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Studies on Genetic variability, correlation analysis, character association and path analysis of phenotypic characteristics of twelve mega varieties of rice and its near-isogenic lines carrying high grain number per panicle QTL *qGN4.1*

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Abstract

In order to evaluate genetic variability of agro-morphological traits and to determine the correlation between grain number and its components in rice lines, twelve near isogenic lines (NILs) along with their recipient parents as check, were grown at IARI, India (2016-18). The experiments were laid out in a randomized complete block design (RCBD) in two replications. High heritability in broad sense (H^2) estimates were obtained for plant height (99.33%), total tillers (90.34%), productive tillers (88.83%), flag leaf length (92.47%), flag leaf width (91.81%), panicle length (97.61%), primary branches in a panicle (91.49%), secondary branches in a panicle (96.76%), number of filled grain (99.90%) and total grain in a panicle (99.73%) for NILs, suggested that the traits were primarily under genetic control. A joint consideration of broad sense heritability (H^2) and genetic advance as percent mean expected (GAM) revealed that filled grain and total grain per panicle combined high heritability and high genetic advance as percent mean expected. Furthermore, high broad sense heritability (H^2) and high GAM recorded in these characters could be explained by additive gene action. However, high estimates (H^2) combined with moderate GAM recorded for plant height, total tillers, productive tillers, flag leaf length, flag leaf width, panicle length, primary branches and secondary branches in a panicle could be due to non-additive gene effect. Grain number per panicle recorded positive correlation with primary branches per panicle (r=0.80191), secondary branches of panicle (r=0.81996) and flag leaf width (r=0.55683). The result indicated that there is a strong possibility that selection based upon primary/ secondary branches per panicle and flag leaf width could be highly effective for improved yield in case of rice.

Keywords: Rice, QTL, qGN4.1, Genetic variability, Broad sense of heritability (H^2), correlation.

Introduction

Rice is one of the most important staple foods and occupies a pivotal place among the cereal crops that feed billion people around the world (1, 2). Yield of paddy is a complex quantitative character controlled by many genes interacting together and with the environment. Selection of parents based on yield alone is often misleading (3, 4). Hence, the knowledge about the relationship between the yield and its contributing characters is required for efficient selection of evolving and economically better yielding varieties. The information about phenotypic and genotypic interactions of yield related traits is immensely important for selection and breeding of different genotypes with increasing yield potential. Grain number per panicle in any crop depends on many component characters which influence yield either jointly or singly and either directly or indirectly through other related characters (5, 6).

Selection for grain number per panicle on the basis of performance alone may not be as effective as that based on component characters associated with it, which is biometrically determined by correlation coefficient and path analysis. The correlation between characters may exist due to various reasons such as pleiotrophy, genetic linkage loci or block of loci governing variability for different characters located on the same chromosomes. The extent of observed relationship between the two characters is known as simple, total or phenotypic correlations (7, 8). Environmental correlation is the measure of environmental influence on the covariance between the two characters. An understanding of the nature and extent of association of the component characters with grain yield and amongst themselves is an essential prerequisite for formulating best breeding programs (9).

In a report published by United Nations, the world population is going to cross 8.5 billion marks by 2030 and 9.7 billion by the end of year 2050 and rice production must be increased by 50% in order to meet the growing demand (1). The development of new genotypes requires some knowledge about the genetic variability presents in the germplasm of the crop to build efficient breeding programme. The knowledge about genetic variability can help to know if these variations are heritable or not. The magnitude of variation due to heritable component is very important because, it would be a guide for selection of parents for crop improvement (11). Therefore, selection for high yield requires knowledge about genetic variability and good understanding of correlation between yield and yield components related to the genetic material of the variety. Genetic variability for agronomic traits is the key component of breeding programme for broadening the gene pool of rice (11).

Heritability estimates provide authentic information about a particular genetic attribute which will be transmitted to the successive generations and constitute an efficient guide for breeders in the choice of parents for crop improvement programmes (12). However, heritability in broad sense alone may not be helpful for selection based on phenotype, because it’s influenced by environment. Thus, estimate heritability along with genetic advance conjointly are reliable in predicting the gain under selection than heritability alone (13). It has been reported that grain yield is a complex trait, quantitative in nature and a combined function of a number of constituent traits (9). Consequently, selection for yield may not be satisfying without taking into consideration yield component traits. Thus, positives correlated between yield and yield components are requires for increasing grain yield in rice (13). So, it is important for plant breeders to understand the degree of correlation between yield and its components.

