Received: 03 Jul 2022 | Revised: 29 Jul 2022 | Accepted: 02 Sep 2022

Electronic Journal of Plant Breeding

Research Article

Identification of anaerobic germination tolerant landraces and validation of molecular marker in rice (*Oryza sativa* L.)

G. Mohanapriya[1], S. Kavitha[2], V. Thiruvengadam[3], M. Raveendran[4] and S. Manonmani[5]*

[1]Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu
[2]Department of Plant Genetic Resources, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu
[3]Agricultural College and Research Institute, Kudumiyanmalai, Tamil Nadu
[4]Department of Plant Biotechnology, Tamil Nadu Agricultural University, Tamil Nadu
[5]Department of Rice, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu
***E-Mai**l : manonmanitnau@gmail.com

Abstract

Poor germination and late seedling establishment are the main obstacles observed for wide spread adoption of direct-seeded rice cultivation in both rainfed and irrigated regions due to the waterlogged condition of the soil. The present experiment was carried out to identify rice landraces with anaerobic germination tolerance for the development of varieties suitable for direct seeded rice cultivation. A total of 53 genotypes of rice landraces were evaluated for anaerobic germination tolerance by following the screening methods of petri plate and tray method. The genotypes namely savudu samba, vellai kavuni, varappu kudaichan and korumbaali recorded the maximum germination percentage (> 90%) under anaerobic conditions and these genotypes also produced high dry matter production. The minimum germination percentage was recorded by malai nellu, surakuruvai and mappillai samba and the remaining landraces observed moderate germination percentage under anaerobic germination. Shoot length recorded for all the genotypes ranged from 0.70 to 6.23 cm under anaerobic germination. Dry matter production of genotypes ranged from 0.031 to 0.265 g/10 seedlings. Based on the screening, the best and least performed genotypes were selected for genotyping using already reported SSR markers. The marker RM 341 produces the polymorphism between tolerant and susceptible genotypes, which can be exploited further for Marker Assisted Breeding programme.

Keywords : Rice, Landraces, Anaerobic, Germination, Validation, Molecular marker.

INTRODUCTION

Rice (*Oryza sativa* L.) is the world's most widely consumed grain, feeding half of the world's population. Rice accounts for about 43% of the country's total food grain production and 46% of its overall cereal production. It is the staple diet of more than 60% of the world's population, particularly in Southeast Asia. In order to recover from the food scarcity problem in the near future, it is necessary to enhance food production. Rice is semi-aquatic, adapting to a broad range of environments like aerobic soils in the uplands, anaerobic as well as flooded fields in the lowlands and even deeply submerged soils in flood-prone places. Diverse rice varieties used to grow in Tamil Nadu, but many of them have now been destroyed as a result of the widespread cultivation of high yielding cultivars (Dhanuja *et al*., 2021). Native rice landraces which are preserved and selected by farmers over generations exhibit lot of variations in the field. Globally in order to fulfil the demands of current and future food grain requirements, the crop yield in marginal farmlands with limited resources should be significantly increased. Rice production has transitioned from transplanting to direct seeding in recent years, particularly in places where irrigation water is limited and has high labour costs. When compared to transplanting, direct seeding (DSR) is more cost-effective than the transplanting technique (TPR) in rice cultivation. Direct seeding can save up to 90 % labour costs and extend crop growth by up to 14 days when compared to transplanting. The direct seeding method has some disadvantages like poor germination and failure of seedling establishment due to damages caused by birds, rats, snails and other biological factors; physical damages caused by flooding, water stress, lodging at maturity and weed infestation (Angaji, 2010). Stagnant water on the soil surface restricts oxygen supply for developing seeds and the entire area seems to be barren due to a lack of adequate germination and crop establishment (Ray *et al*., 2016). Due to a poor germination potential under anaerobic conditions leads to poor crop establishment is the key challenge for DSR in severe climatic conditions. Flooding at the germination stage restricted the availability of oxygen for the developing seeds leads to alcoholic fermentation. During alcoholic fermentation, enzymes act as a source of energy and substitute aerobic respiration, anaerobic germination tolerance is linked to rapid germination and coleoptile elongation (Miro *et al*., 2017). Advancement in molecular genetic mapping and marker study in rice has paved the way for mapping individual genes linked to complex traits which may include anaerobic germination tolerance traits (Ling *et al*., 2004). Rice, unlike all the other cereals, can resist partial flooding and water logging due to the existence of an aerenchyma system that promotes aeration. As a result, flood-tolerant rice germplasm is required for direct seeded rice (DSR) conditions. Farmers cultivating various traditional landraces at different environments contain a considerable amount of genetic diversity and it may serve as good genetic stock for AG potential. Plant breeders are currently engaged for identifying genotypes with anaerobic germination tolerance and incorporating this characters into the background of agronomically adapted variety. The major goal of this work is to identify rice landraces with anaerobic germination tolerance from germplasm accession suitable for direct seeded rice production and the landraces selected by phenotyping methods are further studied for genotyping using an SSR marker for confirmation.

