Int.J.Curr.Microbiol.App.Sci (2017) *6*(5): 580-590

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 5 (2017) pp. 580-590**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.605.067

Designing New Screening Methods and Physiological Dissection of Anaerobic Stress Tolerance in Rice

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Keywords

Rice, anaerobic condition, germination percentage, alcohol dehydrogenase, amylase activity.

Article Info

Accepted:
04 April 2017
Available Online:
10 May 2017

A B S T R A C T

Due to increasing practices of direct seeding in rice under rainfed and irrigated conditions, tolerance to hypoxic or anaerobic condition is becoming an important trait. Considerable genetic diversity is present in our native rice landraces and varieties which is unexploited. Identifying and characterization of contrasting genotypes with acquired tolerance would reveal new donors with improved water logging tolerance for breeding programs which might be suitable for direct seeding systems. Apparently, there is lack of standard screening procedure for anaerobic germination trait in the rice landraces and varieties of Tamil Nadu. The present study designed new methods (Protray and beaker method) to screen for anaerobic germination by assessing simple seed and seedling characters such as germination per cent, greater shoot length by means of coleoptiles elongation and higher vigor index to find the donors for anaerobic stress tolerance. Genotypes differing in their tolerance to anaerobic germination were grouped as: (Highly Tolerant: Anaikomban, Ottadaiyan, Muthuvellai, Rajamannar, CR1009; Moderately tolerant: Improved White Ponni: Highly Susceptible: CO 43 and FR13A). Genotypes showing contrasting behavior to anaerobic stress tolerance were selected to study the physiological basis of anaerobic stress tolerance. The results showed that the tolerant genotypes recorded higher germination per cent, greater shoot length by means of coleoptile elongation and recorded higher vigor index. Further, the tolerant genotypes in this study depicted higher amylase activity, higher Alcohol Dehydrogenase(ADH) activity and lower peroxidase activity highlighting the role of these key enzymes to promote germination and survival under low oxygen conditions.

Introduction

Rice is the most important cereal crop worldwide and is the staple food of Indians. Rice is grown under wide range of ecosystems from marginal dry land to waterlogged wetlands, with lot of modifications in cultural practices to suit the local environmental cues. Rice production systems differ widely in cropping intensity and yield, ranging from single-crop rainfed lowland and upland rice with small yields (1–3 t ha^{-1}), to triple-crop irrigated systems with an annual grain production of up to 15–18 t ha^{-1}. Irrigated and rainfed lowland rice systems account for about 80% of the worldwide harvested rice area and 92% of total rice production. To keep pace with population growth, rice yields in both the irrigated and rainfed lowland environments must increase by 25% over the next 20 years. Currently, upland and flood-prone rice account for less than 8% of the global rice supply, and it is unlikely that production from these systems can be significantly increased in the near future (FAO, 2014). Due to increasing practices of direct seeding in rainfed and irrigated conditions, tolerance to hypoxic or anaerobic condition is becoming an important trait. If heavy rain happens to coincide during direct seeding, the entire crop may be lost. It significantly reduces the yield because of improper crop establishment.

Seed germination and seedling growth under low oxygen stress conditions facilitates an adaptive mechanism to withstand anaerobiosis (Magneschi and Perata, 2009). Climate change projections suggest that temperatures, precipitation, flooding and sea level rise are likely to increase creating adverse impacts on crop yield and farm income (INCCA, 2010). Rice is therefore a very interesting as a plant model to study how plants can escape soil anaerobiosis, through coleoptile elongation (Mapelli *et al*., 1995). Apart from delayed germination and poor seedling establishment, prolonged exposure to hypoxic condition can lead to improper nutrient availability and up takeas a result of altered soil pH. In order to escape these adverse conditions, rice plants have evolved a mechanism called coleoptile elongation to germinate and establish under low oxygen conditions (Huang *et al*., 2003). 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).

Anaerobic stress tolerance in rice is accompanied by a series of biochemical alterations such as, the changes in the enzyme activities of α-amylase, peroxidase and alcohol dehydrogenase. Among these enzymes, it has been reported that α-amylase has a positive role in improving the germination ability of the seed by degrading starch into sugars (Perata *et al*., 1993). These enzymes have been found to accumulate in anaerobic tolerant rice lines whilst absent in intolerant lines. In the absence of α-amylase, starch is not hydrolyzed into sugar and the anaerobic-intolerant lines experience sugar starvation and ultimately fail to (Perata *et al*., 1996). The enzyme alcohol dehydrogenase via anaerobic fermentation metabolism has been reported by several researchers to supply ATP for seedling germination and survival under anaerobic stress condition (Gibbs and Greenway, 1996; Gibbs *et al*., 2000; Jackson *et al*., 1982). Fermentation metabolism might serve to be one of the important mechanisms for the rice seedlings to establish and survive under low oxygen conditions. Another prominent enzyme that is found to be regulated under anaerobic condition is the cell wall peroxidases. This enzyme acts antagonistically to cell wall extensibility thereby inhibiting the cell elongation in mungbean and peanut (Glodberg *et al*., 1987; Zheng and Huystee, 1992; Ismail *et al*., 2009). Thus, the characterization of these biochemical changes under anaerobic germination in rice would reveal the adaptative mechanisms to understand physiological bases of anaerobic stress tolerance.

