Applied Biological Research 23(1): 95-101; (2021)
DOI: 10.5958/0974-4517.2021.00012.4

GERMINATION AND SEEDLING SURVIVALITY OF RICE (*Oryza sativa* L.) UNDER ANOXIA

Deepjyoti Paul, Ranjan Das*, Sangita Das and Hemendra Choudhury

Department of Crop Physiology, Assam Agricultural University, Jorhat – 785 013, Assam (India)
*e-mail: *rdassam19666@gmail.com*

(Received 3 December, 2020; accepted 12 January, 2021)

ABSTRACT

A study was conducted at the Stress Physiology Laboratory, AAU, Jorhat, Assam (India) during *kharif season* 2016-2017. Rice genotypes (146 nos.) collected from different ecotypes were screened for anaerobic tolerance based on their survivality. Of the genotypes evaluated, six genotypes *viz*., 'Ronga Lahi', 'Ronga Bao', 'Bangali Lahi', 'Suwag Moni Horu', 'Asra 123' and 'Asra 8' were selected as anoxia tolerant genotypes on the basis of seed germination, seedling survival and growth. These genotypes were also evaluated for amylase activity. The result revealed a significant variation in genotypes during anoxic germination in terms amylase activity. Higher α- and β-amylase activities were observed in 'Asra 123', followed by 'Asra 8' as compared to the susceptible genotype 'IR-64'. Increased amylases activity in tolerant genotypes might be attributed to the production and maintenance of sufficient amount of soluble sugar under anoxic condition during germination. The study revealed that 'Asra 123', 'Asra 8', 'Ronga Lahi', 'Ronga Bao', 'Bangali Lahi' and 'Suwag Moni Horu' could successfully be germinated and established under anoxic condition.

Keywords: Anoxia, amylase activities, coleoptiles, genotypes, germination, rice, stress tolerance

INTRODUCTION

Poor germination and poor seedling establishment in direct seeding method due to unpredictable flooding events is one of the major problems in rice production in North East region of India. Though rice plants are well known for their adaptation to flood conditions but many rice cultivars are sensitive to low oxygen levels incited by frequent flooding especially during germination period (Yamauchi *et al.,* 1994). Although direct seeding is advantageous over transplanting in terms of labour requirement but many prime qualities of high yielding rice cultivars are not good performer in direct seeding, because of poor development of direct seeding technology for flood-prone area (Tuong *et al.,* 2000). Hence, rice cultivars require to have tolerance to low oxygen levels during germination for adaptation to direct seeding. Angaji *et al*. (2009) opined that flood tolerance during germination of rice is relatively rare. Stagnant water restricts free diffusion of oxygen from air to the germinating seeds (Narsai *et al*., 2015). However, some rice have developed unique mechanism to germinate and elongate its coleoptile under water even in complete absence of oxygen, a phenomenon termed as anaerobic germination ((Magneschi and Perata, 2009, Narsai *et al*., 2015). Umarani et al. (2018) found variation amongst rice genotypes and identified anaerobic germination tolerance. Works on QTL mapping from rice land races and identification of SUBMERGENCE 1

(SUB1) locus, have been undertaken for introgression into rice popular varieties (Septiningsih *et al*., 2013).

The Northeast India is rich in traditional low land rice cultivars that are adapted to the rainfed lowland situation where low oxygen stress prevails during germination and seedling establishment. The aim of present study was to screen some germplasms in terms of germination ability under anaerobic condition using physiological tools. Looking into above, the present study entitled "Germination and seedling survivality of rice (*Oryza sativa* L.) under anoxia" was aimed to evaluate the germination ability and seedling establishment potential of 146 rice genotypes under anaerobic conditions by comparing various physiological and biochemical parameters.

