Asian Journal of Plant Sciences

ISSN 1682-3974

OPEN ACCESS Asian Journal of Plant Sciences
ISSN 1682-3974
DOI: 10.3923/ajps.2021.406.413

Research Article
Correlation and Path Coefficient Analysis of Grain Yield and its Components in Toraja Land-Race Aromatic Rice Mutants Induced by Heavy Ion Beam

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Abstract
Background and Objective: The development of local aromatic rice cultivars has become a distinct segment market for plant breeders and producers. This study aimed to identify the Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV), heritability (h^2bs), a close relationship between traits and the direct and indirect effect of aromatic rice lines based on traits among the population observed. **Materials and Methods:** The field experiment was conducted in Enrekang Regency, South Sulawesi, Indonesia (650 m above sea level), from May-October, 2018. Toraja Local Aromatic Rice "Pare Bau" were irradiated with 300 keV μm^{-1} (10 Gy) Argon ion at RIKEN Nishina Center, Wako-shi, Saitama, Japan. Eighteen aromatic rice lines from M_3 generation and one control (non-irradiated) were transplanted in the paddy field. **Results:** Results showed that the traits observed had a coefficient of variation of moderate to high. All the lines tested showed high heritability associated with the genetic advance as percent of mean (GAM) for all traits, indicating that these traits could be useful to be selected. Based on correlation analysis, the traits that support high yield were the number of panicles, grain weight per panicle and percentage of fertile grain. Moreover, path analysis showed two essential and useful selection traits for grain yield improvement in aromatic rice mutant lines of third generations (M_3), i.e., number of panicles and percentage of fertile grain. In the brief of lines, G_4 is the highest yield per plant. **Conclusion:** It can be concluded that variability observed among lines associated with high yield could be exploited in rice breeding, especially mutant aromatic rice.

Key words: Aromatic rice, heavy ion beam, heritability, irradiation, M_3 generation

Citation: Okasa, A.M., R. Sjahril, M. Riadi, M. Mahendradatta, T. Sato, K. Toriyama, K. Ishii, Y. Hayashi and T. Abe, 2021. Correlation and path coefficient analysis of grain yield and its components in Toraja land-race aromatic rice mutants induced by heavy ion beam. Asian J. Plant Sci., 20: 406-413.

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Tana Toraja is a region in South Sulawesi, Indonesia, with a large diversity of rice germplasm. Until now, farmers nonetheless used to cultivate local varieties in unfavorable ecosystems. Local varieties, consisting of aromatic rice, have unique characteristics such as aroma, higher cooking quality and better taste which also estimate value in socio-economic aspects[1]. Aromatic rice 'Pare Bau' is closely associated with the sociocultural in Toraja and consumed during the funeral ceremony and other celebrations[2]. However, this local variety has low yield potential, tall stems and long harvesting age. The use of indigenous varieties with low yielding capacity also limits rice productivity in Toraja. The potential development of local aromatic rice owned by Toraja is critical to increasing the local aromatic rice production and quality.

Plant breeding is an activity that aimed to improve and enhance the genetic potential of plants so that new varieties that are better than their parents are obtained. One method of plant breeding is by physical mutation. Several types of physical mutagenic sources, such as gamma rays[3], ultra-violet light irradiation[4], neutrons[5] and ion rays[6] have been developed and utilized in plant mutation breeding. In ion beam applications, high-energy irradiation of ion beam has been utilized to induce mutations in many plant species[7,8]. Heavy ion beam irradiation is a useful method for mutation breeding to produce new cultivars[9]. Ion Beam radiation is known to have several effects on plant growth and development. Abe *et al.*[10] argue that ion beams induce mutations at relatively low doses without severely inhibiting growth at a high rate. There has been little information in the selection, genetic variability and utilization variation observed in selected lines for generating new breeding programs that is required in the development of new cultivars[11].

There is considerable interest in the morphological traits that contribute to seed yield because such knowledge is pivotal for breeding. Genotypic Coefficient Variation (GCV), Phenotypic Coefficient Variation (PCV), heritability and Genetic Advance (GA) as genetic parameters may be helpful for the selection of lines with desirable traits. Heritability provides factual information about a specific genetic aspect that is transmitted to the successive generations[12,13]. High heritability values demonstrate that genetic factors play a part in controlling a trait compared to environmental factors. Furthermore, heritability information is crucial for improvement-based selection since it indicates character to future generations[14]. Thus, evaluating heritability, alongside genetic advance is reliable and valuable rather than heritability it self[15].

