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PHENOTYPIC CHARACTERIZATION OF SUPER BASMATI ETHYL METHANE SULFONATE (EMS) INDUCED MUTANTS

Kiran Hameed[1], Muhammad Sarwar Khan[1], Hafeez Ahmad Sadaqat[2] and Faisal Saeed Awan[1,*]

[1]Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad-38040, Pakistan;
[2]Plant Breeding and Genetics, University of Agriculture, Faisalabad-38040, Pakistan.
***Corresponding author's e-mail: faisal.saeed@uaf.edu.pk**

Phenotypic variability in agronomic important traits is prerequisite for the genetic improvement of crop plants. The generation and phenotypic characterization of mutants are helpful to select genotypes with improved agronomic traits. The present study was planned to evaluate the phenotypic variability among mutants for their agronomic important traits in first and second mutagenic generation of super basmati against ethyl methane sulphonate treatments. Data recorded at maturity were statistically concluded using principal component analysis under ten different parameters (germination, number of spikelet/plant, tiller/plant, panicle length, yield/plant, 100 paddy weight, plant height, paddy length, paddy width and paddy length width ratio). The results showed that EMS doses up to 1.5% are the best for generating mutant genotypes of super basmati. Number of spikelet/plant, yield/plant, tiller/plant and 100 paddy weight demonstrated the highest variation in both mutagenic generations of super basmati. Significant correlation and the greatest contribution in total variability were existed among number of spikelet/plant, tiller/plant and plant height during both mutagenic populations of super basmati. First two principle component of bi-plot explained 52.62% and 54.50% of total variance in first and second mutagenic population of super basmati, respectively. It is concluded that ethyl methane sulphonate treatments up to 1.5% might be initiated a considerable level of genetic variability in mutant lines of super basmati. This genetic variability should be used for advance study such as for selection of rice mutant genotypes in mutation breeding program.

Keywords: Cereals, agronomic traits, yield potential, chemical mutagens, genetic diversity, point mutation, principle component analysis.

INTRODUCTION

Rice is second important cereal for world's population due to the staple food (Luz *et al*., 2016). Sustainable rice production must enhance to feed increasing population without extra farmland in changing environment such a rapid climate changes (Tester and Langridge, 2010; Guo *et al*., 2014; Ramchander *et al*., 2015). The yield potential of the crop mostly depends on the climate because climate has deep effects in both positive and negative ways on the crops productivity (Gover and Upadhya, 2014). Broading the genetic base of crop is a significant breeding objective that can be accomplished by induced mutation (Jalata *et al*., 2011). Induced mutation is source of variability creating genotypes with the best agronomic traits adapted to various ecological regions (Serrat *et al*., 2014). Mutation can be induced by physical and chemical mutagens. Ethyl methane sulphonate is usually applied as chemical agent in crop plants. It has ability to build the high and stable nucleotide exchange in different genomes of an organism (Talebi *et al*., 2012). This chemical generates an enormous quantity of point mutations in moderately little mutant populations (Henikoff and Comai, 2003). Multivariate analysis has been reported for genetic divergence analysis in various food crops such as barley (Cross, 1992), sorghum (Ayana and Bekele, 1999), wheat (Hailu *et al*., 2006), peanut (Upadhyaya *et al*., 2009), vineyard peach (Nikolic *et al*., 2010) as well as rice (Sinha and Mishra, 2013; Chakravorty *et al*., 2013). Principal component analysis (PCA) is one of the multivariate statistical approaches. It has been applied in rice for estimation of inherent genetic variability (Rabara *et al*., 2014; Ravikumar *et al*., 2015; Mahendran *et al*., 2015; Mawia *et al*., 2015; Luz *et al*., 2016; Sahu *et al*., 2017). It divides the data into two proportions to expose comparison and association among variables and genotypes based upon percentage of variability and correlations (Chakravorty *et al.*, 2013). The positive and negative trend of coefficients will be useful to categorize the different rice accessions (Mahendran *et al*., 2015). Induced mutation can quickly generate variability in qualitative and quantitative inherited traits of crop plant. Thus, the present study was conducted to develop EMS-induced mutagenic germplasm of super basmati and assess phenotypic diversity among EMS induced mutants of super basmati under agronomic important traits (Plant height, number of tiller/plant, panical length, number of spikelet/plant, 100 paddy weight, yield/plant, paddy length, paddy width, paddy length width ratio) using principle component analysis.

