Oryza Vol. 55 No. 2, 2018 (278-284)
DOI 10.5958/2249-5266.2018.00034.6

Identification of suitable WA-CMS lines using morphological and molecular marker analysis in rice (*Oryza sativa* L.)

Chandan Roy[1]* and Indra Deo[2]

[1]Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

[2]GB Pant University of Agriculture and Technology, Pantnagar, US Nagar, Uttarakhand, India

**Corresponding author e-mail: chandan.roy43@gmail.com*

Received : 05 December 2017 Accepted : 29 May 2018 Published : 27 June 2018

ABSTRACT

Hybrid rice has tremendous potentiality to increase the on farm produce by maximizing the per-capita production. In India, yield potentiality of hybrid rice has not been achieved like China, suggesting the development of more heterotic hybrids adaptable for different agro-climatic region. Development of ideal parental lines is crucial to derive highly heterotic hybrids. Ten cytoplasmic male sterile (CMS) lines were developed into different genetic background of rice and evaluated after five back cross in randomized complete block design with three replications in the year 2010-11 and 2011-12 to identify suitable parental lines for hybrid development. All the lines except IR62871-325-3A showed complete pollen sterility (>85%), among them IR 62871-325-3-1A, UPRM 78-4-1A and UPRM 271-8-5EUI 3-3A were 100% pollen sterile. UPRM 78-4-1A showed 92.1% stigma exertion and early maturity. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis based on molecular marker data grouped UPRM 78-4-1 and IR 62871-325-3-1 into two separate clusters (similarity coefficient 0.54). Considering morphological characteristics and cluster distance UPRM 78-4-1A, UPPRM 271-8-5EUI-3-3A and IR 62871-325-3-1A were identified as most suitable male sterile lines for hybrid rice breeding. Present findings may be useful for the development of new hybrids using these lines as female parent.

Key words: *Hybrid, WA-cytoplasmic, molecular markers, male sterility, rice*

INTRODUCTION

Globally rice is the most important food crop with total production of 472 million tons in the year 2015-16. Almost 90 % of the total rice production is concentrated in the Asia. India is the second largest producer of rice after China with total production of 104 million tons in the year 2015-16 out of 45 Mha areas.

It is estimated that the population pressure is likely to be increased up to 138.89 crores by the end of 2025; to meet the requirement of growing population estimated 130 MT of rice will be required. Acute shortage of land, water and human resources are making the task most challenging to reach the target rice production. These alarming situations necessitate looking other alternative technologies to boost the rice production. Hybrid rice technology has great potentiality to meet the present day food demand. A substantial increase in yield (20-30%) is possible through selective improvement of major yield contributing traits (Siddiq, 1993). Unlike China, adoption of hybrid rice in India is limited by several factors; yield and higher price of hybrid seed are among the major constrain. To improve the yield potential in hybrid rice augmenting the essential plant features into parental line is crucial. Out crossing in WA-CMS line is poor resulting in low production of hybrid seed and parental A-line (Cheng, et al., 2007; Yang et al., 2006). Suitable parental line was effectively identified by evaluating cytoplasmic male sterile (CMS) lines floral traits (Behla et al., 2007; Hasan et al., 2011). Use of morphological traits for evaluation of parental lines is limited by environmental factors. Application of molecular markers along with morphological traits

increases the reliability and effectiveness of parental line selection for heterotic hybrids. Previously, several workers reported the use of molecular markers for genetic relatedness analysis in rice by Sattari et al. (2008); Seeba et al. (2009); Grishma et al. (2012). Molecular markers enhance the effectiveness of selection of traits and can be used for genotyping of the varieties (Grishma et al., 2012). Besides, molecular markers are easy and cost effective for varietal identification and genetic purity test (Nandkumar et al., 2004). In the present investigation WA-cytoplasm male were evaluated using morphological and molecular markers to identify suitable parental lines for hybrid breeding.