In Tamil Nadu, we have a wealth of unexplored germplasm of rice varieties/landraces that evolved several special traits to perform well under any abiotic stress conditions. Apparently, there is lack of standard protocols and methodologies to screen genotypes for anaerobic germination trait in the rice landraces and varieties of Tamil Nadu. Hence, the study was aimed to (i) Design rapid, reliable and repeatable protocols to screen rice lines for improved tolerance to anaerobic conditions (ii) to identify suitable donors for anaerobic stress tolerance among the popular rice varieties and landraces of Tamil Nadu and (iii) to assess the key physiological/biochemical traits and to correlate important enzyme activities to seedling survival under anaerobic conditions.

Materials and Methods

Plant material

The seed materials consist of five popular varieties and fourteen land races grown in Tamil Nadu (Table 1). The seeds were obtained from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore.

Designing screening techniques to identify rice lines for anaerobic stress tolerance based on germination percentage

Initially, anaerobic germination was standardized by adopting two ways

Protray method: Portrays filled with black soil and 15 seeds (3 seeds each for 5 holes) for each of the 19 rice genotypes were sown in portrays and placed inside a concrete submergence tank with 10 cm water level, to check for the germination percentage. Three rows of replications were maintained for each genotype. The germination percentage was recorded at 7 days after sowing. A similar set of genotypes, replicated the same way was kept as control. These portrays were kept inside another submergence tanks without water. The plants were daily watered to maintain optimum moisture content

Beaker method: 15 seeds from each of the genotype were sown in a 500 mL beaker filled with 4cm of soil and water level of 6 cm (Fig 1.). Three replications of 15 seeds per replication for each genotype were maintained. Germination percentage was recorded 7th DAS and shoot length, root length, vigor index and shoot-root ratio of 10 day old seedlings under anaerobic condition were measured since roots of the sensitive lines did not appear until about 7–8 d following sowing.

Methods adopted to assess the morphological traits associated with seedling growth under low oxygen stress

Contrasting genotypes for anaerobic stress tolerance were germinated in the beaker method with three replications each containing 15 seeds per genotype. Seeds were grown until 10 days and the germination percentage, shoot length, root length, root-shoot ratio, vigor index were observed. Germination percentage is measured by calculating the number of seeds germinated to the total number of seeds sown. The root length, shoot length and root-shoot ratio were calculated by pulling out the seedlings without damaging their roots and washing their roots gently. The vigor index was calculated by using the formula *viz.*,(Shoot length + Root length) * germination per cent). Shoot length and root length were measured in centimeters.

Alteration in enzyme activities under anaerobic stress

Enzymes such as α-amylase, peroxidase and alcohol dehydrogenase activity were assessed for the 10 day old seedlings subjected to anaerobic stresses. Total amylase activity was measured in the whole germinated seedlings by following the method of Bernfeld *et al* (1995). The absorption values were read at 540 nm on a standard curve established with increasing amounts of maltose. Total protein concentration was determined following the Bradford method (Bradford, 1976) and the activity expressed in units per milligram protein. One unit of amylase activity is defined as moles maltose produced per minute and specific activity is expressed in terms of units per mg protein.

Crude protein was extracted from 100 mg of tissue by grinding in 600 mL of ice-cold extraction buffer (100 mM TES, pH7.7; 2 mM $MgCl_2.6H_2O$; 1 mM EDTA; 1.25 % (w/v) TritonX-100; 4 mMdithiothreitol). The crude extract was centrifuged at 10 000 g for 10 min at 5°C. Total ADH activity was analyzed using the procedures described in Ella *et al*., (1993) at 340 nm. Bradford's method (Bradford, 1976) was used for total protein assay with bovine serum albumin as a standard.

Peroxidase activity was measured following the procedure of Peru, (1962).One gram of leaf was extracted in 0.1M phosphate buffer (pH 7.0). A known volume of the extract was added to a cuvette containing 3ml phosphate buffer and 3ml pyrogallol was added and the increase in absorbance at 430 nm was recorded. The change in absorbance in minutes was used to calculate the enzyme activity.