Knowledge of interrelationship between yield and its components is obvious for efficient selection of desirable segregants in plant breeding. Unlike the correlation coefficient values which measure the extent of relationship, path coefficient measures the magnitude of direct and indirect effects of characters on yield (7, 8). So, enable the breeders to judge about the important characters during selection

Therefore, the objective of the present study was to assess and evaluate genetic variability of rice near-isogenic lines (NILs) for high grain number per panicle and its recipient lines based on agro-morphological traits and to analyse the relationships between them.

Materials and Methods

The experiments were conducted in the open field condition of Indian Agricultural Research Institute (ICAR-IARI), Delhi (India) from June to November 2016-18. The site is located between 28.080°N and 77.120°E

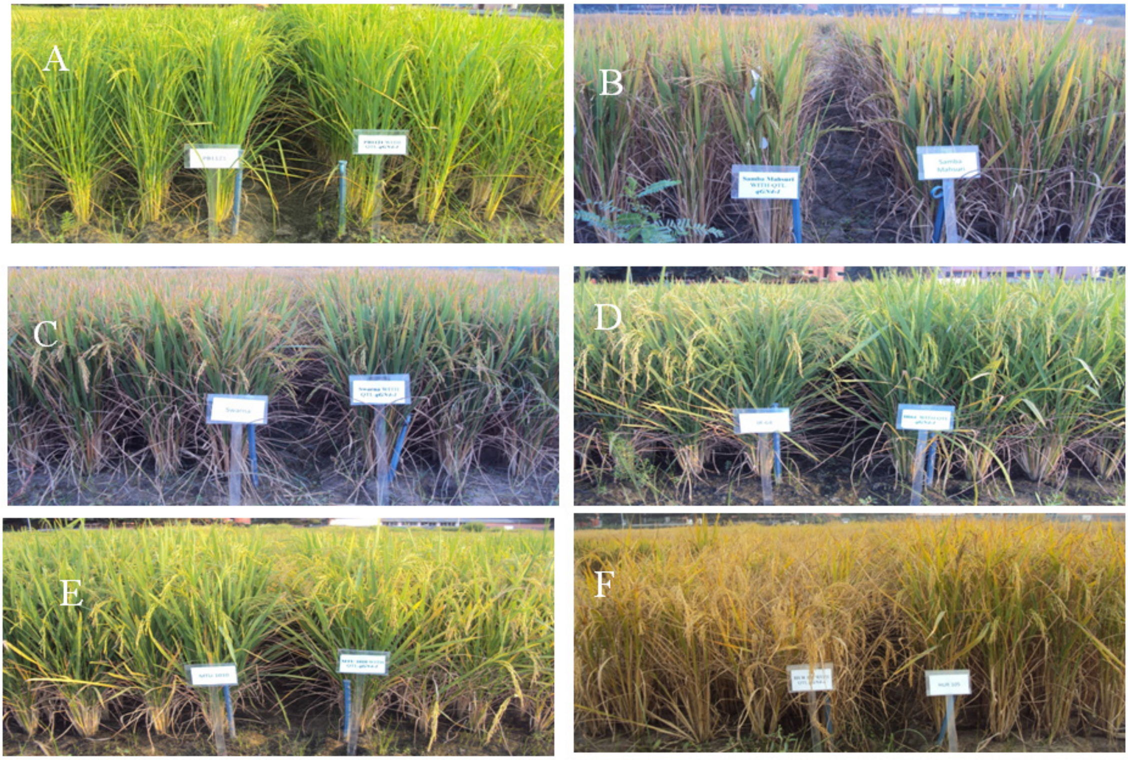

Fig.1. Plant architecture of qGN4.1 QTL-NILs (right side) of rice (*Oryza sativa*)as compare to their recipient parents (left side): (A) Pusa Basmati 1121 (PB 1121); (B)Samba Mahsuri; (C) Swarna; D) IR 64; (E) MTU 1010; (F) HUR 105.