MATERIALS AND METHODS

Evaluation of male sterile lines

WA-cytoplasm was transferred from source material UPRI 95-17A into different genetic back ground. Ten newly developed male-sterile lines, *viz.,* UPRM 78-4-1A, UPRM 78-4-2A, UPRM 271-8-5EUI-3-2A, UPRM 271-8-5EUI-1-1A, UPRM 271-8-5EUI-3-3A, UPRM 271-8-5EUI-6-1A, UPRM 271-8-5EUI-6-3A, UPRM 271-8-5EUI-6-4A, IR 62871-325-3A and IR 62871-325-3-1A along with a check UPRI 95-17A, were evaluated in the year 2010-11 and 2011-12 with aim to identify suitable male sterile lines for hybrid breeding programme. The experiment was conducted at N. E. Borlaug Crop Research Center, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. The genotype was planted in 4 rows plot with 2 meter length following randomized complete block design with three replications. The spacing between row to row was 25 cm and plant to plant 15 cm was maintained. Observations were taken for 17 quantitative traits *viz.,* days to 50% flowering (DTF), flag leaf length (FLL), flag leaf width (FLW), number of tillers per plant (NT), pollen sterility percentage (PS), diameter of pollen (DPL), stigma length (Sgl), plant height (PH), panicle length (PL), number of panicles per plant (pn/pl), days to maturity (DTM), grain length (GL), grain width (GW), numbers of spikelets per panicle (Spk/pl), length of uppermost inter node (LUI), anther length (AL), and stigma exertion percentage (SgEx); five qualitative traits *viz.,* pollen morphology (PM), stigma color (Sgcol), tip color of lemma (TcolL), attitude of flag leaf (AFL) and panicle exertion (PnEx). Data analysis was carried out following Panse and Sukhatme (1961).

DNA isolation and molecular diversity analysis

DNA was isolated from leaf tissues of newly developed male sterile lines following CTAB procedure (Dolye and Dolye, 1990). DNA concentration was measured using Thermo Scientific GENESYS 10S UV-Vis spectrophotometer, USA. Polymeric Chain Reaction (PCR) was carried out using 23 µl PCR Master Mix and 2 µl genomic DNA. Master Mix was prepared using Tdw=16.6 µl, buffer = 2.5 µl, dNTPs = 0.5 µl, Taq Polymerase = 0.34 µl and primer = 1.5 µl each for forward and reverse primer. Thirteen different Simple Sequence Repeat (SSR) markers few of them were linked to fertility restorer gene *Rf4* (RM 6100; RM 171; RM 258) were taken for molecular marker analysis. PCR products were fractionated using horizontal gel electrophoresis assembly containing 3% agarose and gel was documented using Gel Doc system for diversity analysis. Data were scored as 1 (present) and 0 (absent) for all the alleles of each of the SSR locus. Polymorphism information content (PIC) was computed as

$$PIC = 1-\sum_{j=1}^{n} P_{ij}2$$

where, P_{ij} is the frequency of the j^{th} allele at the i^{th} locus summed across all alleles (n). Pair-wise similarity and cluster analysis was done through computer software (NTSYS 2.0). Similarity matrix analysis was performed using 'UPGMA' with Jaccard's coefficient of similarity.

RESULTS AND DISCUSSION

Evaluation of cytoplasmic male sterile lines for morphological traits

Pooled analysis of variance revealed that significant variations were present for all the seventeen morphological traits under study indicating the presence of genetic diversity among the tested lines (Table 1). Most of the traits except number of panicle per plant (Pn/Pl), grain length (GL), and grain width (GW) exhibited non-significant year x treatment interactions. Expression of complete pollen sterility over the year and location is primary criteria for screening of male sterile lines. Complete pollen sterility was recorded for

Table 1. Pooled ANOVA over two years of evaluation for seventeen morphological traits.

Source of Variation	df	flag leaf length (cm)	flag leaf width (cm)	number of tillers	diameter of pollen (mm)	Stigma length (cm)	Plant height (cm)	Panicle length (cm)	Number of panicle per plant
Replication	4	0.64	0.01	4.2	0.01	0.01	6.04	47.7	18.31
Treatments	10	70.45**	0.07**	17.31**	0.07**	0.10**	242.69**	18.02**	6.53**
Year	1	26.17**	0.02ns	2.36ns	0.003ns	0.11**	226.43**	67.97**	0.17ns
Year × Treatment	10	3.55ns	0.002ns	0.52ns	0.04ns	0.03ns	4.29ns	9.11ns	7.10**
Error	40	3.07	0.007	1.71	0.01	0.01	5.01	3.18	1.50
CV (%)		5.37	5.32	10.0	4.30	5.55	2.38	7.29	12.08
CD0.05 Treatment		2.033	0.09	1.51	0.151	0.12	2.59	2.07	1.42
SEm± (Treatment)		0.71	0.03	0.53	0.05	0.04	0.91	0.72	0.50

