

## **Genetic Divergence Studies for Anaerobic Germination Traits in Rice (*Oryza sativa* L.)**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors KS, TS and BNVSRRK designed the study and wrote the protocol. Author KS performed statistical analysis and wrote the first draft of manuscript. Authors TS, BNVSRRK and DPBJ managed the analyses of the study. Author KS managed the literature searches. All authors read and approved the final manuscript.*

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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  $D^2$ . The 107 rice genotypes studied were grouped into nine clusters. Twenty nine genotypes were grouped in cluster II followed by 27, 22, 10, 11 and 5 genotypes each in clusters I, III, IV, VI and cluster VII, respectively. 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

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cluster VII and cluster IX, while intra-cluster distance was noticed to be maximum for cluster VI. Cluster IX had recorded higher cluster mean values for all the traits studied. Further, the traits, namely, shoot length, seedling dry weight and anaerobic response index together accounted for 62.45 per cent of the total genetic divergence in this study.

**Keywords:** Anaerobic response index; genetic divergence; rice.

## 1. INTRODUCTION

Rice crop belongs to the family gramineae and is the world's most important cereal food crop [1]. It is the primary source of food and protein for about half of the mankind and therefore has an enormous nutritional and economic impact. It is the crucial dietary and food security source of many Asian countries. The progressive scarcity of resources like water, energy and human labor as well as the changing climate scenario worldwide has led to marked shift of rice cropping system from puddled transplanting to direct seeding in majority of rice growing areas.

Direct seeding is increasingly becoming popular among the rice farmers due to the ease in cultivation and other economic benefits [2] in addition to early crop maturity and increased yield [3]. This shift of rice cultivation to direct seeding method is alarming as one third of the rice crop worldwide is being cultivated in flood-prone areas [4]. Flooding directly affects germination and seedling establishment leading to tremendous loss and has therefore been identified as the major drawback for large scale adoption of direct seeding. The loss is attributed to rice being extremely sensitive to anaerobic (anoxia / hypoxia) conditions during germination and early growth of embryo [5].

Lack of tolerance to anaerobic germination (AG) results in insufficient energy supply for the growing embryo under oxygen deficit conditions caused by submergence [6]. Some rice genotypes have been reported to germinate under submerged conditions, however, only the tolerant genotypes survive by extending their coleoptiles or shoots above water, under declined oxygen levels [7]. Further, varietal differences for anaerobic germination were also observed and reported earlier [8]. The anaerobic germination processes were also reported to be more efficient in some rice genotypes [9]. The genotypes tolerant to anaerobic stress were reported to exhibit rapid coleoptile elongation after germination resulting in improved seedling survival under anaerobic conditions [10].

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 [11]. However, to execute a breeding programme aimed at the development of rice varieties tolerant to germination under anaerobic conditions, it is essential to study the amount of variability and genetic diversity of the experimental material for the anaerobic germination tolerance traits.

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.

## 2. 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 ARS, Pulla of Andhra Pradesh, India. In addition, germplasm was also 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 [12] 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 (35.5×10×4.5 cm) at about 1 cm soil depth and submerged in tanks by filling water upto 10 cm above the trays. Observations were recorded after 14<sup>th</sup> day of submersion. 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 [13] and anaerobic response index [10] were estimated as per the standard procedures suggested by earlier workers. The data obtained

was subjected to standard statistical procedures. Genetic divergence analysis was done following the  $D^2$  statistics [14]. The analysis was carried out using the software WindowStat Version 8.5.

### 3. RESULTS

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 Figs. 1-2. A perusal of the results on grouping of genotypes (Table 2 and Fig. 1) revealed that the 107 genotypes were grouped into nine clusters based on the relative magnitude of  $D^2$  values such that the genotypes belonging to same cluster had an average smaller  $D^2$  value than those belonging to different clusters. Among the nine clusters, cluster II was largest comprising of 29 genotypes representing collections from different centers, namely, Maruteru and Bapatla of Andhra Pradesh, India, in addition to germplasm from IRRI, Phillipines. Similarly, cluster III comprising of 22 genotypes also included collections from Maruteru and Bapatla of Andhra Pradesh, India and germplasm from IRRI, Phillipines; cluster IV comprising of 10 genotypes included collections from Maruteru and Pulla centre and cluster VII comprising of five genotypes included collections from Maruteru and germplasm from IRRI, Phillipines. However, cluster I comprising of 27 genotypes and cluster VI comprising of 11 genotypes included collections from Maruteru centre only. Further cluster V, cluster VIII and cluster IX were solitary or mono genotypic clusters with zero intra cluster  $D^2$  values. 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 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, as the genotypes in the present study are advanced breeding lines developed at the respective centers, rather than landraces evolved at the respective centers and also due to the increased collaboration between these centers and exchange of material for use in their respective breeding programs.

