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Screening for Tolerance to Anaerobic Germination in Rice (*Oryza sativa* L.)

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A B S T R A C T

The present investigation was carried out during *Kharif* 2017 at Regional Agricultural Research Station, Maruteru, Andhra Pradesh with an objective to screen the rice genotypes for their tolerance to anaerobic conditions during germination and also to identify varieties suitable for direct seeded rice cultivation, using two different methods, namely, test tube method and pro-tray method. The results revealed MTU 1140 to be superior with respect to germination percent, shoot length, root length and seedling dry weight in addition to seedling vigour index and anaerobic response index in both the screening methods and hence, the genotype is identified to be most promising for growing under direct seeded conditions. Among the screening methods, test tube method of screening is inferred to be more useful for evaluating large number of genotypes, while pro-tray method of screening is recommended for greater accuracy of the results.

Introduction

Rice production in the recent years is increasingly shifting from transplanting to direct seeding, particularly under puddled conditions, 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. Flooding due to rainfall or improper leveling of land, resulting in unfavorable anaerobic conditions for the rice seed, leads to poor germination and failure to develop strong and uniform seedlings under direct seeded conditions. It is attributed to the lack of tolerance to anaerobic germination (AG) which results in insufficient energy supply for the growing embryo under oxygen deficit conditions caused by submergence and is identified as the main limiting factor for direct seeding in rice (Yang *et al.*, 2019). However, varietal differences for anaerobic germination were observed by Ismail *et al.,* (2009). Further *et al.,* (2013) reported that unique anaerobic germination processes may be more efficient in some rice genotypes. Hsu and Tung (2015) reported that genotypes tolerant to anaerobic stress exhibited rapid coleoptile elongation after germination resulting in improved seedling survival under anaerobic conditions.

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). In this context, the present investigation was undertaken to identify rice varieties capable of surviving under flooded anaerobic conditions during germination and early growth stages for potential use under direct seeding in standing water under puddled conditions.

Materials and Methods

The experimental material consisted of 107 elite rice genotypes (Table 1). Screening of the rice varieties for their tolerance to anaerobic conditions was undertaken at Regional Agricultural Research Station, Maruteru during *Kharif* 2017 with test tube method detailed by Hsu and Tung (2015) and pro-tray method detailed by Chaitanya (2016). Screening of rice varieties under both methods was carried out in completely randomized design with two replications.

Screening with test tube method was undertaken with three days pre-germinated seeds at pigeon breast stage in glass test tubes (25 mm in diameter and 150 mm in height) filled with 10 cm deep distilled water (Plate 1). In pro-tray method, seeds were sown in pro-trays of (35.5×10×4.5cm) at about 1 cm soil depth and submerged in tanks (Plate 1). Observations were recorded after seven days in the test tube method and after 14th day of submergence in the pro-tray method. Data on number of seedlings survived after 7 or 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 both the methods 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.

Results and Discussion

The results on screening of 107 rice genotypes for their ability to germinate under anaerobic conditions of standing water was evaluated with respect to six anaerobic germination traits, namely germination per cent, shoot length, root length, seedling dry weight, seedling vigour index and anaerobic response index and the range of results obtained are presented in Table 2 and Figure 1. Wide variability was noticed for all the traits studied with respect to response of the rice genotypes under anaerobic conditions under both methods of screening. Germination per cent in the present study ranged from 20 (MTU 1156 and RTCNP 23) to 99 per cent (MTU 1140) in the test tube method of screening and from 25 (MTU 1010, MTU 1156, BPT 3291, MTU 1187, MTU 1064 and Nonabokara) to 95 per cent (MTU 1140 and RTCNP 50) in pro-tray method. Based on germination percentage, the rice genotypes were classified as susceptible (0-50%), moderately tolerant (51-75%) and tolerant (76-100%) in accordance with the classification given by Manigbas *et al.,* (2008).

