

Submergence tolerance in rainfed lowland rice: physiological basis and prospects for cultivar improvement through marker-aided breeding

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Abstract

Two important factors influencing rice plant survival during submergence are limitations to gas diffusion under water, and reduced irradiance that impair photosynthesis and efficient utilization of carbohydrates. Thus, survival during submergence may largely depend on accumulation of high carbohydrate concentrations prior to submergence and a capacity for maintaining energy production through rapid alcoholic fermentation under oxygen shortage. During flash flooding, a third factor thought to affect survival is the aerobic shock during the post-submergence period when floodwaters recede. Changes in the level of antioxidants and enzymes such as superoxide dismutase (SOD) suggest that tolerant rice cultivars develop protective systems to air after exposure to hypoxic or anoxic environments. These responses are similar to other wetland plants.

The capacity to survive submergence depends not only on specific environmental factors, but also on the strategy that plants have evolved for adoption to particular flood-prone environments. In rice the two main strategies are to elongate and escape, or not to elongate and conserve resources. For rainfed lowland rice exposed to flash flooding, elongation growth during complete submergence has major adverse effects on survival, presumably since this competes with maintenance processes which require carbohydrates and energy. Selection for minimal elongation during submergence is currently being exploited as a trait for submergence tolerance by rainfed lowland rice breeders in south and southeast Asia.

Gene mapping for submergence tolerance has been useful in identifying one prominent locus for submergence tolerance. Fine scale gene mapping and sequencing may facilitate further progress in the physiology and genetics of submergence tolerance. Recently published data demonstrate that improving submergence tolerance may be possible through up-regulation of genes for particular traits such as pyruvate decarboxylase (PDC) for alcoholic fermentation. Validation of appropriate mechanisms in other cultivars for target environments, and development and utilization of molecular markers to follow these traits in breeding programs, will therefore be high priorities for future work on submergence tolerance of rice. © 2002 Elsevier Science B.V. All rights reserved.

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1. Rainfed lowland rice

Rainfed lowland rice grows in banded fields that become flooded for at least part of the cropping season to depths that do not exceed 50 cm for more than 10 consecutive days (IRRI, 1993), but also suffers water deficit at various stages of development due to erratic rainfall. From a survey of researchers in eastern India, submergence is identified as the third most important of 42 biotic and abiotic stresses that limit rice production and is surpassed only by drought at anthesis and by weeds (Widawsky and O'Toole, 1990).

Flash flooding and submergence are widespread in south-east Asia, Bangladesh and northeastern India, and affect at least 22 million hectares (16% of world rice lands) including 15 million hectares of potential short-duration flash floods in rainfed lowlands and 5 million hectares of deepwater rice (Khush, 1984). Eastern India alone has approximately 10 million hectares of rice lands affected by flash floods and complete submergence (Reddy and Sharma, 1992). Rice yields in the rainfed lowlands of eastern India are low, averaging 2.4 t/ha (IRRI, 1993), and recent analysis over 1970–1991 revealed that the rate of rice production increased at about 2.7% per year, although this was unevenly distributed across states and districts and was particularly low in Bihar, Assam, Orissa and eastern Madhya Pradesh (Hossain and Laborte, 1996). The same analysis identified a highly significant negative correlation between rainfall and the yield or annual increase in rice yields. Poor drainage and flooding, therefore, is a major constraint to achieving increased yields of rice in eastern India (Hossain and Laborte, 1996) where flooding is common.

Rice has adapted to submergence-prone environments by two strategies:

- (i) Submergence tolerance to flash floods where a rapid increase in water level causes partial to complete submergence for up to 2 weeks;
- (ii) Shoot elongation by types adapted to deepwater areas (>100 cm) where water stagnates for several months and where survival depends on the shoot remaining in contact with the air. This rapid elongation is disadvantageous in flash flood conditions since lodging usually occurs once floodwater recedes. For deepwater and floating

rice, plants have been selected to overcome lodging when the floodwater recedes by further elongation and bending of the upper portions of stems which keeps the panicle upright and off the soil surface (see Catling, 1992).

