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RESEARCH ARTICLE

Appraisal of genetic variability in sodium azide induced rice mutants to identify selection criteria for higher yield using quantitative attributes

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Abstract

Induced mutation is an effective tool in generating variability of crop plants. Identification of efficient genotypes with improved yield requires knowledge of genetic variation in yield and yield contributing traits. Therefore, an investigation was conducted to develop variants through azide mutagenesis and estimation of genetic variability of the mutants to detect selection standards towards higher yield. Mature rice seeds were mutagenized with estimated LD_{50} concentration. A hundred mutants were grown-up accordingly and their quantitative traits were evaluated through multivariate analysis to assess genetic variability. Every assessed trait except grain length exhibited highly significant variation for all mutants. The high genotypic and phenotypic coefficient of variation along with high heritability and genetic advance as a % of mean was accounted for total and filled grains panicle^{-1}. Grain yield was positively and significantly correlated with total grains panicle^{-1}, filled grains panicle^{-1} and straw yield hill^{-1}. Besides, higher variance was also associated with these traits. Ward's Agglomerative clustering grouped the mutants into 7 major clusters. More than 36% of total variation was associated with first 2 principal components (PCs) and was mostly with total grains panicle^{-1} and filled grains panicle^{-1} whereas third and fourth PCs were mainly accounted for straw yield hill^{-1}. Thus, these traits should receive special attention during selection of yield potential genotypes. Presence of genetic variation in mutants was ensured for most of the traits and selection based on greater tillers hill^{-1}, grains panicle^{-1} and straw yield hill^{-1} may open a new avenue towards improved yield and other traits in rice.

Keywords

Azide mutagenesis, quantitative traits, variability analysis, selection standards

Introduction

Rice (*Oryza sativa* L.) becomes one of the most essential cereal crops by serving as the primary source of staple food for more than half of the world's population (1) and it is not only a crop but also a life for the Asiatic population (2). It meets up about 22% of the calorie intake of the world's population and 76% of the calorie of the population of Southeast Asia (3). Albeit several rice varieties with improved yield, quality and stress tolerance have been developed by the Bangladesh Rice Research Institute (BRRI) yet there is still a vital need to advance the existing germplasm towards higher yield to contribute to hunger eradication, global food security, poverty mitigation, economic growth and correspondingly world peace. Improvement of elite genotypes through breeding relies on genetic variation with useful characters. To induce genetic variation with desired attributes, mutation induction has been widely used as a very prolific tool in plant breeding (4). Mutations generate genetic variations that can be released directly as a variety or may serve as a source of raw materials with desirable alleles for the genetic upgrading of crop plants (5).

Variations through mutation were created through physical and/or chemical agents (6). Bringing genetic variation by means of chemical mutagens is fairly cost-effective, robust, quick, verified. Chemical mutagen can mutagenize any genotypes with random distribution in the genome. Furthermore, it provides a large allelic series and does not rely on transformation. Hence, there has been an increasing interest in using chemical mutagenesis (7). A number of mutagenic agents *viz.,* diethyl sulphate (DES), ethylmethanesulfonate (EMS), N-methyl-N-nitrosourea (MNU), sodium azide (SA) (chemical mutagens), fast neutron, and γ-ray (physical mutagens), have been employed to create new mutants for improving rice (8). Several mutants even had attained noticeable success and are providing significant economic contributions (9). The mutagenicity of SA is arbitrated through an organic metabolite (analogous to L-azidoalanine) of the azide compound generated by O-acetylserine sulfhydrylase enzyme (10) and this metabolite creates point mutations by interacting with the genomic DNA after entering the cell nucleus. It causes mainly G/C to A/T transitions (11).

The most common method of mutagenesis in seed-propagated crops is the treatment of seeds (12). Though the genome size of the target individual is not a critical factor in chemical mutagenesis yet its toxicity may show a discrepancy from species to species (13). So the optimal dose (LD_{50}) determination of the mutagen for the species to be mutated is imperative to get a high incidence of anticipated mutants (14) and this is verified by altering the concentrations of the mutagen (15). Variations in rice have already been induced by chemical mutagens (16) and among them, SA has been declared as one of the most potential in creating desired new variants (17).

The productivity of rice is affecting by various biotic and abiotic factors due to its narrow genetic base as most of the improved varieties have been developed through traditional breeding using common ancestors. But there is a lot of opportunities of increasing genetic variations in qualitatively as well as quantitatively inherited traits through induced mutagenesis by SA. Identification and selection of elite mutants to develop high yield potential rice varieties is possible through evaluation of genetic diversity and point out the selection criteria of the mutants. Quantitative traits are used to evaluate the germplasm as this is an easy, rapid, inexpensive and potent method of estimation of genetic diversity. Besides, this technique needs no sophisticated equipment or technical knowledge and can be innate without molecular procedures (18). Hence, the study was undertaken to decipher the diversity of SA induced mutants and to pinpoint selection principles toward higher yield.

Materials and Methods

Location of the study

The present investigation was carried out discreetly at Plant Breeding and Biotechnology Laboratory and at Experimental Farm of Agrotechnology Discipline under Khulna University (latitude 22°79′88″ E, longitude 89°53′44″ N and elevation: 18 m), Bangladesh from 2020 to 2021 (Fig. 1).

Selection of effective dose for mutagenesis

The overnight soaked seeds of BRRI dhan28, a popular rice variety of Bangladesh, were treated for 12 hrs in recurrent shaking (60 rpm) with nine concentrations of SA prepared in potassium phosphate buffer (pH= 3.0) including the control (0, 0.001, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064 and 0.128 M). The seeds were washed under running tap water for an hour after decanting SA solution followed by three times washing with distilled water. The treated seeds were germinated on double-layered filter paper in petri plates under 3000 lux light regime maintaining 16/8 hrs light/dark at 27±1 ^{0}C temperature (19) following Completely Randomized Design (CRD). A concentration of 0.039M SA was determined as LD_{50} through dose-response curve based on germination (%), survivability (%) and mortality (%) of 14 days old seedlings. Further, 500 seeds were mutagenized with LD_{50} concentration to develop mutants.

