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Evaluation of EMS induced Mutant Population using character Associations and Principle Component Analysis in Rice (*Oryza sativa* L)

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ABSTRACT: A research was carried out to evaluate the genetic diversity and establish relationship among yield and its components in rice cultivar BPT 5204. The field experiment was carried out with 30 M1 derived mutant lines treated at 0.75% EMS along with a control. The results of M2 population showed a strong correlation among days to 50% flowering, number of productive tillers and days to maturity upon grain yield. Genotypic path coefficient analysis revealed that productive tillers, sterile grains per panicle and flag leaf length had positive and direct effect on grain yield per hill. Phenotypic path coefficient analysis indicated that number of tillers per hill, sterile grains per panicle, and days to maturity, panicle length and flag leaf length had positive and direct effect on grain yield per hill. Out of 11, four principal components PC1, PC2, PC3 and PC4 exhibited more than 1.00 Eigen value, and 68.6% cumulative variability was observed among the traits studied. Hierarchical clustering analysis categorized 7 major clusters among the mutant lines of which cluster 1 includes 20 lines with superior grain yield. Furthermore, this study will assist the breeder for developing reliable selection indices in rice mutant population for yield enhancement and selection of better yielding varieties.

Keywords: Correlation, mutant population, path coefficient analysis, principal component analysis, Hierarchical clustering analysis.

INTRODUCTION

The primary crop, rice (*Oryza sativa* L.), serves as food for more than 75% of Asian population and more than three billion people worldwide (Sajid *et al.*, 2015; Reetisana *et al.,* 2022). In a person's typical normal diet, it makes up 50% to 80% of their daily caloric consumption and 55% of their protein intake. The primary goals for increasing rice grain output vertically are to introduce new kinds of rice with traits including early heading, low stature, lodging resistance, blast resistance, and increased grain quality. In India, the early Kharif season is when rice is cultivated. The predicted increase in rice output above the previous five years' average of 97.83 million tonnes during the Kharif season 2021–2022 is 9.21 million tonnes. There are attempts being made to break the yield barriers in rice breeding strategies. The demand for food production is always increasing to meet the hunger of raising human population (Kavyashree *et al.,* 2022). The complex nature of grain yield, which depends on several component traits, does not facilitate direct selection. Understanding the relationship between grain yield and its component features will be beneficial for increasing grain production. Correlation studies, which provide information on the kind and degree of relationship between yield and its component traits, can help the breeder decide the magnitude and direction of selection for character development (Vengatesh and Govindarasu 2018; Hari *et al.,* 2022). Plant breeders can rank genetic features based on the contribution they make using route coefficient analysis, which breaks correlation coefficients further into direct and indirect contributions. Therefore, generation of variability through mutagenic treatments is of paramount importance for improvement of aromatic rice. In order to develop high yielding rice genotypes, it is important to understand the nature of the relationship between grain yield and quality traits, the direct and indirect contributions of these components to grain yield, and the best combinations of these yield components to use as selection criteria. This study aims to perform a number of experiments and studies that will be helpful to develop high yielding rice genotypes and understanding the nature of relationship between them.

MATERIALS AND METHODS

The current investigation was carried out at Bagusala farm, which is located in Centurion University of

Technology and Management in Paralakhemundi, Odisha, during the kharif of 2021. The experimental area was quite consistent in terms of both its topography and its level of fertility located 84.139883°E, 18.806853°N. The materials for the experiment include 30 M2 lines along with a control. These lines were planted with a spacing of 20 × 20 cm and three replications after being put out in a randomised block configuration. In order to cultivate a typical crop, the suggested package of practises was adhered to, which included irrigation that consisted of alternating soaking and drying as well as necessary plant protection measures. At various stages of crop development, including the transplanting, early tillering, and booting stages, the main field received an application of fertilisers at a rate of 150:50:50 NPK kg/ha.

Data collection. Randomly five plants were selected from each to serve as samples, and observations were made regarding the plant's height, the number of tillers per hill, the productive tillers per hill, the number of days until 50% flowering, the panicle length, the filled grains per panicle, the sterile grains per panicle, the flag leaf length, the flag leaf width, the number of days until maturity, and the grain yield per hill.