An analysis of inter- and intra- cluster distances (Table 3 and Fig. 2) revealed maximum inter-cluster distance between cluster VII and IX (453.29), followed by cluster IV and VII (274.24) 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 genotypes (MTU 1010, MTU 1156, SM 10, NONA BOKRA, SM 14) of cluster VII and genotype (MTU 1140) of cluster IX is expected to result in greater variability and transgressive segregants. Minimum inter-cluster distance was observed between cluster V and VI (27.45), 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 (V, VIII and IX). The genotypes included in cluster VI exhibited maximum intra-cluster distance (31.06) and are therefore inferred to be more divergent than those in other clusters.

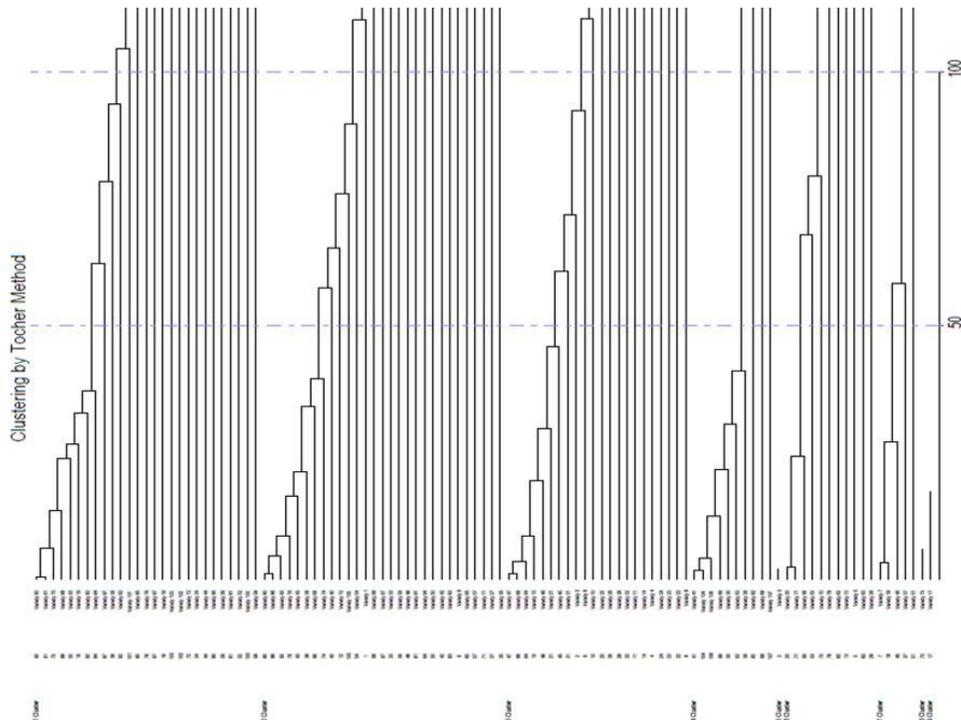
The cluster mean values for anaerobic germination traits studied are presented in Table 4. A perusal of these results revealed considerable differences between the clusters for all characters under study. Cluster means for all the traits studied in the investigation, namely germination per cent, shoot length, root length, seedling dry weight, seedling vigour index and anaerobic response index recorded highest in cluster IX, followed by cluster IV and lowest in cluster VII.

### 4. DISCUSSION

Selection of genotypes from clusters with high mean (IX and IV) is suggested for utilization in hybridization programmes aimed at improvement of the anaerobic germination traits. Further, MTU 1140 of cluster IX is reported to be a high yielding rice variety and also possess tolerance for germination under anaerobic conditions in addition to withstanding of flooding at different stages of crop growth [15]. Therefore, hybridization of genotype of cluster IX (MTU 1140) with genotypes of cluster VII (MTU 1010, MTU 1156, NONA BOKRA, SM 10, SM 14) are expected to result in transgressive segregants due to their high diversity. Among these, hybridization of MTU 1140 genotype with MTU 1010, a high yielding, mega rice variety [16] and MTU 1156 also high yielding rice variety [17] are

