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Research Article

TAGGING OF SEEDLING COLD TOLERANCE IN RICE (*ORYZA SATIVA* L.) WITH MOLECULAR MARKERS

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ABSTRACT: Rice (Oryza sativa L.) is one of the most important food crops and a primary source of food for more than half the world's population. Rice seedlings are particularly sensitive to chilling in early spring in temperate and subtropical zones and in high elevation areas. Cold stress during rabi season at early stage of crop season cause various seedling injuries, delayed heading and yield reduction. Improvement of low temperature (cold) tolerance significantly increases rice production. In the present study 36 diverse and adopted rice genotypes were used to identify seedling cold tolerant varieties and these were genotyped with 32 Rice Microsatellite (RM) markers to identify molecular markers linked to seedling cold tolerance. All 36 genotypes subjected to cold temperatures at seedling stage both in field and at lab. Morpho-physiological traits such as leaf yellowing and SPAD chlorophyll meter readings (SCMR) identified as selection criteria for cold tolerance. SCMR were recorded before and after cold treatment which measures relative chlorophyll content of leaf. Percent decrease in SCMR was considered to identify susceptible and tolerant genotypes. Five genotypes viz., CT376, JGL18270, Chakiya Mao, JGL11470 and JGL1798 showed highest leaf yellowing score and percent decrease in SCMR after cold treatment, hence these are considered as cold susceptible genotypes. Nine genotypes (JGL17004, JGL20171, JGL19607, Tellahamsa, WGL283, BPT5204, IR83222-85, IR83222-174 and CT37) found to be tolerant to cold as they recorded lowest leaf yellowing score and percent decrease in SCMR after cold treatment. Among 32 markers one marker (RM85) showed specific banding pattern in cold susceptible and tolerant genotypes. Cosegregation of this marker will be confirmed using immortal mapping population (RILs: Recombinant Inbred Lines), which is being developed. Cold tolerant linked markers will be more useful in marker assisted pyramiding of biotic and abiotic resistance genes in rice.
Key words: Rice, Seedling, Tagging, Molecular Markers

INTRODUCTION

Low temperature stress is common for rice (*Oryza sativa* L.) cultivation in Northern Telangana Zone. During *rabi* season quite often the temperature drops below 10^{o}c resulting in poor growth of seedlings. At different growth stages low temperature shows different effects such as germination, seedling, vegetative, reproductive and grain maturity [1, 2,3], weakens photosynthetic ability by inducing leaf discoloration, reduces plant height, produces degenerated spikes, delays days to heading, reduces spikelet fertility and poor grain yield. One important strategy for increasing crop productivity is to minimize losses due to abiotic and biotic stresses by developing more stress tolerance varieties [45]. Efforts to develop rice cultivars with improved cold tolerance typically focus on seedling and reproductive stages which have great impact on yield. Since natural incidence of low temperatures is erratic and environmental trait, screening the rice genotypes against cold stress obviously be difficult and such results needs further confirmation. Artificial screening of genotypes in growth chambers enables confirmed results. However, the recently available molecular biology tools of marker assisted selection provide the best method for identification of cold tolerant genotypes irrespective of season and occurrence of low temperature stress.

Genetic analysis of seedling cold tolerance has resulted in the identification of number of genes and QTL that appear to be involved in different responses to stress. Kwak *et. al.* [6] identified a single dominant gene controlling leaf yellowing *Cts1* (t), while Nagamine [7] reported a major gene for leaf withering *Cts2* (t). Many studies suggest that more genes involved in cold tolerance with varying levels of phenotypic effects. QTL analysis gives an idea that cold tolerance is a complex trait involving multiple genes. Misawa *et. al*. [8] found that as many as 13 QTL, located on chromosome 1, 3, 9, 11 which are associated with low temperature response when seedlings at the 2-leaf stage were subjected to 3 days at 4ºC. Zhang *et. al*. [8] identified four putative QTLs for seedling cold tolerance located on chromosome 1, 3, 7, 11 and one QTL on chromosome 11, designated as *qSCT-11* with 30% of the phenotypic variance. Simple sequence repeat (SSR) markers [10] are useful to overcome limitations like cold tolerance. The appropriate screening procedure in evaluating low temperature sensitivity at the seedling stage in rice is an important issue in measuring the trait. This study was also used to locate genetic loci or QTLs associated with rice cold tolerance at the seedling stage measured in a controlled environment facility. DNA markers that co-segregate with the gene as a powerful method to develop a resistant cultivar and also used to conventional phenotypic screening. They are used to estimate overall genetic variability, phenotypically related to a particular trait during multiple rounds of introgression. They are readily accessible through published linkage maps and public databases and they permit differentiation between homozygous and heterozygous individuals and well suited for genotyping. The objective of this study was to identify and analyze seedling cold tolerance in selected genotypes with respect to leaf yellowing score and SPAD chlorophyll meter readings (SCMR) and to identify markers linked to cold tolerance.

