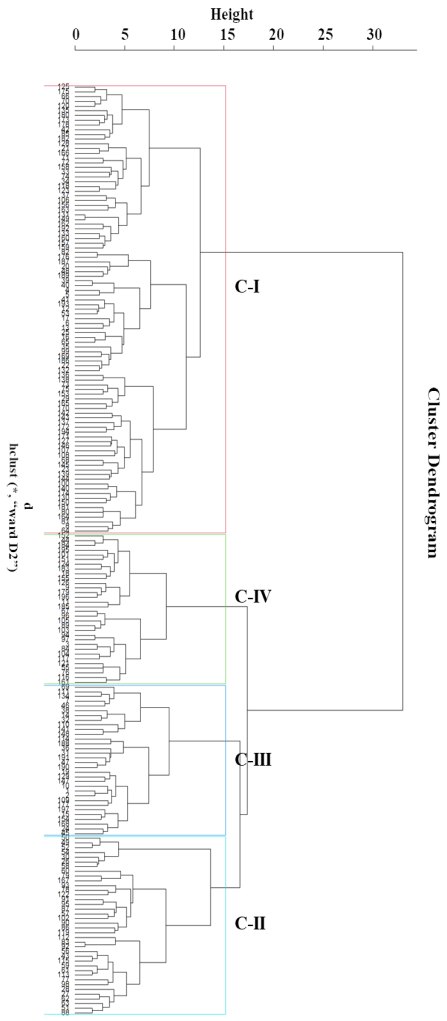
Cluster distance	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	3.94	-	-	-
Cluster II	5.15	4.60	-	-
Cluster III	5.10	5.51	4.47	-
Cluster IV	6.92	6.40	6.43	4.82



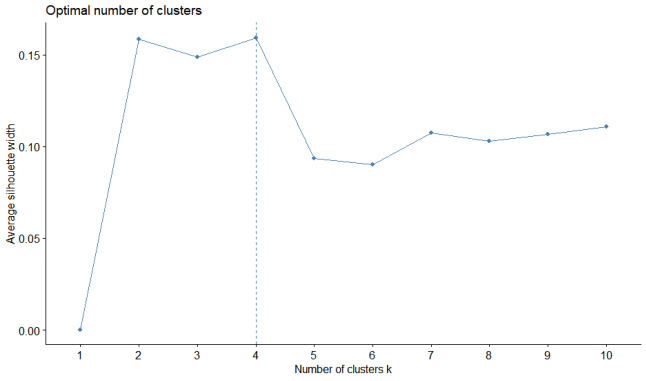
Supplementary Fig. 3. Dendrogram with four clusters generated by SRAP, based on Hierarchical clustering method, Ward.D2 in 197 rice germplasm

Supplementary Table S6. Variables contribution on each Principal components in combined multivariate analysis (Values in bold indicate the most relevant traits with more than 10% variation of the components)

Variables	Dim.1	Dim.2	Dim.3	Dim.4
SRAP1 (ME04 & EM07)	7.9329	5.2288	13.0622	2.5579
SRAP2 (ME05 & EM03)	2.1462	3.1621	0.7598	5.1459
SRAP3 (ME05 & EM10)	7.6052	6.141	2.034	2.8212
SRAP4 (ME05 & EM07)	0.1657	3.3128	1.2553	1.1333
SRAP5 (ME01 & EM10)	3.0874	9.5044	6.8369	9.0769
SRAP6 (ME01 & EM07)	0.8546	4.152	3.1791	3.8357
SRAP7 (ME01 & EM03)	1.4538	1.9528	1.9285	1.7021
SRAP8 (ME10 & EM07)	6.8636	28.6711	13.5317	6.6443
SRAP9 (ME19 & EM03)	15.4772	1.3768	1.9981	1.1781
SRAP10 (ME19 & EM10)	2.2088	4.1936	4.4439	2.0545
TRAP1 (Auxr1 & FT14)	8.6862	5.5265	2.1588	24.4384
TRAP2 (Auxr1 & T03)	14.1165	0.2346	8.9258	7.197
TRAP3 (Auxr1 & FT14)	8.1832	2.5256	8.9201	17.4323
TRAP4 (Cystf& T03)	6.4897	9.9298	22.4708	6.9049
TRAP5 (Auxr1 & T13)	4.0982	0.4186	1.4245	0.8806
TRAP6 (Cystf& FT14)	10.5148	13.64	6.9877	6.734
Plant Height	0.0064	0.0019	0.0007	0.0123
Blade length	0.0002	0.001	0.0005	0
Blade width	0.0006	0.0034	0.0002	0.0264
Days to 50% flowering	0.0013	0.0005	0.0088	0.0029
Stem thickness	0.0005	0.0001	0	0.001
Panicle length	0	0.0003	0.005	0.0002
Panicle number	0.0001	0.0035	0	0.0557
Days to 80% maturity	0.0065	0.0066	0.0067	0.0005
1000 seed weight	0.0001	0.0013	0.0002	0.0592
Decorticated grain length	0.0075	0.0048	0.0085	0.0094
Decorticated grain width	0	0.0005	0.0136	0.0058
Grain Length	0.0021	0.0002	0.0002	0
Grain width	0.0214	0.0048	0.0072	0.0611