MATERIALS AND METHODS

***Plant material collection*:** Seeds of super basmati were collected from Rice Research Institute; Kala Shah Kaku; Gujranwala; Pakistan. The current experiment was carried out in laboratory and field area of Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad (UAF).

***EMS mutagenesis*:** Mutagenic populations were developed by treating the seeds of super basmati with different doses of EMS (0, 0.25, 0.5, 0.75, 1. 0, 1.25, 1.5, 1.75 and 2% (v/v). One hundred and fifty seeds per EMS treatment were soaked in distilled water for 4-5 hours at room temperature. After pour out of water, 10mL working solution of EMS concentration (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2%) was added in falcon and transferred to orbit shaker for 24 hours at 60 rpm. After the application of EMS mutagenesis, distilled water is used to rinse the treated seeds three times for removal of chemical from seed coat. Then, mutagenic seeds were propagated in Belgium compost filled trays under laboratory conditions for calculation of germination percentage and to establish first mutagenic generation (M_1) of super basmati. M_1 progeny were selfed in field to get M_2 generation of super basmati that segregated in 3:1 ratio for wild and mutant genotypes. These progeny raised in clay loamy soil using single seed progeny method in which genotypes were repeated five times with R-R distance (10cm) and P-P distance (8cm). Fertilizer and irrigation application was applied according to recommended agronomic practices.

***Phenotypic characterization of mutants*:** Data was recorded both at seed germination stage (Germination %) and crop maturity (Plant height, number of tiller/plant, panicle length, number of spikelet/plant, 100 paddy weight, yield/plant, paddy length, paddy width, paddy length width ratio).

***Germination (%)*:** Germination percentage was computed by given empirical formula.

GP (%) = No. of seeds germinated/total seeds x 100

***Plant height (cm)*:** The plant height was calculated with the help of measuring rod from the bottom of the plant to the tip of main panicle.

***Tiller per plant (cm)*:** Number of tillers for each plant were counted manually.

***Panicle length (cm)*:** The panicle length was measured from ground base to mother shoot (Main panicle) with the help of measuring tape.

***Number of spikelet per plant*:** Number of spikelet was calculated manually for each selected plant.

***100 Paddy weight (g)*:** A sample of 100 paddy was taken from each genotype and weighted with the help of electronic balance.

***Paddy yield per plant (g)*:** Paddy from each genotype were harvested and weighted in grams by electronic balance.

***Paddy length and Paddy width (mm)*:** Ten random paddy samples were collected to measure paddy dimensions using photographic enlarger

***Paddy length width ratio*:** To obtain the paddy shape, the following equation can be used

$$\text{Length to width ratio (L/W)} = \frac{\text{Average paddy length (mm)}}{\text{Average paddy width (mm)}}$$

The average values of ten selected plant/treatment/replication were used in XLSTAT 2016 software for statistical method of Principal component analysis (PCA). Principal component analysis (Sneath and Sokal, 1973) was used to accesses phenotypic variability in morphological traits among mutant lines of super basmati. This analysis is used to identify the association among traits of distinguishing selected genotypes toward yield and classify these genotypes into separate groups.

RESULTS

***EMS mutagenesis*:** In present study, EMS doses from 0.25-1.5% were more effective for generating mutant genotypes of super basmati with best agronomic traits. No seed was germinated from EMS dose above 1.5% due to the drastic effect of EMS (Fig. 1).