Results and Discussion

Validation of methods adopted to screen rice genotypes for anaerobic stress tolerance

Two screening methods (Portray and Beaker) were designed and all the 19 genotypes were subjected to low oxygen stress by flooding them. Development of different screening methods for anaerobic germination has also been reported by Mapelli *et al*., (1995) for rice and wheat; Ismail *et al*., (2009) for rice. Germination per cent was recorded on 7^{th} day. In both portray and beaker methods, same results were reported. Rice lines that were screened as tolerant and susceptible by portray method also showed tolerance and susceptibility in beaker method. Hence, both the methods can be used as rapid, reliable and repeatable screening methods.

Identification of anaerobic stress tolerant genotypes

Among the nineteen rice genotypes that were germinated under anaerobic condition, the landraces have been found to show higher germination percentage when compared to the popular varieties. Vergara *et al*., (2014) also reported that landraces performed better compared to the cultivated varieties under stagnant flooding conditions. All the genotypes taken for the study recorded 100 per cent germination percentage under control condition created for both the methods (Portray and Beaker).

Fig 2, showed that the genotypes that recorded a germination percentage of more than 90 % under anaerobic condition were regarded as highly tolerant to low oxygen stress, as seed germination is a critical point in seedling establishment and subsequent plant vigor (Manigbas *et al*., 2008; Miro and Ismail, 2013). The genotypes CO 43 and FR13A recorded the lowest germination per cent (10%), and hence were regarded as highly susceptible genotypes. Improved White Ponni was classified as moderately tolerant genotype with germination per cent of 70%. The land races Ottadaiyan and Rajamannar recorded a high germination per cent of 96%; Muthuvellai and CR1009 recorded 94 and 95 per cent germination respectively. The genotype Anaikomban recorded 100 per cent germination. The study clearly stated that there is a wide genetic variation for anaerobic germination in rice. This is in line with the findings of Ismail *et al.,* (2009) and Vergara *et al.,* (2014). Based on germination percentage under low oxygen stress the genotypes were grouped as Highly tolerant (Anaikomban, Ottadaiyan, Muthuvellai, Rajamannar, CR1009), Moderately tolerant (Improved White Ponni) and Highly Susceptible (CO 43 and FR13A)(Fig 3). Thus, the highly tolerant genotypes can be exploited as donors in crop improvement programmes aiming for tolerance to anaerobic stress conditions. Vergara *et al.,* (2014) also reported that variation in tolerance of rice to long-term stagnant flooding that submerges most of the shoot will aid in breeding tolerant cultivars.

Morpho-physiological traits in contrasting rice genotypes subjected to anaerobic stresses

The tolerance/susceptibility of rice lines to anaerobic stresses screened in portray method was confirmed in beaker method using simple morpho-physiological parameters like germination percentage, shoot length, root length, vigor index and root-shoot ratio. Manigbas *et al.,* (2008) has also reported the need for developing a standard screening method for anaerobic seed germination using different rice genotypes. All the parameters were recorded on 7th after sowing in the highly tolerant and highly susceptible genotypes.

All the genotypes under control recorded 100 per cent germination. Under anaerobic stress condition, Anaikomban recorded highest germination percentage of 100 while Co 43, FR 13A were found to be highly sensitive under low oxygen stress with a germination per cent of 12 and 5 respectively. The genotypes Ottadaiyan and Muthuvellai recorded germination per cent of 96, while Rajamannar and CR 1009 recorded a germination per cent of 94 and 92 per cent respectively (Table 2). The genotype Improved White Ponni recorded 72 per cent germination. These results further confirmed the results obtained in the previous experimental set up.

Regarding the shoot and root length, it was observed that the genotype Anaikomban recorded the longest shoot length (27.6 cm), while FR 13A recorded the shortest shoot length (2.0 cm) compared to other genotypes. The tolerant landrace Rajamannar was observed to record a greater shoot length (20.9 cm) and the longest root length (8.3 cm) compared to other genotypes. The results obtained from this study showed that higher the germination per cent greater was the vigor index for seedling establishment. Anaikomban recorded the highest vigor index both under control (2940) and stress (3430) condition while the vigor index was low in the genotype FR13A (control-1530; stress-13). Similar trends were observed for root-shoot ratio with the tolerant genotype recording the lowest root-shoot ratio and vice-versa in the intolerant. The study thus clearly signify the negative correlation between seedling growth, vigor index and root shoot ratio. Hence, morpho-physiological traits attributed for anaerobic stress tolerance are germination per cent, greater shoot length by means of coleoptile elongation and higher vigor index. Since the rice seedlings escape low oxygen stress by coleoptile elongation rather than root emergence, the tolerant genotypes also recorded higher shoot: root ratio (Miro and Ismail, 2013).

Understanding the Physiological basis of anaerobic stress tolerance by assessing the alterations in the enzyme activities

The activities of α-amylase, Alcohol Dehydrogenase and Peroxidase were assessed in 10 days old seedlings both under control and low oxygen stress conditions. Under control conditions the enzyme activities did not show any significant variation among the genotypes. Hence, the enzyme activities of the tolerant and intolerant genotypes under anaerobic stress conditions are alone discussed below (Table 3).

