International *Journal* of *Chemical* Studies

Bhawana Sharma
Scientist, Department of
Genetics and Plant Breeding,
Indira Gandhi Krishi
Vishwavidyalaya, Raipur,
Chhattisgarh, India

Mangla Parikh
Assistant Professor, Department
of Genetics and Plant Breeding,
Indira Gandhi Krishi
Vishwavidyalaya, Raipur,
Chhattisgarh, India

Sandeep Bhandarkar
Senior Scientist, Department of
Genetics and Plant Breeding,
Indira Gandhi Krishi
Vishwavidyalaya, Raipur,
Chhattisgarh, India

Sunil K Nair
Scientist, Department of
Genetics and Plant Breeding,
Indira Gandhi Krishi
Vishwavidyalaya, Raipur,
Chhattisgarh, India

Deepak Gaurha
Scientist, Department of
Genetics and Plant Breeding,
Indira Gandhi Krishi
Vishwavidyalaya, Raipur,
Chhattisgarh, India

Correspondence
Bhawana Sharma
Scientist, Department of
Genetics and Plant Breeding,
Indira Gandhi Krishi
Vishwavidyalaya, Raipur,
Chhattisgarh, India

Evaluation of variations in seed vigour characters of traditional aromatic rice (*Oryza sativa* L.) accessions of Chhattisgarh using multivariate technique

Bhawana Sharma, Mangla Parikh, Sandeep Bhandarkar, Sunil K Nair and Deepak Gaurha

Abstract
Principal component analysis was carried out to determine the variation in seed vigour characters among the fifty traditional aromatic rice genotypes. The wide range was observed for the root length (35.5-162.2 mm) followed by shoot length (70.9 -125.9 mm) and germination % (62.5-100%). The vigour index showed highly significant positive correlations to seed length (r = 0.489), 1000 seed weight (r =0.596), germination % (r =0.460), shoot length (r =0.539), root length (r = 0.686) and shoot dry weight (r =0.572). Out of eight, first three components exhibited more than one eigen value and showed 79.89 % of the total variation among the traits studied for each genotypes. The PC1 had 50.794 %, PC2 showed 16.571% and PC3 exhibited 12.525% variability among the genotypes for the traits under study. It also identified the 1000 seed weight, shoot dry weight, seed length and germination percentage as the characters contributing most of the variation.

Keywords: rice, aromatic, traditional, seed vigour, PCA

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops grown worldwide for more than half of the world population [1]. As the improvement of irrigated condition, implement of efficient herbicide, deployment of new varieties with precocity and high yield, and exaltation of labour cost, direct seeding has become an inevitable trend in rice production [2]. In India, more than 50% of rice areas under rainfed conditions are cultivated as direct seeded rice. Seed vigour is closely related to direct seeding in rice. High seed vigour is important for direct seeding because it can not only enhance crop establishment [3-4] but also increase the plant's ability to compete against weeds [5-6].

Seed vigour comprises those properties that determine the potential for rapid and uniform emergence and development of normal seedlings under a wide range of field conditions. The principal causes of variations in rice seed vigour are aging, cultivars, time of harvest, weather during maturity, nutrition, position of seeds on the panicle, specific gravity, mechanical integrity, storage conditions and pathogens [7].

Seed vigour is a complex trait and root length, shoot length and dry weight of seedling have been identified as good indicators of seed vigour [8-9]. The seed lot showing the higher seed vigour index (SVI) is considered to be more vigorous. Rice is one of the most important food crops in the world, and the cultivars with strong seed vigour are desirable for farmers to get optimum stand establishment in a direct-sowing culture system.

Seed vigour is an important characteristic of seed quality, reflecting potential seed germination, seedling growth, seed longevity, and tolerance to adversity [10]. Seeds with strong vigour may significantly improve the speed and uniformity of seed germination and the final percentage of germination, and lead to perfect field emergence, good crop performance, and even high yield under different conditions [11].