at an altitude of 228.61 m above sea level. Experiments were conducted in open field condition using a randomized complete block design with two replications. Plant of each genotype was planted in 2 m rows in normal row-to-row distance of 30 cm and plant-to-plant spacing of 20 cm (Fig. 1; Fig. 2). The best five plant having most phenotypic similarity with recipient were used for determining plant height, flag leaf length, flag leaf width, counting tillers and grain number per panicle in the BC3F4 generation. A QTL with major effect on grain number per panicle, qGN4.1 was identified on the long arm of rice chromosome 4 in two recombinant inbred line (RIL) populations derived from a new plant type (NPT) Indica rice genotype Pusa 1266 (5, 6). Donors from the NPT source HG28 and HG67 were crossed with 12 different mega varieties of rice namely Pusa Basmati 1121, Samba Mahsuri, Swarna, IR 64, MTU 1010, HUR 105, Sarjoo 52, Pusa 44, CSR 30, Ranjit, CR 1009 and Pusa Basmati 1.

A total of 24 genotypes consisting of 12 recipient varieties along with its near-isogenic inbred lines alternately were planted. Phenotypic measurements were recorded on five plants per row. Thus, morphological and agronomical data were collected for 10 quantitative characters at appropriate growth stage of rice plant following the standard evaluation system indicated by IRRI. The characters that were evaluated included plant height (PH, cm), number of total tillers (TT), number of productive tillers (PT), Flag leaf length (FLL, cm), Flag leaf width (FLW, cm), Panicle Length (PL, cm), Filled grain per panicle (FG) and Total Grain per panicle (TG). The data recorded on 10 morphological and

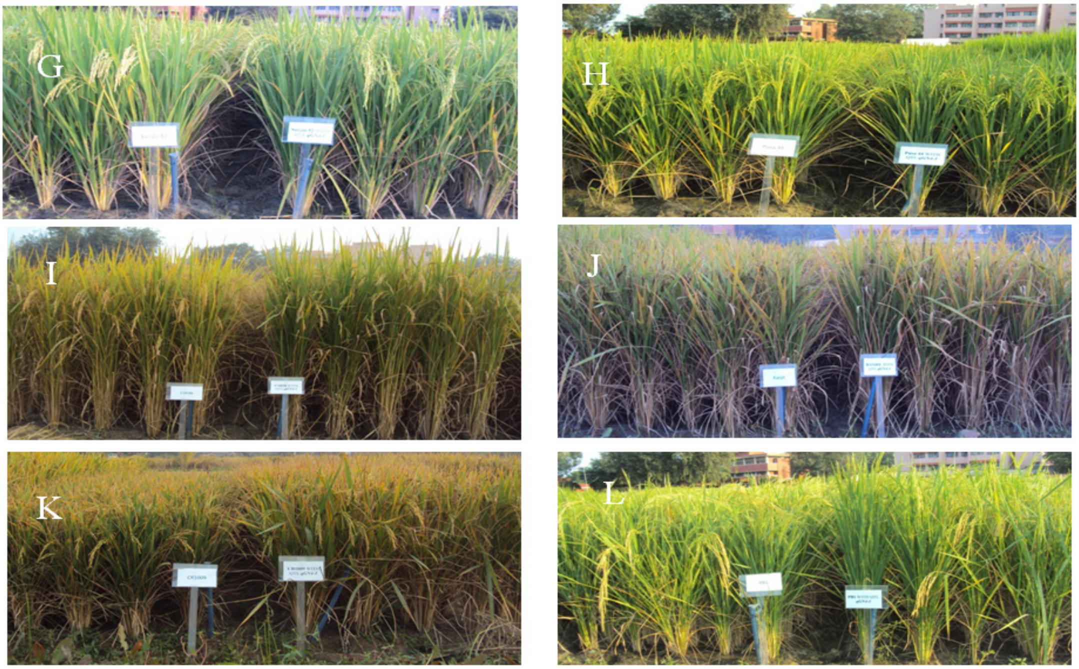

Fig. 2. Plant architecture of *qGN4.1* QTL-NILs (right side) of rice (*Oryza sativa*) as compare to their recipient parents (left side): **(G)** Sarjoo 52; **(H)** Pusa 44; **(I)** CSR 30; **(J)** Ranjit ; **(K)** CR 1009 ; **(F)** Pusa Basmati 1 (PB 1).

