Assessment of Agro-Morphological characters among complete panicle emergence mutants of Samba Mahsuri (BPT-5204)

ABSTRACT

Aims: The aim of the present work was to generate complete panicle emergence mutants of Samba Mahsuri (BPT-5204), using the chemical mutagen, ethyl methane sulphonate (EMS)

Place and Duration of Study: The field evaluation was carried out repeatedly in two locations at Indian Institute of Rice Research, Rajendranagar, and at ICRISAT Patancheru, Hyderabad. The duration of the study was three successive seasons in triplicates namely, june-2014, january-2015 and june-2015.

Methodology: Seedlings of 28-30 days old were transplanted in 5 lines (each line containing 20 plants) into the field. The plant spacing was 20cm by 15cm with density of one hill. The field was irrigated throughout the experiment with average of 10cm water above the soil level. Regular hand weeding was embarked upon to free the plant of inter specific competition. The phenotypic data was recorded for CPE mutants through visual assessment. The characteristics that required measurements were done according to the usual procedure. The Distinctness, Uniformity and stability of the CPE mutant lines have been recorded following (DUS) test guidelines by UPOV guidelines for rice (34) & Yoshida S.(40).

Results: The identified stabilized thirteen complete panicle emergence (CPE) mutants showed superior agro morphological characters, compared with wild type (BPT-5204). The mutants CPE-2 and CPE-3 took 100 days to flowering and CPE-4 and CPE-7 mutants showed superiority for panicle length, and CPE-5 exhibited good performance for grain yield. In correlation analysis CPE trait had a significantly positive correlation with PH and TGW and negative correlation with 50%DFF. The CPE mutants used in the present study exhibited variability for most of the studied traits. Significant variation was detected in the identified stabilized CPE mutants for plant height, panicle length, flag leaf length and width, 50% flowering, different grain types and 1000 grain weight.

Conclusion: The present study discovered adequate genetic divergence in the thirteen stabilized CPE mutants for various qualitative and quantitative traits. The promising mutants identified during the current study have the potential to be used in future breeding programs for getting productive and quality results.

Keywords: Samba Mahsuri (BPT-5204), Mutation, Complete panicle exsertion (CPE), Biotic, Abiotic

1. INTRODUCTION:

Rice (Oryza sativa L.) is an important staple food for more than half of the world's population (20, 29, 28 & 41). In India 70-75% of the people mainly depend on rice. Rice plays an important role in shaping the cultures, diet and economies of millions of people by provide food and nutritional security eradicating poverty (24). Rice is not only the most important food crop but a model plant that has attracted broad interests in basic and applied research (11, 29 & 41). Rice act as a main source of income of many peoples in the world and it occupies a major position in many countries because for its importance in traditional diets (8). In the year 2017 total rice production in the world is 754.6 million tons (10). Many other problems such as unabated growth of population in India, demand of the rice is gradually increasing. To meet the demand of rice consumer's maximization of yield and achieving acceptable grain quality is top most priority in rice Improvement programs. Rice is a global leading food crop and it is an important item to mutagenesis. Food grain production was exceeds, and there are number of constraints like biotic, abiotic stresses, incomplete panicle emergence, lodging etc hampering the yield production (23). For genetic and physiological assessments of yield contributing factors in rice, Induced rice mutants are used as important research tools. Since 1960s, to till date several collections of mutant lines from different species have been isolated and successfully used in many different areas of plant biology and crop breeding (12). Samba Mahsuri (BPT-5204) is one of India's most popular and highly prized rice varieties, cultivated in 1-2 million hectares of land across India. But unfortunately Samba Mahsuri variety is susceptible to many biotic, abiotic stresses and exhibits incomplete panicle emergence, leads to reduction in the 10-20% total crop yield. Complete panicle emergence is economically important trait which influences the emergence of panicle out of flag leaf, resulting in to the complete grain filling and increase crop yield (40). Therefore, mining of superior and desired genotypes within the mutant germplasm of elite rice variety Samba Mahsuri is important for the rice breeders. Hence, assessment of Samba Mahsuri mutants is very important in rice breeding from the standpoint of selection and conservation of different Samba Mahsuri mutant lines for further utilization in crop improvement programs. Induced mutagenesis is one of the powerful tools to increase the extent of variation in the gene pool of rice. Mutation breeding approaches are being widely used throughout the world for biotic, abiotic stresses in various crops (3 & 1). Chemical mutagen EMS is more effective than physical mutagens (5, 6 & 35) and it mainly causes GC to AT transition in the plant genome (35, 16 & 13). Chemical mutagenesis has been widely used to create genetic variability for breeding research and genetic studies (26).

