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MULTIVARIATE ANALYSIS OF RICE GENOTYPES FOR SEEDING TRAITS' CHARACTERIZATION AND EVALUATION

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Abstract

Multivariate analysis has been rarely used for seedling traits characterization of Chinese basmati hybrids. Twenty-two seedling attributes of Basmati and coarse rice germplasm of China and Pakistan origin were analysed using multivariate techniques of Single linkage analysis (SLCA) and Principle component analysis (PCA) and index score method (ISM). Variation among the seedling attributes were recorded by ISM regarding number of genotypes, formation of clusters and superimposition of genotypes in every cluster. Rice genotypes were possibly grouped in six clusters identified through ISM. Highest index scores 68 and 146 were allocated to genotypes of group-I and group-II on morphological basis of seedling attributes. However, the three PCs contributed 87.1 % of the variability among the genotypes and germination rate index, germination %, mean daily germination (MDG), shoot length and root length exhibited maximum positive response in PC1, PC2 and PC3 respectively. PCA ordination on axis I and II were agreed with SLCA. Our study revealed that the agreement in the SLCA and PCA analysis effectively cluster the genotypes for twelve seedlings attribute might be used for the identification of viable genetic material to improve the rice yield potential.

Key words: Index score; Single linkage analysis; Principle component analysis; Seedling traits; Rice.

Introduction

Rice (*Oryza sativa* L.) is not only the staple food of over half of the global production but also the second most widely cultivated cereal over the world. The largest rice producer is China yielding 34.6% of the total grain production with 30% of planting area of the grain crops and accounts for 26% of world's rice production (Shen & Zhang, 2006). The average rice grain yield in China is over 6.3 t/ha that's much higher than the world average 3.7 t /ha. With the new concept of transferring C4 genre from maize into super hybrid rice, the yield potential become 7.35 t/ha in China (Yuan *et al*., 2011). In Pakistan, rice is the major source of exchequer in recent years and accounts about 2.7% of value added and 0.6% of GDP with production of 7,005 tons over estimated area of 2,311 thousand hectares. Among 61 rice producing nations, Pakistan occupies 30th position due to lower yield per unit area as compared to other nations (Safdar *et al*., 2013). Over the globe about 90% of rice is produced and consumed by the small farmers of the developing countries. Due to intense expansion in population, the global rice demand is incessantly growing. To combat with ever increasing population and to gain self-sufficiency in rice production and preserving rice permanence, there is a need for improved genetic recombinants and new genes, either found out in the cultivated varieties or their descendants (Rashid *et al*., 2008). For rice improvement program creation of genetic variability for desired traits is an essential prerequisite. Pakistan rice yield remains lower than many other countries in spite of advances in rice production. Lack of high yielding varieties, a decline in land resources and water scarcity are the main attributes in stagnant yield (Masood *et al*., 2013). Genetic improvement, 62-74% is the main contributors in the hybrid rice yield increment along nutrient application and temperature in China (Wang & Lu, 2006). One of the preeminent preferences to achieve the 20-25% higher yield than pure line varieties is the adoption of hybrid rice technology specifically in the provinces of Pakistan like Punjab, Sindh and Balochistan (Bashir *et al*., 2007). Hybrid rice can be cultivated over an area of 1 million ha, which accounts 40% of the total rice area. In 2007 the hybrid rice covered area improved gradually to 63,000 ha as compared to 8.3% of non-basmati varieties of 400 ha in 2002 (Akhter *et al*., 2007). The rice area projected 4.45 million acre and production was expected to 7.4 million meteric tons in 2019-2020 (Wagan *et al*., 2015). Hybric rice get especially done well in Sindh increased from 35% to 60 % over past few years (Casey Bean, 2019).

The timely sowing of Rabi crops ensured the early maturity of hybrid rice. In the late season hybrid rice can also be planted due to shorter maturity period. Traditionally rice varieties require a greater number of irrigations as compared to hybrid rice. Moreover, hybrid rice can be successfully grown under stressful environment, such as saline, drought and waterlogging (Huang *et al*., 2012).