MATERIALS AND METHODS

A total of 53 genotypes of rice landraces obtained from the Department of Plant Genetic Resources (PGR), Tamil Nadu Agricultural University, Coimbatore were screened for anaerobic germination at the Department of Plant Genetic Resources, Tamil Nadu Agricultural University, Coimbatore during the year 2021-22. Screening methods namely petri plates in the laboratory as well as tray method in shade net were followed for the anaerobic germination test.

In the case of laboratory analysis of anaerobic screening, 150 petridisc of size 150 x15 mm were used for ten seeds of each germplasm and were soaked in tap water and replicated thrice. To maintain the anaerobic environment throughout the germination stage, the petridisc was kept at room temperature and the water level was maintained for 14 days. For the tray method of screening, ten seeds of each germplasm were sown in trays (Tray size: 35.0 cm x 30.0 cm x 8.0 cm) filled with soil mixture stored at Shade net with three replications. The trays were kept at a 5 cm water level for 14 days. In both the methods, the number of germinated seedling, shoot length (coleoptile length), root length, fresh weight and dry weight of seedling data were recorded after 14 DAS. The dry weight of seedling were taken by drying the seedling at 80°C for three days. The germination percentage and vigour index were calculated.

The genotypes which recorded germination percentages of more than 85 per cent and low germinated ones were subjected to molecular analysis using already reported markers namely RM 341 and RM 206 for anaerobic germination and ART 5 for submergence tolerance. The markers RM 341 and RM 206 were linked to traits of anaerobic germination (Reddy *et al*., 2015) and were used for molecular marker analysis targeting Ag tolerant QTL qAG2 on chromosome 2 and qAG11 on chromosome 11, respectively. The marker ART5, a closely linked marker of *Sub1* found in the promoter region of *Sub1C* was also used for genotyping to find out submergence tolerance genotypes. Genomic DNA of genotypes was isolated by the modified CTAB (Cetyl- Trimethyl Ammonium Bromide) method. For PCR reaction gDNA of each genotype was diluted in 50 ng per µl using nanodrop reading. PCR mixture consists of 1µl DNA sample, 2µl primer and 9 µl Master mix and was amplified using Eppendorf thermocycler with the steps:Initial denaturation at 94°C for 5 min. and further cycles of denaturation at 94°C for 30 sec; Annealing at 55°C (primer specific) for 30 sec; and primer extension at 72°C for 1 min., final cycle of extension alone for 7 min. at 72°C. The amplified products were electrophoresed with Ethidium Bromide stained Agarose Gel at 200 volts for 120 min. Gel images were documented under UV light (260 nm) in Bio-Rad gel documentation unit.

RESULTS AND DISCUSSION

In the present study, 53 rice landrace germplasm were screened for anaerobic germination and exhibited a wide range of variability in germination percentage and shoot length under anoxic conditions (**Table 1 and 2**). The genotypes namely savudu samba, vellai kavuni and varappu kudaichan recorded the maximum germination percentage of 100% under petri plate method, whereas the genotypes korumbaali, savudu samba and vellai kavuni recorded maximum germination percentage of more than 90% under tray method. The genotypes malai nellu, surakuruvai and mappillai samba recorded a low germination percentage of less than 10% under petri plate method as well as tray method of screening (**Fig. 1**). For direct seeding, it is critical to choose rice genotypes that can germinate well in anaerobic conditions with high seedling vigour. Tolerant rice genotypes have many adaptive mechanisms which help them to survive under waterlogged conditions, along with the ability to

Table 1. Coleoptile length, root length and dry matter production of rice landraces under anaerobic condition

