

Original Research Article

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Morphological and Molecular Characterization of Complete Panicle Emergence Mutant Lines for Assessing Genetic Relatedness

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ABSTRACT

Keywords

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Morphological characterization of thirteen promising CPE (complete panicle emergence) mutants of Samba Mahsuri was done using thirty four agro-morphological traits by following DUS (Distinctiveness, Uniformity and Stability) guide lines. There was no variation observed in twenty two characters while in 12 characters there was variation among the individual mutants. Among all the traits, Culm attitude showed higher variation but there was no variation in the panicle emergence. Genetic relatedness among the mutants was assessed by the hyper polymorphic SSR markers. Correlation of phenotypic and genotypic data among mutants revealed that three mutants (CPE-1, CPE-9 & CPE-10) showed high genetic similarity with wild type (95%, 98.33% & 96.66%) as well as negligible variation in tested morphological characters. Whereas two mutants (CPE-12 & 13) showed more variation in morphological characters as well as less genetic similarity (86.66 & 86.66 %) with wild type was observed. But all the genotypes used in this study exhibits complete panicle emergence, which is differ from the wild type parent. This detailed characterization of Samba Mahsuri CPE mutants is very important for rice breeding from the standpoint of selection and conservation of different mutants for further utilization in crop improvement programmes and also to seek protection under PPV&FR Act, 2001 of India.

Introduction

Rice (*Oryza sativa* L.) is the world's most important staple food crop for more than 50% of world's population and also a model plant that has attracted broad interests in basic and applied research (Sasaki *et al.*, 2002, Mohapatra *et al.*, 2004, Singh and Singh, 2008). More than 90% of rice consumption is in Asian countries and considered as 'Rice

Basket' of the world (Komala *et al.*, 2017). To meet the need of ever growing population of the world, rice production need to increase. To overcome the yield ceiling issue, diverse genetic resources including near-isogenic lines, land races, wild spices and mutant populations are being used (Jian-Li Wu *et al.*, 2005). Among these, mutant stocks also used to determine the function of genes and their biochemical and metabolic pathways (Jian-Li

Wu *et al.*, 2005). Many national and international research groups are actively working on the production of rice mutants (Hirochika *et al.*, 2004; Leung and an, 2004). The most popular approach is induced mutagenesis and many important varieties of crops belongs to rice, cotton, rapeseed, sunflower, sesame, grapefruit, banana, ornamentals with desired characters were released through mutation breeding and they played a major role in global economic impact (Subodh K. Datta 2012). As per the FAO/IAEA Mutant Varieties Database, 3200 varieties have been released worldwide for cultivation in different countries through mutation breeding. Almost 700 mutant rice varieties were produced through mutation breeding, and maximum mutants were developed in cereals followed by barley, wheat, maize, durum wheat, oat, millet, sorghum and rye (Suprasanna *et al.*, 2015). Ethyl methane sulphonate particularly generates point mutations and it is effective than physical mutagens. EMS proficiently enhances the chemical modifications of nucleotides within the rice genome and it mainly produces random point mutations and the mutants were C/G to T/A transitions type (Reddy 2000; Koornneeff *et al.*, 1982; Rao 1977). EMS generates gene mutation in high frequency and chromosome aberration in low frequency (Van Harten 1998; Lai *et al.*, 2004). EMS induced mutagenesis was chosen for creation of large variants in the popular variety, Samba Mahsuri (Gopi *et al.*, 2013). Characterization of mutant in terms of genetic as well as in morphological characters through distinctiveness, uniformity and stability (DUS) guidelines depicted in PPV & FRA in 2001 is very much necessary for the protection of material as well as use in the breeding programmes. Hence, in the present study the mutants which showed the complete panicle emergence was selected for molecular and morphological characterization.

Materials and Methods

Thirteen complete panicle emergence Samba Mahsuri mutant lines of rice (Suneel *et al.*, 2020) (Table 1) were grown in a RCBD (randomized complete block design) with two replications at IIRR farm, Rajendranagar, Hyderabad, situated at 17.53N latitude and 78.27E longitude, 545 m altitude, with a mean temperature of 31.2°C and mean annual precipitation of 988.3 mm. The material was grown with wild type (BPT-5204) in two replications during Kharif 2016. Thirteen CPE genotypes seed was sowed on nursery beds and 25-30 days old seedlings were transplanted into the field. The plant spacing was 15 cm to 20 cm with density of one hill. The recommended packages of practices were followed to raise the crop in the field and to avoid the interspecific competition in between the plants regular hand weeding was embarked to free the plant. Five plants were selected randomly to take the observations. Selected plants of each genotype were subjected to thirty four morphological traits. Among thirty four (18 essential and 16 additional) quantitative traits, were assessed visually as per the DUS test guidelines by UPOV PPV & FR Act, 2001 for rice. The observations of selected genotype for different characteristics were recorded at different stages of growth.