Multivariate statistical (MS) techniques are being used for summarizing and describing the genetic diversity whether its molecular marker, biochemical and morphological based to classify the germplasm collection (Maji, 2012). Some of the techniques include principle coordinate analysis (PCoA), principle component analysis (PCA) cluster analysis (CA), single linkage cluster analysis

(SLCA) and multiple-dimensional scaling (MDS). These MS techniques at present identify character which deliver maximum variation within the group of genotypes extended to group genotypes similar in one or more characters (Adebisi *et al*., 2013). These techniques are often and thus monitor the selection of parentage for hybridization. Among the MS techniques PCA identifies the plant character that contributes major variation within the group of genotypes, due to common ordination numerical techniques; by removing the inter-correlation among the characters that diminishes the dimension of multivariate data (Chandra *et al*., 2007). Cluster analysis (CA) guide in choosing genotypes for crop improvement, through clustering in one group that exhibited the similarity in one or more than one character. Moreover, CA exposed the relationship pattern between the genotypes within a population (Zia-ul-Qamar *et al*., 2012).

The objective of the canvass was to explore variation and identify promising genotypes of rice by means of index score and multivariate statistical techniques (SLCA and PCA). Moreover, the available rice germplasm of Pakistan and China origin were organized into distinct clusters based on seedling traits and their performance. The outcome of this study would be helpful in choosing suitable genotypes and traits of interest to improve an effective rice-breeding programme.

Materials and Methods

Source of seed: Thirteen rice genotypes were investigated for the study (Table 1). The seeds were obtained from different sources, such as Huazhong Agricultural University (HZAU), Wuhan, China; Rice Research Institute Kala Shah Kaku (KSK) and ICI Pvt Ltd, Pakistan. Genotypes include (Table 1) chinese coarse hybrids (CH1 to CH4) and Chinese basmati hybrid (CH5 to CH7). Pakistani approved fine (Super basmati, Basmati 515, represented PV8, PV9) and coarse varieties (KSK133, KSK434 represented PV10, PV11). Pakistani coarse hybrids (GIR2, and GIR3 represented by PH13 and PH14) of ICI Pvt, Pakistan. The experiment was conducted at National Institute of Agriculture and Biology, Faisalabad, Pakistan during 2014. Three replicated of each genotype arranged in completely randomized design (CRD).

Table 1. Origin of rice genotypes used in present study.

No.	Origin	Code	Genotypes
1.	China	CH1	Chines coarse hybrid
2.	China	CH2	Chines coarse hybrid
3.	China	CH3	Chines coarse hybrid
4.	China	CH4	Chines coarse hybrid
5.	China	CH5	Chines basmati hybrid
6.	China	CH6	Chines basmati hybrid
7.	China	CH7	Chines basmati hybrid
8.	KSK Pakistan	PV8	Super basmati
9.	KSK Pakistan	PV9	Basmati 515
10.	KSK Pakistan	PV10	KSK133
11.	KSK Pakistan	PV11	KSK 434
12.	ICI Pakistan	PH12	GRI 2
13.	ICI Pakistan	PH13	GRI 3

Note: KSK; Kala Shah Kaku, ICI; Imperial Chemical Industries, PV; Pakistan variety, PH: Pakistan hybrid

Seed germination percentage: For test of seed germination two pieces of blotting papers were placed in petri dish. About twenty-five seeds of each genotype were placed on wet blotting paper in labelled petri dishes. The petri dishes were observed every day and germinated seeds were recorded. Within four days after seed setting the maximum number of seeds were germinated. According to Ellis, (1992) the germination percentage (G%), germination rate index (GRI), mean daily germination (MDG) were calculated using the following formulas:

Germination rate: $GR = (n / N) \times 100\ \%$ ---------- (i)

where n stands for no of seed germinated each day and N is total number of seedling germinated at final day.

Mean time of germination: $MGT = \sum (n_i \times d_i) / N$ --- (ii)

Index of germination: $GI = \sum (n_i / d_i)$ ---------- (iii)

where ni stands for number of germinated seeds on day *i*, d is the incubation time (days) and N is the total no of seedling germinated.