Landraces	Coleoptile length (cm)		Root length (cm)		Dry weight (g/10 seedlings)
	Petri plate method	Tray method	Petri plate method	Tray method	
Kallurundaikar	3.90	5.27	2.4	3.4	0.156
Vellimuthu	5.80	3.57	3.9	2.8	0.199
Chennelthondi	3.30	4.77	1.9	1.5	0.145
Athur kichati samba	2.63	2.93	1.6	1.9	0.125
Kanni chennellu	5.57	4.20	4.2	3.1	0.196
Norungan	5.67	4.47	4.7	2.7	0.233
Nootripathu	1.73	2.40	1.1	1.2	0.084
Veliyan	4.37	3.67	2.2	1.9	0.159
Uruni kaima	4.60	5.73	3.1	4.1	0.168
Mahamaya	4.50	4.10	3.7	2.9	0.164
Koduvaliyan	3.37	5.27	2.1	3.3	0.149
Gandakasala	4.03	3.57	3.2	2.8	0.157
Kadaikannan	3.13	2.77	1.6	1.5	0.164
Korumbaali	3.20	4.17	2.6	3.0	0.151
Onamuttan	3.97	3.27	2.2	2.3	0.166
Njavara	4.20	3.93	3.1	2.4	0.176
Thavalakannan	2.60	3.07	1.5	2.9	0.138
Illupai poo samba	3.10	2.80	1.7	1.6	0.166
Raja mudi	4.60	3.93	2.9	2.4	0.167
Chithiraikar	4.13	3.83	3.1	2.9	0.173
Matta thireni	2.50	2.90	1.3	1.7	0.127
Kothandan	4.50	4.23	3.7	1.8	0.186
Paalthondi	3.63	4.70	2.6	3.5	0.165
Mysore malli	1.70	2.10	0.9	2.5	0.076
Okkampunja	0.97	1.30	0.4	0.7	0.031
Jeerakasala	3.67	3.87	2.1	2.1	0.157
Burma black	3.67	2.97	1.8	1.8	0.159
Karimbalan	3.67	3.70	1.9	1.7	0.152
Irunaazhi	3.20	1.90	1.2	1.2	0.143
Chenkazhama	2.43	1.83	1.6	1.2	0.141
Karuppu kavuni	4.57	3.53	3.2	1.4	0.176
Malai nellu	0.00	2.70	0	0.5	0
Poongar	3.23	3.00	1.7	1.7	0.152
Thuyamalli	1.73	1.70	1.1	1.4	0.089
Sivappu chithiraikar	5.13	5.80	3.9	3.9	0.198
Vellai chithiraikar	3.87	3.07	2.1	1.9	0.164
Karuthakar	3.87	4.13	3.2	3.2	0.167
Karnel	5.33	4.63	4.5	1.8	0.196
Vadinell	1.57	1.23	0.3	0.3	0.052
Karunguruvai	6.23	4.30	5.3	2.9	0.265
60th kuruvai	2.97	4.93	1.6	3.7	0.147
Raja mannar	3.87	4.03	2.5	2.5	0.184
Kattu yanam	3.40	2.80	2.5	1.4	0.162
Savudu samba	3.87	5.77	1.4	3.9	0.155
Katta samba	3.30	2.37	2.2	1.1	0.161
Kottanel	3.03	2.10	2.6	1.4	0.17
Ottadiyan	2.83	2.23	2.1	0.9	0.153
Vellai kavuni	3.97	3.90	1.6	1.5	0.172
Varappu kudaichan	4.90	3.20	3.7	2.1	0.194
Karuppu nel	3.17	2.97	1.8	1	0.169
Sura kuruvai	0.70	2.40	0.2	1.4	0.032
Kattarur	2.70	2.07	1.3	1.3	0.168
Mappillai samba	2.45	0.00	1.5	0	0.16
Mean	3.49	3.40	2.28	2.08	0.15
Standard deviation	1.28	1.23	1.17	0.97	0.05

Table 2. Germination percentage, seedling length and vigour index of rice landraces under anaerobic condition

