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Research Article

Multivariate Analysis of Agronomic Traits in M_4 Generation of Aromatic Rice Lines

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Abstract

Background and Objective: Developing rice (*Oryza sativa* L.) varieties with increased yield potential has been a major concern for genetic improvement. This study aimed to evaluate aromatic rice lines and the relationship among their twelve agronomic traits using heatmap Pearson correlation and multivariate analysis to identify high yield lines using grain yield as a marker-trait. **Materials and Methods:** Twelve aromatic rice genotypes (eleven mutant lines and one control) were evaluated in the M_4 generation. The experiment was conducted at Tana Toraja regency following Randomized Complete Block Design (RCBD) with two replications. **Results:** The darker and lighter colour scale produced by heatmap revealed contrasting nature of genotypes. A significant positive correlation observed for yield was the number of fertile grains and grain weight per panicle, while a negative correlation was days to flowering. The first four components account for 83.46% of the total cumulative variation. Cluster analysis grouped 11 lines and one control into three clusters. **Conclusion:** The results concluded that the PB-A.5.3.45 line could be used for hybridization programs to develop high-yielding mutant-derived aromatic rice varieties for further improvement.

Key words: Aromatic rice, cluster analysis, heavy-ion beam, mutation, principal component analysis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indonesia is a country with a wealth of biodiversity that is largely still unexploited. A wide diversity of rice varieties has resulted from a long history of conventional rice cultivation interwoven with an even richer diversity of cultural and spiritual traditions[1]. Local rice that has survived for decades or even hundreds of years is a cultivar that has been selected by natural selection to have desirable traits such as a pleasant aroma and high quality. However, like local rice in general, local rice in Indonesia face obstacles, such as low yields and long life. This is in contrast to the national high-yielding varieties, which are short-lived and high-yielding traits. Nevertheless, the diversity of local rice, which has several advantages, is a potential asset to be utilized and conserved.

One of the efforts to improve plant character is through plant breeding using the plant mutation method. Induced mutations have contributed to the improvement of plant genetics in various parts of the world. In fact, in some cases, they have impacted in improving plant traits[2]. Mutations can occur naturally due to base-pair changes in the DNA sequence[3]. Plant genetic mutations can be induced by using mutagens such as heavy-ion beam irradiation. Heavy-ion beam irradiation has been used on many plant species but local varieties are still very little reported, especially in local varieties of Toraja rice. The objective of mutation induction is to alter one or more essential plant characters while keeping the majority of the original, preferred traits[4]. These changes can be passed on from generation to generation. Thus mutations are the primary source of genetic variation. As the main element of germplasm, genetic diversity of rice is a breeding source to meet existing food needs[5]. Induced mutation can quickly generate variability in qualitative and quantitative inherited traits of crop plants.

Nowadays, mutants have allowed researchers to distinguish important elements for developing high yield potential varieties with desirable traits such as early maturing[6], semi-dwarfism[7] and increased fertile grain[8]. Therefore, continuous germplasm assessment should be conducted to expand the genetic base of species and find additional genes or alternative sources that control a particular trait for crop improvement.

Genetic diversity can describe the variation between individuals in a population[9]. The high genetic diversity and increasing the chances of combining the desired good traits also allow the improvement of plant traits through direct selection. Therefore, information on genetic diversity is needed to obtain the expected new varieties. Osman *et al.*[10] stated that to achieve a selection, it must be known about the agronomic traits so selecting one or more traits can be made.

Several methods exist for analyzing genetic diversity in breeding lines, germplasm accessions and populations. Multivariate analyses simultaneously analyze all random variables measured on each experimental or sampling unit[11]. Principal Component Analysis (PCA), one of the multivariate statistical approaches, divides the data into two proportions to expose comparison and association among variables and genotypes based upon the percentage of variability and correlations[12]. PCA is a technique for reducing the dimensions of a data set with several interconnected variables[13,14]. The cluster analysis, another multivariate statistical tool, is a suitable approach for evaluating genotype relationships. Cluster analysis is a technique for identifying and categorizing variables based on the similarity of the traits they possess[15]. It aims to reduce within-group variance and maximize between-group variance. It is also helpful in the breeding program to select the genotypes that are superior in terms of agronomic traits during the rice improvement. PCA and cluster analysis have previously been used to assess the extent of genetic variation in rice for various agronomic traits[16-18].

