Molecular Characterization and Diversity Analysis for Leaf Folder Resistancein Rice Using Microsatellite Markers

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Abstract

Rice is the staple food for more than one half of the world's population. The major reason for dismal state of rice production and productivity worldwide is due to biotic and abiotic stresses. Among insect pests, rice leaf folder (*Cnapholocrocis medinais*) earlier considered as a minor pest has gained the status of major pest with the widespread cultivation of HYV and the accompanying changes in cultural practices. Lack of reliable resistant donors is forcing the farmers to depend on insecticides to combat the pest. So, there is an urgent need to identify resistant donors and develop resistant varieties against leaf folder. The present study was carried out to study the extent of variability and molecular genetic diversity for leaf folder resistance in 30 genotypes including susceptible check TN1 under natural conditions. Based on the mean damage score (25, 50 and 75 DAT), 21 genotypes were resistant, 8 were moderately resistant and TN1 was susceptible. Correlation studies among different traits revealed that the trait damage score recorded significant positive correlation with leaf width ($r= 0.180^{**}$) and SPAD chlorophyll meter reading ($r= 0.136^{**}$) and non significant negative correlation with leaf length. To study the molecular diversity, 60 microsatellite markers were utilized. UPGMA analysis using NTSYS software grouped the 30 rice genotypes in to three clusters (Cluster I – 1genotype, Cluster II- 11 genotypes and Cluster III – 18 genotypes). UPGMA analysis using Darwin software grouped the 30 rice genotypes in to four clusters (Cluster I – 11 genotypes, Cluster II- 2 genotypes, Cluster III- 7 genotypes and Cluster IV – 10 genotypes). Based on the results it can be concluded that large variability exists in genotypes for leaf folder resistance which can be utilized in breeding programmes to develop rice varieties with resistance to leaf folder coupled with good grain quality and higher yield.

Keywords: Rice, leaf folder, microsatellite marker, molecular diversity

1. Introduction

Rice is the world's second most widely grown cereal crop after wheat, and is the staple food for more than one half of the world's population. To feed the growing world population, of about 9 billion by the year 2050, the production and productivity of rice has to enhance enormously especially in highly populated developing countries. Development of varieties with higher yield potential in combination with tolerance to biotic and abiotic stresses is required to meet the ever growing demand for rice production. In India, the productivity of rice is very low compared to world average which is mainly due to biotic and abiotic constraints. Among biotic constraints, insect pests play a major role in reduction of rice production and productivity. Stem borers, plant hoppers, leaf folder and gallmidge are major insect pests causing huge economic losses to the farmers. Rice leaf folder, earlier a minor pest has now became a destructive and wide spread insect pest throughout the rice growing regions in South and South East Asia. Among the eight leaf folder species recorded in India, *Cnaphalocrocis medinalis* (Guenee) is the most widely spread and the damage ranges from 20% to 60% depending on the stage of crop at the time of infestation. (Ramasamy and Jaliecksono, 1996).This insect feed on the leaf by scrapping the green matter resulting in reduced photosynthesis, consequently leading to reduction in yield. About 5% of total rice growing area of world is affected by rice leaf folder (RLF) and the loss in economic terms is estimated to be $22.4 million (Herdt, 1991).

Conventional methods of pest control often depends upon the use of chemical insecticides, which attracts concern on food safety & environmental pollution. In this context, host-plant resistance (HPR) is a viable alternative to chemical control methods (Khushand Brar 1991).HPR is relatively economical, ecofriendly and compatible with other pest management strategies. Due to lack of resistant donors and varieties, farmers are solely dependent on insecticides to combat leaf folder. So, there is an urgent need to identify resistant donors and develop resistant varieties against leaf folder. Host plant resistance may be due to physiological or biochemical differences or nutritional and allele chemical alterations which make the plant to resist against the phytophagous insects. Several plant morphological characters like flag leaf length& flag leaf width are also associated with RLF infestation (Dhakshayaniet al., 1993).

