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Detection of genetic divergence among Putative Ethyl Methane Sulfonate Mutants of Super Basmati using Microsatellite Markers

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Abstract

Mutation is a resource of generating genetic diversity in crop plant for breeding as well as genetic analysis. Ethyl Methane Sulphonate (EMS) is frequently applied chemical agents in plants. The present study was completed to investigate the mutagenic effects of different concentration of EMS on yield contributing traits of super basmati. The seeds of rice cultivar (Super basmati) were mutagenized with different doses from 0-2% v/v of EMS with difference of 0.25% for determination LD50. The treated and non-treated plants were observed under different agronomic parameters. A total of 48 putative EMS mutants of super basmati were selected randomly to analyze genetic diversity using 25 SSR primers. These markers were located on twelve chromosomes of rice. SSRs analysis revealed that abroad level of genetic diversity was existed among mutants of super basmati. A sum of 91 alleles was identified of which 82 alleles were originated to be polymorphic and the rest of nine alleles were discovered as monomorphic. The range of allele number was 2-10 with mean of 3.64 alleles/locus. The value of polymorphic information content (PIC) was from 0.039 (RM5) to 0.878 (RM44) with mean of 0.439 for each locus. All mutants differentiated from each other in more than two set of primers due to presence or absence of unique bands on chromosomes at definite base pair. The size base pair range was 75-1000bp. Dendrogram located mutant indices into four major groups. Phylogenic analyses exposed 40-96% similarity. It is concluded that EMS induced genetic variability and SSRs markers (RM44, RM154, RM1, RM252, RM334, RM487, RM110 and RM257) could be employed for the selection of rice mutants throughout molecular breeding program.

Background

Rice is a primary food for all world population (Luz et al. 2016). Basmati type of rice is grown in different region of Punjab, Pakistan (Ansari et al. 2015) due to its quantity and quality of grain (Wattoo et al. 2013). Only 40% of national rice production obtains by Basmati (fine) rice according to Food and Agriculture Organization of United Nations. Food production (70%) and rice production (24%) must be increase in 2050 to feed the growing population of nine billion (Naeem et al. 2015). Production of any crop can be improved by changing the inherited genetic makeup of crop. Chemical mutagen (Ethyl methane sulphonate) is most effective than physical agents to create and improve desired genetic variability in crop plant (Bhat et al. 2005; Botticella et al. 2011). Genetic markers have been used for identification of genetic diversity and genetic relationships within and among various species (Matin et al. 2012). Microsatellites are PCR based genetic markers for evaluation of rice genetic diversity (Singh et al. 2013). These markers were firstly expressed in plant genome (Condit and Hubbell 1991). Simple sequence repeats have been employed for identification of genetic diversity in numerous crops such as maize (Senior et al. 1998), sorghum (Smith et al. 2000), cotton (Liu et al. 2000), wheat (Zhu et al. 2011) and rice (Ansari et al. 2015: Allhgholipour et al. 2014; Shamim et al. 2016). Rice SSR markers have been developed for different purposes such as mutation study (Li et al. 2010), genetic diversity (Pervaiz et al. 2010; Zhao et al. 2010), association and QTL mapping (Guo et al. 2010), marker aided selection (Thomson et al. 2009) as well as rice domestication (Sweeney et al. 2007). Rice microsatellites have been recognized to be polymorphic within rice varieties (Kumar and Bhagwat 2012), between rice varieties (Habib et al. 2013; Yesmin et al. 2014) and rice subspecies (Ni et al. 2002). These markers are perfect for analysis of genetic divergence and genetic relationships (Cho et al. 2000).

We predict that EMS as chemical mutagen can enhance desired genetic variability in rice plant. The current study was made to determine the genetic relationship and genetic diversity among 48 EMS putative mutants of super basmati using simple sequence repeats (SSRs) markers and to categorize suitable SSR markers for genetic analysis of rice mutants.

Materials And Methods

Plant materials

Seeds of super basmati (Fine) were collected from RRI (Rice Research Institute; Kala Shah Kaku; Gujranwala; Punjab; Pakistan).

EMS mutagenesis

One hundred and fifty seeds were mutagenized with different EMS doses (0%, 0.25%, 0.5%, 1%, 1.25%, 1.5%, 1.75% and 2% v/v). EMS working solution (10 mL of 0%, 0.25%, 0.5%, 1%, 1.25%, 1.5%, 1.75% and 2% v/v) was added in each falcon and shifted to orbit shaker at 60 rpm for 24 h. Then, treated seeds were washed three to four times by distilled water. Mutagenized seeds were grown in single seed progeny method to get first mutagenic population (M_1). Each treatment was repeated five times with row to row distance (10 cm) and plant to plant distance (8 cm). M1 progeny were selfed in field to get M2 generation of super basmati and in next successive generations selfed M3 generation was collected which showed stable mutated basmati rice population.

Genomic DNA isolation and SSR Analysis

DNA was extracted from 20 days old seedlings using CTAB (Cetyl trimethyl ammonium bromide) method (Zheng et al. 1995). DNA quality was checked by running 0.8% agarose gel. The quantity of DNA was calculated using Nano Drop (ND-1000) spectrophotometer. PCR amplification was carried out using 25 SSR primers covering all twelve chromosomes of rice listed in Table 1. Chromosomes position, repeat motif and primer sequences of all SSR markers can be originated in rice genome databases (http://www.gramene.org). SSR study was done following the method of McCouch et al. (2002) with minor modifications. PCR reaction mixture for amplification was carried out in total volume of 25 µl including template (25 ng), 10x PCR buffer (2.5 µl), Mgcl2 (2.5 mM), dNTPs (200 µM of each set), 0.2 µM of each primer (10 pmol), taq DNA polymerase (0.2 µl; Fermentas Life Sciences) and sterile distilled water. PCR profiling condition was 94˚C for 5 min as initial denaturation step. This was followed by 35 cycles of denaturation (94˚C for 1 min), primer annealing (52–67˚C for 1 min), extension (72˚C for 2 min) and final extension (72˚C for 5 min). Total of 4 µl of amplification product loaded in 7% polyacrylamide gel for electrophoresis and stained with ethidium bromide to visualize the bands under UV light. These photographs were utilized for computational analysis on the basis of absence or presence of amplified products. It was designated as one or zero with presence or absence of amplified product in genotype respectively. A total of 48 EMS putative mutants were characterized using 25 SSR markers. The PIC value, genetic diversity, heterozygosity, major allele frequency were analyzed by PowerMarker version 3.25 (Liu and Muse 2005). POPGENE version 1.32 (Yeh et al. 1999) was used to obtain genetic relationship among mutants