In test tube method of screening, 11 rice genotypes recorded less than 50 per cent germination and were susceptible for anaerobic conditions. Ten rice genotypes recorded 50-75 per cent germination and hence, were classified as moderately tolerant. However, 86 genotypes recorded more than 75 per cent germination and were classified as tolerant for germination under anaerobic conditions. In Pro-tray method of screening, 20 rice genotypes had recorded less than 50 per cent germination and were found to be susceptible for germination under anaerobic conditions, while 53 rice genotypes recorded 50-75 per cent germination and hence were classified as moderately tolerant. However, 34 genotypes recorded more than 75 per cent germination under this method and were classified as tolerant for germination under anaerobic conditions. In both the screening methods, highest germination percentage was recorded by MTU 1140 (95%) and lowest was recorded by MTU 1156 (<26%) High germination percentage of the tolerant genotype, MTU 1140 and others might be due to their rapid breakdown of starch into soluble sugars, useful for growing embryo. Girijarani *et al.,* (2014) also reported MTU 1140 to be able to withstand anaerobic conditions during germination. The results are also in conformity with the reports of Reddy and Girijarani (2018). Further, eight genotypes were observed to be susceptible while four were moderately tolerant to germination under anaerobic conditions. However, 31 genotypes were observed to be tolerant uniformly under both methods of screening (Table 3) and may be recommended for direct seeding under standing water.

Rapid shoot growth is also considered as one of the important traits associated with anaerobic germination tolerance (Ismail *et al.,* 2009). Highest shoot length was exhibited by MTU 1140 (25.65cm) in pro-tray method and RTCNP 28 (3.62cm) in test tube method, indicating their tolerance for anaerobic conditions. The tolerance may be attributed to their rapid shoot elongation when compared with susceptible genotypes so as to reach the water surface resulting in diffusion of air to the growing leaf and root for better seedling establishment. Similar results were reported by Rauf *et al.,* (2019). In the present study also, the susceptible genotypes had recorded low shoot length. Lowest shoot length was exhibited by SM-10 (9.75cm) in pro-tray method and SM-3 (1.12 cm) in test tube method.

Differential responses of root growth were also observed in the genotypes studied in both the screening methods. Root length was observed to range from 0 cm (MTU 1078, MTU 1166, MTU 1187, SM-3, SM 3-1and RTCNP 14) to 4.85 cm (MTU 1032) in the test tube method and from 0.35cm (MTU 1010) to 6.65cm (MTU 1140) in the pro-tray method of screening. MTU 1140, the tolerant line has exhibited maximum germination per cent (95%) and also maximum root length (6.65cm) in the pro-tray method, while minimum germination per cent (25%) and root length (0.35cm) was recorded for MTU 1010, the susceptible line in the pro-tray method of screening, indicating the relationship of germination per cent and root length in tolerant and susceptible lines for anaerobic conditions. The results are in broad agreement with the reports of Bordoloi and Sharma (2018).

The trait, seedling dry weight also exhibited wide variation among the genotypes studied. It ranged from 14 mg (RTCNP14) to 26 mg (RTCNP 15, RTCNP 23, RTCNP 37 and RTCNP 39) in test tube method of screening and from 13.75mg (MTU 1010) to 32mg (MTU 1140) under pro-tray method of screening. The tolerant genotype, MTU 1140 has recorded maximum seedling dry weight of 32 mg under pro-tray method of screening, while the susceptible genotype, MTU 1010 has recorded minimum seedling weight of 13.75 mg under pro-tray method of screening, indicating the relationship between seedling dry weight and tolerance for anaerobic conditions. Similar results were reported by Barik *et al.*, (2019).

Ample variation was also noticed among the genotypes for seedling vigour index, calculated based on germination percentage, shoot length and root length. In the test tube method, seedling vigor index ranged from 0.76 (MTU 1010) to 4.77 (MTU 1140), while in pro-tray method, it ranged from 3.65 (MTU 1010) to 27.00 (MTU 1140). Results of the present study revealed that high germination rate, rapid shoot and root elongation are major traits closely related to seedling vigour and responsible for optimum seedling establishment under submerged conditions. These results are in agreement with earlier findings of Barik *et al.*, (2019).