Rice is one of the very few crop species endowed with rich genetic diversity. An understanding of the extent of genetic diversity is critical for the success of a breeding programme. Traditional methods using morphological characters for establishment of genetic diversity and relationships among accessions are largely unsuccessful due to strong influence of the environment. Recent advances in molecular biology have equipped scientists with a wide choice of marker assisted techniques to know the extent of molecular diversity. Microsatellite markers/SSRs are highly effective in molecular characterization of the genotypes and also in assessing the genetic diversity existing in the genotypes as well as in genetic mapping studies. Molecular Marker based Genetic Diversity Analysis (MMGDA) also has potential for assessing changes in genetic diversity over time and space. In the present investigation, the extent of variability for leaf folder resistance was studied in 30 different rice genotypes including the susceptible check TN1 and these genotypes were characterized to know the extent of molecular diversity using 60 microsatellite markers.

2. Materials and Methods

In the present study, the experimental material consisted of 30 rice genotypes of which 20 (including susceptible check TN1) were from leaf folder screening trial of IIRR, Hyderabad along with ten released varieties from APRRI & RARS, Maruteru. (Table 1). The screening was done under natural conditions during wet season, but to get good pest incidence resurgence chemical (Phorate 10 G) was applied. The data on number of leaf folder damaged leaves and total number of leaves were recorded from ten hills per each genotype at 25, 50 and 75 DAT (days after transplanting).Data was recorded on different traits viz., leaf width, leaf length, SPAD chlorophyll meter reading which have correlation with leaf folder resistance. Based on the % damage the damage scores was estimated as per SES (1996) of IRRI. (Table 2 and 3).

$$\% \text{ damaged leaves} = \frac{\text{Number of damaged leaves}}{\text{Total number of leaves observed}} \times 100$$

The percentage of damaged leaves is converted to figure D and

Table 1: List of genotypes studied

Sl. No	Genotype	Sl. No.	Genotype
1.	Aganni	16.	CO 43
2.	ARC 111289	17.	W 1264
3.	Choorapundy	18.	ADT 46
4.	CR-MR-1523	19.	IC 115737
5.	Gorsa	20.	MTU 1064 (Amara)
6.	GEB 24	21.	MTU 1010 (Cottondo-rasannalu)
7.	INRC 3021	22.	MTU 4870 (Deepthi)
8.	PTB 12	23.	MTU 1061 (Indra)
9.	RP 2068-18-3-5	24.	MTU2077 (Krishnaveni)
10.	TKM 6	25.	MTU 1075(Pushyami)
11.	W 1263	26.	BPT 5204 (SambhaMah-suri)
12.	LF 293	27.	MTU 7029 (Swarna)
13.	IR 36	28.	MTU 2067 (Chaitanya)
14.	TNAU(LFR)831311	29.	MTU 1001 (Vijetha)
15.	SB 319	30.	TN 1

Table 2: Scoring of the entries based on damage

Scale	D
0	No damage
1	1-20%
3	21-40%
5	41-60%
7	61-80%
9	81-100%

Table 3: Classification of the entries based on damage score

Score	Disease reaction
0	Immune (I)
1-3	Resistant (R)
5	Moderately resistant (MR)
7	Susceptible (S)
9	Highly susceptible (HS)

D is converted to 0-9 scale

$$D = \frac{\text{\% damaged leaves in test entry}}{\text{\% damaged leaves in susceptible check}} \times 100$$

To study the molecular genetic diversity, a set of 60 microsatellite markers spanning all the 12 chromosomes were used (Table 4). DNA was extracted from leaf tissue

Table 4: List of Microsatellite markers studied

Sl. No.	Chromosome	Markers
1.	1	RM9,RM14,RM212,RM220,RM243, RM472, RM493,RM495, RM543, RM3412 and RM10793.
2.	2	RM20,RM208,RM3340,RM3865 and RM5101.
3.	3	RM251,RM1319, RM3766 and RM4108.
4.	4	RM559, RM5611, RM3524 and RM3742.
5.	5	RM289,RM334,RM1182 and RM5454.
6.	6	RM400,RM5957, RM7372 and RM8226.
7.	7	RM11, RM248, RM429, RM5100 and RM5711.
8.	8	RM210, RM331, RM1309, RM8266 and RM23048.
9.	9	RM219, RM242, RM3909 and RM3912.
10.	10	RM21, RM216, RM228, RM496 and RM6100.
11.	11	RM206, RM224, RM286 and RM1124.
12.	12	RM1880, RM2854, RM3331, RM5939 and RM6867.

