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### Anaerobic germination tolerance based genetic diversity analysis in rice (*Oryza sativa* L.)

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#### Abstract

The present investigation was carried out to study the inherent genetic diversity of 107 rice genotypes towards development of rice varieties with tolerance for germination under anaerobic conditions for use in wet direct seeded rice cultivation system. The diversity was evaluated using multivariate analysis technique of Mahalanobis D<sup>2</sup>. The 107 rice genotypes studied were grouped into 12 clusters. Cluster I was largest with 25 genotypes followed by 23, 21, 13, 10 and 9 genotypes each in clusters II, III, V, IV and cluster VI, respectively further one genotype each in cluster VII, VIII, IX, X, XI, XII. The pattern of distribution of genotypes into various clusters was observed to be at random with no relation to geographical diversity. Results on inter-cluster distances revealed maximum diversity between genotypes of cluster X and cluster XII, while intra-cluster distance was noticed to be maximum for cluster VI. Cluster II had recorded higher cluster mean values for the trait anaerobic response index. Further, the traits, namely, seedling dry weight, root length, germination per cent and shoot length together accounted for 99.87 per cent of the total genetic divergence in the study.

Keywords: Anaerobic response index, genetic divergence, rice

#### 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)<sup>[1]</sup>. 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 direct seeding in rice (Yang *et al.* 2019)<sup>[2]</sup>. However, varietal differences for anaerobic germination were observed by Ismail *et al.* (2009)<sup>[3]</sup>. The present investigation was undertaken in this context to study the nature and magnitude of genetic diversity for anaerobic germination traits for use in developing rice varieties tolerant to germination under anaerobic conditions.

#### **Material 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), Phillippines (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 test tube method detailed by Hsu and Tung (2015)<sup>[6]</sup> in completely randomized design with two replications. Screening for tolerance to anaerobic conditions during germination with test tube method was undertaken with three days pre-germinated rice seeds at pigeon breast stage in glass test tubes of 25 mm in diameter and 150 mm in height and filled with distilled water to 10 cm depth (Plate 1). Observations were recorded after seven days. Data on number of seedlings survived after seven days. Data on number of seedlings survived server days of cubmargenee was recorded as

after seven 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 both the methods in each replication. Further, seedling vigour index (Kharb *et al.*, 1994) <sup>[5]</sup> and anaerobic response index (Hsu and Tung, 2015) <sup>[6]</sup> were estimated as per the standard procedures suggested by earlier workers. The data obtained was subjected to standard statistical procedures. Principal component analysis was carried out using the software Window Stat Version 8.5.

#### **Results and Discussion**

The results on analysis of variance (ANOVA) for anaerobic germination traits revealed highly significant mean squares due to genotypes for all traits studied, indicating the existence of sufficient variation among the genotypes and therefore an ample scope for effective selection. The results on genetic divergence of the genotypes for anaerobic germination traits are presented in Tables 1-5 and Figures 1-2. A perusal of the results on grouping of genotypes (Table 2 and Fig.1) revealed that the 107 genotypes were grouped into 12 clusters based on the relative magnitude of  $D^2$  values such that the genotypes belonging to same cluster had an average smaller D<sup>2</sup> value than those belonging to different clusters. Among the 12 clusters, cluster I was largest comprising 25 genotypes representing collections from different centres, namely, Maruteru and Bapatla of Andhra Pradesh, India, in addition to germplasm from IRRI, Phillipines. Similarly, cluster II and cluster IV, comprising 23 and 10 genotypes each, included collections from Maruteru of Andhra Pradesh, India, in addition to germplasm from IRRI, Phillipines. Cluster V comprised of 13 genotypes and included collections from Maruteru and Bapatla of Andhra Pradesh, India. However, cluster III comprising of 21 genotypes and cluster VI comprising of nine genotypes included collections from Maruteru centre only. Further, cluster VII, cluster VIII, cluster IX, cluster X, cluster XI and cluster XII were solitary or monogenotypic clusters with zero inter cluster  $D^2$  values. The mode of distribution of genotypes from different geographical regions into various clusters was thus observed to be at random indicating no relation of geographic and genetic diversity. Genotypes chosen from the same eco-geographical region were observed to be present in different clusters as well as in same cluster, while genotypes from diverse geographical regions were included in the same cluster. The findings are in conformity with the reports of Prasad et al.  $(2018)^{[9]}$ .