The genotype Anaikomban recorded the highest (32.18 Units mg^{-1} protein)α-amylase activity under anaerobic stress condition and FR13A and CO43 recorded the lowest (10.45; 8.13 Units mg^{-1} protein) enzyme activity. α-amylases are believed to play a vital role in the breakdown of starch (Magneschi and Perata, 2009)and rice seeds are capable of degrading starch during germination under anoxic condition to generate ATP required for the germinating embryos (Perata *et al*., 1992;Perata *et al*., 1993,Guglielminetti *et al*., 1997). Thus, in line with the above findings, the present study also that the tolerant genotype Anaikomban recorded higher amylase activity highlighting the role in ability to survive and grow faster under low oxygen conditions. On the contrary, the sensitive genotypes recorded very low amylase enzyme activity. Hence, a positive correlation (r^2=0.64) between germination percentage and α-amylase activity was observed in the study (Fig.4). Ismail *et al*., (2009) has also reported that the higher amylase activity during submergence is consistent with the faster growth observed in tolerant genotypes compared with intolerant ones, and is also illustrated by the strong positive correlations with shoot and root lengths during flooding.

The enzyme alcohol dehydrogenase (ADH) via anaerobic fermentation metabolism has been reported by several researchers to supply ATP for seedling germination and survival under anaerobic stress conditions (Gibbs and Greenway, 1996; Gibbs *et al*., 2000; Jackson *et al*., 1982). Alcohol dehydrogenase activity was found to be highest in Muthuvellai (1.93Units $min^{-1}mg^{-1}$ protein) followed by CR1009 (1.71Units $min^{-1}mg^{-1}$ protein) and Anaikomban (1.23Units $min^{-1}mg^{-1}$ protein). The sensitive genotype FR13A was observed to record lowest (0.26Units $min^{-1}mg^{-1}$ protein) ADH activity.

Table.1 List of rice genotypes used for the study

S. No.	Rice varieties/landraces
	Popular varieties
1	CO 43
2	CO 50
3	CR1009
4	IMPROVED WHITE PONNI
5	IR64
	Landraces
6	ANAIKOMBAN
7	APO
8	FL48
9	FR13A
10	KALIYANA SAMBA
11	KALLURNDAIKAR
12	KARTHIGAI SAMBA
13	KODAVARI SAMBA
14	KOMBALAI
15	MUTHUVELLAI
16	NORUNGAN
17	OTTADIAYAN
18	RAJAMANNAR
19	RASACADAM

Table.2 Effect of anaerobic germination on seedling characters in rice genotypes

Genotype	Germination percentage	Shoot length (cm)		Root length (cm)		Vigor Index		Root: shoot ratio	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress
Anaikomban	100±0.334	19.1±0.064	27.6±0.092	10.3±0.034	6.7±0.022	2940	3430	0.5393	0.2428
Ottadaiyan	96±0.334	17.2±0.060	19.4±0.067	8.1±0.028	5.8±0.020	2530	2419	0.4709	0.2990
Muthuvellai	96±0.390	17.4±0.061	18.5±0.064	9.2±0.032	6.4±0.022	2660	2341	0.5287	0.3459
Rajamannar	94±0.327	16.7±0.068	20.9±0.085	10.7±0.043	8.3±0.034	2740	2803	0.6407	0.3971
CR1009	92±0.690	12.9±0.085	20.2±0.134	8.6±0.057	5.2±0.034	2150	2337	0.6667	0.2574
Improved white Ponni	72±0.593	14.8±0.122	10.3±0.126	7.1±0.058	4.4±0.036	2190	1418	0.4797	0.2876
Co 43	12±0.040	10.1±0.034	3.7±0.012	6.8±0.023	2.9±0.010	1690	79	0.6733	0.7838
FR13A	5±0.017	10.3±0.036	2.0±0.007	5.0±0.017	0.6±0.002	1530	13	0.4854	0.3000

Table.3 Effect of anaerobic germination on the enzyme activities (α-amylase, peroxidase and alcohol dehydrogenase) in rice seedlings

	α -Amylase activity (Units mg^{-1} protein)	Alcohol dehydrogenase activity (ADH) (Units min^{-1}mg^{-1} protein)	Peroxidase (POX) (Units min^{-1}mg $^{-1}$protein)
Anaikomban	32.18± 0.108	1.23±0.004	0.38±0.001
Ottadaiyan	20.09±0.070	0.87±0.003	1.12±0.004
Muthuvellai	28.76±0.117	1.93±0.008	2.11±0.009
Rajamannar	22.18±0.077	0.82±0.003	1.77±0.006
CR1009	23.40±0.155	1.71±0.011	2.12±0.014
Improved white Ponni	12.05±0.040	0.63±0.005	9.56±0.079
Co 43	10.45±0.086	0.44±0.001	11.10±0.037
FR13A	8.13±0.028	0.26±0.001	12.49±0.043