**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, 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-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, Phillipines	FL 478, NONA BOKRA, POKKALI



**Fig. 1. Dendrogram showing relationship among 107 rice genotypes in nine clusters based on Mahalanobis  $D^2$  values for anaerobic germination traits**

**Table 2. Clustering pattern of 107 rice genotypes for anaerobic germination traits by Tocher's method**

Cluster No.	No. of genotypes	Genotypes
I	27	RTCNP 17, RTCNP 18, RTCNP 10, RTCNP 43, RTCNP 15, SM 18, MTU 1153, SM 26, RTCNP 38, RTCNP 41, MTU 7029, RTCNP 20, RTCNP 45, RTCNP 39, RTCNP 12, RTCNP1, MTU 1121, RTCNP 47, RTCNP 46, RTCNP 7, MTU 5249, SM 6, RTCNP 42, SM 19, SM 27, SM 15, RTCNP 44, SM 2
II	29	RTCNP 34, RTCNP 40, RTCNP 23, RTCNP 14, SM 4, POKKALI, RTCNP 3, RTCNP 9, SM 1, MTU 1071, RTCNP 49, SM 17, BPT 5204, RTCNP 33, RTCNP 29, MTU 5182, SM 24, SM 11, RTCNP 35, SM 30, SM 13, SM 3, RTCNP 37, MTU 1031, SM 31, SM 23, RTCNP 6, MTU 1229, MTU 5293
III	22	SM 9, SM 3-1, RTCNP 22, MTU 1112, SM 8, MTU 1184, SM 7, MTU 3626, BPT 3291, MTU 1006, MTU 1061, MTU 1224, MTU 2067, MTU 2077, MTU 4870, MTU 1064, MTU 1078, FL 478, MTU 1210, MTU 1194, MTU 1187, MTU 1032
IV	10	SM 2, RTCNP 48, RTCNP 50, RTCNP 28, MTU 2716, RTCNP 21, PLA-1100, RTCNP 36, RTCNP 4, RTCNP 52
V	1	BPT 2231
VI	11	MTU 1166, RTCNP 13, RTCNP 31, SM16, RTCNP5, RTCNP 15, SM 28, MTU 1075, MTU 1001, SM 29, MTU 1226
VII	5	MTU 1010, MTU 1156, SM 10, NONABOKRA, SM 14
VIII	1	RTCNP 8
IX	1	MTU 1140

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

Cluster number	I	II	III	IV	V	VI	VII	VIII	IX
I	<b>12.44</b>	36.40	37.48	116.08	47.40	35.77	74.06	28.24	258.00
II		<b>14.84</b>	75.45	46.66	42.82	27.86	143.40	41.40	136.83
III			<b>15.37</b>	178.03	33.03	54.51	31.93	57.29	331.45
IV				<b>14.90</b>	106.93	79.69	274.24	115.66	43.58
V					<b>0.00</b>	27.45	85.40	75.12	224.63
VI						<b>31.06</b>	112.81	53.33	189.98
VII							<b>23.28</b>	96.04	453.29
VIII								<b>0.00</b>	230.50
IX									<b>0.00</b>

*Diagonal bold values indicate intra cluster distances*

expected to result in transgressive segregants with high yield and tolerance to anaerobic germination.

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 [18]. In the present study, shoot length contributed maximum (22.01%), followed by seedling dry weight (21.18%), anaerobic response index (19.26), germination

per cent (19.01%) and root length (18.52%) towards the total divergence (Table 5). Shoot length of the seedlings under submergence has also been reported to be an important trait contributing to anaerobic germination tolerance as it determines survival of the seedling under anaerobic conditions [7]. Seedling dry weight and anaerobic response index, the next important contributors for genetic divergence in the present study have also been estimated based on shoot length and hence, their contribution towards genetic diversity is also observed to