DNA Extraction

Genomic DNA of CPE individuals and their respective wild type were isolated using modified cetyl trimethyl ammonium bromide (CTAB) method (Devi *et al.*, 2020).

SSR amplification

SSR amplification the veriti PCR system (M/S Applied Bio Systems, USA) was used with following buffer composition. DNA template (30ng/ μ L), 2 μ L of 10 x PCR buffer

(kappa), 0.5 µL of forward and reverse primers each (5pM/ µL), 1µL of dNTP's (2.5mM conc) and 0.1 µL of taq-polymerase (kappa,5U) and the final reaction volume was made up to 20 µL with nuclease free H₂O.

SSR allele scoring and data analysis

Genomic similarity of thirteen CPE promising mutants was carried out with wild type using randomly selected 60 SSR markers spread over 12 chromosomes. The presence or absence of SSR fragment in each mutant genotype was recorded. The SSR amplicons appearing without ambiguity were scored as 1 (present) and 0 (absent) for each primer. The amplified PCR product size was calculated on the basis of its mobility relative to molecular mass of marker (100 bp DNA ladder).

Results and Discussion

A total of 13 M₈ mutant lines (Table 1) along with wild type (BPT-5204) were taken for characterization using 34 DUS characters (Table 2). The Samba Mahsuri each CPE mutant line showed distinctiveness in few morphological traits. Earlier researchers reported the distinctiveness in the morphological traits of rice landraces (Rao *et al.*, (2013); Tirkey *et al.*, 2013); Mondal *et al.*, (2014); Manjunatha *et al.*, 2016); Kalyan *et al.*, (2017); Komala *et al.*, (2017) and Umarani *et al.*, (2017). Quantitative characters of thirteen CPE mutants are presented in Table 4. Among 13 mutants, 7 mutants (CPE-1, 2, 3, 8, 9, 10 and 11) showed dark like wild type, 5 mutants (CPE- 4,5,7,12 & 13) showed medium and 1 mutant (CPE-6) showed light green colour of DUS trait leaf intensity of green colour. Pubescence of blade surface was weak in 2 mutants (CPE-5 & 8), medium in 6 mutants (CPE-1, 2, 3, 4, 9 & 10) which was like wild type, and strong in 5 mutants (CPE-6, 7, 11, 12 & 13). A total of seven mutant genotypes were distinct from

the wild type in the pubescence of blade surface. Culm: attitude was erect in 1 mutant (CPE-6), semi erect in 7 mutants (CPE-1, 2, 3, 4, 9, 10 & 11) which was like wild type, open in 2 mutants (CPE-12 & 13) and spreading in 3 mutants (CPE-5, 7 & 8). While coming to the flag leaf attitude of blade (early observation) among CPE mutants, erect type of flag leaf attitude (early) was observed in CPE-1,2,3,7,9,10 & 11 like wild type, and semi erect type was observed in CPE-4, 5, 6, 8, 12 & 13 which is distinct from the wild type. Density of pubescence of lemma showed weak in 1 mutant, 7 mutants showed medium like wild type and 5 mutants with strong pubescence. Stem thickness was medium (<0.40-0.55 cm) in 7 mutants (CPE-1, 2, 3, 4, 8, 9, & 10) which was like wild type, thin (<0.40 cm) in 3 mutants (CPE-5, 6 & 7), and thick stem (>0.55 cm) was observed in 3 mutants (CPE-11, 12 & 13). While coming to the Flag Leaf attitude (late observation) CPE-1, 3, 5, 6, 7, 9, 10, 11, 12 & 13 were showed semi erect type like wild type and CPE-2, 4 & 8 exhibits horizontal type, which is distinct from wild type. With respect to panicle characters 1 mutant (CPE-2) were of semi straight, 7 mutants (CPE-1, 3, 6, 7, 9, 10 & 11) exhibits deflexed type like wild type, and 5 mutants (CPE-4, 5, 8, 12 & 13) exhibits drooping type. Lemma & Palea: colour was straw in 8 mutants (CPE-1, 2, 3, 5, 6, 7, 9 & 10) and 5 mutants (CPE-4, 8, 11, 12 & 13) showed brown furrows on straw, which is distinct from wild type (Fig. 1).