The present study aimed to evaluate the relationship among their twelve agronomic traits using heatmap Pearson correlation and genetic variation using multivariate techniques. Thus, identify mutants with contrasting characteristics in order to improve local rice breeding in Indonesia.

MATERIALS AND METHODS

Plant material and growth conditions: Eleven M_4 mutant lines, obtained from irradiated local Toraja variety 'Pare Bau' using heavy-ion beam irradiation at 10 Gy and one control (Pare Bau variety) were used in this study. The experiment was carried out at rice fields in Tana Toraja Regency, Gandang Batu Sillanan District, Buntu Limbong Village, with an altitude of 898 m above sea level from December, 2018 to June, 2019. The climate was generally classified with a mean annual maximum temperature of 23°C and a mean annual minimum of 22.4°C. The maximum and minimum relative humidities were 89.1 and 83%. The sunlight intensity was highest (67.3%) in May and lowest (40.5%) in June.

Procedures: The experimental design was a Randomized Complete Block Design (RCBD) with two replications. The experimental plot sizes measured 5 m wide×4 m long. Rice was planted at a spacing of 0.3×0.3 m with one seedling per hill. Sufficient care was taken for plant protection measures, irrigation and weeding at the seedling stage to make seedlings grew well. The plots were kept weed-free by using the hand hoe and chemical for weeding. Control of pests and diseases was carried out chemically. One week after planting, dead seedlings are replanted with healthy seedling. Fertilization was done two times. The first fertilization was carried out, 14 days after planting using NPK fertilizer (16:16:16) at 225 kg ha^{-1} and the second fertilization was applied before panicle initiation stage using urea fertilizer as much as 150 kg ha^{-1}.

Data collection for agronomic traits: Data were collected based on twelve agronomic traits, such as plant height (cm), days to flowering (days from sowing to time when 50% of the plants start to flower), number of tillers, number of panicles, length of flag leaf (cm), width of flag leaf (cm), panicle length (cm), number of fertile grains, grain weight per panicle (g), 100-grain weight (g), grain weight per plant (g) and yield (t ha^{-1}).

Statistical analysis: Mean, range, standard deviation and Coefficient Variation (CV) were determined using descriptive statistics. A correlation analysis was ascertained using the Pearson correlation coefficient for the assessment of relationships between quantitative traits. The PCA was used to determine the genotype relationships[19]. The results of the PCA were shown as biplots of PC1 vs. PC2 for each specific trait, where markers on the biplot represented lines. A Hierarchical Clustering on Principal Components (HCPC) study was used to establish clusters based on agronomic traits. According to the Ward method, hierarchical clustering was performed to understand the patterns of variation among genotypes better[20].