Fig.1 Anaerobic germination screening of rice genotypes by a) portray and b) beaker methods

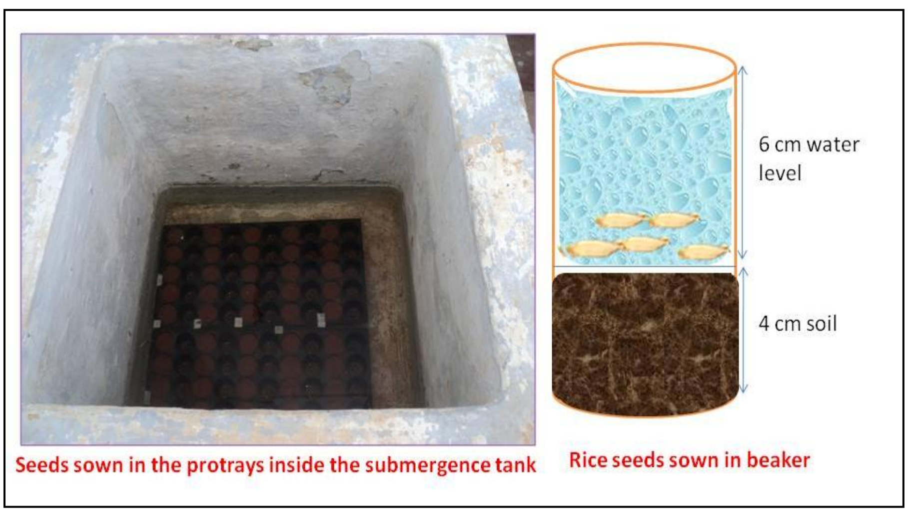


Fig.2 Anaerobic germination percentage (%) of rice genotypes grown in portrays/ beakers

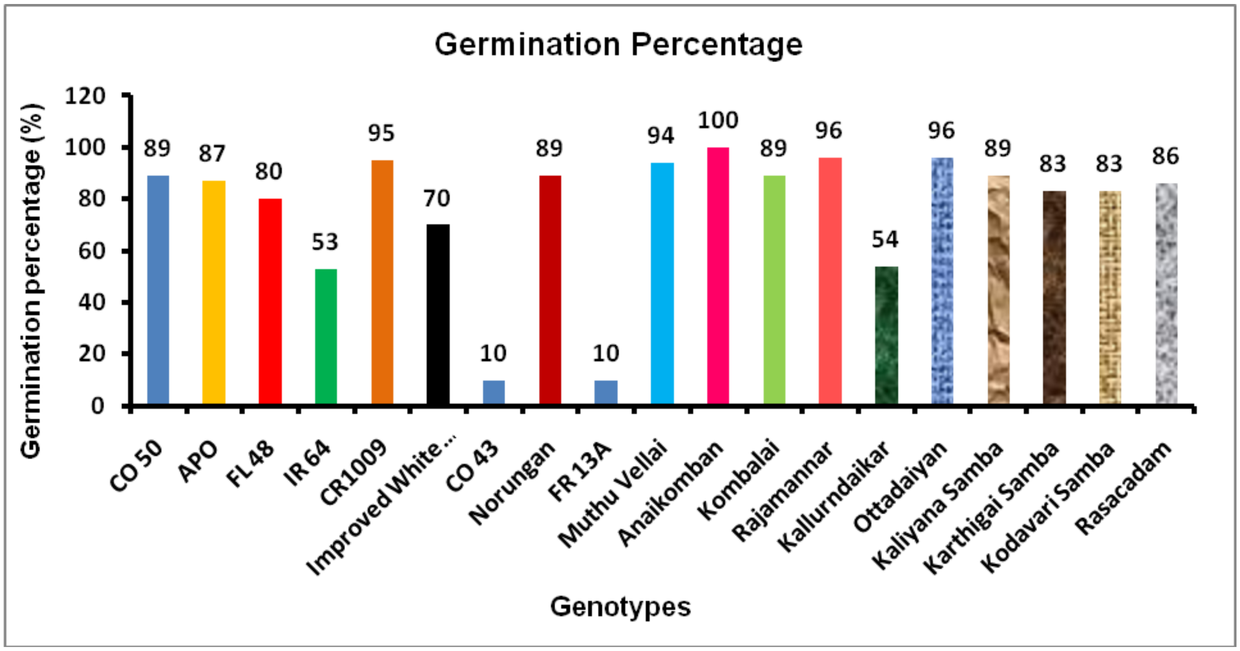


Fig.3 Z- Distribution graph between germination percentage (%) and shoot length (cm) in rice genotypes under anaerobic condition. Quadrant I depicts the highly tolerant genotypes, Quadrant II shows the moderately tolerant genotype and the Quadrant III shows the susceptible genotypes