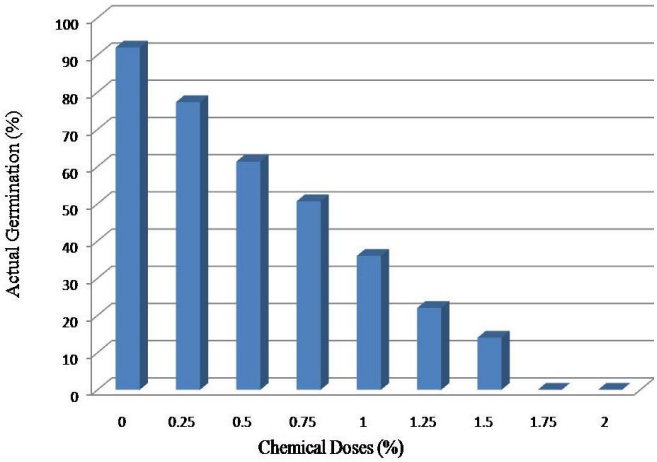

Figure 1. Effect of chemical concentration on seed germination of super basmati.

***Descriptive statistics*:** Total 93 M_1 plants and 80 M_2 plant from all mutagenic population were randomly selected for principle component analysis. Coefficient of variance (CV %) demonstrated that how much variability present in yield contributing variables because it is directly proportional to variability. The largest variation was observed for number spikelet/plant (CV=47.73%; 30.41%), yield/plant (CV=47.39%; 32.85%), tiller/plant (CV=42.79%; 28.70%) and 100 paddy weight (CV=30.77%; 43.88%) during M_1 and M_2 generation of super basmati respectively due to the greater difference between the minimum and maximum values

(Table 1). The minimum (two) and the maximum (34) number of spikelet/plant were recorded in mutant genotypes (M72; M54) respectively during M_1 generation of super basmati. Mutant genotype (M104) was recorded with the highest number of spikelet/plant (24.5) and the genotype (M101) had the lowest one (6) during M_2 (Second mutagenic generation) of super basmati (Table 1). It means that above variables should be used for further study in assortment of rice mutant genotypes due to the highest variability. Paddy length has clarified the smallest variation during both mutagenic generations of super basmati with the CV of 3.313% and 4.018% respectively (Table 1). It means that in collection of rice mutant genotypes, paddy length must not be exploited for further analysis due to least variability in both mutagenic generations of super basmati (Table 1).

***Evaluation of cumulative variability*:** In present study, principal component analysis divided the mutant indices into nine different principal components or factors (F1/PC1 to F9/PC9) in both mutagenic generations of super basmati (Table 2). Among these nine components, only four (M_1) and three (M_2) components had eigen value (bold) greater than unity (Table 2). Eigen value (1) is key criteria as cut off value for assortment of principle factors for further studies. Eigen value greater than one explained that factor reports more variance than one of the original variables. The column of cumulative variability (%) provides the variability (%) for

Table 1. Descriptive statistics for all studied traits during both mutagenic generation of super basmati.

	M_1 of super basmati							
	Obs.	**Mini.**	**Genotypes**	**Maxi.**	**Genotypes**	**Mean**	**S.D**	**CV%**
PH	93	24.000	65,71	56.000	105	44.067	7.418	16.83386
NOSP	93	2.000	72	34.000	54	14.944	7.134	47.73848
PL	93	3.000	12,65,72	12.000	118	8.170	1.726	21.12946
TP	93	6.000	24,78	49.000	54	18.419	7.883	42.79591
100PeW	93	1.230	92	5.090	115	2.600	0.800	30.77817
YP	93	3.060	70	29.760	45	12.126	5.747	47.39608
PeL	93	8.450	121	10.050	30,88,107	9.467	0.314	3.313412
PeW	93	1.690	118	2.030	22	1.909	0.087	4.566219
PeLWR	93	4.400	121	5.900	56	5.000	0.293	5.85616
	M_2 of super basmati							
PH	80	37.12	25	58.250	127	47.289	5.114	10.81353
NOSP	80	6.000	101	24.500	104	14.129	4.297	30.41365
PL	80	6.750	100	10.475	47	8.385	0.751	8.959898
TP	80	7.500	48	28.750	31	16.904	4.852	28.70171
100PeW	80	1.640	100	5.990	115	2.438	1.070	43.88255
YP	80	10.250	129	69.750	25	39.112	12.851	32.85531
PeL	80	7.625	121	9.875	107,108	9.181	0.369	4.018681
PeW	80	1.550	100	2.125	105	1.892	0.119	6.313986
PeLWR	80	3.910	121	6.008	100	4.872	0.381	7.817771