Table1. Contd…

Source of Variation	df	grain length (cm)	grain width (cm)	spikelets per panicle	length of upperrmost internode(cm)	anther length (cm)	pollen sterility (%)	days to flowering	days to maturity	Stigma exertion percentage(%)
Replication	4	0.001	0.00	88.48	47.33	0.01	2.71	6.46	2.32	2.64
Treatments	10	0.01**	0.0004**	904.42**	80.23**	0.18**	289.30**	49.87**	130.44**	271.43**
Year	1	0.0009ns	0.0001ns	210.17ns	-0.008ns	0.007ns	25.78**	5.46**	215.53**	24.17**
Year × Treatment	10	0.009**	0.0004**	93.94ns	4.16ns	0.04ns	2.47ns	4.47ns	6.77ns	1.76ns
Error	40	0.001	0.0001	144.10	8.54	0.04	2.35	2.03	2.59	3.13
CV (%)		4.92	4.63	5.28	8.93	10.05	1.64	1.39	1.10	1.76
CD0.05 Treatment		0.05	0.01	13.93	3.39	0.24	1.78	0.82	0.93	3.54
SEm Treatment		0.01	0.004	4.90	1.19	0.08	0.62	0.58	0.65	1.02

** indicates significance at 1% probability level and ns= Non Significant

all the test entries, except IR62871-325-3, which exhibited partial sterility (<85%). Hundred percent pollen sterility was observed in IR62871-325-3-1A in 2010-11 and UPRM 78-4-1A, UPRM 271-8-5EUI-3-3A and IR62871-325-3-1A in 2011-12. Morphologically, pollen shape was round for UPRM 78-4-1A, UPRM 78-4-2A, UPRM 271 -8-5EUI-1-1A, UPRM 271-8-5EUI-3-2A, UPRM 271-8-5EUI-3-3A, UPRM 271-8-5EUI-6-3A, IR62871-325-3A and UPRM 271 -8-5EUI-6-4A, whereas pollen shape was oval for UPRM 271 -8-5EUI-6-1, UPRM 271-8-5EUI-6-4 and UPRI-95-17A.

Exertion of stigma and panicle exertion percentage in male sterile lines is directly related to extend of out-crossing rate. UPRM 78-4-1A recorded highest stigma exertion percentage (Fig. 1); this may be due to presence of longest stigma among all the tested genotypes. Significant correlation (0.516**) between the stigma exertion percentage and stigma length was also observed (table for correlation analysis data not shown). Most of the lines, except UPRM 271-8-5EUI-3-3, IR62871-325-3-1 and UPRI-95-17A, showed full panicle exertion.

Three newly developed genotypes, *i.e.,* UPRM 78-4-1, UPRM 271-8-5EUI-3-3 and IR62871-325-3-1, were identified as the most suitable CMS lines for use in hybrid rice breeding programs. These genotypes produced higher pollen sterility percentage than the check genotype UPRI 95-17A and also possessed other desirable agronomic traits. Features of these three lines

Fig. 1. Stigma exertion of UPRM 78-4-1

Table 2. Comparison of three male sterile lines with their respective maintainer lines using morphological traits .

