

## Variability, Correlation and Path Analysis for Several Quantitative Traits Derived Multi-Parent Advanced Generation Inter-Cross (Magic) F<sub>2</sub> Population of Rice (*Oryza sativa* L.)

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### Abstract

Eight quantitative traits of an F<sub>2</sub> MAGIC population in rice were subjected to analyze variability, correlation and path analysis. The results of third and fourth degree statistics (skewness and kurtosis) showed that Individual dried straw weight, 1000-seed weight, Individual grain yield, and Number of effective tillers are associated with complementary gene interaction, requiring intense selection, while Plant height, Panicle length, Spikelet fertility percentage, and Harvest index are associated with duplicate (additive x additive) gene interactions, are suitable for mild selection. Leptokurtic distribution of Plant height, Number of effective tillers per plant, Panicle length and Spikelet fertility percentage revealed that the large number of genes in governing the traits. Platykurtic distribution of Individual dried straw weight, 1000-seed weight, Individual grain yield and Harvest index indicated fewer genes controlling the traits. However, when it comes to H<sup>2</sup> and GAM, high values were seen in Number of effective tillers per plant, Individual dried straw weight, Individual grain yield, the traits could be explained by additive gene action and achieved through mass selection. High heritability and lower GAM of Plant height, Panicle length, 1000-seed weight and Harvest index indicated by non-additive genes with limiting the chances of direct selection. For the Pearson correlation, 1000-seed weight showed no correlation with other traits. Individual grain yield highly correlated with Number of effective tillers per plant (0.864\*\*) and Individual dried straw weight (0.828\*\*). The highest total effects on Individual grain yield were Number of effective tillers per plant (2.7437) and Individual dried straw weight (1.0865). Individual dried straw weight had highest direct effect on Individual grain yield at 1.0734, while most of the traits showed high indirect effect on Individual grain yield through Individual dried straw weight.

**Keywords:** variability, heritability, genetic advance, Pearson correlation, direct effects, indirect effects, quantitative traits, MAGIC, F<sub>2</sub> population

### Introduction

Rice (*Oryza sativa* L.) is one of the most important staple foods for global consumption. In 2020, Vietnam ranks fifth in the list of the world's largest rice production. The country produced about 42.69 million tons of rice, accounting for 8.37% of world production (Statista, 2022). Up to October 2013, it released 516 rice varieties to farmers, including 342 pure varieties (Tran Dang Khanh *et al.*, 2021). The most popular conventional rice breeding methods are single crosses, three-way crosses, double crosses and backcrosses have brought certain achievement in rice breeding. However, the number of genes desired to be incorporated into the genome is limited. Besides, modern plant breeding reduces crop genetic diversity which may have consequences for the vulnerability of crops to change in pests, diseases, climate and agricultural practices. Therefore, the compelling need to increase global agricultural production requires new breeding approaches that facilitate exploiting the diversity available in the plant genetic resources. In order to increase the genetic variation in breeding population, more complex crossing schemes involving 6-way, 8-way crosses or diallel selective mating were proposed many years ago but have seldom been used in plant breeding programs. More recently, a multi-parent advanced generation intercross (MAGIC) strategy has been recommended to promote intercrossing and shuffling of the genome (Nonoy Bandillo *et al.*, 2013). In MAGIC designs, multiple inbred founders are intercrossed several times in a well-defined to combine the genetic material of all the founders

in a single line. This leads to a highly diverse population whose genomes are fine-scale mosaics of contribution from all founders. The higher number of parents and recombination events of a MAGIC population are clear advantages compared to a classical biparental population, while for both designs pedigree and genetic structure are well known (Melanie Standlmeier *et al.*, 2018).

Before launching any breeding programme, a breeder should have a thorough knowledge on nature and magnitude of genetic variability, heritability, genetic advance, genetic divergence, correlation and path analysis. Since, genetic variability studies provide basic information concerning the genetic properties of the population based on which breeding methods could be formulated for further improvement of the crop. It can be defined as the genetic makeup of organisms within a population change (Tsegau Senbetay *et al.*, 2020). Heritability is often used by plant breeders to quantify the precision of single field trials or series of field trials. It serves as a reliable indicator for the inheritance of traits to descendants from their parents. Genetic advance remains as other value indexes for the attainment of a desirable outcome on traits concerned in a population pertaining to selection. Genetic advance as percent of mean gives further clear – cut outcome when compared with genetic advance. Kurtosis and skewness stipulate in understanding the mean of genetic effects besides the extent of genes associated with individual traits (Fisher *et al.*, 1932). Correlation analysis guides in the detection of trait positive or negative correlation with yield such that those might be considered during breeding. Correlation accompanying path analysis gives an enhanced vision in determining an association between different characters which would aid deciding viable breeding techniques for the advancement of supreme plant kind. Considering the above viewpoints, the current study was directed for the determination of variability, correlation and path analysis for several quantitative traits derived a Multi-parent Advanced Generation Inter-cross (MAGIC) F<sub>2</sub> population of rice.

