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Screening of Rice Genotypes for Anaerobic Germination Tolerance: Identifying Potential Breeding Lines

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ABSTRACT: Anaerobic germination is an important trait for rice cultivation in flood-prone areas. However, anaerobic germination is a complex process and requires specific adaptations in plant physiology and metabolism to tolerate the low oxygen conditions. In this study, we aimed to identify rice genotypes with high anaerobic germination tolerance through screening of a diverse panel of 103 rice accessions. The screening was conducted at ICAR – NRRI under anaerobic conditions for 21 days, and the percentage of germinated seeds, length of first internode (cm), shoot length (cm), number of leaves, root length (cm), shoot and root biomass (g), and seedling vigour index was recorded. The accessions showed a wide range of anaerobic germination tolerance, with the percentage of germinated seeds ranging from 0% to 94%. Based on the screening, we identified five genotypes Tulasiphula, Panirohi, BJ 1, Chinamali and ARC 14855 with high anaerobic germination tolerance coupled with high seedling vigour. These genotypes had a significantly higher percentage of germinated seeds under anaerobic conditions compared to the other accessions. Our findings provide useful information for rice breeding programs aimed at developing genotypes with high anaerobic germination tolerance. The identified genotypes could be used as parental lines for breeding programs, and the screening methodology could be employed for further evaluation of larger germplasm collections. Overall, the study highlights the importance of anaerobic germination tolerance for rice cultivation in flood-prone areas and provides insights into the genetic diversity of this trait in rice germplasm.

Keywords: Screening, Anaerobic germination tolerance, Length of first internode, seedling vigour.

INTRODUCTION

Rice is a staple food for more than half of the world's population, but its cultivation is often challenged by water logging, which causes a lack of oxygen and leads to anaerobic conditions (Yang *et al.,* 2019). Anaerobic germination is an adaptation mechanism by which rice seeds can germinate even under flooded conditions (Atwell *et al.* 1982; Perata *et al.* 1997). However, excessive waterlogging can lead to poor seedling establishment, yield losses, and reduced grain quality (Angaji *et al.,* 2010). Therefore, there is a need to develop rice varieties that can tolerate anaerobic conditions during germination and early growth stages (Septiningsih *et al*., 2013).

Rice screening for tolerance to anaerobic germination involves the evaluation of rice genotypes under waterlogged conditions to identify varieties that can germinate and grow well in flooded soils. The screening process involves simulating anaerobic conditions in the laboratory or field by submerging the rice seeds in water and monitoring their germination and growth. The screening criteria include the percentage of germination, seedling vigor, and survival rate under waterlogging stress. The advantages of rice screening for tolerance to anaerobic germination are numerous. Firstly, it can help identify rice varieties that can withstand waterlogging stress and ensure better seedling establishment, leading to increased yield potential (Ray *et al*., 2016; Senapati *et al*., 2019). Secondly, it can contribute to the development of climate-resilient rice varieties that can adapt to the changing climate, especially in areas prone to flooding. Thirdly, it can reduce the use of chemicals and fertilizers in rice cultivation, as waterlogging stress can increase nutrient availability in flooded soils.

In conclusion, rice screening for tolerance to anaerobic germination is a valuable tool for developing rice varieties that can withstand waterlogging stress and improve rice production in areas prone to flooding (Joshi *et al.,* 2013, Miro and Ismail 2013; Vijayan *et al.,* 2018). The screening process involves evaluating rice genotypes under flooded conditions to identify varieties that can germinate and grow well in waterlogged soils

(Ismail *et al.,* 2009). The advantages of rice screening for tolerance to anaerobic germination include increased yield potential, development of climate-resilient rice varieties, and reduced use of chemicals and fertilizers in rice cultivation.

MATERIAL AND METHODS

The Experimental material consisted of 103 germplasm accession of Aus population, Odisha land races (Table 1) were utilized for screening for identifying anaerobic germination tolerant cultivars. The experiment was conducted in Kharif 2019 at ICAR-NRRI (National Rice Research Institute) Cuttack under Net house conditions. The experiment was conducted under net house conditions for anaerobic germination tolerance. The seedling tray comprising of 156 wells (13 columns × 12 Rows) was filled with fine soil for seeding. Three seed per well a total Thirty-six dry seeds per entry was sown in each column and covered with 1cm fine soil. The seeded tray was kept in large container and filled with water to the height of 10cm above the seedling tray for a duration of 21 days (Plate 1). Each seedling tray was sown with tolerant and Susceptible checks AG 387 and Cr Dhan 801 respectively. Observations were recorded after 21 days of submergence. Data on number of plants reached above the water surface at DAS was recorded as Anaerobic germination percentage (%). In addition, various an aerobic germination traits i.e., Length of first Internode (cm), Shoot length (cm), Number of leaves, Root length (cm), Shoot biomass (g), Root biomass (g) and seedling vigour Index (SVI) were recorded.