Raising the mutants

A total of 303 mutants were planted in plastic pots (30 l) with single mutant in single pot filled earlier with rice field soil (pH=8.1, OM (organic matter)= 5.38%, total nitrogen= 0.222%, P= 16.36 µg g^{-1} soil, K= 0.34 mg 100g^{-1} soil, S= 31.40 µg g^{-1} soil, Zn= 2.88 µg g^{-1} soil and B= 1.23 µg g^{-1} soil) and finally the number mutants reduced to hundred through selection. An untreated control was also maintained along with mutants. To avoid cross-pollination, an isolation distance of 3 m was maintained and the mutants were grown to maturity following proper agronomic practices. The distribution pattern of rainfall, temperature and relative humidity in the study area during crop growth are presented in Fig. 2.

Experimental design

The mutants were maintained following completely randomized design (CRD) with three replications in a net house that ensured proper sunlight and airflow throughout the crop growth period where the assessment of variations in plant characters were conducted. At biological maturity, the seeds from the panicles of main tillers of each of 100 mutants were harvested, threshed, cleaned, dried and preserved.

Parameters studied

Data were measured for 16 quantitative traits *viz.,* plant height, tillers hill^{-1} (total and effective), days to flowering, days to maturity, panicle length, branches panicle^{-1} (primary and secondary), grains panicle^{-1} (filled and unfilled), 100-grain weight, grain length, grain breadth, straw yield hill^{-1}, grain yield hill^{-1} and harvest index.

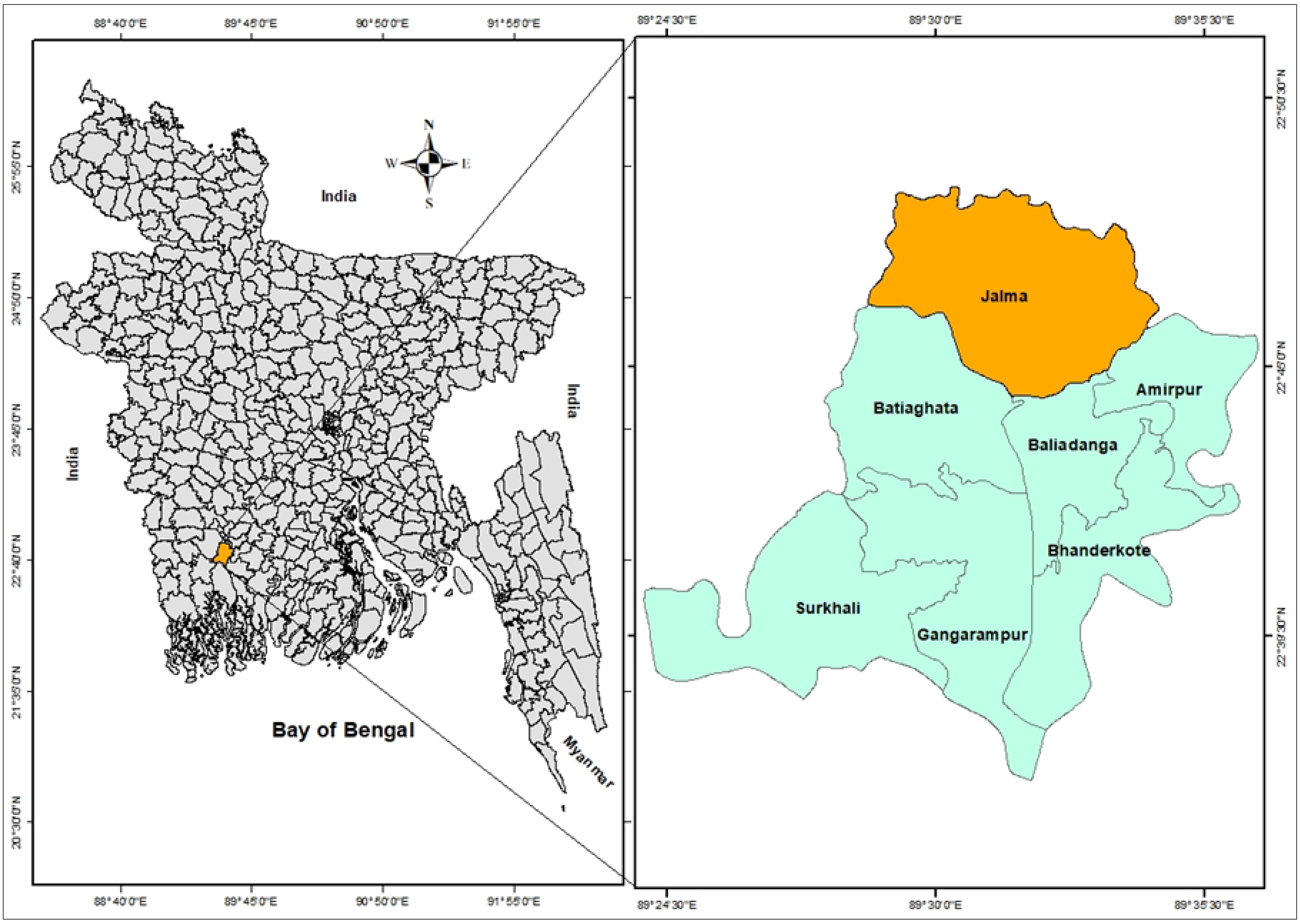

Fig. 1. Geographical location of the study area (highlighted in orange color)