Supplementary Fig. 4. Dendrogram with four clusters generated by TRAP, based on Hierarchical clustering method, Ward.D2 in 197 rice germplasm



Supplementary Fig. 5. Assessment of the quality and optimal number of clusters by Silhouette analysis for PCA analysis of combined phenotypic and genotypic data

Supplementary Table S7. Name and source of the experimental materialsof Northeast India,maintained at ICAR RC NEHR Manipur Centre, Imphal

S. No.	Genotype	Source state
1	Chingphou - I	Manipur
2	Thangmoi	Nagaland
3	Ching Chakhao - I	Manipur
4	Chakhao (Lamhing)	Manipur
5	Mayajang	Nagaland
6	KuntaMah	Manipur
7	MunichsTsok	Nagaland
8	Thekrulha	Nagaland
9	YounyoKangru	Nagaland
10	Mata Maha	Manipur
11	Runya	Nagaland
12	Allechisho	Nagaland
13	Acefuchisho	Nagaland
14	Kezijhum	Nagaland
15	Chupu Wing Rice	Nagaland
16	DesekYouso	Nagaland
17	PhungchamMah	Manipur
18	HaosilMah	Manipur
19	Kapang	Manipur
20	Bhuman	Manipur
21	Rosolia	Nagaland
22	OchuTsokSnapuri	Manipur
23	Apaghi	Arunachal Pradesh
24	Akhanphou	Manipur
25	Makhara -II	Manipur
26	Basmati -370	Manipur
27	Shangshak Local	Manipur
28	MainongKangbu	Nagaland
29	Ccpur	Manipur
30	Chakhao - I	Manipur
31	Ching Chakhao - II	Manipur
32	KemenyaKepeu	Nagaland
33	OngpangMasojang	Nagaland
34	JunguPhek	Nagaland
35	WazhuhoPhek	Nagaland
36	NeikedoUlhaTsia	Nagaland
37	LazaTssok	Nagaland
38	Chakhao - II	Manipur
39	Wainem	Manipur
40	Manen Jang (Wonder Rice)	Nagaland
41	Naga Special-2	Nagaland
42	TssokthiEthonTssok	Nagaland
43	TshngMeki Jang	Nagaland
44	TehumTssok	Nagaland
45	Gambithong	Manipur
46	Chakhao - III	Manipur
47	Azhogihi	Nagaland
48	SitariaMaha	Manipur
49	Kenhoni	Nagaland
50	ManuiKhamei	Manipur
51	RCM-12	Manipur
52	WazuhoPhek	Nagaland
53	Kd-5-3-14	Manipur
54	ZutsokMosta	Nagaland
55	Wonder Rice	Nagaland
56	OtsokKhira	Nagaland
57	Aya Mao Maha	Manipur
58	Ching Chakhao -III	Manipur
59	Chingphou - II	Manipur
60	Meitak N-Special	Nagaland
61	UteibiMah	Manipur
62	Koya Jang	Nagaland
63	Meitidak	Nagaland
64	KbaLaispah	Meghalaya
65	Bali Old	Arunachal Pradesh
66	PumphaMah	Manipur
67	Et Saro	Nagaland
68	Bali White	Arunachal Pradesh
69	MakhapuiKohra	Nagaland
70	Tabusen	Nagaland
71	Naga Special-1	Nagaland
72	KumnupuZunheboto	Nagaland
73	Tsunghi	Nagaland
74	Gum Rice	Manipur
75	WR-1-9-1-1	Manipur
76	KD-5-2-8	Manipur
77	ChangphoiAwangba	Manipur
78	Phat Sen	Nagaland
79	Ankur	Arunachal Pradesh

