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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 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	GCGAAAAC ACAATGCA AAAA	GCGTTGG TTGGACCT GAC	(GA)26
2	RM-05	1	55	TGCAACTT CTAGCTGC TCGA	GCATCCGA TCTTGATG GG	(GA)14
3	RM-44	8	55	ACGGGCAA TCCGAACA ACC	TCGGGAA AACCTACC CTACC	(GA)16
4	RM-70	7	55	GTGGACT TCATTTCA ACTCG	GATGTATA AGATAGTC CC	(ATT)33
5	RM-72	8	55	CCGGCGAT AAAACAAT GAG	GCATCGGT CCTAACTA AGGG	(TAT)5C (ATT)15
6	RM-103	6	55	CTTCCAAT TCAGGCC GGCTGGC	CGCCACAG CTGACCAT GCATGC	(AG)16
7	RM-105	9	63	GTCGTCG ACCCATCG GAGCCAC	TGGTCGA GGTGGGG ATCGGGTC	(CCT)6
8	RM-110	2	52	TCGAAGCC ATCCACCA ACGAAG	TCCGTACG CCGACGA GGTCGAG	(GA)15
9	RM-124	4	67	ATCGTCTG CGTTGCG GCTGCTG	CATGGATC ACCGAGCT CCCCCC	(TC)10
10	RM-152	8	53	CGATAAAA CAATGAG	TCGGTCCT AACTAAGG G	(GCC)10
11	RM-154	2	61	TATATGCC AAGACGGA TG	GGCCAAC GTGTGTAT G	(GA)21
12	RM-161	5	61	TGCAGATG AGAAGCG GCGCCTC	TGTGTCAT CAGACGG CGCTCCG	(AG)20

P.N: Primer Nam**e; C.N**: Chromosome Numbe**r; P.A.T**: Primer Annealing Temperature (T); **R.M**: Repeat Motif

S.N	P.N	C.N	P.A.T	Primer Sequence (F)	Primer Sequence (R)	R.M
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

P.N: Primer Nam**e; C.N**: Chromosome Numbe**r; P.A.T**: Primer Annealing Temperature (T); **R.M**: Repeat Motif

S.N	P.N	C.N	P.A.T	Primer Sequence (F)	Primer Sequence (R)	R.M
25	RM-287	11	55	TTCCCTGT TAAGAGAG AAATC	GTGTATTT GGTGAAA GCAAC	(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).

Table 3

Identification of super basmati mutants based upon absence or presence of unique bands on chromosomes at particular base pairs

Super basmati	M.N	RM 110	RM 161	RM 242	RM 277	RM 287	RM 72	RM 103	RM 105	RM 5	RM 252	RM 1	RM 154	RM 152	RM 222	RM 44	RM 257	RM 174	RM 124	RM 171	RM 229	RM 271	RM 334	RM 487	RM 70	RM 235
	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	150; 175; 200; 250; 275; 400; 500	200; 250	200; 250	225; 250	100; 150	100; 125	100; 125	100; 150	150; 200; 250	130; 160	125
C.D	C.N	2	5	9	12	11	8	6	9	1	4	1	2	8	10	8	9	2	4	10	11	10	5	3	7	12
0.25	M48	A	A:	A	A	A	A	A	A	A	A	A	P	A	A	P	A	P	A	A	A	A	A	A	A	A
	M105	A	A	A	A	A	A	A	A	A	A	A	P	A	A	P	P	A	A	A	A	A	A	A	A	A