Panicle: Secondary branching was strong in 10 mutants (CPE-1, 2, 3, 4, 5, 6, 7, 8, 9 & 10) which was like wild type and clustered in 3 mutants (CPE-11, 12 & 13). While coming to the panicle: attitude of branches 4 mutants (CPE-1, 9, 10 & 11) were showed erect to semi erect type like wild type, 5 mutants (CPE-3, 4, 7, 12 & 13) were showed semi erect type and 4 mutants (CPE-2, 5, 6 & 8) were showed semi erect to spreading type

which is differ from the wild type. The character Leaf: Senescence was early in 2 mutants (CPE-5 & 6), medium in 6 mutants (CPE-1, 3, 4, 7, 9 &10) and late in 5 mutants (CPE-2, 9, 11, 12 & 13).

There was no variation were observed with respect to coleoptile: colour was colourless in all mutants. Basal leaf: sheath colour was green in all mutants. Leaf: anthocyanin colouration and leaf sheath anthocyanin colouration was absent in all mutants. All CPE mutants have leaf auricles and the anthocyanin colouration of auricles was colourless or absent in all mutants. Leaf: Collar was present in thirteen CPE mutants and anthocyanin colouration of Collar was absent in all. Leaf: Ligule was present in all and shape of the ligule was split in all mutants with white in colour. It is important to note that no mutant line exhibited male sterility. Lemma: Anthocyanin colouration of keel and anthocyanin colouration of area below apex was absent in all lines. Spikelet: Colour of Stigma was white in colour in all mutant genotypes. Stem: Intensity of anthocyanin colouration of nodes was not observed. Stem: anthocyanin colouration of internodes was absent in all CPE individuals. Spikelet colour

of tip of lemma was yellowish in all mutants. Panicle: Awns was absent in thirteen mutants and panicle: secondary branching was present in all. Sterile: Lemma colour was straw in all mutant genotypes.

Molecular characterization

Genetic diversity assessment is not only important for crop improvement but also for efficient management and protection of germplasm resources (Yogendra Singh, 2011; Singh and Singh, 2008) and molecular approaches are widely used by researchers time to time (Singh *et al.*, 2013; Koutu *et al.*, 2017; Koutu *et al.*, 2019). Genetic relatedness among the mutants was assessed by the hyper polymorphic SSR markers. Correlation of phenotypic and genotypic data among mutants revealed that three mutants (CPE-1, CPE-9 & CPE-10) showed high genetic similarity with wild type (95%, 98.33% & 96.66; Table 3) as well as negligible variation in tested morphological characters. Whereas two mutants (CPE-12 & 13) showed more variation in morphological characters as well as less genetic similarity (86.66 & 86.66 %) with wild type was observed.

Table.1 List of Samba Mahsuri CPE mutants used in this present study

S. No	Mutant
1	CPE-1
2	CPE-2
3	CPE-3
4	CPE-4
5	CPE-5
6	CPE-6
7	CPE-7
8	CPE-8
9	CPE-9
10	CPE-10
11	CPE-11
12	CPE-12
13	CPE-13

Table.2 Details of 34 DUS characters considered in the present study

S. No	Characteristics	States	Note	Stage of observation
1	Coleoptile: Colour	Colourless	1	2-3days after sowing
		Green	2	
		Purple	3	
*2	Basal leaf: Sheath colour	Green	1	Booting
		Light purple	2	
		Purple lines	3	
		Uniform purple	4	
3	Leaf: Intensity of green colour	Light	3	Booting
		Medium	5	
		Dark	7	
S. No	Characteristics	States	Note	Stage of observation
4	Leaf: Anthocyanin colouration	Absent Present	1 9	Booting
5	Leaf sheath: Anthocyanin colouration	Absent Present	1 9	Booting
*6	Leaf: Pubescence of blade surface	Absent	1	Booting
		Weak	3	
		Medium	5	
		Strong	7	
		Very strong	9	
*7	Leaf: Auricles	Absent	1	Booting
		Present	9	
*8	Leaf: Anthocyanin colouration of auricles	Colourless	1	Booting
		Light purple	2	
		Purple	3	
9	Leaf: Collar	Absent	1	Booting
		Present	9	
10	Leaf: Anthocyanin colouration of Collar	Absent	1	Booting
		Present	9	
11	Leaf: Ligule	Absent	1	Booting
		Present	9	
*12	Leaf: Shape of ligule	Truncate	1	Booting
		Acute	2	
		Split	3	
13	Leaf: Colour of ligule	White	1	Booting
		Light purple	2	
		Purple	3	
14	Culm: Attitude	Erect	1	Booting
		Semi-erect	3	
		Open	5	
		Spreading	7	
*15	Flag leaf: Attitude of blade (early observation)	Erect	1	Beginning of anthesis
		Semi-erect	3	
		Horizontal	5	
		Drooping	7	
*16	Spikelet: Density of pubescence of lemma	Absent	1	Beginning of anthesis
		Weak	3	
		Medium	5	
		Strong	7	
		Very strong	9	