The main objective of the present work was to generate mutants in Samba Mahsuri (BPT-5204) background, using the chemical mutagen ethyl methane sulphonate (EMS), and characterize them for complete panicle emergence mutants of Samba Mahsuri.

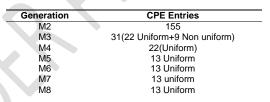
2. MATERIAL AND METHODS:

2.1 Identification of mutants having complete panicle emergence in mutagenized population of

Samba Mahsuri (BPT-5204):

A total of 10,500 individual Samba Mahsuri (BPT-5204) mutant M_2 seeds were sowned on nursery beds and the 28-30 days old seedlings were transplanted in 5 lines (each line containing 20 plants) into the field. The plant spacing was 20cm by 15cm with density of one hill. The field was irrigated throughout the experiment with average of 10cm water above the soil level. Regular hand weeding was embarked upon to free the plant of inter specific competition. The M_2 seeds of 10,500 lines were observed carefully for the identification of useful mutants, which have some agronomic value i.e complete panicle emergence. In M_2 generation, 155 individual potential lines were identified with the desired trait and they were forwarded to M_3 generation and grown in multiple rows to observe uniformity. In M_4 generation 22 entries were selected based on uniform maturity. The selected 22 individual uniform maturity entries were forwarded to M_5 generations. Three replications were transplanted in randomized complete block design (RCBD) mode. In M_5 generation, thirteen uniform maturity entries were selected based on the yield parameters and the selected thirteen entries were forwarded to M_6 generation and grown in three replications each along with checks. The selected thirteen entries were forwarded to M_6 generation and grown in three replications each along with checks. The

Generation wise screening of CPE lines (M2-M8): Table: 1



2.2 Assessment of Samba Mahsuri mutants for complete panicle emergence

In order to characterize complete panicle emergence mutants of Samba Mahsuri, screening carried out at two different locations namely IIRR, Rajendranagar, Hyderabad and at ICRISAT, Patancheru, Hyderabad. Complete panicle emergence in mutants was measured at the time of maturity from the base of panicle to the tip of the last spikelet. The mutant lines panicle internodes were emerged completely from the flag leaf. The mutants with panicles emerged completely from the flag leaf. The mutants with panicles emerged completely from the flag leaf. The mutants with panicles emerged completely from the flag leaf were selected as Samba Mahsuri mutants with complete Panicle emergence. To know, the complete panicle emergence mutants of Samba Mahsuri, this Indica variety (BPT-5204) was extensively screened, for the three seasons in triplicates namely, june-2014, january-2015 and june-2015. Samba Mahsuri (BPT-5204) panicles emerged grain recovery during harvesting. This characters makes Samba Mahsuri is an ideal genotype for identifying mutational changes in traits of panicle exsertion.

2.3 Evaluation of agro-morphological characters of CPE promising mutants

The phenotypic data was recorded for CPE mutants through visual assessment. The characteristics that required measurements were done according to the usual procedure.

The Distinctness, Uniformity and stability of the CPE mutant lines have been recorded following (DUS) test guidelines. The phenotypic data was recorded at maturity stage of the rice crop for characters like panicle morphology and data for quantitative characters like plant height, number of tillers/plant, number of reproductive tillers/plant, flag leaf length, flag leaf width, panicle length, grain type, days to 50% flowering, number of spikelet's/panicle, total plant yield, and 1000 seed weight were also recorded as per the guidelines by UPOV guidelines for rice (34) & Yoshida S.(40) (Table:2).

Agro morphological characters were carried out at the 50% flowering stage and full maturity stage of the plants. In this study 13(M8) complete panicle emergence mutant lines of agro-morphological characters data were recorded for following 12traits. Quantitative characters of CPE mutants are given in table 3.

