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Screening of Rice (*Oryza sativa* L.) Genotypes for Anaerobic Germination

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ABSTRACT

The present investigation was carried out to screen the set of 2000 rice genotypes including germplasm, released varieties, INGER nurseries and elite lines for anaerobic germination trait. Based on initial screening of 2000 genotypes, five hundred lines were selected for further to study the trait based on parameters namely germination percentage, seedling length and vigour index. Frequency distribution was calculated for the three observations under study. Out of five hundred genotypes under study, 16 genotypes were not germinated, while 100 % germination was recorded by 17 genotypes. The seedling length of the germinated seeds was in the range of 1 cm (E 199 and E 282) to 62 cm (E 480), While vigour index was ranged from 20 (E199, E282 and E532) to 4880 (E1777). After repeated screening, 13 entries namely E775, E1810, E596, E1786, E 753, E773, E1846, E1195, E1049, E1772, E1723, E1701 and E1777 were recorded 100% anaerobic germination with high seed vigour index, was identified as tolerant genotypes for the anaerobic germination. These genotypes could be used as parents to introduce anaerobic germination tolerance into improved cultivars to utilize under direct seeded conditions.

Key words: *Germplasm, Genotypes, Oryza sativa, Yield*

INTRODUCTION

Rice (*Oryza sativa* L.) is an important food crop for half of the world's population. It is the only crop that can be grown in most fragile ecosystems and second green revolution is expected only when rice research is designed in such a way to address the specific abiotic and biotic stresses associated with rice production. In order to meet the present and anticipated global food demands, there should be a significant increase in crop productivity in marginal farmlands with minimum resources.

Rice is commonly grown by transplanting, which is labour and energy intensive system. Now a days it is becoming less profitable as these resources are becoming increasingly scarce. It also deteriorates the soil physical properties, adversely affects the succeeding upland crops performance and also contributes to climate change by methane emissions.

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These factors had driven the major shift from transplanting to direct seeding of rice in irrigated ecosystems. It was also evident that wet direct seeding could increase grain yield by 10-15% Islam *et al*[6].

Adoption of direct seeded rice has been held back by different constraints namely, poor crop establishment or a total loss of the crop due to improperly levelled fields, heavy rainfall and poor drainage, which lead to the accumulation of water at varying depths immediately after sowing Angaji *et al*.[2] . The hazards of flooding immediately after sowing or during germination can creates the anoxia or hypoxia conditions, which impairs seedling growth because of high sensitivity of rice to anaerobic germination. Further it, leads to anchorage problem and poor seedling establishment. Even if crop establishes initially but again submergence at later stage may cause heavy losses. Weeds are the major constraint in direct seeded rice, while flooding the field right after the sowing has beneficial effect in weed control.

Therefore, inbuilt tolerance in the rice varieties to anaerobic conditions during germination and early stages is a pre-requisite for effective adoption of direct seeded system under low lying rainfed, flood affected areas and even for intensive irrigated ecosystems. Where flooding immediately after sowing can also suppress the weeds, which will ultimately leads to the reduction in cost of cultivation as opposed to transplanted rice Ismail *et al*[7].
Development of high yielding rice varieties that can withstand anaerobic condition during germination and early seedling growth is essential to alleviate the constraints associated with practicing of direct seeding system. Consequently, characterization and screening of contrasting genotypes would help in evaluating and revealing the various genetic, biochemical regulatory and signalling mechanisms associated with tolerance to anaerobic germination.

The availability of genotypic variation to germinability under oxygen depleted conditions was evidenced from the number of reports. To date, little progress has been made in establishing efficient screening methods to evaluate this trait. Hence, it is proposed to assess and screen the variability among the rice germplasm to identify the genotypes with AG tolerance through standard procedures. This could facilitate breeding of rice varieties suitable for direct seeded conditions and guide efforts for improving water-logging tolerance.

MATERIAL AND METHODS

The present investigation was conducted during *Kharif*, 2015, at ICAR-Indian Institute of Rice Research, Hyderabad, India. An attempt was made to screen a large set genotypes consisting of germplasm, released varieties, INGER nurseries, elite lines to assess the anaerobic germination trait. Based on initial screening of 2000 genotypes, a set of five hundred lines were selected for further screening under lab conditions to assess the occurrence of trait. Before the conduct of the experiment all these genotypes were tested for viability and the genotypes showing 100 percent germination were further tested under anaerobic condition. Screening was done by direct dry seeding in germination trays with a shallow layer of field soil. The trays containing the germination sheets wherein rice seed were placed and covered with thin layer of soil were immersed in larger zinc trays where 40 cm depth of water was maintained for 15 days without any disturbance. Recording of observation on a three metric traits *viz.,* germination percentage (%), seedling length (cm) and vigour index (germination percentage x seedling length) was done on 15th day after seeding as per the standard procedure given by ICAR-IIRR (DRR Newsletter , 10 (4) 2012).