PH: Plant height (cm); NOSP: number of spikelet/plant; PL: Panical length (cm); TP: Tillers/plant; 100PW: 100 Paddy weight (g); YP: Yield/plant (g); PeL: Paddy length (mm); PeW: Paddy width (mm); PeLWR: Paddy length width ratio; M_1: First mutagenic generation; M_2: Second mutagenic generation.

Table 2. Evaluation of cumulative variability of factors during both mutagenic generation of super basmati.

	M_1 of super basmati								
Factors	**PC1/F1**	**PC2/F2**	**PC3/F3**	**PC4/F4**	PC5/F5	PC6/F6	PC7/F7	PC8/F8	PC9/F9
Eigen value	2.774	1.962	1.294	1.148	0.861	0.678	0.194	0.082	0.008
Variability (%)	30.819	21.799	14.382	12.757	9.568	7.528	2.151	0.906	0.089
Cumulative variability (%)	30.819	52.618	67.000	79.757	89.326	96.854	99.005	99.911	100.000
	M_2 of super basmati								
Eigen value	2.522	2.383	1.258	0.919	0.680	0.645	0.537	0.054	0.002
Variability (%)	28.025	26.477	13.974	10.211	7.556	7.170	5.968	0.595	0.025
Cumulative variability (%)	28.025	54.501	68.475	78.686	86.242	93.412	99.380	99.975	100.000

PC: Principle component; F: Factor; M_1: First mutagenic generation; M_2: Second mutagenic generation

first n components. such as, the cumulative variability (%) for the second component is the sum of the variability (%) for the first two components. Principal components (PCs) of Principal component analysis (PCA) are smaller number of uncorrelated variables from a larger set of data. First two PCs contributed the cumulative variability of 52.618% and 54.501% which proved that these two PCs were suitable for sketch of bi-plot due to the highest variability in PC1 (30.819%; 28.025%) and PC2 (21.799%; 26.477%) in M_1 and M_2 population of super basmati, respectively (Table 2).

***Bi-plot depends upon factor loading and contribution of variables*:** Bi-plot (Fig. 2 & 3) was constructed and divided into four groups on the bases the values of factor loading of variables (Table 3). First group of bi-plot represented positive value of factor loading in both factors (F1 and F2) of principle component analysis. Second group of bi-plot showed positive

