



Research Note

The extent of genetic diversity among popular rice cultivars of Andhra Pradesh and Telangana using microsatellite markers

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Abstract

Genetic diversity among 35 rice varieties was investigated using 68 hyper variable polymorphic microsatellite markers to have more insights into the varietal diversity for future selection strategies in development of tailor made rice varieties suitable to different ecological niches of the state. In 68 polymorphic markers, a total of 298 alleles were detected with a range of 2 to 13 with an average allele number (N_a) of 4.3 per locus. The Polymorphic Information Content (PIC) values ranged from 0.36 (RM24015) to 0.97 (RM 25310) with an average of 0.85. Overall estimation of number of alleles (N_a) and genetic diversity index (H_e) indicated low to moderate level of diversity among the cultivars. The research station wise estimation on number of alleles (N_a) and genetic diversity index (H_e) clearly indicated that the varieties developed from Rajendranagar varieties exhibited maximum while, Nellore varieties with minimum followed by Jagtial and varieties from remaining stations showed moderate values for both the parameters. The cluster analysis grouped 35 varieties into six clusters. The cultivars developed from Rajendranagar, Maruteru and Warangal tends to group together and the other station varieties are clustered in different groups. The results of this study revealed that genetic diversity among the varieties released from different research stations is moderate and thus warrants the breeders of particularly ARS, Nellore and RARS, Jagtial to broaden the genetic base by selecting diverse parents while making crosses.

Key words

Rice, hyper variable microsatellite markers, polymorphism, diversity analysis

Rice is the staple food for more than one third of the global population and endowed with rich genetic diversity (Chakravarthi and Naraveni, 2006). In India, Andhra Pradesh and Telangana states are placed among the top paddy producing states. Andhra Pradesh (combined) produced 127.62 lakh metric tons (MT) of paddy for the year of 2013-2014. The Acharya N.G Ranga Agricultural University (ANGRAU) Hyderabad, Andhra Pradesh and Prof. Jayashankar Telangana State Agricultural University (PJ TSAU), Telangana played a vital role in Indian agriculture by releasing many high yielding rice varieties, that is evident from the fact that the 4 mega varieties released from ANGRAU and PJ TSAU *viz*; Swarna, BPT-5204, MTU1010 and MTU-1001, which occupied more than 25% of the rice growing area of the country. A systematic study of estimation of genetic diversity is worthy for identifying genetic divergence among the varieties, landraces, breeding lines and germplasm which in turn helps in development of tailor made designer rice varieties. Understanding the genetic diversity of popular cultivated rice varieties is important for broadening the genetic base. Till now, there is no systematic and comprehensive attempt was made to assess the extent of genetic diversity at molecular level, among the rice varieties released from ANGRAU and PJ TSAU which will be of great significance for their use of in future rice breeding programme.

Molecular markers have demonstrated potential to detect genetic diversity in plant genetic resources (Ford-Lloyd *et al.*, 1997; Song *et al.*, 2003). SSRs (McCouch *et al.*, 1997) markers are useful for assessing the variability and diversity at molecular level (Joshi *et al.*, 2000). Microsatellite markers are PCR- based markers that are technically very efficient and cost effective to use and are abundantly available for rice (Chen *et al.* 1977; Temnykh *et al.*, 2000). In the present study 75hvSSR markers were used (Narshimulu *et al.*, 2011) to detect the genetic diversity among the 35 popular rice varieties released from six different research stations of from ANGRAU and PJ TSAU.

Plant material: The experimental material comprised of 35 rice cultivars (Table 1) that were developed at six different research stations of ANGRAU and PJ TSAU *viz.*, Rice Research Unit (RRU), Bapatla; Andhra Pradesh Rice Research Institute (APRRI), Maruteru; Rice Section (RS), Rajendranagar; Regional Agricultural Research Station (RARS), Warangal; Regional Agricultural Research Station (RARS), Jagtial and Agricultural Research Station (ARS), Nellore. For collection of leaf samples and DNA extraction, 10 seeds from breeder seed lots of each of the 35 varieties collected from the corresponding research stations, were grown and leaf samples of young seedlings were collected after 25 days and kept at -80°C .