from all the genotypes using the modified Cetyl TriMethyl Ammonium Bromide (CTAB) method (Dellaportaet al.1983). The DNA quantification was done by using a Nanodrop spectrophotometer (Thermo Scientific) as well as using known amount Lambda DNA (Bangalore Genei) as standards using agarose gel electrophoresis method. The amplification reaction with microsatellite primers was carried out in a final volume of 10 µl in DNA Thermo cycler (Eppendorf Mastercycler Pro S). Each reaction mixture contained 1.0 µl 10 X reaction buffer containing 1.5 mM $MgCl_2$, 1.0 U of Taq DNA polymerase (Bangalore Genei), 0.1 mM dNTP (Bangalore Genei), 10.0 picomoles each of forward and reverse primer (synthesized by Eurofins) and approximately 50 ng μl^{-1} of template DNA.PCR amplification was carried out on thermal cycler with Initial denaturation at 94 °C for 5 min followed by 45 cycles of denaturation at 94 °C for 30 sec, primer annealing at 55 °C for 45 sec, extension at 72 °C for 1 min and final extension of 72 °C for 7 min.PCR samples were mixed with bromo-phenol blue (0.25% bromo phenol blue and 40% (w/v) sucrose mixed in water) and electrophoresed on a 3% agarose gel (Sigma) containing ethidium bromide (10 mg ml^{-1}) along with the marker 50bp ladder (Genei) at 5.3V cm^{-1} (Bio-Rad Power Pac 300) for one hour in 1x Tris-Acetic acid-EDTA (TAE) buffer (242 g Tris base, 57.1 ml Acetic acid, 100ml 0.5M EDTA mixed and made up the volume to 1 litre with double distilled water and pH adjusted to 8.5). The resolved PCR bands were documented using Syngene Gel Doc System. The scoring of the population was done as presence or absence of the band. Cluster analysis and dendrogram construction was done by UPGMA analysis using Darwin (100 boot straps) and NTSYS softwares.

3. Results and Discussion

3.1. Field reaction of rice genotypes to leaf folder

The data on number of leaf folder damaged leaves and total numbers of leaves indicated that at 25 DAT, the % leaf folder damaged leaves (% LFDL) in all the genotypes ranged from 0.49 to 38.03% with a mean of 11.80% and the susceptible check has recorded 55.56%. The genotype RP 2068-18-3-5 has recorded lowest % of damaged leaves (0.49%) and genotype Swarna (38.03%) recorded highest % of damaged leaves. (Table 5).

At 50 DAT, the % LFDL among the genotypes ranged from 26.98% to 56.31% with a mean of 39.70%. The genotypes which recorded lowest % of LFDL were SB 319 (26.98%), MTU 4870 (27.86%) and Aganni (29.57%). The highest % of LFDL was recorded in MTU 1064(56.31%), MTU 2067 (55.63%) and MTU 2077(53.20 %) and the susceptible check, TN 1 (68.32%). At 75 DAT, the data indicated that the % LFDL ranged from 19.55% to 49.21%. Susceptible check, TN 1 recorded 69.63% of damaged leaves.

In certain genotypes the % damage was highly reduced at 75 DAT when compared to 50 DAT. In the genotype TNAU (LFR) 831311 the % damage was reduced from 44.27% (50 DAT) to 24.98% (75 DAT) and in MTU 1001 also the % damage was reduced from 42.01% (50 DAT) to 21.63% (75 DAT). It was clearly observed that in more than half of the genotypes the % leaf folder damaged leaves at 75 DAT were low when compared to 50 DAT. Similar findings were earlier reported by Kushwaha and Singh (1985) and Sabir et al. (2006) who concluded that the peak incidence of leaf folder was observed during September.

The mean per cent leaf folder damaged leaves were computed per each genotype and were scored as per the SES (1996) of IRRI. The damage scores of all the genotypes indicated that among the 30 genotypes, 21 rice genotypes were categorized as resistant with a damage score 3, eight genotypes were designated as moderately resistant with a damage score of 5 where as TN1 was susceptible (Table 5).

In the released varieties from ANGRAU, MTU 4870, MTU 1010, MTU 1001 and MTU 1075 were found to be resistant with damage score of 3 while, MTU 7029, BPT 5204, MTU 2067, MTU 2077, MTU 1064 and MTU 1061 were found to be moderately resistant with damage score of 5. The results are in agreement with Pillai et al. (1979).

