Estimating Genetic Variability and Diversity for Vein Density, Photosynthesis and Yield in Rice Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Understanding the physiological and leaf anatomical traits having positive association with higher yields is critical to utilize these traits effectively in breeding programs for yield improvements in stable food crops. In rice, this would help in improving the productivity to feed the ever growing population. Therefore, the experiment was conducted to evaluate the variability and diversity of ninety-nine rice genotypes for photosynthesis, vein-related characters, and yield. The field trial was carried out in a wetland in Tamil Nadu Agricultural University, Coimbatore during Rabi, 2020. To investigate the genetic variability, efforts were made to estimate the percent of genotypic and phenotypic variation, heritability, and genetic advance likewise genetic diversity was estimated for twelve traits using Mahalanobis (D^2) statistics. Analysis variance elucidates the existence of significant variation among the genotypes for all the twelve characters studied. The difference between GCV and PCV was narrow for all the characters studied which shows these characters are less influenced by environmental factors. The highest GVC and PCV was observed in yield and biomass simultaneously the high heritability in broad sense along with high genetic advance as percent mean was estimated in biomass, photosynthetic rate, yield, interveinal distance between major vein, leaf width, total number of veins, and total number of minor veins. These characters are

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considered to be inherent in nature and selection for improving these traits may be effective in the genetic improvement program. Based on the D^2 value ninety-nine genotypes were diversified into five clusters in which Cluster II is the largest containing 37 genotypes followed by cluster I. The highest inter-cluster distance was estimated between cluster II and cluster IV whereas the highest intracluster distance was observed in cluster V indicating that genotypes in this cluster are highly diversified. Subsequently, the higher cluster means of most of the characters including vein characters, leaf width, and photosynthetic rate along with optimum yield and biomass fell in cluster II. Whereas in cluster IV the highest cluster means for biomass and yield was recorded. The traits biomass, yield, photosynthetic rate, interveinal distance between major veins, the total number of minor vein is the major contributors of total divergence in this population. Based on the trait contribution percentage for genetic diversity and genetic distance this study suggests that the genotypes from cluster II and cluster IV can be selected as parents for genetic improvement programs. Hence, information on the nature and degree of divergence in this population would help the plant breeder in the selection and hybridization procedure for choosing the right type of parents to improve vein characters, photosynthetic rate, yield, and biomass.

1. INTRODUCTION

Rice is a leading food crop in the world. It is the most staple food for low and lower middle income countries, extended equally across all the income classes. Rice provides nearly 20% of daily calories and 13% dietary protein for more than 3.5 billion human population. Rice is an important cereal crop cultivated across 164.19 million hectares worldwide whereas in India rice occupies an area of 43.78 million hectares with an average production of 118.4 million tonnes with a productivity of 2.98 tonnes per hectare (www.agricoop.nic.in). In addition, the human population is expected to reach 9.1 billion by the year 2050, i.e., 34% higher than today and most of this population will occur in developing countries. Adding to this, per capita consumption in daily diet has increased as a result of economic growth in the world also creates greater demand for food grain but the reality is the growth rate of cereal grain has reduced from 3.2% to 1.5 % since 1960's and 1970's [1]. The yields of major crops including rice have risen by around two-fold since the green revolution years through genetic improvement, management practice, control of pests and diseases, and a greatly increased fertilizer application rate. Consequently, slowing down of compound yield growth is due to the optimization of mechanisms used to improve rice yield potential in the past, such as canopy architecture and Harvest Index (HI). However, it is predicted that further improvements will be required on the existing land area to meet the demand of future populations.

Evidence suggests that to meet the future food demand plant scientist have to improve the photosynthetic efficiency since it is the primary source for yield and biomass production of the crop plants. Therefore identifying the relationship of photosynthetic rate together with leaf anatomical traits is ideal for crop improvement since high-yielding varieties arise from high photoassimilate production and partitioning. In addition, vein density serves as a channel for distribution of photoassimilates, water and nutrients [2]. Moreover studies reports that vein densities are highly correlated with yield [3] and photosynthetic rate [4]. Photosynthesis can be improved by increased vein density. Increased leaf vein density is an essential requirement for C4 photosynthesis. It has been identified as a new trait for introducing C4 photosynthesis into C3 crops [5]. Before planning for the plant breeding programs it is essential to owe knowledge on the magnitude of variability available in the existing population. Thus helps to encounter the donors for leaf vein density and photosynthetic rate for genetic mapping and yield improvement. Generally, genetic diversity is a principal source for the identification of ideal parents or desirable characters for the development of superior recombinants. Moreover, very little attention has been paid to quantifying the diversity of leaf vein traits and photosynthetic rate together with yield. Hence, it is hypothesized that large genetic variability in terms of vein density and photosynthetic rate in association with yield may exist in unexploited rice germplasm. This study was undertaken in diverse germplasm of association mapping panel that comprises of landraces, varieties, exotic genotypes, and improved genotypes that are

collected from 13 countries. Therefore it helps to identify the appropriate parent for genetic crop improvement or plant breeding programs in rice.

