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Microsatellite (SSR) Based Assessment of Genetic Diversity among the Semi-dwarf Mutants of Elite Rice Variety WL112

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ABSTRACT

Success of semi-dwarf varieties in late 1960's lead breeders to depend heavily on these leading to narrow genetic base in today's rice varieties. Analysis of cultivated varieties in tropical Asia revealed *sd1* gene from Dee-geo-woo-gen to be the major dwarfing source. High genetic similarity renders crop genetically vulnerable to potential epidemics. Efforts are underway to broaden the genetic base using alternate sources of dwarfing. Mutation breeding has been used to increase allelic diversity. In this study, twenty radiation induced mutants of rice (*Oryza sativa* L.) and parent variety WL112, showing morphological differences, were screened for variation in Simple Sequence Repeats (SSR). Thirty five polymorphic SSR primers, selected from all chromosomes of rice generated 115 polymorphic bands. Jaccard's similarity coefficient revealed considerable genetic diversity among the mutants. The dissimilarity between the mutants was as high as 65%. The UPGMA based dendrogram showed five major clusters with sub-clusters. Mutant-specific polymorphic SSR markers either alone or in combination, were detected. Twelve mutants could be identified by using specific markers, while combination of two could identify six mutants and parent. The radiation induced mutants revealed diversity and short stature in elite background which could be used in breeding programme.

Key words: Rice, semi-dwarf, mutant, SSR, genetic diversity

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple foods for the world's population ranking third after Wheat and Maize in terms of production and consumption (Akinbile *et al.*, 2011). Asia accounts for over 95% of global rice production with China (194.3 million t) and India (148.3 million t), ranked first and second, respectively are by far the largest producers of rice, producing half of the world's rice (Upadhyay *et al.*, 2011). It is the second most important crop in the world which has an annual cultivation area of 213 Mha, with an annual production of 593 million t and an average productivity of 3.91 t ha^{-1} (Rai, 2003). In India, rice is the most important crop occupying an area of 42 Mha and annual production of 133 mt. Tropical Asia, accounting for over 90% of production and consumption of rice had been growing tall statured lodging prone varieties with very low yields until the advent of the non-lodging, high yielding and semi-dwarf varieties in sixties. The short statured varieties developed using Dee-Geo-Woo-Gen

(DGWG), have enabled many countries in the region to achieve self-sufficiency in rice (Spielmeyer *et al.*, 2002). Up to now more than 60 dwarfing genes have been identified mostly in japonica rice (Nagato and Yoshimura, 1998). Dwarfing genes are often associated with agronomically undesirable traits which restrict their use in rice breeding program. Also, genetic barriers between indica and japonica cultivars restrict transfer of genes. Hence, there is an urgent need to look for alternative sources of semi-dwarfing in indica back ground. Usefulness of radiation induced mutations to alter the culm height has been well documented (Songsri *et al.*, 2011).

The success of DGWG gene based varieties such as IR8 and Taichung (Native) 1 have been excessively used by breeders all over the globe as source of dwarfing. With over 90% of the high yielding varieties in cultivation today having DGWG gene (Cho *et al.*, 1994), the genetic base, as result is quite narrow. Apprehending that high genetic similarity might render the crop genetically vulnerable to biotic or abiotic stresses, many efforts have been made for broadening the genetic base through identification and use of alternate sources of dwarfing genes (Neeraja *et al.*, 2009). There is an urgent need to discover alleles of *sd1* gene or finding non-allelic semi-dwarfing genes. Molecular markers provide a powerful tool for locating and distinguishing key genetic regions on the whole genome and hence differentiating genotypes from each other (Puchooa and Venkatasamy, 2005; Tertivanidis *et al.*, 2008; Yasmin *et al.*, 2006; Nazari and Pakniyat, 2008). A wide range of molecular markers have been applied for genetic diversity studies in rice such as RFLP, microsatellite, RAPD and AFLP (Ndjiondjop *et al.*, 2006). These DNA based molecular techniques helped in detecting genetic differences among mutants and cultivars (Botstein *et al.*, 1980; Williams *et al.*, 1990; Vos *et al.*, 1995). Among these, simple sequence repeat (SSR) markers are efficient, cost-effective and detect a significantly higher degree of polymorphism. These are also highly reproducible, multi-allelic, codominant, relatively abundant and cover whole genome in rice (Guasmi *et al.*, 2008). They are ideal for genetic diversity studies and intensive genetic mapping. The present investigation was aimed at assessing genetic diversity among dwarf and semi-dwarf mutants of rice variety WL112 and to characterize these mutants with the help of microsatellite based SSR markers.