RESULTS AND DISCUSSION

It is prime important to select rice genotypes that can germinate under anaerobic conditions with exceptional seedling vigor for use them in direct seeding. Screening of available germplasm and prioritizing of research strategies to know the various mechanisms of tolerance to anaerobic germination would be necessary to improve the productivity of rice.

Tolerant rice genotypes were developed several adaptive mechanism that allow them to establish under flooded conditions, which includes, their ability to germinate and elongate faster under absence or low oxygen environment, potential to mobilize the stored starch reserves and effective energy generation through fermentation pathways etc, Ismail *et al*[8]. Unravelling the different adoptive mechanisms possessed by tolerant rice genotypes to early flooding will help to design effective management strategies that will allow them to adequately establish under flooded soils by simultaneously suppressing weeds. Among the various adaptive mechanisms, high germination percentage and seedling length are the major characters which inturn closely associated with seedling vigor, which is final determinant of the optimum crop establishment under anaerobic conditions.

In the present study, five hundred rice genotypes were screened for anaerobic germination and they exhibited wide range of variability for all the three traits studied and it could be allowed to classify the genotypes into tolerant, moderate and susceptible groups. Frequency distribution for all the characters under study were computed (Table 1).

In general, seeds with high carbohydrate reserves, such as cereals, are known to be more tolerant for oxygen deprivation during germination Raymond *et al* [11]. Out of all the major cereals, rice is the only crop which has the unique feature to germinate under water, thereafter, growth is limited to only coleoptiles Angaji *et al*[2].

Based on the germination percentage the total genotypes were classified into six categories. Among them 16 genotypes were not germinated under anaerobic conditions. 260 genotypes were having the germination percentage in the range of 1-20 % and these can be treated as susceptible genotypes. 120 genotypes exhibited 20-40 % germination, while 40-60 %, 60-80 % and 80-100% germination percentage was expressed by 64, 23 and 17 genotypes respectively. Among the genotypes germinated under anaerobic conditions, IR 64 was recorded minimum germination percentage (8%).

In case of rice several growth, anatomical and physiological features were identified that are closely associated with germination and survival under flooded soils. The tissues present in germinating seeds of rice will greatly suffer from hypoxia under submerged conditions and which could hinder the activity of the enzymes related to mobilization of stored carbohydrates reserves and generating energy generation system by oxidative pathways. Under anaerobic conditions, germinating rice seeds can retain their ability to break down starch into readily fermentable carbohydrates Atwell and Greenway[3].

Amylases are the class of enzymes believed to play a major role in starch breakdown. Rice seed has the complete set of starch degrading enzymes to support the growth and maintenance of the growing embryo; however, the oxygen status plays a very crucial role in deciding the activities of these enzymes. Previous studies reported that, tolerant genotypes had higher amylase activity in germinating seeds compare to susceptible ones and there is a progressive increase in enzyme activity from sowing to reach several-fold at 3 days after seeding under anaerobic conditions Ismail *et al* [8]. This greater amylase activity was also associated with several essential criteria *viz*., maintenance of higher soluble sugar concentrations in germinating seeds, greater starch depletion, better seedling growth and higher seedling survival.

The genotypes with above 80 % germination were treated as tolerant to anaerobic germination. During our investigation, 100 % germination was recorded by 17 genotypes namely, E372, E596, E747, E 753, E773, E775, E779, E1005, E1049, E1195, E1701, E1723, E1772, E1777, E1786, E1810 and E1846. Further, these genotype are exhibited more tolerance to flooding during germination when compared with sensitive genotypes, seems to have efficient and rapid water uptake during seed imbibitions, better capabilities of breaking starch into simple sugar and faster depletion of starch in their germinating seeds, which might be attributed for their higher germination percentage.

Anaerobic catabolism also significantly increased the activities of both pyruvate decarboxylase and alcohol dehydrogenase within 12 h of imbibitions in tolerant and as well as sensitive genotypes, but it is progressively remained higher in the tolerant genotypes with time, suggesting that anaerobic respiration is probably one of the critical feature contributing to the superior performance of tolerant rice genotypes Ismail *et al*[8].