Genomic DNA isolation: Nucleus seed of 35 rice genotypes were allowed for germination in

petridishes and 3-4 gm of leaf samples were collected from 7 days old seedlings and DNA was isolated following the modified CTAB (Cetyl Try Methyl Ammonium Bromide) method (Murray and Thompson, 1980). The quality and quantity of DNA was estimated in 0.8% agarose gel with lambda (λ) Hind III DNA as standard. Based on the intensity and thickness of genomic DNA bands, as compared to lambda (λ) Hind III DNA, the concentration and quality of DNA in individual samples were determined. After assessing the concentrations these samples were normalized in T₁₀E₁ buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to get final concentration of 5 ng/ μ l for amplification using microsatellite markers.

Microsatellite Markers and PCR amplification: A set of 75 hyper variable rice microsatellite (hvRMs) markers, covering all the 12 chromosomes, were selected from the literature cited by (Narshimulu *et al.*, 2011) to determine the genetic diversity among the 35 rice cultivars used in the present study. Polymerase Chain Reactions were done in a volume of 10 μ l containing 10 x PCR Buffer, 2 mM each of the dNTPs, 5 pmol of each forward and reverse primer, 0.5 unit of Taq DNA polymerase (Genei, Bangalore, India), 10 ng/2 μ l template DNA. Amplification were carried out in GenAmp PCR system 9700 (Applied Biosystem, USA) thermal cycler with the following thermal profile: initial denaturation step at 94°C for 5 min followed by 35 cycles at 95°C for 45 sec, 57°C for 45 sec, and 72°C for 45 sec and a final cycle at 70°C for 10 min. The amplified PCR products were mixed with bromophenol blue and run on a 3% agarose gel along with the 50-bp DNA ladder (New England Biolabs) in 0.5x Tris-Acetic acid EDTA (TAE) buffer. After two hours the gel containing DNA fragments were visualized under UV-Transilluminator and documented using Bio-Rad Molecular Imager Gel Doc XR System.

Data analysis: Only clear and unambiguous bands of SSR markers were scored. The sizes of the amplified fragment were estimated with the help of Bio-Rad Molecular Imager Gel Doc XR System using 50 bp DNA ladder (NEB) as size standard. The amplified bands/alleles were scored as present (1) or absent (0) for each genotype and primer combination. To measure the informativeness of the markers, polymorphism information content (PIC) for each of the SSR markers was computed according to the formula: $PIC = 1 - \sum p_i^2$, Where p_i is the frequency of the i^{th} allele of each locus (Botstein *et al.*, 1980).

Genetic diversity parameters *viz.*, number of alleles (N_a), Shannon Index (I) and Nei's genetic diversity index (H_e) (Nei *et al.*, 1973) were evaluated using POP-GENE version 1.31 (<http://www.ualberta.ca/~fyeh>). The UNJ (Un Weighted neighbor joining Method) cluster

analysis followed by boost strap analysis with 1000 per mutations for total cultivars was carried out using DARwin 5.0.145 (<http://darwin.cirad.fr/>).