Seed vigour has been known as a comprehensive characteristic affected by many factors, such as the genetic background, environmental factors during seed development, and storage stages [10], which makes the genetic analysis of seed vigour very difficult. The comparison of the variation patterns among rice genotypes in respect of seed quality is necessary in seeds of

Aromatic rice genotypes. Multivariate statistical methods have found extensive use in summarizing and describing the inherent variation in a population of crop genotypes. Some of the methods include Principal Component Analysis (PCA), Descriminant Canonical Analysis (DCA) and Cluster Analysis (CA). These statistical techniques identify plant characters that contribute most to the variation within a group of entries. The methods are often extended to genotype grouping in order to cluster entries that show similarity in one or more characters and thus guide in the choice of parents for hybridization [12-13].

PCA is a technique, which identifies plant characters that contribute most to the variation within a group of entries [14]. It is also a common ordinational numerical technique, which reduces the dimensions of multivariate data by removing inter-correlation among variables (characters on which units are to be compared), and enables multi-dimensional relationship to be plotted on two or three principal axes. PCA chooses independent or orthogonal axes, which are minimally correlated and represents linear combination of the original characters [15]. The relative discriminating power for axes and their associated characters are measured by Eigen values and factor scores, respectively.

PCA was used as a descriptive method to show patterns of co-variation of characters among characters with 1.0's in the diagonal of the correlation matrix, it is partitioned into components to account for a maximum amount of variance of the characters. In order to facilitate the interpretation of the result, the components can be rotated mathematically so that a given character tends to show its greatest contribution on a given component [16]. PCA reliably represents large distances between major groups but not between closely spaced units within the groups [17].

Therefore, the objective of this research work was to identify the seed vigour traits that contribute mostly to the variation in seed vigour of traditional Aromatic rice accessions of Chhattisgarh.

Materials and Methods
Seed Source
Seed material for the present investigation consisted of fifty accessions (Table 1) of traditional aromatic rice (*Oryza sativa* L.).

Table 1: The list of the rice genotypes under study.

S. No.	Accession Name
1	Dubraj selection 1
2	Badshahbhog selection 1
3	Tarun bhog selection 1
4	Vishnu bhog Selection 1
5	Kadamphool
6	Tulsimanjari
7	Kargilas
8	Kapoorsar
9	Chinikapoor
10	Kalikamod
11	Chhatri
12	Atmashital
13	Shamjira
14	Jiradhan
15	Suklaphool
16	Elaychi
17	Shayamjira
18	Kubrimohor
19	Javaphool
20	Kevradhul
21	Jatashankar
22	Shrikamal
23	Maharaji
24	Tilkasturi
25	Shitalbhog
26	Samundchini
27	Bagmuchh
28	Chinnor
29	Gangabaru
30	Jaigundi
31	Anterved
32	Samudrafen
33	Tulsiprasad
34	Lahsunbhog
35	Nawabbhog
36	Maidubraj
37	Bantaphool
38	Banspatri
39	Dhawara sawa
40	Tedesi
41	Fundri
42	Loktimachhi
43	Bheisapoochi
44	Lohandi
45	Kasturi
46	Dujai
47	Katrani bhog
48	Jira dhan
49	Ker ghul
50	Kali kamoda

Phenotypic data collection
Seed vigour tests were conducted on seed samples of the 50 traditional aromatic rice accessions of Chhattisgarh. The heading date of each accession was recorded, and seeds were harvested in bulk 45–50 days after flowering. Then the seeds were air-dried under natural conditions. The vigour test evaluation was carried out during June 2016. Seed samples were investigated for seed vigour traits according to the following methods:

Seed length and seed breadth (mm): Ten grains were taken randomly without removing hull and average length and breadth was recorded in mm.

1000-seed weight (g): Weight in grams of 1000 seeds of each genotype was determined.

Germination Percentage (%): For each variety, 100 manually selected plump grains were treated at 50 °C for 5 days to eliminate residual dormancy. The grains were subsequently surface sterilized with 0.6 % sodium hypochlorite solution for 15 min, rinsed three times with tap water. These seeds were used for testing seed vigour. Thirty seeds were sowed in a Petri dish (diameter 9 cm) with two sheets of filter paper, and 10 ml of tap water was added. The petri dishes were placed in germinator. During the cultivation, tap water was added to keep the filter paper moist. Germination count was recorded on the 8th day according to International Seed Testing Association rules [18].