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

	M43	A	A	A	A	A	A	A	A	A	A	P	P	P	A	P	A	P	A	A	A	P	P	P	A	A
	M88	A	P	A	A	A	A	A	A	A	A	P	P	A	A	P	A	A	A	A	A	P	A	P	A	A
	M56	A	P	A	A	A	A	A	A	A	A	P	P	P	A	P	P	A	A	A	P	P	A	P	A	A
	M116	A	P	A	P	A	A	A	P	A	A	P	A	A	A	P	P	A	A	P	P	P	P	A	A	A
	M96	A	P	A	P	A	A	A	A	A	A	P	P	P	P	P	P	P	A	A	A	A	P	A	A	A
	M109	A	A	A	P	A	A	A	P	A	A	P	P	P	P	P	P	P	A	A	A	A	P	A	A	P
	M114	A	A	A	P	A	A	A	A	A	A	P	A	P	A	P	A	10	P	A	A	A	A	A	A	P
	M118	A	A	A	P	A	A	A	A	A	A	P	P	A	A	P	A	A	P	A	A	P	A	A	A	P
	M100	A	A	A	P	A	A	P	A	A	A	P	P	P	A	P	P	A	A	A	A	P	A	A	A	P
	M101	A	A	A	A	A	A	A	A	A	A	P	P	A	A	P	A	P	A	A	A	P	P	A	A	P
0.5	M104	A	A	A	P	A	A	A	A	A	A	P	P	P	A	P	A	A	A	A	A	A	A	A	A	P
	M45	A	A	A	A	A	A	A	A	A	A	P	P	P	A	P	A	P	A	A	A	A	A	A	A	P

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

	M117	P	A	A	A	A	A	A	A	A	A	P	P	A	A	P	A	A	P	P	A	A	A	A	A	P
	M32	A	A	A	A	A	A	A	A	A	A	P	P	P	A	P	A	A	P	A	A	P	A	P	A	A
	M35	A	P	A	P	A	A	A	A	A	A	A	A	P	P	P	P	A	A	A	A	P	A	A	A	A
	M42	A	A	A	P	A	A	A	P	A	A	P	A	P	P	P	P	A	A	A	P	P	A	A	A	A
	M51	A	A	A	P	A	A	A	A	A	P	P	P	P	P	P	P	A	A	P	P	P	A	A	A	A
	M115	A	A	A	P	A	A	A	P	A	A	A	P	P	A	P	P	A	A	A	P	P	A	A	A	A
	M121	A	A	A	A	A	A	A	P	A	A	A	P	P	A	P	P	A	A	A	P	P	A	A	A	A
	M34	A	P	A	A	A	A	A	A	A	P	P	A	A	A	P	P	A	P	A	P	A	A	A	A	A
	M124	A	P	A	A	A	A	A	A	A	A	P	P	A	A	P	P	P	A	P	A	A	P	A	A	P
0.75	M63	A	P	A	A	A	A	A	A	A	A	P	A	A	A	P	P	P	P	A	A	P	P	A	A	P
	M95	A	A	A	A	A	A	A	A	A	P	P	P	A	A	P	P	P	A	A	A	P	P	A	A	P
	M38	A	A	A	A	A	A	A	A	A	A	P	P	A	A	P	A	P	A	A	A	A	A	A	A	P
	M22	P	A	A	P	A	A	A	A	A	A	P	P	P	P	P	A	A	A	P	A	A	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