Table.1 List of genotypes screened in the study for different anaerobic germination traits

S.No.	Genotype	Source	S.No.	Genotype	Source
1	BPT 5204	ARS, Bapatla	34	MTU 5249	RARS, Maruteru
2	BPT 3291	ARS, Bapatla	35	MTU 5293	RARS, Maruteru
3	BPT 2231	ARS, Bapatla	36	MTU 7029	RARS, Maruteru
4	FL 478	RARS, Maruteru	37	NONABOKRA	RARS, Maruteru
5	MTU 1001	RARS, Maruteru	38	PLA-1100	RARS, Maruteru
6	MTU 1006	RARS, Maruteru	39	POKKALI	RARS, Maruteru
7	MTU 1010	RARS, Maruteru	40	SM-1	RARS, Maruteru
8	MTU 1031	RARS, Maruteru	41	SM-2	RARS, Maruteru
9	MTU 1032	RARS, Maruteru	42	SM-3	RARS, Maruteru
10	MTU 1061	RARS, Maruteru	43	SM-4	RARS, Maruteru
11	MTU 1064	RARS, Maruteru	44	SM-6	RARS, Maruteru
12	MTU 1071	RARS, Maruteru	45	SM-7	RARS, Maruteru
13	MTU 1075	RARS, Maruteru	46	SM-8	RARS, Maruteru
14	MTU 1078	RARS, Maruteru	47	SM-9	RARS, Maruteru
15	MTU 1112	RARS, Maruteru	48	SM-10	RARS, Maruteru
16	MTU 1121	RARS, Maruteru	49	SM-11	RARS, Maruteru
17	MTU 1140	RARS, Maruteru	50	SM-13	RARS, Maruteru
18	MTU 1153	RARS, Maruteru	51	SM-14	RARS, Maruteru
19	MTU 1156	RARS, Maruteru	52	SM-15	RARS, Maruteru
20	MTU 1166	RARS, Maruteru	53	SM-16	RARS, Maruteru
21	MTU 1184	RARS, Maruteru	54	SM-17	RARS, Maruteru
22	MTU 1187	RARS, Maruteru	55	SM-18	RARS, Maruteru
23	MTU 1194	RARS, Maruteru	56	SM-19	RARS, Maruteru
24	MTU 1210	RARS, Maruteru	57	SM-23	RARS, Maruteru
25	MTU 1224	RARS, Maruteru	58	SM-24	RARS, Maruteru
26	MTU 1226	RARS, Maruteru	59	SM- 25	RARS, Maruteru
27	MTU 1229	RARS, Maruteru	60	SM-26	RARS, Maruteru
28	MTU 2067	RARS, Maruteru	61	SM-27	RARS, Maruteru
29	MTU 2077	RARS, Maruteru	62	SM-28	RARS, Maruteru
30	MTU 2716	RARS, Maruteru	63	SM-29	RARS, Maruteru
31	MTU 3626	RARS, Maruteru	64	SM-30	RARS, Maruteru
32	MTU 4870	RARS, Maruteru	65	SM-31	RARS, Maruteru
33	MTU 5182	RARS, Maruteru	66	SM-3-1	RARS, Maruteru