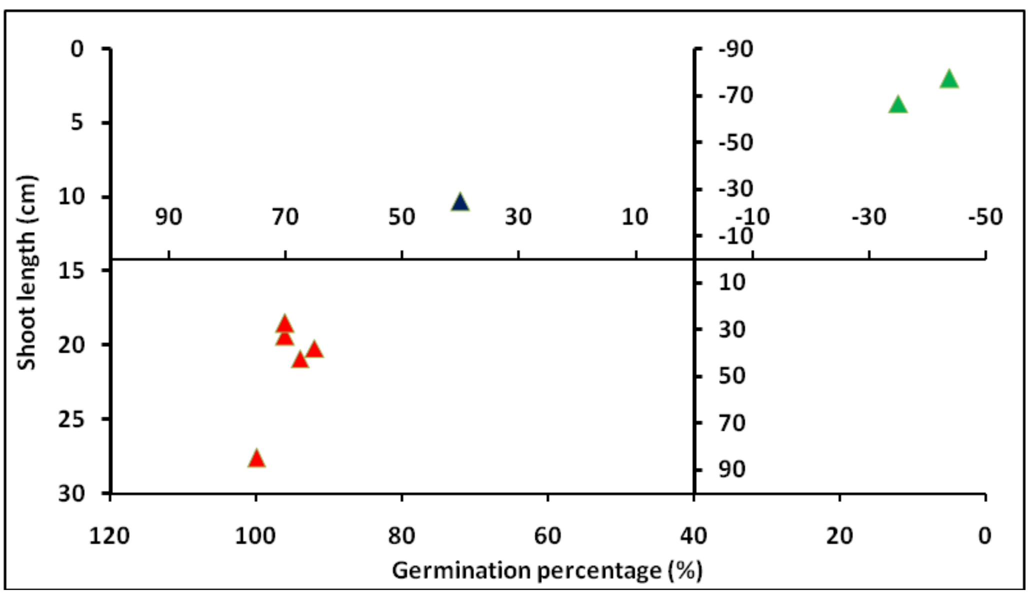


Fig.4 Correlation between germination percentage (%) and α- amylase activity (Units mg–1 protein) of rice seedlings under anaerobic stress

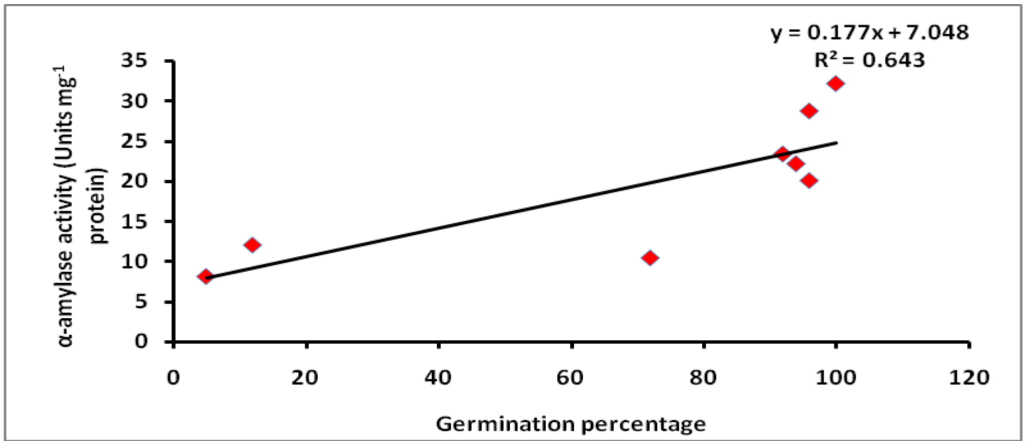


Fig.5 Correlation between germination percentage (%) and ADH activity (Units min–1mg –1protein) of rice seedlings under anaerobic stress