Flash flooding includes three drastic environmental changes: (i) the tendency to a shortage of oxygen restricting respiration during submergence, (ii) a drastic inhibition to the entry of carbon dioxide restricting photosynthesis during submergence, and (iii) the damaging effects of the subsequent reversal back to fully aerobic conditions (post-anoxic shock) when floodwater recedes.

This review focuses on physiological research of these changes which are the basis for submergence tolerance of rainfed lowland rice. Possible options are discussed for the use of genetic markers of those physiological traits for selection and cultivar improvement.

2. Environmental characterization of floodwater

Rice is well adapted to “flooded” ricefields, however, excessive flooding may result in various environmental stresses that accompany partial or complete submergence. Plant survival depends not only on depth and duration of submergence but also on the quality of floodwater, including O₂ and CO₂ concentrations, turbulence and turbidity. The variations in floodwater characteristics across locations induce different responses in various cultivars making the interpretation of research data difficult. In particular, conclusions about flooding tolerance at one site cannot be extrapolated elsewhere without information on floodwater characteristics. Several factors contribute to the adverse effects of submergence in rice, and some may have combined effects (Ito et al., 1999). For example, siltation on leaves may cause mechanical damage, and affect light and gas diffusion (Setter et al., 1995). Current experimental evidence demonstrates that limited gas diffusion (Setter et al., 1988) and underwater irradiance (Palada and Vergara, 1972; Setter et al., 1995; Ram et al., 1999) are, however, the main factors that appear to affect growth, metabolism and survival of rice during submergence.

2.1. Gas diffusion

Among several factors, which affect growth, metabolism and survival of submergence plants, limited gas diffusion is the most crucial component, because gases diffuse 10^4 times more slowly in solution than in air (Armstrong, 1979). Reduced movement of gases to and away from submerged plant surfaces alters the concentration of O_2 , CO_2 and ethylene inside the plants. The depletion of O_2 is a major feature of flooding, inducing hypoxia (low O_2) or anoxia (zero O_2) around the shoot and root tissues or germinating seeds (Kennedy et al., 1992; Collis and Melville, 1974). The importance of reduced gas diffusion during submergence was clearly demonstrated in glasshouse experiments where increase in CO_2 pressure to completely submerged rice (IR42) increased survival from about 10 days to 3 months. Following submergence, these plants survived, flowered and set grain (Setter et al., 1989). Interpretation of the beneficial effects in this experiment is complicated because increased CO_2 supply would increase photosynthesis, increase oxygenation from photosynthesis and from aeration of solutions, and reduce ethylene due to degassing solutions using high CO_2 pressures in air (Setter et al., 1989). Measurement of the gases in floodwater during submergence is therefore relevant to understand the mechanisms of plant death and the potential for cultivar improvement.

Floodwater characteristics were measured over diurnal cycles and water depth for temperature, pH, irradiance, CO_2 and O_2 concentrations and water flow rates in rice fields at various locations in eastern India (Setter et al., 1995; Ram et al., 1999). Similar measurements were made earlier for floodwater in rice fields in Thailand (Setter et al., 1982). At various locations and times, dissolved O_2 concentrations ranged from anoxic to supersaturated, while dissolved CO_2 was invariably greater than that normally present in water equilibrated with air (Ram et al., 1999).

During flash floods, O_2 concentration is often above air-equilibrated levels due to water turbulence and mixing with air, but in stagnant floodwater, it is normally below these levels. In stagnant floodwater, a diurnal cycle is evident showing a peak of O_2 concentration during mid-afternoon rising from the lowest value in the early morning. Floodwater may also become anoxic especially during night and at

depth (near the root zone) when O_2 produced during daytime is presumably consumed in respiration by plants and microorganisms.