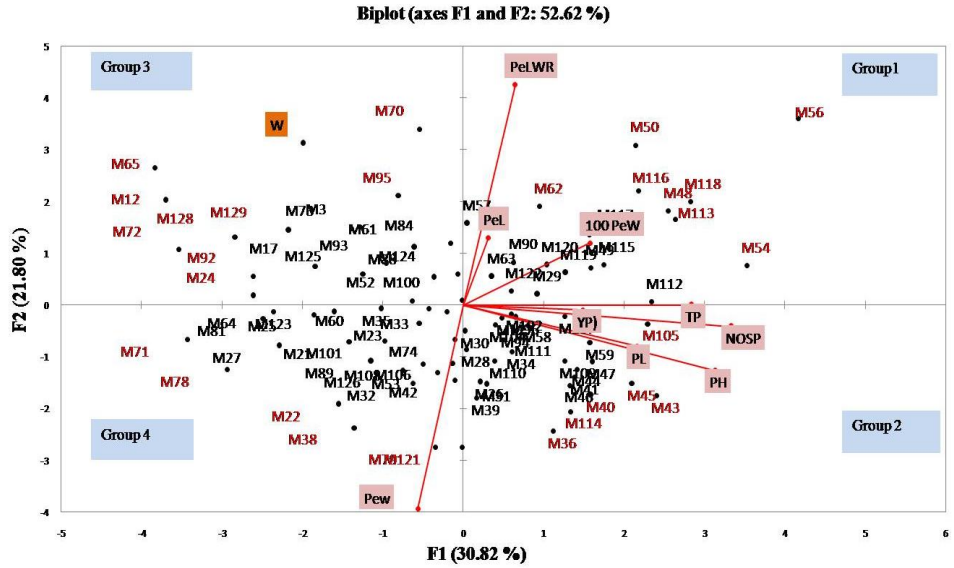

Figure 2. Bi-plot of traits and genotypes for first mutagenic generation of super basmati.

PH: Plant height (cm); NOSP: number of spikelet/plant; PL: Panical length (cm); TP: Tillers/plant; 100PW: 100 Paddy weight (g); YP: Yield/plant (g); PeL: Paddy length (mm); PeW: Paddy width (mm); PeLWR: Paddy length width ratio; PC: Principle component; F: Factor, M: Mutant, W: Wild of super basmati

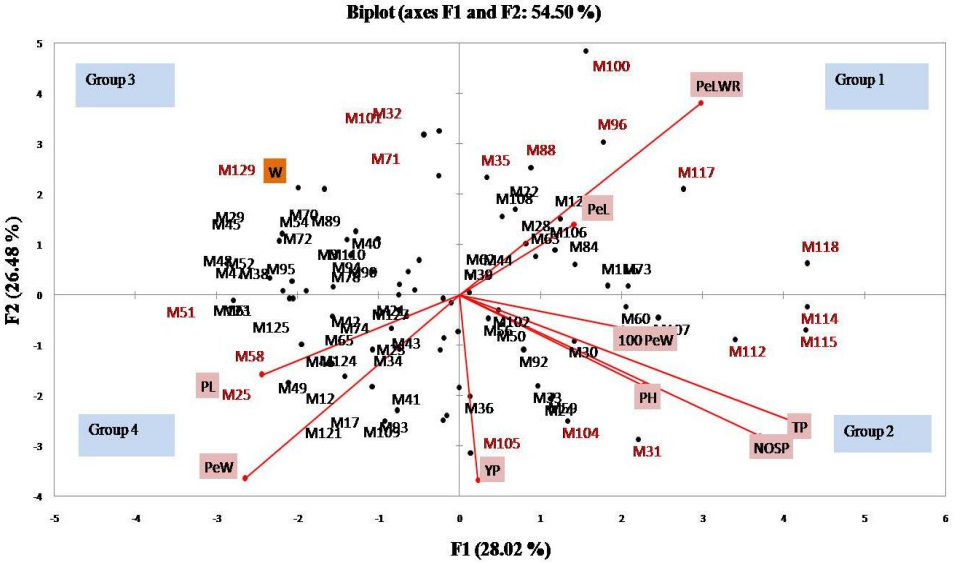

Figure 3. Bi-plot of traits and genotypes for second mutagenic generation of super basmati.

PH: Plant height (cm); NOSP: number of spikelet/plant; PL: Panical length (cm); TP: Tillers/plant; 100PW: 100 Paddy weight (g); YP: Yield/plant (g); PeL: Paddy length (mm); PeW: Paddy width (mm); PeLWR: Paddy length width ratio; PC: Principle component; F: Factor, M: Mutant, W: Wild of super basmati

Table 3. Factor loading and contribution of variables during both mutagenic generation of super basmati.

