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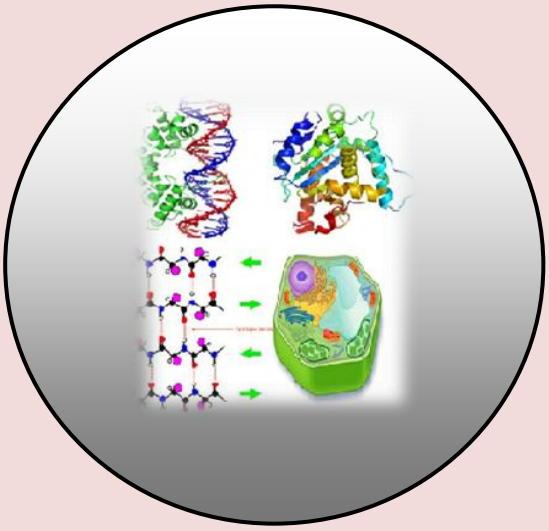

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ABSTRACT

This work was carried out to study the genetic diversity in parental lines of hybrid rice (O. sativa L). The genetic diversity between two parents has been proposed as a possible predictor of F1 performance and heterosis in rice. Genetic diversity is studied by using molecular markers. The role of genetic markers in genetic enhancement is considered in the evaluation of genetic diversity. The more polymorphic the trait the greater its potential value to germplasm management. Genetic diversity in 48 lines was studied. Genomic DNA isolation was carried out. SSR's are capable of detecting a high level of polymorphism among inbreed lines (Liu and Wu; 1998). Therefore identified SSR primers were selected to carry out this work. Polymorphism detection was conducted using 58 primer pairs on each chromosome. Allele diversity was observed among the selected lines. Dendrogram was observed for five different clusters. The average dissimilarity between first and second cluster was 60%, between second and third was 62.42%, between third and fourth was 59.8%, between fourth and fifth was 52.66%, between fifth and first was 50.66%. The data obtained in this study could be utilized in further studies for development of hybrids to exploit heterosis.

Key words: Oryza sativa L, SSR marker, Heterosis, Polymorphism, Genetic diversity and Hybrid.

INTRODUCTION

Hybrid rice is a proven and successful technology for rice production, having contributed significantly toward improving food security, raising rice productivity and farmer's income, and providing more employment opportunities over the past three decades. After China, India is a second country to commercially exploit this technology to benefit of the farming community. Hybrid rice is rice that been created by crossing two different parental strains. Such crosses generally result in an F1 generation that is more robust than either of the parental strains. The enhanced yield performance of F1 generation is referred to as "hybrid vigour'' or "heterosis". The hybrid vigour may result in superior agronomic qualities such as higher yield, stronger resistance to disease, more efficient use of a soil nutrients, and better weed control. Hybrid vigour and other superior qualities arising from crossing genetically different plants have been well known and used by traditional crop breeders for decades.

The level of genetic diversity between two parents has been proposed as a possible predictor of F1 performance and heterosis in rice. This propositions stems from studies by Lin Yuan (1980) Yuan and Cheng (1986). So in order to identify the gene of interest it is necessary to study genetic diversity in hybrid rice. Genetic diversity is studied by using molecular markers like RAPD and SSR which are PCR based markers. The role of genetic markers in genetic enhancement is considered in the evaluation of genetic diversity. Traits that serve as genetic markers are by definition polymorphic. The more polymorphic the trait, the greater its potential value to germplasm management.

