

Full Length Research Paper

Biochemical and molecular characterization of submergence tolerance in rice for crop improvement

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Submergence stress is considered as the third most important limitation of rice production contributing to its low productivity in lowland and rainfed ecosystem. Characterization of genotypes and using them in breeding programme is likely the best option to withstand submergence and stabilize productivity in these environments. However, progress in genotypes characterization has been slow but can substantially be enhanced by using potential of biochemical and molecular markers, to enhance and speedup progress through breeding. Survival percentage, alcohol dehydrogenase (ADH) activity and isozyme profiles were carried out in this investigation. The response to submergence appears to be complex and involves a number of enzymes, therefore, cultivars were also characterized by using other isozymes (Aspartate aminotransferase, AAT; Malate dehydrogenase, MDH; Esterase, EST and Peroxidase, POX) and molecular marker like random amplified polymorphic DNA (RAPD). Dendrogram constructed, using isozymes and RAPD data clustered the rice genotypes into three major groups, submergence tolerant, moderately tolerant and susceptible. The result obtained in this study will help plant breeders in breeding high yielding cultivars for lowland eco-systems, with submergence tolerant cultivars.

Key words: Rice, submergence stress, alcohol dehydrogenase (ADH), isozyme, random amplified polymorphic DNA (RAPD), FR13A.

INTRODUCTION

Submergence stress is a major constraint to rice production during the monsoon flooding season in the rainfed lowlands in south and southeast Asia, which causes annual losses of over US\$1 billion and affects disproportionately the poorest farmers in the world (Dey and Upadhyaya, 1996; Xu et al., 2006). Out of 40 million ha in Asia grown under rainfed lowlands, about 15 million ha are frequently damaged by submergence stress (Huke and Huke, 1997). Recently, the extent of submergence stress has increased due to extreme weather events such as unexpected heavy rains that have inundated wider areas across many regions in Asia.

The onset of flooding leads to the condition of anaerobiosis or oxygen deprivation as gas diffusion from the atmosphere to water is nearly 10^4 times slower as

compared to diffusion in air (Armstrong, 1979). To cope with the reduction in oxygen supply, plants have developed a number of metabolic and morphological adaptations that enable them to survive transient periods of complete or partial submergence (Kende et al., 1998; Drew et al., 2000; Bailey-Serres and Voesenek, 2008). Escape from hypoxia involves shoot elongation, development of aerenchyma and adventitious root formation (Drew et al., 2000; Sauter, 2000; Voesenek et al., 2006; Perata and Voesenek, 2007). Nonetheless, oxygen may ultimately become limiting, necessitating a switch from aerobic respiration to anaerobic fermentation, a key catalytic pathway for recycling NAD^+ to maintain glycolysis and substrate level phosphorylation in the absence of oxygen (Davies, 1980). Enzymes associated with anaerobic fermentation such as alcohol dehydrogenase (ADH) have been considered important in submergence tolerance (Setter, 1992).

The adverse effects of flooding or submergence are

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Table 1. Description of rice cultivars used in this study.

Cultivars	Description
FR13A	Native rice cultivar, source of submergence tolerance gene
Jalashree	Pankaj X FR13A
Jalkunwari	Pankaj X FR13A
Bahadur	Pankaj X Mahsuri
Ranjit	Pankaj X Mahsuri
Luit	Hira X Annada
Keteki	Bahadur X Savitri
Lachit	CRM13-3241 X Kalinga 2
Chilarai	IR24 X CR44-118-1
Mahsuri	(Taichung 63 X Mayung Eboss) X Mayung Eboss.

multiple and complex which leads to death of most rice cultivars under complete submergence for 1 to 2 weeks (Xu et al., 2006; Perata and Voesenek, 2007). However, some cultivars, such as FR13A, a native rice cultivar from eastern India, can survive up to 2 weeks of complete submergence (Jung et al., 2010). But, these tolerant varieties lack many of the desirable traits of the widely grown varieties, referred to as “mega varieties” that are popular in major rice-growing areas of Asia, because of their high yield and grain quality (Mackill et al., 2006). Considerable progress has been made in breeding submergence tolerant cultivars with mega varieties for lowland eco-systems. Therefore, characterization of genotypes and using them in breeding programme is likely the best option to withstand submergence and stabilize productivity in these environments. Biochemical methods are valuable in differentiating and identifying the different genotypes and the development of molecular marker technology in recent years has revolutionized the whole concept of plant breeding.

However, information about these physiological and biochemical parameters in relation to rainfed lowland cultivars in eastern India with different durations of submergence is quite limited. The study aimed to screen rice genotypes for submergence by using biochemical (ADH activity and isozymes) and molecular marker (RAPD; Random Amplified Polymorphic DNA) technique.

In addition, we aim to establishing the relationships between survivals of genotypes under study with the effects of submergence (up to 15 days) stress using ADH activity and its isozymic profiling. The results obtained in this study will help plant breeders in breeding high yielding cultivars for lowland eco-systems, with submergence tolerant genotypes.

MATERIALS AND METHODS

Screening of rice varieties

Ten rice genotypes were collected from RARS, Titabor, Assam, India for the study (Table 1). Ten days old seedlings grown in earthen pots containing soil were completely submerged in a

concrete tank in the greenhouse (28 and 23°C, day and night, respectively) at 0.75 m depth for 10 days. A control set for all the genotypes was also maintained. Scoring was carried out 10 days after total desubmergence and submergence tolerance defined as the ability of a seedling to produce new, growing leaves as described by Singh et al. (2001). The whole experiment was laid out under complete randomized design and replicated three times.

Alcohol dehydrogenase activity and isozyme analysis

Activity and isozymic forms of ADH for both submerged and control plants were studied at 3-day intervals for 15 days of submergence. The leaf tissues were ground in prechilled pestle and mortar with 0.1 M Tris-HCl, pH 7.4 and 10 mM dithiothreitol (DTT) at 4°C. The supernatant obtained after centrifugation (15,000 rpm for 15 min at 4°C) was assayed for the total soluble protein (Lowry et al., 1951) and ADH activity was determined by the Racker method as modified by Stafford and Vennesland (1953). The same extract (60 µg protein) was also used to analyze isozymic forms of ADH (EC 1.1.1.1).