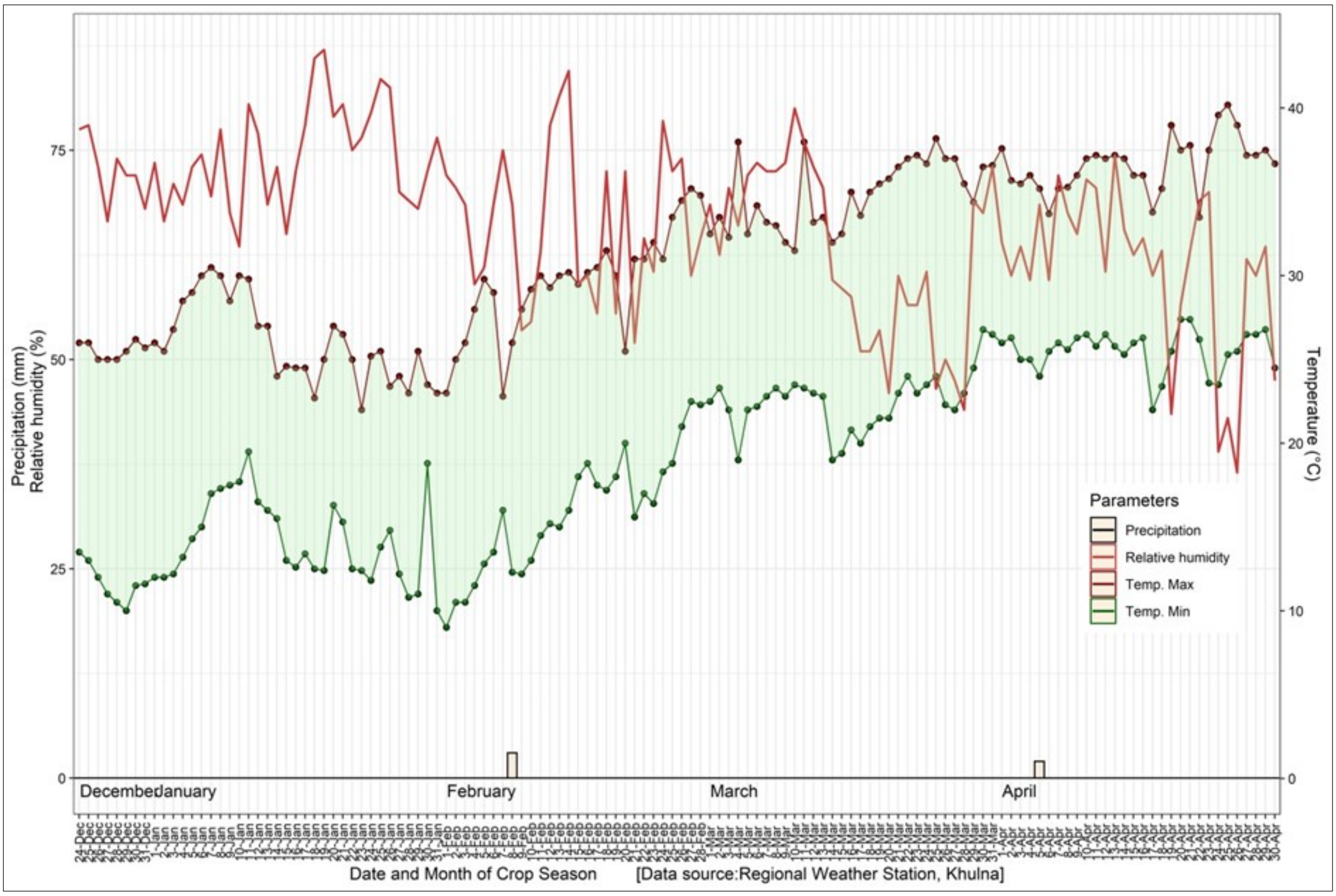

Fig. 2. Manifested weather situation during crop growing period

Statistical analysis

The data were subjected to analysis of variance (ANOVA) by using Statistical Tools for Agricultural Research (STAR) software. The means of the quantitative traits were compared by Duncan's Multiple Range Test (DMRT) (20). To identify the genetic and environmental effects on the studied traits, genetic components were assessed by using the R program language (21) (2.14.0 version).

Genotypic variance: $$\sigma_g^2 = \frac{(MSG-MSE)}{r} \quad \text{……………….…… (22)}$$

Phenotypic variance: $$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 \quad \text{….…………………....(22)}$$

Genotypic coefficient of variation: $$GCV(\%) = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100 \text{ ….(23)}$$

Phenotypic coefficient of variation: $$PCV(\%) = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100 \text{ .(23)}$$

Heritability in broad sense: $$h_b^2 = \frac{\sigma_g^2}{\sigma_p^2} \quad \text{……………....……….....(24)}$$

Genetic advance: $$GA = K \times \sqrt{\sigma_p^2} \times h_b^2 \quad \text{…………….…..(25)}$$

Genetic advance as percentage of mean:

$$GA(\%) = K \times \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times h_b^2 \times 100 \quad \text{…………….....………. (25)}$$

Here, MSG is the mean square of genotypes, MSE is mean square of error, r is the number of replication, σ_e^2 is mean squares of error, σ_p^2 is the phenotypic variance, σ_g^2 is the genotypic variance, $\bar{X}$ is mean of trait, and K is the selection differential (K=2.06 at 5% selection intensity).

The relationships among the traits were also assessed using Pearson's correlation coefficient. The standardized data were subjected to cluster the mutants using Euclidean distance following Ward's Agglomerative clustering method by using 'stats', 'factoextra' and 'cluster' packages of R program to find out the genetic relationships among the variants. The individuals with liked traits were mathematically grouped in the same cluster. Principal component analysis (PCA) based on the Euclidian distance method was also accomplished to simplify the complexity in high-dimensional data while retaining trends and patterns by using 'GGally', 'factoextra' and 'ggfortify' packages, whereas the path coefficient analysis was conducted to evaluate causal models by examining the relationships between a dependent variable and two or more independent variables by using 'lavaan' and 'semPlot' packages of R program.