Allelic diversity of microsatellite markers in rice cultivars: Out of 75 hvRM markers, 7 primer pairs were monomorphic and 68 were polymorphic (90.6% polymorphism). A total of 298 bands/alleles were detected in all 35 cultivars using 68 polymorphic markers. The overall number of alleles per locus ranged from 2 to 13 with an average of 4.38 (Table 2,3,4). This was significantly higher than the average of number of alleles reported by Sajib *et al.* (2012). This higher value in the present study (4.38) could probably be as a result of existence of higher diversity among the cultivars. However, the number of alleles detected in the present study was lower than the average number of alleles reported by Xu *et al.* (2004), Jain *et al.* (2004), Zeng *et al.* (2007) and Speda *et al.* (2004). The locus RM21881 possessed highest number alleles (13). The lowest number of alleles were observed in RM24481, RM11340 and RM11597 loci (2 in each case) and of the all 68 loci, 20 were with 3 alleles, 19 were with 4 alleles, while 15 loci with 5 alleles. Loci with 3 alleles are more frequent followed by 4 and 5 alleles (Fig. 1). The number of alleles detected by a single SSR locus varied from 1 to 31 depending upon the fingerprinting techniques and materials used in the studies (Ni *et al.*, 2002; Blair *et al.*, 2002; Lu *et al.*, 2005; Jayamani *et al.*, 2007; Thompson *et al.*, 2009; Kaushik *et al.*, 2011). In the present study, microsatellite markers with simple di-nucleotide repeat motifs detected a greater number of alleles (average: 4.552) (Table 2) than those with tri-nucleotide and tetra nucleotide repeat motifs (average: 4.39 and 3.50 respectively) (Table 3 and 4). Similar observations were also made by Cho *et al.* (2000) and Jain *et al.* (2004). Significant positive correlation ($r = 0.105$) was found between number of alleles amplified per locus and number of repeats in simple motif of a SSR locus (Table 5) in the present study. Ni *et al.* (2002) and Yu *et al.* (2003) also found positive correlation between number of alleles amplified and number of repeats within a microsatellite marker. The overall H_e values ranged from 0.23 (RM11597) to 0.89 (RM21881) (table 2,3) with an average of 0.60 and more than 40% of the loci were in the frequency of 0.5- 0.6. The overall average H_e values (Genetic diversity index) of the present study are slightly higher than the earlier studies (Gangaprasad chowdary *et al.* 2013). The research station wise estimation of number of alleles (N_a) and H_e values (Genetic diversity index) clearly indicated that the varieties released from Rice Section, Rajendranagar exhibited maximum no. of alleles (3.0145) and genetic diversity index (0.5097), followed by Bapatla (N_a : 2.6522, H_e : 0.4264), Maruteru (N_a : 2.5072, H_e : 0.4184), Warangal (N_a : 2.0725 H_e : 0.3664) and Jagtial (N_a : 1.971, H_e :

0.2965) as represented in Table 4 and Fig. 2. While, Nellore varieties exhibited minimum no. of alleles (1.6087) and genetic diversity index (0.2609) values. These low values for Nellore cultivars might be due to the inclusion of only two popular varieties from this station for this study.

Polymorphism information content of microsatellite markers: The Polymorphism information content (PIC) values provides an estimates of discriminating power of a marker based on the number of alleles at a locus and relative frequencies of these alleles. The PIC values for 68 SSR loci in our study varied from 0.36 (RM24015) to 0.97 (RM 25310) with an average of 0.85 and more than 50% loci in the range of 0.8 - >9.0 (Fig. 1). The estimated average PIC values are relatively higher than the average PIC values as reported by others (Lu *et al.*, 2005; Juneja *et al.*, 2006; Joshi *et al.*, 2010) and thus might be due to higher genetic diversity present among the selected rice cultivars. Moreover, the SSR markers used in the study were selected on the basis of their high PIC values reported earlier (Narshimulu *et al.*, 2011). Microsatellite markers with simple di-nucleotide repeat motifs detected higher polymorphism (Mean: 0.866) than those with tri and tetra nucleotide repeat motifs (Mean: 0.85 and 0.77) (Table 2, 3 and 4). Positive correlation ($r = 0.0832$) was observed between number of repeats of simple motif and PIC value of a SSR locus. Jain *et al.* (2006) had also observed that PIC values showed a positive correlation with total number of alleles at SSR locus ($P = 0.01$). Microsatellite loci with AT-rich di, AAT rich tri-and AGAT rich tetra nucleotide repeat motifs amplified a greater number of alleles and revealed greater polymorphism (Table 2, 3 and 4). One of the microsatellite markers, RM 25310 having (AT)_n repeats amplified 11 alleles and had PIC value of 0.97.