RESULTS

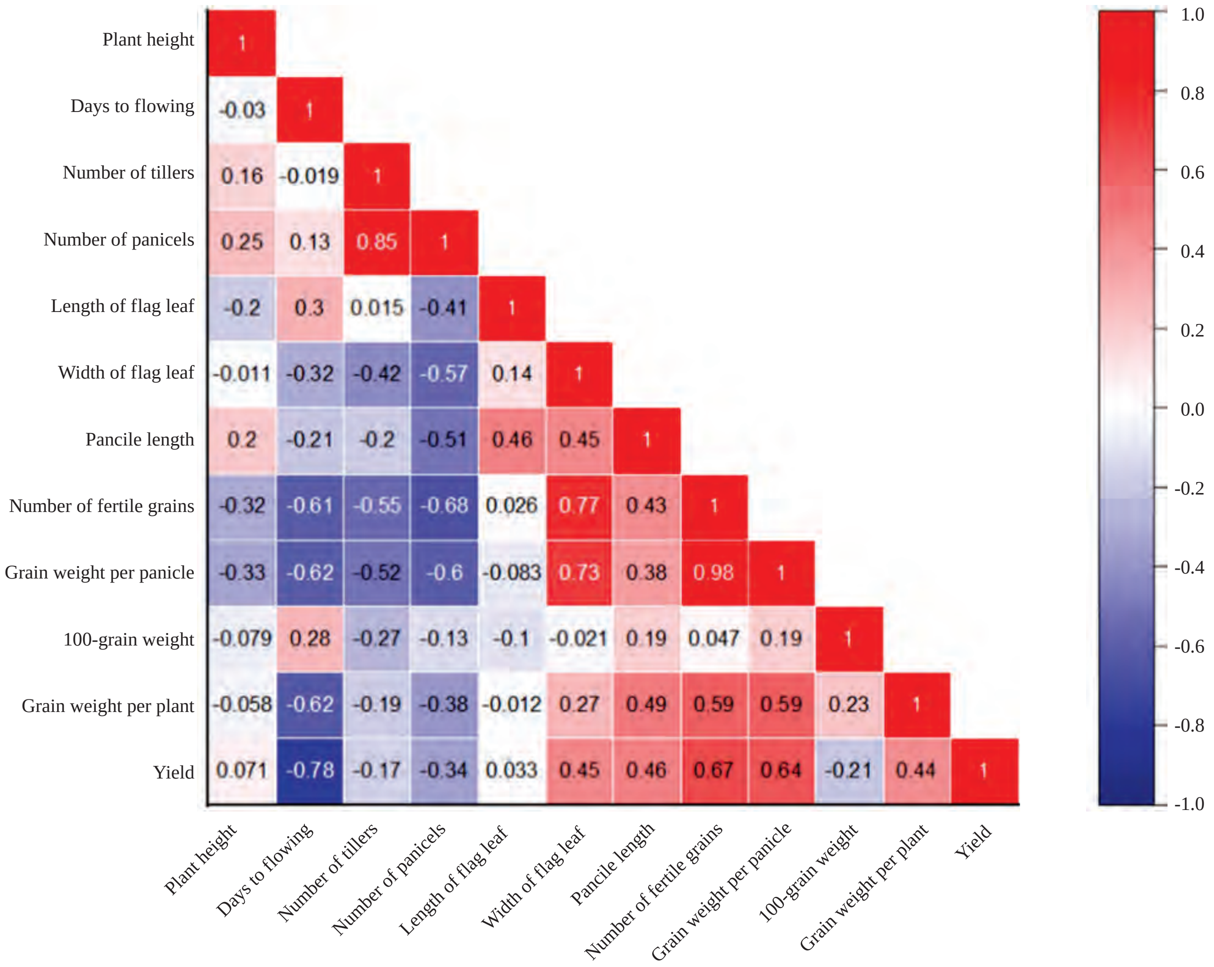
Agronomic traits variation: The mean, standard deviation, range and the coefficient of variation of the traits measured were given in Table 1. The CV ranged from 0.57% for days to flowering to 6.90% for number of fertile grains.

Correlation analysis: Phenotypic correlation analysis was used to determine trait association and the result was shown in Fig. 1. Days to flowering showed negative correlations with the number of fertile grains (-0.61), grain weight per panicle (-0.62), grain weight per plant (-0.62) and yield (-0.78). The number of tillers was highly positive with the number of panicles (0.85). The number of panicles was negatively correlated with the number of fertile grains (-0.68) and grain weight per panicle (-0.60). The width of flag leaf showed a close positive correlation with number of fertile grain (0.77) and grain weight per panicle (0.73). The number of fertile grains was positively correlated with grain weight per panicle (0.98), grain yield per plant (0.59) and yield (0.67). grain weight per panicle was positively correlated with grain weight per plant (0.59) and yield (0.64).