Soluble protein extraction for isozyme studies

Shoots of 7 days old etiolated seedlings were used for aspartate amino transferase (AAT, EC 2.6.1.1), esterase (EST, EC 3.1.1.11), malate dehydrogenase (MDH, EC 1.1.1.37) and peroxidase (POX, EC 1.11.1.7) isozymes studies. For analysis of isozymes except for POX, the sample was ground in prechilled pestle and mortar with 50 mM Tris-Cl buffer (pH 7.6) containing 5 mM β-ME and 5 mM EDTA in the ratio of 1:2 (w/v). For POX, 50 mM Tris-Cl buffer (pH 7.6) alone was used for extraction. The ground mixture was then centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant obtained was immediately used for native polyacrylamide gel electrophoresis (PAGE) (60 µg protein was loaded in each lane) and gels were stained for AAT, EST, MDH, and POX. The relative mobility (Rm) value of each band was computed and stained bands were scored in the format of binary data sets as presence (1) or absent (0).

DNA extraction and RAPD analysis

Genomic DNA of rice cultivars were extracted from 7 day old etiolated seedlings (Dellaporta et al., 1983). RAPD analysis was conducted by using 15 decamer arbitrary primers obtained from Operon Technologies, California. RAPD amplification was performed in 25 µl volume containing 1X Taq DNA Polymerase

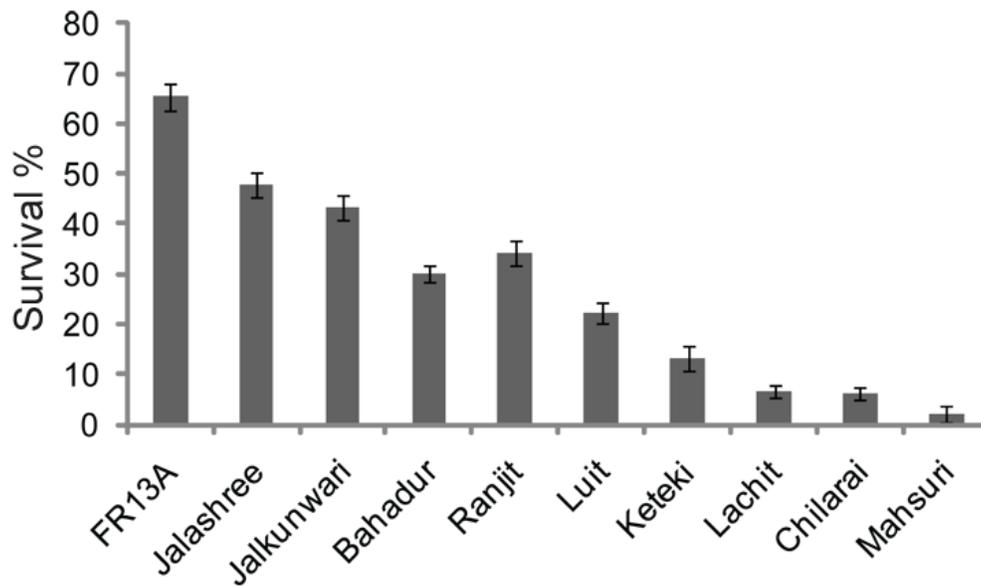


Figure 1. The effect of complete submergence on the survival of rainfed, lowland rice cultivars.

buffer, 200 μ M dNTPs mixture, 0.5 μ M primer, 25 ng of template DNA and 1 U of Taq DNA polymerase (Bangalore Genei, Bangalore, India) in a thermal cycler (Gene Amp® 2400, Applied Biosystems, USA). PCR amplification was carried out with initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 1 min. The 40th cycle was followed by final extension step at 72°C for 5 min. The amplified products were separated by electrophoresis on 1.5% agarose gel and visualized by ethidium bromide (EtBr) staining.

Phylogenetic analysis

Bands were scored as present (1) and absent (0) for isozyme and RAPD profile. An index of genetic similarity using Jaccard's coefficient was calculated. Cluster analyses were performed using UPGMA method and dendrograms were generated using "SPSS for MS Windows Release 16.0".

Data analysis

All the data were analysed statistically with the computer software SPSS and subjected to ANOVA. ADH activity means at each time point under submergence treatment of different rice genotypes were compared to Mahsuri by using least significance difference (LSD) test.

RESULTS

Survival percent

The survival percentage of survived plants is presented in Figure 1. The survival ranged from 1.5 to 65%. The highest survival percentage was recorded in FR13A and the lowest in Mahsuri.

ADH activity during submergence

The ADH enzyme activity in different rice cultivars, as influenced by the different submergence durations, is presented in Figure 2. ADH activity in FR13A, ranged from 0.0076 to 0.328779 units/mg protein during submergence period. ADH activity increased sharply after 6 days of submergence followed by steady increment between 9 and 12 days and then increased sharply from day 12 to reach the maximum (0.328779 units/mg protein) at 15 days of submergence in FR13A. Jalashree (0.0133 to 0.3383 units/mg of protein) and Jalkunwari (0.0064 to 0.3384 units/mg protein) follow almost similar pattern of ADH activity as in FR13A and their ADH activity also reached their maximum at 15 days of submergence. The ADH activity in Bahadur (0.0039 to 0.0488 units/mg of protein) and Ranjit (0.0042 to 0.0384 units/mg of protein) reached maximum at 12 days of submergence followed by a decline at 15 days of submergence. Luit (0.0028 to 0.0582 units/mg of protein), Keteki (0.0033 to 0.0533 units/mg protein), Lachit (0.0012 to 0.0411 units/mg protein), Chilarai (0.0033 to 0.0374 units/mg protein) and Mahsuri (0.0017 to 0.0316 units/mg protein) follow similar pattern of ADH activity. Their ADH activity increased and reached their maximum at 12 days of submergence followed by a sharp decline at 15 days of submergence.