MATERIALS AND METHODS

Plant materials and screening

The present study was conducted on rice (*Oryza sativa* L.) germplasms, collected from different places of North East India. The rice genotypes (146) were screened during *kharif* season (2016-2017) as per the method suggested by Angaji *et al.* (2009) with slight modification. Seeds of particular line were directly sown in small plastic glass (300 mL capacity) filled with soil and placed over trays to maintain the identity of particular lines after germination and kept for 14 days for recording various observations under anoxic condition. The glasses were arranged in a sequence and properly labelled. The soil from rice field (sandy loam) was collected and weed and debris removed. Each glass was filled upto 1/3rd with soil. The seeds were first surface sterilized with 0.1% mercuric chloride for 2 min and washed three times with distilled water to remove adhering mercuric chloride. The seeds were then directly sown in plastic glasses containing a layer of finely ground field soil. A stagnant water level of 5 cm above the soil surface was maintained throughout study period of 14 days. The experiment was conducted in a completely randomized design with three replications. The seeds were observed for germination and seedling survival at 48 h interval from seed sowing date. The coleoptile, radical and plumule characteristic were noticed and tolerance potential of genotype judged on the basis of seedling growth and emergence out of water level.

α-amylase activity

The amylase activity was estimated using the slightly modified method of Bhattacharya and Vijaylaxmi (2016) with slight modification. Ten gram of germinated rice seeds were used for enzyme extraction. The genotypes showing tolerance efficiency were germinated and their amylase contents estimated to assess biochemical basis of tolerance. Germinating seeds were homogenized in cold phosphate buffer (0.01 M, pH 6.8). The homogenate was passed through muslin cloth and filtrate centrifuged at 10000 rpm for 10 min at 4^{o}C. The supernatant was used as enzyme extract. The starch solution (0.1 mL) was mixed with 0.1 mL enzyme extract, the volume made up to 1.0 mL with water and incubated for 5 min at 30^{o}C. The reaction was stopped by the addition of 1.0 mL iodine reagent. Then, 3.0 mL water was added and optical density recorded at 620 nm against a reagent blank. Blank was used by taking 0.1 mL boiled enzyme extract and enzyme units calculated as mg starch hydrolyzed g^{-1} fresh weight (for total activity) or mg protein min^{-1} (for specific activity).

β-amylase activity

Plant material (~ 10.0 g) (germinating seed) was homogenized with 5.0 mL of 0.5 M Tris-HCl buffer pH 7.6 containing 5.0 mM 2-mercaptoethanol. The homogenate was centrifuged at 20000 rpm for 10 min. Supernatants were divided in two parts and one part was heated for 5 min at 70^{o}C in presence of 3.0 mM $CaCl_2$ and cooled at 0^{o}C. To 1.0 mL starch solution, 1.0 mL water and 0.2 mL enzyme extract were added. Incubation was done for 10 min at 30^{o}C. DNS reagent (2.0 mL) was mixed and kept in boiling water. Maltose 100 mg was dissolved in 1000 mL water. The solution had 1000 μg maltose mL^{-1} solution. Standard curve was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1.0 mL of this solution and volume made to 2.0 mL by the addition of respective volumes of water and then the colour was developed.

Statistical analysis

The data was analyzed following the method of analysis of variance (Panse and Sukhatme, 1967). The critical difference between genotypes were calculated at 5% probability level.

RESULTS AND DISCUSSION

Screening of genotypes under anoxia

The germplasms were sown under anaerobic condition (anoxia) for 14 days and germination behaviour were recorded. Result revealed that there was a significant variation among the rice germplasm (Table 1). The germplasms that germinated and survived for 14 days of submergence were screened out. Some genotypes survived very nicely and could come out from the submergence condition within 14 days. Out of the 146 genotypes only 26 genotypes could germinate under anaerobic condition. Again, out of 26 genotypes, six genotypes *viz*., 'Ronga Lahi', 'Ronga Bao', 'Asra 123' (check variety), 'Asra 8', 'Bangali Lahi' and 'Suwag Moni Horu' had quicker germination ability as compared others. In these six genotypes, the germination started from fourth day of submergence and coleoptile emergence was visible on the sixth day onwards. On the eight day of treatment, growth of radicle and plumule was visible with initiation of leaf in these six genotypes. Those seedlings emerged out of the water level on 10^{th} day. While the remaining 20 genotypes could germinate but the coleoptile could not develop, also deterioration of the radicle and plumule started from eight day. Based on the germination behaviour, coleoptile development, radical emergence intensity and radical & plumule deterioration plants were divided into two group *viz*., tolerant and susceptible group. The six screened germplasms that emerged out of the water level were considered as tolerant genotype and were used for further investigation for amylase assay since amylases play important role during germination. The quick germination, growth and development of the germinating seedling could be due to the higher synthesis of α and β amylase, which led to quick breakdown of starch thus providing the energy required for the quick germination and survivability of the genotypes under submergence. After completion of evaluation of genotypes one grouping chart was developed (Table 2).