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and wild based upon Jaccard's similarity coefficient of Nei's UPGMA (Unweighted pair group of arithmetic means) (Nei and Li 1979). Tree View 32 software was used to visualize the dendrogram (Page 1996).

Table 1
SSRs markers used in study

S.N	P.N	C.N	P.A.T	Primer Sequence (F)	Primer Sequence (R)	R.M
1	RM-01	1	55	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	(GA)26
2	RM-05	1	55	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	(GA)14
3	RM-44	8	55	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	(GA)16
4	RM-70	7	55	GTGGACTTCATTTCAACTCG	GATGTATAAGATAGTCCC	(ATT)33
5	RM-72	8	55	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	(TAT)5C (ATT)15
6	RM-103	6	55	CTTCCAATTCAGGCCGGCTGGC	CGCCACAGCTGACCATGCATGC	(AG)16
7	RM-105	9	63	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	(CCT)6
8	RM-110	2	52	TCGAAGCCATCCACCAACGAAG	TCCGTACGCCGACGAGGTCGAG	(GA)15
9	RM-124	4	67	ATCGTCTGCGTTGCGGCTGCTG	CATGGATCACCGAGCTCCCCCC	(TC)10
10	RM-152	8	53	CGATAAAACAATGAG	TCGGTCCTAACTAAGGG	(GCC)10
11	RM-154	2	61	TATATGCCAAGACGGATG	GGCCAACGTGTGTATG	(GA)21
12	RM-161	5	61	TGCAGATGAGAAGCGGCGCCTC	TGTGTCATCAGACGGCGCTCCG	(AG)20
13	RM-171	10	55	AGCTAGGGCTAACGAAC	CCTGGTCAGCCTCTTTC	(GATG)5
14	RM-174	2	57	AGCGACGCCAAGACAAGTCGG	TCCACGTCGATCGACACGACGG	(AGG)7 (GA)10
15	RM-222	10	57	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG	(CT)18
16	RM-229	11	55	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT	(TC)11 (CT)5C3 (CT)5
17	RM-235	12	55	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC	(CT)24/ (GA)24
18	RM-242	9	55	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	(CT)26
19	RM-252	4	55	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	(CT)19 /GA)19
20	RM-257	9	57	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	(CT)24
21	RM-271	10	55	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC	(GA)15
22	RM-277	12	55	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	(GA)11
23	RM-334	5	55	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	(CTT)20
24	RM-489	3	55	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG	(ATA)8
25	RM-287	11	55	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	(GA)21

P.N: Primer Name**; C.N**: Chromosome Number**; P.A.T**: Primer Annealing Temperature (T); **R.M**: Repeat Motif

Results And Discussion

Twenty-five microsatellite markers (Table 1) distributing all twelve rice chromosomes were utilized to access genetic diversity among 48 putative EMS mutants of super basmati (Table 3). Similar number of microsatellite markers previously used as marker set for genetic analysis of rice (Pal et al. 2004; Rahman et al. 2010; Kumar and Bhagwat 2012; Allhgholipour et al. 2014).

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Table 3

Identification of super basmati mutants based upon absence or presence of uniq

Super basmati	M.N	RM 110	RM 161	RM 242	RM 277	RM 287	RM 72	RM103	RM105	RM5	RM 252	RM 1	RM 154	RM152	RM 222
	B.P	80; 300	150	200;225	100;125	100;125	200; 225	425	250;300	225;250	250	75; 100; 200; 300	200;425 450;550 1000	150;250	100; 200; 300
C.D	C.N	2	5	9	12	11	8	6	9	1	4	1	2	8	10
0.25	M48	A	A:	A	A	A	A	A	A	A	A	A	P	A	A
	M105	A	A	A	A	A	A	A	A	A	A	A	P	A	A
	M43	A	A	A	A	A	A	A	A	A	A	P	P	P	A
	M88	A	P	A	A	A	A	A	A	A	A	P	P	A	A
	M56	A	P	A	A	A	A	A	A	A	A	P	P	P	A
	M116	A	P	A	P	A	A	A	P	A	A	P	A	A	A
	M96	A	P	A	P	A	A	A	A	A	A	P	P	P	P
	M109	A	A	A	P	A	A	A	P	A	A	P	P	P	P
	M114	A	A	A	P	A	A	A	A	A	A	P	A	P	A
	M118	A	A	A	P	A	A	A	A	A	A	P	P	A	A
	M100	A	A	A	P	A	A	P	A	A	A	P	P	P	A
	M101	A	A	A	A	A	A	A	A	A	A	P	P	A	A
0.5	M104	A	A	A	P	A	A	A	A	A	A	P	P	P	A
	M45	A	A	A	A	A	A	A	A	A	A	P	P	P	A
	M117	P	A	A	A	A	A	A	A	A	A	P	P	A	A
	M32	A	A	A	A	A	A	A	A	A	A	P	P	P	A
	M35	A	P	A	P	A	A	A	A	A	A	A	A	P	P
	M42	A	A	A	P	A	A	A	P	A	A	P	A	P	P
	M51	A	A	A	P	A	A	A	A	A	P	P	P	P	P
	M115	A	A	A	P	A	A	A	P	A	A	A	P	P	A
	M121	A	A	A	A	A	A	A	P	A	A	A	P	P	A
	M34	A	P	A	A	A	A	A	A	A	P	P	A	A	A
	M124	A	P	A	A	A	A	A	A	A	A	P	P	A	A
0.75	M63	A	P	A	A	A	A	A	A	A	A	P	A	A	A
	M95	A	A	A	A	A	A	A	A	A	P	P	P	A	A
	M38	A	A	A	A	A	A	A	A	A	A	P	P	A	A
	M22	P	A	A	P	A	A	A	A	A	A	P	P	P	P
	M73	P	A	A	P	A	A	A	A	A	A	A	P	P	A
	M70	P	A	A	P	A	A	A	A	P	A	P	P	A	A
	M93	P	A	A	P	A	A	A	A	P	A	A	P	A	A
	M21	P	A	A	P	A	A	A	A	P	A	P	P	P	A
	M60	P	A	A	A	A	A	A	A	P	A	P	P	P	A
	M52	P	A	A	A	A	A	A	A	P	A	P	P	P	A
	M61	P	A	A	A	A	A	A	A	P	A	P	P	P	A