Alcohol dehydrogenase (ADH) isozyme analysis under submergence condition

To investigate the profile of ADH isozyme under different submergence conditions, extract of leaf tissues were

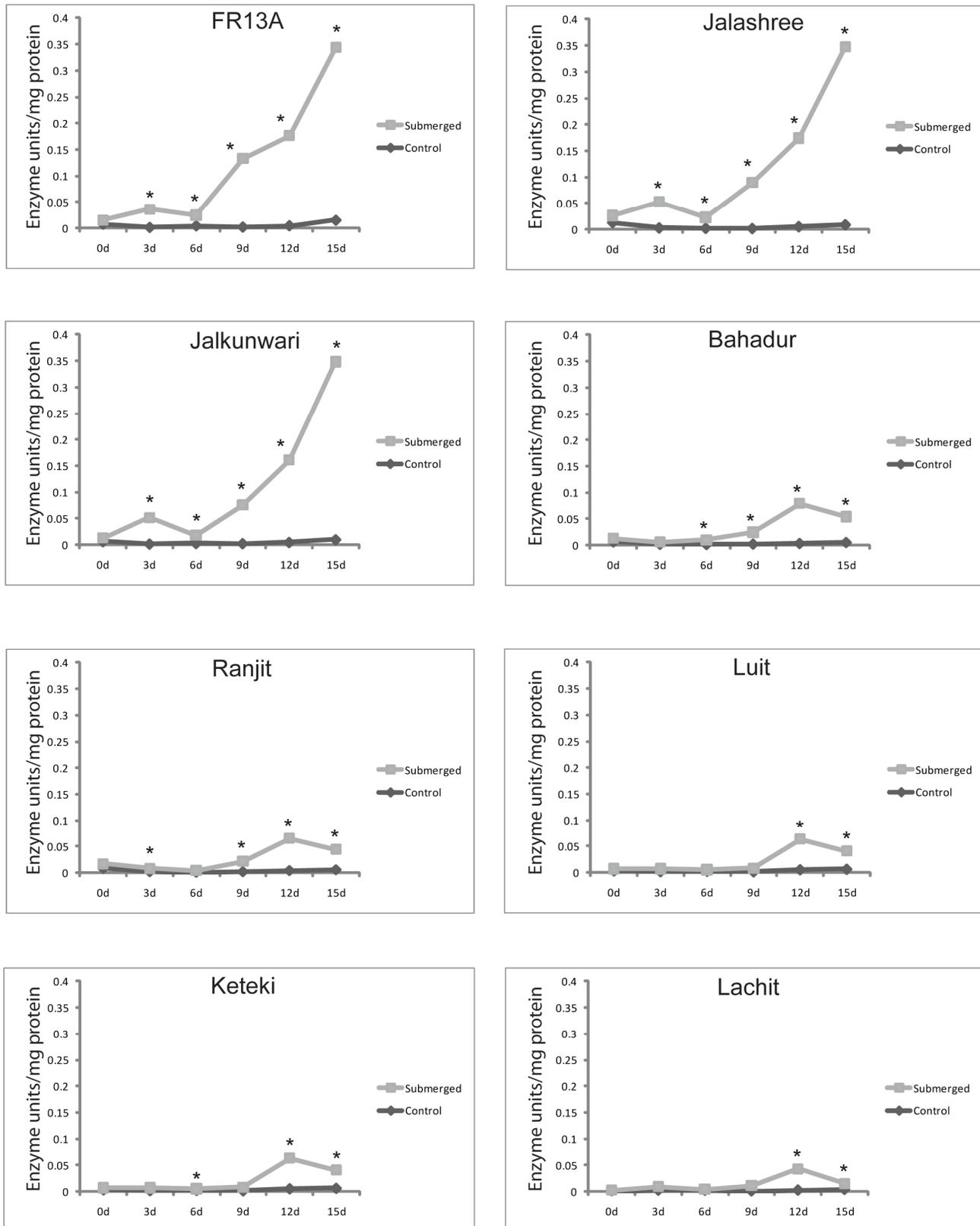


Figure 2. ADH activity of different rice genotypes under stress and control condition. ADH activity was studied at 3-day (d) intervals for 15 days of submergence. ADH activity means at each time point under submergence treatment of different rice genotypes were compared to Mahsuri by using least significance difference (LSD) test. P values are indicated by asterisk ($*p < 0.05$). Error bars have been omitted for clarity of graph.

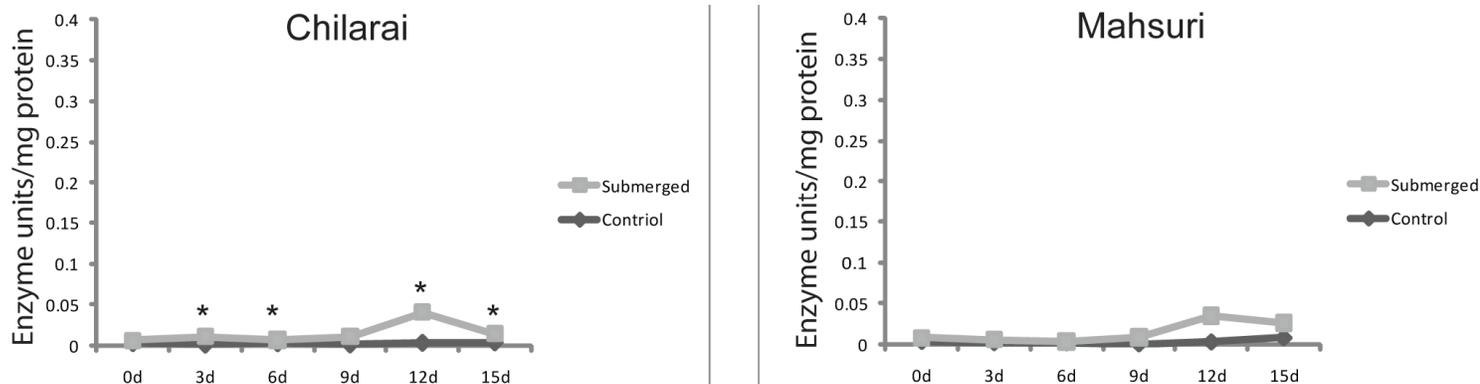


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subjected to native PAGE. Electrophoretic banding pattern of ADH revealed variable banding profile at different submergence durations (Figure 3). Isozyme pattern of ADH at 0 day of submergence showed only one intense band in all rice genotypes investigated. At 3 days of submergence two clear isozymes were observed in FR13A, Jalshree, and Jalkunwari. Second, isozyme has just started to appear in Bahadur and Ranjit, whereas only one isozyme was observed in other genotypes. At 6 and 9 days of submergence, two monomorphic bands were observed in all the genotypes studied. At 12 days of submergence, three isozymes were observed only in FR13A, Jalshree, and Jalkunwari, whereas only two isozymes were identified in all other rice genotypes. At 15 days of submergence, three monomorphic bands were observed in all rice genotypes studied.