Fig: a-Comparison between wild type and CPE mutants



3. RESULTS AND DISCUSSION

(1)Plant Height (cm):

The plant height was refers to the longest distance between the plant base and the tip of the highest leaf (or panicle, whichever is longer) as per the guidelines by UPOV guidelines for rice (34) and Yoshida S. (40). It was measured from the base of the plant to the tip of main panicle. Highly significant difference was observed for plant height, among thirteen CPE mutant lines have shown minimum 83 cm (CPE-1) to maximum 120cm (CPE-7). One mutant CPE-1 showed plant height of 83cm, is equal to wild type plant height (Table: 3). Among thirteen CPE mutants, 84.6% genotypes showed short (91-100 cm) plant height (excluding panicle), and 15% genotypes showed medium (111-130 cm) plant height.

(2)Number of tillers/plant:

Total number of tillers/plant was calculated by physical assessment at the time of maturity stage of the plant as per the guidelines by UPOV guidelines for rice (1985) and Yoshida S.(1981). All thirteen CPE mutants were showed less tillers/plant ranging from minimum 15(CPE-10) to maximum 24(CPE-1).where as in wild type total tiller number/plant is 27(Table: 3).

(3) Number of productive tillers/plant:

Highly significant differences were observed among thirteen CPE mutants for number of productive tillers, and it was ranging from minimum 13 (CPE- 3) to maximum 23 (CPE-1), whereas wild type showed 15 reproductive tillers (Table: 3). For the character number of productive tillers/plant a total of ten genotypes (76.9%) showed medium (11-20) productive tiller number/plant and three genotypes (23.07%) showed many (>20) productive tiller number/plant.

(4) Flag leaf length (cm):

Significant variation was observed among thirteen CPE mutants for flag leaf length. Length of the flag leaf in CPE mutants was less compared with wild type. In CPE mutant's flag leaf length ranging from minimum 26cm (CPE-3 &CPE-9) to maximum 33cm (CPE-11). In case of wild type length of the flag leaf is 35cm(Table:3& fig: b). For the character leaf length, 69.2% accessions exhibited short leaf type (25-30 cm), and 30.7% accessions were of a medium type (31-35 cm).

Fig-b: Measurement of CPE mutant lines flag leaf length



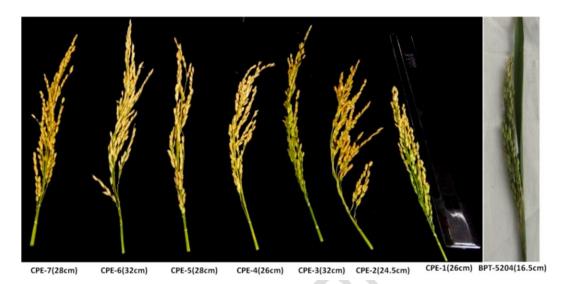
(5)Flag leaf width (cm):

Highly Significant variation was observed among thirteen CPE mutants for flag leaf width. In CPE mutant's flag leaf width ranging from minimum 1.2cm (CPE-1, 9&10) to maximum 2.2cm (CPE-4).In case of wild type width of flag leaf is 1.2cm(Table: 3). For the leaf width, among thirteen CPE accessions, highest number of the accessions 92.30% recorded medium type of leaves and 7.69% accessions recorded broad type of leaves.

(6)Panicle length (cm):

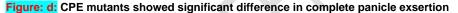
Panicle length was measured in centimeters at the time of maturity stage of the plant. Panicle length was measured from the base of the panicle to the tip of last spikelet prior to harvesting. Stabilized CPE mutant lines showed significant variation in the panicle length. Length of the mutant line panicle ranging from minimum 22cm (CPE-3) to maximum 32 cm (CPE-4 &7). In case of wild type length of the panicle is 16.5cm (Table: 3 &fig: c). Among thirteen CPE mutants highest number of the genotypes (76.9%) fall under the long panicle length (PL) category (26-30 cm), and 23.07 % genotypes fall under medium panicle length (PL) category (21-25 cm).