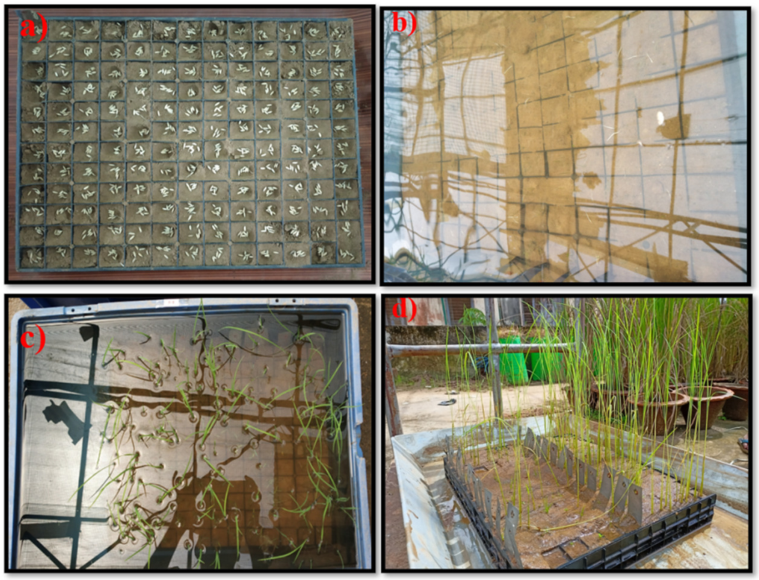

Plate 1. Showing the protocol for screening of rice genotypes for anaerobic germination tolerance. a) seeding of rice genotypes in seedling tray b) emergence of coleoptile from genotypes c) tolerant genotypes reached water surface d) seedling tray showing susceptible and tolerant genotypes on 21st day.

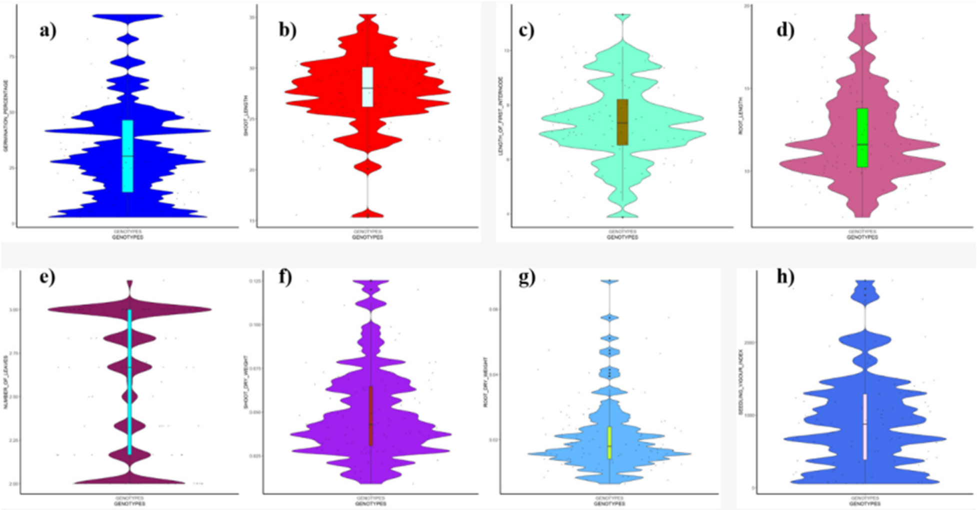

Fig. 1. Frequency distribution genotypes for various traits recorded under anaerobic conditions. a) germination percentage (%), b) shoot length (cm) c) length of first internode (cm), d) root length (cm), e) number of leaves, f) shoot dry weight (g), root dry weight, h) seedling vigour index.