Rice coleoptiles and shoots are one of the few plant organs that could be able to grow under absence or low oxygen conditions and they vary greatly for rates of elongation and alcoholic fermentation. Rapid elongation of coleoptiles and shoots could facilitate, to get into contact with air under anaerobic soils and further to helps to supply oxygen for the growing embryo. Because it is difficult to detect the amount of alcohol under anoxia, hence shoot length of seedlings including the coleoptiles was used as marker to determine anaerobic germination tolerance Ling *et al*[9].

The shoot length of the seeds germinated under 40 cm water submergence was scored at 15 days after germination and wide variability observed among the genotypes (Table 2). Among the total germinated genotypes under study, 25 were just sprouted and 13 genotypes were germinated but did not survived. Even though, excessive shoot elongation but failure to reach the water surface will also lead to exhaustion of total stored carbohydrate reserves and ultimately leading to death of the plant Sarkar *et al.*[3]. Among the germinated seedlings, seedlings lengths were ranged from 1 cm (E 199 and E 282) to 62 cm (E 480) and were grouped into four classes *viz.*, 1-20 cm, 20-40 cm, 41-60 cm and 61-80 cm with frequency distribution of 49.80 %, 32.40%, 6.80 % and 0.20 %, respectively.

Alternatively, flooding induced elongation is one of the escape mechanisms that help submerged plants regain contact with air. There is a interplay of plant hormone ethylene, which promotes rice seedling growth under anaerobic conditions via modifying the effects of GA, ABA and auxins Fukao *et al*[4]. However, the effects of these plant hormones are difficult to predict. Moreover, it was evident from the previous reports that the ethylene was known to enhance sucrose transportation from the scutellum to the growing coleoptile in germinating rice seeds Ishizawa and Esashi[5]. The promoting effect of ethylene on coleoptile growth seems to be manifested at later stages of seedling growth. Hence, it suggests that the role of ethylene is probably more important during coleoptiles and shoot elongation than during germination under hypoxia flooding and low-oxygen stress in germinating seeds.

Seedling vigour plays a key role in the submergence avoidance mechanism Manangkil *et al.*[10]. The seeds which are exhibiting the higher vigour index are considered to be more vigorous. The vigor index was calculated by multiplying seed germination percentage with seedling length Roy and Sharma[12]. The vigour index values were greatly (20-4880) varied among the genotypes under study. Five genotypes namely, E 199 (20), 282 (20), 532 (20) and IR 64 (48) were expressed vigour index of less than 50. While, two entries *viz.*, E 1701 and 1777 recorded highest seed vigour index of 4100 and 4880, respectively.

For instance, under hypoxia or anoxia stress, tolerant rice genotypes will germinate and grow faster with high seedling length and vigour index. They maintain the higher amylase activity and adopt anaerobic respiration to utilize stored starch reserves Ismail *et al*. [7]. In the present investigation, based on the observations recorded, 13 entries namely, E775, E1810, E596, E1786, E 753, E773, E1846, E1195, E1049, E1772, E1723, E1701 and E1777 were having the 100% anaerobic germination with good seed vigour index can be the tolerant genotypes for the anaerobic germination. However, it is unknown whether similar mechanisms as described above are involved in the tolerance of AG lines. Further, it has to be confirmed through intensive research to reveal the associated mechanisms.

Table 1. Frequency distribution rice germplasm lines for anaerobic germination

S. No	Character	State of expression	Number of genotypes	Frequency distribution (%)
1.	Germination percentage (%)	Not Germinated	16	3.20
		1-20 %	260	52.00
		21-40%	120	24.00
		41-60%	64	12.80
		61-80%	23	4.60
		81-100%	17	3.40
2.	Seedling Length (cm)	Not Germinated	16	3.20
		Just Sprouted	25	5.00
		Germinated but not survived	13	2.60
		1-20 cm	249	49.80
		21-40 cm	162	32.40
		41-60 cm	34	6.80
		61-80 cm	1	0.20
3.	Vigour Index	Not Germinated	16	3.20
		Just Sprouted	25	5.00
		Germinated but not Survived	13	2.60
		1-1000	333	66.60
		1001-2000	73	14.60
		2001-3000	21	4.20
		3001-4000	17	3.40
		4001-5000	2	0.40

Table 2. Maximum and minimum mean values observed among the rice germplasm lines for anaerobic germination