3.1.1. Leaf length and width

The leaf length and width were recorded for all the genotypes. The length of the leaf ranged from 29.69 cm (Aganni) to 62.12 (PTB 12) with an average of 46.20 cm (Table 6). In the popular varieties, the leaf length was highest in MTU 1075 (57.01 cm) followed by MTU 1064 (52.13 cm). For the trait width

Table 5: Leaf folder damage at 25 DAT, 50 DAT, 75 DAT and damage score

Sl. No.	Genotype	% LFDL at 25 DAT	% LFDL at 50 DAT	% LFDL at 75 DAT	Average	Damage Score
1.	Aganni	0.73	29.57	38.48	22.93	3
2.	ARC 111289	0.75	48.25	38.69	29.23	3
3.	Choorapundy	0.87	30.71	39.43	23.67	3
4.	CR-MR-1523	31.90	43.76	46.43	40.70	5
5.	Gorsa	0.91	36.08	30.23	22.41	3
6.	GEB 24	37.62	40.35	42.47	40.15	5
7.	INRC 3021	0.79	32.21	36.19	23.06	3
8.	PTB 12	0.66	40.38	37.19	26.08	3
9.	RP 2068-18-3-5	0.49	37.29	37.96	25.25	3
10.	TKM 6	0.94	37.91	27.25	22.03	3
11.	W 1263	0.90	31.62	19.55	17.36	3
12.	LF 293	0.89	33.70	37.52	24.04	3
13.	IR 36	1.03	38.26	47.85	29.05	3
14.	TNAU(LFR) 831311	0.61	44.27	24.98	23.29	3
15.	SB 319	2.76	26.98	30.57	20.10	3
16.	CO 43	1.65	33.86	29.40	21.64	3
17.	W 1264	2.88	31.31	29.73	21.31	3
18.	ADT 46	6.85	33.16	30.13	23.38	3
19.	IC 115737	0.64	35.42	28.79	21.62	3
20.	MTU 1064 (Amara)	34.09	56.31	43.10	44.50	5
21.	MTU 1010 (Cottondorasannalu)	1.23	33.65	29.24	21.37	3
22.	MTU 4870 (Deepthi)	1.64	27.86	31.20	20.23	3
23.	MTU 1061 (Indra)	31.68	45.21	48.96	41.95	5
24.	MTU2077 (Krishnaveni)	32.25	53.20	46.32	43.92	5
25.	MTU 1075(Pushyami)	1.23	44.32	29.62	25.06	3
26.	BPT 5204 (SambhaMahsuri)	31.35	43.25	48.62	41.07	5
27.	MTU 7029 (Swarna)	38.03	36.24	49.21	41.16	5
28.	MTU 2067 (Chaitanya)	32.31	55.63	39.34	42.43	5
29.	MTU 1001 (Vijetha)	1.23	42.01	21.63	21.62	3
30.	TN 1	55.56	68.32	69.63	64.50	7
	Mean	11.80	39.70	36.90	-	-
	SD	+ 16.70	+ 9.40	+ 10.30	-	-

LFDL – Leaf folder damaged leaves, DAT – days after transplanting

of the leaf, MTU 7029 recorded lowest width (0.95 cm) and the highest width was observed in SB 319 (5.53 cm) with an average of 2.70 cm. In the released varieties the leaf width was highest in MTU 2077 (1.89 cm) and lowest in MTU 7029 (0.95 cm).

3.1.2. SPAD Chlorophyll meter reading

MTU 1064 and TN1 recorded lowest SPAD meter reading (12.60) while MTU 7029 recorded highest SPAD reading (34.80) with an average of 23.70. In the popular varieties the SPAD ranged from 12.60 (MTU 1064) to 34.80 (MTU 7029) (Table 6).