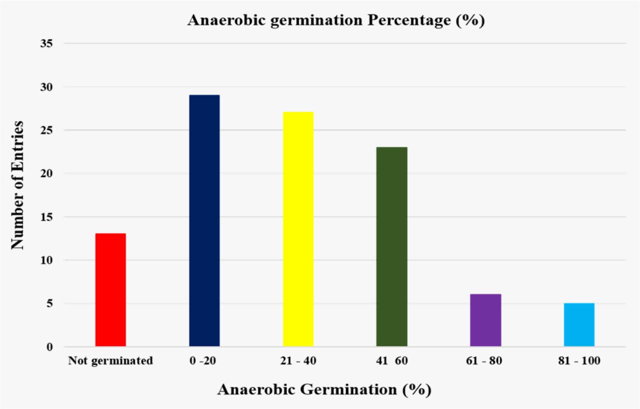

Fig. 2. Frequency distribution of genotypes for germination percentage under anaerobic conditions.

Table 1: List of the germplasm entries utilized in screening for various anaerobic germination tolerance traits.

Sr. No.	Name of the Genotype	Sr. No.	Name of the Genotype
1.	ARC 10958	53.	AUSMERI
2.	ARC 11600	54.	Banda
3.	ARC 14855	55.	Bankoi
4.	ARC 14965	56.	Basantibhog
5.	ARC 5959	57.	Basapatri
6.	ARC 5977	58.	BJ 1
7.	ARC 6000	59.	BORO
8.	ARC 6240	60.	BOWALIA
9.	ARC 7098	61.	BOWALIA 2
10.	ARC 7325	62.	CHAMKA
11.	AS 2	63.	Champa
12.	ASSAM 4(BORO)	64.	Chholaboro
13.	AUS 125	65.	Chinamali
14.	AUS 127	66.	DA 12
15.	AUS 131	67.	Dhala basmati
16.	AUS 151	68.	DHINGHA
17.	AUS 169	69.	Gadakati
18.	AUS 175	70.	Gahamphulla
19.	AUS 204	71.	Goria
20.	AUS 22	72.	IR 64-21
21.	AUS 267	73.	Jabaful
22.	AUS 268	74.	Jangalijata
23.	AUS 273	75.	Jubaphul
24.	AUS 283	76.	Kala kadamba
25.	AUS 29	77.	Kalachampa
26.	AUS 294	78.	Kalajeera
27.	AUS 298	79.	Kalakataki
28.	AUS 309	80.	Kalamkati
29.	AUS 314	81.	Khajara
30.	AUS 317	82.	Krishnabhog
31.	AUS 321	83.	Lajakuli
32.	AUS 335	84.	Lajakuli
33.	AUS 350	85.	Mahakamati
34.	AUS 353	86.	Mukta kiari
35.	AUS 354	87.	MUNSHISHAIL
36.	AUS 361	88.	Nababi
37.	AUS 362	89.	NaliBaunsaGaja
38.	AUS 366	90.	Panirohi
39.	AUS 369	91.	Parbat jeera
40.	AUS 37	92.	Pimpudibasa
41.	AUS 382	93.	RAJ MUNDO
42.	AUS 385	94.	Samudrabali
43.	AUS 391	95.	SANHUANGZHAN NO 2
44.	AUS 417	96.	SATHA
45.	AUS 435	97.	Solari
46.	AUS 46	98.	Swarna
47.	AUS 60	99.	T 1
48.	AUS 62	100.	Tulasiphula
49.	AUS 63	101.	Tupa
50.	AUS 77	102.	**AG 387 (Tolerant check)**
51.	AUS 93	103.	**Cr Dhan 801 (Suceptible check)**
52.	AUS PADDY(WHITE)		

RESULTS AND DISCUSSIONS

The results of 103 rice germplasm accession for their ability to germinate under anaerobic conditions was evaluated with respect to eight anaerobic germination tolerance traits Anaerobic germination percentage (%), Length of first Internode (cm), Shoot length (cm), Number of leaves, Root length (cm), Shoot biomass (g), Root biomass (g) and seedling vigour Index (SVI) and the distribution pattern of genotypes for various traits were presented in (Fig. 1). The wide range of variability has been observed for all the traits studied for the genotypes under anaerobic conditions.