Other isozyme analysis

The isozymic profile of AAT, EST, MDH and POX from 7 days old etiolated seedlings were analyzed in order to differentiate among rice genotypes. Two monomorphic bands were observed in all genotypes for AAT and MDH (Figure 4). EST pattern in rice genotypes yielded 5 bands in total and out of 5 bands, 2 were found to be polymorphic bands (Figure 4). An EST isozyme at Rm 0.80 was present only in FR13A, Jalashree, Jalkunwari, Ranjit and Bahadur, whereas isozyme at Rm 0.85 was found to be common in Luit, Keteki, Lachit, Chilarai, and Mahsuri. Three other EST isozymes were found common in all rice genotypes studied. POX yielded 7 monomorphic and 2 polymorphic bands. Isozymes with Rm 0.42 and 0.90 were present in FR13A, Jalashree, Jalkunwari, Bahadur and Ranjit. However, isozyme with Rm 0.48 was observed in only Mahsuri. The banding patterns of EST and POX isozyme were found to be polymorphic and clearly differentiated rice genotypes used.

Random amplified polymorphic DNA (RAPD) analysis

The banding pattern with all the 15 RAPD primers generated a total of 147 bands, with an average of about 9.8 bands per primer. Out of these amplified fragments 62 were polymorphic. The level of polymorphism was different with different primers among different genotypes. Four primers viz., OPD-06, OPH-07, OPN-04 and OPS-03 (Figure 5) clearly discriminated rice genotypes investigated.

Clustering of genotypes according to their level of submergence tolerance

In order to represent the relationships among genotypes, a cluster analysis (UPGMA) was used to generate dendrogram based on isozyme and RAPD analysis. Isozyme analysis showed two major clusters, FR13A, Jalashree and Jalkunwari were grouped together in one cluster, whereas all other genotypes except Ranjit and Bahadur were grouped together in another cluster. Ranjit and Bahadur were grouped closer to cluster having FR13A, Jalashree and Jalkunwari (Figure 6A). Dendrogram obtained from the RAPD data also showed two major clusters, FR13A, Jalashree and Jalkunwari were grouped together in one cluster, whereas all other genotypes except Bahadur were grouped together in the remaining cluster (Figure 6B).

DISCUSSION

Submergence due to flash flood is the key factor limiting yield of lowland rice which adversely affects grain yield of rice crop (Mohanty et al., 2000). On the whole, improved submergence tolerance is an important trait for rice growing in rainfed lowland areas. Therefore, efforts are being directed towards improving submergence tolerance character without affecting grain yield.

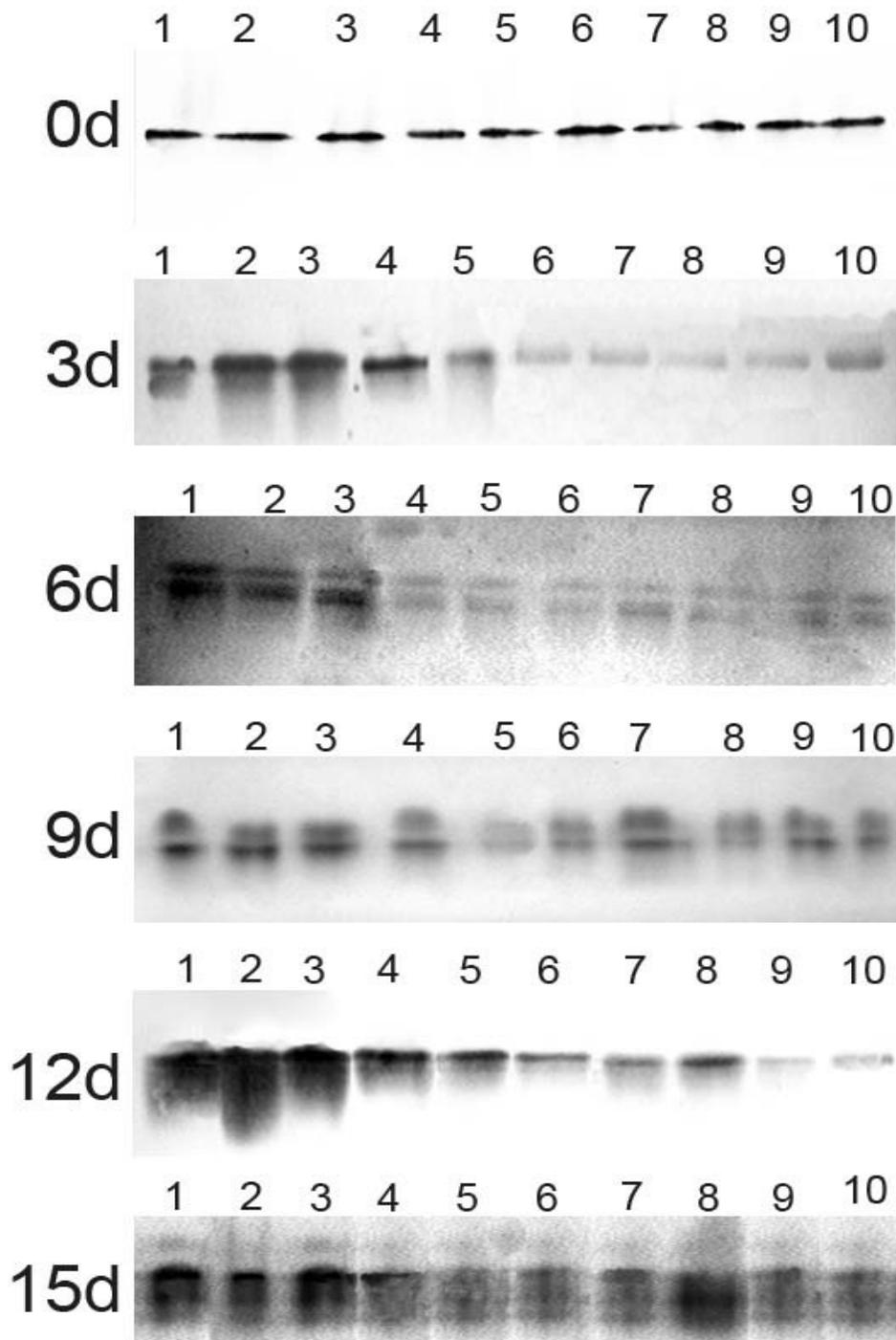


Figure 3. Isozyme profile analyses of ADH at 3-d intervals for 15 days under submergence treatment. Lane 1_FR13A, Lane 2_Jalashree, Lane 3_Jalkunwari, Lane 4_Bahadur, Lane 5_Ranjit, Lane 6_Luit, Lane 7_Keteki, Lane 8_Lachit, Lane 9_Chilarai, and Lane 10_Mahsuri.