The emphasis of this report would be on DNA based molecular techniques and how they can apply in assessing the genetic diversity of parental lines of hybrid rice (*O. sativa L*). For an optimum exploitation of heterosis, parents should be derived from genetically divergent pools, commonly referred to as heterotic groups. Characterisation of genetic variation within the breeding lines could be crucial for effective exploitation of genetic resources for crop improvement programs. The assessment of genetic diversity of a set of parental lines would help to identify the most diverse lines and such lines could be tested for development of hybrids to exploit heterosis. Microsatellites are often multi-allelic because of high mutation rate. Therefore SSR markers were used in this study for theassessment of genetic diversity in 48 parental lines of hybrid rice. The present study has been designed with the objectives, to PCR amplify a set of parental line of hybrid rice using the identified SSR markers and to assay the genetic diversity among these lines utilizing SSR marker polymorphism data.

MATERIALS AND METHODS

Collection of samples

48 parental lines of hybrid rice were collected from Directorate of Rice Research (DRR), Hyderabad for the present study (Table no. 1.1). All 48 rice varieties seeds were soaked in Petri plates in moistened blotting paper at room temperature for proper germination then the leaf samples were collected from 7-10 days rice seedlings after seed germination for genomic DNA extraction.

Preparation of stock solutions

1M Tris HCl (PH 8.0), 0.5M EDTA, 5M NaCl, Bromophenol Blue, 10X TBE.

DNA isolation

Genomic DNA from the leaf samples was isolated using the procedure of Zheng *et al.* (1995) with some modifications. The quantity of extracted genomic DNA from all 48 lines was estimated by agarose gel electrophoresis method.

SSR Markers

SSRs are capable of detecting a high level of polymorphism among inbreed lines. Therefore we have selected SSR primers for this work. For 1-9 and 11-12 chromosome 5 primers each and for 10^{th} chromosome 3 primers were used. In all 58 primers were used for 12 sets of chromosome (Table no. 1.4).

PCR and Electroporesis

Master mix for PCR (10µl): D.W. 3µl, Assay buffer 10X) 1µl, dNTPs0.5µl, Primer forward (20µm) 1µl, Primer reverse (20µm) 1µl, Taq polymerase (0.5U/µl) 0.5µl, DNA sample 3µl. The PCR tubes were then setup in programmable thermal cycler for DNA amplification. The temperature specifications for the denaturation of DNA strands, Annealing of primers and extension steps in SSR were as follows. (Table no. 1.2) PCR amplified products were resolved in 3%agarose gel in 0.5X TBE buffer at 100V for 3.5 hrs in Hoefer super submarine Electrophoresis unit containing 0.5 X TBE buffer solution. Prior to loading, 1/6th volume of gel loading dye (40% sucrose; 0.25% bromophenol blue) was added to the PCR amplified products and ensured proper mixing. The sizes of amplified fragments were determined by comparing with 100 bp ladder (MBI Fermentas). The gels were stained in Ethidium Bromide (10mg/ml) and placed over the UV-transilluminator and documented using ALPHA IMAGER gel documentation system (M/s Alpha innotech) for documentation (Figure 1.1).

Data Analysis

Qualitative multistage traits that depict an array of characters were converted into binary characters (Sneath and Sokal, 1973) based on the variations present. Only the clear and unambiguous bands of SSR markers were scored.

Markers were scored for the presence and absence of the corresponding band among the genotypes. The score 1 and 0 indicates the presence and absence of the bands respectively. A data matrix comprising of '1' and '0' were formed depending upon the character and this data matrix was subjected to further analysis using DARWIN 5.0 analysis software.

RESULTS AND DISCUSSION

The present study aimed to study genetic diversity of parental lines of hybrid rice was carried out using 58 different HRM primers. In rice 12 chromosomes are present for each chromosome HRM primers were tested. The radial type of dendrogram was obtained using DARWIN analysis software version 5.0 (Figure 1.2) also the mathematical values were calculated by factorial analysis (Table no. 1.3).The average dissimilarities between clusters were calculated. In 1st cluster 3 green super lines, 1restorer line, 1maintainer line (IR 79156B) was exists. The maintainer line showed 66% similarity with GSRT–103, whereas restorer line RPHR -1005 showed 63% similarity with GSRT – 103 the average similarity of first cluster was 51%.