3.2. Simple correlations among traits studied

The nature and strength of relationship between traits was analyzed by regressing phenotypic values of one trait on

Table 6: Leaf length, width and SPAD reading in the genotypes

Sl. No	Genotype	Leaf length (cm)	Leaf width (cm)	SPAD Chlorophyll meter reading
1.	Aganni	29.69	5.04	22.50
2.	ARC 111289	46.22	2.53	20.96
3.	Choorapundy	46.82	3.35	24.43
4.	CR-MR-1523	41.44	1.95	32.33
5.	Gorsa	53.54	4.98	29.50
6.	GEB 24	45.37	4.02	24.86
7.	INRC 3021	57.83	5.33	26.76
8.	PTB 12	62.12	5.32	20.06
9.	RP 2068-18-3-5	57.02	3.20	25.30
10.	TKM 6	45.81	2.05	26.80
11.	W 1263	48.94	3.68	19.60
12.	LF 293	41.84	3.66	20.70
13.	IR 36	46.33	2.80	22.93
14.	TNAU(LFR)831311	47.27	3.02	21.46
15.	SB 319	36.41	5.53	29.36
16.	CO 43	49.99	3.74	26.80
17.	W 1264	45.63	4.24	26.53
18.	ADT 46	57.42	2.40	27.60
19.	IC 115737	42.39	2.97	24.00
20.	MTU 1064 (Amara)	52.13	1.08	12.60
21.	MTU 1010 (Cottondorasannalu)	31.98	0.99	24.20
22.	MTU 4870 (Deepthi)	49.42	1.37	16.20
23.	MTU 1061 (Indra)	46.48	1.18	22.40
24.	MTU2077 (Krishnaveni)	34.17	1.89	16.60
25.	MTU 1075(Pushyami)	57.01	1.24	24.20
26.	BPT 5204 (SambhaMahsuri)	47.01	1.09	24.00
27.	MTU 7029 (Swarna)	39.29	0.95	34.80
28.	MTU 2067 (Chaitanya)	42.47	1.04	20.80
29.	MTU 1001 (Vijetha)	50.79	1.26	32.90
30.	TN 1	34.75	0.96	12.60
	Mean	46.20	2.70	23.70
	SD	±8.0	±1.5	±5.3

those of another. The following trait pairs showed significant correlation coefficients. The % damaged leaves at 25 DAT exhibited significant and positive correlation with % damaged leaves at 50 and 75 DAT. (Table 7). The leaf length followed negative correlation (r=-0.021, -0.059 and -0.054 respectively) with % damaged leaves at 25, 50 and 75 DAT. The correlation between leaf length and damage score was negative but not significant. Significant positive association was observed between leaf width and % damaged leaves at 25 and 50 DAT (r=0.248* and 0.240* respectively). The association between the damage score and leaf width is significant and positive (r= 0.180**). The results are in accordance with Punithavalli et al.(2013) and Islam and Karim (1997) who concluded that the leaf folder infestation will be low in genotypes having long and narrow leaves and the damage will be heavy in genotypes with broad leaves. SPAD chlorophyll meter reading was positively associated with % damaged leaves at 50 and 75 DAT and as well as with damage score(r=0.136**). The increase in SPAD

Table 7: Simple correlations among traits studied

Variables	25 DAT	50 DAT	75 DAT	Leaf length	Leaf width	SPAD	Score
25 DAT	1	0.232*	0.438**	-0.021	0.248*	0.165	0.381**
50 DAT		1	0.338**	-0.059	0.240*	0.149	0.777**
75 DAT			1	-0.054	0.177	0.189	0.675**
Leaf length				1	-0.043	-0.023	-0.081
Leaf width					1	0.050	0.180**
SPAD						1	0.136**
Score							1

DAT: days after transplanting; *: significant at α=0.05; **: significant at α=0.01

reading indicates that the leaves are dark green in colour which can attract more insects easily with some exceptions like TN1 which recorded low SPAD reading but still highly susceptible. The most important trait i.e., damage score recorded significant and positive correlation with % damaged leaves at 25, 50 and 75 DAT (r=0.381**, 0.777** and 0.675** respectively).

3.3. Allelic variation

The total number of alleles detected in the study was 127 and all the alleles were polymorphic. The number of alleles detected at a single locus ranged from 2-3 with an average of 2.1 per locus. (Table 8) (Figure 1). A positive correlation was found between the number of alleles per locus and the maximum number of repeats with in a microsatellite marker. The PIC values of the microsatellite markers ranged from 0.620 (RM 9) to 0.061 (RM 18880) with an average PIC of 0.305 (Table 9).