Shoot length and root length (mm): Eight days after sowing, the root length and shoot length were measured, respectively. The average of ten root length and shoot length

with two repeats were calculated, respectively, accurate to 1 mm.

Shoot dry weight: Shoot dry weight of each sample was measured after removing the roots and residual endosperm from the plants, and then drying at 70 °C for 2 days. Shoot dry weight per plant (mg) for each accession was determined for each of the 2 × 10 plants.

Vigour Index (VI): This was calculated using the formula of Kharb et al., 1994 [19], as follows:
Vigour Index (VI) = Germination percentage x (Root length + shoot length) / 100

Data analysis
Multivariate tool used for analysing date was principal component analyse (PCA). The PCA was calculated by using XLSTAT 2018. Those PCs with Eigen values greater than one were selected as proposed by Jeffers (1967) [20]. The principal

component analysis was computed using the following equation:

$$PC1 = \sum_{1}^{p} a_j X_j$$

Where; PC = Principal component, a1j = Linear coefficient – Eigen vectors

Results and Discussion
Execution of the breeding programmes depends largely on the presence of significant genetic variability to permit effective selection. Relative magnitude of variability present in a crop species helps the breeder to handle the breeding population created by hybridizing the selected donors with high yielding base varieties. Descriptive statistics for seed length, seed breadth, 1000 seed weight, germination %, shoot length, root length, shoot dry weight and vigour index is presented in Table 2. The wide range was observed for the root length (35.5-162.2 mm) followed by shoot length (70.9 -125.9 mm) and germination % (62.5- 100%).

Table 2: Discriptive Statistics for various quantitative traits in aromatic rice accessions

S. No.	Traits	Minimum	Maximum	Mean	Standard error of the mean	Std. deviation
1	Seed length (mm)	5.650	10.550	7.354	0.186	1.315
2	Seed breadth (mm)	1.900	5.450	2.343	0.072	0.512
3	1000 seed weight (g)	8.130	37.800	16.527	0.878	6.212
4	Germination %	62.500	100.000	92.500	1.152	8.144
5	Shoot length (mm)	70.900	125.900	95.867	1.913	13.529
6	Root length (mm)	35.500	162.200	88.889	5.046	35.679
7	Shoot dry weight (g)	0.014	0.041	0.022	0.001	0.007
8	Vigour index	5.263	27.280	16.835	0.601	4.252

Pearson's coefficient: Pearson correlation coefficient (r) is a measure of the strength of linear association between two variables. The coefficient of correlation for pairs of characters (P=0.01 and P=0.05) in the aromatic accessions are presented in Table 3. Among the variables studied, the highest correlation corresponded to the 1000 seed weight and shoot dry weight (r = 0.919). Traits presenting highly significant correlation with these two characteristics were seed length (r = 0.840 and 0.740 respectively) and seed breadth (r = 0.373 and 0.393 respectively). The vigour index showed highly significant positive correlations to seed length (r = 0.489),

1000 seed weight (r =0.596), germination % (r =0.460), shoot length (r =0.539), root length (r = 0.686) and shoot dry weight (r =0.572). The results are in agreement with Monajem et al., 2016 [21]. The positive and significant association of these traits will provide plant breeders an understanding to phenotypic traits and their degree of association to be able to plan the breeding scheme. The non significance of some traits may be due to less contribution to their development or might be due to genetic constitution differences in the genotype evaluated.