MATERIALS AND METHODS

Plant materials

The present investigation was conducted at Rice Research Scheme, Regional Agricultural Research Station, Polasa, Jagtial, Karimnagar, Telangana, India. The experimental material comprised of thirty six genotypes which include JGL1798, JGL 3844, JGL3855, JGL11470, JGL11118, JGL17004, JGL18047, JGL19621, JGL20171, JGL17653, JGL18222, JGL18629, JGL18799, JGL19607, KNM110, BPT5204, Tellahamsa, IR64, MTU1010, WGL283, Varalu, HRP2177, Himalaya2, Himalaya741, Chakiya Mao, Changat, CT19, CT37, CT376, China988, IR83222-85, IR83222-114, JGL17970, JGL18270, JGL23710 and JGL23713. For field screening of material for seedling cold tolerance, the material was sown at 15 days interval starting from first November 2011 to fifteenth January 2012. In each set, presoaked and sprouted seeds of each genotype were sown in seedling nursery at 30 cm gap between each entry. The seedlings were regularly observed for yellowing of leaves in comparison with incidence of low temperature. Minolta SPAD chlorophyll meter was used to record the leaf yellowness as it measures the relative leaf chlorophyll content. The data on yellowing score was recorded as per the SES (IRRI, 2002).

SPAD chlorophyll meter readings (SCMR) were recorded for all the genotypes at two different temperature regimes of normal and cold stress and the genotypes with less than 10 percent decrease in SCMR were considered as highly tolerant, 10-20% as tolerant and more than 20% as susceptible.

Artificial screening

For evaluating the entries against cold stress at seedling stage, artificial screening was done. For this study 36 number of rice cultures were sown in trays with three replications during 2012 *kharif* season. The soil was uniformly taken for all trays from ploughed paddy field. Around thirty seedlings were grown per treatment and replication. The seedlings were allowed to grow under normal condition in the open field without any cold stress for two weeks period. The 15 day old seedlings were recorded with SCMR and the seedling trays were transferred to plant growth chamber where the temperatures were set at 15/8^{o}c (day/night). The seedlings were allowed to grow for a period of five days under artificial cold stress. After five days of cold treatment, again the SCMR observation was recorded for all the entries studied. Based on the SCMR values recorded under normal condition and cold stress, the percent decrease was calculated.

DNA extraction and Genotyping

The material was sown in different pots for genomic DNA extraction, leaf tissue from young plants was collected and used for genomic DNA extraction during *rabi* 2013-14. High molecular weight and good quality genomic DNA was isolated from fresh leaf of 15 days old seedlings of each genotype following Cetyl trimethyl ammonium bromide (CTAB) method. The integrity of DNA was judged through gel analysis by casting 0.8% gel in 1X TBE (Tris Borate EDTA) buffer containing 3µl Ethidium Bromide at 100 volts. DNA concentration was carried out by using Nanodrop ND1000 spectrophotometer. The quantification of DNA for each variety is listed in Table.1