Genetic diversity among popular rice cultivars of Andhra Pradesh and Telangana: The un-weighted neighbor joining (UNJ) dendrogram was constructed based on polymorphism data of the 68 hvRMs. In dendrogram, green lines indicated varieties from Rajendranagar, blue line indicated varieties from Bapatla, yellow line indicated varieties from Warangal, brown line indicated varieties from Jagtial, pink line indicated varieties from Nellore and red line indicated varieties from Maruteru (Fig 3). The 35 rice cultivars brought from different rice research stations of ANGRAU and PJTSAU were broadly divided into six clusters. The cultivars developed from Rajendranagar, Maruteru and Warangal tend to group into separate groups. The key reason for their distinct station wise separation can be attributed to the fact of sharing similar parentage or pedigree for the development of varieties within the research stations. As an example, all seven

varieties studied from APRRI, Maruteru showed less diversity as they involved either one or both the parents released from the same research station. Utilizing same station varieties continuously for many years decreased the genetic diversity among themselves. However, the varieties *viz.*, JGL3844, BPT4358, BPT2231 and BPT 5204 were placed in the Nellore group cluster but these varieties do not share the common parentage or pedigree. This might be due to presence of similar alleles corresponds to respective microsatellite loci. Compared to all rice research stations, rice varieties released from Rice section; Rajendranagar found to have more number of alleles and more genetic diversity followed by Rice Research Unit, Bapatla, while, ARS, Nellore varieties showed least genetic diversity.

In conclusion the present study clearly indicated that a low to moderate genetic diversity existed among popular rice cultivars of Andhra Pradesh and Telangana. All the cultivars analyzed could be distinguished from each other. However, this present study warrants the breeders of all research stations of Andhra Pradesh (ANGRAU) and Telangana (PJTSAU) except Rajendranagar, particularly ARS, Nellore and RARS, Jagtial to broaden the genetic base of their parental varieties while planning the future rice breeding programmes by including diverse rice accessions from japonica, javonica, landraces and wild/weedy species.

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Table 1. List of cultivars used for genetic diversity study in rice

S.No.	Genotypes	Pedigree	Year of Release	Research stations
1	RNRC 28	IR64X9994	Pre released	
2	Sugandha Samba	RNR19994/RNR M7	2010	
3	Chandan	SonaX Manoharsali	1989	
4	Sumathi	ChandanXPak-basmati	2002	
5	RNR 2354	RNR M7/RNR 1994	Minikit	Rice Section, Rajendranagar
6	Early Samba	Mutant of Samba Mahsuri	1999	
7	RNR 2458 (Krishna)	Chandan X BPT 5204	2012	
8	RNR 23064 (Taramati)	Tellahamsa/ BPT 5204	2009	
9	Tellahamsa	HR12 X TN1	1971	
10	Satya	Tellahamsa X Rasi	1989	
11	MTU1010 (Cottondora Sannalu)	Krishnaveni X IR 64	2000	
12	MTU 4870 (Deepthi)	Sowbhagya X ARC 6650	1997	
13	MTU 1075 (Pushyami)	PLA 1100 X MTU 1010	2006	APRRI, Maruteru
14	MTU 1061 (Indra)	PLA 1100 X MTU 1010	2006	
15	MTU 7029 (Swarna)	Vasista X Mahsuri	2011	
16	MTU 1001 (Vijetha)	Vajram X MTU7014	1995	
17	MTU 1064 (Amara)	PLA 1100 X MTU 1010	2009	
18	JGL 384 (Polasaprabha)	Samba Mahsuri X Kavya	2011	
19	JGL 11470 (Jagtial Mahsuri)	JGL 418/ Gedongibeton	2012	
20	JGL 3855 (Karimnagar Samba)	BPT 5204/ARC5984//Kavya	2012	RARS, Jagtial
21	JGL 1798 (Jagtial Sannalu)	Samba Mahsuri X Kavya	2002	
22	JGL 3844(Jagityal Samba)	Samba Mahsuri X ARC 5984	2010	
23	NLR 145(Swarnamukhi)	CICA4/IR625-23-3-1//Tetep	2011	ARS, Nellore
24	NLR 34449 (Nellore Mahsuri)	IR72 X Samba Mahsuri	2010	
25	BPT 4358(Surya)	Sona Mahsuri X ARC6650	2000	
26	BPT 5204(Samba Mahsuri)	GEB24/T(N)1//Mahsuri	1986	
27	BPT 2231(Akshya)	Surya X IR64	2010	
28	BPT 2270 (Bhavapuri Sannalu)	Samba Mahsuri/CR15MR1523	2010	RRU, Bapatla
29	BPT 2295	BPT 1768/NLR 33641	2012	
30	BPT 3291(Sona Mahsuri)	SONA/Mahsuri	1984	
31	BPT 1768 (Bapatla Sannalu)	BPT 3301 X Mutant Mahsuri	2003	
32	WGL 14 (Warangalsamba)	Samba Mahsuri/ARC5984// BPT 3291	2005	
33	WGL 2047 (Erramallelu)	BC-5-55/W12708	1991	RARS, Warangal
34	WGL 32100 (Warangal Sannalu)	Divya X BPT 5204	2011	
35	WGL 48684 (Kavya)	WGL27120/WGL17672// Mahsuri/Surekha	1991	