agronomical traits from the genotypes used, were subjected to statistical analysis. Analysis of variance (ANOVA) was carried out to access the genotype effect and their interaction using R program package. The correlation analysis was performed using the same software to determine the degree of correlation between yield and its components. In order to assess and quantify the genetic variability among the genotypes for the characters under study the variance components, values of heritability and genetic advance were estimated following the formula illustrated and validated by several scientific studies (14, 15, 16, 17).

Phenotypic, genotypic and environmental variances were estimated using the following formula:

- Environmental variance (Ve) = MSE/r

Where,

MSE: Mean squared error

r: No. of replications

- Genotypic variance (Vg) = (MSG - MSgl)/rl

Where,

Vg: Genotypic variance

MSG: Mean square of genotypes

MSgl: mean square of genotype by location interaction

l: No. of locations

- Phenotypic variance (Vp) = Vg + Vgl +Ve

Where,

Vg: Genotypic variance

Vgl: Genotype by location interactions

Ve: Environmental variance

Both genotypic and phenotypic coefficients of variability were estimated using the formula below:

- GCV= /X × 100
- PCV=/X × 100

Where,

GCV: Genotypic coefficient of variability

PCV: Phenotypic coefficient of variability

: Genotypic standard deviation

: Phenotypic standard deviation

X: General mean of the character.

- Heritability in broad sense (H^2) = Vg/ Vp
- Genetic advance (GA) = H^2k
- Genetic advances as per cent of mean (GAM) = (GA/X)100

Where,

k: Selection differential which is equal to 2.06 at 5% intensity of selection

Results

Analysis of variance

The results of combined analysis of variance for all the characters are summarized in Table 1. Significant effects of genotype were observed for all the characters under study. High significant effect of year for all the characters except the flag leaf width (FLW) was observed

Table 1. Mean square of combined analysis of variance for all the characters studies among twelve rice varieties and its NILs.

Parameters	Analysis of variance for different phenotypic characters under investigated									
	PH	**TT**	**PT**	**FLL**	**FLW**	**PL**	**PB**	**SB**	**FG**	**TG**
Replications	0.01	6.75	7.68	1.63	0.02	0.09	0.04	0.44	15.64	4.08
Genotype	334.9*	21*	16.4*	60.81*	0.26*	23.36*	12.65*	268.59*	14407.5*	14768.58*
Std. error	± 1.1	± 1.06	±0.9	±2.37	±0.01	±0.28	±0.56	±4.42	±7.34	±19.6

Table 2. Genotypic (Vg) and phenotypic variance (Vp), genotypic coefficient (GCV) and phenotypic coefficient of variance (PCV), broad sense heritability (H^2), genetic advance (GA) and genetic advance as per cent of mean (GAM) for all investigated traits.

Characters	Min	Max	Means	Vg	Vp	GCV	PCV	H^2 (%)	GA	GAM
PH	103.8	157.2	122.4	166.9	168.1	10.6	10.6	99.3	26.5	21.7
TT	9.4	23	14.2	10	11	22.2	23.3	90.3	6.2	43.4
PT	9.2	21.4	13.3	7.7	8.7	20.9	22.1	88.8	5.4	40.5
FLL	20.6	43.3	31	29.2	31.6	17.5	18.1	92.5	10.7	34.6
FLW	1.2	2.8	1.8	0.1	0.1	19.7	21.2	91.8	0.7	40.2
PL	19.9	33	25.8	11.5	11.8	13.2	13.3	97.6	6.9	26.8
PB	9.6	20.8	13.5	6	6.6	18.2	19	91.5	4.8	35.8
SB	32.4	88.8	57.7	132.1	136.5	19.9	20.3	96.8	23.3	40.4
FG	74.8	495	196.7	7200.1	7207.4	43.1	43.2	99.9	174.7	88.8
TG	96.6	515.2	219.9	7374.5	7394.1	39.1	39.1	99.7	176.7	80.4

from this analysis.