Table.1 continued

S.No.	Genotype	Source	S.No.	Genotype	Source
67	RTCNP 1	RARS, Maruteru	88	RTCNP 31	RARS, Maruteru
68	RTCNP 3	RARS, Maruteru	89	RTCNP 33	RARS, Maruteru
69	RTCNP 4	RARS, Maruteru	90	RTCNP 34	RARS, Maruteru
70	RTCNP 5	RARS, Maruteru	91	RTCNP 35	RARS, Maruteru
71	RTCNP 6	RARS, Maruteru	92	RTCNP 36	RARS, Maruteru
72	RTCNP 7	RARS, Maruteru	93	RTCNP 37	RARS, Maruteru
73	RTCNP 8	RARS, Maruteru	94	RTCNP 38	RARS, Maruteru
74	RTCNP 9	RARS, Maruteru	95	RTCNP 39	RARS, Maruteru
75	RTCNP 10	RARS, Maruteru	96	RTCNP 40	RARS, Maruteru
76	RTCNP 12	RARS, Maruteru	97	RTCNP 41	RARS, Maruteru
77	RTCNP 13	RARS, Maruteru	98	RTCNP 42	RARS, Maruteru
78	RTCNP 14	RARS, Maruteru	99	RTCNP 43	RARS, Maruteru
79	RTCNP 15	RARS, Maruteru	100	RTCNP 44	RARS, Maruteru
80	RTCNP 17	RARS, Maruteru	101	RTCNP 45	RARS, Maruteru
81	RTCNP 18	RARS, Maruteru	102	RTCNP 46	RARS, Maruteru
82	RTCNP 20	RARS, Maruteru	103	RTCNP 47	RARS, Maruteru
83	RTCNP 21	RARS, Maruteru	104	RTCNP 48	RARS, Maruteru
84	RTCNP 22	RARS, Maruteru	105	RTCNP 49	RARS, Maruteru
85	RTCNP 23	RARS, Maruteru	106	RTCNP 50	RARS, Maruteru
86	RTCNP 28	RARS, Maruteru	107	RTCNP 52	RARS, Maruteru
87	RTCNP 29	RARS, Maruteru			

Table.2 Maximum and minimum mean values recorded for 107 rice genotypes with respect to anaerobic germination traits in the screening methods studied

S.No.	Character	Test tube method			Pro-tray method		
		Maximum	**Minimum**	**Mean**	**Maximum**	**Minimum**	**Mean**
1	Germination (%)	MTU 1140 (99.00)	MTU 1156 (20.00), RTCNP 23 (20.00)	82.94	MTU 1140 (95.00), RTCNP 50 (95.00)	MTU 1010, MTU 1156, BPT 3291, MTU 1187, NONABOKRA, MTU 1064, BPT 3291 (25.00)	64.96
2	Shoot length (cm)	RTCNP 28 (3.62)	SM-3 (1.12)	2.49	MTU 1140 (25.65)	SM-10 (9.75)	16.42
3	Root length (cm)	MTU 1032 (4.85)	MTU 1078, MTU 1166, MTU 1187, SM-3, SM 3-1, RTCNP 14 (0.00)	1.40	MTU 1140 (6.65)	MTU 1010 (0.35)	4.04
4	Seedling dry weight (mg)	RTCNP 15, RTCNP 23, RTCNP 37, RTCNP 39 (26.00)	RTCNP 14 (4.00)	13.60	MTU 1140 (32.00)	MTU 1010 (13.75)	21.48
5	Seedling vigour index	MTU 1140 (4.77)	MTU 1010 (0.76)	2.73	MTU 1140 (27.00)	MTU 1010 (3.65)	11.85
7	Anaerobic response index	MTU 1140 (2.28)	MTU 1156 (0.26), SM-10 (0.26)	1.17	MTU 1140 (4.85)	MTU 1010 (0.60)	2.12

Table.3 Tolerant and susceptible genotypes identified for anaerobic germination in the screening methods studied

S.No.	Classification	Number of genotypes	Pro-Tray and Test tube method of screening
1	Susceptible	8	MTU 1156, MTU 1010, SM-10, SM-14, SM 3-1, SM-7, SM-27, NONABOKRA
2	Moderately tolerant	4	MTU 1166, MTU 7029, MTU 5293, RTCNP 45
3	Tolerant	31	MTU 1031, MTU 1071, POKKALI, SM-4, SM-16, RTCNP 7, RTCNP 33, RTCNP 34, RTCNP 49, SM-24, SM 30, RTCNP 4, RTCNP 9, RTCNP 36, RTCNP 40, RTCNP 46, RTCNP 48, SM-2, SM-23, SM-31, RTCNP 5, RTCNP 15,RTCNP 21, RTCNP 29, RTCNP 35, RTCNP 37, MTU 2716, RTCNP 28, PLA-1100, MTU 1140, RTCNP 50.