The present investigation mainly attempts to characterize different rice genotypes on the basis of their submergence tolerance using biochemical and molecular techniques.

Screening of rice genotypes for submergence tolerance

Identification of submergence tolerant genotypes has

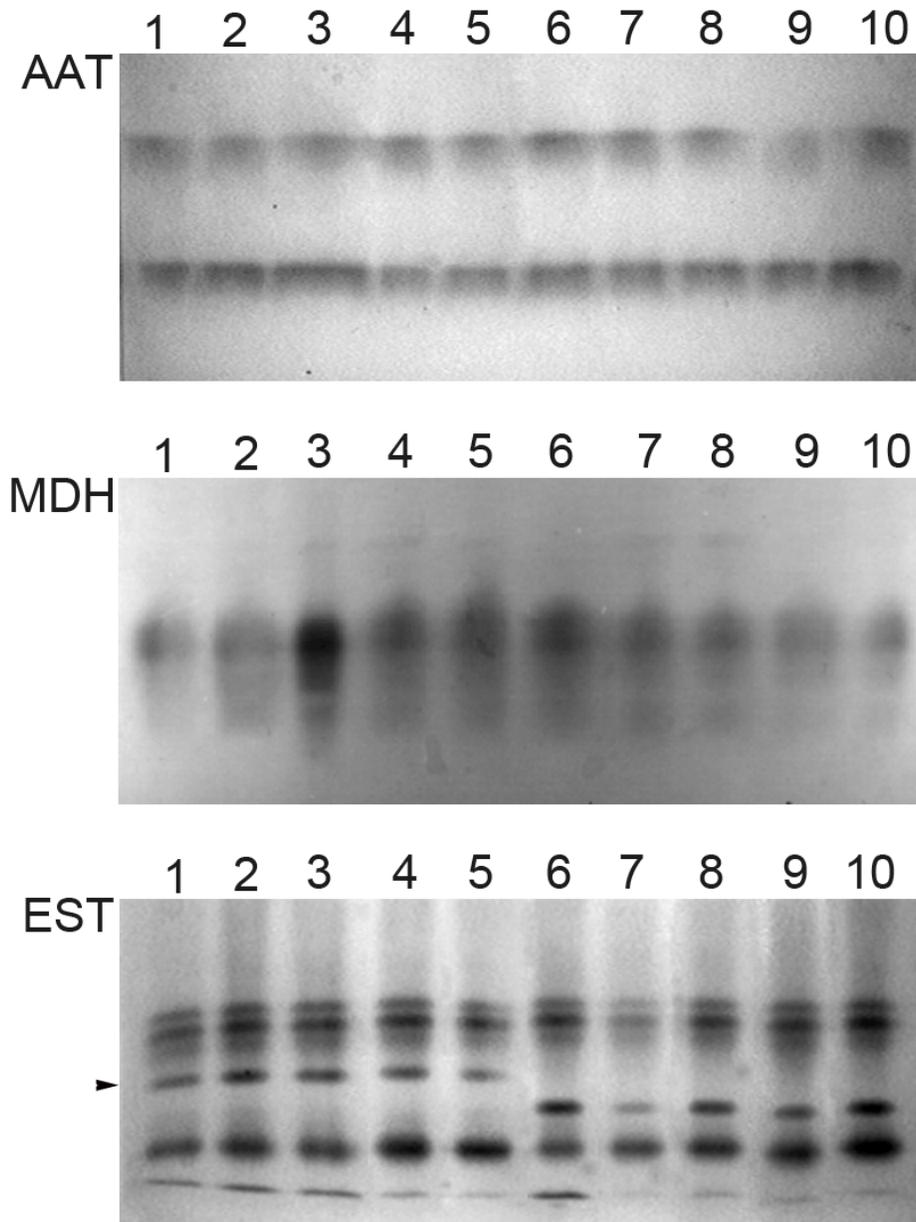


Figure 4. Isozyme profile analyses of AAT, MDH and EST of 7 days old etiolated seedlings. Lane 1_FR13A, Lane 2_Jalashree, Lane 3_Jalkunwari, Lane 4_Bahadur, Lane 5_Ranjit, Lane 6_Luit, Lane 7_Keteki, Lane 8_Lachit, Lane 9_Chilarai, and Lane 10_Mahsuri.

required the use of stress-specific screens, because direct evaluation of tolerance is not as simple as it might seem. Therefore, in the present investigation, 10-day-old seedlings were submerged for 10 days at a depth of 0.75 m water to check their ability to withstand submergence.

The highest survival percentage was observed in FR13A, while the lowest was recorded in Mahsuri. This may be because of high level of ADH activity in FR13A whereas, least ADH activity was observed in Mahsuri throughout the period of submergence.

These results were in consistency with previous report

(Singh et al. 2001) where FR 13 A showed the least damage and the best survival, whereas, the poorest survival was observed in Mahsuri at the end of the submergence treatment.

ADH activity during submergence

Anaerobic fermentation is one of the major metabolic adaptations that plants assume when they are submerged or faced with lack of oxygen (Sachs et al.,