An analysis of inter- and intra- cluster distances (Table 3 and Fig. 2) revealed maximum inter-cluster distance between cluster X and XII (561.07), followed by cluster VII and XI (531.81) indicating that genotypes from these clusters were highly divergent meriting their consideration in selection of parents for hybridization. The greater the distance between two clusters, the wider would be the genetic diversity between the genotypes. Therefore, hybridization between the genotype RTCNP 14 of cluster X and genotype RTCNP 23 of cluster XII is expected to result in greater variability and transgressive segregants. Minimum inter-cluster distance was

observed between cluster X and XI (58.76), indicating their close relationship and similarity with regards to the characters studied for most of the genotypes in the two clusters. Further, intra-cluster distance was observed to be zero for the monogenotypic clusters (VII, VIII, IX, X, XI and XII). The genotypes included in cluster VI exhibited maximum intra-cluster distance (142.77) and are therefore inferred to be more divergent than those in other clusters.

The cluster mean values for anaerobic germination traits studied are presented in Table 4. A perusal of these results revealed considerable differences between the clusters for all characters under study. Cluster mean for germination per cent was highest in cluster IX (77.11%) and lowest in cluster IV (33.28%), while for shoot length, it was highest in cluster VIII (3.38 cm) and lowest in V (1.90 cm). Similarly, root length was highest in cluster IX (1.07 cm) and lowest in VI (2.07 cm), while seedling dry weight was highest in cluster VII (26.00 mg) and cluster XII (26.00 mg) and lowest in cluster X (4.00 mg). Similarly, seedling vigour index mean value was highest in cluster VI (3.56) and lowest in cluster IV (1.09). Further, anaerobic response index was highest in cluster II (1.58) and lowest in cluster XII (0.39). Selection of genotypes from clusters with high mean for the respective traits is suggested for utilization in hybridization programmes aimed at improvement of the respective traits. A perusal of these results also revealed that there was no single cluster with all the desirable traits, which ruled out the possibility of direct selection of genotypes for immediate use. Therefore, judicious combination of all the targeted traits requires hybridization between the selected genotypes from divergent clusters.

Information on the relative contribution of various anaerobic germination characters towards divergence was also reported to aid the breeder in choice of parents for hybridization and effective selection (Prasad *et al.*, 2018) <sup>[9]</sup>. In the present study, anaerobic response index contributed maximum (27.24%), followed by seedling dry weight (24.63%), root length (22.78%), germination per cent (16.05%) and shoot length (9.17%) towards the total divergence (Table 5). Contribution of seedling vigour index to the total divergence was however, relatively low (0.12%). Therefore anaerobic response index, seedling dry weight, root length, germination per cent and shoot length contributing to 99.87 per cent of the total divergence need to be stressed in selection of parents for hybridization. Similar results have been reported earlier by Srilakshmi (2016)<sup>[10]</sup> and Ravikanth (2018)<sup>[11]</sup>.

S. No.	Centre of Collection	Genotypes obtained
1	Maruteru, Andhra Pradesh, India	<ul> <li>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 36, RTCNP 37, RTCNP 29, RTCNP 39, RTCNP 40, RTCNP 41, RTCNP 42, RTCNP 43, RTCNP 44, RTCNP 45, RTCNP 46, RTCNP 47, RTCNP 48, RTCNP 49, RTCNP 50, RTCNP 52, 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-30, SM-31, SM-31</li> </ul>
2	Bapatla, Andhra Pradesh, India	BPT 2231, BPT 3291, BPT 5204
3	Pulla, Andhra Pradesh, India	PLA-1100
4	IRRI, Phillipines	FL 478, NONA BOKRA, POKKALI