Data presented in mean (n = 24) and ± SE; Degree of freedom (df)= (n-1) = 23

Abbreviations: (**PH**: Plant height); (**TT**: Total numbers of tillers); (**PT**: Productive no. of tillers); (**FLL**: Flag leaf length); (**FLW**: Flag leaf width); (**PL**: Panicle length); (**PB**: Primary branches per panicle); (**SB**: Secondary branches per panicle); (**FG**: Filled grain per panicle); (**TG**: Total grain per panicle); (*: Significant at 1% level probability).

Estimate of genetic parameters

Estimates of genotypic (Vg) and phenotypic variances (Vp), genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), broad sense heritability (H^2), genetic advance (GA) and genetic advance as percentage of the mean (GAM) are shown in Table 2.

Abbreviations: (**PH**: Plant height); (**TT**: Total numbers of tillers); (**PT**: Productive no. of tillers); (**FLL**: Flag leaf length); (**FLW**: Flag leaf width); (**PL**: Panicle length); (**PB**: Primary branches per panicle); (**SB**: Secondary branches per panicle); (**FG**: Filled grain per panicle); (**TG**: Total grain per panicle).

High genotypic and phenotypic variances were recorded with filled grain, 7200.08 & 7207.42 and total grain per panicle 7374.45 & 7394.12, respectively. High genotypic and phenotypic variances were equally observed for plant height 166.93 & 168.06, and secondary branches per panicle 132.09 & 136.5, respectively. The low values of genotypic and phenotypic variances were observed with the characters total tillers (9.97 & 11.04), productive tillers (7.73 & 8.70), flag leaf length (29.22 & 31.59), flag leaf width (0.12 & 0.14), panicle length (11.54 & 11.82) and primary branches per panicle (6.04 & 6.60), respectively. In general, the phenotypic variances were higher than genotypic variances for all the characters.

Genotypic coefficients of variance (GCV) ranged from 10.56% for plant height to 43.13% for filled grain, whereas phenotypic coefficients of variance (PCV) ranged from 10.59% for plant height to 43.16% for filled grain. According to several research publications the PCV and GCV values more than 20% are regarded as high, whereas values less than 10% are considered to be low and values between 10 and 20% to be moderate (16). Based on this delineation, GCV and PCV values were moderate for plant height, FLL, PL and PB; high for TT, PT, FG and TG. The FLW and SB were recorded with moderate GCV and high PCV. In addition, PCV values were higher than theirs corresponding GCV values

Table 3. Correlation coefficients among agronomical and morphological characters studied in twelve rice varieties and its NILs.

Characters	Correlation coefficient for different agronomical and morphological characters under investigation								
	PH	**TT**	**PT**	**FLL**	**FLW**	**PL**	**PB**	**SB**	**FG**
PH									
TT	-0.2097								
PT	-0.13836	0.9752**							
FLL	0.67621**	-0.21367	-0.2087						
FLW	0.07222	-0.21515	-0.23151	0.18968					
PL	0.18125	-0.1256	-0.14493	0.38601	-0.28244				
PB	-0.34538	-0.11295	-0.15874	-0.27756	0.521768*	-0.32654			
SB	-0.41655	-0.12105	-0.18786	-0.17626	0.51614*	-0.19007	0.88367**		
FG	-0.21245	-0.24084	-0.22807	-0.0699	0.55683**	-0.12944	0.80191**	0.81996**	
TG	-0.22877	-0.23213	-0.22226	-0.08113	0.5575**	-0.14153	0.81223**	0.82062**	0.99791**

Table 4. Path coefficient of increase in grain number per panicle for contributing traits in twelve rice varieties and its NILs.