M_1 of super basmati	**PC1/F1**		**PC2/F2**	
	Factor loading	**Contribution (%)**	**Factor loading**	**Contribution (%)**
PH	0.833	25.044	-0.283	4.089
NOSP	0.887	28.377	-0.094	0.451
PL	0.575	11.924	-0.179	1.629
TP	0.755	20.535	-0.001	0
100 PeW	0.42	6.346	0.267	3.642
YP	0.396	5.656	-0.023	0.026
PeL	0.083	0.251	0.291	4.317
PeW	-0.149	0.803	-0.881	39.599
PeLWR	0.172	1.065	0.953	46.247
M_2 of super basmati				
PH	0.455	8.191	-0.35	5.15
NOSP	0.738	21.567	-0.543	12.366
PL	-0.485	9.321	-0.308	3.976
TP	0.815	26.312	-0.482	9.732
100 PeW	0.402	6.411	-0.128	0.684
YP	0.046	0.083	-0.711	21.196
PeL	0.283	3.17	0.271	3.087
PeW	-0.527	11.005	-0.707	20.987
PeLWR	0.593	13.94	0.737	22.823

F: Factors; PC: Principle component; M_1: First mutagenic generation; M_2: Second mutagenic generation; PH: Plant height (cm); NOSP: number of spikelet/plant; PL:Panical length (cm); TP: Tillers/plant; 100PW: 100 paddy weight (g); YP: Yield/plant (g); PeL:Paddy length (mm); PeW:Paddy width (mm); PeLWR:Paddy length width ratio; PC: Principle component; F: Factor; M_1: First mutagenic generation; M_2: Second mutagenic generation

and negative value of factor loading in factor one and factor two respectively. Third group of bi-plot has negative value of factor loading in F1 and positive value of factor loading in F2. Negative value of factor loading in both factors (F1 and F2) created fourth group of bi-plot (Fig. 2 & 3). On the whole, first and second group considered as positive group of bi-plot. Third and fourth group collectively called as negative group of bi-plot (Figure 2; Figure 3). Factor loading represented the orientation of variables in one of four groups depend upon its charges. Plant height, number of spikelet/plant, tiller/plant and yield/plant have oriented in second group of bi-plot (Fig. 2 & 3) because these variables have positive charge in F1 and negative charge in F2 (Table 3) during both mutagenic generations of super basmati. Negative value of both factor (Table 3) represented the orientation of paddy width in fourth group of bi-plot (Fig. 2 & 3) during both mutagenic generations of super basmati. On PC1, high loading was observed for number of spikelet/plant (0.887; 0.738), tiller/plant (0.755; 0.815) and plant height (0.833; 0.455) (Table 3) explaining (28.377%; 21.567%), (20.535%; 26.312%) and (25.044%; 8.191%) of contribution (Table 3) with variance (30.819%; 28.025%) in total variability (Table 2) in M_1 and M_2 generation of super basmati respectively. In the 2nd PC, only paddy length width ratio had the high loading (0.953; 0.737) (Table 3) explaining (46.247%; 22.823%) of contribution (Table 3) and variance (21.79%; 26.477%) in total variability (Table 2) during M_1 and M_2 generation of super basmati, respectively. It means that these variables must be used for advance study in evaluation of rice mutant genotypes in mutation breeding program.

***Bi-plot depends upon correlation of variables*:** Correlation demonstrated that how much one variable correlated to others. Correlation is an easy and efficient means to determine the association among different traits. Correlation matrix demonstrated significantly positive or non-significant negative correlation of one variable to another variable. Correlation between variables is described as angle between vectors (right, acute as well as obtuse angle). Acute angle less than 90° showed positive correlation whereas acute angle less than 45° showed strong positive correlation. Moreover, right angle (90°) showed independence or no correlation. Obtuse angle more than 90° showed negative correlation whereas obtuse angle of greater than 135° and less than 180° showed strong negative correlation. Length of vector showed the discrimination power of traits for differentiation of genotypes. Correlation of variables is directly proportional to the length of vector of variables and inversely proportional to cosine angle (acute angle). Bi-plot (Fig. 2 & 3) showed clearly that vectors of the number of spikelet/plant, tiller/plant, plant height, paddy length width ratio, paddy width and panicle length were the most discriminative and informative for evaluation of mutant genotypes in both mutagenic generations of super basmati as their vector length was the longest relative

Table 4. Correlation coefficient among various traits in both mutagenic generation of super basmati.