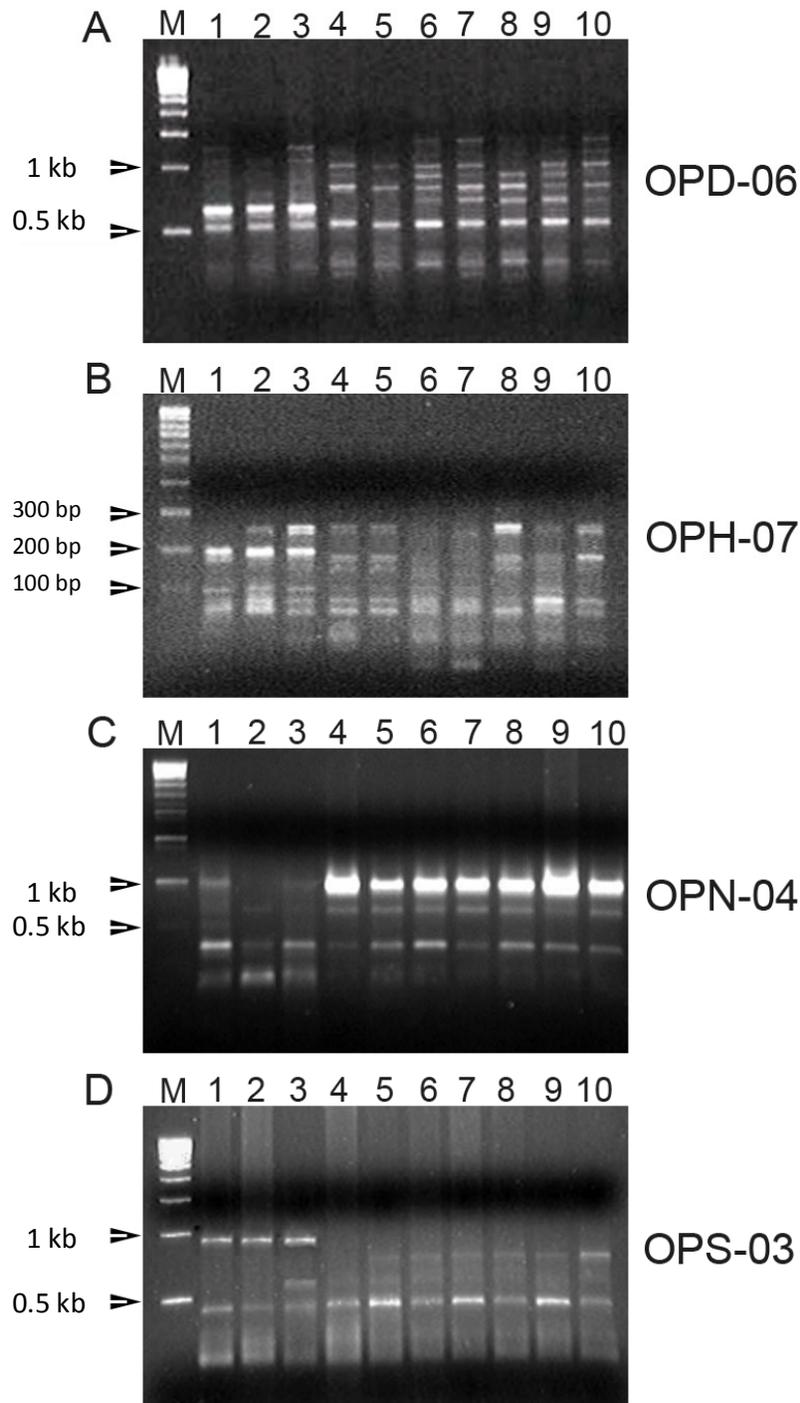


Figure 5. RAPD pattern of rice cultivars using OPD-06 (A), OPH-07 (B), OPN-04 (C), and OPS-03 (D) primers. Lane M_DNA ladder, Lane 1_FR13A, Lane 2_Jalashree, Lane 3_Jalkunwari, Lane 4_Bahadur, Lane 5_Ranjit, Lane 6_Luit, Lane 7_Keteki, Lane 8_Lachit, Lane 9_Chilarai, and Lane 10_Mahsuri.

1996). Higher levels of ADH, an enzyme associated with anaerobic fermentation and ethanol production during anaerobiosis have been reported for flood tolerant plants (Tripepi and Mitchell, 1984, Kato-Noguchi and Kugimiya, 2003). In all rice genotypes investigated, the ADH activity

under submerged condition increased as the duration of submergence increased. A sharp increase in the ADH activity was observed after 9 days of submergence. In FR13A, Jalashree, Jalkunwari and Bahadur, the increase in the activity continued till 15 days of submergence.

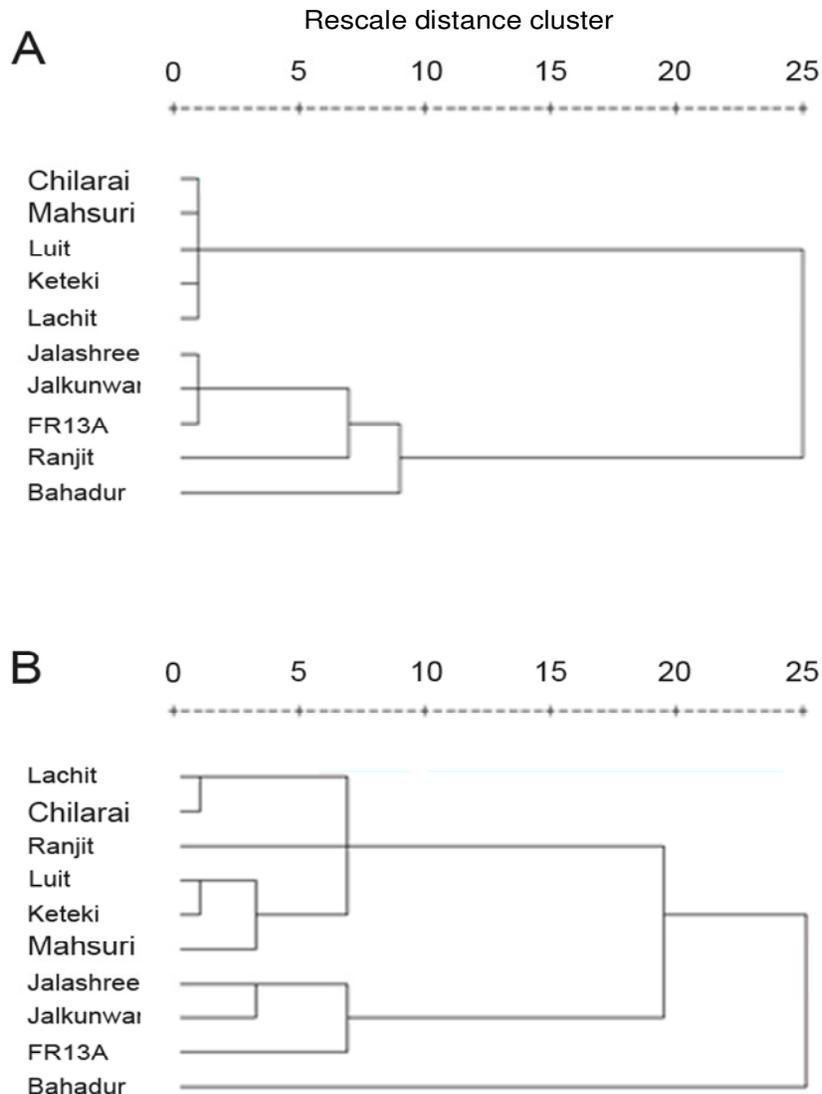


Figure 6. Cluster analysis dendrogram of rice varieties based on (A) Isozymes, and (B) RAPD data.