S. No	Characteristics	States	Note	Stage of observation
17	Male sterility	Absent	1	Anthesis half way
		Present	9	
18	Lemma: Anthocyanin colouration of keel	Absent or very weak	1	Anthesis half way
		Weak	3	
		Medium	5	
		Strong	7	
		Very strong	9	
*19	Lemma: Anthocyanin colouration of area below apex	Absent	1	Anthesis half way
		Weak	3	
		Medium	5	
		Strong	7	
		Very strong	9	
20	Lemma: Anthocyanin colouration of apex	Absent	1	Anthesis half way
		Weak	3	
		Medium	5	
		Strong	7	
		Very strong	9	
*21	Spikelet: Colour of stigma	White	1	Anthesis half way
		Light green	2	
		Yellow	3	
		Light purple	4	
		Purple	5	
22	Stem: Thickness	Thin (<0.40 cm)	3	Milky stage of plant
		Medium (0.40 - 0.55)	5	
		Thick (>0.55 cm)	7	
*23	Stem: Anthocyanin colouration of nodes	Absent	1	Milky stage of plant
Present	9			
24	Stem: Anthocyanin colouration of internodes	Absent	1	Milky stage of plant
Present	9			
*25	Flag leaf: Attitude of blade (late observation)	Erect	1	Maturity stage
		Semi-erect	3	
		Horizontal	5	
		Deflexed	7	
*26	Panicle: Curvature of main axis	Straight	1	Maturity stage
		Semi straight	3	
		Deflexed	5	
		Drooping	7	
*27	Spikelet: Colour of tip of lemma	White	1	Maturity stage
		Yellowish	2	
		Brown	3	
		Red	4	
		Purple	5	
		Black	6	
28	Lemma and Palea: Colour	Straw	1	Maturity stage
		Gold and Gold furrows on straw back ground	2	
		Brown spots on straw	3	
		Brown furrows on straw	4	
		Brown(tawny)	5	
			6	

		Reddish to light purple	7	
		Furrows on straw		
		Purple	8	
		Black	9	
*29	Panicle: Awns	Absent	1	Maturity stage
		Present	9	
30	Panicle: Presence of secondary branching	Absent	1	Maturity stage
		Present	9	
31	Panicle: Secondary branching	Weak	1	Maturity stage
		Strong	2	
		Clustered	3	
32	Panicle: Attitude of branches	Erect	1	Maturity stage
		Erect to semi erect	3	
		Semi erect	5	
		Semi erect to spreading	7	
		Spreading	9	
33	Leaf: Senescence	Early	3	Maturity stage
		Medium	5	
		Late	7	
*34	Sterile lemma: Colour	Straw	1	Maturity stage
		Gold	2	
		Red	3	
		Purple	4	

Table.3 Assessment of similarity of CPE mutants with wild type

S.no	Genotype	No. of markers tested	No. of markers monomorphic	% similarity with wild type
1	CPE-1	60	57	95.00
2	CPE-2	60	54	90.00
3	CPE-3	60	55	91.66
4	CPE-4	60	54	90.00
5	CPE-5	60	55	91.66
6	CPE-6	60	54	90.00
7	CPE-7	60	56	93.33
8	CPE-8	60	53	88.33
9	CPE-9	60	59	98.33
10	CPE-10	60	58	96.66
11	CPE-11	60	55	91.66
12	CPE-12	60	52	86.66
13	CPE-13	60	52	86.66