Table 3: Correlation coefficient among different quantitative traits

Correlation matrix (Pearson (n)):								
	Seed length	Seed breadth	1000 seed weight	Germination %	Shoot length	Root length	Shoot dry weight	Vigour index
Seed length	1	0.072	0.840**	-0.031	0.484**	0.505**	0.740**	0.489**
Seed breadth		1	0.373**	0.129	0.224	0.067	0.393**	0.181
1000 seed weight			1	0.115	0.546**	0.483**	0.919**	0.596**
Germination %				1	0.152	-0.247	0.078	0.412**
Shoot length					1	0.233	0.642**	0.539**
Root length						1	0.413**	0.686**
Shoot dry weight							1	0.572**
Vigour index								1

**= r value, 0.361 at 1%; *= r value, 0.279 at 5%

Principal component analysis (PCA)
The results of the principal component analysis substantially confirms the pattern of character co-variation among the genotypes studied. It also identified the characters that contribute most to the variation within a group of entries [14]. The biological meaning of the principal components can be assessed from contribution of the different variables to each principal component according to the Eigen vectors [22]. The

results of the principal component analysis show that different characters contributed differently to the total variation as indicated by their Eigen vectors as well as their weight and loading on the different principal axes. Each component score obtained is a linear combination of the traits similar to an index, such that the maximal amount of variance is shown on the first principal component, second maximal amount is

shown on the second component, third maximal amount is shown on the third component and so on.

The extent of genetic variation in seed vigour and related traits of the 50 aromatic rice accessions was assessed using multivariate techniques. Principal component analysis was performed to reveal the pattern of data matrix for determination and identification of selection criteria. The result of PCA explained the genetic diversity among the rice accessions. The results of the PCA are presented in Table 4. According to Brejda *et al.* (2000) [23], data were considered in each components with Eigen value >1 which determined at least 10% of the variation. The higher Eigen values were considered as best representative of system attributes in principal components. Statistically, first few principal components usually account for most of the variation in the original set of data. The total variance is simply the sum of variances of these variables [24]. The arithmetic sign of the coefficient is irrelevant since a common rule of thumb for determining the significance of a trait coefficient is to treat coefficient greater than 0.3 as having a large enough effect to be considered important [25]. Traits having less than 0.2 coefficient value were considered to be of no effect to the overall variation observed in the present study.

Table 4: Eigen values, percent of total variation accounted for, cumulative percent and eigen vectors of major seed vigour traits.

	Principal components (PCs)							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Eigenvalue	4.064	1.326	1.002	0.783	0.554	0.180	0.051	0.041
Variability (%)	50.794	16.571	12.525	9.783	6.931	2.245	0.643	0.508
Cumulative variability (%)	50.794	67.365	79.890	89.673	96.604	98.849	99.492	100.000
Traits	Factors loadings after Varimax rotation							
	F1	F2	F3	F4	F5	F6	F7	F8
SL	0.825	-0.269	0.007	-0.312	-0.264	0.274	-0.072	-0.019
SW	0.368	0.407	0.654	0.506	0.013	0.119	-0.020	-0.002
1000 Sw	0.930	-0.017	0.168	-0.114	-0.243	-0.074	0.141	0.094
Ger	0.156	0.868	-0.406	-0.034	-0.216	0.040	0.046	-0.071
Shl	0.709	0.191	0.026	-0.293	0.605	0.080	0.045	-0.013
RL	0.632	-0.522	-0.298	0.473	0.040	0.025	0.075	-0.091
Sh dry wt	0.911	0.026	0.236	-0.150	-0.057	-0.273	-0.078	-0.083
VI	0.792	0.155	-0.486	0.288	0.090	-0.039	-0.100	0.105

Figures in bold represents highly loaded factor/traits in respective PC

The result of the PCA explained the genetic diversity of the aromatic acessions of rice. Within each PC, only highly loaded factors or traits were retained for further explanation. The results revealed that only three of the principal components had eigen values greater than 1.0 and exhibited 79.890 % cumulative variability, therefore these three PCs were used for further explanation. The first PC accounted for 50.794 % of the variability and was related to thousand seed weight (0.930), Shoot dry weight (0.911) and seed length (0.825), so it must be considered for direct selection. The second principal component accounted for 16.571 % of the total variation and was dominated by germination percentage (0.868). Likewise, third PC accounted for 12.525 % of the total variation and was related to seed breadth (0.692). Eigen value and variance associated with each principal, decreased gradually and stopped at 0.041 and 0.508%, respectively. Adebisi *et al.,* 2013 and Adekoya, 2008 [26-27] reported that the first three principal components were the most important in reflecting the variation patterns among accessions and the characters highly associated with these should be used in differentiating accessions.