This finding is similar to that of Bertani et al. (1980) who observed that ADH undergoes a rise in activity upon imposition of anaerobiosis which then tends towards a plateau. Increased level of ADH activity during O_2 deficiency and its level measured at any point of time has been function of rate of transcription and on its transcript stability (Feri et al., 1980; Dennis et al., 1984, 1985; Rowland and Strommer, 1986). The decline in the level of ADH activity in the susceptible genotypes after initial induction might be attributed to the decline in the energy metabolism under anoxia condition.

ADH isozyme in submerged rice genotypes

Increased rice ADH enzyme activity under submergence condition prompted us to see the change in isozymic

profiles in different under submergence stress. The number of ADH isozyme observed in rice genotypes varied from 1 to 3. Plant ADH enzymes were considered to be dimers and the two subunits of ADH are encoded by two unlinked genes (Gottlieb, 1982; Sachs and Ho, 1986). The products of these genes dimerize randomly to yield three electrophoretically distinct isozymes: ADH1-ADH1 homodimer, ADH1-ADH2 heterodimer and ADH2-ADH2 homodimer (Gottlieb, 1982; Newman and VanToai, 1991).

In this study, early appearance of 2nd and 3rd isozyme at 3 and 12 days of submergence in FR13A, Jalshree, and Jalkunwari indicated that the increase in ADH activity lead to increase in enzyme synthesis. Increased rice ADH enzyme activity has been shown by a change in isozymic profiles of the ADH protein (Rivoal et al., 1989; Xie and Wu, 1989). Hence, the results obtained in our

study strengthen the hypothesis that change in isozymic profiles may be used to distinguish submergence tolerant and susceptible rice genotypes.

Other isozymes

Response to submergence appears to be complex and involves a number of enzymes. Hence, we investigated AAT, EST, MDH, and POX to see the differences, if any, between the cultivars under study. Isozyme profile of AAT and MDH were found to be monomorphic, whereas EST and POX pattern were polymorphic and able to differentiate cultivars under study. Such differences may not be a mere coincidence, as EST and POX has been previously shown to be a useful induction of submergence and other abiotic stress tolerance in rice (Mandal et al., 2004; Zhang et al., 1988).

The differences in the isozyme binding pattern were due to variation in the amino acid content of the molecule, which in turn was dependent on the sequence of nucleotides in DNA (Micales et al., 1986). Different bands obtained indicate different electrophoretic mobilities of the isozymes, which were coded by different alleles or separate genetic loci.

Random amplified polymorphic DNA (RAPD) analysis

Genetic markers such as morphological markers and biochemical markers were more prone to environment effect and limited by small number of loci (Tanksley et al., 1989). The use of PCR based assays having advantage of being quick, easy to use and refractory to many environmental influences can complement traditional and biochemical methods of cultivars characterization. The RAPD technique for detecting genetic variation among cultivars and identifying germplasm is well established (Cao and Oard, 1997; Gorji et al., 2010).

In this study, four primers viz.; OPD-06, OPH-07, OPN-04 and OPS-03 were found to clearly discriminate rice genotypes used in this study. OPH-07 primer was found to be linked with Sub 1 locus of chromosome number 9 and produced bands associated with either tolerant or susceptible F₃ families in rice (Xu and Mackill, 1996). Dendrogram generated based on RAPD analysis, FR13A, Jalashree and Jalkunwari were grouped together in one cluster, whereas all other genotypes except Bahadur were grouped together in the remaining cluster.

Conclusion

With the identification of physiological traits, enzyme activity, isozymes, and DNA markers associated with submergence tolerance, the prospects for breeding suitable rice cultivars for rainfed lowlands have been

improved. The characterization of genotypes based on survival percentage, ADH activity, isozymes, and RAPD analysis for its survival under submergence has again been well established in our study. Dendrogram generated by isozymes and RAPD analyses were able to discriminate genotypes under study. The genotypes grouped in each cluster showed similar pattern of ADH activity and its isozymic profile and were well correlated with survival percentage under different period of submergence. The rice genotypes used in the present study can be grouped into three categories:

- (I) Submergence tolerant (FR13A, Jalashree and Jalkunwari),
- (II) Moderately submergence tolerant (Bahadur and Ranjit),
- (III) Submergence susceptible (Luit, Keteki, Lachit, chilarai and Mahsuri).

Thus, the results of this study clearly indicate the utility of biochemical and molecular markers for the characterization of rice genotypes for submergence tolerance. Therefore, such studies are useful in identifying and characterizing submergence tolerant genotypes in rice and information obtained can be of use for breeders.

REFERENCES

- Armstrong W (1979). In: Advances in Botanical Research. Woolhouse, H. W. (ed.) Acad. Press, New York, 7: 225-332.
- Bailey-Serres J, Voeselek LACJ (2008). Flooding stress: acclimations and genetic diversity. *Annu. Rev. Plant Biol.*, 59: 313-339.
- Bertani A, Brambilla I, Manegus F (1980). Effects of anaerobiosis on rice seedlings: Growth, metabolic rate, and fate of fermentation products. *J. Exp. Bot.*, 31: 325-331.
- Cao D, Oard JH (1997). Pedigree and RAPD based DNA analysis of commercial US rice cultivars. *Crop Sci.*, 37: 1630-1635.
- Davies DD (1980). Anaerobic metabolism and the production of organic acids. In: Davies DD, ed. *The biochemistry of plants*. Vol 2. New York: Academic Press, 581-611.
- Dellaporta SL, Wood J, Hicks JB (1983). Maize DNA minipreps. *Maize Genet. Crop Newsl.*, 57: 26-29.
- Dennis ES, Gerlach WL, Pryor AJ, Bennetzen JL, Inglis A, Llewellyn D, Sachs MM, Ferl RJ, Peacock WJ (1984). Molecular analysis of the alcohol dehydrogenase (Adh1) gene of maize. *Nucleic Acids Res.*, 12: 3983-4000.
- Dennis ES, Sachs MM, Gerlach WL, Finnegan EJ, Peacock WJ (1985). Molecular analysis of the alcohol dehydrogenase 2 (Adh2) gene of maize. *Nucleic Acids Res.*, 13: 727-743.
- Dey MM, Upadhyaya HK (1996). Yield loss due to drought, cold and submergence in Asia. In: Everson RE, Herdt RW, Hossain M, eds. *Rice research in Asia: progress and priorities*. Manila, The Philippines: IRRI, pp. 291-303.
- Drew MC, He CJ, Morgan PW (2000). Programmed cell death and aerenchyma formation in roots. *Trends Plant Sci.*, 5: 123-127.
- Ferl RJ, Brennan M, Schwartz D (1980). *In vitro* translation of maize ADH: evidence for the anaerobic induction of mRNA. *Biochem. Genet.*, 18: 681-691.
- Gorji AH, Davish F, Esmailzadehmoghadam M, Azizi F (2010). Application RAPD Technique for Recognition Genotypes Tolerant to Drought in some of Bread Wheat. *Asian J. Biotechnol.*, 2: 159-168.
- Gottlieb LP (1982). Conservation and duplication of isozymes in plants. *Science*, 216: 373-380.