Results

Variability among the mutants

Sodium azide induced mutants presented greatly significant variation ($p \leq 0.05$) for all traits (Supplementary Table 1). The analysis of variance showed that SA mutagenesis had brought substantial modifications on the quantitative traits. The estimates of genetic components of mutants for quantitative traits are presented in SupplementaryTable 2. A higher value for GCV and PCV was observed for all the parameters. The highest value of GCV (24.51) and PCV (33.71) were recorded for filled grains panicle^{-1} and that of lowest GCV (2.31) for grain breadth and PCV (2.81) for days to maturity. Most of the traits (10 out of 16) exhibited high heritability. Among the parameters, plant height, total tillers hill^{-1}, effective tillers hill^{-1}, total grains panicle^{-1} and 100-grain weight exhibited high heritability while panicle length, grain length and grain breadth exhibited low heritability specifying more impact of environment on these parameters. The ultimate genetic advance as percentage of mean was recorded for total grains panicle^{-1} (38.53) followed by filled grains panicle^{-1} (36.71) though the lowest assessment was for grain breadth and grain length.

Interrelations among the parameters

Character association analysis showed that the majority of the parameters had significant ($p \leq 0.001$) and positive associations with one another. Additionally, the genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients, indicating that the environmental effects reduced resilient association at the phenotypic level (SupplementaryTable 3). Grain yield hill^{-1} had significant and positive correlations with total grains panicle^{-1}, filled grains panicle^{-1}, 100-grain weight, grain breadth, straw yield hill^{-1}, and harvest index at genotypic level and it had significant and positive correlations with secondary branches panicle^{-1}, total grains panicle^{-1}, filled grains panicle^{-1}, 100-grain weight, grain length, grain breadth and straw yield hill^{-1} at phenotypic level. Grain yield hill^{-1} was significantly and positively associated with total grains panicle^{-1}, filled grains panicle^{-1}, 100-grain weight, grain breadth and straw yield hill^{-1} in both genotypic and phenotypic levels.

Path analysis

Partitioning of the correlation coefficients of the traits towards grain yield was assessed through path analysis to know their direct and indirect effects (Fig. 3). Path coefficient analysis revealed that all the traits had direct positive effects on grain yield hill^{-1} whereas the panicle length and primary branches panicle^{-1} showed negative effects on the road to grain yield. Among the traits, grain breadth (2.09)

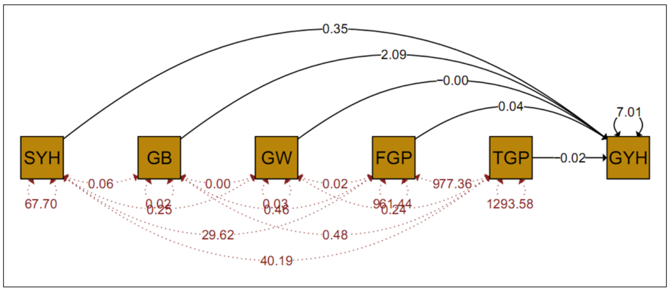

Fig. 3. Path diagram of traits on grain yield hill^{-1}. Single-headed arrows (paths) denote causal relationships, with the variable at the arrow's tail causing the variable at the arrow's head. The covariance between the two variables is represented by the double headed arrows pointing to them, and the variances associated with that variable are represented by the single headed arrow pointing to that variable. (Residual= 0.578).

had the maximum effects followed by straw yield hill^{-1} (0.35). The highest variance was associated with total grains panicle^{-1} (1293.58) followed by filled grains panicle^{-1} (961.44) whereas the variance associated with grain breadth was the minimum (0.02).

Cluster analysis

The standardized data were subjected to cluster the mutants using Euclidean distance following Ward's agglomerative clustering method and grouped the mutants into seven major clusters (Fig. 4). The distribution array publicized a maximum number of genotypes including the parent (28) under cluster I. The second largest group having 21 genotypes was cluster III. Each of clusters IV and VI comprised 14 mutants and cluster II contained low number of mutants (5).