Table 1: DNA concentration of different genotypes

S.No.	GENOTYPES	DNA concentration	S.No.	GENOTYPES	DNA concentration
1	JGL 1798	334.3ng/µl	19	HIMALAYA741	1083.8ng/µl
2	JGL 3844	150.4ng/µl	20	IR-64	167.1ng/µl
3	JGL 3855	349.9ng/µl	21	IR-83222-85	2085.6ng/µl
4	JGL 11118	1135.3ng/µl	22	IR-83222-114	1817.9ng/µl
5	JGL 11470	292.4ng/µl	23	CT-19	937.8ng/µl
6	JGL 17004	1140.6ng/µl	24	CT-376	1664.9ng/µl
7	JGL 17653	460ng/µl	25	KNM-110	3431.9ng/µl
8	JGL 17970	3573ng/µl	26	WGL-283	2025ng/µl
9	JGL 18047	730.2ng/µl	27	CHINA-988	2871.1ng/µl
10	JGL 18222	268.9ng/µl	28	MTU-1010	3541.2ng/µl
11	JGL 18270	2543.7ng/µl	29	HRP-2177	1140ng/µl
12	JGL 18629	1015ng/µl	30	BPT-5204	1410.3ng/µl
13	JGL 18799	1043.6ng/µl	31	CHAKIYAMAO	3354ng/µl
14	JGL 19607	387.9ng/µl	32	CHANGAT	4165.8ng/µl
15	JGL 19621	1253.8ng/µl	33	TELLAHAMSA	126.2ng/µl
16	JGL 20171	552.1ng/µl	34	VARALU	90.5ng/µl
17	JGL 23712	1009.8ng/µl	35	JGL 23710	546.2ng/µl
18	HIMALAYA-2	93.7ng/µl	36	CT-37	455ng/µl

Genotyping

In order to identify marker linked or co segregating with seedling cold tolerance, the cultivars were first being screened with DNA markers to establish polymorphism between them. For this purpose 36 genotypes are screened with selected 32 SSR primers.

SSR primers (Eurofins) for polymerase chain reaction (PCR) were taken based on the information available in www.gramene.org, MC Couch [9]. PCR amplification was carried out with 20µl reaction mixture having 20ng DNA, 10X PCR buffer (Tris with 15mM $Mgcl_2$), 50mM dNTP mix (2.5mM each dNTP), 5pM of each forward and reverse primer, and 0.5 unit Taq DNA polymerase enzyme (Genei). Amplification was performed in a thermal cycler (Eppendorf, USA) and the PCR performed with the following thermal cycle profile: initial denaturation at 94ºC for 5 min, cyclic denaturation at 94ºC for 2 minutes, primer annealing at 50-54ºC (vary from marker to marker) for 1 min and primer extension at 72ºC for 20min. The cycle was repeated 40 times and ended with the final extension step at 72ºC for 10 min. The amplified PCR products were resolved in gel electrophoresis on 3.0% Seakem®LE agarose gel (Lonza, USA) along with 50bp molecules and documented using gel documentation system (Alpha Innotech, USA). The genotypic dataset was generated based on the PCR amplification profile by scoring presence and absence of specific allele with specific base pair (bp) size for all the samples. The set of primers used to screen 36 genotypes are listed in table 2. The total number of alleles were observed in each genotype with specific primer are listed in table 4.

RESULTS AND DISCUSSION

Phenotypic data

The cold tolerance of rice seedling was difficult to measure in a single test because of complexity of injuries and symptoms caused by low temperature stress. Therefore the genotypes were subjected to phenotyping at the seedling stage following artificial and field screening methods.

In natural screening, the material sown on 15th December was fortunately exposed to two different temperature regimes and SCMR between these two periods enabled to calculate percent decrease in SCMR. A set of 36 genotypes were also evaluated for cold tolerance in a controlled environment with 15/8ºC (day/night) temperatures. The scale used to score cold tolerance was based on the leaf yellowing and SPAD chlorophyll meter readings (SCMR). SCMR were recorded before and after cold treatment which measures relative chlorophyll content of leaf. Leaf yellowing scores were classified as either tolerant (1-3) or susceptible (4-7). Percent decrease in SCMR was considered to identify susceptible and tolerant genotypes.

The percent decrease in SCMR in field and artificial conditions along with yellowing score was presented in Table 3. The data clearly showed that wide variation among the entries in field and artificial screening with respect to percent decrease in SCMR. In field screening, the percent decrease in SCMR ranged from 2.9 to 42.8 whereas in artificial screening it was from 1.1 to 50.9. The yellowing score ranged from 1 to 7 which clearly indicate the existence of variation for seedling cold tolerance among the genotypes studied.