The anaerobic germination percentage in the present study has ranged from 0.00 % (BOWALIA 2) to 94 % (Tulasiphula, Panni rohi) followed by tolerant check AG 387 (93%) (Table 2) (Fig. 2). The mean germination percentage of the panel is 33%. Based on germination percentage, the rice genotypes were classified as susceptible (0 - 50%), moderately tolerant (51 – 75 %) and tolerant (51–75 %) in accordance with the classification given by Manigbas *et al.* (2008) (Table 3). Out of 103genotypes, thirteen entries have not germinated and 76 genotypes recorded less than 50 percent of germination and were categorized as susceptible for anaerobic germination. Nine genotypes were found to moderately tolerant and five entries were identified as tolerant for germination under anaerobic conditions. The susceptible check Cr Dhan 801 and tolerant check AG 387 has recorded 19.44% and 94.44% of germination respectively under anaerobic conditions. The five tolerant entries identified in this experiment could utilized as donor for various breeding programmes. There might be due to rapid break down of starch into soluble sugars and efficient utilization for proper growth of embryo and seedling establishment in tolerant genotypes compared to the susceptible genotypes and the results were conformity with Reddy and Girijarani (2018); Sudeepthi *et al.* (2019).

The length of the first internode is an important trait for anaerobic germination tolerance in rice. During anaerobic germination, rice plants elongate their first internode to reach the water surface and obtain oxygen, which is essential for growth and survival. Studies conducted by Miro Berta *et al.* (2017) shown that rice varieties with longer first internodes are more tolerant to anaerobic conditions and have a higher survival rate than those with shorter internodes. Longer internodes allow the plant to reach the water surface faster and obtain oxygen more efficiently, which is important for early seedling growth and development. The maximum length of first internode was exhibited by Mahakamati (11.32cm), followed by ARC 6000 (10.14cm), while minimum length of first internode was exhibited by ARC 10958 (3.88cm) and followed by AUS 93 (4.48cm). The mean internode length of the panel is (7.32cm). The mean length of first internode of tolerant genotype and moderately tolerant genotypes was (9.70cm) and (7.87cm) respectively, which is higher the panel mean. While susceptible genotypes exhibited mean of (7.19cm). The genotype Tulasiphula, panirohithat has exhibited highest germination percentage has showed higher the length of first internode of (9.91cm) and (9.49cm). There results were confirmatory with reports (Miro Berta *et al.,* 2017) on length of first internode under anaerobic conditions.

The shoot elongation is one of the most critical physiological changes that occur in the plant (Ismail *et al.*, 2009; Rauf *et al.,* 2019). The shoot length of rice under anaerobic germination is variable and depends on various factors such as cultivar, duration of submergence, and other environmental factors. The starchy seeds like rice have been found to possess a remarkable ability to withstand anaerobic conditions, as they can sustain a high level of energy metabolism even in the absence of oxygen, in contrast to fatty seeds. However, it is important to note that there exists a significant difference in the anoxia tolerance levels among starchy seeds when it comes to their ability to germinate. For example, while oat and barley germination (which begins with root emergence) is adversely affected by decreasing oxygen concentrations, rice behaves differently, exhibiting a pattern where root growth is inhibited while shoot growth increases as oxygen concentrations decrease (Tsuji, 1973; Alpi and Beevers 1983). The highest shoot length was exhibited by AS 2 (35.28cm) followed by AUS 125 (34.27cm) and the lowest shoot length was exhibited by Aus 60 (15.33cm) and followed by ARC 10958 (20.10cm). The mean shoot length of the panel is (27.82cm). The mean shoot length of tolerant and moderately tolerant entries was found to be (29.10cm) and (28.85cm) higher the mean of panel, while the shoot length of susceptible genotype was (27.67cm) lower the panel mean. The genotype TulasiPhula, PaniRohi that has exhibited highest germination percentage has exhibited the shoot length of (28.29cm) and (29.30cm) while, the susceptible genotype has exhibited shoot length of AUS353 (15.56cm). Under anaerobic conditions for shoot length similar results were reported by (Miro Berta *et al.,* 2017).

The trait number of leaves that has reached the water surface on 21st day is considered as the important trait under anaerobic conditions, indicates the higher-level photosynthesis helps in good seedling establishment after the stress condition. The maximum number of leaves was observed to be three in all tolerant genotypes (Tulsa Phula, Panirohi, BJ-1, Chinamali and AG 387(tolerant check)). The minimum number of two leaves is observed in most of the genotypes of susceptible group and Cr dhan 801 (susceptible check). The genotype with highest germination percentage has developed three leaves and the genotype with the lowest germination percentage AUS353 has developed two leaves. The panel doesn't exhibit much variation for the trait number of leaves.