In 2nd cluster has further subdivided into 3 sub cluster. Only 2 genotypes were exists in 2 (a) cluster that were green super line GSRT-107 and 1 check variety SABHAGI DHAN showed 51% similarity. The average similarity of second cluster was 76%whereas 2nd (b) cluster has 4 genotype.TJ-18, NWGR-3045, IR-58025B, TJ-52. 2 restorer lines and 1 green super line GSRT -106 showed 49% similarity. While check variety JAYA showed 55% similarity with GSRT-106. The average similarity of this cluster was 49%. In 2nd (c) cluster 2 tropical japonica lines, 1 maintainer line IR58025B existed showed 64% similarity with each other. The average similarity of this line IR58025B existed showed 64% similarity with each other. The average similarity of this cluster was 64%. In cluster 3 (a) 2 maintainer lines, 1 restorer line DR 714-2R were exists showed 49% similarity. The average similarity of cluster was 50%. Cluster 3 (b) contains 4 genotypes. 2 restorer line showed 48% similarity. The average similarity of this cluster was 62%. In cluster 3 (c) 3 genotypes were exists. 2 restorer lines and 1 green super line GSRT-104 showed 77% similarity with RPHR-1004. The average similarity of cluster was 67%. In cluster 3 (d) 5 genotypes were exists.2 maintainer line,1 green super line GSRT-102 showed 53% similarity with APMS 6B. Tropical japonica line TJ-11 and IBL 51 showed 39% similarity. TJ-11 had 61% similarity with maintainer line IR 68897B. Average similarity of this cluster was 63%.

Only tropical japonica lines were present in cluster 4. The average similarity between them was 57%. The average similarity of this cluster was 57%.

In cluster 5 (a) 5 genotypes were exists. 2 maintainer lines showed 64% similarity. 2 restorer lines were exists ICRD 16-4-2 and IR 40750R which showed 54% similarity. The average similarity of this cluster was 49%.

In 5 (b) cluster 8 genotypes were exists. 2 green super lines showed 68% similarity. 1tropical japonica line was exists which showed 41% similarity with DRR10B. The average similarity of this cluster was 52%.

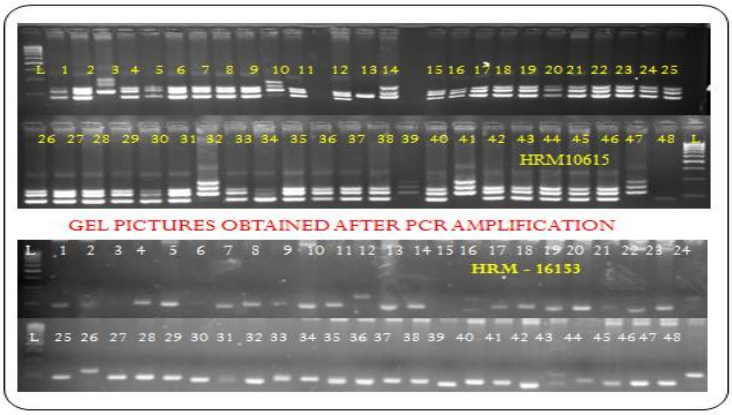

Figure 1.1. Gel pictures showing amplification of SSR primers.