2. MATERIALS AND METHODS

The present investigation was carried out during Rabi 2020 with a set of diverse genotypes containing 98 rice accessions. All the accessions belong to an association mapping panel which are collected from different countries like Argentina, Bangladesh, Brazil, Bulgaria, Uruguay, China, Indonesia, Philippines, Colombia, Taiwan, Venezuela, and the US including India. The experiment was conducted in a randomized block design (RBD) with three replications in wetland, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. All the genotypes were sown in a raised bed and transplanted 14days old seedlings in the field with a spacing of 25cm between rows and plants. The crop was maintained with a standard package of practices recommended by TNAU. The observations on 12 quantitative traits were documented on the 5th leaf of primary tiller from randomly selected plants of each replication. The data recorded in 12 traits are photosynthetic rate, leaf width, leaf thickness, the total number of veins, major and minor vein width, the interveinal distance between major and minor vein, yield, and biomass.

The data obtained from each replication of the genotypes were subjected to basic statistical analysis that gives maximum, minimum, mean, standard deviation, and analysis of variance (ANOVA) using SPSS Statistics version 16.0 (SPSS Inc., Chicago, Ill., USA). The genetic parameters like genotypic variance, phenotypic variance, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), genetic heritability, genetic advance were calculated by the procedure given by Burton and De Vane (1953) and Johnson et al. [6]. The formulas for these genetic parameters were given below

i) Genetic variance (GV)

$$\frac{\text{(Mean square of genotypes-Mean square of error)}}{\text{Number of replication}}$$

ii) Phenotypic variance (PV)

Genetic variance + Mean squares of error

iii) Phenotypic coefficient of variance (PCV %)

$$\frac{\sqrt{PV}}{\text{Mean of trait}} \times 100$$

iv) Genotypic coefficient of variance (GCV %)

$$\frac{\sqrt{GV}}{\text{Mean of trait}} \times 100$$

v) Heritability (broad sense)

$$\frac{\text{Genotypic variance}}{\text{Phenotypic variance}}$$

vi) Genetic advance (GA %)

K × PV × Heritability × 100

While genetic diversity among 98 genotypes was quantified by Mahalanobis D^2 statistics and genotypes were grouped into clusters by following the procedure given by Rao (1952).

3. RESULTS AND DISCUSSION

The analysis of variance showed significant differences among the genotypes for all twelve characters indicating the existence of genetic variability in the population studied (Table 1.). In the current study, the maximum range of variation was observed in yield (4.40-82.16), biomass (20.77-93.37), total number of minor veins (25.67-68.67), total number of veins (37.33-79.67), interveinal distance between major vein (33.09-73.77) and photosynthetic rate (13.39-36) (Table. 2). Priyanka et al. [7] has also recorded high variability for yield per plant and Jahn et al. [8] reported similar photosynthetic variation in diverse rice varieties. Similar variability in vein characters was reported by Nawarathna et al. [3]. The range and mean value of all the characters reveal the possibility of diversity among ninety-nine genotypes. Such magnitude of diversity in the population gives the scope for further improvement in the crop and this population could be a source for the donor of desirable traits.

In the current study, the phenotypic coefficient of variation (PCV) has ranged from 9.85 to 38.74 % and the genotypic coefficient of variation (GCV) has ranged from 6.70 to 36.25 % (Table2). The result shows that PCV is higher than the GCV

(Table 2). However, the difference between PCV and GCV is narrow indicating that the variation is due to the inherent nature of the genotypes and negligible influence of environmental factors. Less difference between PCV and GCV was recorded in all the characters studied suggesting the minimal influence of environmental factors. Therefore the selection of genotypes based on the phenotypic performance of all the characters studied is effective in genetic improvement. This result was in agreement with Praveen et al. [9] for yield and biomass. According to the GCV and PCV classification given by Sivasubramanian and Madhavamenon [10], the highest genotypic and phenotypic coefficient of variation was recorded in yield (33.33% and 38.74%) and biomass (36.25 and 36.67%). Similar results were reported by Bupesh Kumar et al. [11] for yield and Abebe et al. [12] for biomass. While the lowest GCV and PCV were observed in the total number of major veins (6.70 and 9.85%) and interveinal distance between minor veins (7.73 and 9.89% respectively) this is due to the variation among all the genotypes for the total number of major veins (6.67 - 12.67) and interveinal distance between minor veins (13.57 - 22.27) were low (Table 2).