Ten seedlings were randomly selected 30 days after sowing and were used to measure the shoot length (SL), root length (RL), fresh shoot weight (FSW), fresh root weight (FRW) and root shoot ratio (RSR) in centimeters. Randomly selected five seedlings were placed in the ventilated oven for 30 mins at 105°C for dry shoot weight (DSW) and dry root weight (DRW). The weight was determined until the constant weight achieved. Total length vigour index (LVI) and total weight vigour index (WVI) were recorded on randomly selected ten seedlings from each genotype. SLVI and SWVI of seedling were calculated as:

Length vigour index

$$SLVI = \frac{\text{Seedling length X Germination percentage}}{100}$$

Weight vigour index

$$SWVI = \frac{\text{Seedling weight X Germination percentage}}{100}$$

Statistical analysis

Multivariate analysis of mean data was subjected for single linkage cluster analysis (SLCA) and principle component analysis (PCA) (Corliss *et al*., 1974). Using the MINITAB 13.2 the data was transofrmed to SLCA and PCA (Lesik & Lesik, 2009). SLCA explored the genotype position into dendrogram for seedling character variations. The component score of genotypes were calculated using character loading. Two-dimensional ordination of genotypes used to extract the first two components. Analysis of variation (ANOVA) through statistcal analysis was performed for all the seeding characteristics (Kéry, 2010).

Table 2. Principle components (PCs) for seedling traits in 13 rice genotypes.

	PC1	PC2	PC3	PC4	PC5
Eigenvalues	6.81	2.35	1.28	0.73	0.45
Proportion	0.57	0.11	0.11	0.06	0.04
Cumulative	0.57	0.76	0.87	0.93	0.97
Eigen vectors					
G%	0.11	0.60	-0.192	-0.10	0.17
GRI	0.26	-0.03	-0.10	-0.80	-0.16
MDG	0.12	0.60	-0.11	0.18	-0.15
SL	-0.25	0.01	0.65	-0.21	-0.04
RL	-0.33	-0.05	0.063	-0.18	0.71
RSR	-0.33	-0.05	-0.40	-0.18	0.23
FSW	-0.35	0.10	0.08	-0.16	-0.38
FRW	-0.33	-0.01	-0.24	-0.30	-0.35
DSW	-0.35	0.06	-0.25	0.01	0.06
DRW	-0.34	-0.15	0.023	0.32	-0.30
LVI	-0.20	0.44	-0.09	0.06	0.04
WVI	-0.34	-0.34	-0.21	0.07	-0.06

Note: G%; Germination %, GRI Germination rate index, MDG; Mean daily germination, SL; Shoot length, RL; Root length, RSR; Root shoot ratio, FSW; Fresh shoot weight, FRW; Fresh root weight, DSW; Dry shoot weight, DRW; Dry root weight, LVI; Length vigour index, WVI; Weight vigour index

Results

Principle component analysis (PCA): Multivariate analysis technique was used to assess magnitude of genetic variation in seedling characteristics of the Chinese and Pakistani rice genotypes. PCA performed on the seedling characteristics of rice genotypes expressed the negative and positive arithmetic sign with the value of coefficients. Coefficients having value greater than 0.3 were considered important regardless to sign as a common rule of thumb and coefficient value less than 0.2 were considered to be of no effect on overall genetic variation. The first three components i.e., PC1, PC2, and PC3 had eigenvalues greater than 1.0 (Table 2). The first three PCs, with eigenvalues of 6.81, 2.35, and 1.28 respectively, collectively contributed for 87.1% of the total genotypic variability. The first PC explained the 0.57% of the variableness and was more associated to G%, GRI, and MDG. The second PC described 0.76% of total change and was subjected for G%, MGD, SL, FSW, DSW and LVI. The third PC exhibited 0.87% of additional variability with positive effects for SL, RL, FSW and DRW. The first two PCs accounted to 76.4% of variation and were plotted to describe the relationship between the clusters (Fig. 1). The separate clustering pattern of Chinese coarse hybrids and Pakistani approved varieties; Super basmati (CH1, 2, 4 and PV8) come forward in first, Pakistani basmati 515 (PV9), and coarse varieties KSK 434 (PV11) appeared in second quadrate. Chinese basmati hybrid (CH3, CH5 and CH7) and Pakistani coarse varieties (KSK133) and hybrids GIR2 exposed in third, Chinese (CH6) and Pakistani coarse hybrid GIR3, (PH13) appeared in forth quadrate.