Landraces	Germination percentage (%)		Seedling length (cm)		Vigour index		Reaction of genotype on anaerobic germination tolerance
	Petri plate method	Tray method	Petri plate method	Tray method	Petri plate method	Tray method	
Kallurundaikar	80	733	6.3	8.7	504.0	635.6	Moderately tolerant
Vellimuthu	90	66.7	9.7	6.4	873.0	424.4	Tolerant
Chennelthondi	83	66.7	5.2	6.3	431.6	355.1	Moderately tolerant
Athur kichati samba	70	60.0	4.2	4.8	296.3	290.0	Moderately susceptible
Kanni chennellu	90	73.3	9.8	7.3	879.0	535.3	Tolerant
Norungan	97	76.7	10.4	7.2	1005.6	549.4	Tolerant
Nootripathu	93	83.3	2.8	3.6	263.5	300.0	Tolerant
Veliyan	97	83.3	6.6	5.6	637.0	463.9	Tolerant
Uruni kaima	83	73.3	7.7	9.8	639.1	721.1	Moderately tolerant
Mahamaya	83	73.3	8.2	7.0	680.6	513.3	Moderately tolerant
Koduvaliyan	93	83.3	5.5	8.6	508.4	713.9	Tolerant
Gandakasala	80	76.7	7.2	6.4	578.7	488.1	Moderately tolerant
Kadaikannan	86	76.7	4.7	4.3	407.1	327.1	Moderately tolerant
Korumbaali	93	93.3	5.8	7.2	539.4	668.9	Tolerant
Onamuttan	83	73.3	6.2	5.6	511.8	408.2	Moderately tolerant
Njavara	93	73.3	7.3	6.3	678.9	464.4	Tolerant
Thavalakannan	90	60.0	4.1	6.0	369.0	358.0	Tolerant
Illupai poo samba	76	63.3	4.8	4.4	364.8	278.7	Moderately susceptible
Raja mudi	86	73.3	7.5	6.3	645.0	464.4	Moderately tolerant
Chithiraikar	80	70.0	7.2	6.7	578.7	471.3	Moderately tolerant
Matta thireni	66	56.7	3.8	4.6	250.8	260.7	Moderately susceptible
Kothandan	90	63.3	8.2	6.0	738.0	382.1	Tolerant
Paalthondi	86	66.7	6.2	8.2	536.1	546.7	Moderately tolerant
Mysore malli	83	63.3	2.6	4.6	215.8	291.3	Moderately tolerant
Okkampunja	33	40.0	1.4	2.0	45.1	80.0	Susceptible
Jeerakasala	97	73.3	5.8	6.0	559.4	437.6	Tolerant
Burma black	36	33.3	5.5	4.8	196.8	158.9	Susceptible
Karimbalan	93	63.3	5.6	5.4	517.7	342.0	Tolerant
Irunaazhi	80	63.3	4.4	3.1	352.0	196.3	Moderately tolerant
Chenkazhama	80	63.3	4.0	3.0	322.7	192.1	Moderately tolerant
Karuppu kavuni	93	83.3	7.8	4.9	722.3	411.1	Tolerant
Malai nellu	0	3.3	0.0	3.2	0.0	10.7	Susceptible
Poongar	90	76.7	4.9	4.7	444.0	360.3	Tolerant
Thuyamalli	60	53.3	2.8	3.1	170.0	165.3	Moderately susceptible
Sivappu chithiraikar	96	83.3	9.0	9.7	867.2	808.3	Tolerant
Vellai chithiraikar	86	73.3	6.0	5.0	513.1	364.2	Moderately tolerant
Karuthakar	86	73.3	7.1	7.3	607.7	537.8	Moderately tolerant
Karnel	83	73.3	9.8	6.4	816.2	471.8	Moderately tolerant
Vadinell	43	36.7	1.9	1.5	80.3	56.2	Moderately susceptible
Karunguruvai	93	83.3	11.5	7.2	1072.6	600.0	Tolerant
60th kuruvai	93	76.7	4.6	8.6	424.7	661.9	Tolerant
Raja mannar	83	76.7	6.4	6.5	528.4	500.9	Moderately tolerant
Kattu yanam	86	73.3	5.9	4.2	507.4	308.0	Moderately tolerant
Savudu samba	100	90.0	5.3	9.7	526.7	870.0	Tolerant
Katta samba	96.6	76.7	5.5	3.5	531.3	265.8	Tolerant
Kottanel	86	70.0	5.6	3.5	484.5	245.0	Moderately tolerant
Ottadiyan	86	76.7	4.9	3.1	424.3	240.2	Moderately tolerant
Vellai kavuni	100	93.3	5.6	5.4	556.7	504.0	Tolerant
Varappu kudaichan	100	86.7	8.6	5.3	860.0	459.3	Tolerant
Karuppu nel	93	86.7	5.0	4.0	461.9	343.8	Tolerant
Sura kuruvai	3	6.6	0.9	3.8	2.7	25.3	Susceptible
Kattarur	86	80.0	4.0	3.4	344.0	269.3	Moderately tolerant
Mappillai samba	10	0	4.0	0.0	39.5	0.0	Susceptible
Mean	79.67	70.35	5.77	5.47	492.66	392.42	
Standard deviation	23.39	25.14	2.40	2.11	246.93	197.29	