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B.P: Base pairs; **A**: Absence of unique bands; **P**: Presence of unique bands

Super basmati	M.N	RM 110	RM 161	RM 242	RM 277	RM 287	RM 72	RM103	RM105	RM5	RM 252	RM 1	RM 154	RM152	RM 222
	B.P	80; 300	150	200;225	100;125	100;125	200; 225	425	250;300	225;250	250	75; 100; 200; 300	200;425 450;550 1000	150;250	100; 200; 300
1	M24	P	A	A	A	A	A	A	A	P	A	P	P	A	A
	M125	P	P	A	A	A	A	A	A	A	A	P	P	A	A
	M17	P	A	A	P	A	A	A	A	P	A	P	P	A	P
	M92	P	A	A	P	P	A	A	A	A	P	P	P	A	P
1.25	M123	P	A	A	A	A	A	A	A	A	P	P	P	A	A
	M12	P	P	A	A	A	A	A	A	A	P	A	P	A	A
	M25	P	P	A	A	A	A	A	A	A	A	A	P	A	A
	M78	A	A	A	A	A	A	A	A	A	A	P	P	A	A
	M129	P	A	A	A	A	A	A	A	A	A	P	P	A	A
	M3	A	A	P	A	A	P	A	P	A	A	A	P	P	A
1.5	M71	P	A	A	A	A	A	A	A	A	A	A	P	P	A
	M72	P	A	A	A	A	A	A	A	P	A	P	P	A	A
	M65	P	A	A	A	A	A	A	A	P	P	P	P	A	P

C.D: Chemical Doses; **M.N**: Markers Name; **B.P**: Base pairs; **A**: Absence of unique bands; **P**: Presence of unique bands

A broad range of genetic variability was discovered among different mutants of super basmati for 23 SSR markers (Table 2). The remaining two markers (RM5 and RM103) were monomorphic (Table 2). A sum of 91 alleles was identified of which nine alleles (9.89%) were considered as monomorphic and remaining 82 alleles (90.10%) were established to be polymorphic with average of 85.6 bands per primer (Table 2). Seventeen SSR primers produced 100 polymorphic bands. The level of polymorphism was calculated by the PIC value of each marker loci. The PIC value differed from locus to locus and ranged from 0.04 (RM5) to 0.88 (RM44) with the average of 0.44 for each locus (Table 2). The highest PIC value was examined in RM44 (0.88) followed by RM154 (0.87), RM1 (0.79), RM252 (0.71), RM334 (0.64), RM487 (0.63), RM110 (0.59) and RM257 (0.59) based upon SSRs data of mutant genotypes (Table 2). PIC value is a mirror of allelic variability among and within varieties which was not always higher for every tested SSRs loci (Kumar et al. 2012; Kumar and Bhagwat 2012; Allhgholipour et al. 2014). It has been reported by (Yesmin et al. 2014) that the higher PIC value of marker designated the higher polymorphism which helped to select best marker in phylogenic analysis. According to report of (Rahman et al. 2010; Allhgholipour et al. 2014), low PIC value may be the product of closely related genotypes as well as the high PIC value might be the effect of diverse genotypes. The high PIC values suggested that microsatellites were polymorphic and suitable to detect the genetic variation in rice cultivar at DNA level. Markers were categorized as informative when PIC value was greater than 0.5 (Taheri et al. 2016; Kumar et al. 2012). The range of PIC value was in series of PIC value of existing outcome (Jain et al. 2004; Thomson et al. 2009; Pervaiz et al. 2010; Matin et al. 2012). The PIC value for all SSRs loci ranged from 0.36–0.98 in rice genotypes according to Kumar et al. (2010) reported that eight SSRs primers produced 100% polymorphic bands from total of 20 SSRs primers. According to (Kumar and Bhagwat 2012), all SSRs primer were distributed overall 12 chromosome of rice were found to be polymorphic with PIC range of 0.125 (RM208) and 0.68 (RM1) across 20 dwarf, semi dwarf mutant and wild (WL112) of rice variety. Mean value of alleles (3.29) and PIC (0.47) were comparable to the finding of current study. The present PIC range is also comparable with that previously reported by Patel et al. (2014) with value of 0.36–0.78 among colored and white rice genotypes. They also reported that nine SSRs marker were found to be polymorphic from 14 SSRs markers with 129 bands. According to study of Sahu et al. (2017) 83 loci (24.02%) revealed polymorphism out of 343 loci between rice genotypes. Microsatellites have 28.98% (51) polymorphic ratio. The highest polymorphism about 41.67% was occurred on second chromosome as well as twelve chromosome numbers had lowest polymorphic % age of 6.67. The PIC range of the recent conclusion was lower than previous observation reported in rice (Jain et al. 2003) and higher then (Singh et al. 2004; Johsi and Behera 2006; Xu et al. 2004; Brondani et al. 2006; Rahman et al. 2008; Rahman et al. 2010) respectively due to different selected SSRs markers as well as diverse genotypes of rice.