	PH	NOSP	PL	TP	100 PeW	YP	PeL	PeW	PEWR
M_1 of super basmati									
PH	1	0.664*	0.667*	0.464	0.176	0.253	0.118	0.129	-0.055
NOSP	0.664*	1	0.285	0.877*	0.198	0.240	-0.033	-0.069	0.052
PL	0.667*	0.285	1	0.068	0.237	0.153	0.094	0.038	-0.010
TP	0.464	0.877*	0.068	1	0.154	0.099	-0.040	-0.123	0.094
100 PeW	0.176	0.198	0.237	0.154	1	0.264	-0.006	-0.234	0.178
YP	0.253	0.240	0.153	0.099	0.264	1	-0.006	-0.025	0.010
PeL	0.118	-0.033	0.094	-0.040	-0.006	-0.006	1	0.117	0.453
PeW	0.129	-0.069	0.038	-0.123	-0.234	-0.025	0.117	1	-0.822*
PeLWR	-0.055	0.052	-0.010	0.094	0.178	0.010	0.453	-0.822*	1
M_2 of super basmati									
PH	1	0.376	0.427	-0.002	0.025	0.178	0.067	-0.008	0.042
NOSP	0.376	1	0.937*	-0.117	0.285	0.294	0.033	-0.011	0.023
TP	0.427	0.937*	1	-0.179	0.222	0.282	0.074	-0.096	0.117
PL	-0.002	-0.117	-0.179	1	-0.282	0.145	-0.055	0.346	-0.338*\
100 PeW	0.025	0.285	0.222	-0.282	1	0.125	-0.102	-0.113	0.054
YP	0.178	0.294	0.282	0.145	0.125	1	-0.015	0.420	-0.357
PeL	0.067	0.033	0.074	-0.055	-0.102	-0.015	1	-0.029	0.531*
PeW	-0.008	-0.011	-0.096	0.346	-0.113	0.420	-0.029	1	-0.860*
PeLWR	0.042	0.023	0.117	-0.338	0.054	-0.357	0.531*	-0.860*	1

*Significant at 0.05 (>0.5), ** Significant at 0.01(>2)

PH: Plant height (cm); NOSP: number of spikelet/plant; PL:Panical length (cm); TP: Tillers/plant; 100PW: 100 paddy weight (g); YP: Yield/plant (g); PeL:Paddy length (mm); PeW:Paddy width (mm); PeLWR: Paddy length width ratio; M_1: First mutagenic generation; M_2: Second mutagenic generation

to other vectors. Paddy length, 100 paddy weight and yield/plant were the shortest relative to others vectors in both mutagenic generations of super basmati. Number of spikelet/plant reflected the high, positive correlation with tiller/plant (0.877*; 0.937*) followed by plant height (0.664*; 0.376) in both mutagenic generations of super basmati (Table 4) because these variables have less cosine angle as well as longer length of these vectors of variables (Fig. 2 & 3). But number of spikelet/plant negatively correlated with paddy width (-0.069; -0.011) (Table 4) suggesting that higher number of spikelet/plant is expected to be accompanied by increased number of tiller/plant, plant height and reduced the value of paddy width on PC1 and PC2 (Fig. 2 & 3). It was also suggested that plant height and tiller/plant would be considered as an interesting index for number of spikelet/plant in experimental situations during both mutagenic generations of super basmati.

***Bi-plot of genotypes*:** All the genotypes and traits were widely scattered across different quarters of bi-plot (Fig. 2 & 3). Bi-plot had characteristic of grouping the mutant genotypes into four distinct groups. The finding of current research explained that mutant genotypes (marked as red color) that were farther away from the origin in positive direction of trait showed their better performance in first and second group of bi-plot on the base of significant traits (Fig. 2 & 3). Mutant genotypes (marked as red color) that were away from the origin in negative direction demonstrated as poor performing mutant genotypes of super basmati in third and fourth group of bi-plot on the bases of non-significant traits (Fig. 2 & 3).