Table 2. Details of di nucleotide repeat microsatellite markers used in the study besides their corresponding Na, PIC and He Values

Sl. No.	SSR Locus	Motif	Number of Repeats	Chr. No.	Number of alleles (<i>N_a</i>)	PIC Values	He Values Genetic diversity index	<i>I</i>
1	RM10167	AT	43	1	6	0.82	0.55	1.03
2	RM12031	AG	48	1	3	0.83	0.51	0.85
3	RM11340	AT	42	1	2	0.67	0.38	0.57
4	RM11597	AT	42	1	2	0.61	0.23	0.40
5	RM12292	AT	38	1	4	0.93	0.73	1.42
6	RM13659	AT	50	2	7	0.94	0.73	1.51
7	RM13131	AT	32	2	5	0.92	0.64	1.18
8	RM15679	AC	59	3	4	0.81	0.37	0.72
9	RM15630	AG	55	3	3	0.80	0.42	0.72
10	RM15580	AT	50	3	5	0.93	0.64	1.18
11	RM15831	AT	57	3	4	0.85	0.58	1.00
12	RM14778	AT	37	3	4	0.92	0.69	1.29
13	RM14270	AT	46	3	4	0.85	0.61	1.09
14	RM16153	AC	56	3	3	0.81	0.46	0.73
15	RM15379	AT	46	3	3	0.82	0.49	0.81
16	RM18939	AT	46	5	4	0.93	0.72	1.34
17	RM18704	AT	47	5	5	0.90	0.67	1.25
18	RM20710	AT	46	6	6	0.93	0.76	1.58
19	RM20583	AT	46	6	5	0.83	0.74	1.47
20	RM21693	AT	44	7	6	0.95	0.73	1.51
21	RM23237	AC	57	8	4	0.90	0.60	1.12
22	RM22273	AT	35	8	3	0.84	0.55	1.09
23	RM24542	AT	51	9	3	0.86	0.60	0.98
24	RM25453	AT	45	10	5	0.94	0.72	1.37
25	RM25310	AT	42	10	11	0.97	0.86	2.18
26	RM25355	AT	60	10	6	0.92	0.76	1.56
27	RM26109	AT	45	11	3	0.82	0.50	0.74
28	RM27323	AT	47	11	7	0.92	0.77	1.61
29	RM28067	AT	48	12	5	0.92	0.62	1.18
Total					132	25.14	17.63	33.48
Mean					4.551	0.866	0.607	1.154