Sl. No.	Characters	UPRM 78-4-1A	UPRM 78-4-1B	UPRM 271-8-5EUI-3-3A	UPRM 271-8-5EUI-3-3B	IR62871 -325-3-1A	IR62871 -325-3-1B	SE (±)
1	Flag leaf length (FLL)	31.33	31.33	26.83	28.49	33.74	40.665	1.27
2	Flag leaf width (FLW)	1.78	1.53	1.45	1.66	1.50	1.6	0.09
3	Number of tillers (NT)	12.99	8.66	15.60	10.99	13.77	14.16	1.09
4	Diameter of pollen (DPL)	2.88	3	3.62	3.54	2.99	3.13	0.11
5	Stigma length (SgL)	2.22	2.06	2.30	2.00	1.91	1.80	0.10
6	Plant height (PH)	97.22	72.00	89.44	54.33	98.22	55.66	1.61
7	Panicle Length (PL)	25.54	24.50	24.65	22.46	27.11	24.00	1.09
8	Number of panicle/Plant (Pn/Pl)	8.00	8.99	10.22	7.16	8.44	9.33	1.05
9	Grain Length (GL)	0.86	0.89	0.85	0.86	0.95	0.97	0.02
10	Grain width (GW)	0.24	0.25	0.22	0.21	0.21	0.22	0.009
11	Number of spikelets/ panicle (Spk/Pn)	190.94	210.11	238.59	229.10	220.16	23.41	11.92
12	Length of upper most inter node (LUI)	36.12	29.33	30.55	22.33	38.89	27.33	2.39
13	Anther length (AL)	2.09	2.13	2.62	2.40	2.09	2.06	0.18
14	Days to flowering (DTF)	94.33	104.50	111.00	117.10	107.00	111.50	1.12
15	Days to maturity (DTM)	132.67	136.20	151.20	154.00	146.67	157.00	0.97
16	Stigma exertion percentage (SgEx)	92.10	1.30	27.10	0.50	29.16	0.80	1.13
17	Pollen sterility percentage (PS)	100.00	1.80	100.00	0.90	100.00	1.30	0.04
18	Pollen morphology (PM)	round	round	round	Round	round	round	-
19	Stigma color (Sgcol)	black	black	white	White	white	white	-
20	Tip color of lemma (TcolL)	red	red	red	Red	white	white	-
21	Attitude of flag leaf (AFL)	erect	erect	erect	Erect	erect	erect	-
22	Panicle exertion (Pn Ex)	full	full	partial	Full	partial	full	-

along with their corresponding maintainer lines are in Table 2.

Molecular data analysis

Overall, 33 alleles were amplified, with maximum of 6 alleles for the primer RM258, followed by 4 alleles each for the primers RM1 and RM3873 (Table 3). The average number of alleles per locus was 2.54. Three primers, *viz.,* RM3233, RM171 and RM334, produced monomorphic bands. PCR amplification results of the RM 6100 and RM 1 are given in Fig. 2. Highest PIC value of 0.69 was detected for the primers RM3873 and RM1. Highest gene diversity of 0.45 recorded for

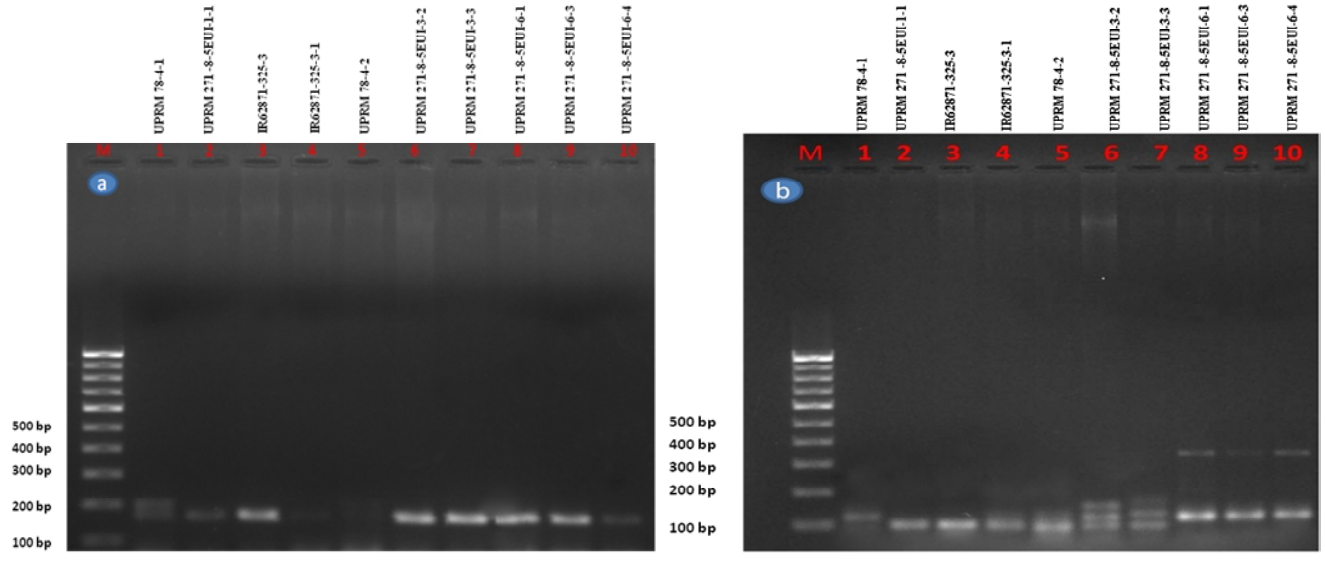

Fig. 2. PCR amplified fragments by marker locus RM 6100 (a) and RM1(b)

Table 3. Data on the number of alleles, allele size range, frequency allele, gene diversity and polymorphism information content (PIC) found among 10 male sterile genotypes for 13 microsatellite markers.