Even in turbulent water, submerged rice plants may be exposed to hypoxia or anoxia because of diffusion barriers. Oxygen deficiency will tend to accelerate breakdown of carbohydrates (Setter et al., 1987b) if the so-called Pasteur effect is induced. As a result, a higher rate of photosynthesis would be required to compensate for the additional carbon loss. Unlike O_2 , CO_2 concentrations show a contrasting diurnal cycle, with high levels during late night (respiratory CO_2) and low levels during daytime due to consumption in photosynthesis by submerged plants and algae. Low CO_2 concentrations inside plants may reduce photosynthesis, even though the floodwater itself is always rich in CO_2 . Smith and Walker (1980) demonstrated that underwater photosynthesis in aquatic species increased until the CO_2 concentration in the bulk solution reached a concentration above $1\text{--}2\text{ mol m}^{-3}$ compared to the approximately $15\text{ }\mu\text{M}$ present in water equilibrated with air at pH 7 and $30\text{ }^\circ\text{C}$. The maximum photosynthetic rate decreased during 7 days submergence of a tolerant cultivar (FR13A), but plants still maintained 32% of the dry weight gain of non-submerged controls. In contrast, a submergence-intolerant cultivar (IR42) did not gain dry weight (Smith et al., 1987). Photosynthesis in rice can be increased by CO_2 enrichment of floodwater, and growth up to 100% of non-submerged plants has been reported (Setter et al., 1989).

2.2. Light

Light is another important environmental factor which affects growth and survival of rice during submergence. Variable light profiles in floodwater were obtained in Thailand (Setter et al., 1987a) and India (Ram et al., 1999). In the brightest profile, photosynthesis was 50% of the maximum rate at 0.75 m water depth from the surface, while the most turbid profile reduced photosynthesis to the compensation point at 0.25 m water depth (Setter et al., 1987a). Floodwater turbidity reduces light transmission and deposits silt on the leaves of submerged plants. Irradiance in turbid water can be 0 cm \times 40 cm of depth (Table 1). Whitton et al. (1988) observed that low irradiance in floodwater in Bangladesh was due to surface algal colonies as well as turbidity.

Table 1

Photosynthetically active irradiance (PAR) during submergence in clear and turbid water and survival of 14-day-old rice seedlings during 7 days submergence in clear and turbid water at Kumarganj, India^a

Water depth (cm)	Irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR)	
	Clear water	Turbid water
2	285 \pm 5	217 \pm 24
20	120 \pm 21	30 \pm 6
40	73 \pm 19	0
60	42 \pm 5	0
80	24 \pm 6	0
Cultivar	Survival (%)	
	Clear water	Turbid water
FR13A	87 \pm 12	53 \pm 12
Vaidehi	77 \pm 6	53 \pm 12
Pankaj	30 \pm 0	26 \pm 6
IR42	20 \pm 6	16 \pm 6
Mahsuri	27 \pm 6	20 \pm 6

^a Irradiance data are averages of four measurements at 1100 h \pm S.E.M.; survival data are means of 10 replicates \pm S.E.M. (Ram et al., 2000).

Palada and Vergara (1972) observed lower light transmission (40% that in air) and decreased survival of rice seedlings submerged in turbid water. Submergence in turbid water was confirmed to be more detrimental than in clear water for several cultivars, however, the submergence period is often too long and the differences are insignificant to confirm this in intolerant cultivars (Table 1). Irradiance under water is a major factor affecting the O₂ and CO₂ concentrations, hence photosynthesis and respiration that influence the balance between gain and loss of carbohydrate (Section 3.2.1).

2.3. Temperature

Temperature is a further factor affecting the survival of plants during submergence. High temperature (30 °C) accelerates plant mortality, whereas low temperature (20 °C) improves survival. Submergence-tolerant (FR13A) and intolerant (IR42 and Mahsuri) cultivars responded similarly when submerged for 7 days at 20 \pm 2 °C (100% survival), although during the post-submergence recovery phase of 10 days, the intolerant cultivars developed severe leaf tip burning

Table 2

Effect of floodwater temperature during submergence on survival of 30-day-old seedlings of rainfed lowland rice submerged for 7 days at Kumarganj, India^a

Cultivar	Survival (%)	
	20 \pm 2 °C	30 \pm 2 °C
FR13A	100 \pm 0 ^b	87 \pm 12 ^c
Vaidehi	100 \pm 0 ^b	77 \pm 12 ^c
Pankaj	100 \pm 0 ^c	30 \pm 6 ^d
IR42	100 \pm 0 ^c	20 \pm 6 ^d
Mahsuri	100 \pm 0 ^c	27 \pm 6 ^d

^a Data are the means of four replicates \pm standard errors (Ram et al., 2000).