Principal component analysis: To extract the important information from the data table and simplify the data set definition, we used Principal Component Analysis (PCA), a multivariate statistical technique. The first four principal component axes represented 81.46% of the total cumulative variation (Table 2). The PC1 accounted for 44.46% of variability, which was mainly explained by days to flowering (-0.60), number of tillers (-0.78), number of panicles (-0.81), width of flag leaf (0.77), number of fertile grains (0.96), grain weight per panicle (0.94), grain weight per plant (0.65) and yield (0.71). The PC2 accounted for 16.76% of variability and

Table 1: Mean values, standard deviation, minium value, maximum value and coefficients of variation of agronomic traits of aromatic rice lines

Traits	Mean±SD	Minimum value	Maximum value	Coefficient of variation (%)
Plant height (cm)	152.75±2.49	148.00	156.00	1.81
Days to flowering (day)	124.17±1.80	121.00	127.00	0.57
Number of tillers	9.67±0.89	8.00	11.00	6.45
Number of panicles	9.33±0.89	7.00	10.00	6.24
length of flag leaf (cm)	40.86±1.14	39.13	42.37	2.30
Width of flag leaf (cm)	1.88±0.09	1.79	2.11	3.87
Panicle length (cm)	31.60±0.78	29.86	32.79	1.72
Number of fertile grains	150.58±14.30	134.00	186.00	6.90
Grain weight per panicle (g)	4.99±0.47	4.42	6.02	6.76
100-grain weight (g)	3.34±0.07	3.22	3.49	1.71
Grain weight per plant (g)	45.58±2.88	40.67	49.42	4.59
Yield (t ha^{-1})	4.52±0.27	4.25	5.03	4.70



	Plant height	Days to flowing	Number of tillers	Number of panicels	Length of flag leaf	Width of flag leaf	Pancile length	Number of fertile grains	Grain weight per panicle	100-grain weight	Grain weight per plant	Yield
Plant height	1											
Days to flowing	-0.03	1										
Number of tillers	0.16	-0.019	1									
Number of panicels	0.25	0.13	0.85	1								
Length of flag leaf	-0.2	0.3	0.015	-0.41	1							
Width of flag leaf	-0.011	-0.32	-0.42	-0.57	0.14	1						
Pancile length	0.2	-0.21	-0.2	-0.51	0.46	0.45	1					
Number of fertile grains	-0.32	-0.61	-0.55	-0.68	0.026	0.77	0.43	1				
Grain weight per panicle	-0.33	-0.62	-0.52	-0.6	-0.083	0.73	0.38	0.98	1			
100-grain weight	-0.079	0.28	-0.27	-0.13	-0.1	-0.021	0.19	0.047	0.19	1		
Grain weight per plant	-0.058	-0.62	-0.19	-0.38	-0.012	0.27	0.49	0.59	0.59	0.23	1	
Yield	0.071	-0.78	-0.17	-0.34	0.033	0.45	0.46	0.67	0.64	-0.21	0.44	1

Fig. 1: Pearson correlation coefficients for 12 agronomic traits of 12 genotypes of aromatic rice mutant

Strength of a particular association between two traits is indicated by the color assigned to a point in the heat map grid. Positive correlation is indicated by red, while negative correlation is indicated by blue

Table 2: Eigenvalues, proportion of variability and agronomic traits that contributed to the first four principal components of aromatic rice mutant lines