Index score (IS): Index score (IS) technique (Table 3) grouped thirteen diverse rice genotypes into six separate clusters based on the relative disposition (Table 4). Among the 13 genotypes maximum index score was noted for group II that included Chinese coarse (CH3) and basmati hybrid (CH5, CH6) along with basmati 515 (PV9) and two coarse varieties KSK 133 and KSK 434 (PV10, PV11) of Pakistan. Group-I included three genotypes (PV1, 2 and 4) having index score of 68. Group-IV having Super basmati (PV8) scored minimum index score of 18. Discrete groups (III, V and VI) were established for Chinese basmati hybrid (CH7) and Pakistani coarse hybrid (GIR2 and GIR3) on the basis of relative character of genotypes.

Means and standard deviation for each cluster were expressed in (Table 5). Cluster-I have maximum GRI. Cluster-II has high G % and MDG. Cluster-III shows high FSW< FRW< DSW and WVI. Cluster-V possessed maximum SL, DSW and LVI, and genotypes. Cluster-VI revealed maximum RL, RSR and DRW whereas the genotypes in Cluster-IV showed minimum average for SL, RL, RSR, FSW and LVI respectively.

Table 3. Score of the seedling traits of 13 rice genotypes.

Genotype	G%	GRI	MDG	RL	SL	RSR	FRW	FSW	DRW	DSW	LVI	WVI	Total
CH1	2	3	2	2	2	2	2	2	2	2	2	2	25
CH2	2	3	2	2	2	2	2	2	1	1	2	1	22
CH3	2	2	2	2	2	2	2	2	2	2	2	2	24
CH4	1	2	1	2	2	2	2	2	2	2	1	2	21
CH5	2	2	2	2	3	2	2	2	2	2	2	1	24
CH6	2	2	2	2	3	2	2	2	2	2	2	2	25
CH7	2	2	2	2	2	3	3	3	2	3	2	3	29
PV8	2	2	2	1	1	1	1	1	2	2	1	2	18
PV9	3	2	2	2	2	2	2	2	2	2	2	2	23
PV10	2	2	2	2	2	2	2	2	2	2	2	2	24
PV11	3	2	3	2	2	2	2	2	2	2	2	2	26
PH12	2	2	2	3	3	3	2	3	2	3	3	3	31
PH13	1	2	1	3	2	2	3	2	3	3	2	2	28

Note: G%; Germination %, GRI Germination rate index, MDG; Mean daily germination, SL; Shoot length, RL; Root length, RSR; Root shoot ratio, FSW; Fresh shoot weight, FRW; Fresh root weight, DSW; Dry shoot weight, DRW; Dry root weight, LVI; Length vigour index, WVI; Weight vigour index.

Cluster analysis (ca): The genotypes (CH1-7, PV8-11 and PH12, 13) correspond the later 1-13 (Table 1) and dendrogram (Fig. 2). At the 30.03% level of similarity, six clusters were observed. Maximum genotypes were grouped in cluster-II having basmati approved varieties (Basmati 515) and two coarse varieties (PV10, PV11). Beside Chinese basmati hybrid (CH5, CH6) Chinese coarse hybrid (CH3) were also clubbed in cluster-II. Three Chinese coarse hybrids (CH1, CH2, CH4) were grouped together within cluster-I. Four independent clusters, III, IV, V, VI, possess genotype CH7, PV8, PH12 and PH13 respectively. Analysis of variance (ANOVA) showed the significant variation for all the seedling characteristics of 13 rice genotypes (Table 7).

Table 4. Metroglyph technique for cluster number, index score in each cluster of rice genotypes.