Scree plot (Fig.1) explained the percentage of variance associated with each principal component obtained by drawing a graph between eigen values and principal component numbers. The PC1 showed 50.794 % variability with eigen value 4.064 which then declined gradually. From the graph, it is clear that the maximum variation was observed in PC1 in comparison to other than 7 PCs (Fig 1).
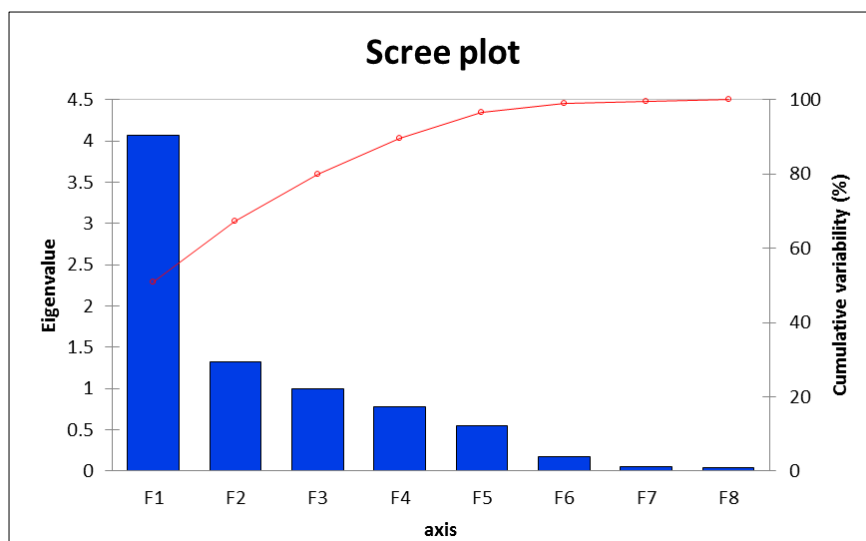
Fig 1: Scree plot showing Eigen value and percentage of cumulative variability