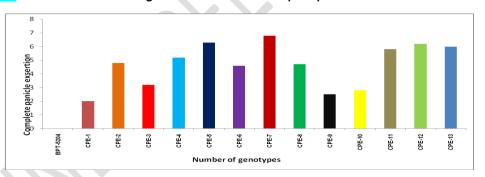
Figure-c: CPE mutants showed significant variation in panicle length



7).Complete panicle exsertion (CPE):

Complete panicle exsertion was measured in centimeters at the maturity stage of the plant.CPE was measured from the flag leaf to the panicle base using a ruler.CPE mutants showed significant difference in panicle exsertion ranging from maximum CPE-7(6.8cm) to minimum CPE-1(2cm).But in case of wild type 10-20% panicle choking was observed. In Samba Mahsuri 4-5cm panicle was present within the flag leaf (Table: 3 & fig: d).





(8) Grain type:

Four different grain types were identified among thirteen CPE mutant lines. In the current study, grain lengths were recorded according to Hailiang Mao (21). The mutants with different grain types like long slender (L/B>3.0), long bold (L/B<3.0), medium bold (L/B<2.5), and medium slender (L/B 2.5-3.0) were identified. Eight mutant lines (CPE-1,3,7,9,10,11,12&13) showed medium slender grain(MS) like wild type, two mutant lines(CPE-5&6) showed long slender grain(LS),two mutant lines (CPE-2&4) showed medium bold (MB) and one mutant line CPE-8 showed long bold(LB) grain type(Table: 3 & fig: e).

Comment [DL1]: Methodology

(9) Days to 50% flowering:

Significant variation was observed among thirteen mutants for days to 50% flowering, minimum 100days (CPE-2&3) to maximum 115days (CPE-1,CPE-9&CPE-10). Among thirteen genotypes three genotypes showed 115days to 50% flowering like wild type(Table: 3). For the character time of heading (50% of plants with panicles) were observed and noticed that 76.9% genotypes were of medium type (91-110days), and 23.07% genotypes fall under late type (111-130 days).

(10) Number of filled grains/panicle:

Significant variation was observed among thirteen mutants for number of grains per panicle, CPE-13 had the lowest number of filled grains/panicle (202), and CPE-11 had the highest number of filled grains/panicle (225). In case of wild type number of filled grains per panicle is 200, which is lower than the CPE mutant lines (Table: 3).

(11) Single plant yield (gr):

Highly significant difference was observed among thirteen mutants for plant yield. The highest grain yield per plant was observed in the CPE-5 (26.4gr) and lowest in the CPE-3 (20gr), which is higher than the wild type 15.8gr (Table: 3).

Fig-e: CPE mutant lines with different grain types



(12) 1000 seed weight (gr):

Among thirteen genotypes significant variation was observed for 1000 seed weight. It was ranging from maximum 23.99gr (CPE-5) to minimum14.12gr (CPE-1). Among 13 CPE mutants CPE-1 showed less (14.12 gr) 1000 seed weight, compare with wild type. Test weight of wild type is 15.2gr (Table: 3). For the character grain weight of 1000 fully developed grains, highest number of the genotypes (76.9%) showed low grain weight (15-20 g) and 23.0% genotypes showed medium grain weight (21-25g).

3.1 Correlation data between agro-morphological characters of CPE mutants:

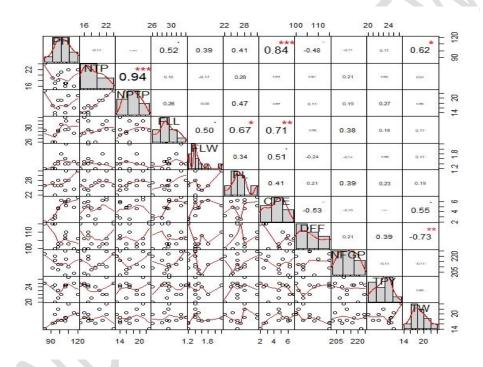
Comment [DL2]: Place after the observed result

The degree of correlation between the traits is significant in plant breeding. It can be used as tool for indirect selection. Correlation studies help the plant breeder during selection and provide the understanding of yield components.