## Materials and Methods

The experiment was conducted at the Experimental Station, Field Crops Research Institute, Hai Duong, Vietnam during wet season 2021. The study materials comprised of 8 parental varieties (ST25, BT7 KBL-03, Huang Viet 3, NB01, Thien Uu 8, IRBB7, Dai Thom 8, BC15-02) and an *Indica* MAGIC F<sub>2</sub> population (the order of hybridization ST25/BT7 KBL-03// Huang Viet 3/NB01/// Thien Uu 8/ IRBB7//Dai Thom 8/BC15-02). The eight parents were intercrossed using structured matings to ensure that the population would have balanced representation from each parent. The F<sub>1</sub> plants were harvested individually and forwarded to the F<sub>2</sub> generation. Segregants were planted successively without replication, while the parents were replicated three times. The experiment was transplanted at the spacing of 20 x 20 cm, single seedling per hill.

Eight quantitative traits recorded at maturity stage, including: Plant height (cm), Number of effective tillers per plant, Panicle length (cm), Spikelet fertility percentage (%), Individual dried straw weight (SW), 1000-seed weight (g), Individual grain yield (g), Harvest Index (%). Data were subjected to analyse skewness, kurtosis, phenotypic coefficient of variance, genotypic coefficient of variance, broad-sense of heritability, genetic advance, genetic advance as percent of mean, Pearson correlation and path analysis by using Excel and IBM SPSS Statistic 20.

Skewness ( $\beta_1$ ) and kurtosis ( $\beta_2-3$ ) was calculated using the frequency distribution of the traits mentioned (Kapoor, 1981).

The skewness for a normal distribution is zero. Traits with symmetric data could be in the absence of gene interaction, have skewness near zero. Traits with negative skewness indicates that data are skewed left and these traits is in the presence of duplicate interactions. Traits with positive skewness indicate that data are skewed right and these traits are in the presence of average complementary interactions.

Gene interaction can be detected by studying fourth degree statistics, kurtosis ( $\beta_2-3$ ). It is positive only in the presence of gene interaction (Choo and Reibergs, 1982). Positive kurtosis (leptokurtic),  $\beta_2-3 > 0$ , and negative kurtosis (platykurtic)  $\beta_2-3 < 0$ . The left panel shows that a distribution with positive kurtosis has heavier tails and a higher peak than the normal, whereas the right panel shows that a distribution with negative kurtosis has lighter tails and is flatter. The normal distribution has a kurtosis of 3, and  $\beta_2-3$  is often used to that the reference normal distribution has a kurtosis of zero ( $\beta_2-3$ ) is sometimes denoted as  $\gamma_2$  (Lawrence, 1997).

Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were computed utilizing the method in recommendation with Burton (1952). An array of variations were classified to be low (less than 10%), moderate (10-20%), high (above 20%). Since segregants could not be assessed in replicated trials, the environmental variance was determined from parents raised in replicated trials. Where, Genotypic variance ( $\sigma_g^2$ ), Phenotypic variance ( $\sigma_p^2$ ), Environmental variance ( $\sigma_e^2$ ), MSG (Mean square of genotype), MSE (Mean square of error), r (Number of replications),  $\bar{x}$  (General mean).

$$\sigma_g^2 = \frac{MSG - MSE}{r}$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

$$GCV (\%) = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100$$

$$PCV (\%) = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100$$

Broad-sense heritability ( $H^2$ ) was worked out applying the formula stated by Lush (1940) and categorized to be low (less than 30%), moderate (30-60%) and high (above 60%).

$$H^2 (\%) = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Genetic advance (GA) was estimated as per formula given by Allard (1960). Where, K is standardized selection differential constant (2.06)- at 5% selection intensity.