S. No	Mean value (Range)	Character	Name of the genotype
1.	Minimum	Germination percentage (%)	IR 64 (8 %)
		Seedling length (cm)	E 199 and E 282 (1 Cm)
		Vigour Index	E 199 and E 282 (20)
2.	Maximum	Germination percentage (%)	E372, E596, E747, E 753, E773, E775, E779, E1005, E1049, E1195, E1701, E1723, E1772, E1777, E1786, E1810 and E1846 (100 %)
		Seedling length (cm)	E 480 (62 Cm)
		Vigour Index	E 1777 (4880)

* The values in the parenthesis indicates the minimum and maximum mean values observed for the traits under study

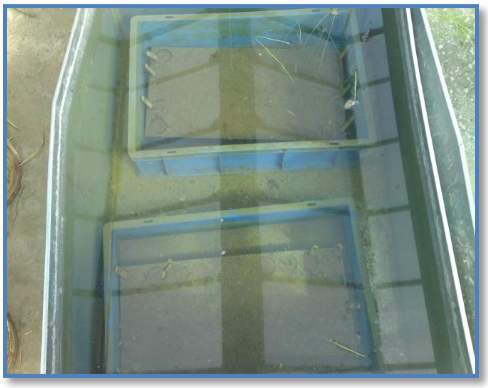

Fig. 1: Genotypes failed to germination under water

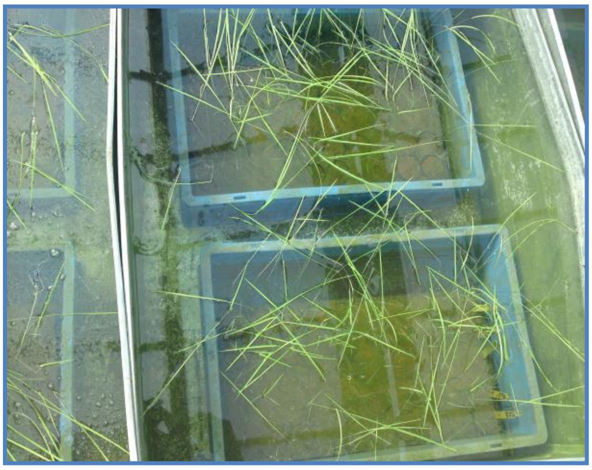

Fig. 2: Genotypes with >90% anaerobic germination

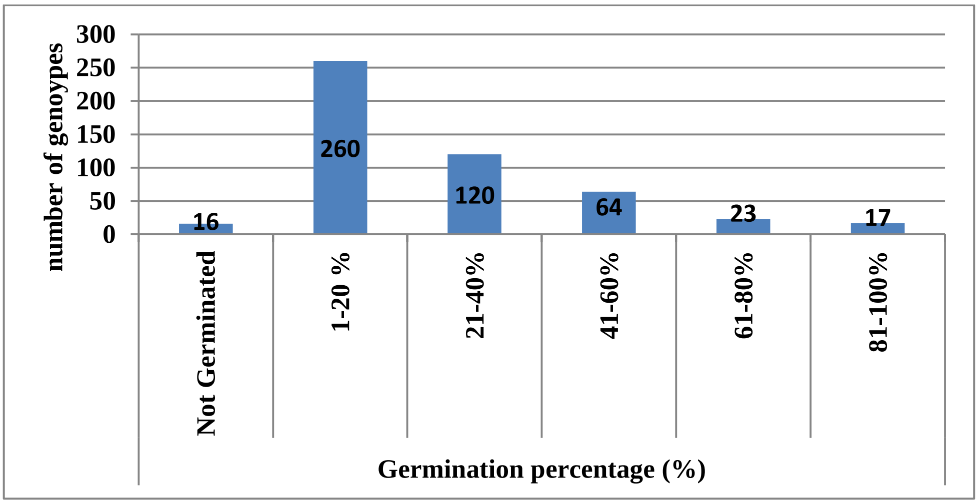

Fig 3. Frequency distribution rice germplasm lines for germination percentage (%)