DISCUSSION

***EMS mutagenesis*:** In present study, EMS dose up to 1.5% was the best for generating desired agronomic traits of super basmati because no seeds were germinated above 1.5% of EMS. Previous studies (Wu *et al*., 2005; Till *et al*., 2007) explained that EMS doses up to 1.5% were helpful in developing rice mutagenic generations. Every mutant genotypes illustrated zero seed germination at 1.25% of EMS in rice (Talebi *et al*., 2012) and over 1.25% in mustard (Yadav *et al*., 2016).

***Descriptive statistics*:** In current study, the largest variation was observed for number spikelet/plant, yield/plant, tiller/plant and 100 paddy weight during M_1 and M_2 generation of super basmati, respectively. These results concurrence with results of Maji and Shaaibu (2012), Gana *et al*. (2013), Chakravorty *et al.* (2013), Kumar *et al*. (2015), Mawia *et al*. (2015) and Sahu *et al*. (2017). They proposed that wider variation was observed in different traits among different accessions of rice by the high value of coefficient of variance and range.

Evaluation of cumulative variability: First two PCs gave the cumulative variability of 52.618% and 54.501% in current M_1 and M_2 population of super basmati respectively. The

importance of eigen value and first two principle components is already described (Brejda *et al*., 2000; Kumar *et al*., 2014; Aslam *et al*., 2014; Maqbool *et al*., 2015; Sahu *et al*., 2017). First two principle components explained 36.98, 69.32, 41.11, 91.3, 62.43, 34.087 and 31.489% of cumulative variability among different set of rice accessions, respectively (Rabara *et al*., 2014; Ravikumar *et al*., 2015; Mahendran *et al*., 2015; Mawia *et al*., 2015; Luz *et al*., 2016; Gour *et al*., 2017; Ojha *et al*., 2017).

***Bi-plot depends upon factor loading and contribution of variables*:** In present investigation, high factor loading was observed on PC1 for number of spikelet/plant, tiller/plant and plant height and PC2 for paddy length with greatest contribution of total variability in both mutagenic generations of super basmati. Similar results were published in articles of Zahid *et al*. (2006) and Rabara *et al*. (2014). They proposed that number of tillers/plant contributed the maximum direct effect on yield with the value of high factor loading indicating this trait must be used in selection of high yielding cultivars of rice. In further support to current findings, Mahendran *et al*. (2015) explained that total tiller/plant, total productive tiller and yield/plant accounted maximum variability in PC2/F2.

***Bi-plot depends upon correlation of variables*:** Tiller/plant of super basmati would be considered as a remarkable index for number of spikelet/plant due to the high value of correlation in present study. Naeem *et al*. (2015) proposed that a majority of the traits associated significant correlation but some traits was non-significant in mutated and non-mutated rice varieties. Correlation coefficient with more than 0.7 is considered to be highly significant because at this value each trait controls to the other traits by more than 50% (Snedecor and Cochran, 1989). The present results found such significant correlation between number of spikelet/plant and tillers/plant. Similar results for significance of tiller/plant and effective tiller was reported by Kumar *et al*. (2014) and Ravikumar *et al*. (2015) among rice cultivars.

***Bi-plot of genotypes*:** Better or poor performing genotypes were to be found in positive or negative direction of differentiating traits in present bi-plot analysis. The study of Aslam *et al*. (2014) explained that diverse genotypes were plotted away from origin in positive or negative direction of discriminating traits showed better or poor performance, respectively.

***Conclusions*:** The considerable amount of phenotypic variability was identified within mutant lines of super basmati. Principal component analysis suggested that EMS treatments were efficient for producing variability in agronomic important traits of super basmati. It is also concluded that both significant traits and mutant genotypes might be utilized in rice mutation and molecular breeding program to develop superior mutants with high yield contributing trait.

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