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Table 2
Genetic polymorphism revealed by SSRs (PCR) analysis among genotypes of super basmati

S.N	M*	C.N*	P.R*	MAF*	A.N*	TNAP*	TNL*	TNPA*	TNMA*	P (%)*	G.D*	H*	PIC*
1	RM110	2	80–300	1.36	4	62	2	4	0	100	0.7408	1.24	0.586
2	RM161	5	150–180	0.89	2	85	1	2	0	100	0.1958	0.22	0.1766
3	RM242	9	200–225	0.51	2	48	1	2	0	100	0.4998	0.98	0.3749
4	RM277	12	125–150	0.67	2	65	1	2	0	100	0.4422	0.66	0.3444
5	RM287	11	100–125	0.5	2	49	1	1	1	50	0.5	1	0.375
6	RM72	8	200–225	0.5	2	49	1	1	1	50	0.5	1	0.375
7	RM105	9	90–200	1.9	4	95	2	2	2	50	0.18	0.09	0.1638
8	RM5	1	94–250	1.98	4	110	2	4	0	100	0.0396	0.04	0.0392
9	RM252	4	75–300	2.3	6	96	3	6	0	100	0.8764	1	0.7075
10	RM1	1	75–600	2.42	6	72	3	6	0	100	0.934	0.68	0.7879
11	RM154	2	175–1000	3.39	8	112	3	8	0	100	1.005	0.94	0.8641
12	RM44	8	80–900	4.42	10	270	5	10	0	100	0.9964	0.12	0.8781
13	RM222	10	140–275	1.42	4	64	3	4	0	100	0.756	0.96	0.6012
14	RM152	8	80–700	2.4	4	68	2	4	0	100	0.7244	0.96	0.5747
15	RM257	9	150–500	1.4	4	149	2	4	0	100	0.7376	1.16	0.5852
16	RM174	2	200–300	1.65	3	66	1	2	1	66.66	0.455	0.7	0.3515
17	RM124	4	250–275	1.46	4	63	1	4	0	100	0.5948	0.96	0.4654
18	RM171	10	300–400	0.82	2	54	1	2	0	100	0.2952	0.2	0.2516
19	RM229	11	100–125	0.91	2	78	1	2	0	100	0.1638	0.18	0.1504
20	RM271	10	100–125	0.78	2	87	1	2	0	100	0.3432	0.32	0.2843
21	RM334	5	100–200	1.33	4	74	2	3	1	75	0.807	1.26	0.635
22	RM487	3	150–300	1.43	4	129	2	3	1	75	0.779	0.9	0.6206
23	RM70	7	130–160	0.5	2	79	1	1	1	50	0.5	1	0.375
24	RM103	6	240–425	0.96	2	50	1	1	1	50	0.0768	0.08	0.0739
25	RM235	12	100–132	0.68	2	66	1	2	0	100	0.4352	0.64	0.3405
Total				36.58	91	2140	44	82	9	2166.66	13.578	17.29	10.982
Mean				1.4632	3.64	85.6	1.76	3.28	0.36	86.66	0.5431	0.691	0.439

M*: Markers; **C.N***: Chromosome Number; **P.R***: Product range; **MAF***: Major allele Frequency; **A.N***: Allele Number; **TNAP***: Total number of allele in population; **TNL***: Total number of loci; **TNPA***: Total number of polymorphic allele; **TNMA***: Total number of Monomorphic allele; **P***: Polymorphism %; **G.D***: Genetic Diversity; **H***: Heterzygosity; **PIC***: Polymorphism Information Content

PIC value showed positive signification correlation with allele numbers in current study. The maximum numbers of alleles was ten in RM44 marker with the highest PIC value of 0.88. Markers (RM161, RM242, RM277, RM287, RM72, RM171, RM229, RM271, RM70, RM103 and RM235) have minimum two numbers of alleles (Table 2) with mean value of 3.64 alleles/locus. The overall amplified product range was from 75 bp (RM1; RM252) to 1000 bp (RM154). SSR markers demonstrated many bands that common among mutants and wild of super basmati. Correlation of allele number and their PIC value might also depends upon repeat number and repetitive sequence of microsatellites (Yu et al. 2003; Temnykh et al. 2000; Temnykh et al. 2001; Ni et al. 2002). The present range and mean value of allele numbers were similar to those reported by (Pal et al. 2004) among basmati and non-basmatic rice varieties. They proposed that range of alleles was 1–8 with mean value of 3.51 alleles/locus. RM252 marker showed the high PIC value (0.8) with the maximum eight allele numbers. The mean PIC vale for all SSRs loci was 0.4 that were similar to the current findings in rice. According to conclusion of (Rahman et al. 2008), marker (RM1 and RM334) were found to be polymorphic among rice varieties of Bangladesh with the PIC value of 0.862 and 0.863 respectively.

Interestingly, several mutant loci exhibited presence or absence of discrete bands by different set of SSR markers (Table 3). These unique alleles were not produced in wild used. Every mutant genotypes distinguished from each other either alone or combined set of SSR primers. Genotypic variations were existed in putative mutants at a particular base pair of definite chromosomes (Table 3) because the selected primers sequence located from particular chromosomes. These genetic modifications occurred either in the form of discrete alleles or non-amplified PCR product due to mutation in primer sequence. For instance, phenotypic putative mutants (xantha and albino; M56) (Fig. 1) showed genetic changes in various microsatellites with respect to particular base pair such as RM161 (150 bp), RM1 (75 bp; 100 bp; 200 bp; 300 bp), RM152 (150 bp; 250 bp), RM44 (150 bp; 175 bp; 200 bp; 250 bp; 275 bp; 400 bp and 500 bp), RM257 271(100–125 bp) and RM487(150 bp; 200 bp; 250 bp) on 5, 1, 8, 8, 9, 11, 10 and 3 chromosome of rice