Heritability describes the reliability of the character to the environmental factors. According to Johnson et al. (1955) heritability was categorized into 3 classes as low (<30%), medium (30-60 %), and high (>60 %). Based on this characterization, heritability in a broad sense was recorded in all twelve characters. High heritability values were recorded in almost all the characters (ranging from 61.17 of Interveinal distance between minor veins to 97.52% for biomass) except the total number of major veins (46.24%) and leaf thickness (58.47%) that comes under the category of medium heritability (Table 2). Therefore these characters are considered to be less influenced by environmental factors. This result provides information about the phenotypic performance of these twelve characters to the environmental factors for the better selection of the genotypes from this population.

Heritability estimate alone is not sufficient to be effective for selection programs. Therefore, heritability along with genetic advance predicts the information about the genetic gain after the selection on the trait of interest. In the present study, the genetic advance was ranged from 0.27 % of leaf width to 35.73% for

biomass. Moreover, genetic advance as a percent mean gives more accurate information than genetic advance. According to Johnson et al. [6], genetic advance as percent mean was classified into low (<10%), medium (10-20%), and high (>20%). In the present study genetic advance as percent mean among 12 characters has ranged from 9.38 % (total number of major vein) to 73.75% (biomass) (Table 2).

The highest genetic advance as percent mean was recorded in biomass, yield, interveinal distance between major vein, photosynthetic rate, total number of minor veins, leaf width, and the total number of veins while the estimates on moderate genetic advance as percent mean was recorded in minor vein width, major vein width, interveinal distance between minor vein and leaf thickness (Table.2).The above traits also fell into the category of high and moderate heritability. Shrivastava et al. [10] recorded similar results on high heritability with high genetic advance as percent mean in yield and biomass and Acevedo-Siaca et al. [13] for the photosynthetic trait. In this research, leaf architecture characters like total number of veins, total number of minor veins, interveinal distance between major vein and leaf width show high heritability along with high genetic advance as percent mean. These traits are considered as principle plant traits in maintaining hydraulic conductivity and photo-assimilate transport for the normal functioning of plants. Generally, rice leaves are diverse in leaf architecture and have strong genetic control over variation [14]. Therefore these characters are found to be effective for the selection process for genetic improvement.

In the present study, D^2 statistics was performed among ninety-nine genotypes, and based on the level of genetic distance genotypes were classified into five clusters by using the Tochers method. The clustering of ninety-nine genotypes into five groups was included in Table 3. The maximum number of genotypes was grouped into Cluster II with 37 genotypes and cluster I with 29 genotypes. Compared to other clusters size, cluster III is the smallest containing 2 genotypes while cluster IV has 18 genotypes and cluster V has 13 genotypes. These results reveal that the genotypes with closer genetic distance/ association fall on the same clusters which are less divergent and the genotypes with diverse distance fall on different clusters.

Table 1. Analysis of Variance for 12 characters in rice genotypes

Mean Sum of Squares													
Source of variation	df	Total Major vein	Total Minor vein	Total number of vein	Major vein width	Minor vein width	IVD b/w Major vein	IVD b/w Minor vein	Leaf width	Leaf thickness	Pn	Yield	Biomass
Replication	2	0.15	11.58	10.47	0.33	0.08	87.36	2.90	0.01	1.71	2.15	46.66	8.51
Genotypes	98	1.77*	121.50*	141.07*	1.21*	0.32*	196.74*	6.14*	0.08*	4.53*	46.96*	600.92*	933.25*
Error	196	0.50	12.84	14.91	0.19	0.05	12.47	1.07	0.01	0.87	2.88	62.92	7.88
Mean Sum of Squares													
Source of variation	df	Total Major vein	Total Minor vein	Total number of vein	Major vein width	Minor vein width	IVD b/w Major vein	IVD b/w Minor vein	Leaf width	Leaf thickness	Pn	Yield	Biomass
Replication	2	0.15	11.58	10.47	0.33	0.08	87.36	2.90	0.01	1.71	2.15	46.66	8.51
Genotypes	98	1.77*	121.50*	141.07*	1.21*	0.32*	196.74*	6.14*	0.08*	4.53*	46.96*	600.92*	933.25*
Error	196	0.50	12.84	14.91	0.19	0.05	12.47	1.07	0.01	0.87	2.88	62.92	7.88