Several studies have been published on the heritability of mutation in rice[16], wheat[17], corn[18] and soybean[19]. However, little is known about heritability and selection criteria for aromatic mutant rice. This could be the first research report related to heritability in local aromatic mutant lines "Pare Bau" using heavy ion beam irradiation to the author's knowledge. It is crucial to distinguish cultivars with better results and desired agronomic traits for increasing the potential of local aromatic rice yields. The study of correlation is necessary to design a suitable selection strategy for genetic improvement in yield and other traits. Several researchers have studied the relationship among yield as well as its fundamental components in mutant rice[20-22]. The information on the direct and indirect effect of each component's traits toward yield will offer breeders to define the sufficient criteria in selecting desirable lines in population. Given this, a study was undertaken to determine the most important traits for breeding programs by exploiting genotypic and phenotypic coefficient of variation, heritability value, close relationship between traits and direct and indirect effect of yield components among rice lines.

MATERIALS AND METHODS

Study area: Field experiment was carried out at Enrekang Regency, South Sulawesi, at an altitude 650 m above sea level (S: 3°19'47.44", E: 119°50'1.57"), from April-October, 2018. In this study, the materials used were 18 lines of M_3 generation from irradiated local aromatic rice "Pare Bau" from Toraja agriculture office induced by heavy-ion beam irradiation. Argon ions with an irradiation dose of 10 Gy (300 keV μm^{-1}) were used as ion irradiation at the RIKEN Nishina Center, Wako-shi, Saitama, Japan.

Research procedure: The experiment used a design without replication. Fifty seeds from each M_3 selected line were immersed one night for germination. The seeds of each line were sown into trays containing 1:1 (v/v) mixture soil and manure. The seedlings age 21 days of each line were then transplanted in the paddy field using a single seedling per hill with a plant spacing of 30 and 30 cm, respectively. Lines were transplanted together with unirradiated controls. The experimental field was irrigated with 10 cm water above the ground surface level. Fertilizers used were Urea at a dose 100 kg ha^{-1}, SP-36 (200 kg ha^{-1}) and KCl (100 kg ha^{-1}) at 7 days after planting (DAP) and followed by Urea at a dose 200 kg ha^{-1} at 45 DAP, respectively. Weeding was treated with herbicides for broad leaves, whereas narrow leaves were conducted manually.

Data collection for agronomic traits: Data were collected on five quantitative traits, i.e., number of panicles, grain weight per panicle (g), percentage of fertile grain per panicle (%), panicle density and grain yield per plant (g). Sampling was recorded from ten plants for each line.

Data analysis: Data were analyzed through several stages. The significance test was performed using t-test with a standard deviation. The GCV and PCV were computed using the formula as suggested by Burton and Devane[23]. According to Terfa and Gurmu[24], GCV and PCV values were categorized as low (0-10%), moderate (10-25%) and high (25% and above). Estimation of heritability in a broad sense was computed following the formula Allard[25]. The heritability value was classified as low (<0.2), moderate (0.2-0.5) and high (>0.5)[26]. Genetic advance as a percentage of the mean (GAM) was computed using Assefa *et al.*[27] method and was classified as low (0-10%), moderate (10-20%) and high (>20%)[28]. Selection intensity (K) was considered to be 20%. Phenotypic correlation coefficients were estimated using the standard procedure suggested by Miller *et al.*[29]. The path analysis used was according to Dewey and Lu's method[30].

RESULTS

T-test: T-test showed significant difference in yield and yield component traits among mutant lines (Table 1). The results further informed that most of the traits exhibited a vast extend of variation among 18 lines. The variability observed wide range for the number of panicles (8-11), grain weight per panicle (1-4.6 g), percentage of fertile grain (21.4-74.6%), panicle density ratio (5.6-8.4) and grain yield per plant (8.2-48.1 g). The number of panicles showed that G_4 and G_{15} lines were significant different compared to control. Grain weight per panicle and percentage of fertile grain showed that most of the lines had high significant differences compared to control, except G_1, G_{14}, G_{15}, G_{16}, G_{17} and G_{18}. Panicle density showed that G_8, G_9, G_{11}, G_{12} and G_{17} lines had significant differences, while G_1, G_2, G_7 and G_{14} lines had high significant differences compared to control. Grain yield per plant showed that most of the lines had high significant differences compared to control, except for G_1, G_{14}, G_{16}, G_{17} and G_{18}.