Table.4 Promising tolerant genotypes identified for anaerobic germination in the screening methods studied

S.No.	Genotypes	Germination (%)		Shoot length (cm)		Root length(cm)		Seedling dry weight (mg)		Seedling vigour index		Anaerobic response index	
		TT	PT	TT	PT	TT	PT	TT	PT	TT	PT	TT	PT
1	MTU 1140	99.00	95.00	3.60	25.65	3.04	6.65	23.45	32.9	4.77	27.00	2.28	4.85
2	MTU 2716	91.00	91.00	3.17	22.85	4.16	5.25	12.70	27.70	4.00	22.69	1.28	3.85
3	SM-2	97.00	89.00	2.79	23.35	1.18	6.20	19.00	28.40	3.29	23.74	1.68	3.55
4	RTCNP 28	95.00	93.00	3.62	23.75	1.29	5.85	14.5	29.55	4.06	23.97	1.62	3.90
5	RTCNP 29	91.00	90.00	1.98	20.25	1.55	5.60	12.5	23.55	2.46	18.64	1.13	3.05

TT: Test Tube method; PT: Pro-tray method

Test tube method of screening

Pro-tray method of screening

Plate.1 Screening methods adopted for studies on anaerobic germination traits in 107 genotypes of rice (*Oryza sativa* L.)