$$GA = K \times \sigma_p \times H^2$$

The genetic advance as percentage of mean (GAM) known as expected genetic gain was assessed by embracing the technique provided by Johnson *et al.* (1995) wherein categorized to be low ( $\leq 10\%$ ), moderate (10 – 20%) and high ( $\geq 20\%$ ).

$$GAM (\%) = \frac{GA}{\bar{x}} \times 100$$

## Results and Discussion

### Variability and frequency distribution

Quantitative traits are controlled by several small-effect genes that are difficult to distinguish and controlled by quantitative trait loci (Bernardo, 2008). The genetics of the quantitative traits can be better understood using third and fourth degree statistics i.e. skewness and kurtosis in segregating generations (Savitha and Usha Kumari, 2015), which provides information on nature of gene action (Fisher *et al.*, 1932) and number of genes controlling the traits (Robson, 1956) and selection can be done based on these values. The coefficient of variation, skewness and kurtosis along with the frequency distribution of several quantitative traits are presented in Table 1 and Figure 1.

A wide variation exhibited by Number of effective tillers per plant (29.7%), Individual dried straw weight (33.39%) and Individual grain yield (37.1%). Many quantitative traits of interest to plant breeders are often genetically correlated, which can complicate progress from selection. Improving multiple traits may be enhanced by identifying parent combinations – an important breeding step – that will deliver more favorable genetic correlation. Within a simulated plant breeding program employing indirect selection, it is possible to choose crosses based on the predicted cross mean (Jeffrey *et al.*, 2019). The real genetic potentially can be detected only in progeny performance of the individual selection, since the mean of progeny is more reliable than the individual values of the selected parents (Savitha and Usha Kumari, 2015). It is noticeable that the average values of traits in progeny were usually higher than those in their parents, except for Number of effective tillers per plant. Besides, in the traits viz. Plant height, Panicle length, Spikelet fertility, their only upper-limit values of the parents fell outside the ranges of its progeny. While the lower-limit and upper-limit values of the parents on the traits as Number of effective tiller per plant, Individual dried straw weight, Individual grain yield and and Harvest index lied inside these ranges of its progeny, the opposite was true of 1000-seed weight. Transgressive segregation was seen in most of the traits (except for 1000-seed weight) where their value of several individuals fall outside the range of their parents. Only a small proportion of  $F_2$

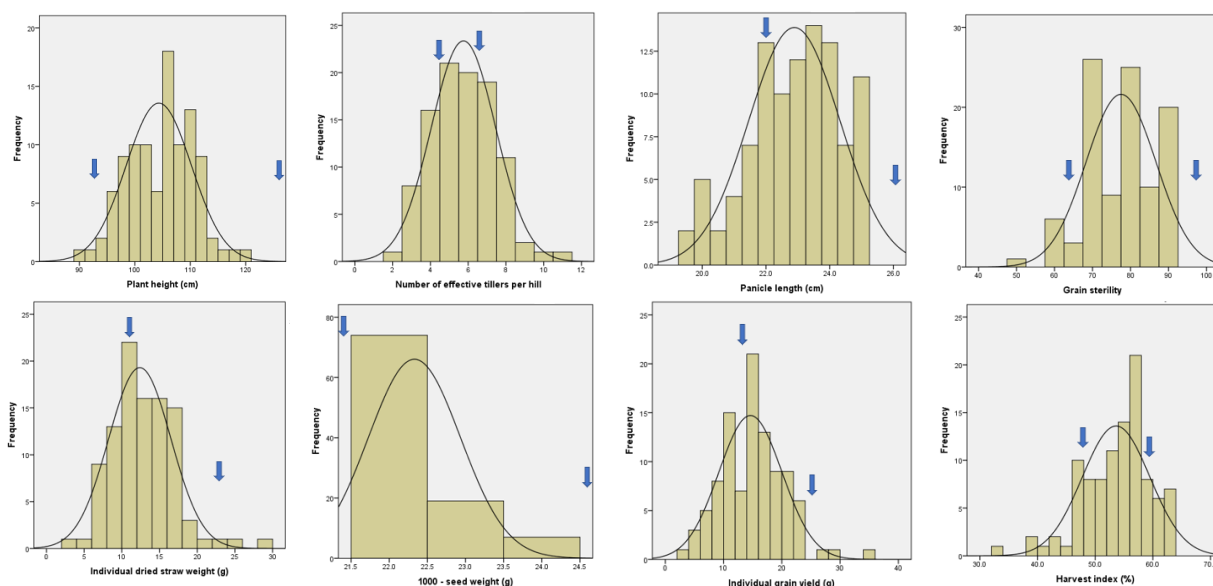
population were transgressive, but it occurs frequently enough that plant breeding works as a matter of routine. This proportion suggests that the cumulative effect of genetic complex influencing the traits.