Phenotypic and genotypic coefficient of variation: The GCV, PCV, heritability and GAM were presented in Table 2. The GCV

Table 1: Mean performance of 18 putative aromatic rice mutant lines for five traits

Lines	Number of panicles	Grain weight per panicle (g)	Percentage of fertile grain (%)	Panicle density	Grain yield per plant (g)
Control	9±1.9^{ns}	1.6±0.7	29.4±16.7	6.2±1.1	14.1±4.7
G_1	9±2.2^{ns}	2.6±2.2^{ns}	37.9±15.0^{ns}	8.4±2.2**	23.9±14.4^{ns}
G_2	9±1.2^{ns}	3.6±1.2**	53.6±13.9**	7.5±1.2**	33.5±16.0**
G_3	9±0.8^{ns}	3.8±0.8**	60.8±11.4**	6.7±0.8^{ns}	35.1±11.3**
G_4	11±1.3*	4.5±1.3**	74.6±9.8**	6.9±1.3^{ns}	48.1±11.8**
G_5	9±1.7^{ns}	4.2±1.7**	67.2±4.4**	7.7±1.7^{ns}	39.5±11.8**
G_6	9±1.4^{ns}	3.3±1.4**	54.0±12.8*	6.4±1.4^{ns}	29.6±7.3**
G_7	9±1.1^{ns}	4.6±1.1**	66.9±13.4**	7.6±1.1**	41.9±16.1**
G_8	8±1.8^{ns}	4.5±1.8**	58.3±12.9**	7.7±1.8*	25.5±10.5**
G_9	9±1.2^{ns}	4.0±1.2**	60.2±10.6**	7.4±1.2*	36.1±14.3**
G_{10}	10±0.9^{ns}	3.4±0.9**	57.3±12.3**	6.5±0.9^{ns}	33.1±8.4**
G_{11}	8±0.8^{ns}	4.2±0.8**	60.4±15.0**	7.8±0.8*	30.8±10.4**
G_{12}	10±1.9^{ns}	3.4±1.9**	48.3±12.7*	7.5±1.9*	33.4±14.8**
G_{13}	9±1.3^{ns}	3.9±1.3**	57.4±14.3**	7.0±1.3^{ns}	35.5±20.3*
G_{14}	9±1.7^{ns}	3.1±1.7^{ns}	51.0±26.9^{ns}	7.5±1.7**	28.1±24.7^{ns}
G_{15}	10±1.9*	2.0±1.9^{ns}	28.1±9.6^{ns}	6.2±1.9^{ns}	20.6±7.2*
G_{16}	8±0.7^{ns}	1.8±0.7^{ns}	33.9±18.6^{ns}	6.8±0.7^{ns}	21.5±8.0^{ns}
G_{17}	8±1.1^{ns}	2.2±1.1^{ns}	35.4±20.0^{ns}	7.2±1.1*	17.3±13.9^{ns}
G_{18}	8±1.0^{ns}	1.0±1.0^{ns}	21.4±17.4^{ns}	5.6±1.0^{ns}	8.2±7.4^{ns}

Data shown are Mean±Standard deviation, ns: Non significant, *Significant at level 5%, **Significant at level 1%

Table 2: Estimation of coefficient of variation, heritability and genetic advance for five traits in putative aromatic rice mutant lines

		Coefficient of variation (%)							
Traits	Mean	GCV	Criteria	PCV	Criteria	h^2b	Criteria	GAM (%)	Criteria
NP	9.09	14.35	Moderate	25.24	High	0.57	High	53.04	High
GWP(g)	3.34	40.92	High	45.51	High	0.90	High	89.55	High
PFG	53.11	19.49	Moderate	36.95	High	0.53	High	61.81	High
PD	7.12	14.31	Moderate	20.81	Moderate	0.69	High	52.96	High
GYP(g)	30.50	50.74	High	53.02	High	0.96	High	99.72	High

NP: Number of panicles, GWP: Grain weight per panicle, PFG: Percentage of fertile grain, PD: Panicle density, GYP: Grain yield per plant, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, h^2b: Heritability and GAM: Genetic advance as percent of mean

Table 3: Phenotypic correlation coefficient between five traits in putative aromatic rice mutant lines

Traits	NP	GWP	PFG	PD	GYP
NP	1.000	0.257^{ns}	0.333^{ns}	-0.107^{ns}	0.546*
GWP		1.000	0.964**	0.575**	0.888**
PFG			1.000	0.461*	0.936**
PD				1.000	0.433^{ns}
GYP					1.000

ns: Non-significant, *Significant at level 5%, **Significant at level 1%, NP: Number of panicles, GWP: Grain weight per panicle, PFG: Percentage of fertile grain, PD: Panicle density, GYP: Grain yield per plant