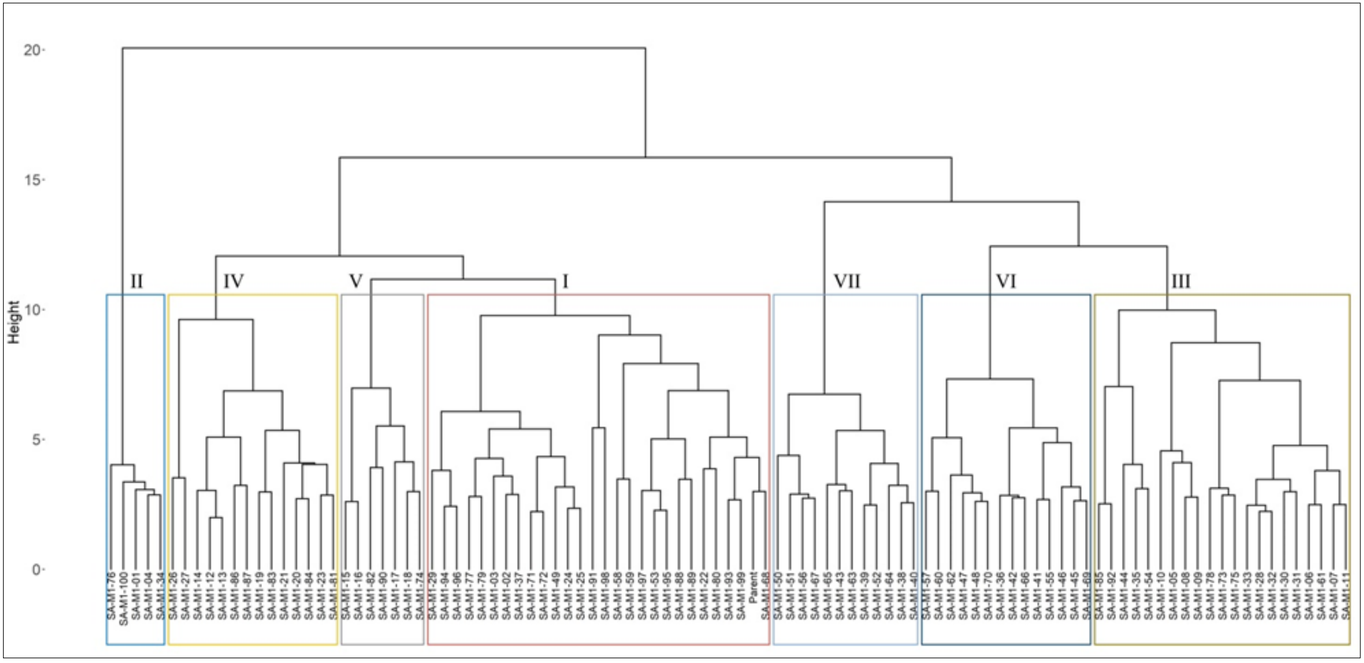

Fig. 4. Dendrogram constructed for SA induced rice mutants with their parent using the data obtained from morphological traits.

Cluster V had the highest average value of six traits *viz.,* plant height, secondary branches panicle^{-1}, total grains panicle^{-1}, filled grains panicle^{-1}, grain length and grain breadth followed by cluster II and cluster IV each of which had the highest mean values of four traits (Table 1). The maximum inter-cluster distance (8.71) was between cluster II and cluster VI and that of minimum (5.06) was between cluster VI and cluster VII whereas the highest (4.69) and the lowest (3.33) intracluster distance were for cluster I and cluster II, respectively (Table 2).

Principal component analysis (PCA)

First six principal components (PCs) which had Eigen values greater than one, accounted for 74% of total variation. Of which 22.70% of variation could be explained by the first PC followed by 13.95% and 12.12% variation by second and third PC, respectively (Table 3). The first PC was associated mostly with total grains panicle^{-1}, filled grains panicle^{-1}, secondary branches panicle^{-1}, grain length, primary branches panicle^{-1} and effective tillers hill^{-1} due to their high loadings which are entirely the yield contributing traits. The second PC was principally directed by total tillers hill, effective tillers hill^{-1} and days to maturity that are also the yield contributing characters (Fig. 5).

The cluster analysis and PCA were further confirmed by plotting them together (Fig. 6). The mutants under the distant clusters (cluster II and cluster VI) were plotted far away from each other indicating a higher genetic variability among the genotypes while the clusters with minimum distance (cluster VI and cluster VII) came closer to each other indicating comparatively homogeneity among the mutants.

Discussion

Variability among the mutants

The existence of genetic diversity is one of the most important principles to conduct an efficient selection program from a mix population for superior yield. Highly significant differences ($p \leq 0.01$) of the traits revealed the presence of wide variability in the mutants. This might be due to the existence of variation in their genetic makeup and/or environmental influences. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were grouped as low (0-10%), moderate (10-20%) and high (>20%) according to the classification (26). The level of PCV estimations was higher for all the traits compared to GCV, indicating the impact of environment on the expression of all traits (27). But the actual strength of variability can be reached by comparing their coefficient of variation. There was a substantial difference between GCV and PCV for panicle length, primary branches panicle^{-1}, total grains panicle^{-1}, filled grains panicle^{-1} and grain yield hill^{-1} which indicates greater influence of environmental factors in the expression of these traits. But the high value of heritability and genetic advance for total grains panicle^{-1} indicated more contribution of genetic components for the total observable variation of this trait. The degree of differences between GCV and PCV were fairly low for the rest of the traits signifying a slight effect of environment on their

Table 1. Cluster means for characters in 100 SA induced rice mutants and their parent

Characters	I	II	III	IV	V	VI	VII
PH	84.85	95.03	90.64	80.43*	96.38**	88.88	88.37
TTH^{-1}	28.43	30.83**	13.89*	26.83	24.53	21.83	28.55
ETH^{-1}	21.94	24.17**	11.06*	20.50	18.50	16.85	22.33
DF	104.45*	114.95**	109.06	108.78	107.00	109.24	108.98
DM	130.91*	138.72**	131.45	133.81	135.87	135.67	133.74
PL	20.05*	23.25	22.36	23.85**	22.53	22.47	21.90
PBP^{-1}	9.15	9.22	5.78*	10.28**	8.82	8.22	9.93
SBP^{-1}	19.57	19.28	9.95	21.11	22.22**	19.76	19.04*
TGP^{-1}	112.50	102.06	58.17*	135.47	146.90**	111.44	96.78
FGP^{-1}	87.96	84.61	43.56*	106.50	114.65**	83.95	76.40
GW	1.92	1.96	2.03	1.97	2.00	2.09**	1.90*
GL	8.28	8.02	7.31*	8.15	8.32**	7.94	8.04
GB	2.27	2.30	2.19*	2.20	2.31**	2.28	2.22
SYH^{-1}	37.77	33.97	29.84*	39.45	31.99	42.44**	32.13
GYH^{-1}	18.23	16.22	13.98	19.61**	15.63	18.90	13.02*
HI (%)	32.44	32.32	32.10	33.17**	32.69	30.95	29.14*

*: The lowest cluster mean; **: The highest cluster mean; PH: Plant height (cm); TTH^{-1}: Total tillers per hill; ETH^{-1}: Effective tillers per hill; DF: Days to flowering; DM: Days to maturity; PL: Panicle length (cm); PBP^{-1}: Primary branches per panicle; SBP^{-1}: Secondary branches per panicle; TGP^{-1}: Total grains per panicle; FGP^{-1}: Filled grains per panicle; GW: 100 grain weight (g); GL: Grain length (mm); GB: Grain breadth (mm); SYH^{-1}: Straw yield per hill (g); GYH^{-1}: Grain yield per hill (g); HI (%): Harvest index (%).