https://doi.org/10.37992/2022.1303.110

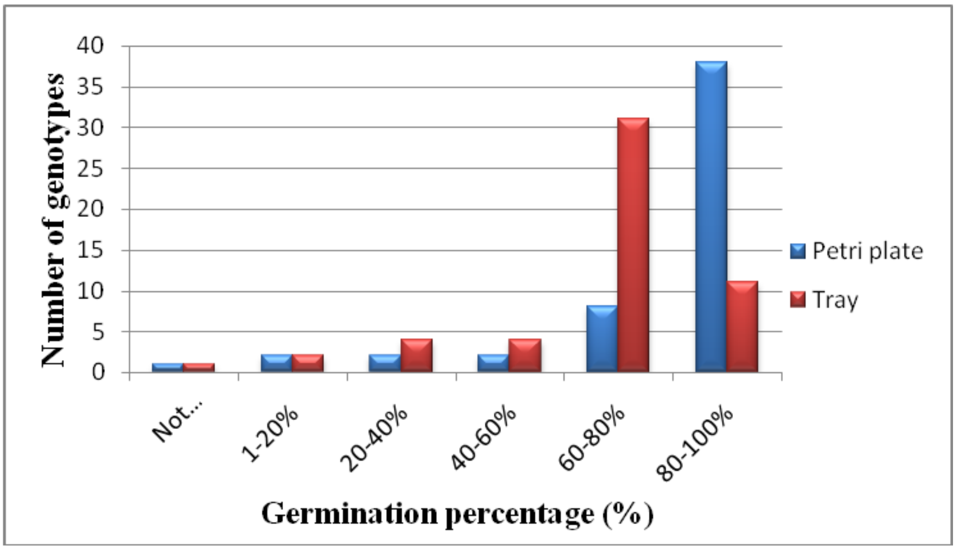

Fig. 1. Frequency distribution of rice landraces for germination percentage

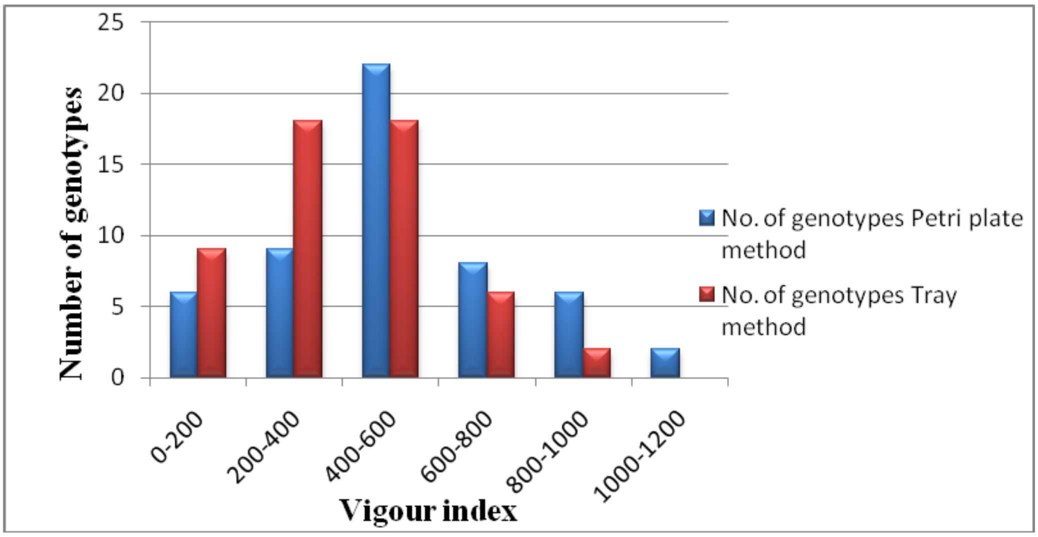

Fig. 2. Frequency distribution of rice landraces for vigour index

germinate and elongate faster in the lack or decreased oxygen environment, the capacity to move stored starch reserves and effective energy production via fermentation pathways etc. (Ismail *et al.,* 2009). Reddy *et al*. (2017) also identified >70% of survived genotypes as tolerant genotypes for anaerobic germination.

Rice is the only major cereal crop that has the distinct potential to germinate beneath the water and subsequent growth limiting with only coleoptiles (Angaji *et al.,* 2010). Germinating rice seeds can preserve their capacity to break down starch into easily fermentable carbohydrates under anaerobic conditions (Atwell and Greenway, 1987). Earlier findings have shown that tolerant genotypes had greater amylase activity in germinating seeds than susceptible genotypes, and that enzyme activity increases gradually from sowing to several-fold at three days after sowing under anaerobic conditions(Ismail *et al.,*2009).

Shoot length ranged from 0.70 cm (surakuruvai) to 6.23 cm (karunguruvai) in petri plate method and from 1.23 cm (vadinell) to 5.80 cm (sivappu chithiraikar) in tray method. The genotypes namely karunguruvai (6.23 cm), vellimuthu (5.80 cm) and norungan (5.67 cm) recorded maximum shoot length and the genotypes *viz*., okkampunja (0.97 cm), vadinell (1.57 cm) and surakuruvai (0.70 cm) recorded minimum shoot length in petri plate method. In tray method, the land races sivappu chithiraikar (5.80 cm), savudu samba (5.77 cm) and uruni kaima (5.73 cm) were found to have more shoot elongation, while the genotypes vadinell (1.23 cm), thuyamalli (1.70 cm) and okkampunja (1.30 cm) observed lower shoot elongation (**Fig. 2**). In general, rapid elongation of coleoptiles and shoots may make it easier for them to comes into contact with air in anaerobic soils and so provide oxygen to the growing embryo. Ling *et al.* (2004) reported that detecting the quantity of alcohol under anoxia is a complicated one, the seedling shoot length including the coleoptiles was employed as a marker to evaluate anaerobic germination tolerance.