Sl. No	Marker	Sequence	No. of allele	Size range (bp)	Allele frequency(%)	Gene diversity	PIC Value
1	RM6100	TTCCCTGCAAGATTCTAGCTACACC **F** TGTTCGTCGACCAAGAACTCAGG **R**	3	165-200	0.1-1.0	0.17	**0.54**
2	RM3233	GAAATTCGAAATGGAGGGAGAGC **F** GGTGAGTAAACAGTGGTGGTGAGC **R**	1	180	0.9	0.18	0
3	RM3873	GCTATAGACGCCTCCTCCTTATCC **F** AAAGCTAGCTAGGACCGACATGC **R**	4	180-400	0.2-1.0	0.32	0.69
4	RM1	GCGAAAACACAATGCAAAAA **F** GCGTTGGTTGGACCTGACG R	4	100-330	0.2-0.8	0.38	0.69
5	RG140	GTACATAGTAGCACCTGCTC **F** TCCCTAGTTTGTGCTACTC **R**	2	80-850	0.6-1.0	0.24	0.47
6	RM6344	ACACGCCATGGATGATGAC **F** TGGCATCATCACTTCCTCAC **R**	2	75-150	0.9-1.0	0.09	0.50
7	RM258	GCATGGCCGATGGTAAAG **F** TGTATAAAACCACACGGCCA **R**	6	80-610	0.1-0.9	0.20	0.68
8	RM7003	GGCAGACATACAGCTTATAGC **F** TGCAAATGAACCCCTCTAGC **R**	2	100-300	0.1-0.9	0.18	0.18
9	RM171	AACGCGAGGACACGTACTTAC **F** ACGAGATACGTACGCCTTTG **R**	1	350	1.0	0	0
10	RM 334	CCACGAACCCTTTGCATC **F** GTGATGATGCGTCGGTTG **R**	1	100	0.8	0.32	0
11	RM 151	GGCTGCTCATCAGCTGCATGCG **F** TCGGCAGTGGTAGAGTTTGATCTGC **R**	3	150-180	0.1-1.0	0.43	0.53
12	RM 13	TCCAACATGGCAAGAGAGAG F GGTGGCATTCGATTCCAG **R**	2	130-150	0.2-0.7	0.37	0.35
13	RM 206	CCCATGCGTTTAACTATTCT **F** CGTTCCATCGATCCGTATGG **R**	2	130-180	0.4-0.7	0.45	0.46
	Mean		2.53			0.25	0.39

RM 206 with an average gene diversity of 0.25. Large variation was found for different bands amplified by the all SSR markers. Higher variation for allele size recorded for RG140, RM258, RM1 and RM3873 with no variation for RM3233, RM171 and RM334.

Genetic similarity and UPGMA cluster analysis

Pair-wise genetic similarity matrices were analyzed; the lowest similarity of 48% was recorded between the genotypes IR62871-325-3 and UPRM 78-4-2; UPRM 271 -8-5EUI-1-1 and IR62871-325-3; IR62871-325-3 and UPRM 271 -8-5EUI-6-1. Highest genetic similarity of 88% was detected between genotypes UPRM 78-4-2 and UPRM 271-8-5EUI-1-1. The UPGMA cluster analysis of 10 male sterile genotypes grouped them into two distinct classes; UPRM 78-4-1 and IR62871-325-3 were grouped into one cluster and the other eight genotypes were grouped into cluster 2 (Fig. 3). Cluster 2 formed two sub-groups; genotype IR62871-325-3-1 formed a separate group as cluster 3 and the rest of the genotypes formed group 4. Cluster 4 was further divided into three sub-sub-groups, where genotypes UPRM 78-4-2, UPRM 271 -8-5EUI-1-1and UPRM 271 -8-5EUI-6-1 formed one group, genotypes UPRM 271-8-5EUI-3-2 and UPRM 271-8-5EUI-3-3 fell in another group, and genotypes UPRM 271 -8-5EUI-6-3 and UPRM 271 -8-5EUI-6-4 formed another group.