Charac-ters	Contributing trains for path coefficient of increase in grain number per panicle							
	PH	TT	PT	FLL	FLW	PL	PB	SB
PH	**-0.06318**	0.01325	0.008742	-0.04273	-0.00456	-0.01145	0.021822	0.026319
TT	0.318029	**-1.51656**	-1.47895	0.324047	0.326293	0.190479	0.171295	0.183575
PT	-0.20278	1.429238	**1.465584**	-0.30587	-0.3393	-0.21241	-0.23265	-0.27533
FLL	0.049499	-0.01564	-0.01528	**0.0732**	0.013885	0.028256	-0.02032	-0.0129
FLW	0.009595	-0.02858	-0.03076	0.025199	**0.132849**	-0.03752	0.069315	0.068568
PL	0.024286	-0.01683	-0.01942	0.051723	-0.03785	**0.133994**	-0.04375	-0.02547
PB	-0.12685	-0.04149	-0.0583	-0.10194	0.191633	-0.11993	**0.367284**	0.324557
SB	-0.22104	-0.06423	-0.09969	-0.09353	0.273885	-0.10086	0.468914	**0.530645**

for all the characters considered. However, this difference was low for all the characters except the PT and FLW.

Heritability analyses estimate the relative contributions of differences in genetic and non-genetic factors to the total phenotypic variance in a population. It is an important concept in quantitative genetics, particularly in selective breeding. The heritability in broad sense (H^2) estimate varied from 88.83% to 99.90%, respectively for PT and FG. All the characters studies had high heritability (>85%). This result indicates that these characters could be easily improved by selection. Genetic advance (GA) under selection refers to the improvement of characters in genotypic value for the new population compared with the base population under one cycle of selection at a given selection intensity (19). The high value of GA was recorded with total grain (176.7) and the low (0.7) with Flag leaf width.

Genetic advance as per cent mean expected (GAM) had a general range between 21.7% for PH and 88.8% for FG. Among the characters high values of GAM (>20%) were recorded for all the character studied in this case. Filled grain and total grain per panicle had high heritability and high GAM, whereas plant height, total number of tillers, productive tillers, flag leaf length and width, panicle length, primary and secondary branches in a panicle had high heritability but moderate genetic advance as percent mean expected.

Abbreviations: (**PH**: Plant height); (**TT**: Total numbers of tillers); (**PT**: Productive no. of tillers); (**FLL**: Flag leaf length); (**FLW**: Flag leaf width); (**PL**: Panicle length); (**PB**: Primary branches per panicle); (**SB**: Secondary branches per panicle); (**FG**: Filled grain per panicle); (**TG**: Total grain per panicle); (*: Significant at 5% level probability; **: Significance at 1% level probability).

Correlation

The degree of correlation between the traits is important in plant breeding. It can be used as tool for indirect selection. Correlation studies help the plant breeder during selection and provide the understanding of yield components. The results of correlation analysis are showed in Table 3, it reveals that there was positive and highly significant correlation between filled grain per panicle and total number of grain per panicle (r=0.99791), total number of tillers with productive tillers (r=0.9752), primary branches of panicle with secondary branches in a panicle (r=0.88367), secondary branches in a panicle with total grain (r=0.82062), panicle length with total grain (r=.81223) and filled grain (r=0.80191), plant height with flag leaf length (r=0.67621), flag leaf width with primary branches in a panicle (r=0.52176), secondary branches in a panicle (r=0.51614), filled grain (r=0.55683) and total number of grain in a panicle (r=0.5575). The characters which had positive relationship with plant height were flag leaf width (r=0.07222) and panicle length (r=0.18125), flag leaf length with flag leaf width (r=0.18968) and panicle length (r=0.38601). Among these characters only flag leaf width, primary and secondary branches in a panicle have significant positive correlation with grain number per panicle in a plant. On the contrary plant height (r=-0.21245), total tillers (r=-0.24048), number of productive tiller (r=-0.22807), flag leaf length (r= -0.0699) and panicle length (r=-0.12944) were inversely related. Plant height had negative correlation with all the characters except flag leaf length, flag leaf width and panicle length. Similarly flag leaf length had negative correlation with almost all the characters except flag leaf width and panicle length.

Residual effect (0.17); Diagonal & bold indicates direct effects

Abbreviations: (**PH**: Plant height); (**TT**: Total numbers of tillers); (**PT**: Productive no. of tillers); (**FLL**: Flag leaf length); (**FLW**: Flag leaf width); (**PL**: Panicle length); (**PB**: Primary branches per panicle); (**SB**: Secondary branches per panicle).