Data analysis. Using a number of different packages, the information gathered from the 21 quantitative and quality attributes was analysed in "Studio (4.1.2)". The 'Variability' software was utilised in order to do the analyses of correlation and path coefficient.

RESULTS AND DISCUSSION

Correlation analysis. Correlation estimated helps the plant breeder during selection by providing a better understanding on yield and yield components. The phenotype of a plant is result of interaction between many contributing factors. The final yield of a plant depends on the combined effects of several characters. Among all the characters yield is the most important complex phenotypic performance of a plant. Majority of the times yield is influenced by genetic and environmental factors and their interactions. Genotypic and Phenotypic correlations coefficient values of various traits are listed in Table 1 and 2. In general, the genotypic correlation coefficients were greater than the respective phenotypic correlation coefficients which might be from the modifying effect of environment on the association of characters at phenotypic level.Similar results also reported by Keerthiraj *et al*. (2020). Under the present investigation, the phenotypic and genotypic correlation coefficients were worked out for grain yield per plant and yield related characters. The correlations of grain yield per plant were negative and significant with characters *viz*., days to 50% flowering and days to maturity. These results were in accordance with the results of Kole *et al*. (2010); Kishore *et al*. (2015); Edukondalu *et al*. (2017); Saha *et al*. (2019); Keerthiraj *et al*. (2020).

Table 1: Genotypic correlation between grain yield and its component characters in rice.

Traits	PH	NTH	PTH	UGP	50%F	DM	PL	FGP	FLL	FLW	GYH
PH	1	-0.222*	-0.144	-0.01	-0.118	-0.157	-0.046	0.389**	0.013	0.122	-0.202
NTH		1	1.011**	0.165	0.315**	0.358**	0.071	-0.135	-0.071	0.320**	0.068
PTH			1	0.223*	0.151	0.155	0.227*	-0.181	-0.092	0.353**	0.068
UGP				1	0.117	0.142	0.400**	0.114	0.023	0.139	0.175
50%F					1	1.071**	0.01	-0.124	0.346**	0.384**	-0.269**
DM						1	-0.009	-0.146	0.365**	0.403**	-0.291**
PL							1	-0.068	0.059	0.270**	0.145
FGP								1	-0.123	0.400**	-0.164
FLL									1	0.199	-0.052
FLW										1	-0.097
GYH											1

* Significant at 1% ** Significant at 5%

PH: Plant Height (cm); NTH: No. of Tillers per Hill; PTH: Productive Tillers per Hill; UGP: Unfilled grains per plant; 50%F: Days to 50% Flowering; DM: Days to Maturity; PL: Panicle Length (cm); FGP: Filled Grains per Panicle; FLL: Flag Leaf Length (cm); FLW: Flag Leaf Width (cm); GYH: Grain Yield per Hill (g).

Table 2: Phenotypic correlation between grain yield and its component characters in rice.

Traits	PH	NTH	PTH	UGP	50%F	DM	PL	FGP	FLL	FLW	GYH
PH	1	-0.108	-0.137	-0.035	-0.13	-0.087	-0.033	0.223*	-0.004	0.103	-0.169
NTH		1	0.710**	0.142	0.118	0.092	0.005	-0.138	-0.107	0.233*	0.026
PTH			1	0.188	0.118	0.119	0.195	-0.082	-0.016	0.306**	0.007
UGP				1	0.059	0.036	0.321**	0.082	0.001	0.108	0.164
50%F					1	0.938**	-0.007	-0.026	0.171	0.286**	-0.134
DM						1	0.015	-0.015	0.168	0.281**	-0.124
PL							1	0.06	-0.053	0.189	0.076
FGP								1	-0.104	0.230*	-0.09
FLL									1	0.153	0.01
FLW										1	-0.078
GYH											1

* Significant at 1% ** Significant at 5%

PH: Plant Height (cm); NTH: No. of Tillers per Hill; PTH: Productive Tillers per Hill; UGP: Unfilled grains per plant; 50%F: Days to 50% Flowering; DM: Days to Maturity; PL: Panicle Length (cm); FGP: Filled Grains per Panicle; FLL: Flag Leaf Length (cm); FLW: Flag Leaf Width (cm); GYH: Grain Yield per Hill (g).

