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ESTIMATION OF GENETIC DIVERSITY FOR ANAEROBIC GERMINATION TRAITS USING PRINCIPAL COMPONENT ANALYSIS IN RICE (*Oryza sativa* L.)

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ABSTRACT

Principal component analysis was carried out to assess the diversity among 107 rice genotypes with regards to anaerobic germination traits. Results revealed two principal components with eigen value > 1. These components contributed for a total variability of 77.662 per cent. Component 1 (PC 1) had contributed maximum (57.897 %) while component 2 (PC 2) had contributed to 19.764 per cent towards the total variability. The characters, namely, germination per cent, shoot length, anaerobic response index and root length explained maximum variance in PC 1. The PCA scores for 107 genotypes were plotted in graph to get two dimensional scatter diagram. The results of 2D scatter diagram revealed maximum diversity between the genotypes number (MTU 1140) and (MTU 1010) and hybridization of these diverse genotypes is therefore predicted to result in transgressive segregants with high yield and tolerance to germination under anaerobic conditions.

INTRODUCTION

Rice production in the recent years is increasingly shifting from transplanting to direct seeding, due to reduction in cost of cultivation and early maturity of the direct sown crop (Pandey and Valesco, 2002). However, poor seedling establishment under direct seeding in standing water has prevented its large-scale adoption. It is attributed to the lack of tolerance to anaerobic germination (AG) caused by submergence and is identified as the main limiting factor for popularisation of direct seeding in rice (Yang *et al*., 2019). However, varietal differences for anaerobic germination were observed by Ismail *et al*. (2009). Development of rice cultivars tolerant to anaerobic conditions during germination coupled with early seedling vigor was reported to be an important objective under direct-seeding (Joshi *et al*., 2013; Miro and Ismail, 2013 and Vijayan *et al*., 2018). Studies on genetic diversity for anaerobic germination traits are therefore essential for devising effective breeding strategies aimed at the development of rice varieties tolerant to anaerobic conditions during germination for wet direct seeding under puddled conditions. In this context, multivariate analysis tools such as principal component analysis (PCA) and cluster analysis have been reported to be effective for evaluating the phenotypic diversity in addition to identifying genetically distant clusters of genotypes and selecting important traits contributing to the total variation in the genotypes. These analyses provide information that could help in better selection of parental genotypes with specific traits and in devising breeding strategies for trait improvement. Principal component analysis (PCA) allows natural grouping of the genotypes and is precise indicator of differences among genotypes. The main advantage of using PCA over cluster analysis is that each genotype can be assigned to one group only (Mohammadi, 2002). Principal component analysis is a variable reduction procedure and useful when breeders have obtained data on a number of variables (possibly a large number of variables), and believe that there is some redundancy in those variables (Ray *et al*., 2014). Principal component analysis (PCA) was used to identify redundancy of the genotypes with similar characters and their elimination (Adams, 1995), while two-way cluster analysis is useful for identification and separation of core subset of genotypes with distinct phenotypic traits. Therefore, Principal component analysis (PCA) and two-way cluster analysis are two important statistical programs that aid in selection of elite genotypes. Previous workers namely (Sharma *et al*., 2018) and (Barik *et al*., 2019) also studied the genetic diversity for anaerobic germination traits using Principal component analysis. The present investigation was undertaken in this context to study the nature and magnitude of genetic diversity for anaerobic germination traits using Principal Component analysis towards development of anaerobic germination tolerant varieties.

MATERIALS AND METHODS

The experimental material consisted of 107 rice genotypes collected from Regional Agricultural Research Station (RARS), Maruteru; Agricultural Research Station (ARS), Bapatla; and erstwhile, ARS, Pulla of Andhra Pradesh, India, in addition to germplasm obtained from International Rice Research Institute (IRRI), Philippines (Table 1). Screening of these genotypes for tolerance to anaerobic conditions during germination was undertaken at Regional Agricultural Research Station, Maruteru during Kharif 2017 with pro-tray method detailed by Reddy *et al*. (2017) in completely randomized design with two replications. The screening was undertaken with three days pre-germinated seeds at pigeon breast stage. The seeds were sown in pro-trays of ($35.5 \times 10 \times 4.5$cm) at about 1cm soil depth and submerged in tanks by filling water upto 10 cm above the trays. Observations were recorded 14th day of submergence. Data on number of seedlings survived after 14 days of submergence was recorded as germination percentage (%). In addition, shoot length (cm), root length (cm) and seedling dry weight (mg) were recorded for each variety in each replication. Further, seedling vigour index (Kharb *et al*., 1994) and anaerobic response index (Hsu and Tung, 2015) were estimated as per the standard procedures suggested by earlier workers. Principal component analysis was carried according to procedure described by Banfield (1978) and detailed by Gomez and Gomez (1984). PCA can be performed on two types of data matrices *viz*., variance-covariance matrix and correlation matrix. With characters of different scale a correlation matrix standardizing the original data set is preferred. If the characters are of same scale, a variance – covariance matrix can be used. In the present study, PCA was performed on the correlation matrix of traits, thereby removing the effects of scale (Jackson, 1991). A data matrix of 107 rice genotypes over six variables for anaerobic germination tolerance of each genotype was prepared and used for analysis. A covariance matrix derived from the data matrix was converted to correlation matrix.