Cluster No.	Genotype	Index score
I	CH1, CH2, CH4	68
II	CH3, CH5, CH6, PV9, PV10, PV11	146
III	CH7	29
IV	PV8	18
V	PH12	31
VI	PH13	28

Discussion

In our study the first three PCA had the greater (1.0) eigenvalues and contributed maximum towards the total variability among the genotypes, this was in accordance with Habtamu, (2013) who described that the first three PC were the mostly imperious in focusing variation pattern that were highly associated for genotypes characterization. Similar findings were reported by earlier workers on rice seedling experiment (Matsuo & Mochizuki, 2009). Among the first three PCs, PC3, which was the weighted average of the characters, had five characters which contributed positively to discriminate among the 13 genotypes. Similarly, the earlier workers (Maji, 2012; Chakravorty, 2013) reported that the seedling traits were effectively used for genotypes classification. Genotypes having high G%, MDG, GRI, FRL and LVI emphasize on increase biomass production and complete their vegetative growth earlier as supported by earlier workers (Jiangbo Zhou, 2012; Chakravorty, 2013).

Multivariate analyses an effective system to deal with the collection of germplasm and their clustering towards effective breeding programme (Nisar *et al*., 2007). In present study the variation in genotypes, seedling traits such as GRI, G%, MDG divides 13 genotypes into different groups. Similar findings were reported by Pongprayoon *et al*., (2019) and 24 genotypes were grouped differently for seedling traits based on genotypic variation. However, varietal difference in genotypes for seedling traits (emergence and seed germination) has been specified previously (Adebisi *et al.*, 2009).

In our study the clustering of genotypes for GRI, G%, MDG, and other seedling traits (SL, RL) is possible. The cluster with superior seedling attributes was identified that might be suitable to exploit the genetic potential of genotypes. However, due to lack of substantial contribution in variation few attributes were dropped. Index score (Table 3) was allocated to each character of the thirteen rice genotypes which specified the worth of the genotypes about seedling traits. Similar finding of (Rashid *et al*., 2008; Kumar *et al*., 2014) projected that index score method would be suitable for grouping of rice genotypes (Table 6). The genotypes were grouped in to the six clusters based on the index score (Table 4) in ascending order. Similarly, Tuhina – Khatun *et al*., 2015 evaluated 43 genotypes of rice for morphological and seedling variation using index score method in upland rice, resulted to cluster these differently. Similar to our study Prasad, (2013) also reported seedling traits, G%, GRI, etc. and showed their importance for genetic divergence in rice. Therefore, higher score was allocated to these genotypes, expressed low magnitude of that character.

In this study the major difference in the seedling characteristics of 13 rice genotypes quantified through means square of ANOVA. Similarly, Singh *et al*., (2013) reported that the 30 rice genotypes had significant mean squares for 6 quantitative character. Significant differences among mean square of seedling characteristics of rice genotypes directed the need to assemble them into cluster for identification of divergent groups.

Table 5. Mean and standard deviation in 13 rice genotypes for six cluster bases on 12 seedling traits.

Variables	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster-V1
G%	82.89 ± 6.74	90.50 ± 7.52	88.67 ± 00	86.67 ± 00	88 ± 00	73 ± 00
GRI	104.55 ± 3.66	45.23 ± 2.43	26.01 ± 00	66.87 ± 00	24.13 ± 00	17.00 ± 00
MDG	11.87 ± 0.87	13.27 ± 0.63	13.00 ± 00	13.17 ± 00	12.57 ± 00	10.43 ± 00
SL	28.71 ± 1.58	30.48 ± 2.32	27.85 ± 00	22.52 ± 00	33.95 ± 00	32.10 ± 00
RL	7.10 ± 0.52	7.69 ± 0.50	8.10 ± 00	5.70 ± 00	10.22 ± 00	10.72 ± 00
RSR	0.22 ± 0.01	0.24 ± 0.02	0.34 ± 00	0.21 ± 00	0.29 ± 00	0.35 ± 00
FSW	0.31 ± 0.05	0.47 ± 0.11	0.80 ± 00	0.09 ± 00	0.69 ± 00	0.63 ± 00
FRW	0.19 ± 0.01	0.22 ± 0.02	0.43 ± 00	0.07 ± 00	0.25 ± 00	0.37 ± 00
DSW	0.10 ± 0.02	0.14 ± 0.02	0.25 ± 00	0.11 ± 00	0.25 ± 00	0.24 ± 00
DRW	0.03 ± 0.01	0.04 ± 0.01	0.06 ± 00	0.03 ± 00	0.05 ± 00	0.07 ± 00
LVI	2968.63 ± 190.10	3541.36 ± 118.17	3278.22 ± 00	2604.47 ± 00	3886.80 ± 00	3126.53 ± 00
WVI	11.11 ± 1.50	17.17 ± 1.74	28.00 ± 00	12.00 ± 00	27.33 ± 00	22.67 ± 00