Path coefficient analysis. Path coefficient analysis separates correlation coefficients into direct and indirect effects of various characters influencing on grain yield. It offers a useful method for determining both the direct and indirect causes of an association, delivers a critical analysis of the particular forces at work in a given correlation, and quantifies the relative weight of each causative element. In the present study, the phenotypic and genotypic correlation coefficient between grain yield per plant and yield components were partitioned to the corresponding direct and indirect effects through path analysis in rice are depicted in Table 3 (genotypic path analysis) and 4 (phenotypic path analysis).

In genotypic path analysis out of ten, three characters had positive and direct effect on grain yield per plant *viz*., productive tillers per hill (0.81417), sterile grains per plant (0.1821) and flag leaf length (0.04059), while rest of eight had negative and direct effect on grain yield per plant *viz.,* plant height (-0.2576), No. of tillers per hill (-0.7694), days to 50% flowering (-0.1837), days to maturity (-0.03), panicle length (-0.071), filled grains per panicle (-0.0624) and flag leaf width (-0.0138).

In phenotypic path analysis out of ten, five characters had positive and direct effect on grain yield per plant *viz.,* No. of tillers per hill (0.05997), sterile grains per plant (0.1718), days to maturity (0.10287), panicle length (0.03593) and flag leaf length (0.04088), while rest of eight had negative and direct effect on grain yield per plant *viz*., plant height (-0.1753), productive tillers per hill (-0.0776), days to 50% flowering (-0.2631), filled grains per panicle (-0.0611), flag leaf width (-0.0217). These results are in agreement to the earlier finding of Kole *et al*. (2010); Kishore *et al.* (2015); Edukondalu *et al.* (2017); Saha *et al.* (2019); Keerthiraj *et al*. (2020).

Principal component analysis. In the present inquiry, principal component analysis (PCA) was carried out on 31 genotypes of rice, each of which had 11 yield and yield-attributing variables. The principal components (PC) with Eigen values greater than one and which explained at least 5% of the variation in the data was taken into consideration for this analysis. These criteria were established by Brejda *et al*. (2000). It was determined that the PC that had Eigen values that were higher and variables that had high factor loading were the most accurate representatives of system characteristics. Out of a total of 11, four principal components (PCs) demonstrated more than 1.00 Eigen value (Fig. 1), including PC1 with 2.755, PC2 with 1.911, PC3 with 1.585, and PC4 with 1.289. This indicated that there was approximately 68.6% cumulative variability among the qualities that were investigated (Table 5). Similar results were observed by Krishna *et al*. (2022).

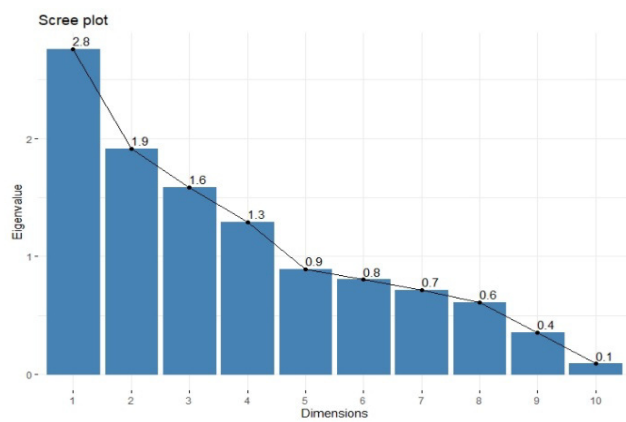

Fig. 1. Graphical representation of Eigen values screen plot for Principal component analysis in rice lines

Therefore, these four primary components were accorded the appropriate amount of priority for the expansion of the explanation. The proportion of variation exhibited by PC1 was 25.00%, the proportion exhibited by PC2 was 17.4%, the proportion exhibited by PC3 was 14.4%, and the proportion exhibited by PC4 was 11.7%. The overall cumulative variability was 68.6%. After the first PC, a semi-curve line was obtained. There was little difference noticed in each PC indicated, and as a result, selecting characters under the first PC would be desirable.