The eigen value and eigen vector pairs created from data matrix were utilized to identify the principal components. The eigen value and eigen vector pairs created from data matrix were utilized to identify the principal components. Eigen values define the amount of total variation that is displayed on principal components. The proportion of variation accounted for each principal component (PC) is expressed as the eigen value divided by the sum of the eigen values.

$$\text{Percent variance explained for PCI} = \frac{\text{Eigen value(PCI)}}{\text{Sum of eigen values}}$$

The analysis was carried out using the software WindoStat Version 8.

RESULTS AND DISCUSSION

PCA measures the importance and contribution of each component to the total variance, whereas each coefficient of vector indicates the degree of contribution of every original variable with which each principal component is associated. The higher the coefficients, regardless of the direction (positive or negative), the more effective they will be in discriminating between the genotypes (Yumnam *et al*.,2015). The results on analysis of variance (ANOVA) for anaerobic germination traits revealed highly significant differences among the genotypes for all the characters studied, indicating the exsistence of sufficient variation among the genotypes and therefore opportunity for plant breeder to undertake further breeding activities like hybridization program. In the present study, first three principal components contributed to 88.335% towards the total variability (Table 2). The first principal component (PC 1) contributed 57.897 per cent towards total variability.

Table 1: Details of the material studied

S.No.	Centre of Collection	Genotypes
1	Maruteru, Andhra Pradesh, India	MTU 1001, MTU 1006, MTU 1010, MTU 1031, MTU 1032, MTU 1061, MTU 1064, MTU 1071, MTU 1075, MTU 1078, MTU 1112, MTU 1121, MTU 1140, MTU 1153, MTU 1156, MTU 1166, MTU 1184, MTU 1187, MTU 1194, MTU 1210, MTU 1224, MTU 1226, MTU 1229, MTU 2067, MTU 2077, MTU 2716, MTU 3626, MTU 4870, MTU 5182, MTU 5249, MTU 5293, MTU 7029, RTCNP 1, RTCNP 3, RTCNP 4, RTCNP 5, RTCNP 6, RTCNP 7, RTCNP 8, RTCNP 9, RTCNP 10, RTCNP 12, RTCNP 13, RTCNP 14, RTCNP 15, RTCNP 17, RTCNP 18, RTCNP 20, RTCNP 21, RTCNP 23, RTCNP 28, RTCNP 29, RTCNP 31, RTCNP 33, RTCNP 34, RTCNP 35, RTCNP 36, RTCNP 37, RTCNP 38, RTCNP 39, RTCNP 40, RTCNP 41, RTCNP 42, RTCNP 43, RTCNP 44, RTCNP 45, RTCNP 46, RTCNP 47, RTCNP 48, RTCNP 49, RTCNP 50, RTCNP52, SM-1, SM-2, SM-3, SM-4, SM-6, SM-7, SM-8, SM-9, SM-10, SM-11, SM-13, SM-14, SM-15, SM-16, SM-17, SM-18, SM-19, SM-23, SM-24, SM-25, SM-26, SM-27, SM-28,SM-29, SM-30, SM-31, SM-3-1
2	Bapatla, Andhra Pradesh, India	BPT 2231, BPT 3291, BPT 5204
3	Pulla, Andhra Pradesh, India	PLA-1100
4	IRRI, Philipines	FL 478, NONA BOKRA, POKKALI

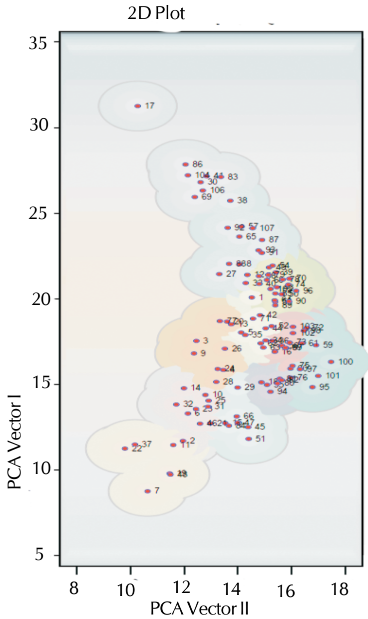

Figure 1: Two dimensional graph showing relative positions of 107 rice genotypes based on PCA scores