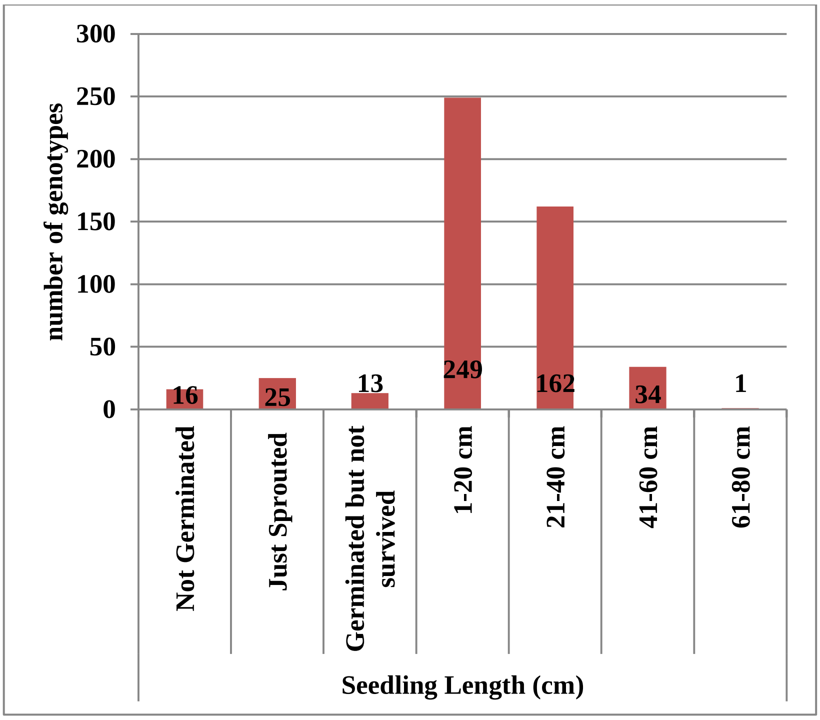

Fig 4. Frequency distribution rice germplasm lines for seedling length (cm)

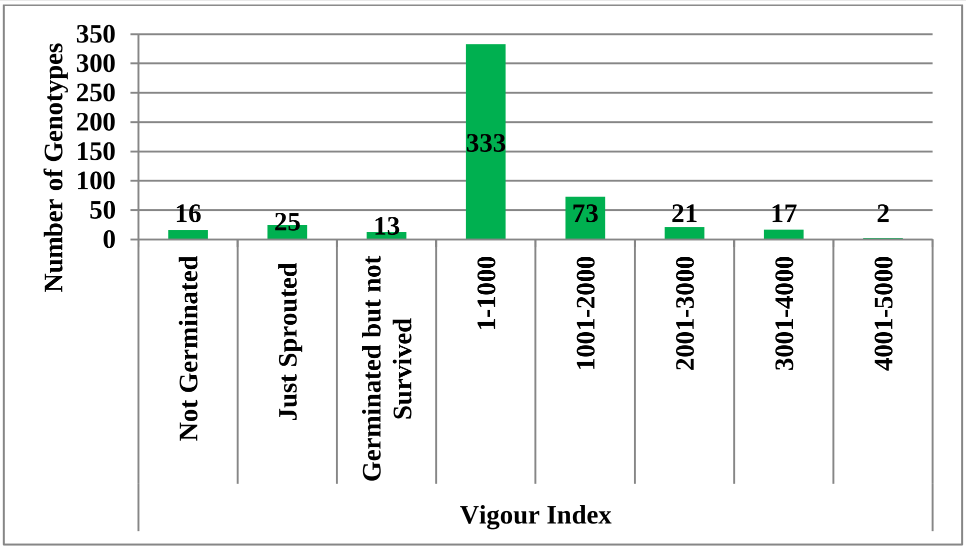

Fig 5. Frequency distribution rice germplasm lines for vigour index

CONCLUSIONS

Improving tolerance to submergence during germination in rice will facilitate the use of direct seeding in lowland rice ecosystems, which brings numerous benefits to farmers by reducing the labour costs, effective water management, early harvest and less reliance on herbicides. Several studies revealed that rice has enormous exploitable variation in tolerance of flooding during germination, while very little progress was made in understanding the basis of tolerance.

The present investigation is the first step towards elucidating the morphological, genetical and physiological mechanisms underlying the vigorous growth of rice seedlings under anaerobic conditions. High germination percentage, longer seedling length and high seed vigour index are very critical for a high seedling establishment rate and these could be targeted while selecting rice genotypes suitable for anaerobic conditions. Therefore, high germination percentage and high seed vigour index of 13 tolerant genotypes *viz*., E775, E1810, E596, E1786, E 753, E773, E1846, E1195, E1049, E1772, E1723, E1701 and E1777 are important criteria for breeding cultivars with strong early growth under anoxia or hypoxia. Hence, these traits could be introduced into elite cultivars through various breeding programmes to improve their establishment rates in direct seeding cultivation. However, substantial efforts are still needed to reveal the signalling mechanisms that regulate the metabolic pathways involved in anaerobic tolerance of these genotypes.

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