Table 4: Path coefficients analysis of agronomic traits on the direct and indirect effects of the number of panicles, grain weight per panicle, percentage of fertile grain and panicle density on grain yield of aromatic mutant lines

		Indirect effect				
Trait	Direct effect	X_1	X_2	X_3	X_4	Total effect
X_1	0.289**		-0.043	0.314	-0.013	0.546*
X_2	-0.169^{ns}	0.074		0.911	0.072	0.888**
X_3	0.945**	0.096	-0.163		0.058	0.936**
X_4	0.125^{ns}	-0.031	-0.097	0.436		0.433^{ns}
Residual effect	0.232					

ns: Non-significant, *Significant at level 5%, **Significant at level 1%, X_1: Number of panicles, X_2: Grain weight per panicle, X_3: Percentage of fertile grain, X_4: Panicle density

values computed for five traits ranged from 14.31% for panicle density to 50.74% for grain yield per plant. The PCV ranged from 20.81-53.02%.

Heritability and genetic advance: Heritability is one of the genetic parameters used for the selection criteria in a population. In this study, heritability ranged from 0.53-0.96, respectively. The genetic advance as percent of mean ranged from 52.96% in panicle density to 99.72% in grain yield per plant. High heritability and genetic advance were estimated for all morphological traits, such as the number of panicles, grain weight per panicle, percentage of fertile grain, panicle density and grain yield per plant.

Correlation: The estimates of correlation coefficients were computed between 5 quantitative traits among 18 putative aromatic rice mutant lines in Table 3. The number of panicles were significant and positively correlated with grain yield per plant (0.546), whereas, grain weight per panicle with percentage was highly significant and positively correlated with fertile grain (0.964), panicle density (0.575) and grain yield per plant (0.888), percentage of fertile grain were also highly significant and positively correlated with panicle density (0.461) and grain yield per plant (0.936).

Path coefficient analysis: The result of path coefficient analysis, presented in Table 4, was calculated to get an insight into direct and indirect effects on yield traits. Percentage of fertile grain showed the highest positive direct effect (0.945), followed by the number of panicles (0.289) and panicle density (0.125). Grain weight per panicle had a negative direct effect on grain yield per plant (-0.169) while indirectly increased the yield via grain yield per plant through the percentage of fertile grain (0.911). The residual effect of direct trait was 0.232.

DISCUSSION

The present study determines traits variance among 18 aromatic rice mutant lines which were highly significant indicating considerable genetic variability. The percentage of fertile grain was one of the most critical factors of yield and possibly this character would help break the yield plateau. Enhancement in the number of fertile grains should be linked with effective carbohydrates translocate from the leaves (sources) to the spikelets (sinks), resulting an increase in grain yield[31]. The more fertile grain, the more grain yield per plant, if other environmental conditions were not limiting. The same result was achieved by Immanuel *et al.*[32]. Grain yield per plant showed that G_4 line had the highest weight and highly significant differences compared to control. This higher grain weight may be the result of mutation. Several studies have been reported using heavy-ion beam irradiation to increase crop yields, including Sjahril *et al.*[33], who also achieved early maturing lines.

A relationship between GCV and PCV was found in all the traits. Expression of traits as an influence of the environment, PCV values were slightly higher than GCV. Similar studies were earlier recorded by Barik *et al.*[34], Rashmi *et al.*[35] and Akinwale *et al.*[36]. The magnitude of genetic variability percentage for a trait was determined by the genotypic coefficient of variation but did not evaluate the number of genetic variations, which is heritable. However, the number of panicles, percentage of fertile grain and panicle density showed moderate levels of GCV. Sabri *et al.*[37] declared moderate levels of GCV for the number of panicles, while Behera *et al.*[38] reported the percentage of fertile grain and Sanghera *et al.*[39] showed a result for panicle density. The high to moderate genotypic coefficients of variation indicated adequate genetic variability for the traits, which might also encourage the selection[40].

Traits with high heritability associated with the action of the additive gene have a high selection response. Srujana *et al.*[41] argued that rice breeders could cultivate wide varieties of rice plants with superior genotypes by selecting cultivars with desirable phenotypic traits. In this study, all traits showed high heritability and genetic advance. In addition to high heritability and high genetic advance, higher GCV provided better indicators and can be used as selection criteria. Heritability combined with genetic advance was a more dependable indicator for selections of traits. These suggest the selection could be easily practiced using these traits to improve grain yields in putative aromatic rice mutant lines. Current result support the findings of Sala and Shanthi[42], Sandeep *et al.*[43], who also reported such type of heritability in rice. Therefore, selection can also be deferred to the next generations for these traits.