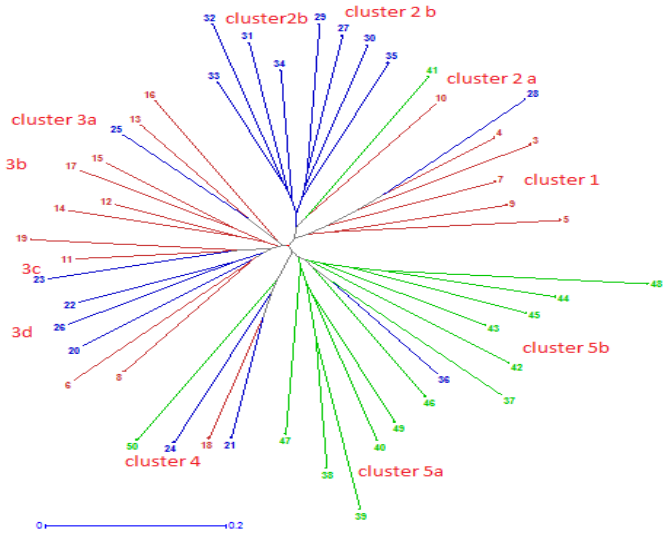

Figure: 1.2. Radial Dendrogram.

Table No. 1.1. List of Parental lines used.

Sr. no.	Parental Line	Sr. no.	Parental Line
Green super lines		26	TJ 33
1	GSRT-101	27	TJ 34
2	GSRT-102	28	TJ 36
3	GSRT-103	29	TJ 40
4	GSRT-104	30	TJ 52
5	GSRT-105	**Restorer lines**	
6	GSRT-106	31	RPHR 1005
7	GSRT-107	32	DR 714-1-2R
8	GSRT-108	33	BCW 56
9	GSRT-109	34	RPHR 1096
10	GSRT-110	35	IBL 57
Maintainer lines B		36	KMR 3
11	IR58025 B	37	GQ 70
12	IR79156 B	38	IR 40750R
13	PUSA5 B	39	RPHR 1004
14	IR68897 B	**Poor Restorer lines**	
15	IR68888 B	40	UPRI 2973
16	APMS 6 B	41	CN 1272
17	DRR 9 B	42	CB 06-137
18	DRR 10 B	43	RP 4092
19	IR80555 B	44	NWGR 30-45
20	IR80561 B	45	ICRD 16-1-4-2-1
Tropical japonica lines		**Checks**	
21	TJ-11	46	SABHAGI DHAN
22	TJ-12	47	IR 64
23	TJ-18	48	JAYA
24	TJ-20		
25	TJ-21		

Table No. 1.2. Dendrogram Analysis data.

Sn	Group	No. of Genotypes	No. designated in dendrogram	Name of line
1	1	6	5	GSRT-105 (Green super lines)
			9	GSRT-108
			3	GSRT-103
			7	IR 79156 B (Maintainer line)
			4	RPHR 1005 (Restorer line)
			28	TJ 21 (Tropical Japonica line)
2	2(a)	2	41	GSRT-107 (Green super line)
			10	SABHAGI DHAN (Check 1)
	2 (b)	4	27	JAYA (Check 1)
			29	IR 80561 B (Maintainer line)
			35	UPRI 2973 (Poor restorer line)
			30	GSRT-106 (Green super line)
	2 (c)	4	32	NWGR 3045(Poor restorer line)
			31	IR 58025 B (Maintainer line)
			34	TJ 52 (Tropical Japonica line)
			33	TJ 18 (Tropical Japonica line)
3	3 (a)	3	16	IR 68888 B (Maintainer line)
			25	DRR 9 B (Maintainer line)
			13	DR 714 2R (Restorer line)
	3 (b)	4	14	GSRT-109 (Green super line)
			17	RP 4O92 (Poor restorer line)
			12	CB-06-137 (Poor restorer line)
			15	IR 64 (Check 1)
	3 (c)	3	23	RPHR 1004 (Restorer line)
			19	RPHR 1096 (Restorer line)
			11	GSRT-104 (Green super line)
	3 (d)	5	20	IR 68897 B (Maintainer line)
			6	APMS 6B (Maintainer line)
			8	IBL 57 (Restorer line)
			26	TJ 11 (Tropical Japonica line)
			22	GSRT-102 (Green super line)
4	4	4	50	TJ 12 (Tropical Japonica line)
			24	TJ 34 (Tropical Japonica line)
			18	TJ 20 (Tropical Japonica line)
			21	TJ 36 (Tropical Japonica line)
5	5 (a)	5	47	PUSA 5B (Maintainer line)
			38	IR 80555B (Maintainer line)
			39	ICRD 16-1-4-2-1 (Poor restore)
			40	IR 40750R (Restorer line)
			49	TJ 33 (Tropical Japonica line)
	5 (b)	8	46	GSRT-110 (Green super line)
			36	GSRT-101 (Green super line)
			37	KMR 3 (Restorer line)
			42	BCW 56 (Restorer line)
			43	GQ 70 (Restorer line)
			45	CN 1272 (Poor Restorer line)
			44	DRR 10B (Maintainer line)
			48	TJ 40 (Tropical Japonica line)