A further understanding was obtained by plotting the PC scores for individual observations in relation to the axes of PC1 and PC2 (Figure 2). Genotype 36 (Maidubraj) was the most distinct from the others. Likewise Genotype 7 (Kargilas) and 40(Tedasi) are also distinct from core group. The main components which discriminated between genotypes for PC1 (Y-axis) were 1000 seed weight (TSW), shoot dry weight (SDW) and shoot length SL) and for PC2 (x-axis) were germination percentage (GP).
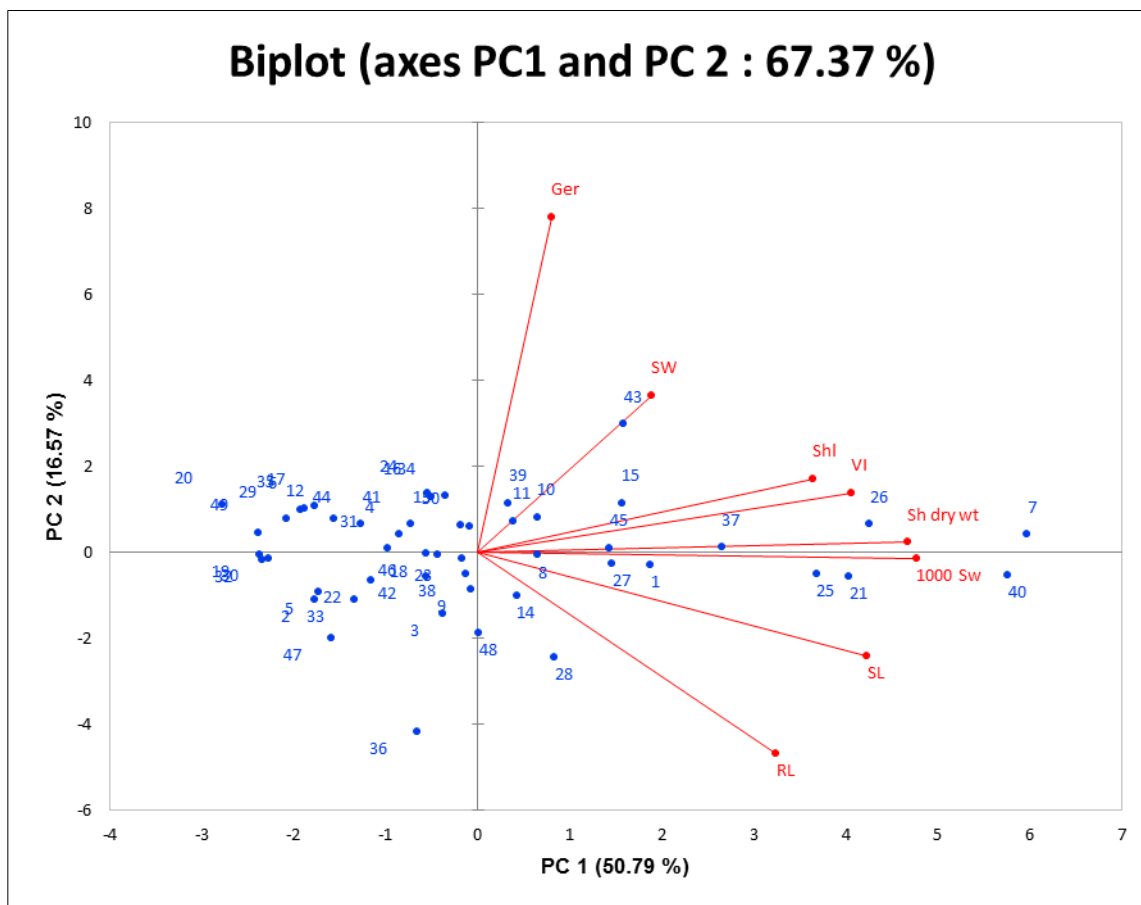
Fig 2: Distribution and grouping of 50 aromatic rice accessions across first two components based on PCA

This study, which used multivariate techniques to assess the extent of genetic variation in seed vigour of fifty aromatic rice accessions, was a first step in gaining an insight into the germplasm divergence, which is an important step towards an efficient exploitation of genetic resources of rice genotypes.

The principal component analysis identified 1000 seed weight, shoot dry weight, shoot length and germination percentage as characters that mainly described the variation within the rice genotypes. However, some components did not contribute appreciably to the variation within the entries and could be dropped in similar analysis, in order to improve the quality of result from the technique.

References

1. Sasaki T, Burr B. International rice genome sequencing project: the effort to completely sequence the rice genome. Curr Opin Plant Biol 2000; 3:138-141.
2. Dang X, Thi TGT, Dong G, Wang H, Edzesi WM, Hong D. Genetic diversity and association mapping of seed vigor in rice (*Oryza sativa* L.). Planta 2014; 239:1309-1319.
3. Wang ZF, Wang JF, Bao YM, Wang FH, Zhang HS. Quantitative trait loci analysis for rice seed vigor during the germination stage. Biomed and Biotechnol 2010; 11(12):958-964.
4. Yamauchi M, Winn T. Rice seed vigor and seedling establishment in anaerobic soil. Crop Sci. 1996; 36:680-686.
5. Dingkuhn M, Johnson DE, Sow A, Audebert AY. Relationship between upland rice canopy characteristics and weed competitiveness. Field Crop Res. 1999; 61:71-95.
6. Rao an, Johnson De, Sivaprasad B, Ladha JK, Mortimer AM. Weed management in direct-seeded rice. Adv Agron. 2007; 93:153-255.
7. Seshu DV, Krishnasamy V, Siddique SB. Seed vigor in rice. Rice seed health. International Rice Research Institute, Los Banos, 1988; 315-329.
8. Regan KL, Siddique KHM, Turner NC, Whan BR. Potential for increasing early vigor and total biomass in spring wheat. II. Characteristics associated with early vigor. Aust J Agric Res. 1992; 43:541-553.
9. Redonˇa ED, Mackill DJ. Genetic variation for seedling vigor traits in rice. Crop Sci. 1996; 36:285-290.
10. Sun Q, Wang JH, Sun BQ. Advances on seed vigor physiological and genetic mechanisms. Agric. Sci. China. 2007; 6(9):1060-1066.
11. Foolad MR, Subbiah P, Zhang L. Common QTL affect the rate of tomato seed germination under different stress and nonstress conditions. Int. J Plant Genomics. 2007, 97386.