Presence of inherit difference is pre-requisite to select suitable male sterile lines for hybrid breeding programme. Significant variations for genotypes and year depicted the presence of substantial genetic variability and also the effect of different environment over the year for the expression of characters. The existence of significant year x treatment interactions for number of panicle per plant, grain length and grain width, when the data were pooled represented the differential response of genotypes over the year of evaluation for these traits. However, non-significant effect of environment was previously reported on grain length and grain width by Xu et al. (2014); Laxmi, et

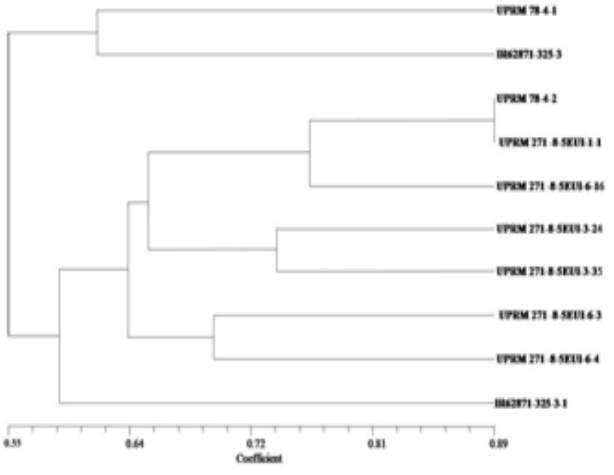

Fig. 3. UPGMA cluster diagram of new CMS genotypes using SSR markers.

al. (2014) reported significant genotype x environment interactions for panicle length however grain size was found less affected by the environment or agronomic practices (Borrell et al., 1999; Kibanda and Kihupi, 2007).

Expression of male sterility over the year is a prerequisite for its commercial use. In the present study, most of the lines were found to be completely pollen sterility except IR62871-325-3. However, the expression of 100% pollen sterility by three genotypes UPRM 78-4-1, UPRM 271-8-5EUI-3-3 and IR62871-325-3-1 in both years helps in the selection of suitable lines based on the other traits. Pollen sterility along with floral characteristics decides extend of outcrossing in rice. A wide range of out-crossing (0-44%) has been reported (Athwal and Virmani 1972) and several traits has positive influence on out-crossing rate. Higher rate of stigma exertion percentage in UPRM 78-4-1 may provide a greater advantage for out crossing than other strictly cleistogamous lines during seed production programme of hybrid and parent. Panicle exertion affects the rate of seed production in hybrid and parents. Exertion of complete panicle favored maximum out-crossing (Sidharthan et al., 2007; Youssef et al., 2011). Most of the test genotypes showed complete panicle exertion from the flag leaf, except UPRM 271-8-5EUI-3-3, IR62871-325-3-1 and UPRI 95-17A. WA-Cytoplasm is associated with partial panicle exertion as reported by Behla et al. (2007). It was also observed that WA-cytoplasm reduces plant height by shortening the upper most inter node length (Kadoo et al., 2002; Behla et al., 2007). In the present study plant height has increased after the transfer of WA-cytoplasm into their respective maintainers with increasing the length of upper most internode in UPRM 78-4-1; UPRM 271-8-5EUI-3-3 and IR62871-325-3-1. This may affect the parental line seed production by reducing the efficiency of out crossing, suggesting few agronomic practices for increasing the height of maintainer line during seed production. Most of the hybrids released in India are of medium to late maturity duration group; earliness is one of the most desirable traits as it can facilitate the use of rice-wheat and rice-pulses cropping system in the Indo-Gangetic plain of India (Timisana et al., 2001; Khush, 1995). The genotype UPRM 78-4-1 could be a viable alternative for producing early maturing hybrids.