S. No.	Primer name	Phy. loci	Sequence	Base
1	HRM10615_1F	9.793	CGCCCTAACAACTTAGGGAACAGC	25
	HRM10615_1R		ATTGGCTGAAAGATGAAGGGTTCTCC	26
2	HRM10066_1F	13.105	TCGCCATCTCAATCCAATCTAGG	23
	HRM10066_1R		GTCTGAAGCATAGGTTTGTCTGAAG	26
3	HRM10936_1F	15.7	ACGGTTTGGAAGTGTTCGTAGG	22
	HRM10936_1R		TGGTACTGCATAATCTCAGCATCG	24
4	HRM11114_1F	20.42	CATGGCCCTGTTGCTGTATGG	21
	HRM11114_1R		GCGGTTCAAGAACAACTCATGG	22
5	HRM11997_1F	38.68	TGTTGTACCAGATGCCCATGTACC	24
	HRM11997_1R		CCGCGTTCACATAACGATGC	20
6	HRM12349_2F	9.77	CCCGATTAGCGATTGATATGGAGTAGG	26
	HRM12349_2R		AGTGCACAGCCATGGAATTATGC	23
7	HRM13154_2F	15.32	GGTACTTAGCGTGCAATTTAACC	24
	HRM13154_2R		TAGGTAACTAGACGAAGCGATAGAGG	26
8	HRM13659_2F	25.75	GAACAGATTCTTGCCAATGTGC	22
	HRM13659_2R		AGCGAGAAAGAACAGGAAGTGC	22
9	HRM13867_2F	29.33	AATGCCTAGCACTCATCCTTGC	22
	HRM13867_2R		AGGCACCTACGATGAAATAGTGG	24
10	HRM12690_2F	60	CCTCCTGAAGGGTAAAGGATTGG	23
	HRM12690_2R		TCCACACATGATCGCTACATCG	22
11	HRM14250_3F	19.46	GATTACTGCCCGATTCGATAGC	21
	HRM14250_3R		AAATGGGACATGTTCTCTCG	20
12	HRM15337_3F	20.83	CTTTCGGGAGATGGTGTTTGC	21
	HRM15337_3R		CTCCTTCCATTCCCTTCCATAGG	23
13	HRM15626_3F	25.8	TGGGTTTCGGGATACAAATGC	21
	HRM15626_3R		CCCGTTTAGGTTGATGGTTACG	22
14	HRM15630_3F	25.88	AACTCGAAGGATCTCGCCCAACC	23
	HRM15630_3R		ACCCACCTCCTCACGCTGTACG	22
15	HRM16006_3F	32.01	CCCGAGTCTTCATAGAGATATTCC	24
	HRM16006_3R		ATCCCTAGCTAGCCTTCCTTCC	22
16	HRM16592_4F	11.36	CTTAGCACGGACACTCATATTTGG	24
	HRM16592_4R		CACAATACGTTTGATGGCTTGC	22
17	HRM16801_4F	18.34	CGTTCAAGGAGCTTGTGTTGATCC	24
	HRM16801_4R		GGACCGATTTAAGTGAACGTTGATGG	26
18	HRM17405_4F	29.8	GGTGTACGTATTAGCAGGTTTCG	23
	HRM17405_4R		CGAACTACCAACTCAAATCACC	22
19	HRM17600_4F	33.8	CCTCGAAATGAATTGCAGTCGAACG	25
	HRM17600_4R		GTCTTGTGCCTTGTGCCGATGG	22
20	HRM16652_4F	13.65	TGACATTAGTTGTGGCAGATCC	22
	HRM16652_4R		CCTAGAATCTCATCTGTCTTCTGG	24
21	HRM18939_5F	24.61	CCAATATACGGGTGAAATCC	20
	HRM18939_5R		AGCTAGCTACGTGTGTGACG	20
22	HRM17950_5F	34.9	GGAAATGTGCATAGGTAGTTCAGG	24
	HRM17950_5R		GAGTTGGGAACTGCTACAAACG	22