The failure of some rice genotypes to germinate and emerge out of the water level might be because of their inability to cope up with limited gas diffusion and low irradiance in submerged condition. Environmental characteristics are the most important factors contributing to plant mortality. In germinating seeds, rate of coleoptile elongation during anoxia are highly correlated with rates of alcoholic fermentation and carbohydrate supply for energy production (Setter *et al*., 1997).

α-amylase activity

A common factor related to tolerance in limited gas diffusion may be responsible for genetic differences in the submergence tolerance of rice. Significant increment in α-amylase activity due to the submergence treatment was noticed. The highest α-amylase activity (0.52 μg mg^{-1}) was noted in in submerged condition while in normal condition it was 0.50 μg mg^{-1} (Fig. 1.). Likewise, a significant difference in terms of α-amylase activity was found amongst the genotypes tested. Among the interaction, highest α- amylase activity was recorded in check variety 'Asra 123' (0.61 μg mg^{-1}), which was at par with 'Ronga Bao' (0.61 μg mg^{-1}). The lowest α-amylase activity was recorded in susceptible genotype 'IR 64' (0.35 μg mg^{-1}), followed by 'Suwag Moni Horu' (0.42 μg

Table: 1 Screening of the different genotypes under anaerobic condition

Genotypes	Submergence time (days)							Obser-vation
	2	4	6	8	10	12	14	
A. Highly susceptible								
'Kakuwa Bao', 'Disang', 'Happy Bao', 'Kehjoreh', 'Kushal', 'Jalashree', 'TTB-303-2-23', 'Negeri Bao', 'Adeng', 'Jalashree', 'TTB-303-2-23', 'Negeri Bao', 'OM 5451', 'Jahinga', 'Punjab Hao', 'Satya Ranjan', 'Jalakuwari', 'Tejai Longpi', 'Horu Jangiya', 'Dhan', 'Dubri Bao', 'Nayan Moni', 'Bira Bhonga', 'Ranga Sali', 'Mala', 'Tarabali', 'Isingneh', 'Aemo', 'Bishnu Prasad', 'Papri Bora', 'Moniram', 'TTB-303-7-42', 'Bhogali Bora', 'Bahadur', 'Ampatti', 'TTB-404', 'Merut Amkel', 'Kranti', 'Bao 25', 'Kukuwa Bao Dhan', 'Maipholong', 'Seni Kumol Sali', 'Chilarai', 'Kehneh', 'Aam Keh Leh Bora', 'Bash Rice', 'Asra EX', 'Prafulla', 'Bora-131', 'Muliga', 'Aijong', 'Bijor', 'Keh Tulu', 'Luit', 'Sokcherop', 'Cachar Sali', 'Kerjorg Ker', 'Gitesh', 'Khongsa Longpi', 'Bora 133', 'Lachit', 'Keh Borah', 'Suwak Moni', 'Soksu Ajoja', 'Athabu Chedo', 'Soklanglu', 'Kalijira', 'Nolsuti Sali', 'Asra 57', 'Maloken', 'Sokpi', 'IR 50', 'Patungneh', 'Joha 13', 'Kolong', 'Piyolee', 'Joldubi', 'Soksu Abara', 'Bokul Joha', 'Sokcherop', 'Sana', 'Koubru', 'Purnima', 'Saidrum', 'Thangjing', 'Itanagar', 'Khow Noya Say', 'Kheraijam Diren', 'Khow Namtow', 'Aghoni Sali',' Khow Nowpan', 'Jaria', 'Lal aus', 'Terabali', 'Khoiri', 'Thoibi', 'Khamba', 'Saiamara', 'Baumurali', 'Katak Tara', 'Jatra', Chakhao Amubi', 'Kaya Boro', 'Kouja Puri', 'Kartica', 'Tepi Bor', 'Choyamora', 'Moka Boro', 'Mayamati', 'Biyoi Bao', 'Boka', 'Bor Joha', 'Darum' and 'Rangai'	-	-	-	-	-	-	-	Did not germinate, so highly susceptible
B. <u>Showed germination after 6 DAS but susceptible</u>								
'Tombung', 'Lahi 26', 'Ranjit, Rakhi (III)', 'Sangatik', 'Jampa', 'Joha', 'Chakhao', 'Angangbi', 'Nurin', 'Niphu Thokpi', 'RCM-9', 'Mohon', 'Andeng', 'Hasna Kumari', 'Pori Chaya Bora' and 'Kala Boro'	-	-	Germinated	Coleoptile emerged but not developed	Deterio-ration of radicle and plumule	Seed-ling death	-	Could not withstand submer-gence so susceptible
C. Showed germination after 4 DAS but susceptible								
'Asra 151', 'Leima, Asra 3' and 'Punshi'	-	Germinated	Coleo ptile emerg ed	Retarded radicle and plumule growth observed	Deterio-ration of radicle and plumule	Seed-ling death	-	Showed deterio-ration during later phase so susceptible
D. Showed germination after 4 DAS but tolerant								
'Bangali Lahi', 'Asra 8', 'Ronga Bao', 'Suwak Moni Horu', 'Asara 8', 'Ronga Lahi' and 'Asra 123'	-	Germinated	Coleo ptile nicely emerg ed out	Better growth of radicle and plumule with leaf initiation	The seedling emerged out of the water level	No death	No death	Quick germi-nation, could emerge out of the water level so tolerant to anoxia