Table.4 Classification of CPE mutants based on DUS characters

S.no	Characteristics	CPE-1	CPE-2	CPE-3	CPE-4	CPE-5	CPE-6	CPE-7	CPE-8	CPE-9	CPE-10	CPE11	CPE-12	CPE-13	BPT-5204
1	Coleoptile: Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1
*2	Basal leaf: Sheath colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	Leaf: Intensity of green colour	7	7	7	5	5	3	5	7	7	7	7	5	5	7
4	Leaf: Anthocyanin colouration	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	Leaf sheath: Anthocyanin colouration	1	1	1	1	1	1	1	1	1	1	1	1	1	1
*6	Leaf: Pubescence of blade surface	5	5	5	5	3	7	7	3	5	5	7	7	7	5
*7	Leaf: Auricles	9	9	9	9	9	9	9	9	9	9	9	9	9	9
*8	Leaf: Anthocyanin colouration of auricles	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	Leaf: Collar	9	9	9	9	9	9	9	9	9	9	9	9	9	9
10	Leaf: Anthocyanin colouration of auricles	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	Leaf: Ligule	9	9	9	9	9	9	9	9	9	9	9	9	9	9
*12	Leaf: Shape of ligule	3	3	3	3	3	3	3	3	3	3	3	3	3	3
*13	Leaf: Colour of ligule	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	Culm: Attitude	3	3	3	3	7	1	7	7	3	3	3	5	5	3
*15	Flag leaf: Attitude of blade (early observation)	1	1	1	3	3	3	1	3	1	1	1	3	3	1
*16	Spikelet: Density of pubescence of lemma	5	5	5	5	7	7	7	3	5	5	5	7	7	5
17	Male sterility	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	Lemma: Anthocyanin	1	1	1	1	1	1	1	1	1	1	1	1	1	1

	colouration of keel														1
*19	Lemma: Anthocyanin colouration of area below apex	1	1	1	1	1	1	1	1	1	1	1	1	1	1
*20	Lemma: Anthocyanin colouration of apex	1	1	1	1	1	1	1	1	1	1	1	1	1	1
*21	Spikelet: Colour of stigma	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	Stem: Thickness	5	5	5	5	3	3	3	5	5	5	7	7	7	5
*23	Stem: Anthocyanin colouration of nodes	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	Stem: Anthocyanin colouration of internodes	1	1	1	1	1	1	1	1	1	1	1	1	1	1
*25	Flag leaf: Attitude of blade (late observation)	3	5	3	5	3	3	3	5	3	3	3	3	3	3
*26	Panicle: Curvature of main axis	5	3	5	7	7	5	5	7	5	5	5	7	7	5
*27	Spikelet: Colour of tip of lemma	2	2	2	2	2	2	2	2	2	2	2	2	2	2
28	Lemma and Palea: Colour	1	1	1	4	1	1	1	4	1	1	4	4	4	1
*29	Panicle: Awns	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	Panicle: Presence of secondary branching	9	9	9	9	9	9	9	9	9	9	9	9	9	9
31	Panicle: Secondary branching	2	2	2	2	2	2	2	2	2	2	3	3	3	2
*32	Panicle: Attitude of branches	3	7	5	5	7	7	5	7	3	3	3	5	5	3
33	Leaf: Senescence	5	7	5	5	3	3	5	7	5	5	7	7	7	5
*34	Sterile lemma: Colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Bold denotes the variation in the trait

Note: (*) Essential characters

Fig.1 variation in the DUS characters among CPE mutants: (a) Leaf: Auricles, (b) Leaf: Collar, (c) Leaf: Shape of Ligule, (d) Spikelet: colour of Stigma, (e) Stem: Thickness, (f) Stem: Nodes & internodes, (g) Flag leaf: Attitude of blade (late observation), (h) Panicle: Curvature of main axis, (i) Lemma & Palea: colour, (j) Spikelet: Colour of tip of lemma, (k) Panicle: Secondary branching



Among all mutant genotypes CPE-3 showed variation in only one character i.e. attitude of branches, which is differ from wild type. Among 34 quantitative characters Culm: attitude was showed higher variation. Among 13 CPE mutants CPE-2, CPE-4 & CPE-6 showed 90% similarity with wild type, followed by CPE-3, CPE-5 & CPE-11 (91.66%), CPE-7 (93.33%), and CPE-8 (88.33%) respectively. But all the genotypes used in this study exhibits complete panicle emergence, which is differ from the wild type parent.

In conclusion, the present investigation, an effort was made to assess the 13 CPE mutants of rice for genetic and morphological

relatedness with the wild type. Uniformly spread 60 SSR markers were employed to assess their genetic diversity. CPE-1, 9 & 10 showed high similarity with the wild type. The Samba Mahsuri CPE promising mutants identified in this study will be useful for plant breeders and researchers for getting productive and quality results in the plant breeding programs.

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Competing interests

Authors have declared that no competing interests exist.

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