	M73	P	A	A	P	A	A	A	A	A	A	A	P	P	A	P	A	A	P	P	A	A	P	P	A	P
	M70	P	A	A	P	A	A	A	A	P	A	P	P	A	A	P	P	A	A	P	P	A	P	A	A	A
	M93	P	A	A	P	A	A	A	A	P	A	A	P	A	A	P	A	A	P	A	P	A	A	A	P	A
	M21	P	A	A	P	A	A	A	A	P	A	P	P	P	A	P	A	A	A	P	A	A	A	A	A	A
	M60	P	A	A	A	A	A	A	A	P	A	P	P	P	A	P	A	A	A	A	A	A	A	P	A	P
	M52	P	A	A	A	A	A	A	A	P	A	P	P	P	A	P	A	A	A	A	A	A	A	A	A	P
	M61	P	A	A	A	A	A	A	A	P	A	P	P	P	A	P	A	P	A	A	A	A	A	A	A	P
1	M24	P	A	A	A	A	A	A	A	P	A	P	P	A	A	P	A	A	P	A	A	A	P	A	A	P
	M125	P	P	A	A	A	A	A	A	A	A	P	P	A	A	P	A	P	A	P	A	P	P	P	A	A
	M17	P	A	A	P	A	A	A	A	P	A	P	P	A	P	P	A	P	A	A	A	P	A	A	A	A
	M92	P	A	A	P	P	A	A	A	A	P	P	P	A	P	P	A	A	A	P	A	A	A	P	A	A
1.25	M123	P	A	A	A	A	A	A	A	A	P	P	P	A	A	P	A	A	P	P	A	A	P	P	A	A
	M12	P	P	A	A	A	A	A	A	A	P	A	P	A	A	P	A	A	A	P	A	A	A	A	A	A

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

	M25	P	P	A	A	A	A	A	A	A	A	A	P	A	A	P	A	A	P	P	A	P	A	A	A	A
	M78	A	A	A	A	A	A	A	A	A	A	A	P	P	A	A	P	A	P	A	A	A	A	A	A	P	A
	M129	P	A	A	A	A	A	A	A	A	A	A	P	P	A	A	P	A	A	A	A	A	A	A	A	P	A	A
	M3	A	A	P	A	A	P	A	P	A	A	A	A	P	P	A	P	A	P	A	A	A	P	A	P	A	A
1.5	M71	P	A	A	A	A	A	A	A	A	A	A	A	P	P	A	P	A	A	A	A	A	A	A	P	A	A	A
	M72	P	A	A	A	A	A	A	A	P	A	P	P	P	A	A	P	A	A	A	P	A	A	P	P	A	A
	M65	P	A	A	A	A	A	A	A	P	P	P	P	P	A	P	P	A	A	A	A	A	A	A	P	A	A	A

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.

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	**RM 110**	2	80–300	1.36	4	62	2	4	0	100	0.7408	1.24	0.586
2	**RM 161**	5	150–180	0.89	2	85	1	2	0	100	0.1958	0.22	0.1766
3	**RM 242**	9	200–225	0.51	2	48	1	2	0	100	0.4998	0.98	0.3749
4	**RM 277**	12	125–150	0.67	2	65	1	2	0	100	0.4422	0.66	0.3444
5	**RM 287**	11	100–125	0.5	2	49	1	1	1	50	0.5	1	0.375
6	**RM 72**	8	200–225	0.5	2	49	1	1	1	50	0.5	1	0.375
7	**RM 105**	9	90–200	1.9	4	95	2	2	2	50	0.18	0.09	0.1638
8	**RM 5**	1	94–250	1.98	4	110	2	4	0	100	0.0396	0.04	0.0392
9	**RM 252**	4	75–300	2.3	6	96	3	6	0	100	0.8764	1	0.7075
10	**RM 1**	1	75–600	2.42	6	72	3	6	0	100	0.934	0.68	0.7879
11	**RM 154**	2	175–1000	3.39	8	112	3	8	0	100	1.005	0.94	0.8641
12	**RM 44**	8	80–900	4.42	10	270	5	10	0	100	0.9964	0.12	0.8781
13	**RM 222**	10	140–275	1.42	4	64	3	4	0	100	0.756	0.96	0.6012