Fig. 3. Phenotypic screening of land races

Vigour index is minimum in the landrace of surakuruvai (2.7) and maximum in karunguruvai (1072.6). Vigour index of all genotypes varied from 0 (mapillai samba) to 1100 (karunguruvai) (**Fig. 3**). Revealing the various adaptive mechanisms carried by rice genotypes tolerant to initial flooding would allow the development of efficient management approaches that will allow them to establish properly under flooded soils with weed control. High germination percentage and seedling length are the main aspects among the various adaptive mechanisms, which are in turn closely associated with seedling vigour, which is the final factor of successful crop establishment under anaerobic conditions. Seeds having significant carbohydrate stores, like grains, are thought to be more tolerant of oxygen deprivation during germination (Raymond *et al.,*1985). The promising genotypes for high germination percentage, vigour index and dry matter production under anaerobic germination were identified and depicted in **Table 3**.

A higher value of dry matter production indicates that the landrace has high vigour which results in higher shoot elongation under anaerobic conditions. Therefore dry matter production is an important trait to be studied for withstanding the power of genotype under adverse conditions. Flooding-induced elongation, on the other side, is one of the escape strategies that help submerged plants to establish contact with the air. The plant hormone ethylene reacts with other plant hormones to enhance rice seedling development under anaerobic conditions by altering the actions of GA, ABA and auxins (Fukao *et al.,* 2003). However, it is difficult to predict the effects of these plant hormones. Furthermore, prior studies had shown that ethylene improved the sucrose transfer from the scutellum to the developing coleoptile in germinating rice seeds (Ishizawa and Esashi, 1998). Tolerance rice genotypes, for instance, germinate and develop faster under hypoxia or anoxia stress, with greater seedling length and vigour index. It will keep the greater amylase activity and shift to anaerobic respiration to make use of the starch stores (Ismail *et al.,* 2012).

A total of twenty landraces were selected for genotyping study based on the performances in phenotyping. Landraces *viz*., vellai kavuni and varappu kudaichan showed tolerance on anaerobic germination with allele size of 160 bp in genotyping. Landraces *viz*., norungan, karuppu kavuni and karimbalan also had the same allele size as that of the former one which confirms their tolerance capacity in anaerobic germination (**Fig. 4**). The other AG reported marker RM 206 marker was not amplified (**Fig. 5**). The genotypes were further screened with gene specific marker ART5 along with the submergence

Table 3. Promising genotypes identified under anaerobic germination

Characters	**Name of the promising genotypes**
Germination percentage	Varappu kudaichan, Vellai kavuni, Savudu samba, Karunguruvai and Norungan
Vigour index	Karunguruvai, Varappu kudaichan, Norungan, Savudu samba and Vellimuthu
Dry matter production	Karunguruvai, Norungan, Varappu kudaichan, Vellimuthu and Kothandan