Table 2. List of primers used for genotyping

S. No.	Primer Name	Forward Primer	Reverse primer	Expected product size (bp)
1	RM444	GCTCCACCTGCTTAAGCATC	TGAAGACCATGTTCTGCAGG	162
2	RM527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG	233
3	RM5526	TCAGCCTGGCCTCTCTTATC	ATGATCCTCCACCCACTAGC	171
4	RM6208	TCGAGCAGTACGTGGATCTG	CACACGTACATCTGCAAGGG	143
5	RM5926	ATATACTGTAGGTCCATCCA	AGATAGTATAGCGTAGCAGC	176
6	RM23901	TCAGCTATTGAGACAACGCAAACACC	TCGTAGGCAGGTGGCTATGACG	446
7	RM17503	CCAGATCATCCAGGCATAACATCACC	CGGCGCTGGTAAACTCCATTCC	100
8	RM22709	CGCGTGGGCGAGACTAATCG	CCTTGACTCCGAGGATTCATTGTCC	196
9	RM22685	ATGGGCTTCCAGGCTCAATCTCG	CCCACTCTCACGTCTCCTCTCTTCC	251
10	RM17508	CGGAATCACCAATTTCTCTCTCAGC	CGCAAGAAACGGAAACGAAACC	282
11	RM28706	GGTTCCCGGTCATCATATTTCC	ACTTTACCCACGCGCTTTGC	343
12	RM6094	TGCTTGATCTGTGTTCGTCC	TAGCAGCACCAGCATGAAAG	182
13	RM7654-H	CTCATGGTTGTGTCGTGGTC	GTGCAGTGCCAGTGGTACG	173
14	RM23956	GTCTCTCCCTCTCTCATCTTGTCG	CCCTATTCATGTGCAATGGAACC	623
15	RM23914	GAGGATCCTTACCATCAAACTTCG	CCAAGAACCTGCATTCTTCAAGG	196
16	RM28574	TAGTTTGGTGAAGTGGCATTGG	ATAGTAGGGCAAGGATTCAGAAGAGG	494
17	RM22551	CTTCGATCTCCTCGTCCTCTTCC	GAGCATGAGATGATGCATGACG	138
18	RM6759	CCCGAGTCTTCATAGAGATATTC	ATCCCTAGCTAGCCTTCCTTCC	324
19	RM1233	ATGGGCACGTGTAATTCATTCG	ATCCTCCGAAAGTAGGAGTAGGAAA	175
20	RM3472	CACACACTCTCTCAATCTCAACACC	AGAAGCGAGAGGAGGGAGATAGC	215
21	RM144	CATGTTGTGCTTGTCCTACTGC	AGCTAGAGGAGATCAGATGGTAGTGC	237
22	RM85	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC	107
23	RM335	GTACACACCCACATCGAGAAGC	TCCATGGATATACGAGGAGATGC	104
24	RM214	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA	112
25	RM244	CCGACTGTTCGTCCTTATCA	CTGCTCTCGGGTGAACGT	163
26	RM591	CTCATAGGTGGGTTAGTTTCTTGG	GCTGGTTTACAACTTGCTACTCTACC	258
27	RM261	CTACTTCTCCCCTTGTGTCG	TGTACCATCGCCAAATCTCC	125
28	RM307	GTACTACCGACCTACCGTTCAC	CCTGCTCTGCATGAACTGCTC	174
29	RM173	CCTACCTCGCGATCCCCCCCTC	CCATGAGGAGGAGGCGGCGATC	186
30	RM50	ACTGTACCGGTCGAAGACG	AAATTCCACGTCAGCCTCC	201
31	RM105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	134
32	RM6340	GCATGATGCAACGGAGCTCG	CTTCCTCATCTCCCTCACCTTCC	149

The field screening data revealed that the genotypes *viz.*, CT 37, IR 83222-85, BPT 5204, Tellahamsa, JGL 17653, JGL 17004, WGL 283, IR 83222-174, JGL 20171 and JGL 19607 were recorded with less (<10) decrease in SCMR whereas, the genotypes, Chakiyamao, CT 376, JGL 18270, JGL 11470, JGL 1798, JGL 18222 had highest percent decrease in SCMR. The filed screening data was in accordance with the artificial screening results. The yellowing score was lowest for the genotypes JGL 19607, BPT 5204, CT 37, IR 8322-85, JGL 23710, JGL 23713, whereas, high for CT 376, JGL 18270, Chakiyamao, JGL 11470. The data of percent decrease in SCMR and yellowing score revealed that the genotypes, CT 37, IR 83222-85, BPT 5204, Tellahamsa, JGL 17653, JGL 17004, WGL 283, IR 83222-174, JGL 20171 and JGL 19607 were identified as highly tolerant to cold stress whereas the genotypes Chakiyamao, CT 376, JGL 18270, JGL 11470, JGL 1798, JGL 18222 as highly susceptible (Fig.1). Yellowing of rice genotypes due to cold was also observed by Peterson [11].