Table 2: Characteristic of tolerant and susceptible group

Characters	Tolerant	Susceptible
Germination	Good germinating ability with speedy germination	No germination and slow germination
coleoptiles	Distinct coleoptiles	Germinate but there no coleoptiles or deteriorated
Radicle	Distinct and better radicle with proper in size	Deterioration of radicule start from 8-10 DAS in some case no radicle growth observed
Plumule	Distinct plumule with proper in size	Deterioration of plumule start from 8-10 DAS; in some case no plumule growth observed.
Survivability	Could withstand submergence	Could not withstand submergence due to after 8-10 due to seedling death
ß -amylase	More active in germinating seeds	Less active in germinating seeds
α-amylase	More active in germinating seeds	Active in germinating seeds

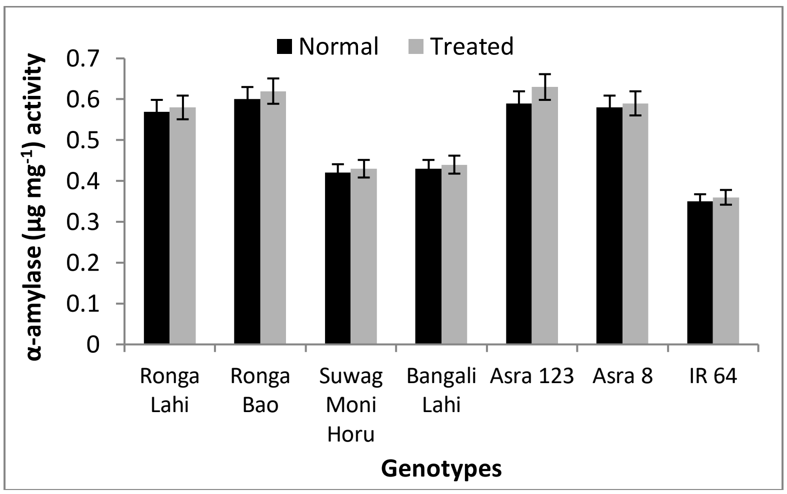

Fig. 1: Effect of submergence on α-amylase activity of germinating rice seeds (µg mg^{-1})