Path coefficient analysis

Direct and indirect effect of different characters on grain number at phenotypic level is given in Table 4. From the path coefficient analysis, the highest positive and direct effect on grain yield was exhibited by number of productive tillers (1.4656) followed by secondary branches in a panicle (0.530645), primary branches in a panicle (0.3673), panicle length (0.1340), flag leaf width (0.1328) and flag leaf length (0.0732) indicating these are the main contributors to yield. Negative direct effect was recorded by total number of tillers in a plant (-1.5166) and plant height (-0.06318). The residual effect of 0.17 is low since the traits contributed more to the variability in grain yield of rice.

Discussion

Genetic diversity in plant breeding is aimed for crop improvisation. More genetic variability in population leads to high survival and productivity. In the present study, results from ANOVA showed highly significant difference among the genotypes for all the characters, indicating huge genetic variability among the genotypes. So, the parents used for crossing were genetically different.

The current study suggests that phenotypic variance (Vp) and phenotypic coefficient variance (PCV) were higher than theirs corresponding genotypic variance (Vg) and genotypic coefficient of variance (GCV) respectively for all the characters studied, indicating that the expression of these characters were influenced by environment. Similar results have been reported by several independent research works carried out on rice (11, 16, 20). It is interesting to note that this difference was low for all the characters except filled grain and total grain per panicle, indicating that these characters were less influenced by environment. It also suggests that selection based on these characters would be effective for further crossing. However, the other traits like flag leaf width and number of productive tillers which showed a higher difference between phenotypic and genotypic coefficient indicated that environmental effect on the expression of these traits was higher.

The most important function of the heritability in the genetic study of quantitative characters is its predictive role to indicate the reliability of the phenotypic value as a guide to breeding value (3, 4). High heritability estimates for all the character indicated a high response to selection in these traits which is in line with the research findings of several research workers (4, 11, 21). Heritability in broad sense and the genetic advance are also important selection parameters. It is more useful as a selection tool when considered jointly with heritability. The estimates of genetic advance can help to understand the type of gene action underlining various polygenic characters. It has been suggested that high heritability estimates along with the high genetic advance is more helpful in predicting gain under selection than heritability estimates alone (15). Thus, the heritability estimates will be reliable if accompanied by high genetic advance. The present study revealed high heritability accompanied with high genetic advance as percent of the mean for total grain and filled grain per panicle; high heritability and moderate genetic advance as percent of the mean for plant height, total number of tillers, productive tillers, flag leaf length and width, panicle length, primary and secondary branches in a panicle. These results could be explained by additive gene action and their selection may be done in early generations.

Selection of traits contributing simultaneously to a character will improve it in subsequent segregation population (22). Thus, the correlation analysis is therefore necessary to determine the direction of selection and the numbers of characteristics needed to be considered in improving any character such as grain number per panicle. The present study showed that there was a highly significant correlation between grain number per plant with flag leaf width, primary and secondary branches per panicle at 1% level indicating that simultaneous selection for these characters would result in improvement of yield. Also, plant height exhibits a significant positive correlation with flag leaf length. Plant height has not been significantly correlated with yield per plant, which was in accordance to the study of several research workers (21, 23), while others have obtained contrasting results (9, 24) of negative correlation between plant height and yield per plant.

Conclusion

The present study highlighted the existence of diversity among the 12 rice near-isogenic lines and their parents as check variety. High heritability in broad sense recorded for plant height, total tillers, productive tillers per plant, flag leaf length, flag leaf width, panicle length, primary branches in a panicle, secondary branches per panicle, filled grain and total grain per panicle demonstrates that these characters could be successfully transferred to offspring's if their selection is performed in hybridization programme. The correlation analysis revealed that agronomical traits such as filled grain per panicle with total number of grain per panicle, total number of tillers with productive tillers, primary branches of panicle with secondary branches in a panicle, secondary branches in a panicle with total grain, panicle length with total grain and filled grain, plant height with flag leaf length, flag leaf width with primary branches in a panicle, secondary branches in a panicle, filled grain and total number of grain in a panicle have positive contribution to grain number for plant. Among these characters, filled grain and total grain showed significant correlation with grain yield. So, these two traits may be considered as the selection criteria for the improvement of grain yield in rice.

Conflict of Interests

The authors declare to have no conflict of interest among them.

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