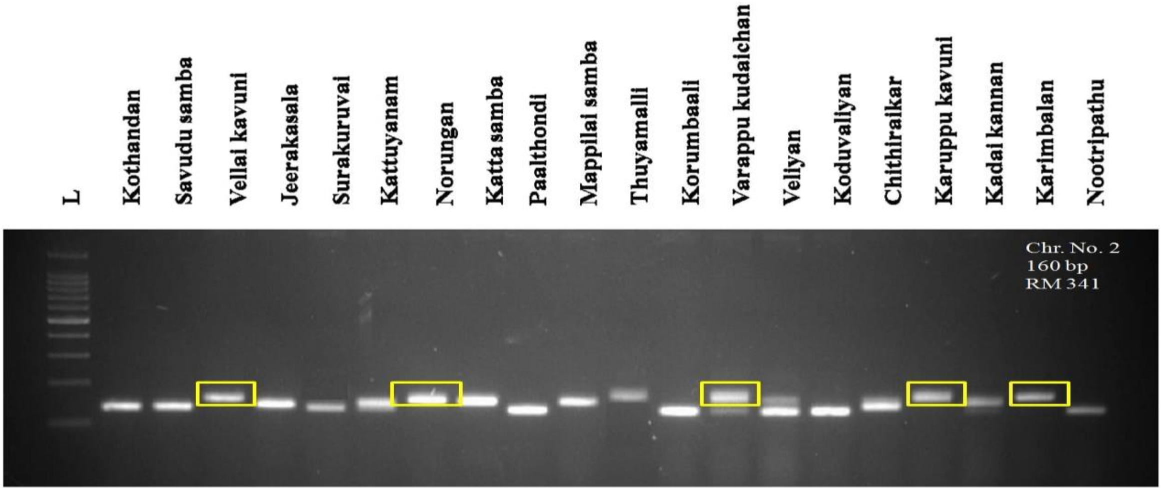

Fig. 4. Screening of rice landraces with microsatellite marker RM 341

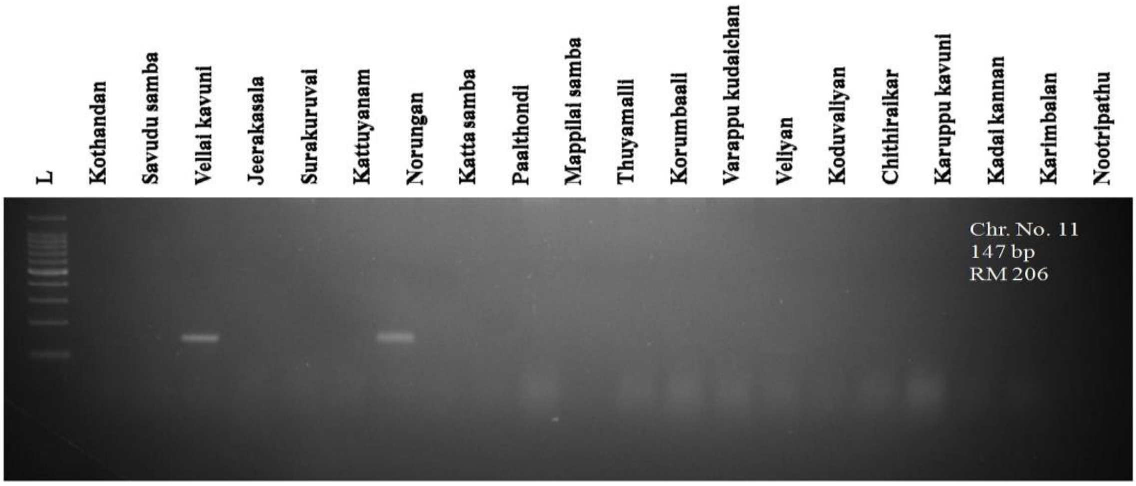

Fig. 5. Screening of rice landraces with microsatellite marker RM 206

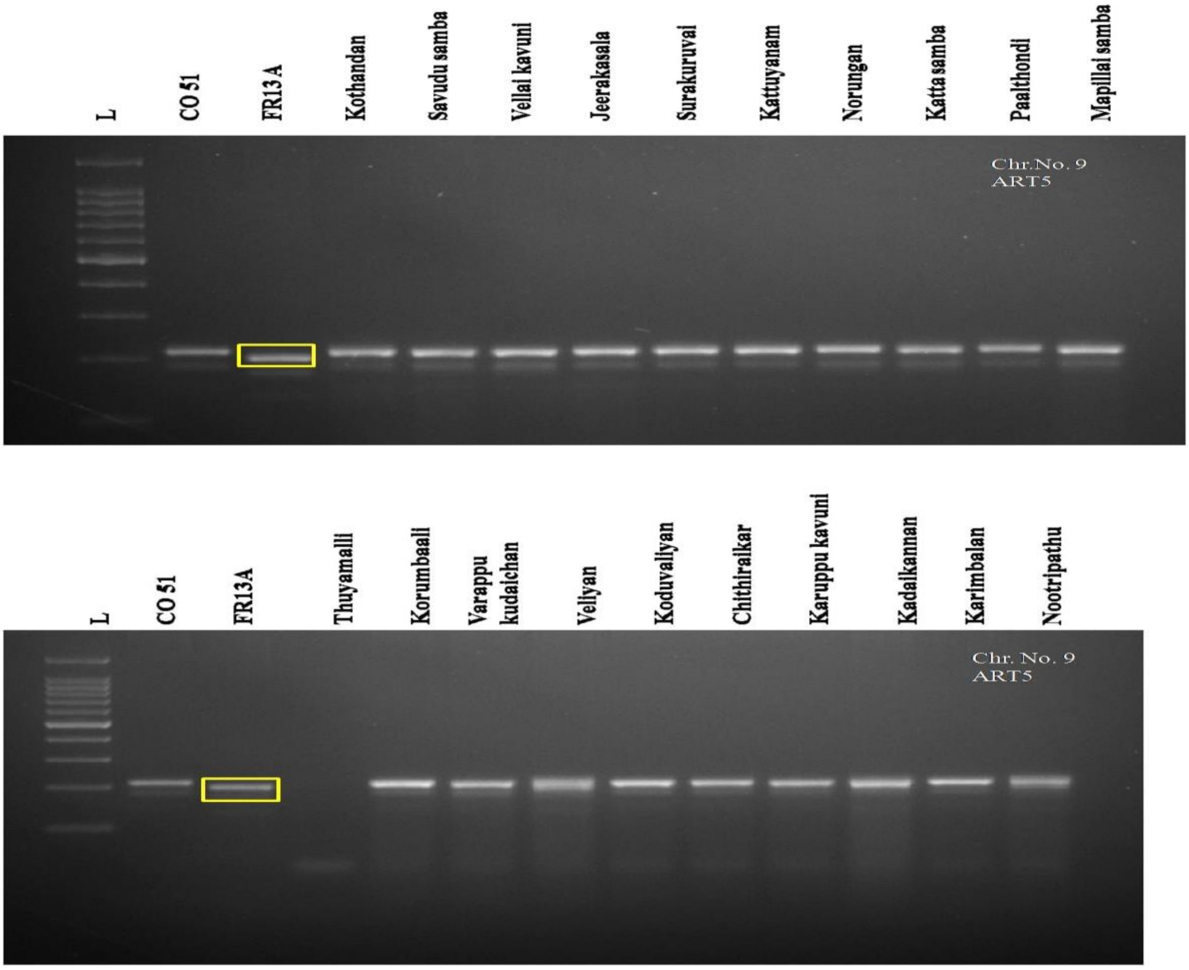

Fig. 6. Screening of rice landraces with ART5 *Sub 1C*

tolerant genotype FR13A and submergence susceptible genotype CO 51, all the landraces which were used for genotyping showed similar band size of the susceptible genotype CO51 (**Fig. 6**). It indicates that the submergence tolerance gene is different from anaerobic germination.

In the present study, it was concluded that the land races namely vellai kavuni, varappu kudaichan, norungan and karuppu kavuni showed anaerobic germination (AG) tolerance in both phenotypic and genotyping studies. Petriplate method of screening was found to be the best one for screening anaerobic germination. The marker RM 341 was found to be the best marker for screening anaerobic germination tolerance. AG tolerance genotypes are highly suitable for direct seeded rice (DSR) cultivation and the tolerant genotypes can be used in the further breeding programme to transfer AG ability to desirable cultivars.

REFERENCES

Angaji, S.A., Septiningsih, E.M., Mackill, D.J. and Ismail, A.M. 2010. QTLs associated with tolerance of flooding during germination in rice (*Oryza sativa* L.). *Euphytica*,**172**: 159–168. [Cross Ref]

Atwell, B.J. and Greenway, H.1987. Carbohydrate metabolism of rice seedlings grown in oxygen-deficient solution. *Journal of Experimental Botany*, **38**: 466– 478. [Cross Ref]

Dhanuja, N., Ganesamurthy, K., Pushpam, R., Amutha, K. and Uma, D. 2021.Studies on genetic diversity in selected rice (*Oryza sativa* L.) landraces of Tamil Nadu. *Electronic Journal of Plant Breeding,* **12**(3):990 – 997. [Cross Ref]

Fukao, T., Kennedy, R.A., Yamasue, Y. and Rumpho, M.E. 2003.Genetic and biochemical analysis of anaerobically- induced enzymes during seed germination of *Echinochloa crusgalli* varieties tolerant and intolerant of anoxia. *Journal of Experimental Botany*. **54**: 1421–1429. [Cross Ref]