3.4. Cluster analysis

UPGMA analysis according to NTSYS software has grouped the 30 rice genotypes in to two major clusters and one minor cluster. (Figure 2), (Table 10). Cluster I had only one genotype i.e., TN1, the susceptible check. Cluster II had eleven genotypes viz., MTU 1010, MTU 7029, MTU 2067, MTU 2077, BPT 5204, MTU 1075, MTU 1001, MTU 1061, MTU 1064, MTU 4870 and Gorsa. The Cluster III is the largest one which had 18 genotypes viz., TNAU (LFR) 831311, PTB 12, ADT 46, IR 36, GEB 24, CRMR 1523, Choorapundy, ARC 111289, IC 115737, RP 2068-18-3-5, SB 319, CO 43, LF 293, W 1264, W 1263, TKM 6, INRC 3021 and Aganni. The similarity coefficient ranges from 36 to 88%. The similarity coefficient between two major clusters was 50%. Interestingly all the released varieties from ANGRAU were placed in second cluster as most of them are related to each other. The genotypes MTU 2067 and MTU 2077 which are sister cultures from same parents (Sowbhagya and ARC 6650) showed 80% similarity. Similarly, the varieties MTU 1061 and MTU 1064 which are sister cultures of the cross PLA 1100/MTU 1010 showed 70% similarity.

UPGMA analysis according to Darwin software has grouped the 30 rice genotypes in to four major clusters (Figure 3),

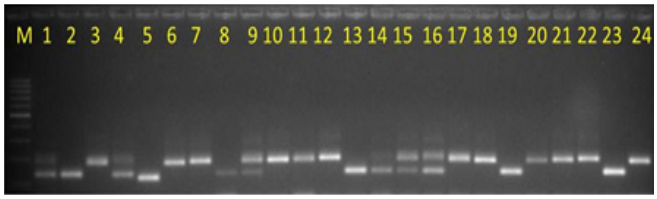
Table 8: Allele variation for Microsatellite loci (SSR) studied in 30 rice genotypes

Sl. No.	SSR locus	Chromo-some	No. of alleles	Amplicon size (bp)
1.	RM 9	1	3	180,200,210
2.	RM 14	1	2	400,850
3.	RM 212	1	2	120, 150
4.	RM 220	1	2	100,110
5.	RM 243	1	2	100,110
6.	RM 472	1	2	300, 350
7.	RM 493	1	2	210,250
8.	RM 495	1	2	170,180
9.	RM 543	1	2	800,900
10.	RM3412	1	2	200,240
11.	RM10793	1	2	200,140
12.	RM207	2	2	300,500
13.	RM208	2	2	170,190
14.	RM3340	2	3	100,130,140
15.	RM3865	2	2	200,220
16.	RM5101	2	2	130,160
17.	RM251	3	3	130,150,180
18.	RM1319	3	2	130,150
19.	RM3766	3	2	130,180
20.	RM4108	3	2	200,210
21.	RM559	4	2	160,170
22.	RM5611	4	2	180,200
23.	RM3524	4	2	100,150
24.	RM3742	4	2	150,160
25.	RM289	5	3	100,140,190
26.	RM334	5	2	190,200
27.	RM1182	5	2	220,250
28.	RM5454	5	2	190,210

Continue...

Sl. No.	SSR locus	Chromo-some	No. of alleles	Amplicon size (bp)
29.	RM400	6	2	230,260
30.	RM5957	6	2	180,190
31.	RM7372	6	2	150,180
32.	RM8226	6	2	250,280
33.	RM11	7	2	120,150
34.	RM248	7	2	100,150
35.	RM429	7	2	160,180
36.	RM5100	7	2	200,220
37.	RM5711	7	2	160,180
38.	RM210	8	3	120,140,160
39.	RM331	8	2	200,210
40.	RM1309	8	2	200,210
41.	RM8266	8	3	200,290,800
42.	RM23048	8	2	560,600
43.	RM219	9	2	200,240
44.	RM242	9	2	200,240
45.	RM3909	9	2	190,200
46.	RM3912	9	2	150,190
47.	RM 21	11	2	150,190
48.	RM216	10	2	140,160
49.	RM228	10	2	120,150
50.	RM496	10	2	280,300
51.	RM6100	10	2	160,180
52.	RM206	11	2	150,200
53.	RM224	11	2	130,150
54.	RM286	11	2	100,130
55.	RM1124	11	2	160,180
56.	RM1880	12	2	320,410
57.	RM2854	12	2	350,400
58.	RM3331	12	2	200,290
59.	RM5939	12	3	180,190
60.	RM6867	12	2	200,210