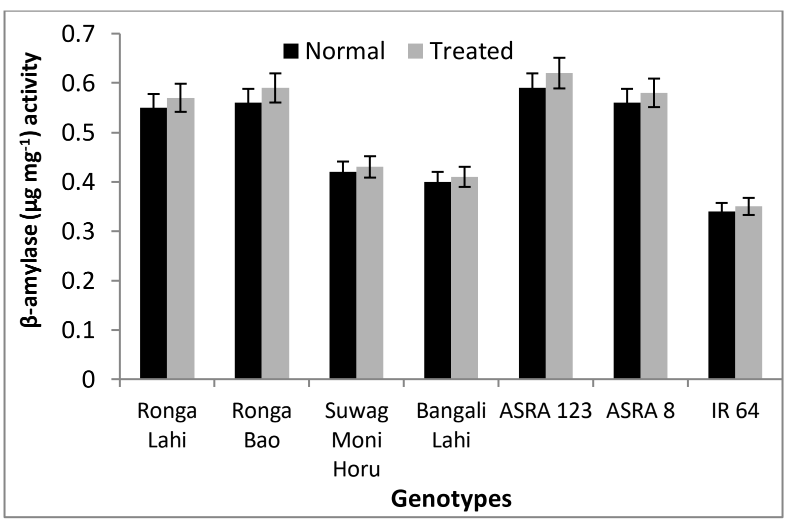

Fig. 2: Effect of submergence on β-amylase activity of germinating rice seeds (µg mg^{-1})

mg^{-1}). In rice, α-amylase isozymes aid in the formation of storage starch which nourish the developing seedling, thus directly affects the plant growth and yield (Damaris *et al.*, 2019). During cereal seed germination, α-amylase in aleurone layer plays an important role in hydrolyzing the endospermic starch into the metabolizable sugars, which provides energy for the growth of roots and shoots (Akazawa and Hara-Mishimura, 1985, Beck and Ziegler, 1989). Previous studies have revealed that α-amylase expression in aleurone layer occurs when gibberellin biosynthesis commences in the embryo and is transported from embryo to the aleurone layer (Fincher, 1989), which triggers the expression of α-amylase (Gubler *et al.*, 1995). The α-amylase is secreted from aleurone layer into endosperm; this enhances the hydrolysis reaction of stored starch. Our results are in confirmity with those of Perata *et al*. (1992) and Guglielminetti *et al*. (1995) who have reported higher α-amylase activty in rice grown under anoxia conditions.

Plate 1: Germinating rice seeds under anoxia

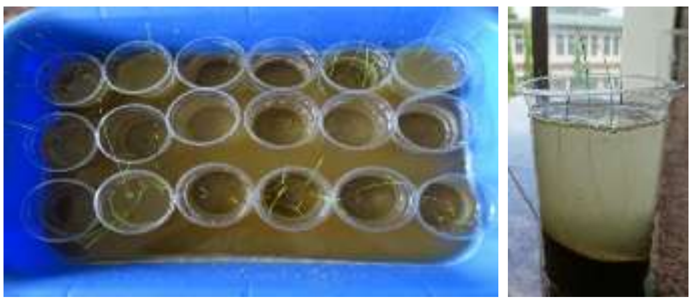

Plate 2: Seedlings emerged above the water level

β-amylase activity

In six tolerant genotypes significant increment in β-amylase activity was noticed due to the submergence. The highest β-amylase activity was recorded in submerged condition (0.50 μg mg^{-1}) while in normal condition it was 0.49 μg mg^{-1} (Fig. 2). Likewise, significant difference in terms of β-amylase activity was noted in the genotypes. The highest β-amylase activity was recorded in check genotype cv. 'Asra 123' (0.60 μg mg^{-1}), followed by 'Ronga Bao' (0.57 μg mg^{-1}); however, a significant difference was noted between them. In contrast, low β-amylase activity was found in susceptible cv. 'IR 64' (0.34 μg mg^{-1}), followed by 'Bangali Lahi' (0.40 μg mg^{-1}). A significant difference was noted among them. β-amylase is an important starch degrading enzyme in germination and β-amylase activity is considered an index for germination potential in rice (Nandi., 1995). Livesley and Bray (1991) have concluded that amylase plays a crucial role during anoxia as no other starch degrading enzymes could intiate the process under anoxia. In our study, the tolerant rice germplasms *viz*.. 'Ronga Lahi', 'Ronga Bao', 'Suwag Moni Horu', 'Bangali Lahi', 'Asra 123' and 'Asra 8' could survive anoxia and emerged out from the submergence of water (Plate 1 and 2).