Comment [DL3]: Not important in the result

The results of correlation analysis reveals that CPE trait showed positive and highly significant correlation with plant height (r=0.84), flag leaf length (r=0.71), test weight(r=0.55), and flag leaf width(r=0.51) respectively. However, number of reproductive tillers/plant(r=0.94) showed highly positive and significant correlation with number of tillers/plant. Among eleven characters panicle length showed positive correlation with flag leaf length (r=0.67), and plant height(r=0.62) showed positive correlation with flag leaf length(r=0.52) and panicle length(r=0.41).Flag leaf length showed positive significant correlation with flag leaf width(r=0.50) (fig: f).

Fig-f: Pearson's correlation coefficients among thirteen CPE mutants



PH=plant height, PL=panicle length, FLL=flag leaf length, NTP=number of tillers/plant, NRT= number of reproductive tillers/plant, FLW=flag leaf width, SPY= single plant yield, DFF=days to 50% flowering, TW= 1000 test weight, NFG= number of filled grains/panicle.

However, days to 50% flowering showed highly significant negative correlation with CPE (-0.53), test weight (-0.73), plant height (-0.43), flag leaf length (-0.08), and flag leaf width (-0.24) respectively.

Assessment of CPE mutants through various morphological traits is an important step for assessment of its genetic potential. Improve in grain yield potential is the most important goal of the rice breeders. To enhance the yield and yield contributing traits in rice, mutation breeding is the best available choice. Samba Mahsuri is a popular rice variety with good cooking quality characteristics. It is susceptible to many biotic and abiotic stresses and it exhibits incomplete panicle emergence. To overcome this problem, increase the extent of variation in gene pool of rice by using mutation breeding is the potent way. To develop a rice variety with medium slender grain, good cooking quality, and early duration with high yielding than Samba Mahsuri is the biggest task for plant breeders. So, in this current study Samba Mahsuri was used to create genetic variation through EMS mutagenesis (Mutation breeding).Stabilized thirteen CPE mutants were analyzed separately for different agronomic traits. Compare with Samba Mahsuri a notable variation was observed among thirteen CPE mutant lines for yield and yield contributing traits.

In this present study, among thirteen CPE mutants, eleven genotypes showed semi dwarf plant height (80-110cm) and exhibits higher single plant yield. Plant height is a complex trait in rice crop (4, 2 & 7),.Reduction in plant height may reduce yield losses and enhance their resistance to lodging (1)Semi dwarf plant height is a key feature for higher yield production and it has been extensively utilized in the improvement of rice throughout the world(14 & 18). The genotypes, which were produced more number of effective tillers/plant that showed higher grain yield in rice (9 &17). Yaqoob et al (37) and Tiwari et al (33) were observed significant variations among genotypes for days to maturity, plant height, panicle length, 50% flowering, spikelets/panicle, number of fertile spikelet's and grains yield/plant. Similarly in this current study all the genotypes had significant variation with each other.Joshi et al (15) described that, test weight (1000 grain weight) have been used by several researchers for characterizing rice varieties. In this current study we found that number of filled grains/panicle (NFGP) has highest positive direct effect on yield. According to Yolanda and Das (39) and Zahid et al (43) number of filled grains panicle-1 has highest positive direct effect on yield. It implies that the number of filled grains per panicle could be considered as critical criteria for yield improvement Samonte et al (25) and Mahto et al (19). Correlation studies for a number of agronomic traits with panicle exsertion (PE) have been reported by Yang et al (37) and Hori et al (14). Earlier researchers reveal that the panicle exsertion was positively correlated with PH, PL, and TGW (43 & 44). It implies that PE plays a crucial role in the regulation of grain weight. Similarly in this present study we found that CPE had a significantly positive correlation with PH and TGW. It had a significant positive correlation with FLL, and FLW. However, in this present study highest correlation was corresponded to the total tillers and effective tillers. Flag leaf length was positively correlated with plant height, flag leaf width and panicle length. Plant height showed positive correlation with effective tillers and panicle length .It signifying the role of plant height in enhancing the panicle length by large stem reserve mobilization (42). Similar results were also reported by Shishir Sharma et al (30) and Wattoo et al (36). Rajeswari and Nadarajan (27), Surek and Beser (31) It is well known that 50% flowering (DFF) is one of the most important agronomic trait in rice (22&31). Current study reveals that DFF had a highly significantly negative correlation with CPE, which was similar to the results reported by (43 & 44). However, in this current study we found that DFF had significantly negative correlation with PH, TGW, FLL and FLW. The CPE mutants used in the present study exhibited variability for most of the studied traits. Significant variation was detected in the identified stabilized CPE mutants for plant height, panicle length, flag leaf length and width, 50% flowering, different grain types and 1000 grain weight.