MATERIALS AND METHODS

Rice material: WL112 is an important rice variety in India which is extra tall type (plant height >150 cm) and extremely lodging susceptible especially during late monsoon rains which can result in complete loss. With an aim to get dwarf or semi-dwarf mutants, seeds of parent variety WL112 were earlier subjected to gamma ray irradiation with 250 Gy dose in Gamma Cell 220 irradiator. Twenty dwarf and semi-dwarf mutants were identified in M_2 generation and were stabilized in subsequent generations. These were stable as judged from their uniformity in morphological traits. The mutants used in this study are listed in Table 1. The parent variety WL112 and its mutants were grown in Trombay location in Kharif 2009 and 2010. Standard agronomic practices were followed. Observations were recorded for various morphological and agronomic traits. Data were taken from five randomly selected plants and averaged. GA_3 sensitivity test was carried out by spraying 12 day old seedlings with GA_3 at 10^{-4} M concentration. Control plants were sprayed with distilled water. Seedling height was measured after five days of GA_3 spray. The symbol + or - in Table 1 indicates GA_3 responsiveness or non-responsiveness, respectively. Reaction to rice blast disease was monitored under field conditions at a hot spot location in Rice Research Station, Ratnagiri. Scoring was based on 0-5 scale (Mohanta *et al.*, 2003).

Table 1: Morphological traits of parent WL112 and dwarf mutants

Mutant/parent	Plant height (cm)	GA_3 response	Days to flower	No. of tillers	Yield (g) per plant	Reaction to rice blast
D1	27.0	-	52	15.0	7.36	Moderate
D2	30.2	-	75	15.2	15.28	Moderate
D3	33.2	+	77	19.8	20.74	Moderate
D4	36.0	-	74	18.8	21.50	Moderate
D5	47.0	+	87	19.2	18.23	Moderate
D6	47.4	+	86	19.0	16.13	Moderate
D7	48.8	+	83	27.0	25.78	Moderate
D8	49.2	+	86	15.6	30.97	Moderate
D9	51.6	+	88	20.0	17.35	Moderate
D10	51.6	+	112	20.4	22.58	Moderate
D11	52.0	-	78	20.0	18.20	Moderate
D12	54.9	-	74	15.2	8.34	Moderate
D13	54.8	-	75	8.2	15.46	Moderate
D14	70.0	+	90	17.0	29.49	Moderate
D15	71.2	-	115	13.2	16.50	Moderate
D16	71.4	+	103	14.0	47.86	Moderate
D17	83.0	+	89	17.6	18.48	Susceptible
D18	84.2	+	121	16.8	25.25	Susceptible
D19	88.4	-	97	8.8	23.09	Resistant
D20	90.0	-	120	9.4	12.87	Resistant
WL112	160.0	+	120	14.1	22.95	Susceptible

+: Positive, -: Negative

Primer selection: A total of thirty five SSR primer pairs, synthesized by M/s Allied Scientific Products, Kolkata, India, were used for PCR amplification. These primers were selected based on their uniform distribution on all the 12 linkage groups and their polymorphic information content (PIC) value (http://gramene.org/markers/microsat/50_ssr.html). Sequences of the SSR primers used in this study and their allelic information are given in Table 2.