Leptokurtic distribution with negative skewness was found in traits viz. Plant height (-0.276; -0.065), Panicle length (-0.445; -0.445) and Spikelet fertility percentage (-0.409; -0.345), while platykurtic distribution with positive skewness was found in traits viz. Individual straw weight (1.625; 0.722), 1000-seed weight (1.679; 1.668), Individual grain yield (1.196; 0.528). Number of effective tillers positively skewed (0.288) with leptokurtic distribution (-0.003), whereas Harvest index negatively skewed (-0.786) with platykurtic distribution (0.732).

The traits with positive skewness are associated with complementary gene interaction. In order to maximize the genetic gain in respect of these traits requires intense selection from the existing variability. These traits with negative skewness are associated with duplicate (additive x additive) gene interactions. The genes controlling the traits with skewed distribution tend to be predominantly dominant irrespective of whether they have increasing or decreasing effect on the trait. Genetic gain in respect of all the above mentioned traits showing negatively skewed distribution will be rapid under mild selection from the existing variability. Selection intensity could be higher under complementary than under duplicate relationship. Probably, progress in improving population performance may be greater under complementary interaction than under duplicate gene interaction (Choo and Reinbergs, 1982).

**Table 2:** Descriptive statistics for eight quantitative traits of an Indica F<sub>2</sub> MAGIC population

Traits	CV%	Skewness	Kurtosis	Progeny			Parent		
				Min	Max	Aver.	Min	Max	Aver.
Plant height (cm)	5.6	-0.065	-0.276	90.0	120.0	104.3	93.6	128.4	108.1
Number of effective tillers per plant	29.7	0.288	-0.003	2.0	11.0	5.8	4.6	6.8	5.6
Panicle length (cm)	9.4	-0.445	-0.445	19.5	25.0	22.9	22.0	26.0	24.2
Spikelet fertility percentage (%)	11.9	-0.345	-0.409	50.0	90.0	77.5	63.8	95.8	74.6
Individual dried straw weight (g)	33.4	0.722	1.625	3.0	28.0	12.4	11.6	23.4	15.9
1000-seed weight (g)	2.7	1.668	1.679	22.0	24.0	22.3	18.9	26.2	23.4
Individual grain yield (g)	37.1	0.528	1.196	3.0	35.0	14.6	13.8	25.4	19.3
Harvest index (%)	10.9	-0.786	0.732	33.3	63.6	53.6	48.4	58.9	54.8



**Figure 1:** Histogram for several quantitative traits of Indica MAGIC population (two green arrows in each graph represent for lowest and highest parents)

Kurtosis is any measure of the “peakness” of the probability distribution of a real valued random variable. Studies on the amount of gene interaction are undoubtedly needed so as to increase the efficiency of our selection and breeding programs. Leptokurtic distribution is suggesting that the large number of genes in governing the traits. Whereas, platykurtic distribution is suggesting that these traits were controlled by fewer genes.




In the study of Mayank Patel *et al* (2020), Number of grains per panicle, straw yield per plant was showed normal distribution in segregating population. Productive tillers per plant, grain yield per plant and harvest index considered to be governed by complementary gene interaction and days to flowering, plant height and panicle length was governed by duplicate gene interaction.

### Heritability, Genetic advances

Selection is the basic activity of a plant breeding program in an effort to obtain new superior varieties. Selection will be effective if a breeding population used by breeders have a broad genotypic variation. In other words, the measurement and evaluation of variability are essential steps in determining the success of a selection activity. A breeding population with a wide variety of genotypes will increase the chances of obtaining individuals with desirable traits (Sulistyo and Mejaya, 2018). Genetic variability analysis is an essential criterion in the selection of crops for improvement programmes and components like PCV, GCV,  $H^2$ , GAM are useful for the exploitation of variability. Since plant characters are complex in nature and is often environments biased and controlled by several genes in different environments, PCV and GCV is useful not only for the comparison of the phenotypic and genotypic variation but also acts as a tool to measure the effectiveness of the appropriate selection procedure for improvement. Variance, coefficient of variance, heritability in broad sense, genetic advance and genetic advance in percent of mean for several quantitative traits in  $F_2$  MAGIC population are presented in Table 2.