The rotated component matrix showed that the PC1 factor, which accounted for 25% of the total variation, was primarily associated with characteristics such as the number of tillers per hill, the number of productive tillers per hill, the number of days until 50% flowering, the number of days until maturity, and the width of the flag leaf. In the second PC2, the traits that were taken into consideration were the number of tillers per hill, the number of productive tillers per hill, the number of unfilled grains per panicle, the number of days until 50% flowering, the number of days until maturity, the length of the panicle, the length of the flag leaf, and the grain yield per hill. The PC3 was more related to characteristics such as the height of the plant, the number of filled grains per panicle, and the breadth of the flag leaf. The PC4 was more related to the number of tillers that were produced per hill, the number of unfilled grains that were produced per panicle, the length of the panicle, the length of the flag leaf, and the grain yield that was produced per hill. According to the PCA, the majority of critical yield and yield-attributing variables were contained in PC2 and PC4, respectively (Table 6, 7, 2 and 3).

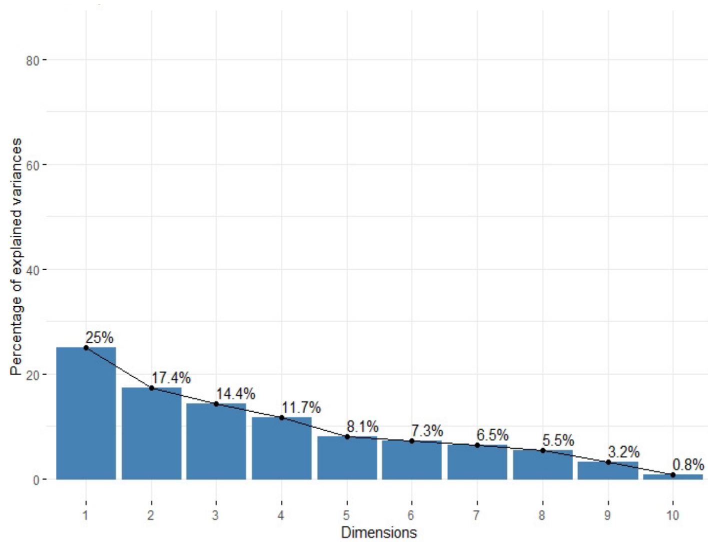

Fig. 2. Graphical representation of Principal Component Analysis.

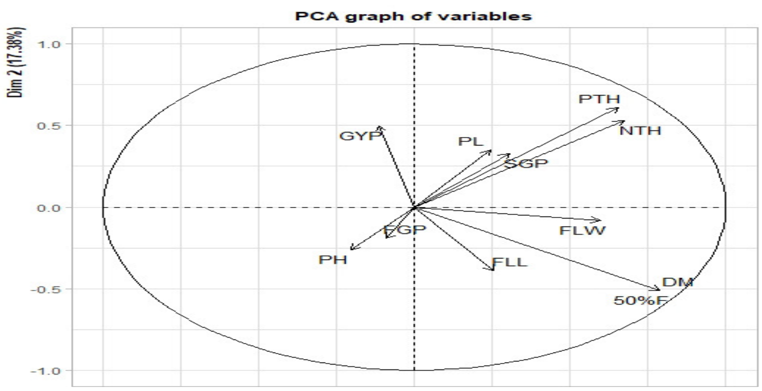

Fig. 3. Rotated component matrix for traits studied in rice.

Table 3: Genotypic path coefficient showing direct and indirect effects of different contributing characters on grain yield per hill in rice.