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respectively (Table 3). Correspondingly, microsatellites (RM110, RM154, RM152; RM44 and RM334) showed molecular variations on chromosome 2, 2, 8, 8 and 5 with range of 75-1000 bp (Table 3) in viridis and albino mutants (M71) of super basmati respectively (Fig. 1). The current outcomes pointed out that wild and mutants were genetically dissimilar to each other. A major purpose of molecular analysis is to sort out a marker which can discriminate a desired genotype from control and rest of other genotypes used. Kumar and Bhagwat (2012) described that distinct alleles were identified for 18 rice mutants either in a single or combination of two SSRs markers. A sum of 19 unique alleles was detected from 17 genotypes of weedy rice with the size range of 200 to1300bp (Choudhary et al. 2011). Results of Gealy et al. (2009) explained that presence or absence of discrete SSRs loci was exploited to differentiate white rice from US weedy rice. According to Yu et al. (2005), gene flow was caused by existing of unique bands in weedy rice. It was also reported that regular occurrence of gene flow from domesticated to weedy rice was happened due to presence of discrete alleles (Chen et al. 2004). Ethyl methane sulphonate (EMS) has been reported to be the most potent in producing chlorophyll mutation among chemical mutagens in rice (Kawai and Sato 1965) and other crops (Jacob 1965). Chlorophyll mutation were group into three classes including albino (white), xantha (yellow), viridis (light green) (Cheema and Atta 2003). Detectable mutation is valuable in mutant populations as clue of variability (Wu et al. 2005).

In the current study, putative mutants that decreased or increased in their agronomic features showed less or more in the similar cluster (Fig. 2). Dendrogram divided into four main clusters with sub-clustering. Cluster one, two, three and four has 2, 38, 2, 6 genotypes of super basmati respectively (Fig. 2). Phylogenic study exposed a pair-wise similarity ranging from 40% (M45 and M116) to 96% (M105 and W1) at chemical dose of (0.25% and 0.5%) and (0.25% and 0%) respectively (Fig. 2). Previous finding of (Kumar et al. 2010) also supported the present study that similarity coefficient varied from 0.40–0.96 among genotypes of rice. Similarity index varied from 0.56–0.95 among red and white rice genotypes for SSRs markers that described by (Patel et al. 2014). According to finding of (Kumar and Bhagat 2012), similarity index was 35% as establishment of genetic diversity among dwarf mutant genotypes. It was also reported by (Herrera et al. 2008) that mean similarity was 0.48 among eighteen genotypes of all SSRs loci using microsatellite markers. Results of (Naeem et al. 2015) revealed a pair wise similarity range from 0.39–0.89 was observed after mutation in rice varieties. The recent results are in synchronization with those of (Arif et al. 2005) who examined an increase or decrease in genetic diversity of mutated genotypes as compared to their control genotypes. Jaccard's genetic similarity ranged from 0.04–0.92 according to (Tu et al. 2007; Wong et al. 2009). According to finding of (Pal et al. 2004), genetic relationship among 13 basmati and non-basmati rice varieties demonstrated that these genotypes similar to each other by 0.65–0.94 pair-wise similarity index. The high degree of diversity was observed among rice cultivar with 21–86% similarity that previously reported by (Matin et al. 2012). According to statement of (Oladosu et al. 2015; Tu et al. 2007; Wong et al. 2009), difference between the highest and lowest value of similarity coefficient indicated a high level of genetic diversity among rice genotypes depend upon different set of SSRs markers and diverse selection of rice genotypes. The high genetic diversity was observed in dendrogram coefficient among mutant rice varieties due to effect of induced mutation (Oladosu et al. 2015).

It was also suggested from current study that more diverse mutants existed using high EMS doses. Phenotypic and genotypic data correlated to each other indicating that EMS might be brought desired genetic variability in putative mutants of super basmati. These genetic changes might be useful in selection of desired mutant genotype of rice in future. According to (Babaei et al. 2010; Domingo et al. 2007; Oladosu et al. 2015), induced genetic variability was verified through phenotypic and genetic study of quantitative traits in rice. Moreover, results of (Elayaraja et al. 2005; Luzi-Kihupi et al. 2008) showed that induced mutation could produce a significant quantity of genetic changes for development as well as diversification of crop. These genetic changes might also be helpful in improvement of mutant lines with improved traits, which are deficient in wild (Shehata et al. 2009).

Conclusion

The considerable amount of phenotypic variability identified in the mutated rice population (73) and based on these observations the genetic variability was also identified by genetic analysis of SSRs markers within mutant lines of super basmati. These genetic changes indicated that EMS might be helpful for the development of desired genetic changes in rice. It was also recommended that based on these genetic analysis the functional gene analysis can be done precisely by identifying the causal mutation in the basmati rice population more over current polymorphic SSRs markers could be used for molecular breeding of new basmati rice cultivars.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

"The authors declare that they have no conflict of Interest in the publication."

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Authors' contributions

KH and FSA were involved in planning executing and manuscript write-up, BS, MH and ZQ were contributed in the data analysis.