Note: G%; Germination %, GRI Germination rate index, MDG; Mean daily germination, SL; Shoot length, RL; Root length, RSR; Root shoot ratio, FSW; Fresh shoot weight, FRW; Fresh root weight, DSW; Dry shoot weight, DRW; Dry root weight, LVI; Length vigour index, WVI; Weight vigour index

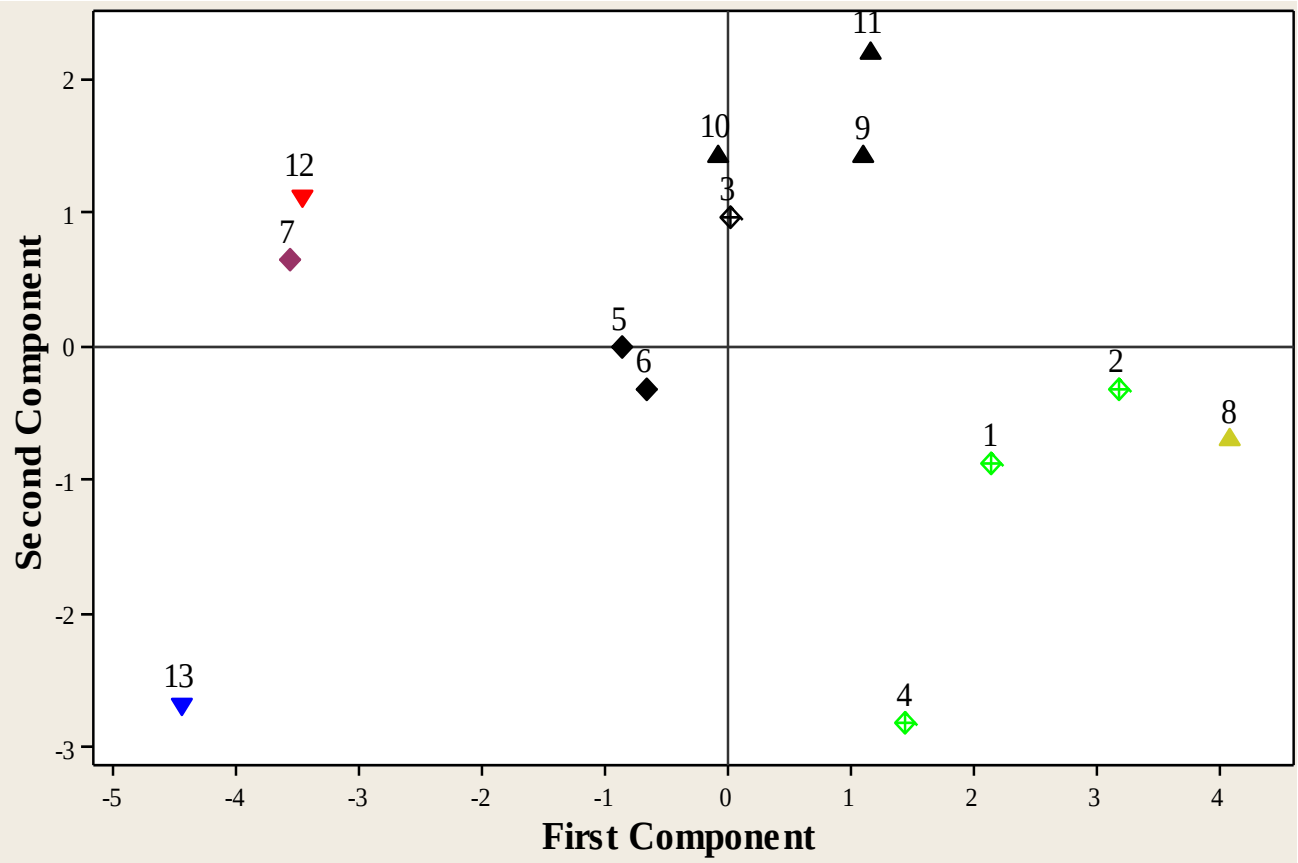

Fig. 1. Principle component axis I and II of 13 rice genotypes in two-dimensional ordination.