^b The severity of the leaf burning in the various cultivars during recovery is \sim 25% leaf injury.

^c The severity of the leaf burning in the various cultivars during recovery is 25–60% leaf injury.

^d The severity of the leaf burning in the various cultivars during recovery is $>$ 60% leaf injury.

(Tables 2 and 3). In contrast, submergence at 30 \pm 2 °C for 7 days drastically reduced survival in intolerant rice cultivars (Table 3). High temperature decreases O₂ and CO₂ solubility in floodwater and accelerates anaerobic respiration leading to faster starvation and faster death of plants.

Wheat, which is much less intolerant to submergence than rice, also showed 100% survival following 7 days submergence at 18 \pm 2 °C (Lal, 1998). However, the extent of visible leaf injury during the

Table 3

Soluble carbohydrate concentrations of shoots (mg soluble carbohydrate (g dry wt.)⁻¹) before (BS) and after (AS) 7 days submergence of 14-day-old seedlings of rice cultivars grown under various irradiances. Plants were grown and submerged in pots at Kumarganj, India (Ram et al., 2000)

Genotype	Irradiance (% of incident)					
	100		50		25	
	BS	AS	BS	AS	BS	AS
FR13A	78	33	50	31	37	25
Vaidehi	70	35	46	33	36	26
TCA-95-4	58	17	34	15	32	07
TCA-95-5	63	29	41	15	35	11
Pankaj	56	21	28	16	21	08
IR42	61	21	39	16	23	11
LSD 0.05	BS = 1.4, AS = 1.2					

recovery phase was variable across wheat cultivars. This clearly indicates that temperature plays a vital role, along with other environmental factors, in plant survival during submergence. Adkins et al. (1990) and Siebel and Blom (1998) observed that at high water temperature, plants became more intolerant to submergence. However, in the work of Adkins et al. (1990) the LD₅₀ among rice cultivars were similar at the temperatures between 20 and 30 °C. At all ages, depths, temperatures and irradiance, the submergence-tolerant cultivar FR13A maintained its ranking as most tolerant and IR42 remained the most submergence intolerant cultivar.

3. Submergence tolerance: success and uncertainties

Rainfed lowland rice research in eastern India during last decade has identified two physiological traits:

- (1) minimal underwater elongation during submergence,
- (2) high storage carbohydrate concentration prior to submergence.

A number of publications have established the validity of these physiological traits (Chaturvedi et al., 1995, 1996; Setter et al., 1994, 1997, 1998; Mallik et al., 1995; Greenway and Setter, 1996; Setter and Laureles, 1996; Singh et al., 2001) and they are now being used for screening. Prospects for further use of these traits in breeding are now discussed in relation to morphological adaptations and metabolic adaptations for improving submergence tolerance.

3.1. Morphological adaptations

3.1.1. Aerenchyma

The presence of gas-filled spaces, known as aerenchyma, in roots of numerous plant species is considered to be an important anatomical adaptation for survival under flooded conditions (Justin and Armstrong, 1987). Ample evidence has been presented to show that aerenchyma provide a diffusion path of low resistance for the transport of oxygen from aerial plant parts to roots or rhizomes in a waterlogged, O₂ deficient environment (Armstrong, 1971; Kawase and Whitmoyer, 1980; Laan et al., 1989).

Aerenchyma also provide a path for diffusion of volatile compounds such as ethylene, methane, CO₂, ethanol, and acetaldehyde that are produced in anaerobic soil and plant tissues during flooding (Vartapetian and Jackson, 1997; Visser et al., 1997). Aerenchyma in some plants is constitutive while in others it is induced in response to flooding. Aerenchyma are formed in roots, but shoots of some plants also have similar structures (lacunae) which provide an interconnected continuum for gas diffusion in tissues (Vartapetian and Jackson, 1997). Oxygen is supplied through aerenchyma for root respiration and oxygenation of rhizosphere facilitating detoxification of chemically reduced iron, manganese, and hydrogen sulfides (Gambrell et al., 1991) and may also support nitrifying bacteria in conversion of ammonia to nitrate (Blom et al., 1994).