	Components			
Traits	1	2	3	4
Plant height	-0.20	-0.50	0.47	0.36
Days to flowering	-0.60**	0.74**	0.16	0.09
Number of tillers	-0.78**	-0.49	0.13	-0.03
Number of panicles	-0.81**	-0.44	0.16	0.08
length of flag leaf	0.11	0.55	0.65**	-0.34
Width of flag leaf	0.77**	0.11	0.14	-0.13
Panicle length	0.56	0.06	0.73**	0.24
Number of fertile grains	0.97**	-0.04	-0.14	-0.10
Grain weight per panicle	0.94**	-0.06	-0.23	0.05
100-grain weight	0.10	0.40	-0.14	0.85**
Grain weight per plant	0.65**	-0.28	0.07	0.39
Yield	0.71**	-0.48	0.21	-0.18
Eigen value	5.33	2.01	1.41	1.25
Variability (%)	44.46	16.76	11.79	10.45
Total variance (%)	44.46	61.22	73.01	83.46

**Eigen values ≥0.59 are significant

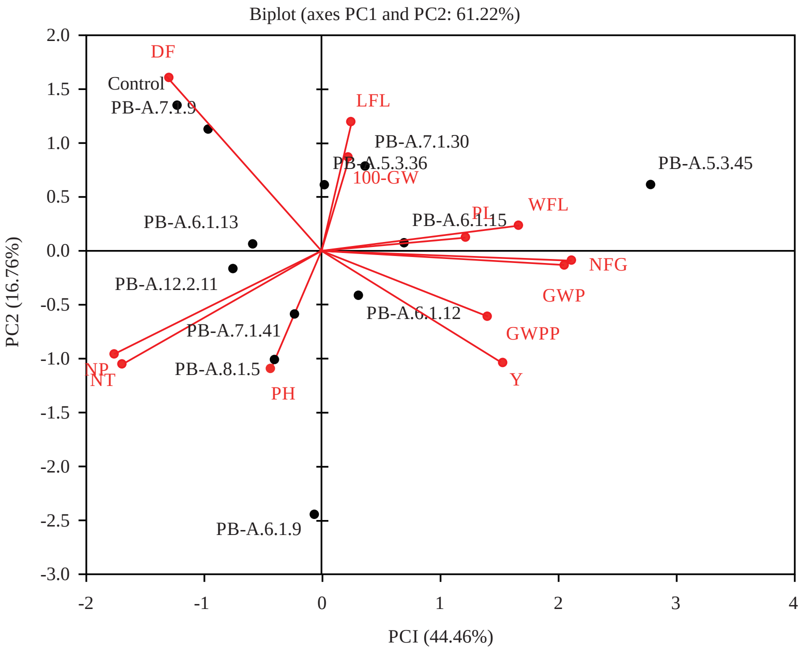


Fig. 2: Biplot drawn based on the first and second components obtained from principal component analysis

PH: Plant height, FD: Days to flowering, NT: Number of tillers, NP: Number of panicles, LFL: Length of flag leaf, WFL: Width of flag leaf, PL: Panicle length, NFG: Number of fertile grains, GWP: Grain weight per panicle, 100-GW: 100 grain weight, GWPP: Grain weight per plant, Y: Yield

Table 3: Groups of 12 genotypes according to cluster analysis from twelve agronomic traits

Cluster number	Number of lines	Percent	Lines
I	1	8.33	PB-A.5.3.45
II	4	33.33	PB-A.7.1.9, control, PB-A.8.1.5, PB-A12.2.11
III	7	58.33	PB-A.5.3.36, PB-A.6.1.15, PB-A.7.1.30, PB-A.7.1.41, PB-A.6.1.9, PB-A.6.1.12, PB-A.6.1.13

Table 4: Mean values of twelve agronomic traits for five groups revealed by cluster analysis among 12 genotypes

	Clusters		
Traits	I	II	III
Plant height (cm)	152.00	153.26	152.68
Days to flowering (day)	122.00	125.38	123.36
Number of tillers	7.56	10.09	9.57
Number of panicles	7.34	9.92	9.44
length of flag leaf (cm)	42.37	41.75	40.14
Width of flag leaf (cm)	2.11	1.84	1.88
Panicle length (cm)	32.79	31.51	31.47
Number of fertile grains	186.22	139.48	152.02
Grain weight per panicle (g)	6.02	4.55	5.09
100-grain weight (g)	3.33	3.30	3.36
Grain weight per plant (g)	49.42	43.63	46.14
Yield (t ha^{-1})	5.03	4.41	4.51

was largely influenced by days to flowering (0.74). The PC3 accounted for 11.79% of the variability, mainly explained by the length of flag leaf (0.65) and panicle length (0.73). The PC4 accounted for 10.45% of the variability, which was explained by 100-grain weight (0.85).