Table 3. SCMR and yellowing scores under artificial and field screening conditions.

Sl. No.	Genotypes	Artificial Screening			Field Screening			YS
		SCMR_ Normal	SCMR_ Cold stress	% Decrease	SCMR_ Normal	SCMR_ Cold stress	% Decrease	
1	**JGL1798**	23.4	16.8	28.1	25.6	17.5	31.6	4
2	**JGL3844**	22.9	21.8	4.7	23.1	19.0	17.7	2
3	**JGL3855**	20.9	15.1	27.6	23.5	19.2	18.3	3
4	**JGL11470**	21.6	15.1	30.2	22.5	15.1	32.9	5
5	**JG 11118**	23.4	21.5	8.1	23.2	19.8	14.7	2
6	**JGL17004**	22.3	21.4	4.0	25.4	23.1	9.1	2
7	**JGL18047**	23.6	21.2	10.2	24.9	21.9	12.0	2
8	**JGL19621**	21.4	17.1	20.1	24.3	19.4	20.2	2
9	**JGL20171**	25.1	23.2	7.6	22.2	20.1	9.5	2
10	**JGL17653**	23.5	20.1	14.5	24.2	22.1	8.7	2
11	**JGL18222**	22.4	17.1	23.7	23.2	16.1	30.6	3
12	**JGL18629**	23.9	19.2	19.7	21.0	15.4	26.7	3
13	**JGL18799**	20.5	17.2	16.1	21.5	18.4	14.4	3
14	**JGL19607**	22.6	20.4	9.7	24.5	22.1	9.8	1
15	**KNM110**	23.5	17.7	24.7	24.2	18.6	23.1	3
16	**BPT5204**	22.1	19.9	9.8	22.0	20.6	6.4	1
17	**Tellahamsa**	27.4	24.8	9.5	21.6	19.8	8.3	2
18	**IR64**	24.7	21.3	13.6	25.8	22.6	12.4	3
19	**MTU1010**	23.2	18.9	18.5	23.2	19.2	17.2	3
20	**WGL283**	23.1	20.8	9.7	20.8	18.9	9.1	2
21	**Varalu**	25.4	22.6	11.0	22.1	19.1	13.6	2
22	**HRP2177**	20.9	14.5	30.9	28.8	23.9	17.0	3
23	**Himalaya2**	21.0	14.6	30.5	20.6	16.9	18.0	3
24	**Himalaya741**	22.0	15.8	28.1	29.2	23.9	18.2	2
25	**Chakiya Mao**	21.5	11.3	47.4	15.2	8.7	42.8	5
26	**Changat**	22.6	20.1	11.1	21.4	17.7	17.3	2
27	**CT19**	24.2	19.3	20.2	23.7	17.2	27.4	4
28	**CT37**	25.2	23.2	7.9	20.6	20.0	2.9	1
29	**CT376**	22.6	11.1	50.9	24.8	15.4	37.9	7
30	**China988**	20.4	17.2	15.7	20.5	16.9	17.6	2
31	**IR83222-85**	25.8	23.2	9.9	27.6	26.7	3.3	1
32	**IR83222-174**	23.4	23.1	1.1	24.1	21.8	9.5	2
33	**JGL17970**	20.1	11.6	42.3	23.2	17.6	24.1	3
34	**JGL18270**	18.7	12.9	30.7	20.5	13.6	33.7	6
35	**JGL23710**	25.9	22.4	13.5	26.2	23.1	11.8	1
36	**JGL23713**	24.7	20.6	16.6	25.7	21.4	16.7	1
	Minimum	**18.7**	**11.1**	**1.1**	**15.2**	**8.7**	**2.9**	**1**
	Maximum	**27.4**	**24.8**	**50.9**	**29.2**	**26.7**	**42.8**	**7**
	Range	**18.7-27.4**	**11.1 – 24.8**	**1.1 – 50.9**	**15.2 – 29.2**	**8.7 – 26.7**	**2.9 – 42.8**	**1-7**