Table 2 : Eigen values, proportion of the total variance represented by first three principal components, cumulative per cent variance and component loading of different characters in rice for anaerobic germination traits

	PC 1	PC 2	PC 3
Eigen Value (Root)	3.473	1.185	0.64
% Var. Exp.	57.897	19.764	10.673
Cum. Var. Exp.	57.897	77.662	88.335
Germination (%)	0.499	0.171	0.091
Shoot length (cm)	0.484	-0.081	0.189
Root length (cm)	0.437	0.363	0.276
Seedling dry weight (mg)	0.338	-0.351	-0.814
Seedling vigour index	0.048	-0.835	0.464
Anaerobic response index	0.455	-0.1	-0.011

The characters, namely, germination per cent (0.499), shoot length (0.484), anaerobic response index (0.455), root length (0.437) and seedling dry weight (0.338) explained maximum variance and positive loadings in this component. The second principal component (PC 2) contributed to 19.764 per cent of total variance. The characters namely, root length (0.363) and germination per cent (0.171) explained maximum variance and positive loadings in this component. The third principal component was characterized by 10.673 per cent contribution towards the total variability. The characters, namely, seedling vigour index (0.464), root length (0.276) and shoot length (0.189) explained maximum variance in this component. The PCA analysis thus identified the maximum contributing traits towards the existing variability as germination per cent, shoot length, anaerobic response index and root length.

The PCA scores for 107 rice genotypes in the first three principal components were computed and were considered as three axes as X, Y and Z and squared distance of each genotype from these three axes were calculated (Table 3). These three PCA scores for 107 genotypes were plotted in graph to get two dimensional scatter diagram (Fig. 1). A perusal of these results revealed genotype number 17, *i.e.*, MTU 1140, a known anaerobic germination tolerant genotype to be widely diverse from other genotypes studied in the present

Table 3: PCA scores for 107 rice genotypes

Geno type number	Genotype name	PC I X vector	PC II Y vector	PC III Z vector
1	BPT 5204	20.075	14.521	-10.523
2	BPT 3291	11.697	11.961	-10.78
3	BPT 2231	17.541	12.463	-14.102
4	FL 478	15.847	13.46	-12.327
5	MTU 1001	18.027	14.125	-10.366
6	MTU 1006	13.295	12.148	-11.98
7	MTU 1010	8.756	10.649	-9.578
8	MTU 1031	22.017	14.066	-10.749
9	MTU 1032	16.811	12.369	-13.491
10	MTU 1061	14.38	12.797	-10.944
11	MTU 1064	11.454	11.602	-9.617
12	MTU 1071	21.393	14.358	-10.106
13	MTU 1075	18.511	13.757	-11.349
14	MTU 1078	14.772	11.997	-11.581
15	MTU 1112	12.733	13.535	-9.702
16	MTU 1121	16.906	15.375	-9.233
17	MTU 1140	31.253	10.283	-8.741
18	MTU 1153	15.121	14.877	-9.913
19	MTU 1156	9.798	11.471	-9.417
20	MTU 1166	18.666	13.603	-13.42
21	MTU 1184	12.715	12.974	-9.602
22	MTU 1187	11.245	9.807	-12.521
23	MTU 1194	13.559	12.444	-13.045
24	MTU 1210	15.899	13.253	-12.444
25	MTU 1224	14.046	12.917	-12.31
26	MTU 1226	17.084	13.518	-11.539

Table 3: Continue..

S.NO.	GENOTYPE	PC I X vector	PC II Y vector	PC III Z vector
27	MTU 1229	21.44	13.312	-13.206
28	MTU 2067	15.148	13.202	-10.737
29	MTU 2077	14.815	13.996	-9.932
30	MTU 2716	26.81	12.619	-8.946
31	MTU 3626	13.695	12.892	-11.364
32	MTU 4870	13.82	11.716	-11.748
33	MTU 5182	20.917	14.295	-11.23
34	MTU 5249	17.571	15.033	-10.128
35	MTU 5293	17.887	14.279	-11.373
36	MTU 7029	17.539	15.28	-10.08
37	NONABOKRA	11.486	10.181	-10.55
38	PLA-1100	25.728	13.725	-7.47
39	POKKALI	21.545	15.419	-8.62
40	SM1	20.866	14.794	-9.088
41	SM2	27.16	12.843	-8.284
42	SM 3	19.035	14.828	-11.796
43	SM 4	20.672	15.454	-8.055
44	SM 6	18.27	15.038	-10.479
45	SM 7	12.499	14.387	-9.17
46	SM8	12.701	12.597	-10.478
47	SM 9	12.76	13.991	-9.152
48	SM 10	9.729	11.498	-9.449
49	SM 11	21.831	15.161	-10.664
50	SM 13	20.262	15.633	-9.792
51	SM 14	11.812	14.406	-8.587
52	SM 15	18.395	15.262	-8.943
53	SM16	19.822	15.393	-11.818
54	SM 17	21.933	15.293	-9.21