23	HRM18704_5F HRM18704_5R	20.39	GAGTGATGGCATTGCTTGAGAGC CGAGCCATCATACCTCCGTCTAGC	23 24
24	HRM18799_5F HRM18799_5R	21.97	CTCGCTCTTACAACTTTCAAGC CACTTACCTCCACTTCTCAACC	22 22
25	HRM18770_5F HRM18770_5R	21.43	TCATATACAAGCACGCACACACC GCTCCAATCAAACGACCATTCC	23 22
26	HRM20583_6F HRM20583_6R	27.56	GAAGCTACTCCAAGTTCAGTCATTGC TTAGTACGTATGCACATCCCATCC	26 24
27	HRM20096_6F HRM20096_6R	17.21	CGGTAAGCCATAAATAGATCCCAAGG TTTGAACAGCGACACGGTTTCC	26 22
28	HRM20672_6F HRM20672_6R	29.121	GAGATCGAAATGCTGAATGC GAGTTATTTGAGAGGACCTACCG	20 23
29	HRM20060_6F HRM20060_6R	16.06	CACACATGAGTGGTTAGGTAAGATGC CAGTGACAAGAGCGAAATGATCC	26 23
30	HRM19697_6F HRM19697_6R	7.554	AACAACCTGAGAACACCTCTTGG AACAACCTGAGAACACCTCTTGG	23 22
31	HRM20818_7F HRM20818_7R	4.59	AGATGCAGATAGATGCATGTCACG ACCGATCATCCACGATCCTACG	24 22
32	HRM20866_7F HRM20866_7R	1.18	TATTCCCGGGAGATCCAACAGC AAGATCCAGTCGATTTGGTTCAGG	22 24
33	HRM20948_7F HRM20948_7R	2.52	GCAAGCTGGAAGAACATCGTACC TGCTTATGGTTCTGGTCACTTCG	23 23
34	HRM21258_7F HRM21258_7R	7.21	TATCATTCCGGTCCAAAGTGTCG TCCGGTCCAAAGTCTCATTTGC	23 22
35	HRM22131_7F HRM22131_7R	28.61	GACTCGTCACTGACACTGATACG CTTGTTAGGAAGAGCATTCTGC	23 22
36	HRM22299_8F HRM22299_8R	1.2	ACGCTTCACATTGTAACACACAGG GATCGATTGATCGGTGCTTTCC	24 22
37	HRM22585_8F HRM22585_8R	6.088	CACCGATTATTGTCGTATGG AGTGAGGAAGGGAAGAATACG	20 21
38	HRM22732_8F HRM22732_8R	9.88	TCTTTGAAGGTCATTCCTGGAACC CGCCCTTAGCTGTGTTATTGTAATCG	24 26
39	HRM22892_8F HRM22892_8R	14.65	GAACATGTCTTGGGTGTGATACAGG TATGTTTAAACGGGCTCCAACC	25 22
40	HRM23578_8F HRM23578_8R	27.46	AGCGATTCAGAACGAATCAACG TGCCAAAGCTACACAAATCTGACC	22 24
41	HRM24017_9F HRM24017_9R	9.54	CCTGCTATTGTACCTGCTCTAATGC CGTCAGATTACAGTGTCGCATCC	25 23
42	HRM24217_9F HRM24217_9R	13.04	CAGAATCCAATAGGCTCCACACG GTCAACGGCCACTTCAAGCTACC	23 23
43	HRM24542_9F HRM24542_9R	18.11	ATCCACAAGAGCACCGATGAGG TGACCTGGTAGTGGTGAGTGTGC	22 23
44	HRM24654_9F HRM24654_9R	19.9	TTGCTAGGTTAGCATCCGGTACG TGGTCTTTGCGAATCTGAATCC	23 22