The trait root length has exhibited much variation for root growth under anaerobic conditions. Rice can germinate under hypoxic or anoxic conditions, but only tolerant genotypes have the ability of fast coleoptile elongation and root formation under submerged conditions in the field (Ismail *et al.,* 2009). Conversely, the coleoptile growth is slow in sensitive genotypes, and they fail to develop further. The maximum root length was observed in the genotype AUS 391 (19.48cm) from the susceptible group and minimum root length was exhibited by Chinamali (7.21cm). The mean root length of the panel was 12.10cm. The mean root length of tolerant group was found to be 13.73cm which is higher than the panel mean root length. The moderately tolerant and susceptible group has exhibited mean root length of (11.26cm) and (12.10cm) respectively that is clustering around than panel mean. The genotype with maximum (TulasiPhula) and minimum (AUS 353) germination percentage has showed root length of 13.97cm and 7.98cm. As per the results of (Miro Berta *et al.,* 2017); Bordoloi and Sharma (2018); the tolerant genotypes has showed higher root length compared to susceptible genotypes. Similar results were found in the present investigation seedling biomass can affect establishment under anaerobic conditions is by increasing the rate of photosynthesis. A larger seedling biomass means more chlorophyll and a greater ability to capture light, which can lead to higher rates of photosynthesis and carbon fixation. This can help seedlings to produce the energy they need to grow and develop, even under anaerobic conditions. Additionally, Seedling biomass can also play a role in nutrient uptake. A higher biomass can mean a larger root system, which can help seedlings to access nutrients that are otherwise limited in anaerobic soils. Additionally, a larger biomass can help seedlings to compete with other plants for resources, such as light and nutrients, which can be limited in flooded or waterlogged soils. In case of present investigation ample amount of variation for shoot and root biomass is existing among the genotypes in the panel. The shoot biomass was ranged from 0.0090 g (Chholaboro) to 0.1253g (AUS 350) and mean shoot biomass of panel was found to be 0.0497g. The tolerant genotypes have exhibited mean shoot biomass of 0.1040g higher than the mean of panel. While moderately tolerant and susceptible genotypes have showed 0.0399g and 0.0473g respectively. The trait root biomass has ranged from 0.0065g (AUS PADDY(WHITE)) to 0.0688g (AUS 175) with mean root biomass of 0.0210g. The tolerant and moderately tolerant group mean root biomass was found to be 0.0151g and 0.0155g lower than the mean of panel. The susceptible group has exhibited mean root biomass of 0.0220g higher than the panel mean. The results were confirmatory with the report of Miro Berta *et al*. (2017) for shoot biomass and contradictory for root biomass.

Seedling vigour plays a key role in the submergence avoidance mechanism (Manangkil *et al*., 2008). The seeds which are exhibiting the higher vigour index are considered to be more vigorous. The vigour index was calculated by multiplying seed germination percentage with seedling length (shoot length + root length) Roy and Sharma 2014. The lowest vigour index was reported from the genotype ARC 10958 (55.75) and the highest was identified in BJ 1 (2855.73) and the mean vigour index of panel was found to be 924. The tolerant and moderately tolerant group has exhibited seedling vigour of 2574.67 and 1786.13 respectively which is higher than the panel mean, while susceptible group has showed the vigour index of 712.93.

Table 2: Maximum and minimum mean values observed among the rice germplasm entries for various anaerobic germination tolerance traits

Sr. No.	Character	Maximum	Minimum	Mean
1.	Germination_percentage	93.88	2.76	32.87
2.	Shoot_length	35.28	15.33	27.77
3.	Length_of_first_internode	11.32	3.87	7.35
4.	Root_length	19.48	7.21	12.10
5.	Number_of_leaves	3	2	3
6.	Shoot_dry_weight	0.1253	0.0090	0.0497
7.	Root_dry_weight	0.0688	0.0065	0.0210
8.	Seedling_vigour_index	2855.73	55.75	923.69

Table 3: Tolerant, moderately tolerant and susceptible genotypes identified for germination percentage under anaerobic condition.