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References

1. Allhgholipour M, Farshdfar E, Rabiei B (2014) Molecular characterization and genetic diversity analysis of different rice cultivars by microsatellite markers. Genetika 46:187–198
2. Abe A, Kosugi S, Yoshida K, Natsume S, Takagi H, Kanzaki H, Matsumura H, Yoshida K, Mitsuoka C, Tamiru M, Innan H (2012 Feb) Genome sequencing reveals agronomically important loci in rice using MutMap. Nature biotechnology 30(2):174–178
3. Ansari MR, Shaheen T, Bukhari SA, Husnain T (2015) Genetic improvement of rice for biotic and abiotic stress tolerance. Turk J Bot 39:911–919
4. Arif M, Kousar S, Ajwa MA, Arif A, Zafar Y (2005) Genetic diversity among rice genotypes of Pakistan through random amplified polymorphic DNA (RAPD) analysis. Pak J Bot 37:585–592
5. Babaei A, Nematzadeh GA, Avagyan V, Hamidreza S, Petrodi H (2010) Radio sensitivity studies of morpho-physiological characteristics in some Iranian rice varieties (Oryza sativa L.) in M1 generation. Afric J Agr Res 5:2124–2130
6. Bhat TA, Khan AH, Parveen S (2005) Comparative analysis of meiotic abnormalities induced by gamma rays, EMS and MMS in Vicia faba L. J Indian Botanical Soc 84:45–58
7. Botticella E, Sestili F, Hernandez-Lopez A, Phillips A, Lafiandra D (2011) High resolution melting analysis for the detection of EMS induced mutations in wheat Sbellagenes. BMC Plant Biol 11:156
8. Brondani C, Borba TCO, Rangel PHN, Brondani RPV (2006) Determination of genetic variability of traditional varieties of Brazilian rice using microsatellite markers. Genet Mol Biol 9:676–684
9. Cheema AA, Atta BM (2003) Radiosensitivity studies in basmati rice. Pak J Bot 35:197–207
10. Chen LJ, Lee DS, Song ZP, Shu HS, Lu BR (2004) Gene flow from cultivated rice (Oryza sativa) to its weedy and wild relatives. Ann Bot 93:67–73
11. Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch SR, Park WD, Ayers N, Cartinhour S (2000) Diversity of microsatellites derived from genomic libraries and GeneBank sequences in rice (Oryza sativa L.). Theor Appl Genet 100:713–722
12. Choudhary N, Ahuja U, Chawla V, Jain RK, Kumari P, Batan KR (2011) Morphological and molecular variability in weedy rice of Haryana. Asian J Agri Res 5:250–259
13. Condit R, Hubbell SP (1991) Abundance and DNA sequencing of two-base repeat regions in tropical tree genome. Genome 34:66–71
14. Domingo C, Andrés F, Talón M (2007) Rice cv. Bahia mutagenized population: a new resource for rice breeding in the Mediterranean basin. Spanish J Agri Res 5:341–347
15. Elayaraja K, Prakash M, Saravanan K, Kumar SB, Ganesan J (2005) Studies on variability, heritability and genetic advance for certain quantitative characters in mutant population of rice (Oryza sativa L.). Crop Res-Hisar 1:134–137
16. Gealy DR, Agrama HA, Eizenge GC (2009) Exploring genetic and spatial structure of U.S. weedy red rice in relation to rice relatives worldwide. Weed Sci 57:627–643
17. Guo Y, Cheng B, Hong D (2010) Construction of SSR linkage map and analysis of QTLs for rolled leaf in japonica rice. Rice Sci 17:28–34
18. Habib M, Bhat KA, Bhat MA, Parray GA, Dar ZA, Zaffar G, Sabina A (2013) Genetic diversity of important rice cultivars of Kashmir valley using microsatellite markers. Afric J Biotechnol 12:658–664
19. Hameed K, Khan MS, Sadaqat HA, Awan FS (2019) Phenotypic characterization of super basmati ethyl methane sulfonate (EMS) induced mutants. Pakistan Journal of Agricultural Sciences. Apr 1;56(2)
20. Herrera TG, Duque DP, Alemeida IP, Numez GT, Pieters AJ, Masrtinez CP, Tohme JM (2008) Assessment of genetic diversity in Venezuelan rice cultivar using simple sequence repeats markers. Electron J Biotechnol 11. 10.2225/vol11-wssue5-fulltext-6
21. Jacob M (1965) Mutagenesis of some monofunctional alkylating agents. In: Arabidopsis Res Rep Int Symp University Gottingen
22. Jain S, Jain RK, McCouch SR (2004) Genetic analysis of Indian aromatic and quality rice (Oryza sativa L.) germplasm using panels of fluorescently-labeled microsatellite markers. Theor Appl Genet 109:965–977
23. Jain S, Mitchell SE, Jain RK, Kresovich S, McCouch SR (2003) DNA fingerprinting and phylogenetic analysis of Indian aromatic high quality rice germplasm using panels of fluorescent labeled microsatellite markers. In: Brar DS, Hardy B (eds) Advance in rice genetics, Khush, GS. IRRI, Philippine, pp 162–165
24. Joshi RK, Behera L (2006) Identification and differentiation of indigenous non-Basmati aromatic rice genotypes of India using microsatellite markers. Afric J Biotechnol 6(4):348–354
25. Kawai T, Sato H (1965) Studies on artificial induction of mutations in rice by chemicals 1. Chlorophyll mutations by ethyl imine and ethylene oxide treatments. Bull Nat Inst Agri Sci 13:133–162