The formation of aerenchyma occurs either through lysigeny via cell lysis, or through schizogeny via separation of cells during tissue development (Smirnov and Crawford, 1983). Root porosity increases in proportion to the volume of aerenchyma and is thought to facilitate the diffusion of gases in and out of the plants. Poor aeration promotes the development of aerenchyma in wetland and amphibious species such as rice (Justin and Armstrong, 1987), *Rumex crispus* (Laan et al., 1989) and willow (Jackson and Attwood, 1996). In spite of the promotion of aerenchyma formation (often measured by increased porosity) during flooding, positive correlation between survival and porosity are not often observed. In our experiments with double haploid (DH) rice lines, root porosity increased in about half the lines exposed to submergence for 7 days, however, there was little or no relationship between survival during submergence and porosity before ($r^2 = 0.00$) or after ($r^2 = 0.29$; $n = 16$) submergence. Such results need to be interpreted carefully, however, since doubled haploid populations are only representative of the progeny from one particular cross.

The formation of aerenchyma in rice roots has little or no requirement for ethylene, and rice roots normally form aerenchyma in well-aerated conditions (Jackson et al., 1985). There is, however, variation in promotion of aerenchyma formation in adventitious rice roots by ethylene across cultivars (Justin and Armstrong, 1991). For other plants, the requirement of ethylene for aerenchyma formation in maize during hypoxia

has been proved conclusively (He et al., 1996; Vartapetian and Jackson, 1997; Drew et al., 1979).

3.1.2. Shoot elongation

The mechanisms of plant adaptation to excessive flooding depend on the water regime. In deepwater areas with >100 cm water depth for 2–3 months, cultivars with sufficient capacity for internode elongation maintain their foliage above the water surface to sustain leaf photosynthesis and oxygen transport, leading to better survival. In contrast to deepwater rice, rainfed lowland rice is subjected to flash floods (>50 cm water depth), ranging from 1 to 2 weeks, where elongation growth is not sufficient for leaves to regain contact with the air. Furthermore, when water levels recede the elongated growth would lead to serious lodging.

Survival of rainfed lowland rice cultivars is negatively correlated with underwater elongation growth by leaves during complete submergence (Jackson et al., 1985) and varies with seedling age (Fig. 1A). Elongation was a maximum in 14-day-old seedlings followed by 21 and 30 days, respectively. Survival was least in young seedlings and improved with seedling age (Fig. 1A). Setter and Laureles (1996), using five rice cultivars, also showed a negative correlation ($r = -0.81$) between survival and shoot elongation growth. This negative relationship between submergence tolerance and elongation during submergence was confirmed using IRRI gene bank database on 903 cultivars (Setter and Laureles, 1996). Of a total population, 23 cultivars had a submergence tolerance score of 1 (100% survival relative to FR13A; IRRI, 1988) and elongated only slightly underwater, whereas 69 cultivars with vigorous elongation had a poor submergence tolerance score (9). There were cultivars (49% of the total population) which had low (7–9) elongation score but poor submergence tolerance. However, none of the cultivars or crossbreds had vigorous elongation combined with high submergence tolerance (score 1–3; Setter and Laureles, 1996).

Rapid leaf elongation during submergence competes with maintenance respiration for carbon sources leaving less assimilates available to support maintenance required for survival during submergence (Setter and Laureles, 1996; Greenway and Setter, 1996; Singh et al., 2001). Plants actively growing during submergence are more susceptible than dormant or