12. Ariyo OJ. Multivariate Analysis and Choice of Parents Hybridization in Okra (*Abelmoschus esculentus* L.). Theoretical and Applied Genetics. 1987; 74(3):361-363.

13. Nair NV, Balakrishan R, Sreenivasan TV. Variability for Quantitative Traits in Exotic Hybrid Germplasm of Sugarcane. Genetic Resources and Crop Evolution. 1998; 45(5):459-464.

14. Ogunbodede BA. Multivariate Analysis of Genetic Diversity in Kenaf (*Hibiscus cannabinus* L.). African Crop Science Journal 1997; 5(2):127-133.

15. Clifford HT and W. Stephenson W. An Introduction to Numerical Classification. Academy Press, London, New York, 1975, 229 pages.

16. Rhodes AM, Martins FW. Multivariate Studies of Variation in Yams (*Dioscorea alata* L.). Journal of American Society of Agricultural Science. 1972; 97: 685-688.

17. Akoroda MO. Principal Component Analysis and Metroglyph of Variation among Nigerian Yellow Yam. Euphytica 1983; 32(2):565-573.

18. ISTA. International Rules for Seed Testing. Seed Science and Technology. 1985; 13:307-513.

19. Kharb RPS, Lather BPS, Deswal DP. Prediction of Field Emergence through Heritability and Genetic Advance of Vigour Parameters. Seed Science and Technology. 1994; 22:461-466.

20. Jeffers JNR. Two case studies in the application of principal component analysis. Appl. Stat. 1967; 16:225-236.

21. Monajem S, Zeinali E, Ghaderi FF, Soltani E and Hosseini CM. Evaluation of the seed vigour diversity of rice genotypes (*Oryza sativa* L.). Electronic Journal of crop production 2016; 8(4):121-142.

22. Lezzoni AF and M. P. Pritts MP. Application of Principal Component Analysis to Horticultural Research. Hort Science. 1991; 26(4):334-338

23. Brejda JJ, Moorman TB, Karlen DL, Dao TH. Identification of regional soil quality factors and indicators. I. Central and Southern High- Plains. Soil Sci. Soc. Am. J. 2000; 64:2115-2124.

24. Ray K, Dutta J, Banerjee H, Biswas R, Phonglosa A, Pari A. Identification of principal yield attributing traits of Indian mustard [*Brassica juncea* (L.) Czernj and cosson] using multivariate analysis. The Bioscan. 2014; 9(2):803-809.

25. Raji A. Assessment of Genetic Diversity and Heterotic Relationships in African Improved and Local Cassava (*Manihot esculenta* Crantz) Germplasm, Ph.D. Thesis, Obafemi Awolowo University, Ile Ife, 2003, 120.

26. Adebisi MA, Okelola FS, Ajala MO, Kehinde TO, Daniel IO, Ajani OO. Evaluation of Variations in Seed Vigour Characters of West African Rice (*Oryza sativa* L.) Genotypes Using Multivariate Technique. American Journal of Plant Sciences, 2013; 4:356-363.

27. Adekoya MA. Evaluation of Variation, Inter-Character Correlation and Performance of Okra (*Abelmoschus esculentus*). MA Dissertation, University of Agriculture, Abeokuta, 2008, 111.