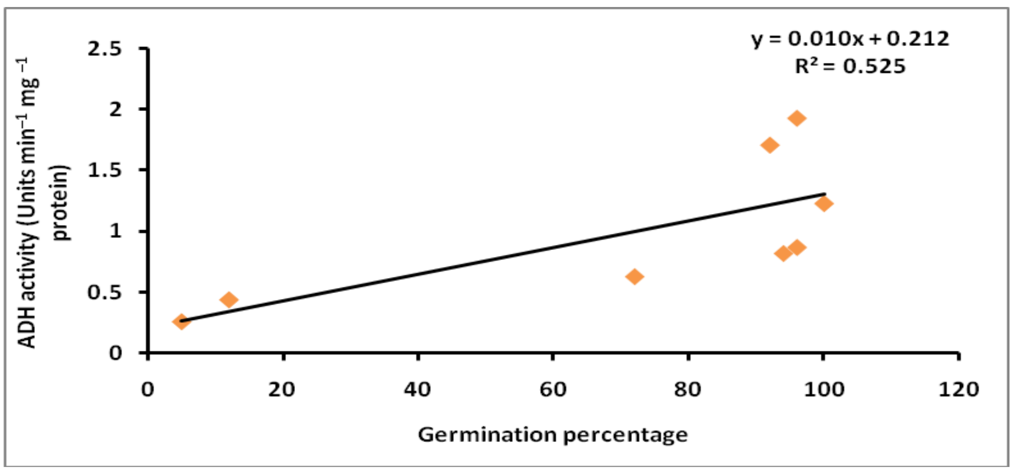
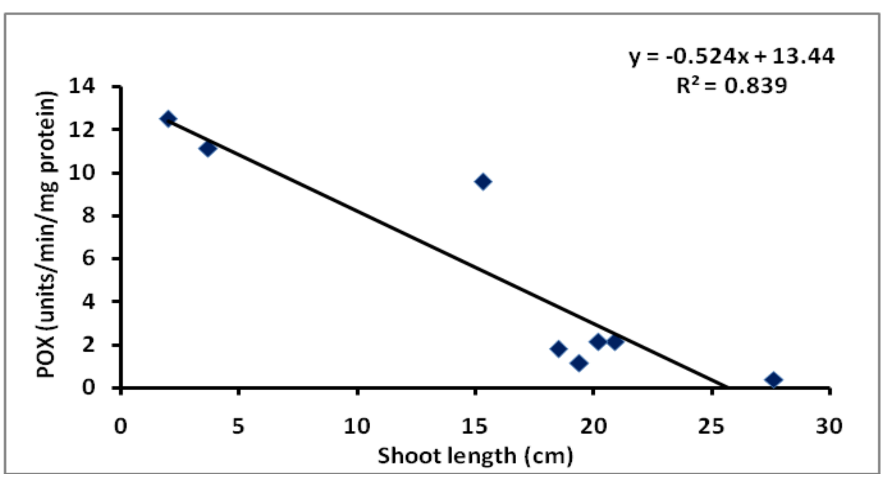


Fig.6 Correlation between shoot length (cm) and peroxidase activity (Units min–1mg –1protein) of rice seedlings under anaerobic stress



A positive correlation between the germination percentage and the ADH activity (Fig.5) with the correlation coefficient of r^2= 0.53 was reported in this study similar to the findings of Miro and Ismail, (2013). This explains that higher the ADH activity higher will be the germination per cent and higher will be the survival rate and seedling establishment. Also, the ability of the seedlings to maintain an active formative metabolism is very crucial to survive under the anoxic conditions (Magneschi and Perata, 2009).

Generally it was observed that peroxidase activity was low in the tolerant genotypes (Anaikomban-0.38Units $min^{-1}mg^{-1}$ protein; Ottadaiyan-1.12Units $min^{-1}mg^{-1}$ protein) and highest in the sensitive genotypes (FR13A-12.49 Units $min^{-1}mg^{-1}$ protein; CO43-11.10Units $min^{-1}mg^{-1}$ protein). Peroxidases are reported to inhibit the cell wall extension which promotes the coleoptile elongation (Ismail *et al*., 2009). In line with the above findings, a strong negative correlation was observed for the shoot length and peroxidase enzyme activity (r^2=0.84) in the present study. Lee and Lin, (1996) have also reported a negative correlation between shoot elongation and peroxidase activity (Fig.6). Germination percentage was found to be positively correlated with the α-amylase and ADH activity but negatively correlated with peroxidase activity.

In conclusion, a more reliable, rapid and repeatable method using the protrays/beakers to screen the genotypes for anaerobic stress tolerance was designed in this study. The study has also led to the identification of three landraces namely Anaikomban, Muthuvellai and Rajamannar and a variety CR1009 as donors for anaerobic stress tolerance that embed this trait to germinate under anaerobic condition. The important physiological mechanisms underlying stress tolerance was identified as higher α-amylase and ADH activity which are positively correlated to higher germination per cent under low oxygen stress. Higher POX activity in rice seedlings were negatively correlated to germination per cent under low oxygen stress. Thus, genetic variability in acquired tolerance to anaerobic germination in landraces of rice reveals new sources of donors for anaerobic germination under direct seeding.

Acknowledgement

The authors thank the Department of Crop Physiology for providing the necessary facilities for this work.

References

Bernfeld, P. 1955. Amylases, alpha and beta. *Meth. Enzymol.*, 1: 149–58

Bradford, M.M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann. Biochem.*, 72: 248–254.