Table 2. Average intra (Diagonal) and intercluster distances of 100 SA induced rice genotypes with their parent

Cluster	I	II	III	IV	V	VI	VII
I	4.69						
II	7.82	3.33					
III	5.46	6.89	4.62				
IV	5.30	8.18	5.36	4.55			
V	5.52	7.37	5.88	5.64	4.50		
VI	5.32	8.71	5.14	5.26	6.43	3.96	
VII	5.14	7.54	5.58	5.54	6.50	5.06	3.84

expression (28). Hence, there is an auspicious possibility of bringing anticipated genetic upgrading in successive generations through selection (29, 30).

The GCV does not offer full scope to estimate the variation that is heritable in nature and therefore, estimation of heritability becomes necessary. The broad sense heritability was grouped into three major classes based on the classification system of (22, 31) as low (<30%), medium (30-60%) and high (>60%). The high heritability for most of the traits indicating the possibility of upgrading those parameters through selection. But heritability alone does not always point toward the genetic gain unless it is studied in combination with genetic advance which comprises the effect of both additive and non-additive genes. Genetic advance is used to compute the type of gene action in polygenic traits and indicates the prospected progress as

Table 3. Eigen values, percentage of variation and standard deviation of the first five principal components of corresponding 16 characters in SA induced mutants

Variables	PC1	PC2	PC3	PC4	PC5	PC6
PH	-0.16	0.15	0.40	-0.03	0.37	-0.08
TTH^{-1}	-0.32	-0.46	0.01	-0.11	0.01	0.20
ETH^{-1}	-0.29	-0.48	-0.02	-0.12	-0.03	0.24
DF	0.05	0.09	0.26	-0.53	-0.32	0.15
DM	-0.15	0.32	0.34	-0.31	-0.19	0.12
PL	-0.08	0.21	0.16	-0.32	0.16	0.23
PBP^{-1}	-0.29	-0.24	-0.01	-0.13	-0.17	-0.02
SBP^{-1}	-0.39	-0.01	0.11	-0.01	0.23	-0.10
TGP^{-1}	-0.40	0.26	0.02	0.17	-0.17	-0.30
FGP^{-1}	-0.39	0.25	0.02	0.18	-0.27	-0.19
GW	0.01	0.22	-0.25	-0.18	0.29	-0.12
GL	-0.34	-0.08	0.05	0.19	0.02	-0.13
GB	-0.17	0.16	0.03	0.16	0.56	0.45
SYH^{-1}	-0.16	0.11	-0.51	-0.37	0.10	-0.14

GYH^{-1}	-0.20	0.23	-0.54	-0.14	-0.08	0.24
HI (%)	-0.04	0.24	-0.05	0.39	-0.33	0.60
Eigen value	3.63	2.23	1.94	1.71	1.26	1.07
Variance (%)	22.70	13.95	12.12	10.69	7.87	6.68
Cumulative (%)	22.70	36.65	48.77	59.46	67.32	74.00
SD	1.91	1.49	1.39	1.31	1.12	1.03

PH: Plant height (cm); TTH^{-1}: Total tillers per hill; ETH^{-1}: Effective tillers per hill; DF: Days to flowering; DM: Days to maturity; PL: Panicle length (cm); PBP^{-1}: Primary branches per panicle; SBP^{-1}: Secondary branches per panicle; TGP^{-1}: Total grains per panicle; FGP^{-1}: Filled grains per panicle; GW: 100 grain weight (g); GL: Grain length (mm); GB: Grain breadth (mm); SYH^{-1}: Straw yield per hill (g); GYH^{-1}: Grain yield per hill (g); HI (%): Harvest index (%); SD: Standard deviation.