PCR amplification, data scoring and analysis: DNA was extracted as per the protocol developed by Nalini *et al.* (2004). The DNA was quantified using Cintra 2.2 UV-Vis spectrophotometer (GBC) at 260 nm wavelength. The DNA was diluted to 25 ng μL^{-1} and used as working stock. PCR was conducted in a total reaction volume of 15 μL per sample. The reaction mixture contained 10X PCR buffers 1.5 μL (10 mM tris-HCl pH 9.0, 50 mM KCl and 15 mM $MgCl_2$), dNTP's (2.5 mM) 1.5 μL, Taq DNA polymerase (3 U μL^{-1}) 0.17 μL, forward and reverse primer (10.5 μM μL^{-1}) 1 μL each and DNA 25 ng. Thermal cycling conditions were as follows: Initial denaturation for 3 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C (annealing temperature) and 1 min at 72°C. Annealing temperature was varied depending upon the specific requirements of the SSR primer pairs. PCR was performed on Eppendorf master gradient thermal cycler. The PCR amplified products were resolved on 6% denaturing polyacrylamide gel (acrylamide:Bisacrylamide (30:1) 80 mL, urea (7.2 M) 20 mL, 10% ammonium per sulphate 400 μL and TEMED 170 μL). The gel was pre run at 60 W (40 mA; 1500 V) with pre run dye for 60 min or until the gel temperature reached 55°C in 1X TAE. Sample preparation was done by adding 7.5 μL denaturing buffer (95% Formamide in 1X TAE, 0.05% xylene cyanol (w/v), 0.05% bromophenol blue (w/v). To 200 μL of this mix, 30% formamide in glycerol was added to 15 μL PCR

Table 2: List of SSR primers used in the present investigation

SSR primer	Forward primer	Reverse primer	Linkage group	Annealing (°C)	Product range	No. of alleles	PIC
RM 5	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	1	57	94-138	3	0.240
RM 431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC	1	55	233-261	3	0.521
RM 1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	1	55	67-119	5	0.689
RM 237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC	1	55	105-153	3	0.522
RM 495	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	1	55	148-160	2	0.353
RM 283	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG	1	61	130-176	3	0.325
RM 312	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC	1	55	86-106	4	0.562
RM 283	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG	1	61	130-176	3	0.546
RM 259	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT	1	55	133-186	4	0.453
RM 452	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG	2	61	192-213	5	0.522
RM 154	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	2	61	148-230	3	0.563
RM 338	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC	3	55	178-184	3	0.501
RM 514	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG	3	55	229-278	5	0.587
RM 55	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG	3	55	216-247	3	0.431
RM 489	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG	3	55	223-289	2	0.453
RM 124	ATCGTCTGCGTTGCGGCTGCTG	CATGGATCACCGAGCTCCCCCC	4	67	257-289	2	0.421
RM 507	CTTAAGCTCCAGCCGAAATG	CTCACCCTCATCATCGCC	5	55	234-257	4	0.501
RM 161	TGCAGATGAGAAGCGGCGCCTC	TGTGTCATCAGACGGCGCTCCG	5	61	154-187	2	0.375
RM 413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	5	53	71-114	3	0.463
RM 178	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC	5	69	112-131	4	0.502
RM 162	GCCAGCAAAACCAGGGATCCGG	CAAGGTCTTGTGCGGCTTGCGG	6	61	191-244	5	0.626
RM 510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC	6	57	99-127	4	0.634
RM 133	TTGGATTGTTTTGCTGGCTCGC	GGAACACGGGGTCGGAAGCGAC	6	63	226-237	4	0.617
RM 125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	7	63	105-147	2	0.240
RM 152	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG	8	53	133-157	4	0.579
RM 408	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC	8	55	112-128	2	0.215
RM 44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	8	53	82-132	3	0.471
RM 105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	9	63	100-141	2	0.465
RM 215	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	9	55	126-161	2	0.435
RM 474	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG	10	55	216-288	5	0.567
RM 171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	10	55	307-347	3	0.325
RM 484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC	10	55	286-298	3	0.219
RM 552	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC	11	55	167-258	3	0.584
RM 287	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	11	55	82-118	4	0.587
RM 277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	12	55	104-121	3	0.497
Total						115	16.591
Mean						3.29	0.474