Ella, E.S., A.P. Valdez, R.V. Reyes, H. Greenway and Setter, T.L. 1993. Importance of several enzymes in limitation of alcoholic fermentation of rice under anoxia. *Proceedings of the 6th Annual Meeting of the International Program on Rice Biotechnology*, Chiang Mai, Thailand.

Gibbs, J., and Greenway, H. 2003. Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. *Functional Plant Biol*., 30: 1–47

Gibbs, J., S. Morrell, A. Valdez, T.L. Setter and Greenway, T. 2000. Regulation of alcoholic fermentation in coleoptiles of two rice cultivars differing in tolerance to anoxia. *J. Exp. Bot.*, 51: 785–796

Goldberg, R., M. Liberman, C. Mathieu, M. Peirron and Catesson, A.M. 1987. Development of epidermal cell wall peroxidase along the mung bean hypocotyls: possible involvement in the cell wall stiffening process. *J. Exp. Bot.*, 38: 1378–1390.

Guglielminetti, L., Y. Wu, E. Boschi, J. Yamaguchi, A. Favati, M. Vergara, P. Perata, and Alpi, A. 1997. Effects of anoxia on sucrose degrading enzymes in cereal seeds. *J. Plant Physiol*., 150: 251–258

Huang, S.B., H. Greenway and Colmer, T.D. 2003. Anoxia tolerance in rice seedlings: exogenous glucose improves growth of an anoxia-'intolerant', but not of a 'tolerant' genotype. *J. Exp. Bot.*, 54: 2363–2373

INCCA (Indian Networkfor Climate Change Assessment). 2010. Climate change and India - A 4X4 Assessment, A Sectoral and Regional Analysis for 2030s: Ministry of Environment and Forests, Government of India

Ismail, A.M., E.S. Ella, G.V. Vergara and Mackill, D.J. 2009. Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*). *Ann. Bot.,* 103: 197-209

Jackson, M.B., B. Herman and Goodenough, A. 1982. An examination of the importance of ethanol in causing injury to flooded plants. *Plant Cell Environ*., 5: 163–172.

Lee, T.M., and Lin, Y.H. 1996. Peroxidase activity in relation to ethylene-induced rice (*Oryza sativa* L.) coleoptile elongation. *Botanical Bull. Academia Sinica*, 37: 239–245.

Magneschi, L., and Perata, P. 2009. Rice germination and seedling growth in the absence of oxygen. *Ann. Bot.,* 103: 181-196

Manigbas, N.L., O.S. Renando, V.B. Wilhelmina, J.N. Angelo, C.A. Emily, F.P. Thelma and Rolando, T.C. 2008. Development of screening methods for anaerobic germination and seedling vigor in direct seeded wet seeded rice culture. *Philippine J. Crop Sci*., 33(3): 34-44.

Mapelli, S., F. Locatelli and Bertani, A. 1995. Effect of anaerobic environment on germination and growth of rice and wheat: endogenous levels of ABA and IAA. Bulgarian *J. Plant Physiol.,* 21: 33–41.

Miro, B., and Ismail, M. 2013. Tolerance of anaerobic conditions caused by flooding during germination and early growth in rice (*Oryza sativa* L.). *Frontiers Plant Sci*., 4: 1-18.

Perata, P., N. Geshi, J. Yamaguchi and Akazawa, T. 1993. Effect of anoxia on the induction of alpha-amylase in cereal seeds. *Planta*, 191: 402–408

Perata, P., L. Guglielminetti and Alpi, A. 1996. Anaerobic carbohydrate metabolism in wheat and barley, two anoxia-intolerant cereal seeds. *J. Exp. Bot.*, 47: 999–1006.

Perata, P., J. Pozuetaromero, T. Akazawa and Yamaguchi, J. 1992. Effect of anoxia on starch breakdown in rice and wheat seeds. *Planta*, 188: 611–618.

Peru, N.G. 1962. Measurement of peroxidase activity in plant tissues. *Curr. Sci.,* 31: 71-81

Vergara, G.V., Y. Nugraha, M.Q. Esguerra, D.J. Mackill and Ismail, A.M. 2014. Variation in tolerance of rice to long-term stagnant flooding that submerges most of the shoot will aid in breeding tolerant cultivars. *AoB Plants*, 6: 1-16.

Zheng, X., and van Huystee, R.B. 1992. Peroxidase-regulated elongation of segments from peanut hypocotyls. *Plant Sci.,* 81: 47–56.

How to cite this article:

Partheeban, C., S. Srividhya, M. Raveendran and Vijayalakshmi, D. 2017. Designing New Screening Methods and Physiological Dissection of Anaerobic Stress Tolerance in Rice. *Int.J.Curr.Microbiol.App.Sci.* 6(5): 580-590. doi: https://doi.org/10.20546/ijcmas.2017.605.067