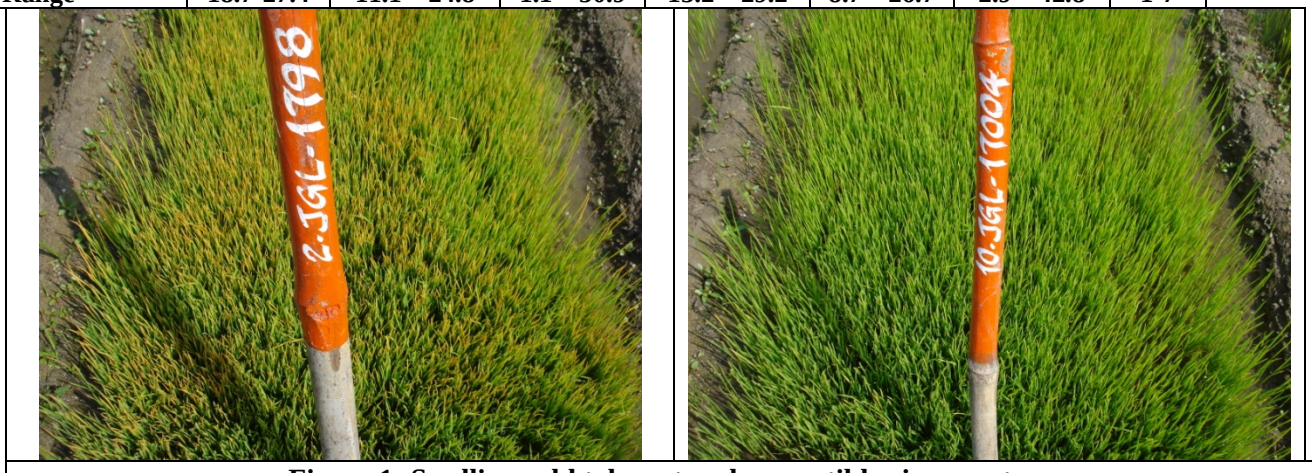

Figure 1: Seedling cold tolerant and susceptible rice genotypes

Genotyping

A total of 32 SSR markers were used for genotyping 36 rice cultures which were phenotyped for seedling cold tolerance. Number of alleles ranged from one to three in tested genotypes for different markers (Table 5). Among the SSR markers used RM85 showed a specific banding pattern in susceptible and tolerant cultures (Fig.2). This marker can be considered as putatively linked to cold tolerance in rice. Further studies are under way to know the exact linkage between the marker and the gene through development of recombinant inbred lines and phenotyping.

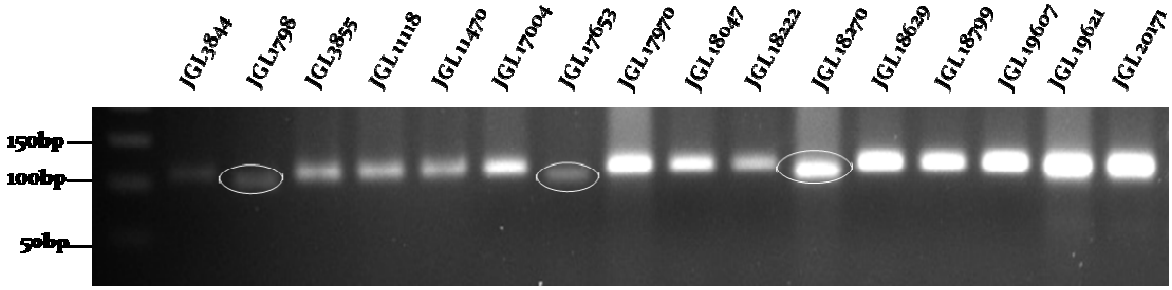

Figure-2. Amplification pattern of RM85 in different rice cultures

Table 5. Number of alleles in tested rice cultures for different SSR markers.