80	Gumdhan	Nagaland
81	TonakenaPhek	Nagaland
82	Hotung	Nagaland
83	PurmhiMakhrei	Manipur
84	JenilKajiePhek	Nagaland
85	LangphouAngouba	Manipur
86	Teke	Nagaland
87	Keda	Nagaland
88	TsukNyiko	Nagaland
89	GP-D	Manipur
90	MoibroTssok	Nagaland
91	Khangamra	Manipur
92	Maya Masce Jang	Nagaland
93	ThemyouhKhangru	Manipur
94	Shangshak Local	Manipur
95	YenjoEpya	Nagaland
96	Kongyouh	Manipur
97	Yenglo	Nagaland
98	KhumtiaTssok	Nagaland
99	Ngodzu	Nagaland
100	TangsekKangbo	Nagaland
101	RCM-10	Manipur
102	TaoreiMah	Manipur
103	WR-3-2-1	Manipur
104	Wonder Rice	Nagaland
105	Ereima	Manipur
106	Noin	Manipur
107	Ehunyo (MakhapuiKalry)	Nagaland
108	Arunachal II	Arunachal Pradesh
109	JakSemla	Nagaland
110	Chandel Exhibition	Manipur
111	MakharaMasuta	Manipur
112	Sanaphou	Manipur
113	Shang Bhuman	Manipur
114	ThumpakTssok	Nagaland
115	Chakhao - III	Manipur
116	Daramphou	Manipur
117	MoirangphouKhongnembi	Manipur
118	Samphai	Manipur
119	Kunda	Manipur
120	KD	Manipur
121	Chingphou - III	Manipur
122	Sanayanbi	Manipur
123	Korphan	Manipur
124	Atra	Manipur
125	Leimaphou	Manipur
126	KD-62	Manipur
127	RCM-16	Manipur
128	Ginphou	Manipur
129	RCM-17	Manipur
130	Chapali	Manipur
131	Ruchitra	Manipur
132	Nungshang Phou	Manipur
133	Forfour	Manipur
134	Niphuthokpi	Manipur
135	Nenetsuk	Nagaland
136	RCM-11	Manipur
137	Zunheboto	Nagaland
138	Pariphou	Manipur
139	Matruri	Manipur
140	Tamphaphou	Manipur
141	RCM-9	Manipur
142	Bhumap	Manipur
143	Charongphou	Manipur
144	Khata Jang	Nagaland
145	MachawKaoyeng	Manipur
146	Hundung Collection	Manipur
147	Kishegui	Nagaland
148	Chakhao Amubi	Manipur
149	MachangKaoyeng	Manipur
150	ShonburEpyoTsuk	Nagaland
151	ZunhebotoGhisul	Nagaland
152	ChingtuiMakarei	Manipur
153	Kezu	Nagaland
154	MukhokMujeh	Nagaland
155	MephongTssokl	Nagaland
156	HaosilMah	Manipur
157	Ripu Dune	Nagaland
158	RCM-21	Manipur
159	Gilele	Meghalaya
160	Longmai	Meghalaya
161	Lakang	Meghalaya

162	Sohkyrleh	Meghalaya	180	PhourenChaobi	Manipur
163	Bapnan	Meghalaya	181	Kohriiro	Manipur
164	Khonemma	Manipur	182	Naga-Special	Manipur
165	Makra	Manipur	183	Mikrotho	Manipur
166	Moirangphou	Manipur	184	Roziia Kate	Nagaland
167	Lephama	Manipur	185	Tolen Phou	Manipur
168	Changat	Manipur	186	Desha	Manipur
169	Phouren	Manipur	187	Chakhao Poreiton (Thoubal - I)	Manipur
170	Nagara	Manipur	188	Chakhao (Chandel)	Manipur
171	Khongnem	Manipur	189	Chakhao Poreiton (Thoubal - II)	Manipur
172	Changlei	Manipur	190	Chakhao Angoubi (Chandel)	Manipur
173	Bhalum - 1	Meghalaya	191	Chakhao (Ccpur)	Manipur
174	Bhulum - 3	Meghalaya	192	Chakhao Amubi (Thoubal - I)	Manipur
175	Bhulum - 4	Meghalaya	193	Chakhao Poreiton	Manipur
176	PhourenAmubi	Manipur	194	Chakhao (Senapati)	Manipur
177	Phouring Kate	Manipur	195	Chakhao (Tamenglong)	Manipur
178	RodziiaKakra	Manipur	196	Chakhao (Ukhrul)	Manipur
179	Tarasang	Manipur	197	Tamphaphou	Manipur