In the microsatellite markers analysis the size difference for the smallest and the largest alleles for a given SSR locus were higher. The maximum size difference reflected higher efficiency in fingerprinting and diversity analysis. Clustering of genotypes using SSR marker data based on UPGMA cluster analysis grouped UPRM 78-4-1 and IR 62871-325-3-1 into two separate clusters; IR 62871-325-3-1 and UPRM 271-8-5EUI-3-3 into two sub-clusters within cluster 2 that indicated the parental genotypes were genetically diverse. Eight genotypes were grouped into the cluster 2 indicating there were genetic similarities among the genotypes. Information of genetic similarity within the cluster 2 may be useful to avoid redundancy while use of same parents for new CMS lines development.

On the basis of morphological analysis it could be concluded that three genotypes UPRM 78-4-1, UPPRM 271-8-5EUI-3-3 and IR 62871-325-3-1 were suitable for hybrid breeding programme. These genotypes were also distinct enough based on molecular marker diversity.

REFERENCES

Athwal DS and Virmani SS (1972). Cytoplasmic mal sterility and hybrid breeding in rice. In: Rice Breeding. pp. 615-620. IRRI, Manila, Philippines

Behla RS, Ranga A and Bharaj TS (2007). Floral and morphological traits of some A, B and R line of rice. Crop Improvement 34 (1): 24-26

Borrell AK, Garside AL, Fukai S and Reid DJ (1999). Grain quality of flooded rice is affected by season, nitrogen rate, and plant type. Australian Journal of Agricultural Research 50(8): 1399-1408

Cheng SH, Zhuang JY, Fan YY, Du JH and Cao LY (2007). Progress in research and development on hybrid rice, a super-domesticate in China. Ann Bot. 100: 959-966

Doyle JJ and Doyle JL (1990). Isolation of plant DNA from fresh tissues. Focus 12: 13-15

Grishma S, Sasidharan N, Chakraborty S, Trivedi R, Ravikiran R and Davla D (2012). Genetic diversity and molecular analysis for fertility restorer genes in Rice (*Oryza sativa* L.) for wild abortive (WA) cytoplasm using microsatellite markers. Journal of Agricultural Technology 8(1): 261-271

Hasan MJ, Kulsum U, Rahman MH, Ali MH and Julfiquar AW (2011). Genetic variability of some cytoplasmic male sterile lines (cms) of rice (*Oryza sativa* L.) Genotypes. Bangladesh J. Agril. Res. 36(2): 263-270

Kadoo NY, Zaman FU, Singh AK and Deshmukh PS (2002). Effect of male sterility inducing cytoplasm on morpho-physiological and bio chemical characters in rice (*Oryza saliva* L.). Indian J. Genet. 62: 300-304

Khush GS (1995). Breaking yield frontier of Rice. Geo Journal 35(3): 320-332

Kibanda JMN and Kihupi AL (2007). Influence on genotype and genotype X environmental interactions on qualiy of rice grain. African crop Sci. Journal15(4): 173-182

Nandkumar N, Singh AK, Sharma RK, Mohapatra T, Pravu KV and Zaman FU (2004). Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. Euphytica 136: 257-264

Panse VG and Sukhatme PV (1961). Statistical methods for agricultural workers. ICAR, New Delhi

Sattari M, Kathiresan A, Gregorio GB and Virmani SS (2008). Comparative genetic analysis and molecular mapping of fertility restoration genes for WA, Dissi, and Gambiaca cytoplasmic male sterility system in rice. Molecular Breeding 168: 305- 315

Siddiq EA (1993). Rice Production Strategy for 21 st Century. Oryza 30: 180-196

Sidharthan B, Thiyagarajan K and Manonmani S (2007). Cytoplasmic male sterile lines for hybrid rice production. J. App. Sci. Res. 3: 935-937

Timisana J and Connor DJ (2001). Productivity and management of rice-wheat cropping systems: issues and challenges. Field Crops Research 69(2): 93-132

Xu FF, Tang FF, Shao YF, Chen YL, Tong C and Bao JS (2014). Genotype × Environment Interactions for Agronomic Traits of Rice Revealed by Association Mapping. Rice Science 21(3): 133-141

Yang SH, Cheng BY, Wu JL, Shen WF and Cheng SH (2006). Review and prospects on rice breeding and extension in China. Rice Sci. 13(1): 1-8

Youssef SA, Dora SF, Deraz AAM, Abo-Shosha AA, Khalil MAA and El-Sayed (2011). Estimating the genetic purity in cytoplasmic male sterile (CMS) lines of Egyptian rice. Australian Journal of Crop Science 5(3): 251-261