PIC: Polymorphic information content

product. The samples and ladders were denatured for 3 minutes at 94°C in the thermocycler and immediately placed on ice. About 6 μL of sample was loaded quickly and the gel was run at 60 W for 80-100 min at 50-55°C. The gel was silver stained using improved modified protocol by Creste *et al*. (2001). Bands on silver stained gels were scored as 1 (presence) or 0 (absence). Data were entered in binary data matrix as discrete variables. Data were analyzed for dissimilarity using DARwin 5.0 software (Perrier *et al*., 2003). Pair wise genetic similarity were estimated using Jaccard's similarity co-efficient. Unweighted pair group method using arithmetic averages (UPGMA) was employed for cluster analysis generating dendrogram. Mutant specific polymorphic

Table 3: Identification of dwarf and semi-dwarf mutants and parent WL 122 with SSR markers

Mutant/parent	SSR Marker	Unique band
D1	RM 413	+
D2	RM 5	+
D3	RM 5 and 162	+
D4	RM 162	+
D5	RM 510	+
D6	RM 287	+
D7	RM 474 and 408	+
D8	RM 507	+
D9	RM 455	-
D10	RM 5 and 55	+
D12	RM 162	+
D13	RM 514	+
D14	RM 514 and 5	+
D15	RM 287	+
D17	RM 510	+
D18	RM 452 and 510	+
D19	RM 474 and 510	+
D20	RM 552	+
WL112	RM 338 / 171	+

Mutants D11 and D16 could not be differentiated, +: Present, -: Absent

bands were identified either singly or in combination of two markers to identify and differentiate it from the rest of mutants and the parent (Table 3). Only clear and unambiguous bands were taken into consideration for differentiating mutants and parent variety.

RESULTS

Phenotypic diversity among genotypes: Morphological traits of each genotype were recorded on five randomly chosen plants in each replication. Data on some agronomic traits such as plant height, days to flowering, number of tillers per plant, yield per plant, GA_3 response and reaction to rice blast disease of the mutants and parent were recorded (Table 1). Mutants exhibited significant variability for these traits. Plant height ranged from 27.0 to 90.0 cm among mutants as compared to parent WL112 which was 160 cm. Extreme dwarf mutants D1 and D2 were shortest with height 27.0 and 30.2 cm, respectively. Figure 1 shows field view of three of the short culm mutants (mutant D5, D13 and D20) and the parent. The parent WL112 flowered in 120 days. Earliest flowering among the mutants was recorded in mutant D1 (52 days) followed by mutant D12 (74 days). Most of the mutants were significantly earlier in flowering except mutants D10, D15, D16, D18 and D20 which were at par with the parent. Variability was also observed for tillering ability. Sparse tillering with average 8.2 and 8.8 tillers per plant was observed for mutants D13 and D19, respectively. Parent WL112 showed moderate number of tillers with average 14.1 tillers per plant. Dwarf mutant D7 showed very high tillering with 27.0 tillers per plant. Mutant D9, D10 and D11 also exhibited increase in tiller number with 20 tillers per plant. Some of the dwarf mutants were relatively non-responsive to GA_3 such as D1, D2, D4, D12, D13, D15, D19 and D20, remaining mutants were responsive. Parent WL112 and mutant D17 and D18 showed high susceptibility to rice blast disease. Mutants D19 and D20 were found to be highly resistant, rest of the mutants were moderately resistant. Grain yield per plant also exhibited