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

S.N	M*	C.N*	P.R*	MAF*	A.N*	TNAP*	TNL*	TNPA*	TNMA*	P (%)*	G.D*	H*	PIC*
14	**RM 152**	8	80–700	2.4	4	68	2	4	0	100	0.7244	0.96	0.5747
15	**RM 257**	9	150–500	1.4	4	149	2	4	0	100	0.7376	1.16	0.5852
16	**RM 174**	2	200–300	1.65	3	66	1	2	1	66.66	0.455	0.7	0.3515
17	**RM 124**	4	250–275	1.46	4	63	1	4	0	100	0.5948	0.96	0.4654
18	**RM 171**	10	300–400	0.82	2	54	1	2	0	100	0.2952	0.2	0.2516
19	**RM 229**	11	100–125	0.91	2	78	1	2	0	100	0.1638	0.18	0.1504
20	**RM 271**	10	100–125	0.78	2	87	1	2	0	100	0.3432	0.32	0.2843
21	**RM 334**	5	100–200	1.33	4	74	2	3	1	75	0.807	1.26	0.635
22	**RM 487**	3	150–300	1.43	4	129	2	3	1	75	0.779	0.9	0.6206
23	**RM 70**	7	130–160	0.5	2	79	1	1	1	50	0.5	1	0.375
24	**RM 103**	6	240–425	0.96	2	50	1	1	1	50	0.0768	0.08	0.0739
25	**RM 235**	12	100–132	0.68	2	66	1	2	0	100	0.4352	0.64	0.3405

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

S.N	M*	C.N*	P.R*	MAF*	A.N*	TNAP*	TNL*	TNPA*	TNMA*	P (%)*	G.D*	H*	PIC*
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 (200 bp; 250 bp), RM229 (100–125 bp), RM271(100–125 bp) and RM487(150 bp; 200 bp; 250 bp) on 5, 1, 8, 8, 9, 11, 10 and 3 chromosome of rice 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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Figures