45	HRM24842_9F HRM24842_9R	22.54	CGTCATCTGAATTGTTGCTTACCC TATGCACAGCCGGGTACATAATCC	24
46	HRM25310_10F HRM25310_10R	12.88	GGCGCCATGATTTAATTTGC GATCACGACGTTGACTTCAATCC	20 23
47	HRM24954_10F HRM24954_10R	1.91	CGAATCTTGGAACACATCAACG GGGAGGAGTGCTGTGAGAGG	22 20
48	HRM25796_10F HRM25796_10R	20.655	GAAGCTTCCTCCTACGCTTTCC TCAAGACTCAAGAGCCACAGTGC	23 22
49	HRM26329_11F HRM26329_11R	7.47	TAACCGGGACTAAAGATAGAGC CTACGTCGAAATCGTAACTAGC	22 22
50	HRM26829_11F HRM26829_11R	18.58	GAGAAGGCCTGATGAGTACAAGG GATTATTGTGCAGGTGAGAAGTGG	23 24
51	HRM27034_11F HRM27034_11R	22.3	AGGCCCTCGCGTGTACATACC ATCCGACCCACGGTAATCTGAGG	21 23
52	HRM26213_11F HRM26213_11R	4.72	GCCACAGGAGACAGCAAGAACC CGATCCAATTCCAGCCTAGATAGC	22 24
53	HRM27310_11F HRM27310_11R	27.35	TTACCAACCGGGACTAAAGATCG CAATTCATAACGTCGGTCCTTCC	23 23
54	HRM27814_12F HRM27814_12R	7.46	CTGGAGTGGAGAAGAGAGAACAGG TCTCCGCTCGGTTTCATCTAGG	24 22
55	HRM28157_12F HRM28157_12R	17.43	GCTTAATTTCTGACAGACCAGTGC GATCTAAACACAGCCTTCCTTGG	24 23
56	HRM28616_12F HRM28616_12R	24.91	CACCGGAGTTCCCTCAACTTACC TACGTATGGCCAATTCAGACTGG	23 23
57	HRM27406_12F HRM27406_12R	0.22	TGGTAGGTGTGCAATAGAAGTAGG AATGCATGCAAACACAGTGG	24 20
58	HRM28424_12F HRM28424_12R	22.4	TCCACACACTTCGCCAATAAACC CCGCCACCACTCCTCTATCC	23 20

Table No. 1.4. PCR conditions kept.

Sr.No	Steps	Temperature	Time
1.	Initial denaturation	94^{O} C	5 min.
2.	Denaturation	94^{O} C	30 sec.
3.	Annealing	55^{O} C	30 sec.
4.	Extension	72^{O} C	1 min.
5.	Final extension	72^{O} C	7 min.
6.	Cooling	4^{O} C	α

CONCLUSION

Average dissimilarity between all clusters was observed as follows.
The average dissimilarity between 1st and 2nd cluster was 60%
The average dissimilarity between 2nd and 3rd cluster was 62.42%.
The average dissimilarity between 3rd and 4th cluster was 59.8%
The average dissimilarity between 4th and 5th cluster was 52.66%
The average dissimilarity between 5th and 1st cluster was 50.66%.

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