bp: base pairs



M-100 bp ladder

Figure 1: Molecular profile of rice genotypes with RM 10793 on chromosome 1

Table 9: PIC values of the individual SSR markers used in the study

Sl. No	Marker	Chromosome	PIC value
1.	RM 9	1	0.620
2.	RM 14	1	0.420
3.	RM 212	1	0.480
4.	RM 220	1	0.358
5.	RM 243	1	0.124
6.	RM 472	1	0.124
7.	RM 493	1	0.064
8.	RM 495	1	0.491
9.	RM 543	1	0.124
10.	RM3412	1	0.180
11.	RM10793	1	0.444
12.	RM207	2	0.064
13.	RM208	2	0.231
14.	RM3340	2	0.464
15.	RM3865	2	0.480
16.	RM5101	2	0.358
17.	RM251	3	0.540
18.	RM1319	3	0.358
19.	RM3766	3	0.491
20.	RM4108	3	0.462
21.	RM559	4	0.064
22.	RM5611	4	0.064
23.	RM3524	4	0.444
24.	RM3742	4	0.278
25.	RM289	5	0.184
26.	RM334	5	0.358
27.	RM1182	5	0.124
28.	RM5454	5	0.320
29.	RM400	6	0.464
30.	RM5957	6	0.500
31.	RM7372	6	0.064
32.	RM8226	6	0.358
33.	RM11	7	0.124
34.	RM248	7	0.391
35.	RM429	7	0.064
36.	RM5100	7	0.231
37.	RM5711	7	0.480
38.	RM210	8	0.123
39.	RM331	8	0.064

Continue...

Sl. No	Marker	Chromosome	PIC value
40.	RM1309	8	0.480
41.	RM8266	8	0.227
42.	RM23048	8	0.124
43.	RM219	9	0.491
44.	RM242	9	0.064
45.	RM3909	9	0.231
46.	RM3912	9	0.124
47.	RM 21	11	0.358
48.	RM216	10	0.491
49.	RM228	10	0.444
50.	RM496	10	0.124
51.	RM6100	10	0.500
52.	RM206	11	0.491
53.	RM224	11	0.391
54.	RM286	11	0.491
55.	RM1124	11	0.480
56.	RM1880	12	0.061
57.	RM2854	12	0.498
58.	RM3331	12	0.124
59.	RM5939	12	0.438
60.	RM6867	12	0.124

Table 10: Clustering pattern among 30 rice genotypes (NTSYS software)

Clusters	No. of genotypes	Details of the genotypes
I	1	TN-1.
II	11	MTU 1010, MTU 7029, MTU 2067, MTU 2077, BPT 5204, MTU 1075, MTU 1001, MTU 1061, MTU 1064, MTU 4870 and Gorsa.
III	18	TNAU(LFR)831311, PTB 12, ADT 46, IR 36, GEB 24, CRMR 1523, Choorapundy, ARC111289, IC 115737, RP 2068-18-3-5, SB 319, CO 43, LF 293, W 1264, W 1263, TKM 6, INRC 3021 and Aganni.

(Table 11). Cluster I had 11 genotypes which includes all the major varieties of ANGRAU viz., MTU 1001, MTU 1061, MTU 1064, Gorsa, MTU 4870, MTU 2067, MTU 2077, MTU 7029, BPT 5204, MTU 1075 and MTU 1010. Cluster II had only two genotypes (GEB 24 and CRMR 1523). Cluster III had seven genotypes (TNAU(LFR)831311, PTB 12, TN 1, ADT 46, IR 36, IC 115737 and RP 2068-18-3-5) and cluster IV had ten