Table 3. Details of tri nucleotide repeat microsatellite markers used in the study besides their corresponding Na, PIC and He Values

S.NO	SSR Locus	Motif	No. of Repeats	Chr	No. of alleles (Na)	PIC Values	He Values Genetic diversity index	I
1	RM11111	AAT	31	1	3	0.80	0.58	0.96
2	RM11229	AAT	42	1	6	0.85	0.77	1.63
3	RM10886	AAG	13	1	3	0.84	0.55	0.90
4	RM11313	AAT	23	1	4	0.88	0.53	0.97
5	RM12690	AAT	42	2	3	0.53	0.61	1.01
6	RM13154	AAT	34	2	3	0.80	0.43	0.77
7	RM13238	AAT	49	2	5	0.94	0.72	1.38
8	RM12353	AAT	26	2	3	0.84	0.54	0.86
9	RM13155	AAC	39	2	3	0.87	0.54	0.89
10	RM13867	AAT	34	2	5	0.72	0.69	1.33
11	RM16006	AAT	80	3	3	0.85	0.59	0.96
12	RM14250	AAT	41	3	5	0.93	0.70	1.33
13	RM15626	AAT	39	3	6	0.90	0.76	1.58
14	RM16913	AAT	51	4	7	0.89	0.83	1.85
15	RM19208	AAT	19	5	4	0.89	0.57	1.02
16	RM18639	AAT	17	5	4	0.92	0.68	1.25
17	RM18384	AAG	22	5	5	0.84	0.33	0.66
18	RM20615	AAT	63	6	5	0.80	0.72	1.43
19	RM21881	AAT	25	7	13	0.95	0.89	2.36
20	RM21539	AAT	32	7	4	0.89	0.56	1.05
21	RM20979	AAG	15	7	3	0.86	0.60	1.01
22	RM23017	AAT	18	8	4	0.92	0.69	1.26
23	RM23362	AAG	19	8	3	0.86	0.58	0.95
24	RM23146	AAT	66	8	4	0.91	0.65	1.20
25	RM22250	AAT	30	8	5	0.94	0.71	1.38
26	RM22554	AAT	20	8	3	0.85	0.55	0.92
27	RM22688	AAT	28	8	3	0.74	0.24	0.47
28	RM24044	AAG	11	9	4	0.90	0.63	1.16
29	RM23741	AAT	28	9	4	0.93	0.72	1.33
	RM25408	AAT	22	10	4	0.88	0.56	0.93
	RM25969	AAG	18	11	5	0.95	0.76	1.52
	RM28800	AAT	32	12	5	0.95	0.75	1.44
	RM27840	AAT	37	12	4	0.56	0.51	0.75
Total					145.00	28.18	20.54	38.51
Mean					4.39	0.85	0.62	1.17

Table 4. Details of tetra nucleotide repeat microsatellite markers used in the study besides their corresponding Na, PIC and He Values

S.NO	SSR Locus	Motif	Number of Repeats	Chr	Number of alleles (Na)	PIC Values	He Values Genetic diversity index	I
1	RM10936	AGAT	37	1	4	0.84	0.46	0.84
2	RM22565	ACAT	15	8	5	0.90	0.52	0.84
3	RM23036	AGAT	15	8	4	0.90	0.67	1.19
4	RM24015	AGAT	9	9	3	0.36	0.51	0.75
5	RM24481	AGAT	23	9	2	0.74	0.48	0.67
6	RM26190	AGAT	13	11	3	0.87	0.62	1.02
Total					21.00	4.61	3.26	5.31
Mean					3.50	0.77	0.54	0.89

Table 5. Correlation coefficients

S.No.	Attributes (Table)	Correlation coefficient (r)
1	Number of repeats - Number of alleles	0.105
2	Number of repeats - Polymorphic information content	0.0832
3	Number of alleles - Polymorphic information content	0.4426