The biplot of the first two components (PC1 on the X-axis and PC2 on the Y-axis to assess the association of the lines and agronomic traits) were illustrated in Fig. 2. The biplot of the mean performance explained 61.22% of the total variation. Across the 11 tested rice lines and one control, the yield was positively associated with the number of fertile grains, grain weight per panicle and grain weight per plant, while negatively correlated with days to flowering. The distance between the genotype and the origin of the biplot is a one-of-a-kind measure of the genotype, a hypothetical genotype with an average level for all traits represented by the biplot origin. Therefore PB-A.5.3.45 line with a long vector had extreme value for one or more traits.

Cluster analysis: Ward's hierarchical cluster method was split into three clusters for the 11 lines and control (Table 3, Fig. 3). The first major group was cluster III consisting of 7 lines, followed by cluster II (3 lines and one control) and cluster I (1 line). Mean values of twelve different traits for five groups among 12 genotypes were presented in Table 4. Mean values of the traits in cluster I showed high yield and early flowering. The representative line of cluster I can be used as parents for hybridization.

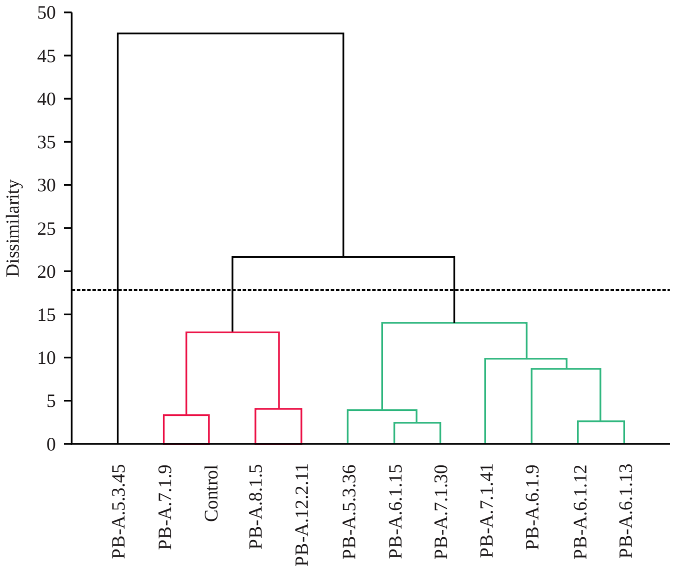


Fig. 3: Cluster analysis showing the relationship among 12 genotypes of aromatic rice based on twelve agronomic traits

DISCUSSION

The main objective of plant breeding is to create new plant types with improved characteristics that provide a high yield. Yield is one of the most important and complex characteristics of rice. These characters are controlled by genes and influenced by external environmental factors[21]. In rice, yields are determined by indirect properties such as plant height, growth period, tiller production capacity, panicle length as well as direct properties such as number of panicles, number of grains contained per panicle and grain weight per panicle[22,23].

The days to flowering had a highly significant negative correlation with yield (-0.78**). The negative correlation coefficient between traits indicates that two variables are changing in opposite directions. However, it indicates that the lines with early flowering tend to have higher yield. This was corroborated by the findings from Abarshahr *et al.*[24] and Asante *et al.*[25]. On the contrary, the grain weight per panicle and grain weight per plant positively correlates with yield. It indicated that these traits should be emphasized as the best selection criteria in selecting high-yielding lines. This relationship indicated that these properties affect the final rice yield. This character is considered a suitable outcome predictive factor and is very important in further studies in the selection process. Breeding with these traits may be useful for developing high-yielding lines.