*Significant at 5% level; df = Degrees of freedom
IVD b/w Major vein= Interveinal distance between major vein; IVD b/w Minor vein= Interveinal distance between minor vein; Pn= Photosynthetic rate

Table 2. Estimation of genetic variability parameters for leaf anatomical traits, photosynthetic rate, yield and biomass

Character	Mean	Range	Genotypic Variance	Phenotypic variance	GCV%	PCV%	Heredity in broad sense	Genetic advance	Genetic advance as % mean
Total Number of Major Vein	9.71	6.67 - 12.67	0.43	0.92	6.70	9.85	46.24	0.914	9.38
Total Number of Minor Vein	40.01	25.67 - 68.67	36.22	49.06	15.00	17.46	73.82	10.65	26.54
Total Number of Veins	49.88	37.33 - 79.67	42.05	56.96	13.00	15.13	73.83	11.48	23.02
Major Vein Width	7.00	4.84 - 9.03	0.34	0.53	8.37	10.42	64.92	0.97	13.87
Minor Vein Width	3.28	2.41 - 4.30	0.11	0.11	9.33	11.26	66.41	0.67	15.66
Interveinal Distance between Major Vein	45.70	33.09 - 73.77	61.41	73.90	17.15	18.81	83.12	14.72	32.21
Interveinal Distance between Minor Vein	16.82	13.57 - 22.27	1.69	2.76	7.73	9.89	61.17	2.10	12.46
Leaf Width	1.12	0.80 - 1.60	0.025	0.03	13.91	16.36	72.45	0.27	24.53
Leaf Thickness	14.44	11.67 - 17.74	1.22	2.09	7.65	10.10	58.47	1.74	12.05
Photosynthetic rate	25.67	13.39 – 36.00	14.69	17.57	14.96	16.35	83.62	7.23	28.17

Table 3. Clustering of 99 rice genotypes into five clusters by Mahalanobis D2 statistics

Cluster	Genotypes	No of genotypes
Cluster I	G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, G16, G17, G18, G19, G20, G21, G22, G23, G24, G25, G26, G27, G116, G132	29
Cluster II	G28, 29, G30, G31, G32, G33, G34,G35, G36, G37, G38, G39, G40, G41, G42, G43, G44, G45, G46, G47, G48, G49, G50, G71, G72, G74, G76, G83, G85, G87, G88, G91, G93, G94, G95, G118, G126	37
Cluster III	G134, G137	2
Cluster IV	G96, G97, G98, G100, G101, G111, G112, G113, G114, G115, G117, G119, G120, G121, G122, G123, G124, 135	18
Cluster V	G125, G127,G128, G129, G130, G131, G133, G136, G138, G139, G140, G141, G147	13

Table 4. Inter and Intra-Cluster Distance (bold) among Five Clusters of Rice Genotypes

	I	II	III	IV	V
I	9.51	11.26	7.78	12.26	11.52
II		11.01	9.30	12.52	11.95
III			3.00	11.120	9.47
IV				11.842	12.32
V					12.29

Inter-cluster and Intra-cluster distance D value arranged in diagonal and bold

Table 5. Cluster mean values of twelve characters of 99 rice genotypes

Cluster	Total Major vein	Total Minor vein	Total number of vein	Major vein width	Minor vein width	IVD b/w Major vein	IVD b/w Minor vein	Leaf width	Leaf thickness	Pn	Yield	Biomass
Cluster I	9.644	37.425	47.069	6.854	3.147	41.469	16.400	1.053	14.625	24.323	48.068	42.185
Cluster II	9.973	44.559	54.532	7.211	3.458	52.067	17.297	1.228	13.927	27.087	37.892	48.249
Cluster III	8.833	35.667	44.500	6.707	2.905	41.763	16.258	0.983	14.873	28.647	32.688	40.780
Cluster IV	9.537	38.815	48.352	6.850	3.268	43.178	16.637	1.076	14.913	23.489	39.501	58.469
Cluster V	9.718	36.051	45.769	6.961	3.156	41.149	16.721	1.044	14.752	26.930	31.178	50.444

IVD b/w Major vein = Interveinal distance between major vein; IVD b/w Minor vein = Interveinal distance between minor vein; Pn = Photosynthetic rate

Table 6. Percent contribution of twelve characters for total divergence in 99 rice genotypes