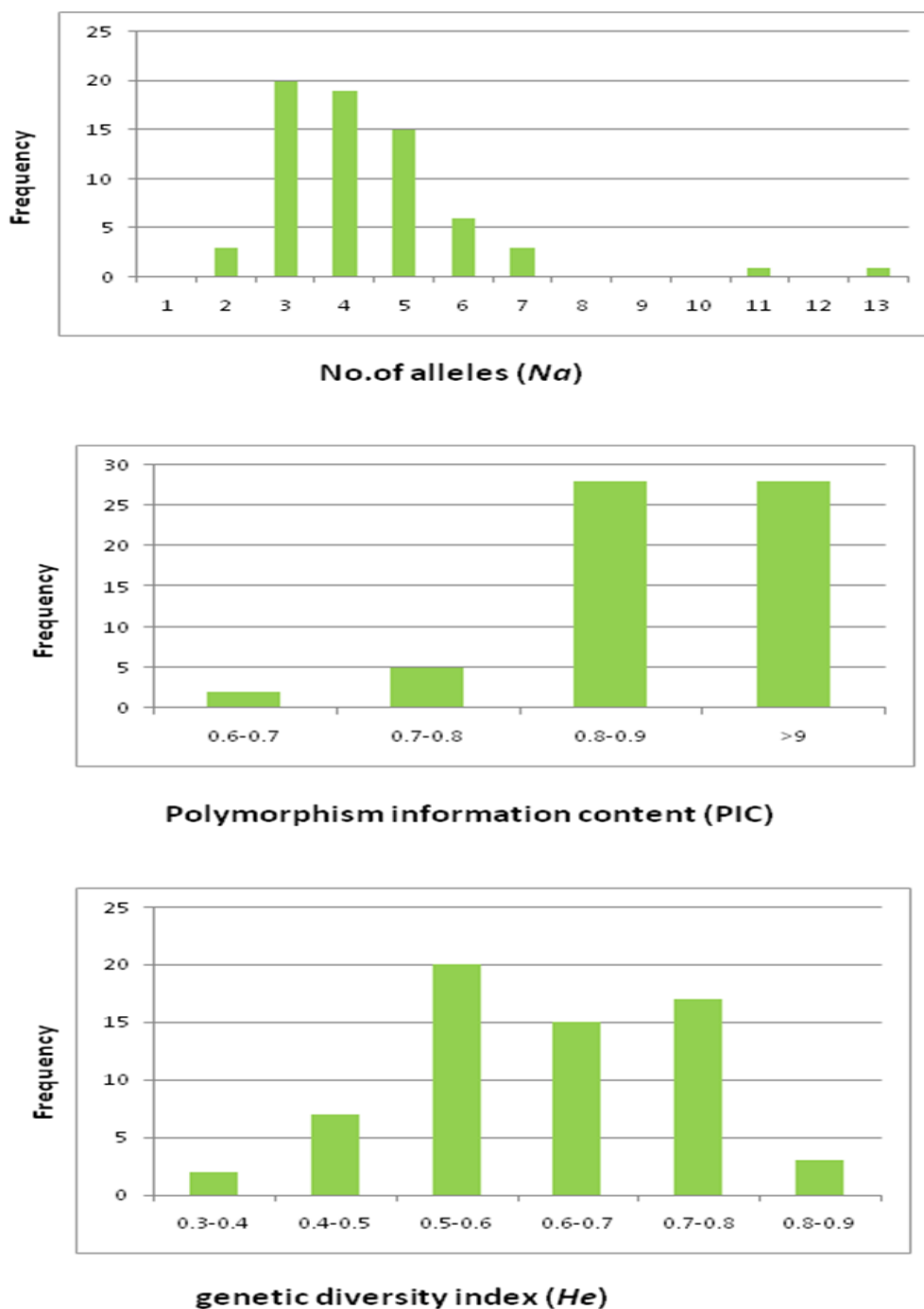


Fig 1. Average allele number (Na), Polymorphic information content (PIC) and Genetic diversity index (He) among the six rice research station cultivars of ANGRAU and PJTASU