The number of panicles also played an important role in determining production and it was found that both the environment and cultivation strongly influenced the main yield components. However, this is not always the case. In this study, the number of panicles had a significant negative correlation with the number of fertile grains and grain weight per panicle, suggesting that the increase in one trait would reduce the other. It would be challenging to identify rice lines with a higher number of panicles simultaneously with a higher number of fertile grain and grain weight per panicle. Results assured that the rice crops with fewer tillers will have a better canopy structure for light interception to produce fertile grains than those with more tillers. When light is a limiting factor, fewer tillers are important for maintaining a high canopy photosynthetic rate[26].

The PCA revealed that total variation was largely influenced by the number of fertile grains, grain weight per panicle and yield, indicating that the traits were useful in identifying the mutant lines. These traits could be selected based on how important they are in distinguishing the lines and their inter-relationships. Grain yield improvement by indirect selection of related traits is well-known and commonly used[27-28]. High-loading traits from the PC are critical for selection because they can better distinguish between lines than traits with lower contributions[29-31].

The graphic dispersion demonstrated the separation of mutant lines into groups and can be used as a strategy to select different families for artificial crosses in breeding programs. The groups formed by the biplot (Fig. 2). The visualization of interrelationships among traits is aided by a vector drawn from the origin of each trait in the biplot. Its vector length measures the magnitude of a trait's effects on yield. According to this theory, two traits are positively correlated if the angle between their vectors is acute and negatively correlated if the angle is obtuse[32]. These associations could be confirmed from Pearson correlation coefficients between any two traits.

Lines plotted in the positive quadrants of the axis can be selected as genetic resources for improving yield. All the lines and traits were widely scattered across a different group of biplots. Biplot had the characteristic of grouping the mutant genotypes into four distinct groups. The finding of current research explained that mutant genotypes that were farther away from the origin in the positive direction of trait showed better performance in the first and second biplot groups based on significant traits. Current results suggested that lines PB-A.5.3.45 were far from the center, indicating that the line can be parents for some useful traits. The study of Gedam *et al.*[33] explained that superior genotypes in a particular trait are the ones that are closer to the vector of that trait but further plotted away from origin in the direction of that particular trait vector. The biplot has been utilized to study trait relation and genotype evaluation in several crop species, including rice[34], wheat[35], Maize[36] and Sorghum[37].

Cluster analysis based on twelve agronomic traits groups in three clusters indicates that each cluster's lines had some distinct traits. The heterotic effect in cross progenies could be increased by grouping the lines into separate distinct clusters and lines with desired traits could be selected from these for use in crossbreeding programs to obtain higher hybrid vigor and improved segregants. The characterization of the present study leads to some promising lines for specific traits. This corroborated with the finding of several researchers about the different number of clusters using agronomic traits in rice lines[38,39].

Results revealed that, among 11 mutant lines, only three mutants cluster with control and mutant PB-A.5.3.45 formed individual clusters. These findings confirmed that mutations play a significant role in the development of genetic variations in crop plants. Lines with distant clusters could be used as parents for the hybridization program to increase heterosis.

CONCLUSION

Heavy-ion beam treatments play a significant role in generating genetic variation and created line with superior traits. Significant correlation between the number of fertile grains, grain weight per panicle and yield showed that simultaneous selection for the traits would be highly effective. The days to flowering associated negatively with yield indicating that the lines with early flowering tend to have higher yield. The multivariate approach utilized in this study could help breeders to make better selections on which lines should be recommended to be released based on high yields. The combining potential of the high yielding line from cluster I could be investigated further. Thus, the genotypes observed can be hybridized to create desirable traits with higher heterotic potential.

SIGNIFICANCE STATEMENT

This study discovers the performance of tested aromatic mutant lines for the 12 traits considered. This study will help researchers to identify high-yield mutant lines. Thus, the selected lines may come up as a recommendation for superior parent for hybridization, which can boost the productivity of aromatic rice.

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