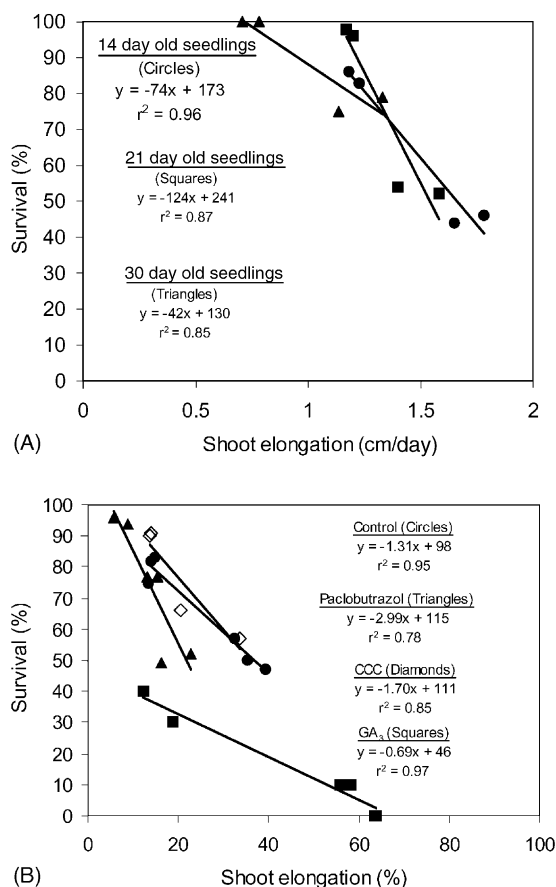


Fig. 1. Relationships between shoot elongation and survival (A) at various seedling ages 14–30 days; (B) in seedlings exposed to gibberellic acid or gibberellin biosynthesis inhibitors. Plants were grown at Kumarganj, India, and submerged 7 days; each symbol is a different cultivar consisting of 10 replicates. In (B), seedlings were 14 days at submergence.

slowly growing plants (Vartapetian and Jackson, 1997).

Attempts to manipulate leaf elongation during submergence by the application of GA₃, paclobutrazol (PB, an inhibitor of kaurene oxidation in the gibberellin biosynthesis pathway, Rademacher, 1992) and cycocel (CCC) have further confirmed the hypothesis that minimum underwater elongation is associated with increased survival (Fig. 1B). Foliar spray of gibberellin 24 h prior to submergence increased underwater elongation during submergence by 6% in FR13A and 24% in IR42 in comparison to control and decreased survival by 53 and 47% in FR13A and

IR42, respectively (data not presented). Treatments with PB and CCC, reduced elongation during submergence and improved survival; they also showed an improved relationship between elongation growth and survival ($r^2 = 0.78$ for PB and 0.85 for CCC) relative to control ($r^2 = 0.95$). GA₃ worsened this correlation. Setter and Laureles (1996) showed similar effects for GA₃ and PB, and showed that the inhibitory effects of PB were reversible with addition of GA₃. Finally, Tan-Ginbozu, a gibberellin deficient rice mutant has submergence tolerance similar to FR13A when seedlings of equal dry weight and carbohydrate content are submerged for 10 days (Setter and Laureles, 1996). These results are consistent with the hypothesis that elongation growth competes for carbon used for maintenance processes for survival, and therefore that reduced elongation growth increases survival during complete submergence of rice.

The possible mechanism for submergence-induced shoot elongation was mediated by an interaction of ethylene and GA (Musgrave et al., 1972). The requirement of GA for internodal growth of deepwater rice was also demonstrated by Suge (1985). In deepwater rice, internode elongation during submergence is controlled by the ratio of gibberellin and ABA (Hoffman-Benning and Kende, 1992) and enhancement of gibberellin activity by ethylene (Raskin and Kende, 1984; Suge, 1985). Submergence-induced ethylene accumulation increases the responsiveness of the internode to GA (Jackson, 1982; Raskin and Kende, 1984) through decrease in endogenous ABA levels (Hoffman-Benning and Kende, 1992; Azuma et al., 1995). Thus, rapid internodal elongation of deepwater rice may result from an ethylene-mediated increase in the ratio of an endogenous growth promoter (GA) and a growth inhibitor (ABA). Leaf elongation in rainfed lowland rice may be regulated similarly.