Sl. No.	Genotypes	Number of alleles																														
		RM444	RM527	RM5526	RM23901	RM17503	RM22709	RM22685	RM17508	RM28706	RM6094	RM23956	RM23914	RM28574	RM22551	RM7654-H	RM5926	RM6208	RM6759	RM1233	RM3472	RM85	RM244	RM261	RM307	RM173	RM50	RM105	RM6340	RM335	RM144	RM591	RM214
1	JGL1798	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	0	1	2	2	1	1
2	JGL3844	1	1	0	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	0	1	1	1	1	0	2	1	1
3	JGL3855	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	2	2	1	1
4	JGL11470	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	JG 11118	1	1	0	1	1	1	1	2	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1
6	JGL17004	1	1	1	1	1	1	1	1	0	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1
7	JGL18047	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	0	0	1	2	2	1	1
8	JGL19621	1	1	1	1	1	1	1	1	0	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	0	1	1	2	2	1	1
9	JGL20171	1	1	1	1	1	1	1	2	1	1	1	1	0	1	2	1	1	1	1	1	1	1	1	1	1	0	1	1	1	2	1	1
10	JGL17653	1	1	2	1	1	1	1	2	1	1	1	2	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1
11	JGL18222	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	2	1	1	1	1	1	1	1	1	1	1	2	2	0	1
12	JGL18629	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1
13	JGL18799	0	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	0	1	1	1	2	1	1
14	JGL19607	0	1	1	0	1	1	1	1	1	1	1	1	0	1	3	1	1	0	1	1	1	1	2	1	1	1	1	1	1	2	1	2
15	KNM110	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	0	1	2	2	1	1
16	BPT5204	1	1	1	1	1	2	1	1	1	1	1	1	0	1	3	1	1	1	1	1	1		1		1	1	1		1	2	1	
17	Tellahamsa	0	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	0								1	2		
18	IR64	1	1	2	1	1	1	1	2	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	0	1	1	3	3	1	1
19	MTU1010	1	1	2	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1		1	1	1	1	1	1	2	2	1	2
20	WGL283	0	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	0	1	1	2	2	1	1
21	Varalu	0	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	1	1	0								1	2		
22	HRP2177	2	1	2	1	1	1	1	1	0	1	0	2	1	1	2	1	1	1	1	1	1		1		1	0	0		1	2	1	
23	Himalaya2	1	1	2	1	1	1	1	1	1	1	0	1	1	0	3	1	1	1	1	1	1	1	1	1	1	1	0	0	2	3	0	1
24	Himalaya741	1	1	1	1	1	1	2	1	1	1	0	1	1	0	3	1	1	1	1	1	1	1	1	1	1	1	0	1	2	3	1	1
25	Chakiya Mao	1	1	1	0	1	1	1	1	0	1	1	1	1	2	3	1	1	1	1	1	1		1						2	1	1	
26	Changat	1	1	2	1	1	1	1	1	1	1	1	1	1	2	3	1	1	1	1	1	0		1						0	2	1	
27	CT 19	1	1	2	1	1	1	1	2	1	1	1	1	1	2	2	1	1	2	2	1	1	1	1	1	1	0	0	1	1	2	1	1
28	CT 37	1	1				1	1	1	1	1	1	1				1		1	1	1	0		1						2	2		
29	CT 376	0	1				1	1	1	1	1	1	1				1		1	2	1	1	1	1	1	1	0	1	1	2	2	1	1
30	China988	0	1				1	1	1	1	1	1	1				1		1	1	1	1	1	1	1	1	1	1	1	2	2	1	1
31	IR83222-85	1	1				1	1	1	1	1	1	1				1		0	2	1	1		1	1	1	1	1	2	2	2	1	1
32	IR83222-174	1	1				1	1	1	1	1	1	1				1		1	1	1	1	1	1	1	1	1	1	1	2	2	1	1
33	JGL17970	1	1				1	1	1	1	1	1	1				1		1	1	1	1	1	1	1	1	1	1	1	2	2	1	1
34	JGL18270	1	1				1	1	1	1	1	1	1				1		1	1	1	1	1	1	1	1	0	1	1	2	2	1	1
35	JGL23710	1	2				1	1	1	1	1	1	1				1		1	1	3	0								2	1		
36	JGL23713	1	2				1	1	1	0	1		1				1																

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