Traits	PH	NTH	PTH	UGP	50%F	DM	PL	FGP	FLL	FLW	Correlation GYP
PH	-0.2576	0.17055	-0.1174	-0.0017	0.02166	0.00472	0.0033	-0.0243	0.00052	-0.0017	-0.20196
NTH	0.05709	-0.7694	0.82336	0.02978	-0.058	-0.0107	-0.005	0.00844	-0.0029	-0.0044	0.06828
PTH	0.03715	-0.778	0.81417	0.04025	-0.0277	-0.0047	-0.0162	0.01131	-0.0037	-0.0049	0.06771
UGP	0.00247	-0.1271	0.18185	0.18021	-0.0215	-0.0043	-0.0284	-0.0071	0.00093	-0.0019	0.17518
50%F	0.03037	-0.2427	0.12278	0.02107	-0.1837	-0.0321	-0.0007	0.00776	0.01403	-0.0053	-0.26863**
DM	0.04055	-0.2752	0.12606	0.02558	-0.1968	-0.03	0.00066	0.00913	0.01482	-0.0056	-0.29078**
PL	0.01196	-0.0545	0.18522	0.07208	-0.0019	0.00028	-0.071	0.00424	0.00238	-0.0037	0.14507
FGP	-0.1003	0.10405	-0.1476	0.02052	0.02284	0.00439	0.00482	-0.0624	-0.005	-0.0055	-0.16421
FLL	-0.0033	0.05488	-0.0751	0.00415	-0.0635	-0.011	-0.0042	0.00767	0.04059	-0.0028	-0.05246
FLW	-0.0315	-0.2459	0.28755	0.02497	-0.0706	-0.0121	-0.0192	-0.025	0.00809	-0.0138	-0.09733

— Underlined values are direct effects

PH: Plant Height (cm); NTH: No. of Tillers per Hill; PTH: Productive Tillers per Hill; UGP: Unfilled grains per plant; 50%F: Days to 50% Flowering; DM: Days to Maturity; PL: Panicle Length (cm); FGP: Filled Grains per Panicle; FLL: Flag Leaf Length (cm); FLW: Flag Leaf Width (cm); GYH: Grain Yield per Hill (g).

Table 4: Phenotypic path coefficient showing direct and indirect effects of different contributing characters on grain yield per hill in rice.

Traits	PH	NTH	PTH	UGP	50%F	DM	PL	FGP	FLL	FLW	Correlation GYP
PH	-0.1753	-0.0065	0.01059	-0.006	0.03409	-0.009	-0.0012	-0.0136	-0.0002	-0.0022	-0.169
NTH	0.01887	0.05997	-0.055	0.02438	-0.0309	0.00951	0.00019	0.00846	-0.0044	-0.0051	0.026
PTH	0.02394	0.04255	-0.0776	0.03234	-0.031	0.01223	0.00702	0.00499	-0.0007	-0.0066	0.007
UGP	0.00607	0.00851	-0.0146	0.1718	-0.0155	0.00368	0.01154	-0.005	-0.0002	-0.0023	0.164
50%F	0.02272	0.00705	-0.0091	0.01011	-0.2631	0.09651	-0.0003	0.00161	0.00698	-0.0062	-0.134
DM	0.01527	0.00554	-0.0092	0.00614	-0.2468	0.10287	0.00055	0.00089	0.00685	-0.0061	-0.124
PL	0.00573	0.00031	-0.0152	0.05516	0.00189	0.00157	0.03593	-0.0037	-0.0022	-0.0041	0.076
FGP	-0.0391	-0.0083	0.00634	0.01403	0.00694	-0.0015	0.00216	-0.0611	-0.0043	-0.005	-0.090
FLL	0.00072	-0.0064	0.00128	-0.00007	-0.0449	0.01723	-0.0019	0.00636	0.04088	-0.0033	0.010
FLW	-0.018	0.01399	-0.0237	0.01856	-0.0754	0.02891	0.00678	-0.014	0.00627	-0.0217	-0.078

— Underlined values are direct effects

PH: Plant Height (cm); NTH: No. of Tillers per Hill; PTH: Productive Tillers per Hill; UGP: Unfilled grains per plant; 50%F: Days to 50% Flowering; DM: Days to Maturity; PL: Panicle Length (cm); FGP: Filled Grains per Panicle; FLL: Flag Leaf Length (cm); FLW: Flag Leaf Width (cm); GYH: Grain Yield per Hill (g).

Table 5: Eigen values, % variance and cumulative variance of rice line.