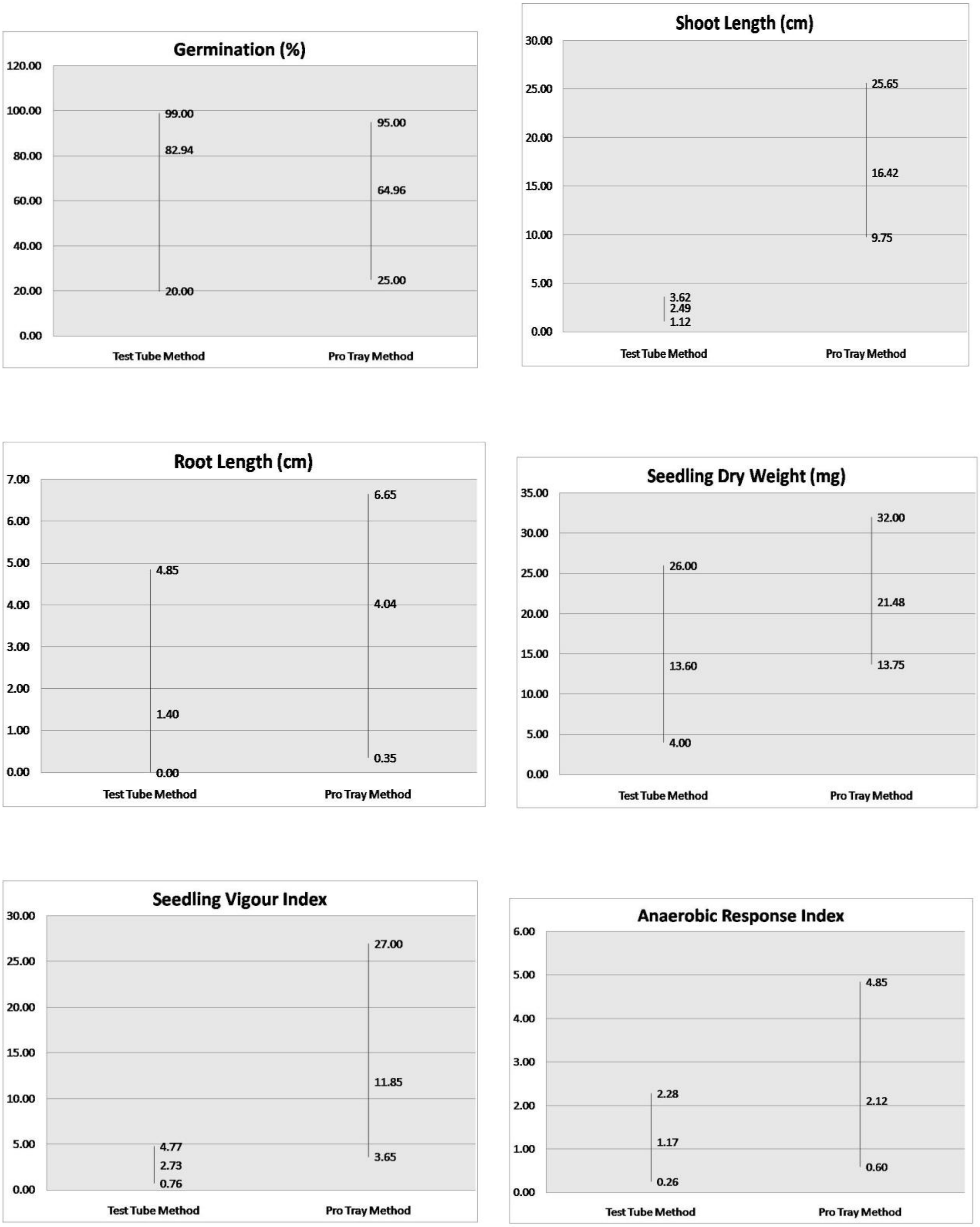

Fig.1 Range for anaerobic germination traits studied in the present investigation for two different screening methods

The trait anaerobic response index (ARI) is one of the crucial parameter that helps to distinguish between tolerant and susceptible entries. Genotypes with the ability to elongate the shoot rapidly under submerged condition along with high germination percentage exhibit higher anaerobic response index. MTU 1156 (0.26) and SM-10 (0.26) exhibited low anaerobic response index and MTU 1140 (2.28) recorded high anaerobic response index in test tube method. In pro-tray method MTU 1010 (0.60) recorded low ARI and MTU 1140 recorded the highest ARI (4.85). The genotypes which exhibited high germination percentage, shoot length and root length exhibited high anaerobic response index in test tube method (MTU 1121, SM-15 and MTU 1140) and pro-tray method (MTU 2716, MTU 1140, POKKALI, PLA-1100, SM-23, SM-31, RTCNP 14, RTCNP 21, RTCNP 29, RTCNP 36, RTCNP 48 and RTCNP 50). These results are in conformity with Hsu and Tung (2015) and Chaitanya (2016).

In general, test tube method of screening resulted in higher mean germination per cent (82.94%), compared to pro-tray method of screening (64.96%) and was found to be effective in categorization of genotypes as tolerant or susceptible for anaerobic germination. This method is therefore inferred to be useful for evaluating large number of genotypes and rapid identification of genotypes tolerant to anaerobic conditions. Roy and Sharma (2014) also reported test tube method of screening to be more simple and rapid method of bioassay for screening large number of genotypes for anaerobic germination. However, with regard to all other traits, namely shoot length (16.42 cm *vs* 2.49 cm), root length (4.04 cm *vs* 1.40 cm), seedling dry weight (21.48 mg *vs* 13.60 mg), seedling vigour index (11.85 *vs* 2.73) and anaerobic response index (2.12 *vs* 1.17) pro-tray method of screening had recorded higher values compared to test tube method of screening. Hence, pro-tray method of screening is recommended for greater accuracy and reliable results for identification of potential genotypes tolerant to anaerobic conditions and use under direct seeding method of rice cultivation in standing water under puddled condition.

The promising genotypes tolerant to anaerobic conditions and suitable for direct seeding under standing water conditions of puddled soil identified in the present study are presented in Table 4. Among these, MTU 1140 possessing AG gene (Girijarani *et al.*, 2014) and superior germination per cent, shoot length, root length and seedling dry weight in addition to seedling vigour index and anaerobic response index was identified as the most promising genotype for direct seeding under both methods of screening studied. Other tolerant genotypes identified *viz.*, MTU 2716, SM-2, RTCNP 28 and RTCNP 48 also exhibited high values for most of the traits in both the methods of screening. These genotypes need to be analyzed further for innate study of their physiological mechanisms for their tolerance to anaerobic conditions under direct seeded cultivation towards development of diverse cultivars tolerant to anaerobic conditions.

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