Sr. No.	Classification	Number of genotypes	Name of the genotypes
1.	Tolerant	4	Tulasiphula, Panirohi, AG 387, BJ 1 Chinamali
2.	Moderately Tolerant	9	ARC 14855, Basapatri, Parbat jeera, T 1 ARC 14965, ARC 5977, ARC 11600, DHINGHA, Bandha
3.	Susceptible	89	Dhala basmati, Khajara, Gadakati, Kalakataki, RAJ MUNDO, CHAMKA, Goria, Gahamphulla, Jabaful, Swarna, Lajakuli, Krishnabhog, Pimpudibasa, Basantibhog, Lajakuli, Champa, Mahakamati, Jubaphul, Kalajeera, Samudrabali, Bankoi, ARC 7098, Kala kadamba, Kalachampa. Chholaboro, AUS 204, Cr Dhan 801, BORO, AUS 314, AUS 175, ARC 6000, AUS 151, NaliBaunsaGaja, Mukta kiari, AUS 350, Tupa, Jangalijata, Nababi, SANHUANGZHAN NO 2, IR 64-21, AUS 354, Solari, Kalamkati, MUNSHISHAIL, ASSAM 4(BORO), AUS 267, AUS 366, ARC 7325, AUSMERI, AS 2, ARC 6240, ARC 5959 AUS 435, AUS 63, SATHA, DA 12, AUS PADDY(WHITE), AUS 29, AUS 298, AUS 169, AUS 268, AUS 77, AUS 131, AUS 417, AUS 309, AUS 60, AUS 362, AUS 273, BOWALIA, AUS 93, AUS 127, AUS 391, AUS 62, AUS 125, ARC 10958, AUS 353, BOWALIA 2, AUS 22, AUS 37, AUS 46, AUS 283, AUS 294, AUS 317, AUS 321, AUS 335, AUS 361, AUS 369, AUS 382, AUS 385

Table 4: Promising tolerant genotypes identified under screening studies for anaerobic germination tolerance.

Genotype	Germination Percentage	Shoot Length	Length of First Internode	Root Length	Number of Leaves	Shoot Dry Weight	Root Dry Weight	Seedling Vigour Index
Tulasiphula	93.88	28.29	9.91	13.97	3	0.1250	0.0106	2655.26
Panirohi	93.55	29.30	9.49	14.80	3	0.1128	0.0193	2743.12
BJ 1	91.91	31.08	9.93	14.20	3	0.0887	0.0166	2855.73
Chinamali	82.83	28.92	9.79	12.20	3	0.0949	0.0121	2023.42

CONCLUSIONS

The present investigation on screening rice genotypes for anaerobic germination tolerance have shown that there is considerable genetic variability in rice varieties, with some genotypes being more tolerant to anaerobic conditions than others. The tolerance of rice genotypes to anaerobic germination is an important trait in rice breeding programs, particularly in flood-prone regions, as it ensures that the crop can germinate and grow even under waterlogged conditions. The genotypes with higher germination percentage and high seedling vigour (TulasiPhula, Panirohi, BJ 1, Chinamali, ARC 14855) are crucial good seedling establishment rate and these genotypes could be selected for introgression of anaerobic germination tolerance traits into the elite cultivars through various breeding programmes to improve their adaptability seedling establishment rates in direct seeded rice (DSR) cultivation. The screening of rice genotypes for anaerobic germination tolerance is a time-consuming and labour-intensive process, but it is crucial for identifying genotypes that can withstand adverse environmental conditions. The use of molecular markers can facilitate the identification of specific genes that confer tolerance to anaerobic conditions, making the screening process more efficient.

In conclusion, the screening of rice genotypes for anaerobic germination tolerance is an important step in rice breeding programs, as it can lead to the development of rice varieties that are better adapted to flood-prone regions. The use of modern biotechnological tools, such asmolecular markers, can further enhance the efficiency of the screening process, leading to the identification of genes that confer tolerance to anaerobic conditions.

FUTURE SCOPE

Anaerobic germination screening studies have potential to develop flood-tolerant rice varieties. Future scopes include developing high-throughput screening methods, identifying molecular markers associated with anaerobic germination tolerance, understanding physiological and biochemical mechanisms, utilizing genome editing technologies, and exploring other crops. These efforts could lead to the development of new varieties that are better adapted to waterlogged soils and can withstand floods and waterlogging.

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