Principal components	Eigen values	Proportion	Cumulative Proportion	Principal components	Eigen values	Proportion	Cumulative Proportion	Principal components	Eigen values	Proportion	Cumulative Proportion
PC1	2.755	25	25	PC1	2.755	25	25	PC1	2.755	25	25
PC2	1.911	17.4	42.4	PC2	1.911	17.4	42.4	PC2	1.911	17.4	42.4
PC3	1.585	14.4	56.8	PC3	1.585	14.4	56.8	PC3	1.585	14.4	56.8
PC4	1.289	11.7	68.6	PC4	1.289	11.7	68.6	PC4	1.289	11.7	68.6
PC5	0.89	8.1	76.6	PC5	0.89	8.1	76.6	PC5	0.89	8.1	76.6
PC6	0.803	7.3	83.9	PC6	0.803	7.3	83.9	PC6	0.803	7.3	83.9
PC7	0.717	6.5	90.5	PC7	0.717	6.5	90.5	PC7	0.717	6.5	90.5
PC8	0.607	5.5	96	PC8	0.607	5.5	96	PC8	0.607	5.5	96
PC9	0.354	3.2	99.2	PC9	0.354	3.2	99.2	PC9	0.354	3.2	99.2
PC10	0.089	0.8	100	PC10	0.089	0.8	100	PC10	0.089	0.8	100

Table 6: Principal Component values of rotation component matrix.

Principal components	Eigen values	Proportion	Cumulative Proportion	Principal components	Eigen values	Proportion	Cumulative Proportion	Principal components	Eigen values	Proportion	Cumulative Proportion
PC1	2.755	25	25	PC1	2.755	25	25	PC1	2.755	25	25
PC2	1.911	17.4	42.4	PC2	1.911	17.4	42.4	PC2	1.911	17.4	42.4
PC3	1.585	14.4	56.8	PC3	1.585	14.4	56.8	PC3	1.585	14.4	56.8
PC4	1.289	11.7	68.6	PC4	1.289	11.7	68.6	PC4	1.289	11.7	68.6
PC5	0.89	8.1	76.6	PC5	0.89	8.1	76.6	PC5	0.89	8.1	76.6
PC6	0.803	7.3	83.9	PC6	0.803	7.3	83.9	PC6	0.803	7.3	83.9
PC7	0.717	6.5	90.5	PC7	0.717	6.5	90.5	PC7	0.717	6.5	90.5
PC8	0.607	5.5	96	PC8	0.607	5.5	96	PC8	0.607	5.5	96
PC9	0.354	3.2	99.2	PC9	0.354	3.2	99.2	PC9	0.354	3.2	99.2
PC10	0.089	0.8	100	PC10	0.089	0.8	100	PC10	0.089	0.8	100

Table 7: Interpretation of rotated matrix for the traits studied in rice.

PC1	PC2	PC3	PC4
No. of tillers per hill	No. of tillers per hill	plant height	No. of tillers per hill
productive tillers per hill	productive tillers per hill	filled grains per panicle	productive tillers per hill
days to 50% flowering	unfilled grains per panicle	flag leaf width	unfilled grains per panicle
days to maturity	days to 50% flowering	-	panicle length
flag leaf width	days to maturity	-	flag leaf length
-	panicle length	-	grain yield per hill
-	grain yield per hill	-	-

Hierarchical clustering analysis. There are seven clusters which could be framed from cluster analysis. Out of 30 mutated lines and one control line, the cluster 1 is made of seven individuals G6, G14, G3, G21, G10, G20 and G26. Cluster 2 has only two individuals which are G7 and G24, cluster 3 is formed with three individuals Table 8 and Fig. 4.

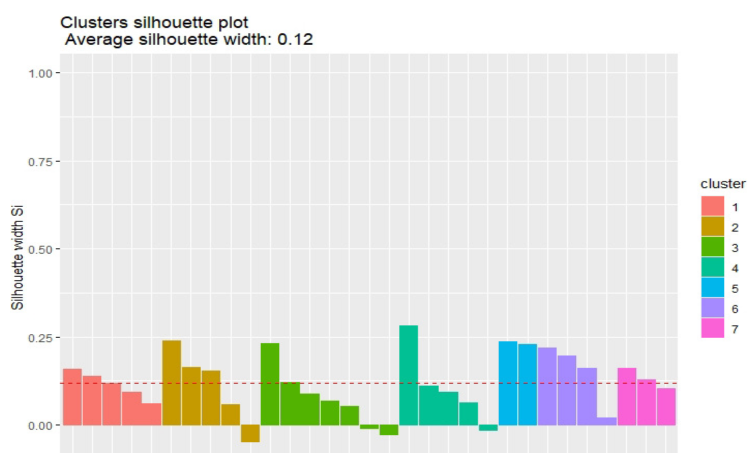

Fig. 4. Hierarchical clustering analysis for the traits studied in rice.