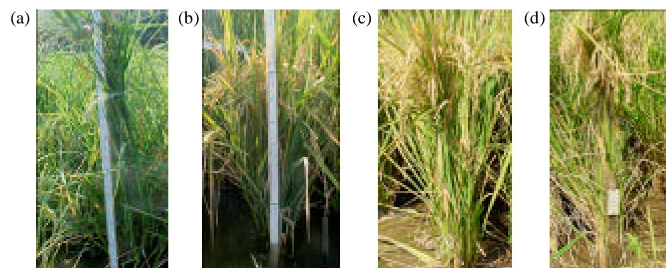

Fig. 1(a-d): Field view of the parent variety (a) WL112, (b) Mutant D5, (c) Mutant D13 and (d) Mutant D20

significant variation among dwarf mutants. Average yield for WL112 was 22.95 g per plant. Mutant D16 recorded almost double the yield of the parent variety with 47.9 g seed yield per plant. Mutants D8 and D14 also exhibited higher yield than parent with 30.9 and 29.4 g seed yield per plant. Mutants D1 and D12 recorded low yield with 7.4 and 8.3 g seeds per plant.

Microsatellite polymorphism and genetic variability analysis among genotypes: A total of 35 SSR loci were used to detect polymorphism across 20 dwarf and semi-dwarf mutants and parent rice variety WL112. The 35 primer pairs were well distributed over all the 12 linkage groups of rice and were found to be highly polymorphic. The level of polymorphism among these 21 genotypes of rice was evaluated by calculating allele number and PIC values for each of the 35 SSR loci. The SSR primer pairs used for genetic diversity analysis, the number of alleles for each SSR locus and PIC values are given in Table 2. Average number of alleles was estimated to be 3.29 and ranged from two (RM 125) to five (RM 474, RM 162, RM 514, RM 452 and RM 1). The PIC values, a reflection of allele diversity and frequency among the varieties, were not uniformly high for all the SSR loci tested. The average PIC value was 0.474 and it ranged from 0.215 for RM 208 to 0.689 for RM 1. Similarity matrices were constructed based on shared allele analyses which revealed that the average genetic similarity between genotypes was 0.52 with SSR markers. Maximum similarity was observed between D7 and D9 (0.8108) and minimum between D12 and D20 (0.2666).

Identification based on presence of unique bands: Most of the genotypes used in the present study could be differentiated from each other by using a single marker or a combination of two markers. Out of twenty dwarf and semi-dwarf mutants, twelve mutants could be differentiated by the presence of specific band with specific SSR primer (Table 3). Remaining six mutants i.e. D3, D7, D10, D14, D18, D19 and parent WL112 could be differentiated and identified by a combination of two SSR primers. Mutants D11 and D16 were not distinguishable.

Genetic relationship between dwarf mutants and parent WL112: Cluster analyses were used to group mutants and parent to construct dendrogram based on SSR (Fig. 2). The dendrogram separated all the 21 genotypes into five distinct clusters. The first major cluster, cluster 1 consisted of eight mutants with three sub-clusters. Cluster 2 consisted of five dwarf mutants and was subdivided into two sub-clusters; cluster 3 contained only one mutant D19. Cluster 4 and 5 were of equal size with three mutants each. Parent WL112 was included in cluster 4 along with mutant D17 and D18. Mutant D20 was not included in any of the clusters.

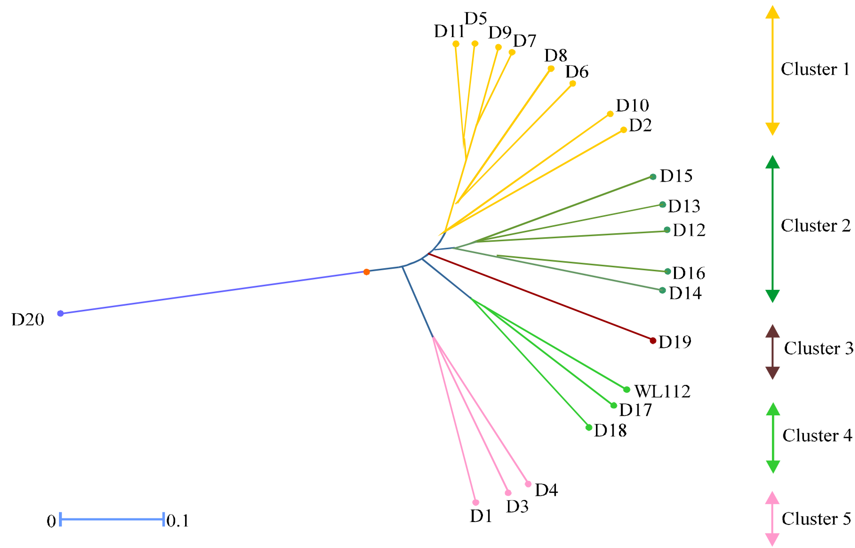

Fig. 2: Dendrogram resulting from UPGMA cluster analysis of 20 dwarf mutants and parent WL112