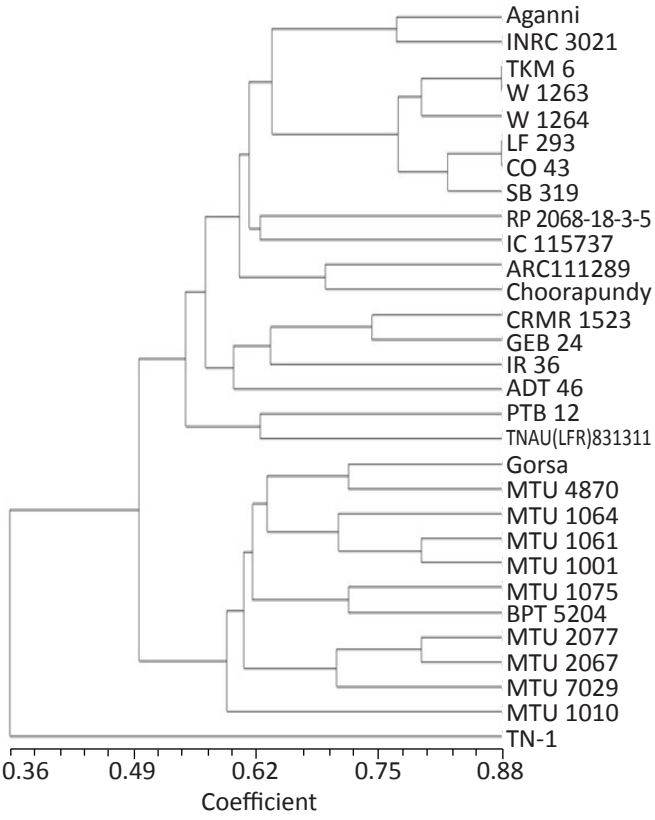


Figure 2: Dendrogram of 30 rice genotypes using 60 Microsatellite markers using NTSYS software

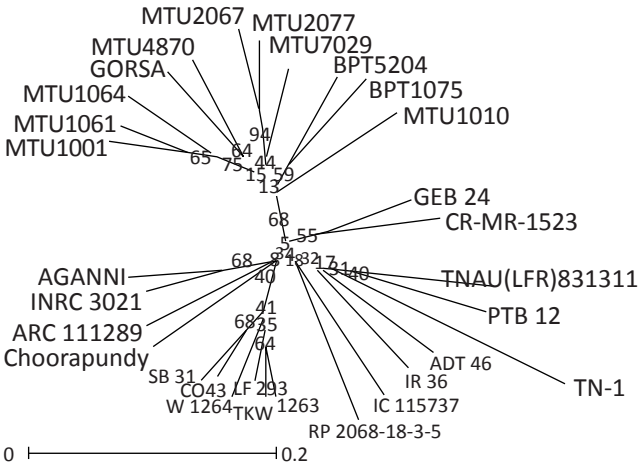


Figure 3: Dendrogram of 30 rice genotypes using 60 Microsatellite markers using DARWIN software

genotypes viz., Aganni, INRC 3021, ARC 111289, Choorpundy, SB 319, CO 43, W 1264, LF 293, TKM 6 and W 1263. When Darwin was used the sister cultures MTU 2067 and MTU 2077 had 94 % similarity while MTU 1061 and MTU 1064 had 75% similarity. The grouping pattern of the genotypes studied into different clusters is almost similar using both the softwares. The observed level of genetic diversity from the SSRs and its distribution pattern were generally consistent with those of most previous studies based on much larger samples (Li and

Table 11: Clustering pattern among 30 rice genotypes (Darwin software)

Clusters	No. of genotypes	Details of the genotypes
I	11	MTU 1001, MTU 1061, MTU 1064, Gorsa, MTU 4870, MTU 2067, MTU 2077, MTU 7029, BPT 5204, MTU 1075 and MTU 1010.
II	2	GEB 34 and CR MR 1523.
III	7	TNAU(LFR)831311, PTB 12, TN 1, ADT 46, IR 36, IC 115737 and RP 2068-18-3-5.
IV	10	Aganni, INRC 3021, ARC 111289, Choorpundy, SB 319, CO 43, W 1264, LF 293, TKM 6 and W 1263.

Rutger, 2000). Hence, it can be concluded that molecular characterization and genetic diversity studies for leaf folder resistance and its contributing traits can be utilized in breeding programmes to develop rice varieties with strong resistance to leaf folder coupled with good grain quality and higher yield for typical tropical irrigated ecosystem.

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