This study cluster 6 has 4 mutated lines while cluster 4, 5 and 7 have five lines on each cluster along with one check which fall on the cluster 5. From the analysis, cluster 1 has more number of mutated line, whereas clusters 2 has only 2 mutated line which is the smallest of all the seven clusters. Mutated lines in cluster 5 showed similar characterization to that of control line as it fell on the 5th cluster. The cluster means of plant height range from 86.26 cm to 92.07 cm; number of tillers per hill from 21.63 to 31.96; number of productive tillers per hill from 15.80 to 31.23; sterile grains per panicle from 17.75 to 34.05; Days to 50% flowering from 97.16 days to 105.90 days; Days to maturity from 132.16 days to 140.90 days; Panicle length from 18.48 cm to 26.25 cm; Filled grains per panicle from 156.88 to 175.38; Flag leaf length from

22.95 cm to 30.19 cm; Flag leaf width from 1.08 cm to 1.56 cm; Grain yield per hill from 30.17 g to 38.22 g. Wider range of cluster means values among the studied germplasm lines indicated the presence of genetic variation among the lines. From this observation only number of productive tillers per hill and sterile grains per panicle have wide range of mean values, thus higher genetic variance would be present.

Table 8: Interpretation of Hierarchical clustering analysis for the traits studied in rice.

Cluster	No. of lines	Lines	PH	NTH	PTH	UGP	50% F	DM	PL	FGP	FLL	FLW	GYP
1	7	6,14,3,21,10,20,26	92.01	27.78	25.43	24.34	105.90	140.90	23.10	169.40	29.57	1.56	32.63
2	2	7,24	90.55	27.84	27.04	23.67	97.17	132.17	26.25	175.38	24.25	1.40	38.22
3	3	11,25,29	86.42	31.97	31.23	34.05	102.56	137.56	21.84	163.47	25.54	1.36	36.88
4	5	23,2,5,9,30	88.97	24.90	19.83	21.12	104.00	139.00	21.95	156.88	26.33	1.08	35.29
5	5	1,31,16,13,22	86.27	30.00	25.71	17.75	101.67	136.67	18.49	159.32	29.58	1.30	36.86
6	4	15,18,8,27	92.07	21.64	15.81	27.10	98.33	133.33	22.08	170.55	30.20	1.27	37.37
7	5	12,19,4,17,28	90.32	25.34	21.53	21.25	99.13	134.13	19.43	171.51	22.95	1.21	30.17

CONCLUSIONS

The present study revealed that there were direct positive associations between yield per plant and days to 50% flowering, plant height, panicle length, number of productive tillers, spikelets per panicle, selection for which would be effective to enhance the yield potential. The correlations of grain yield per plant were negative and significant with characters *viz*., days to 50% flowering and days to maturity. For all traits, the phenotypic correlation is higher than the genetic correlation, which reveals environment has greater effect on the traits which is taken for story. In genotypic path analysis out of ten, three characters had positive and direct effect on grain yield per plant while rest of eight had negative and direct effect on grain yield per plant Principal component analysis explains 4 PCs whose has the cumulative effect of 68.6%. It explains the presence of vital genetic diversity of the mutated lines under study. Hierarchical clustering showed the presence of 7 clusters in the mutated lines, on which first cluster has 7 out of 31 mutated lines and remaining line were distributed among rest of the clusters with cluster 2 having least of only 2 mutated lines.

FUTURE SCOPE

The results of the present study revealed high level of genetic variation existing in the population along with the traits contributing to this diversity, which can find immense applications in rice improvement programme. Findings from the present investigation will help in utilizing the mutant lines for genetic improvement of indica rice varieties.

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Conflicts of Interest. None.

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