#### Table 2: Clustering pattern of 107 genotypes of rice

Cluster No.	No. of genotypes	Name of the genotypes
Ι	25	MTU 1064, MTU 1210, BPT 3291, MTU 1061, MTU 1184, RTCNP 41, SM 16, RTCNP 3, RTCNP 7, RTCNP
		9, MTU 1006, MTU 1032, SM 13, MTU 1075, MTU 1001, RTCNP 8, FL 478, BPT 5204, RTCNP 47, SM 6,
		RTCNP 13, RTCNP 29, SM 11, SM 24, SM 4
Π	23	MTU 1071, MTU 1078, MTU 1187, MTU 1224, PLA 1100, SM 30, RTCNP 17, MTU 5249, SM 19, SM 15,
		RTCNP 5 MTU 2077, POKKALI, MTU 1112, SM 18, RTCNP 42, RTCNP 33, RTCNP 40, SM 8, SM 23, MTU
		1031, MTU 1229, SM 2
	21	SM 26, RTCNP 12, RTCNP 44, MTU 7029, SM 28, RTCNP 49, SM 31, MTU 1194, RTCNP 20, RTCNP 21,
III		RTCNP 36, RTCNP 46, MTU 4870, SM 17, RTCNP 48, RTCNP 28, MTU 5293, RTCNP 18, RTCNP 38,
		RTCNP 50, RTCNP 34
IV	10	SM 14, RTCNP 1, MTU 1156, RTCNP 10, SM 7, MTU 1010, SM 3-1, SM 10, NONA BOKRA, RTCNP 22
v	13	MTU 1166, SM 29, SM 9, SM 3, RTCNP 52, RTCNP 6, BPT 2231, SM 25, RTCNP 4, RTCNP 35, RTCNP 45,
		RTCNP 43, MTU 3626
VI	9	MTU 2716, MTU 5182, MTU 1153, MTU 2067, MTU 1121, MTU 1226, MTU 1140, RTCNP 15, RTCNP 37
VII	1	RTCNP 39
VIII	1	SM 1
IX	1	SM 28
Х	1	RTCNP 14
XI	1	RTCNP 31
XII	1	RTCNP 23

Table 3: Average intra-and inter –cluster D<sup>2</sup> values of 107 rice genotypes.

Cluster number	Ι	II	III	IV	V	VI	VII	VIII	IX	Х	XI	XII
Ι	50.28	109.60	188.38	322.02	135.82	169.05	208.49	395.54	419.54	160.56	243.65	413.65
II		48.60	151.84	282.85	126.90	249.94	238.31	281.58	319.68	118.80	173.62	423.53
III			89.120	187.63	187.07	192.27	251.29	116.20	171.29	187.58	130.73	195.25
IV				84.870	267.29	361.60	463.32	230.17	387.42	166.11	152.31	337.14
V					125.02	261.40	179.03	362.20	332.49	207.39	284.40	356.90
VI						142.77	214.66	308.97	303.50	326.26	304.76	240.17
VII							0.00	433.29	209.16	487.43	531.81	200.96
VIII								0.00	110.70	300.12	123.76	178.03
IX									0.00	522.53	370.16	73.40
Х										0.00	58.76	561.07
XI											0.00	419.23
XII												0.00

Diagonal bold values indicate intra cluster distances

#### Table 4: Mean values of twelve clusters by Tocher's method for 107 rice genotypes

Cluster Number	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling dry weight (mg)	Seedling vigour index	Anaerobic response index
Ι	74.33	2.25	1.84	12.60	3.04	1.40
II	75.55	2.88	1.25	12.06	3.13	1.58
III	65.78	2.67	1.44	14.85	2.72	0.82
IV	33.28	2.09	1.29	9.43	1.09	0.55
V	65.77	1.90	1.31	14.63	2.15	1.06
VI	71.07	2.90	2.07	18.33	3.56	1.36
VII	75.99	2.31	1.48	26.00	2.88	1.39
VIII	65.27	3.38	1.23	15.50	3.01	0.58
IX	77.11	3.06	1.07	25.00	3.18	0.62
Х	53.51	2.68	1.46	4.00	2.22	1.38
XI	57.65	3.08	1.47	6.00	2.62	0.98
XII	60.00	2.49	1.58	26.00	2.45	0.39

Table 5: Contribution of different characters towards genetic divergence among 107 genotypes of rice

Character	% Contribution towards divergence
Germination (%)	16.05
Shoot length (cm)	9.17
Root length (cm)	22.78
Seedling dry weight (mg)	24.63
Seedling vigour index	0.12
Anaerobic response index	27.24



Fig 1: Dendrogram showing relationship among 107 rice genotypes in 12 clusters based on Mahalanobis D<sup>2</sup> values



Fig 2: Intra-and Inter-cluster distances of 107 rice genotypes in 12 clusters  $\sim$  470  $\sim$ 



Fig 3: Seeds in petriplate at pigeon breast stage



Fig 4: Pre-germinated seeds transferred to test tubes with 10 cm water

#### Conclusions

A perusal of the results suggest hybridization between RTCNP 14 of cluster X and the genotype, RTCNP 23 of cluster XII for realization of transgressive segregants towards development of varieties with high degree of tolerance for germination under anaerobic conditions for use under wet direct seeding in puddled conditions.

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