Table 3: Continue..

S.NO.	GENOTYPE	PC I X vector	PC II Y vector	PC III Z vector
55	SM 18	14.971	15.09	-9.713
56	SM 19	17.259	15.644	-8.636
57	SM 23	24.212	14.138	-10.469
58	SM 24	21.321	14.789	-11.517
59	SM 25	17.282	16.907	-7.333
60	SM 26	15.919	15.963	-8.595
61	SM 27	17.396	16.384	-7.358
62	SM 28	17.398	14.855	-10.54
63	SM 29	17.149	14.95	-9.473
64	SM 30	19.914	15.372	-11.452
65	SM 31	23.628	14.056	-11.225
66	SM 3-1	13.124	13.963	-9.166
67	RTCNP1	17.127	15.783	-8.574
68	RTCNP 3	21.102	15.084	-9.897
69	RTCNP 4	25.94	12.399	-11.557
70	RTCNP5	21.171	15.917	-11.247
71	RTCNP 6	18.837	14.567	-11.988
72	RTCNP 7	18.297	16.569	-8.22
73	RTCNP 8	17.439	15.927	-7.141
74	RTCNP 9	20.825	15.881	-9.395
75	RTCNP 10	16.086	16.036	-9.645
76	RTCNP 12	15.393	15.971	-7.837
77	RTCNP 13	18.696	13.335	-13.348
78	RTCNP 14	21.12	15.672	-8.1
79	RTCNP 15	21.392	15.13	-9.905
80	RTCNP 17	15.056	15.479	-10.191
81	RTCNP 18	15.326	15.555	-9.947
82	RTCNP 20	15.242	15.632	-8.167

Table 3: Continue..

S.NO.	GENOTYPE	PC I X vector	PC II Y vector	PC III Z vector
83	RTCNP 21	27.111	13.369	-8.155
84	RTCNP 22	12.583	13.667	-9.644
85	RTCNP 23	20.304	15.394	-8.785
86	RTCNP 28	27.845	12.058	-8.924
87	RTCNP 29	23.434	14.9	-8.235
88	RTCNP 31	22.037	13.679	-13.123
89	RTCNP 33	19.618	15.392	-9.396
90	RTCNP 34	19.843	15.904	-8.744
91	RTCNP 35	22.685	14.898	-10.127
92	RTCNP 36	24.146	13.624	-10.942
93	RTCNP 37	22.834	14.773	-10.983
94	RTCNP 38	14.571	15.216	-9.052
95	RTCNP 39	14.838	16.775	-7.667
96	RTCNP 40	20.465	16.168	-8.173
97	RTCNP 41	15.887	16.314	-8.171
98	RTCNP 42	18.138	16.465	-8.452
99	RTCNP 43	17.139	15.765	-10.428
100	RTCNP 44	16.312	17.468	-6.619
101	RTCNP 45	15.493	16.993	-7.497
102	RTCNP 46	17.995	16.054	-9.584
103	RTCNP 47	18.357	16.055	-9.003
104	RTCNP 48	27.211	12.148	-8.604
105	RTCNP 49	20.571	15.213	-9.905
106	RTCNP 50	26.322	12.702	-9.18
107	RTCNP 52	24.124	14.555	-11.377

investigation. The results also revealed the genotypes number 17 (MTU 1140) and 7 (MTU 1010) to be most diverse. MTU 1010 (genotype number 7) is a short duration, mega rice variety with high yield potential (Anila *et al.*, 2018), while MTU 1140 (genotype number 17) is a flood and submergence tolerant variety also reported to possess tolerance for germination under anaerobic conditions (Girijarani *et al.*, 2014). Hybridization of these diverse genotypes is therefore predicted to result in transgressive segregants with high yield and tolerance to germination under anaerobic conditions for use in the breeding programmes aimed at the development of high yielding varieties coupled with anaerobic germination tolerance for wet direct seeding under puddled conditions.