- Huke RE, Huke EH (1997). Rice area by type of culture-in South, Southeast, and East Asia. A revised and updated data base. IRRI, Los Baños, Philippines.
- Jung KH, Seo YS, Walia H, Cao P, Fukao T, Canlas PE, Amonpant F, Bailey-Serres J, Ronald PC (2010). The submergence tolerance regulator Sub1A mediates stress-responsive expression of AP2/ERF transcription factors. *Plant Physiol.*, 152: 1674-1692.
- Kato-Noguchi H, Kugimiya T (2003). Preferential Induction of Alcohol Dehydrogenase in Coleoptiles of Rice Seedlings Germinated in Submergence Condition. *Biol. Plant*, 46: 153-155.
- Kende H, van der Knaap E, Cho HT (1998). Deepwater rice: a model plant to study stem elongation. *Plant Physiol.*, 118: 1105-1110.
- Lowry OH, Rosenbought NJ, Ferr AL, Randall RJ (1951). Protein measurement with the Folin-phenols reagent. *J. Biol. Chem.*, 193: 265-275.
- Mackill DJ, Collard BCY, Neeraja CN, Rodriguez RM, Heuer S, Ismail AM (2006). QTLs in rice breeding: examples for abiotic stresses. In: Brar DS, Mackill DJ, Hardy B (eds) Rice genetics 5: proceedings of the international rice genetics symposium. International Rice Research Institute, Manila, pp. 155-167.
- Mandal S, Mandal N, Hazra K, Mukherjee SK, Das PK (2004). Submergence tolerant androgenic rice dihaploids derived from intra- and interspecific crosses: I. Seed Storage Protein and Isozyme Pattern Through Gel Electrophoresis. *Plant Tissue Cult.*, 14: 25-35.
- Micales JA, Bonde MR, Peterson GL (1986). The use of isozyme analysis in fungal taxonomy and genetics. *Mycotaxonomy*, 27: 407-449.
- Mohanty HK, Mallik S, Grover A (2000). Prospects of improving flooding tolerance in lowland rice varieties by conventional breeding and genetic engineering. *Curr. Sci.*, 78(2): 132-140.
- Newman KD, VanToai TT (1991). Developmental regulation and organ-specific expression of soybean alcohol dehydrogenase. *Crop Sci.*, 31: 1253.
- Perata P, Voesenek LA (2007). Submergence tolerance in rice requires Sub1A, an ethylene-response-factor-like gene. *Trends Plant Sci.*, 12: 43-46.
- Rivoal J, Ricard B, Pradet A (1989). Glycolytic and fermentative enzyme induction during anaerobiosis in rice seedlings. *Plant Physiol. Biochem.*, 27: 43-52.
- Rowland LJ, Strommer JN (1986). Anaerobic treatment of maize roots affects transcription of *Adh1* and transcript stability. *Mol. Cell Biol.*, 6: 3368-3372.
- Sachs MM, Ho T-HD (1986). Alteration of gene expression during environmental stress in plants. *Annu. Rev. Plant Physiol.*, 32: 363-376.
- Sachs MM, Subbaiah CC, Saab IN (1996). Anaerobic gene expression and flooding tolerance in maize. *J. Exp. Bot.*, 47: 1-15.
- Sauter M (2000). Rice in deep water: how to take heed against a sea of troubles. *Naturwissenschaften*, 87: 289-303.
- Setter TL (1992). Important physiological mechanisms of submergence tolerance in rice. In: Kuo, G.C. (ed.) Adaptation of food crops to temperature and water stress. Proc. Int. Symp., Taiwan. 13-18th August, 1992. pp. 220-230.
- Singh HP, Singh BB, Ram PC (2001). Submergence tolerance of rainfed lowland rice: search for physiological marker traits. *J. Plant Physiol.*, 158: 883-889.
- Stafford HA, Vennesland B (1953). Alcohol dehydrogenase of wheat germ. *Arch. Biochem. Biophys.*, 44: 404-414.
- Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989). RFLP mapping in plant breeding: New tools for old science. *Biotechnology*, 7: 257.
- Tripepi RR, Mitchell CA (1984). Metabolic response of river birch and european birch roots to hypoxia. *Plant Physiol.*, 76: 31-35.
- Voesenek LA, Colmer TD, Pierik R, Millenaar FF, Peeters AJ (2006). How plants cope with complete submergence. *New Phytol.*, 170: 213-226.
- Xie Y, Wu R (1989). Rice alcohol dehydrogenase genes: anaerobic induction, organ specific expression and characterization of cDNA clones. *Plant Mol. Biol.*, 13: 53-58.
- Xu K, Mackill DJ (1996). RAPD and RFLP mapping of a submergence tolerance locus in rice. pp. 244. Rice Genetic Newsletter, Vol. 12.
- Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ (2006). Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature*, 442: 705-708.
- Zhang ZH, Zheng ZL, Gao HY, Cao HX (1988). Breeding, evaluation and utilization of anther cultured varieties "Zin Xion" and "Hua Han Zoo" in rice (*Oryza sativa* L.). In : IRRI and Academia Sinica (eds.) Genetic manipulations in crops, Cassel Tycooly, Philadelphia, pp. 36-37.