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26. Kumar GR, Sakthivel K, Sundaram RM, Neeraja CN, Balachandran SM, Rani NS, Viraktamath BC, Madhav MS (2010) Allele mining in crops: prospects and potentials. Biotechnol Adv 28:451–461
27. Kumar R, Singh AK, Kumar A, Radha (2012) Evaluation of genetic diversity in rice using simple sequence repeats (SSR) markers. Afric J Biotechnol 11:14956–14995
28. Kumar V, Bhagwat SG (2012) Microsatellite (SSRs) based assessment of genetic diversity among the semi-dwarf mutants of elite rice variety WL112. Int Plant Breed Genet. doi:10.3923/IJPBG
29. Li W, Wu J, Weng S, Zhang D, Zhang Y, Shi C (2010) Characterization and fine mapping of the glabrous leaf and hull mutants (gl1) in rice (Oryza sativa L.). Plant cell Rep 29:617–627
30. Liu K, Muse SV (2005) PowerMarker: an intregrated analysis environment for genetic marker analysis. Bioinformatics 21:2128–2129
31. Liu S, Cantrell RG, McCarty JC, Stewart JMD (2000) Simple sequence repeat-based assessment of genetic diversity in cotton race stock accessions. Crop Sci 40:1459–1469
32. Luz VKD, Silveira SFDS, Fonseca GMD, Groli EL, Figueiredo RG, Baretta D, Kopp MM, Junior AMDM, Maia LCD, Oliveira ACD (2016) Identification of variability for agronomically important traits in rice mutant families. Bragantia Campinas 75:41–50
33. Luzi-Kihupi A, Shao-Mwalyego F, Zakayo JA, Mkuya M (2008) Mwangaza-a new early maturing, RYMV resistant rice mutant released in the United Republic of Tanzania. Plant Mut Rep 2:13–15
34. Matin S, Ashrafuzzaman M, Islam MdM, Sikdar SU, Zobayer N (2012) Molecular marker based (SSR) genetic diversity analysis in deep water rice germplasms of Bangladesh. Int J Biosci 2:64–72
35. McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.). DNA Res 9:199–207
36. Naeem M, Ghouri F, Shahid MQ, Iqbal M, Baloch FS, Chen L, Allah S, Babar M, Rana M (2015) Genetic diversity in mutated and non mutated rice varieties. Genet Mol Res 14:17109–17123
37. Nei M, Li WH (1979) Mathematical model of studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:s5269–5276
38. Ni J, Colowit PM, Mackill DJ (2002) Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Sci 42:601–607
39. Oladosu Y, Rafii MY, Abdullah N, Malek MA, Rahim HA, Hussin G, Ismail MR, Latif MA, Kareem I (2015) Genetic variability and diversity of mutant rice revealed by quantitative traits and molecular markers. Agrociencia 9:249–266
40. Page RDM (1996) TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358
41. Pal S, Jain S, Saini N, Aarti, Jain RK (2004) Identification of microsatellite markers for differentiating some basmati and non basmati rice varieties. Indian J Biotechnol 3:519–526
42. Patel S, Ravikiran R, Chakraborty S, Macwana S, Sasidharan N, Trivedi R, Aher B (2014) Genetic diversity analysis of colored and white rice genotypes using microsatellite (SSR) and insertion-deletion (INDEL) markers. J Food Agri 26:497–507
43. Pervaiz ZH, Rabbani MA, Khaliq I, Pearce SR, Malik SA (2010) Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice landraces. Electron J Biotechnol 13:1–12
44. Rahman L, Islam MN, Rahman MS, Islam MS, Shah-e-Alam M, Bashar MK (2008) Characterization of 94 rice (Oryza sativa L.) varieties of Bangladesh based on microsatellite loci. Bangladesh J Agri Sci 35:97–112
45. Rahman MS, Sohag MKH, Rahman L (2010) Microsatellite based DNA fingerprinting of 28 local rice (Oryza sativa L.) varieties of Bangladesh. J Bangaladesh Agri Univ 8:7–17
46. Sahu VK, Sunil, Nair K, Vishwakarma AK, Verulkar SB, Chandel G (2017) QTL hotspots detected for yield contributing traits in rice (Oryza sativa l.) using composite interval mapping analysis. Biosci Biotechnol Res Asia 14:329–341
47. Senior ML, Murphy JP, Goodman MM, Stuber CW (1998) Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci 38:1088–1098
48. Shamim MZ, Manzar H, Sharma VK, Kumar P (2016) Microsateliite marker based characterization and divergence analysis among rice varieties. Indian J Biotechnol 15:182–189
49. Shehata SM, Ammar MH, Abdelkalik AF, Zayed BA (2009) Morphological, molecular and biochemical evaluation of Egyptian jasmine rice variety and its M5 derived mutants. Afr J Biotechnol 8:6110–6116
50. Singh N, Choudhury DR, Singh AK, Kumar S, Srinivasan K, Tyagi RK, Singh NK, Singh R (2013) Comparison of SSR and SNP markers in estimation of genetic diversity and population structure of indian rice varieties. PLoS ONE 8:e84136
51. Singh RK, Sharma RK, Singh AK, Singh VP, Singh NK, Tiwari SP, Mohapatra T (2004) Suitability of mapped sequence tagged microsatellite site markers for establishing distinctness, uniformity and stability in aromatic rice. Euphytica 135:135–143
52. Smith JSC, Kresovich S, Hopkins MS, Mitchell S, Dean RE, Woodman WL, Lee M, Porter K (2000) Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. Crop Sci 40:226–232
53. Sweeney MT, Thomson MJ, Cho YG, Park YJ, Williamson SH, Bustamante CD, McCouch SR (2007) Global dissemination of a single mutation conferring white pericarp in rice. PLoS Genet 3:e133
54. Taheri S, Abdullah TL, Ahmad Z, Sahebi M, Azizi P (2016) Phenotypic and molecular effects of chronic gamma irradiation on curcuma alismatifolia. Eur J Horti Sci 81:137–147