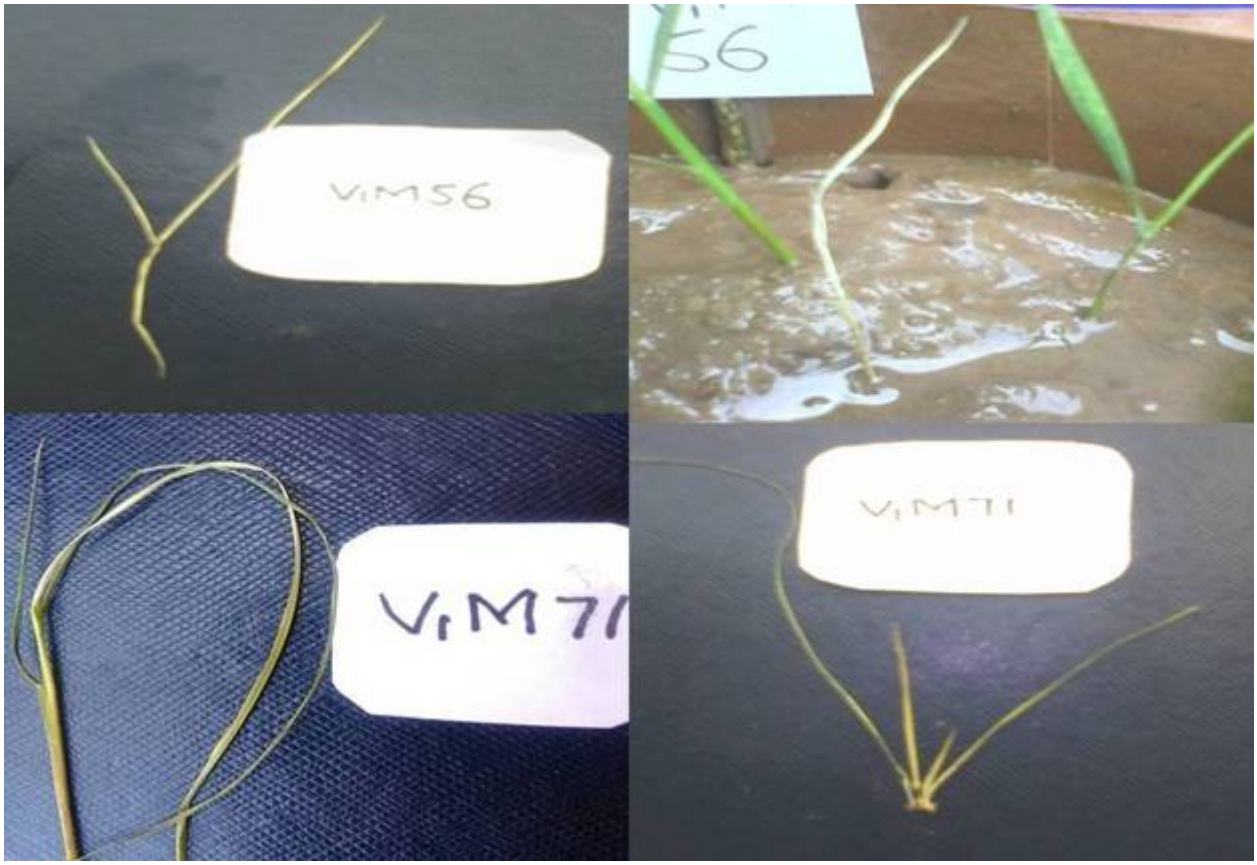

Figure 1

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.

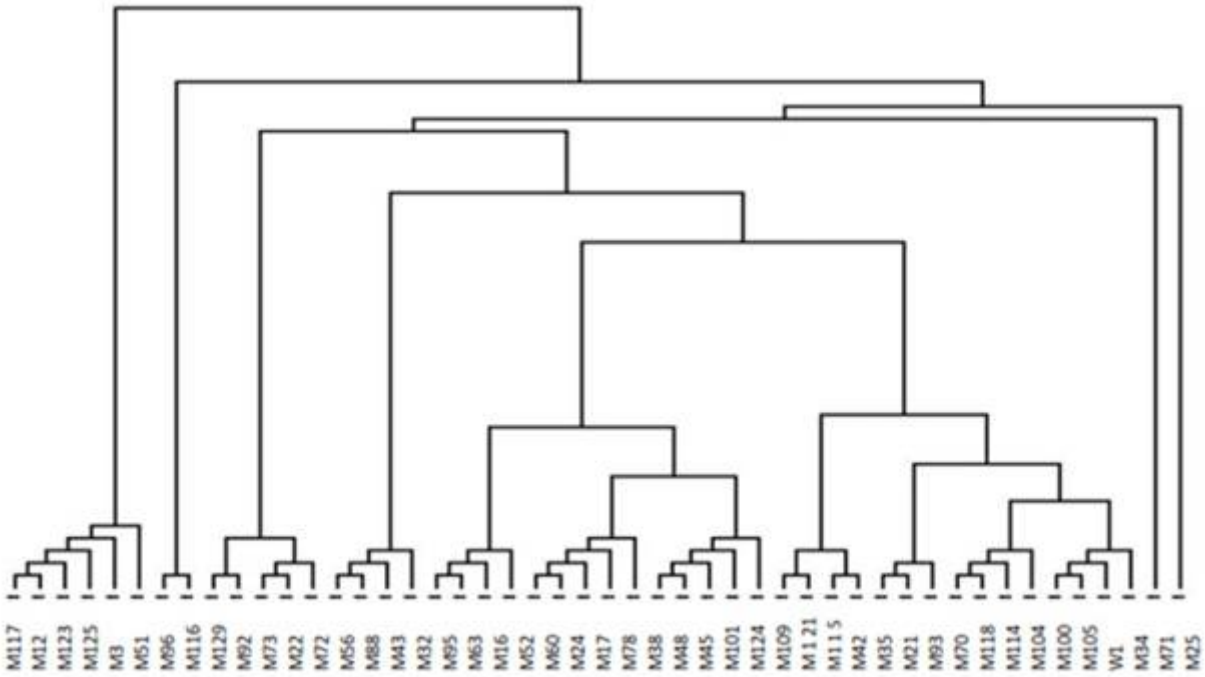

Figure 2

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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