**Table 2:** Variability, heritability and expected genetic advance for several quantitative traits of *Indica* MAGIC population

Traits	Plant height (cm)	Number of Effective tillers per plant	Panicle length (cm)	Spikelet fertility percentage (%)	Individual dried straw weight (g)	1000-seed weight (g)	Individual grain yield (g)	Harvest index (%)
Genotypic variance	26.12	2.36	1.44	81.02	12.38	0.31	28.65	29.02
Phenotypic variance	34.20	2.90	2.06	85.04	16.95	0.36	29.39	34.14
PCV	5.61	29.62	6.26	11.90	33.26	2.70	37.11	10.89
GCV	4.90	26.71	5.24	11.61	28.42	2.51	36.64	10.04
$H^2$ (%)	76.38	81.33	70.10	95.28	73.00	86.05	97.49	85.01
GA	9.20	2.85	2.07	18.10	6.19	1.07	10.89	10.23
GAM (%)	8.82	49.63	9.04	23.35	50.02	4.79	74.52	19.08

(Variation array for GCV, PCV,  $H^2$  and GAM  Low  Moderate  High)

There are variations between the individuals in the same population, because the traits are probably influenced by environment, genes, and the interaction between the genes and environment. In this study, the differences between phenotypic variance and genotypic variance of all traits are low, together with high heritability (greater than 70%), which reveals that environment has minimal effects on the phenotypic performance of the traits. Hence, the selection is relatively confident and useful.

The study suggests that the PCV were higher the corresponding GCV for all quantitative traits observed. This means environmental effects of any trait represented by the magnitude of the differences between the genotypic and phenotypic coefficients of variation. The higher differences the higher environmental effect. The small differences were seen in the study reveal a high genetic influence. In other hand, the selection based on these characters would be effective for further crossing programmes. Low GCV were seen at 1000-seed weight (2.51%), Plant height (4.9%) and Panicle length (5.24%), while Number of effective tillers per plant, Individual dried straw weight and Individual grain yield had high GCV at 26.71, 28.42, 36.64, respectively. Harvest index, Spikelet fertility percentage presented moderate GCV values at 10.04% and 11.61%, subsequently.

Since high heritability does not always indicate a high genetic gain, heritability with genetic advance considered together should be used in predicting the ultimate effect of selecting superior varieties (Ali et al., 2002). A joint consideration of GCV,  $H^2$  and GAM revealed that Number of effective tillers per plant (26.71%; 81.3%; 49.63%), Individual dried straw weight (28.42%; 73%; 50.02%), Individual grain yield

(36.64%; 97.49%; 74.52%) were high, which could be explained by additive gene action and their improvement could be achieved through mass selection. Besides, the GAM of Plant height, Panicle length, 1000-seed weight and Harvest index just varied from 4.79% to 19.08%. These traits could be indicated by non-additive genes with limiting the chances of improvement of these traits through direct selection in its heritance and heterosis breeding may be useful.

### Pearson correlation

In a breeding program, the correlation between yield, yield components and other economic traits is important for selection, especially when the breeder needs to improve several traits simultaneously. Pearson's correlation is a correlation coefficient commonly used in linear regression to measure how strong a relationship is between two traits. Positive correlation increase selectivity for both traits, whereas negative correlation decreases selectivity. Therefore, it is important for breeders to identify correlations that have a genetic basis or reflect environmental factors. The Pearson correlation for the studied traits is shown in Table 3. It is noticeable that most traits correlated each other, while 1000-seed weight showed no correlation with any traits.

Table 3: Pearson correlation for several quantitative trait of Indica MAGIC population

	Plant height (cm)	Number of effective tillers per hill	Panicle length (cm)	Spikelet fertility percentage (%)	Individual dried straw weight (g)	1000 - seed weight (g)	Individual grain yield (g)	Harvest index (%)
Plant height (cm)	1	0.179	0.167	0.433**	0.481**	0.126	0.367**	-0.046
Number of effective tillers per hill		1	0.190	0.258**	0.765**	-0.047	0.864**	0.360**
Panicle length (cm)			1	0.248*	0.284**	0.066	0.395**	0.301**
Spikelet fertility percentage (%)				1	0.372**	0.005	0.449**	0.194
Individual dried straw weight (g)					1	-0.006	0.828**	-0.064
1000 - seed weight (g)						1	0.003	-0.080
Individual grain yield (g)							1	0.474**
Harvest index (%)								1

Individual grain yield highly correlated with Number of effective tillers per plant. It also showed medium correlation with Harvest index (0.474\*\*), Spikelet fertility percentage (0.449\*\*), Panicle length (0.395\*\*) and Plant height (0.367\*\*). Hence, yield improvement should be carried out with aid of an indirect selection of the traits contributing towards yield.