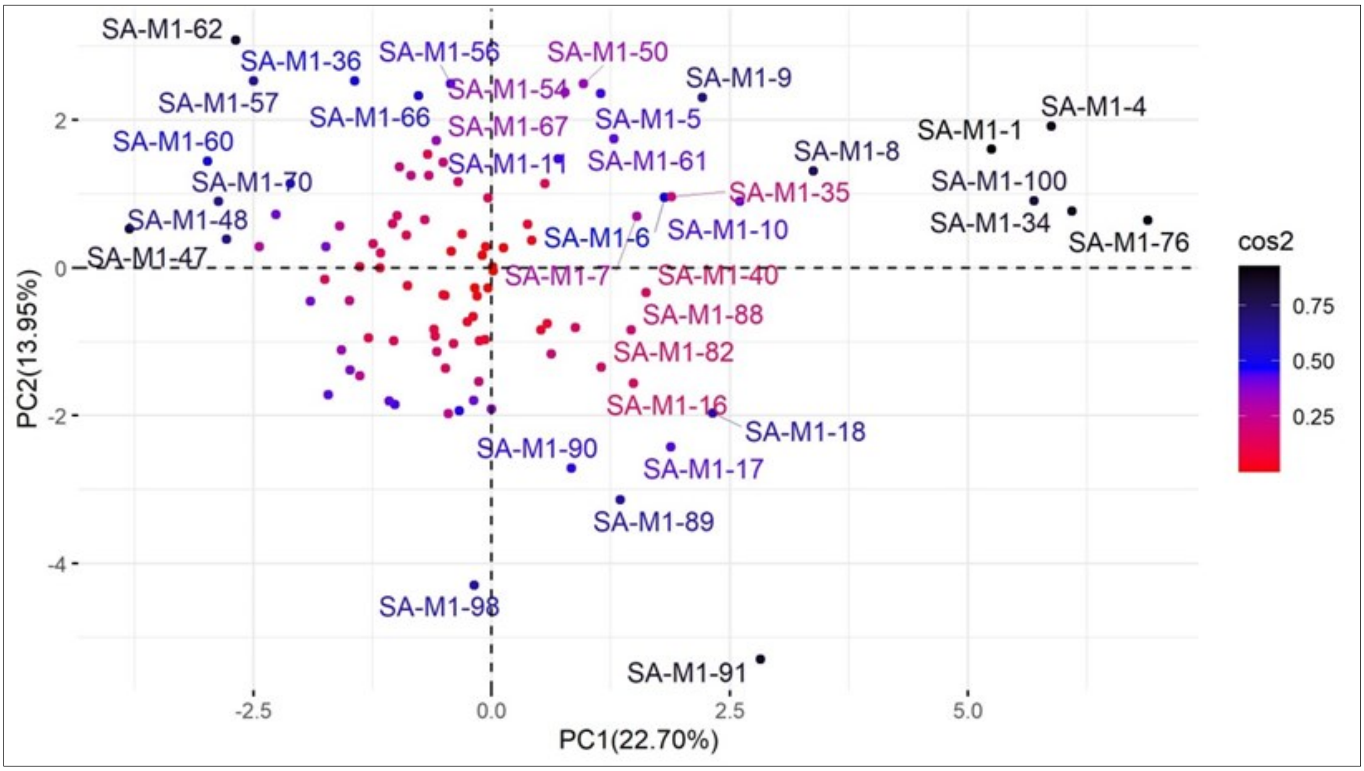

Fig. 5. Two dimensional PCA plot showing the distribution of mutant individuals according to their degree of morphologic variation.

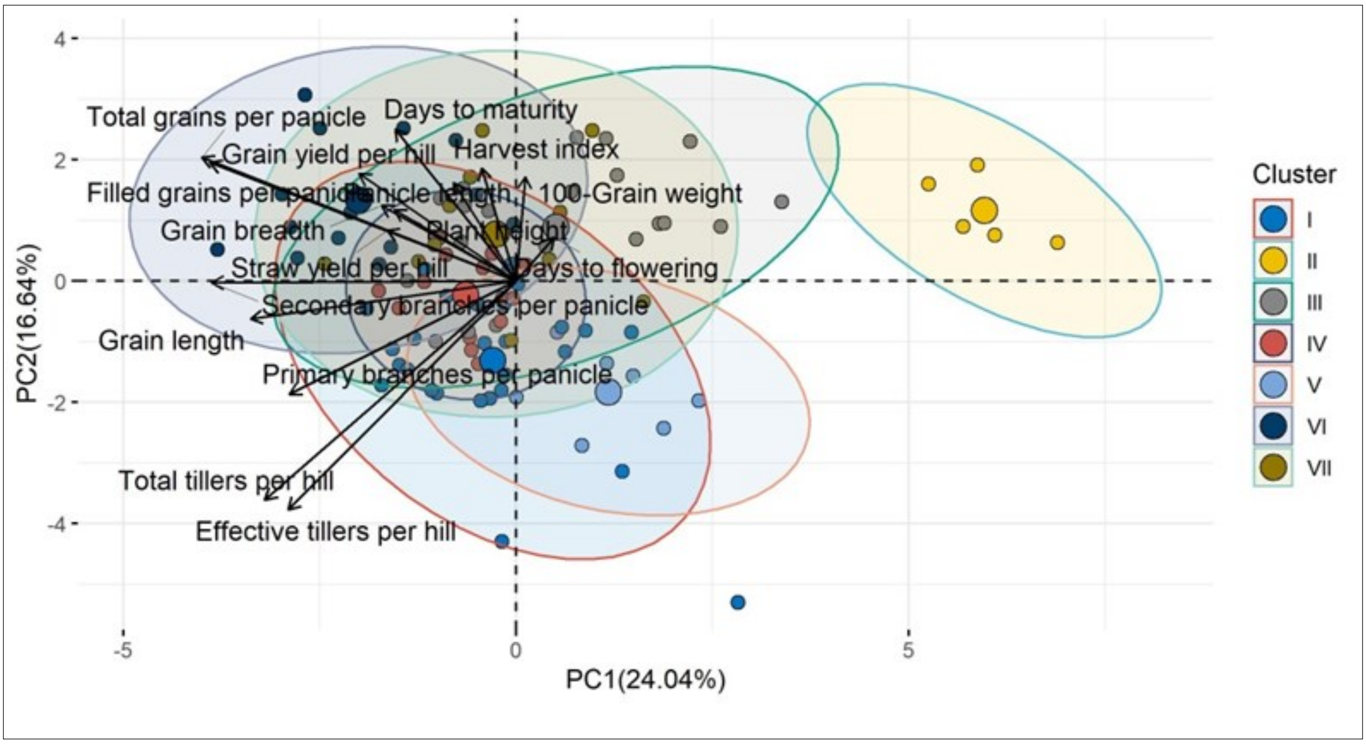

Fig. 6. PCA plot showing the distribution of mutants in clusters and contribution of traits (loading vectors) towards variation.

the result of selection (22, 32). Heritability and genetic advance are inexorable in selecting elite genotypes based on the performance of quantitative traits (33). Genetic advance as a % of mean offer a more specific result compared to just genetic advance (34). Genetic advance as % of mean was considered as low if the value was >10%, average > 10-20% and high > 20% (22, 35). High heritability with high genetic advance as a % of mean offers better clues for selecting suitable traits. In this study, high genetic advance as a % of mean together with high heritability were obtained for plant height, total tillers hill^{-1}, effective tillers hill^{-1}, secondary branches panicle^{-1}, total grains panicle^{-1} and straw yield hill^{-1} which reported the preponderance of additive gene action in phenotypic expression of these traits and were less affected by environmental variations. Hence, selection based on the phenotypic performance of these traits would upgrade rice (36). Besides high heritability with high genetic advance as a % of mean, total grains panicle^{-1} had also high genotypic coefficient of variation that made this trait more and more considerable in selection program. These findings were corroborated (37). Average genetic advance with high heritability was observed for 100-grain weight indicating the action of both additive and non-additive gene action on this trait and there is a possibility of improvement of these traits through selection also (38).