S.No	Character	Contribution %
1	Total Number of Major Vein	0.8452
2	Total Number of Minor Vein	2.0614
3	Total Number of Veins	0.0000
4	Major Vein Width	1.0513
5	Minor Vein Width	1.6698
6	Interveinal Distance between Major Vein	4.2672
7	Interveinal Distance between Minor Vein	0.2268
8	Leaf Width	1.1132
9	Leaf Thickness	0.7215
10	Photosynthetic rate	6.2461
11	Yield	10.5133
12	Biomass	71.2843

Inter and intracluster distance were computed and included in Table 4. In this investigation, the intracluster distance ranges from 3.004 to 12.290. The highest intracluster distance was recorded in cluster V with a D value of 12.290 followed by cluster IV with a D value of 11.842. This result indicates the presence of genetic diversity among the genotypes in cluster V and also in cluster IV. While the minimum intracluster distance was observed in Cluster III with 3.004 which intimates the closer relationship among the genotypes in this cluster. The highest inter-cluster distance was observed between cluster IV and cluster II (12.52) followed by cluster V and cluster IV (12.32) (Table 4). This result indicates the existence of greater genetic divergence between the genotype in these clusters. From the present study, the result infers that the selection of parents from these diverse clusters (IV &II; V & IV) for hybridization programs would give maximum heterogeneity in the segregating population. Chaturvedi and Maurya [15] recommend selecting the parent from the clusters with high inter-cluster distance to get more variability and heterotic effect in the segregating generation. In contrast, the lower inter-cluster distance was noticed between cluster III and I (3.00) which suggests that the genotypes in these clusters are relatively closer than the other clusters. Therefore the selection of parents from these homogenous clusters for crossing programs may be avoided.

Analysis of cluster means for 12 characters were included in Table 5. The maximum cluster means for leaf anatomical traits like total number of major veins, the total number of minor veins, total number of veins, interveinal distance between major vein and interveinal distance between minor vein, major vein width, minor vein width, leaf width falls on the same cluster II which has optimum yield and biomass. However, leaf thickness, photosynthetic rate, yield, and biomass are relatively distributed. Cluster IV has recorded the highest cluster mean value for leaf thickness and biomass but lower cluster means for photosynthesis. This result reveals that the genotypes with higher leaf thickness would have higher biomass because the volume of leaf tissues and cell wall thickness will be more for these leaves. Though leaf thickness is an important constituent of leaf mass but a higher volume of leaf tissues and thickened cell wall causes resistance to diffusion of CO_2 that would result in reduced photosynthetic rate. Similar findings were reported by Zang et al. (2020).

In the case of yield per plant cluster, I possess a higher cluster mean of 48.068 followed by cluster IV with 39.501, and cluster III was observed to have a higher cluster for photosynthetic rate and lower cluster mean for minor veins, the total number of veins, interveinal distance between minor vein, major vein width, minor vein width, and leaf width. These results indicate that the genotype in this cluster has narrow leaf width, as a result of that, the vein counts, interveinal distance between minor veins, and width of the veins were low. A significant reduction in the number of minor and major veins for narrow leaves was documented by Kubo et al. [16]. Whereas in the case of cluster V minimum cluster mean of interveinal distance between major vein (41.149) and yield (31.178) was noticed. These findings serve as a diverse source for selecting the desirable genotypes for hybridization programs.

Percent contribution of individual characters of ninety-nine genotypes for total diversity is given in Table 6. Among the traits studied, biomass has the highest percent (71.2843%) of contribution for total divergence followed by yield per plant (10.5133%), photosynthetic rate (6.2461%), the interveinal distance between

major vein (4.2672%), and the total number of minor vein (2.06145). whereas other traits like the total number of veins, interveinal distance between minor veins, leaf thickness, and the total number of major veins have contributed least to total genetic divergence among ninety-nine genotypes [17-20].

4. CONCLUSION

In this study considering the genetic distance and relative contribution of individual traits to the genetic diversity the genotypes in cluster II and cluster IV can be utilized for an effective crossing program to get a high heterotic effect on segregating population. These clusters have major contribution factors of divergence and the highest cluster mean for vein characters together with high photosynthetic rate and yield. It is well known that crosses between divergent parents usually produce greater heterotic effect than crosses between closely related ones. Considering the importance of genetic distance and relative contribution of characters towards total divergence, the present study indicated that genotypes in clusters II and IV could be selected for photosynthetic rate, vein characters, yield, and biomass could be used in crossing programmes to identify desirable segregants for isolating improved rice cultures with enhanced productivity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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