Ishizawa, K. and Esashi, Y.1988. Action mechanism of ethylene in the control of sugar translocation in relation to ricecoleoptiles growth. I. Sucrose metabolism. *Plant, Cell and Environment*, **29**: 131–141.

Ismail, A.M., David E. Johnson, D.E., Ella, E.S., Vergara, V.G and. Baltazar, M.A. 2012. Adaptation to flooding during emergence and seedling growth in rice and weeds, and implications for crop establishment. *Aob plants*, 1-18. [Cross Ref]

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* L.). *Annals of Botany*, **103**: 197– 209. [Cross Ref]

Ling, J., Ming-yu, H., Chun-ming, W. and Jian-min, W. 2004. Quantitative trait loci and epistatic analysis of seed anoxia germinability in rice (*Oryza sativa* L.). *Rice Science*, **11**(5-6): 238-244.

Miro, B. and Ismail, A.M. 2013. Tolerance of Anaerobic conditions caused by flooding during germination and early growth in Rice (*Oryza sativa* L.). *Frontiers in Plant Science*, (4) http://www.frontiersin.org/Plant_Science/editorialboard. [Cross Ref]

Miro, B., Longkumar, T., Entila, F.D., Kohli, A. and Ismail, AM.2017. Rice seed germination Underwater: Morpho- Physiological responses and the bases of differential expression of Alcoholic fermentation enzymes. *Frontiers in Plant Sciences*, **8**: 1857. [Cross Ref]

Ray, S., Vijayan, J. and Sarkar, R.K. 2016. Germination Stage Oxygen Deficiency (GSOD) : An emerging stress in the era of changing trends in climate and Rice cultivation practice. *Frontiers in Plant Science,*(7): 671. [Cross Ref]

Raymond, P., Al-Ani, A. and Pradet, A. 1985.ATP production by respiration and fermentation, and energy charge during aerobiosis and anaerobiosis in twelve fatty and starchy germinating seeds. *Plant Physiology,* **79**: 9879–9884. [Cross Ref]

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

Reddy, B.S. and Rani, M.G. 2017. Evaluation of Near Isogenic Lines (NILs) of rice for major abiotic stresses of coastal areas. *Electronic journal of Plant breeding*, **9**(3): 808-814. [Cross Ref]