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55. Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch SR (2001) Computational and experimental analysis of microsatellites in rice (Oryza sativa L.), frequency, length variation, transposon associations and genetic marker potential. Genome Res 11:1441–1452
56. Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, shiiand T, McCouch SR (2000) Mapping and genome organization of SSRs sequences in rice (Oryza sativa L.). Theor Appl Genet 100:697–712
57. Thomson MJ, Polato NR, Prasetiyono J, Trijatmiko KR, Silitonga TS, McCouch SR (2009) Genetic diversity of isolated populations of Indonesian landraces of rice (Oryza sativa L.) collected in East Kalimantan on the island of Borneo. Rice 2:80–92
58. Tu M, Lu BR, Zhu Y, Wang Y (2007) Abundant within-varietal genetic diversity in rice germplasm from Yunnan Province of China revealed by SSR fingerprints. Biochem Genet 45:789–801
59. Wattoo JI, Aslam K, Shah SM, Shabir G, Sabar M, Naveed SA, Waheed R, Samiullah, Muqaddasi QH, Arif M (2013) Ethyl methane sulphonate (EMS) induced mutagenic attempts to create genetic variability in basmati rice. Afric J Water Conserve Sustain 1:045–048
60. Wong SC, Yiu PH, Bong STW, Lee HH, Neoh PNP, Rajan A (2009) Analysis of Sarawak Bario rice diversity using microsatellite markers. Am J Agri Biol Sci 4:298–304
61. Wu JL, Wu C, Lei C, Baraoidan M, Bordeos A, Madamba MRS, Ramos-Pamplona M, Mauleon R, Portugal A, Ulat VJ, Bruskiewich R, Wang G, Leach J, Khush G, Leung H (2005) Chemical and irradiation-induced mutants of indica rice IR64 for forward and reverse genetics. Plant Mol Biol 59:85–97
62. Xu YB, Beachell H, McCouch SR (2004) A marker-based approach to broadening the genetic base of rice in the USA. Crop Sci 44(6):1947–1959
63. Yeh FC, Yang RC, Boyle T (1999) POPGENE VERSION 1.31: Microsoft window-based free software for population genetic analysis. ftp://ftp.microsoft.com/softlib/HPGL.EXE
64. Yesmin N, Elias SM, Rahman Md S, Haque T, Hasan AKMM, Seraj ZI (2014) Unique genotypic differences discovered among indigenous Bangladeshi rice land races. Int J Genomics ID:210328
65. Yu GQ, Bao Y, Shi CH, Dong CQ, Ge S (2005) Genetic diversity and population differentiation of Liaoning weedy rice detected by RAPD and SSR markers. Biochem Genet 43:261–270
66. Yu SB, Xu WJ, Vijayakumar CHM, Ali J, Fu BY, Xu JL, Jiang YZ, Marghirang R, Domingo J, Aqino C, Virmani SS, Li ZK (2003) Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program. Theo Appl Genet 108:131–140
67. Zhao K, Wright M, Kimball J, Eizenga G, McClung A, Kovach M, Tyagi W, Ali ML, Tung CW, Reynolds A, Bustamante C, McCouch SR (2010) Genomic diversity and introgression in O. sativa reveal the impact of domestication and breeding on the rice genome. PLoS One 5:e10780
68. Zheng KL, Huang N, Bennett J, Khush GS (1995) PCR-based phylogenetic analysis of wide compatibility varieties in Oryza sativa L. Theor Appl Genet 88:65–69
69. Zhu Y, Hu J, Han R, Wang Y, Zhu S (2011) Fingerprinting and identification of closely related wheat (Triticum aestivum L.) cultivars using ISSR and fluorescence-labeled TP-M13-SSR markers. Afric J Crop Sci 5:846–850

Figures

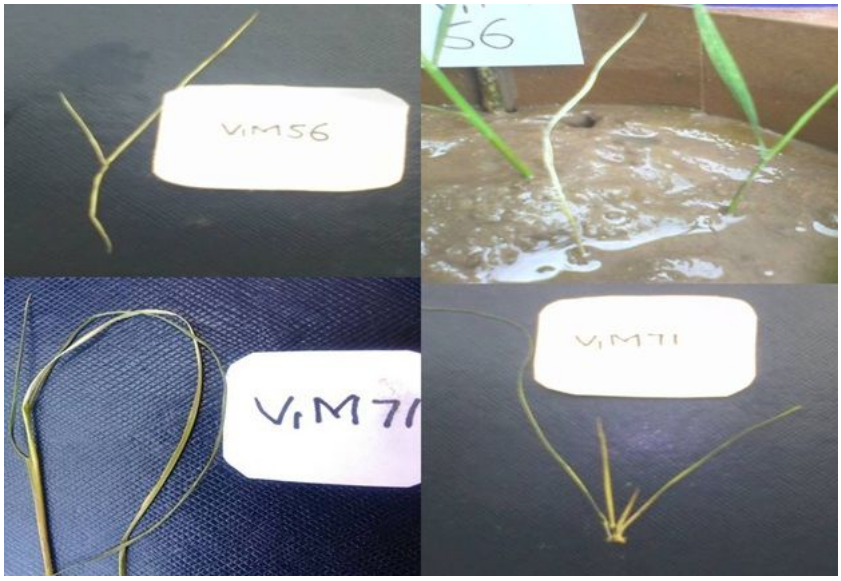


Figure 1

Effect of chemical treatment on mutant seedling (M56 and M71) of super basmati. V1: wild type super basmati M: Mutant. The general variation at premature phase of growth, included slow growth, plant architecture, pigmentation cholorphyll mutation (albino, xanatha and viridis), dwarfism.

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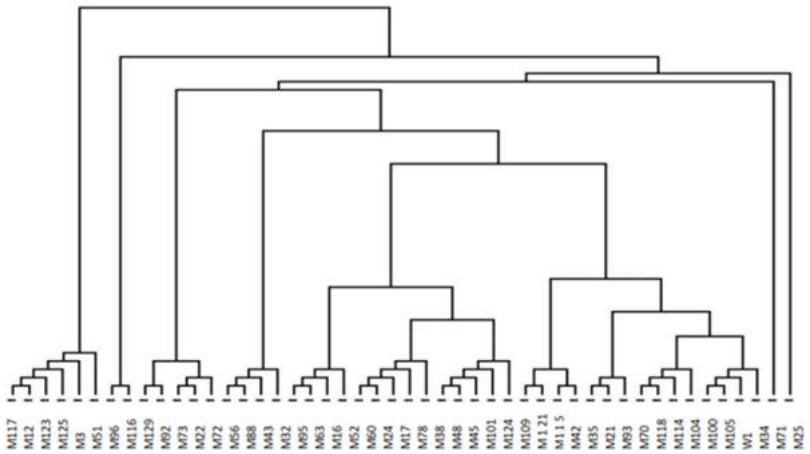


Figure 2

Dendrogram among mutant genotypes of super basmati based on Nei's Genetic Distance: Method = UPGMA Modified from NEIGHBOR procedure of PHYLIP Version 3.5. M: Mutant; W1: Wild of super basmati; V1: Variety one of super basmati

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