DISCUSSION

The present attempt was made to characterize 20 gamma ray induced short stature mutants and assess diversity among the mutants for their efficient utilization in breeding programme.

Phenotypic variability: The mutants exhibited significant variability for plant height which ranged from 27.0 to 90.0 cm as compared to parent WL112 which was 160 cm. Significant reduction in plant height was achieved. Similar reduction in plant height was also reported earlier (Jana and Roy, 1973). The mutants were either responsive or non responsive to spray of 10^{-4} M GA_3 at early seedling stage. The responsive dwarf mutants could be defective in the GA biosynthetic pathway, which could be rescued by the application of GA_3. Dwarf stature could also be a result of other reasons. For instance, the D1 type dwarf is reported to be defective in α-subunit of the heterotrimeric G protein which affects gibberellin signal transduction (Ueguchi-Tanaka *et al.*, 2000). Likewise, loss of function mutation in the rice homeobox gene *OSH15* is reported to affect the architecture of internodes resulting in D6 dwarf plants (Sato *et al.*, 1999). Characterization of dwarfs of D16 mutants revealed a protein kinase with high similarity to a putative brassinosteroid receptor in *Arabidopsis* to be linked to prevention of internode elongation (Yamamuro *et al.*, 2000). Some of the dwarf mutants used in this study (D1, D2, D4, D11, D12, D13, D15, D19 and D20) were insensitive to gibberellin spray indicating mutation in pathway other than GA synthesis.

Some of the mutants exhibited earliness in flowering by 20-70 days. This trait can be useful in reducing the duration of crop particularly in India where rice is cultivated in monsoon season (Kharif) which is followed by season which is important for winter (Rabi) crops.

The induced dwarf mutants D1 to D16 also exhibited moderate resistance to rice blast disease while mutants D19 and D20 were highly resistant to blast. The isolation of possible blast resistant mutants in adapted material is of agronomic importance. Similar mutants with early flowering and resistance to rice blast disease are reported (Wu *et al.*, 2010). Mutation in rice is known to change the tiller number per plant. High tillering mutation ht1 in japonica rice variety Xindao18 was

reported with tiller number three times of the parent and was mapped on chromosome number 10 with SSR markers (Li *et al.*, 2010). In the present study, mutant D7 exhibited almost double the tiller number of the parent variety WL112. Increase in tiller number was also reported in mutant D9, D10 and D11. Increase in tillering is ascribed to reduced tiller bud dormancy at the prophyll nodes as reported for fc1 mutation in rice (Goto *et al.*, 2005).

Among the mutants, significant variation for seed yield per plant was observed. As a complex agronomic trait, grain yield per plant of rice is determined by three component traits: Number of panicles per plant, number of grains per panicle and grain weight. These traits in turn are controlled by many correlated traits such as number of tillers, number of spikelets, grain dimension, degree and rate of grain filling etc. Earlier studies have revealed presence of QTLs for grain yield on almost all the rice chromosomes (Xing and Zhang, 2010). Mutant D16, D8 and D14 exhibited significant change in grain yield per plant. Dwarf mutant D16 showed almost double the yield of parent variety. This major change is probably due to increased number of spikelets and more number of grains per panicle as grain dimension and filling rate was more or less unchanged.