Interrelations among the parameters

Identification and selection of highly correlated traits is essential (39) to improve complex plant characters like yield through direct selection of traits as the traits are quantitatively inherited and influenced not only by genotypes but by the interaction between the genotype and environment also. Significant, positive and high correlation coefficients of total grains panicle^{-1}, filled grains panicle^{-1}, 100-grain weight, grain breadth and straw yield hill^{-1} at both genotypic and phenotypic levels in this study was a clue that selection of these traits would be suitable to obtain desired genotypes with better heterosis (40). Ample grains panicle^{-1} with plenty filled grains along with higher grain weight sums up to produce grain yield hill^{-1} and more vegetative growth sinks more photosynthates. Therefore, strong and positive correlation coefficients have resulted for these traits with grain yield (30). Straw yield hill^{-1} had the best relationship with yield production in the present study which is in accordance with the results reported (41). Hence, more attention is to be concentrated to this trait for final yield determination of the mutants.

Path analysis

The correlation offers only degree and direction of association between traits but not the true contribution of them towards yield (42) whereas the path coefficient analysis separates the direct and their indirect effects through other attributes by dividing up the correlations (43) for better interpretation. Among the 5 traits that showed positive and significant correlation with grain yield hill^{-1}, were also showed positive effect with maximum for straw yield hill^{-1}. Similar results were reported in rice (44). Positive direct effects of these traits on grain yield specified their significance in determining this complex character. The direct positive effect and positive significant correlation of these traits on grain yield indicate that selection considering these traits would be more effective towards the improvement of grain yield (45). The residual effect of 0.578 indicated more than 42% of the variability of grain yield hill^{-1} was contributed by the 5 characters studied in the path analysis. This high residual effect is the indication of the impact of other attributes on grain yield which were not considered and/or there might have environmental influences (46).

Cluster analysis

The genetic diversity analysis based on quantitative traits can be used to categorize the mutants (47). Ward's agglomerative clustering dendrogram broadly clustered the mutants into seven major groups that revealed the effectiveness of quantitative traits in grouping the mutants. The seven clusters imply that SA mutagenesis had brought high level of variability in mutants. Among the traits selected on the basis of genotypic and phenotypic correlation in accordance with path analysis, rice mutants with the highest mean performance for total grains panicle^{-1}, filled grains panicle^{-1} and grain breadth were gathered into cluster V whereas the genotypes with maximum 100-grain weight and straw yield hill^{-1} were assembled into cluster VI. This specified the greatest contribution of these five traits headed for the variability of mutants grouped in the cluster from mutants found in cluster V and cluster VI (48). To exploit hybrid vigor, parents should be selected from the clusters with higher inter-cluster distance (49) as parents with high yield potential along with great genetic diversity increase the chance to yield superior progeny (50). The inter-cluster distance between cluster V and cluster VI was also higher and this might be due to the presence of mutants having extensive genetic variability and selection of promising mutants from these 2 clusters might be rewarding.

Principal component analysis (PCA)

Eigenvalues were used to find out the number of factors to retain (51). According to Gatten's lower bound principal, eigenvalues less than one were ignored (52). The morphological inconsistency among the mutants was authenticated by PCA as it redirects the significance of greater contributor to the total variation at each axis of differentiation (53). The PCA results specified that the overall distinction was honestly distributed through all the quantitative traits studied rather than the contribution of some specific traits. The greater eigenvectors proportions of traits in the principal components indicated that these traits had higher involvement in the total variability of the population into clusters. Among the selected traits from correlation and path analysis, total grains panicle^{-1} and filled grains panicle^{-1} had their higher eigenvectors percentage in the first as well as second principal components whereas 100-grain weight and straw yield hill^{-1} had their higher eigenvectors in the third and fourth principal components indicating their importance towards genetic diversity. It was found only twelve PCs more than 0.5 eigenvalues and 93.87% total variability (54). The cosine of the angle between the vectors of 2 traits indicates the strength of relationship between the traits (55). The 2-dimensional plot of the first 2 principal components that contributed to 36.65% of the variance measured the relationships among the characters and showed a distinguished relationship of grain yield hill^{-1} with total grains panicle^{-1}, filled grains panicle^{-1} and straw yield hill^{-1} as indicated by the small angle between their vectors (α = 15°58′1″, 16°19′48″ and 14°28′ 57″ respectively) whereas 100-grain weight had a weak relationship with grain yield hill^{-1} (α = 43°36′42″). Selection of genotypes having greater grain producing capacity with more filled grains panicle^{-1} and profuse vegetative growth may be effective for rice improvement.

Conclusion

Azide mutagenesis had brought significant genetic changes in rice mutants and could serve as a great genetic source for multiple aspects. Correlation and cluster analysis were complementary to each other while cluster and principal component analysis showed a little inconsistency in respect of extracting the % of variation. Evaluation of genetic parameters of the quantitative traits of the mutants specified that selection of mutants based on total tillers hill^{-1}, effective tillers hill^{-1}, total grains panicle^{-1}, filled grains panicle^{-1} and straw yield hill^{-1} might be pleasing to improve rice towards high yield, and this assessment could be workable in developing feasible selection exponents for important quantitative traits in rice mutants.

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Authors contributions

MMI and SKA developed the concept of the research and AAM carried out the laboratory as well as field research. AAM recorded and analysed the data with the assistance of MSS and prepared the manuscript. The manuscript was corrected and edited by MMI, SKA and MSS. Finally, all authors read and approved the manuscript for publication.

Compliance with ethical standards

Conflict of interest: **:** For the current study, the authors declare no conflicts of interest.

Ethical issues: None.

Supplementary data

Supplementary Table 1. . Mean squares of analysis of variance of the morphological traits of SA induced mutants of rice

Supplementary Table 2. Estimation of genetic parameters of the sixteen morphological traits among SA induced mutants of rice

Supplementary Table 3. Genotypic correlation (r_g, above diagonal) and phenotypic correlation (r_p, below diagonal) for the morphological traits of SA induced mutants

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