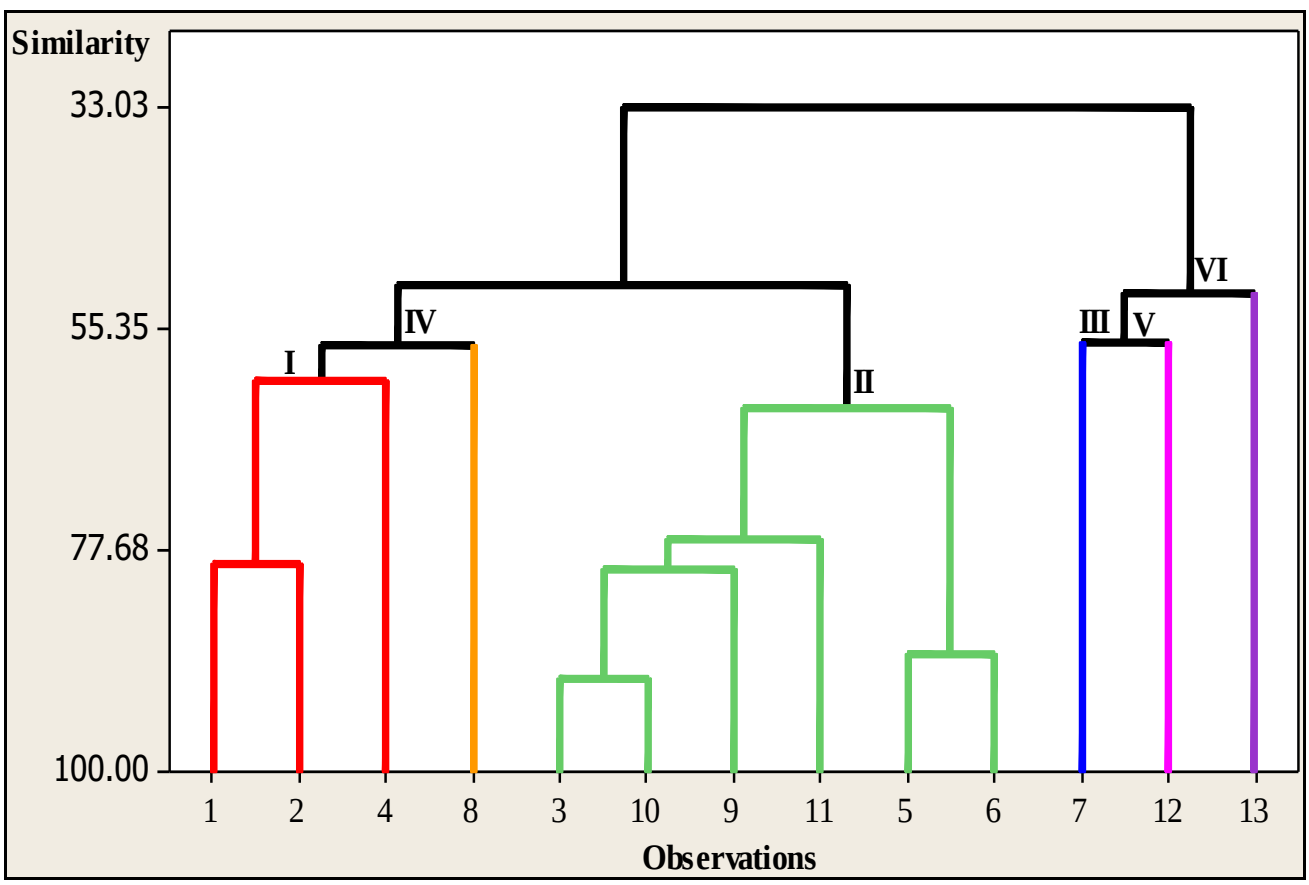

Fig. 2. Dendrogram expressing single linkage cluster analysis (SLCA) of 13 rice genotypes.

Table 6. Range of mean and index score for seedling traits of 13 rice genotype.

Traits	Range of mean	Score-I	Score-II	Score-III
		Value more	Value from	Value less
G%	94.39-79.15	96.33	94.33-80.33	73.00
GRI	95.71- 14.91	132.50	74.04-17.00	17.0
MDG	13.68- 11.60	14.10	13.67-12	10.43
		Value less than	Value from	Value more than
SL	32.78- 26.52	22.52	32.10-27.30	33.23
RL	9.22 -6.50	5.70	8.32-6.73	10.22
RSR	0.30 - 0.21	0.21	0.29-0.21	0.34
FSW	0.65- 0.26	0.09	0.63-0.26	0.69
FRW	0.32- 0.14	0.07	0.25-0.18	0.37
DSW	0.21 - 0.10	0.08	0.16-0.10	0.24
DRW	0.06- 0.03	0.02	0.06-0.03	0.07
LVI	3685.48- 2937.60	2604.47	3886.8-3029.0	3886.8
WVI	23.23 - 11.59	9.667	22.667-12.00	27.333

Note: G%; Germination %, GRI Germination rate index, MDG; Mean daily germination, SL; Shoot length, RL; Root length, RSR; Root shoot ratio, FSW; Fresh shoot weight, FRW; Fresh root weight, DSW; Dry shoot weight, DRW; Dry root weight, LVI; Length vigour index, WVI; Weight vigour index

Table 7. Analysis of variance and mean square for 13 genotypes of rice.

SOV	df	G%	GRI	MDG	SL	RL	RSR	FSW	FRW	DSW	DRW	LVI	WVI
Replication	2	78.77	6.26	0.66	0.39	0.48	0.0048	0.013	0.0033	0.00090	0.000026	108259	21.26
Genotypes	12	174.2**	4896.56**	3.24**	29.32**	5.53**	0.00634*	0.117**	0.024**	0.00944**	0.00073**	419504**	101.6**
Error	24	17.52	1.06	0.16	0.94	0.46	0.0022	0.0112	0.00191	0.00074	0.0001720	31281	8.8

Note: G%; Germination %, GRI Germination rate index, MDG; Mean daily germination, SL; Shoot length, RL; Root length, RSR; Root shoot ratio, FSW; Fresh shoot weight, FRW; Fresh root weight, DSW; Dry shoot weight, DRW; Dry root weight, LVI; Length vigour index, WVI; Weight vigour index. * Significant at 0.05 probability level** highly significant, 0.01 probability level df (degree of freedom)

Conclusion

It is concluded that genetic improvement of seed and seedling traits like GRI, G %, MDG, SL, RL is possible. However, some components could be dropped due to lack of appreciable contribution in variations. Results of SLCA accord with PCA ordination on I and II axis and Index score provided the 6 groups of rice genotypes. The pre-eminently performing cluster or groups of genotypes may be preferred for seedling traits and could be used for potential breeding programme. This study implies the need for breeders to quantify germplasm with precise tools in order to epitomize and evaluate it accurately into their distinct groupings. Clusters with superior seedling traits were acknowledged for hybridization and to upgrade the potential yield of the rice crop through transfer of the desirable genes.

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