Genotypic variability among dwarf mutants and parent: The assessment of genetic diversity is essential for germplasm characterization and utilisation. The results derived from analyses of genetic diversity at the DNA level could be used for designing effective breeding programs. Microsatellite diversity analysis of 21 genotypes with 35 SSR primers pairs indicated a high level of genetic diversity (0.65) and higher average number of alleles per primer of 3.29. These results support the finding that microsatellite markers are informative and powerful to assess the genetic variability among closely related mutants (Xu *et al.*, 2004). Majority of the SSR primers used in this study had the GA-dinucleotide repeat motif which has been reported to display high level of variation among the rice genotypes (Temnykh *et al.*, 2000).

The present attempt was made to identify alternate sources to *sd1* by characterizing 20 induced (gamma rays) dwarf and semi dwarf genotypes along with parent WL112 at molecular level. Genetic variability was observed among the dwarf mutants and the parent using the SSR markers. Among the 21 genotypes the average genetic similarity of 0.52 (based on similarity matrix in DARwin) indicated creation of genetic diversity among the dwarf mutants. Herrera *et al.* (2008) also reported average genetic similarity of all SSR loci for 18 genotypes using 45 SSR primers to be 0.48.

SSR markers were highly informative and polymorphic as evident from its PIC value of 0.474 (Chen *et al.*, 2011). SSR markers are normally hyper variable and hence the polymorphism estimate is generally higher than other loci in the genome. It was seen that PIC values were relatively higher for markers RM 1 (0.689), RM 510 (0.634) and RM 162 (0.626) having GA and AC repeats which is because of the fact that these repeats are highly variable and polymorphic in nature. The structure and length of simple sequence repeats are considered to be one of the major factors affecting microsatellite variability. In general, SSR loci such as RM 452, RM 1, RM 514, RM 162 and RM 474 with more repeats tend to be more polymorphic and had larger amplitude of variation (Schug *et al.*, 1998).

An important application of molecular diversity study is to identify a marker which can differentiate a desirable genotype from the rest. In the present study, unique alleles were identified for twelve mutants and combination of two primers could distinguish the rest of the six genotypes (Choudhary *et al.*, 2011). Generation of data base for morphological and molecular variation of the mutants will help in their identification and utilization in future.

UPGMA clustering: The UPGMA cluster analysis showed that all 20 dwarf mutants of rice variety WL112 could be distinguished based on the information generated by the 35 SSR markers. Clusters generated showed a close association with the morphological traits under study. The dendrogram indicated five major clusters with different sub-clusters. Most of the semi-dwarf, mid-early maturity with moderate resistance to rice blast mutants were included in cluster 1 and 2. The mutant D19 which showed high resistance to rice blast disease was the only component of cluster 3. Cluster 4 contained all the entries susceptible to blast which also included parent variety WL112. Extreme dwarf mutants D1, D3 and D4 were included in cluster 5 and genetic distance of about 0.65 showed wide genetic divergence from the rest of mutants. Kumar and Bhagwat (2009) also reported distinct SSR clustering among the dwarf mutants of rice variety Basmati 370. Similar SSR based clustering has been reported in basmati and non-basmati rice varieties (Nagaraju *et al.*, 2002). Our results showed that the SSR markers could distinguish genetically close breeding lines and mutants and the information generated can be used in the characterization and utilization of rice mutant germplasm.

CONCLUSION

Although, it is common to obtain reduced height mutants in rice, they are often associated with reduced plant vigour. Some of the dwarf mutants in the present study have been found to have good phenotypic and agronomic attributes especially dwarf mutant D16 with high grain yield/plant and also earliness in maturity. This mutant could be used directly for further evaluation of grain yield. Other mutants (mutant D8, D14 and D16) could also serve as alternate source(s) of dwarfing with desirable agronomic and yield attributes for use in breeding programme. SSR markers proved to be effective in estimating genetic diversity among the mutants. These mutants could help in increasing the genetic diversity and allelic richness. Parent identification for a hybridization programme on the basis of estimated diversity is likely to be more effective than choosing parents randomly. Understanding of genetic relationship together with analysis of their agronomic performance will help in efficient utilization of these induced mutants in rice improvement programme.

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