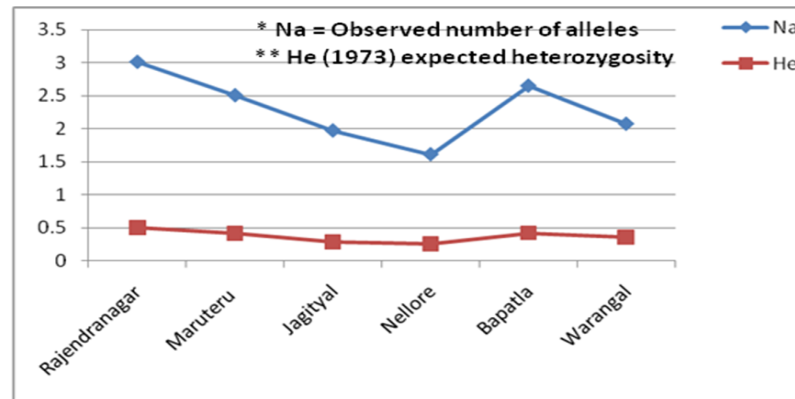


Fig 2a. Research stationwise number of alleles (Na) and expected heterozygosity or genetic diversity (He) of ANGRAU released rice varieties.

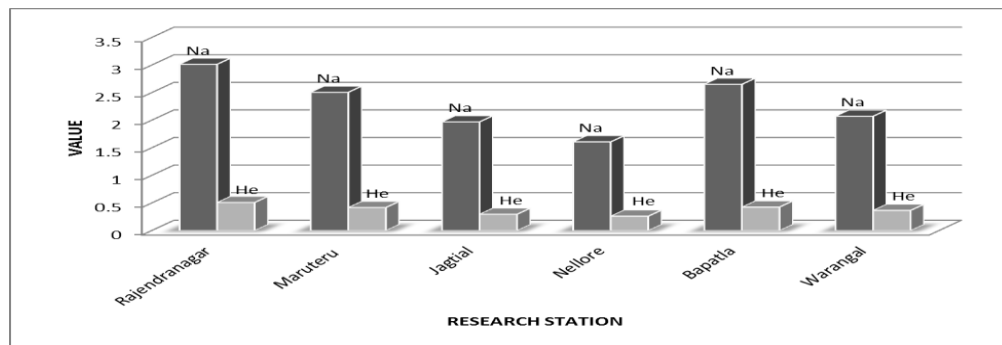


Fig 2b. Comparisons of average allele number (Na) and Genetic diversity index (He) among the six rice research station cultivars of ANGRAU and PJTASU

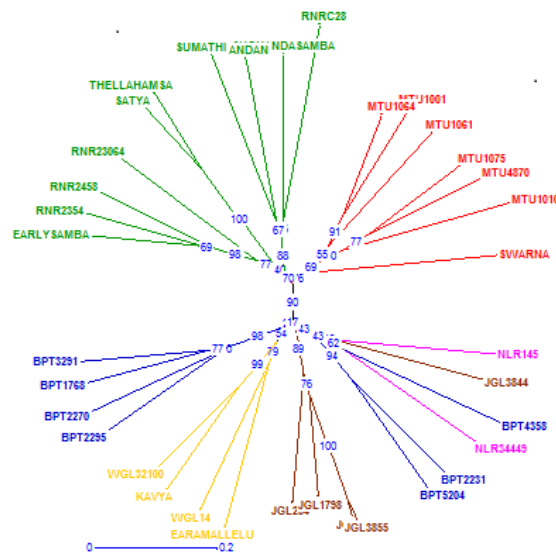


Fig 3. Un-weighted Neighbor Joining (UNJ) tree showing genetic relationship among 35 rice cultivars released from six rice research stations. Green: varieties from Rajendranagar, Blue: varieties from Bapatla, Yellow: varieties from Warangal, Brown: varieties from Jagtial, pink: varieties from Nellore, red: varieties from Maruteru.