Conclusion: Tolerant genotypes could maintain higher α & ß amylase activity under submerged condition that facilitated the easy metabolization of starch by the action of amylases which helped in breakdown of starch to simple sugar making them available for germinating embryo for quick germination. This was evident in our present study. The role of amylases in germination is that it helps in hydrolyzing the endosperm starch which provide the energy required for the development of roots and shoots of tolerant genotypes. These tolerant genotypes *viz*., Ronga Lahi, Ronga Bao, Asra 123, Asra 8, Bangali Lahi and Suwag Moni Horu can be used for breeding matarial for near future in stress resistant breeding

REFERENCES

Akazawa, T. and Hara-Mishimura, I. 1985. Topographic aspects of biosynthesis, extracellular section and intracellular storage of proteins in plant cells. *Annual Review of Plant Physiology*, **70**: 441-472.

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**(2): 159-168.

Beck, E, and Ziegler, P. 1989. Biosynthesis and degradation of starch in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology***, 40**: 95-117.

Bhattacharya, A. and Vijaylaxmi. 2016. Carbohydrates. pp. 167-220. **In**: *Biochemical Aspects of Plant Physiology.*, Mew India Publishing Agency, New Delhi, India.

Damaris, R. N., Lin, Z., Yang, P. and He, D. 2019. The rice alpha-amylase, conserved regulator of seed maturation and germination. *International Journal of Molecular Science*, **20**(2): 450. [*doi: 10.3390/ijms20020450*].

Fincher, G.B. 1989. Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**: 305-345.

Gubler, F., Kalla, R., Roberts, J.K. and Jacobsen, J.V. 1995. Gibberellin-regulated expression of a Myb gene in barley aleurone cells: Evidence for Myb transactivation of a high-pI alpha-amylase gene promoter. *Plant Cell*, **7**: 1879-1891.

Guglielminetti, L., Yamaguchi, J., Perata, P. and Alpi, A. 1995. Amylolytic activities in cereal seeds under aerobic and anaerobic conditions. *Plant Physiology*, **109**(3): 251-260.

Livsley, M.A. and Bray, C.M. 1991. The effect of ageing upon α-amylase production and protein synthesis by wheat aleurone layers. *Annals of Botany*, **68**(1): 69-73.

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

Nandi, S., Das, G. and Sen-Mandi, S. 1995. β-amylase activity as an index for germination potential in rice. *Annals of Botany*, **75**(5): 463-467.

Narsai, R., Edwards, J.M., Roberts, T.H., Whelan, J., Joss, G.H. and Atwell, B.J. 2015. Mechanisms of growth and patterns of gene expression in oxygen deprived rice coleoptiles. *Plant Journal*, **8**: 25-40.

Panse, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research, New Delhi, India.

Perata, P., Pozueta-Romero, J., Akazawa, T. and Yamaguchi, J. 1992. Effect of anoxia on starch breakdown in rice and wheat seeds. *Planta*, **188**(4): 611-618.

Septiningsih, E.M., Ignacio, J.C.I., Sendon, P.M.D., Sanchez, D.L., Ismail, A.M. and Mackill, D.J. 2013. QTL mapping and confirmation for tolerance of anaerobic conditions during germination derived from the rice landrace Ma-Zhan Red. *Theoretical Applied Genetics*, **126:** 1357-1366.

Setter, T.L., Ellis, M., Laureles, C.V., Ella, E.S., Senadhira, D. and Mishra, S.B. 1997. Physiology and genetics of submergence tolerance in rice. *Annals of Botany*, **79**: 67-77.

Tuong, T.P., Pablico, P.P., Yamauchi, M., Confesor, R. and Moody, K. 2000. Increasing water productivity and weed suppression of wet-seeded rice: effect of water management and rice genotypes. *Experimental Agriculture*, **36**: 71-89.

Umarani, E., Hemalatha V., Bhadana, V.P., Senguttuvel, P., Subbarao, L.V., Neeraja, C.N., Yugandhar, R.P. and Kota, S. 2018. Screening of rice (*Oryza sativa* L.) genotypes for anaerobic germination. *International Journal of Pure and Applied Bioscience***, 6**(5): 1318-1325.

Yamauchi, M., Herradura, P.S. and Aguilar, A.M. 1994. Genotype difference in rice post germination growth under hypoxia. *Plant Science*, **100**: 105-113.