The findings confirmed that positive and significant traits with grain yield could increase grain yield. These traits were given importance while selection as they expressed a positive and significant correlation with grain yield. Therefore, these traits would increase rice yield. Hossain *et al.*[44] argued that the number of panicles is an important characteristic for improving rice lines. Hence, similar associations were already reported for the number of panicles[45], grain weight per panicle[46] and percentage of fertile grain[47]. Selection for one trait would directly affect other traits shown by the significant and positive correlation of the traits facilitating effective selection for breeding program.

In the present investigation, grain weight per panicle was highly significant and positively correlated with the percentage of fertile grain and panicle density. Therefore, grain weight per panicle can be increased if more percentage of fertile grain. Current result is supported by the previous finding in the percentage of fertile grain rice presented by Naseer *et al.*[48].

Correlation values could not describe the causal relationship among characters to their direct and indirect effect through other characters. Path analysis, measuring the direct and indirect effects, was applied to partition the correlation coefficient between grain yield per plant and its four component traits. The use of path coefficient analysis is restricted to considering the causal factor's route as the dependent variable and analyzing the direct and indirect effect that leads to the correlation between the traits[49]. In the present study, the path coefficient analysis was performed at the phenotypic level. The direct effect indicates the direct variance magnitude of a character influencing the main character variance[50]. The main factor for their relationship with grain yield per plant is the high direct effects on their traits. This means that the number of panicles and percentage of fertile grain were suitable as the best secondary traits, whereas the traits that have negative direct effect will decrease grain yield per plant. This corroborates the works by Prasad *et al.*[51]. Surprisingly as it might seem, the direct effect of percentage of fertile grain on plant yield is more significant than the effect of the number of panicles. For these traits, this may be a result of the relative amount of genotypic variation. Moderate positive direct effect but was non-significance, were observed for the traits of panicle density.

Panicle density showed high positive indirect effect towards grain yield via percentage of fertile grain. In the rest of the traits, the correlation was mainly due to indirect selection. Using these associated traits may be useful for breeders in formulating appropriate breeding plans for selection of lines. According to Saleh *et al.*[52], if the direct effect's value is negative, the correlation coefficient will be significant due to high value of indirect effect. With residual effect, the significance of the selected traits used in the study could be calculated. The residual effect of the yield of aromatic rice lines showed that 76.8% of the genotypic level variability was the selected independent traits. This might be due to a significant positive correlation between the number of panicles, grain weight per panicle and the percentage of fertile grain with plant yield. This study demonstrated that the most important determining factor of plant yield was the number of panicles and percentage of fertile grain and it should be possible to breed for increased reproductive system efficiency.

CONCLUSION

The traits had genotypic and phenotypic coefficient of variation from moderate to high in M_3 generation of aromatic mutant lines. Lines exhibited high heritability coupled with genetic advance as percent of the mean for all traits, indicating selection may be effective for these traits. Based on correlation analysis, the traits that support high yield were a number of panicles, grain weight per panicle and percentage of fertile grain. By improving these traits in a desirable direction, one can also improve the yield of the plant. Based on path analysis, two essential and useful traits will be rewarding for grain yield improvement in aromatic rice mutant lines of third generations (M_3), i.e., number of panicles and the percentage of fertile grain. In the brief of lines, G_4 is the highest yield per plant, followed by G_7. These lines can be exploited for commercial cultivation.

SIGNIFICANCE STATEMENT

The present study revealed an effective method of improving local Toraja aromatic rice traits from Indonesia, namely Pare Bau, by using heavy ion beam irradiation. As a reference point for local aromatic rice breeders, specifically in Indonesia, the genetic parameters of Pare Bau cultivars have not been reported. The emphasize of the findings of this study is number of panicles and the percentage of fertile grain that resulted as the most significant components of direct yield during simultaneous selection to increase grain yield in rice. Our findings will help breeders develop efficient strategies for breeding aromatic-grade rice cultivars and this line can be used as a donor parent of superior genotypes.

ACKNOWLEDGMENT

The authors are highly thankful to the Indonesian Ministry of Research, Technology and Higher Education for funding this research under the integrated Master-Doctor program for excellent undergraduate students (Contract Number: 149/SP2H/LT/DPRM/2018).

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