Table: 2. CPE promising mutant's quantitative traits methodology:

List of quantitative traits										
Denotation	Method of evaluation									
PH(cm)	The average height from the base to the tip of the main panicle									
NT(number)	Count the number of tillers per plant									
NPT(number)	Count the number of productive tillers per plant									
FL (cm)	measure the length of the flag leaf									
FLW(cm)	Calculate the width of the flag leaf									
PL(cm)	Measure the panicle length from the node below the lowest branch on the panicle to the top of first superior spikelet									
CPE(cm)	Measure from the flag leaf to the panicle base using a ruler									
GT	Record the different grain types in the all the accessions									
DFF(days)	Count the number of days from seeding to flowering									
NFG/P(number)	Count the number of spikelet's per panicle									
Y/P(gm)	Weigh total grains produced per plant									
1000GW(gm)	Weigh any 1000 filled grains									
	Denotation PH(cm) NT(number) NPT(number) FL (cm) FLW(cm) PL(cm) CPE(cm) GT DFF(days) NFG/P(number) Y/P(gm)									

Table: 3. Agro-morphological data of CPE mutants:

Trait/	Wild type	COMPLETE PANICLE EMERGENCE MUTANTS												
Characters	BPT-5204	CPE-1	CPE-2	CPE-3	CPE-4	CPE-5	CPE-6	CPE-7	CPE-8	CPE-9	CPE-10	CPE-11	CPE-12	CPE-13
PH	83.0	83.0	116.0	95.0	107.0	110.0	98.0	120.0	98.0	92.0	95.0	106.0	102.0	106.0
NTP	27.0	24.0	16.0	17.0	17.0	22.0	20.0	22.0	18.0	19.0	15.0	16.0	21.0	17.0
NPTP	15.0	23.0	16.0	13.0	17.0	21.0	19.0	21.0	16.0	17.0	14.0	14.0	19.0	14.0
FLL	35.0	29.3	28.5	26.0	32.0	29.6	29.3	32.0	29.0	26.0	28.0	33.0	31.0	29.5
FLW	1.2	1.2	1.5	1.4	2.2	1.4	1.4	1.4	1.5	1.2	1.2	1.4	1.8	1.6
PL	16.5	26.0	24.5	22.0	32.0	28.0	28.0	32.0	26.0	27.0	26.0	28.0	26.0	24.0
CPE	0.0	2.0	4.8	3.2	5.2	6.3	4.6	6.8	4.7	2.5	2.8	5.8	6.2	6.0
GT	MS	MS	MB	MS	MB	LS	LS	MS	LB	MS	MS	MS	MS	MS
DFF	115.0	115.0	100.0	100.0	110.0	103.0	101.0	106.0	103.0	115.0	115.0	106.0	103.0	108.0
NFGP	200.0	222.0	204.0	220.0	217.0	210.0	212.0	224.0	207.0	209.0	216.0	225.0	212.0	202.0
TPY	15.8	25.0	25.0	20.0	24.4	26.5	23.0	22.0	21.0	24.0	24.5	24.5	22.0	24.0
TW	15.2	14.1	20.6	19.7	18.4	24.0	21.8	19.9	17.5	16.3	16.5	18.5	17.5	17.2

PH: Plant height; NTP: Number of tillers/plant; NPTP: Number of productive tillers/plant; FLL: Flag leaf length(cm); FLW: Flag leaf width(cm); PL: Panicle length(cm); CPE: Complete Panicle exsertion; GT: Grain type; DFF: Days to 50% flowering; NFGP: Number of filled grains/panicle; TPY: Total plant yield(gm); TW: 1000 seed weight(gm).

4. CONCLUSION

In the present study the CPE accessions showed ample range of divergence in the various qualitative and quantitative traits. The promising mutants identified during the current study have the potential to be used in future breeding programs for getting productive and quality results. These phenotypic characters were played key role in crop improvement. This material can use as donor material, which provides wide scope for selection of superior and desired genotypes for breeders to transfer into another genetic background for further improvement.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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