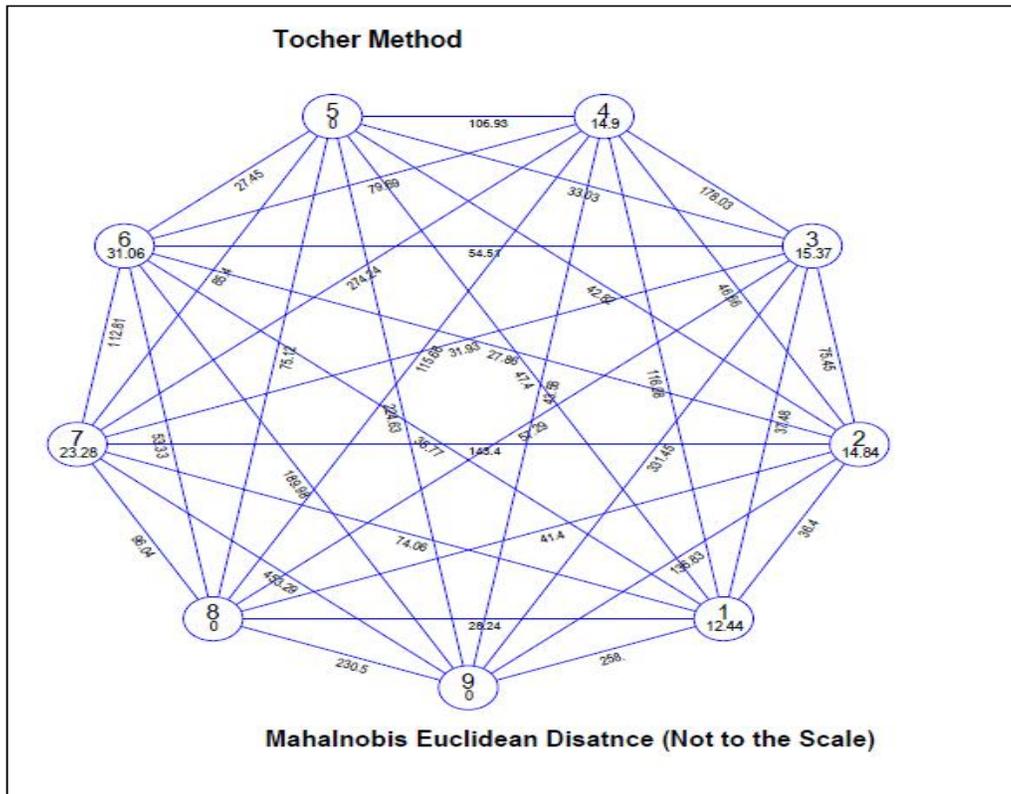


Fig. 2. Intra- and Inter- cluster distances of 107 rice genotypes in nine clusters for anaerobic germination traits

Table 4. Mean values of nine clusters for anaerobic germination traits 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
I	55.17	15.15	4.23	18.16	10.71	1.68
II	63.41	17.88	4.88	23.67	14.47	2.67
III	38.93	14.02	2.42	19.20	6.42	1.43
IV	79.34	22.04	5.61	28.36	21.96	3.48
V	47.87	16.12	2.90	27.00	9.11	1.92
VI	56.94	16.81	4.14	24.23	11.96	2.17
VII	32.08	11.55	1.53	14.85	4.20	0.94
VIII	44.98	16.85	4.65	15.10	9.70	2.85
IX	83.51	25.65	6.65	32.90	27.00	4.85

Table 5. Contribution of different anaerobic germination characters towards genetic divergence among 107 genotypes of rice

Character	% Contribution towards divergence
Germination (%)	19.01
Shoot length (cm)	22.01
Root length (cm)	18.52
Seedling dry weight (mg)	21.18
Seedling vigour index	0.04
Anaerobic response index	19.26

be relatively high. Contribution of seedling vigour index to the total divergence was however, relatively low (0.04%). The results are in agreement with the reports of earlier workers [19]. Therefore, shoot length, seedling dry weight and anaerobic response index, contributing to 62.45 per cent of the total divergence need to be stressed in selection of parents for hybridization.

## 5. CONCLUSION

A perusal of the results revealed existence of genetic diversity in the experimental material studied for the anaerobic germination tolerance traits. MTU 1140, tolerant to anaerobic germination and flooding was observed to most diverse from other genotypes studied and formed a separate cluster with high means for most of the anaerobic germination traits studied. Hybridization between the high yielding genotypes, MTU 1140 of cluster IX with the genotypes, MTU 1010 and MTU 1156 of cluster VII exhibiting wide genetic diversity and maximum inter cluster distance is suggested for realization of transgressive segregants towards development of high yielding rice varieties coupled with high degree of tolerance for germination under anaerobic conditions for use under wet direct seeding in puddled conditions to help the rice farmer in overcoming the problem of acute labour shortage and reducing the cost of cultivation.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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