Although Harvest index moderately correlated with Individual grain yield (0.474\*\*), Number of effective tillers per plant (0.360\*\*), Panicle length (0.301\*\*), it showed no correlation with other traits. Similarly, Plant height had medium correlation with Individual dried straw weight (0.841\*\*), Spikelet fertility percentage (0.433\*\*) and Individual grain yield (0.367\*\*).

### Direct and Indirect effect

Plant breeders routinely select on multiple traits, but progress can be complicated by genetic correlations. If two traits are favorably correlated, selection can simultaneously improve both by tandem selection, indirect selection, or a trait index (Bernardo 2010). Unfavourable correlations, meanwhile, are common and often the bane of the breeder (Jeffrey et al., 2019). The directions of such correlation imply an unfavourable response in one trait when selecting on another and the underlying causes will impact the prospects of long-term improvement.

A path coefficient analysis is highly capable to simplify the exact interrelationship and contribution of each component effect on highly dependent variable like yield. Therefore, breeders are inclined to wisely focus on the set of components which shows high direct effect on yield and selection for those traits with positive moderate indirect effects to synchronize the selection efficiency (Shaheen et al., 2022)

Table 5: Direct and Indirect effect of all traits to yield

Trait	Plant height (cm)	Number of effective	Panicle length (cm)	Spikelet fertility	Individual dried straw weight (g)	1000 - seed	Harvest index (%)	Total effect	Pearson correlation
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		tillers per plant		percentage (%)		weight (g)			
Plant height (cm)	<b>-0.0426</b>	0.0102	0.0026	0.0219	0.394	0.0069	0.023	0.3379	0.367**
%	8.50	2.04	0.52	4.37	78.61	1.38	4.59		
Number of effective tillers per plant	-0.0082	<b>0.1808</b>	0.0011	0.013	0.6259	-0.0025	0.1781	2.7437	0.864**
%	0.81	17.91	0.11	1.29	61.99	0.25	17.64		
Panicle length (cm)	-0.0087	0.0043	<b>0.0353</b>	0.0094	0.1055	0.0045	0.1312	0.6587	0.395**
%	2.91	1.44	11.81	3.14	35.30	1.51	43.89		
Spikelet fertility percentage (%)	-0.0199	0.0147	0.0026	<b>0.0297</b>	0.3046	0.0002	0.0961	0.2638	0.449**
%	4.25	3.14	0.56	6.35	65.11	0.04	20.54		
Individual dried straw weight (g)	-0.0221	0.0436	0.0018	0.0188	<b>1.0734</b>	-0.0003	-0.0318	1.0865	0.828**
%	1.85	3.66	0.15	1.58	90.07	0.03	2.67		
1000 - seed weight (g)	-0.0058	-0.0026	0.0012	0.0002	-0.0051	<b>0.4870</b>	-0.0394	0.0241	0.003
%	1.07	0.48	0.22	0.04	0.94	89.97	7.28		
Harvest index (%)	0.0021	0.0205	0.0037	0.0098	(0.0525)	-0.0043	<b>0.4573</b>	0.4382	0.474**
%	0.38	3.73	0.67	1.78	9.54	0.78	83.12		

Determination of relationship amidst the attributes utilising correlation analysis might not convey an exact vision of the comparative significance of yield contributing characters. Hence, path analysis was utilized for the verdict of indirect and direct impacts to disclose the relationship among yield and its attributable traits. Similar to the Pearson correlation, Number of effective tillers per plant and Individual dried straw weight showed high total effect on Individual grain yield at 2.7437 and 1.0865, respectively. The highest direct effect was seen in Individual dried straw weight (1.0734), followed by 1000-seed weight (0.4870) and Harvest index (0.4573). Then, Individual grain yield of rice could be very effectively enhanced through direct selection of Individual dried straw weight, 1000-seed weight and Harvest index.

Plant height, Number of effective tillers per plant, Spikelet fertility percentage and Harvest index had highest indirect effect on Individual grain yield through Individual dried straw weight, whereas Panicle length, 1000-seed weight had highest indirect effect on Individual grain yield through Harvest index.

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