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REFERENCES

Anila, M., Swamy, M.H.K., Kale, R. R. 2018. Breeding lines of the Indian mega-rice variety, MTU 1010, possessing protein kinase OsPSTOL (Pup 1), show better root system architecture and higher yield in soils with low phosphorus. *Molecular Breed*. **38:** 147.

Adams M.W. 1995. An estimate of homogeneity in crop plants with special reference to genetic vulnerability in dry season. *Euphytica*. **26:** 665-679.

Banfield, C.F. 1978. Principal component analysis for genstat. *J. of Static. Computer Simulations.* ***6:*** *211-222.*

Barik, J., Kumar, V., Lenka, S.K and Panda, D. 2019. Genetic potentiality of lowland indigenous indica rice (*Oryza sativa* L.) landraces to anaerobic germination potential. *Indian J. of Plant Physiology*. 1-13.

Girijarani, M., Satyanarayana, P.V., Suryanarayana, Y., Rao, R.P.V, Neerajakshi, Chamundeswari, N., Ravikumar, B.N.V.S.R, Vishnuvardhan, K.M and Bharathalakshmi, M. 2014. Development of new flood tolerant rice varieties for coastal Andhra Pradesh. Life *Sci. Int. Research J.* **1(1):** 143-147.

Gomez, K. A. and Gomez, K.K. 1984. Statistical analysis for agricultural research, Second Edition, John Wiley & Sons, New York.

Hsu, S.K. and Tung, C.W. 2015. Genetic mapping of anaerobic germination-associated QTLs controlling coleoptile elongation in rice. *Rice*. **8(1):** 1-12.

Ismail, A. M., Ella, E.S., Vergara, G.V and Mackill, D.J. 2009. Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*). Annals of Bot.**103:** 197-209.

Joshi, E., Kumar, D., Lal, B., Nepalia, V., Gautam, P and Vyas, A.K. 2013. Management of direct seeded rice for enhanced resource use efficiency. *Plant Knowledge J.* **2(3):** 119-134.

Kharb, R.P.S., Lather, B.P.S., Deswal, D.P. 1994. Prediction of field emergence through heritability and genetic advance of vigour parameters. *Seed Sci and Techn*. **22:** 461-466.

Miro, B and Ismail, M.A. 2013. Tolerance of anaerobic conditions caused by flooding during germination and early growth in rice (*Oryza sativa* L.). *Frontiers in Plant Science*. **4(269):** 1-18.

Mohammadi SA. 2002. Statistical methods in genetics, 6th International Conference of Statistics, University of Tarbiatmodares, Iran,

Pandey, S. and Velasco, L. 2002. Economics of direct seeding in Asia: patterns of adoption and research priorities. p. 3-8. In:. Pandey, S., Mortimer, M., Wade, L., Lopez, K., Hardy, B (Eds.) Direct seeding: research strategies and opportunities. International Rice Research Institute, Los Banos, Philippines.

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

Reddy, V.A., Girija Rani, M., Satyanarayana, P.V., Suryanarayana, Y.Chamundeswari, N., Ravikumar, B.N.V.S.R., Ramana, R,P.V and Vishnuvardhan, K.M. 2015. Physiological and molecular response of rice genotypes for different types of flooding. *Current Biotica*. **8(4):**345-350.

Sharma, B., Parikh, M., Bhandarkar, S., Nair, S.K and Gaurha, D. 2018. Evaluation of variations in seed vigour characters of traditional aromatic rice (*Oryza sativa* L.) accessions of Chhattisgarh using multivariate technique. *International J. of Chemi. Studies*. **6(5):** 348-353.

Vijayan, J., Senapati, S., Ray, S., Chakraborty, K., Molla, K. A., Basak, N. 2018. Transcriptomic and physiological studies identify cues for germination stage oxygen deficiency tolerance in rice. Environ. and Experimen. Botany. **147:** 15-248.

Yang, J., Li, D.K.S., Luo, L., Liu, Y., Huang, M., Yang, G., Liu, H., Wang, H., Chen, Z and Guo, T. 2019. Identification of stable QTLs and candidate genes involved in anaerobic germination tolerance in rice via high-density genetic mapping and RNA-Seq. BMC Genomics. **20(355):** 1-15.

Y**umnam, S., Sawarkar, A., Patil, S.G and Senapati, B.K. 2015.** Multivariate analysis of recombinant inbred lines (F6) of basmati derivative. *The Ecoscan*. **9(1&2):** 289-294.

Jackson, J. E. 1991. A User's Guide to Principal Components. John Wiley and Sons, New York.