A study of the genetic basis for submergence-promoted internodal elongation in deepwater rice revealed that this trait is controlled by a number of minor and perhaps as few as two major genes (Xu and Mackill, 1996). GA regulates, directly or indirectly, the expression of growth-related genes. Three genes whose function in growth is unknown but that appear to be of particular interest encode a leucine-rich repeat receptor-like protein kinase (Os-TMK), a putative type 1a plasma membrane receptor (Os-DD₃) and a putative transcription factor or activator (Os-DD₄; Van

der Knaap, 1998). The expression of all three genes is enhanced by GA and occurs in growing regions of the plants. The model of deepwater rice may be helpful in explaining elongation growth in leaves of rainfed lowland rice also but with differential regulation of gene expression in tolerant and intolerant cultivars.

It has been shown that response of rice is similar to that of the temperate dicot *Rumex palustris* and related species. Submergence here induces ethylene-(Voese-nek et al., 1993) and gibberellin-mediated (Blom et al., 1994; Voese-nek et al., 1996) elongation growth for survival during submergence. In other species, e.g. *Regnellidium diphyllum* and *Ranunculus sceleratus*, auxin rather than gibberellin appear to be needed for the ethylene-mediated submergence response (Cookson and Osborne, 1978; Rijnders et al., 1996).

3.2. Metabolic responses/adaptations

The majority of plants may encounter oxygen shortage at least once during their life and thus must cope with its adverse effects. For example, the oxygen supply to imbibed seeds is frequently restricted (Martin et al., 1991), and a metabolic adaptation mechanism with respect to energy production is easily induced through activation of fermentation process. Although ethanolic and lactate fermentation do occur, in most cases they will not lead to long-term flooding tolerance (Laan and Blom, 1990) although the most anoxia-tolerant species so far reported (*Potamogeton pectinatus*) has an unusually fast rate of anaerobic fermentation (Summers et al., 2000). A number of reviews have assessed the metabolic responses to oxygen deficiency in plants (Drew, 1983, 1997; Jackson and Drew, 1984; Vartapetian and Jackson, 1997; Perata et al., 1998).

3.2.1. Sustained sugar supply and energy metabolism

Submergence tolerance is related to high carbohydrate supply during submergence. The role carbohydrate plays in submergence tolerance is presumably through energy supply needed for maintenance processes. Hence, factors affecting carbohydrate supply and depletion during submergence may also influence submergence tolerance. This was tested by experiments using rainfed lowland and a DH mapping population on: (a) cultivar comparisons at different seedling ages, (b) light and dark treatments prior to

and during submergence, (c) time of the day of submergence, (d) grading seeds for weight and specific gravity and (e) glucose feeding.

Old seedlings tend to have large carbohydrate reserves and therefore good survival during submergence (Adkins et al., 1990; Chaturvedi et al., 1995, 1996). Culms of submergence-tolerant plants contained starch even after being submerged for 7 days, whereas the reserves of submergence intolerant rice cultivars were exhausted during the same period (Malik et al., 1995; Singh et al., 2001).

Underwater elongation during submergence is usually greatest in plants grown under low light. The greater elongation presumably reduces carbohydrate reserves, resulting in poor survival. Tolerant cultivars, FR13A and Vaidehi, with high initial soluble sugars at three different levels of irradiance survived better despite significant reduction in carbohydrate after 7 days submergence (Table 3). Intolerant cultivars, IR42 and Mahsuri, with lower concentrations of initial carbohydrate and greater depletion rate on submergence showed poor survival. It seems that intolerant cultivars were unable to maintain a minimum threshold carbohydrate level to sustain energy production for maintenance processes.

Further evidence for the importance of carbohydrate on plant survival during submergence came from experiments in the phytotron at International Rice Research Institute, Los Baños, Philippines. The effect of seedling age and the time of day of submergence (0600 and 1800 h) were studied on shoot carbohydrate status and plant survival of the submergence intolerant cultivar IR42. Seedlings that were 8 and 10 days still had carbohydrate supply from the endosperm, and showed 100 and 90% survival, respectively, after 12 h submergence in the dark starting at 0600 h. However, seedlings that were 12–20 days had little or no carbohydrate supply from the seed, and they did not survive when submergence commenced at 0600 h (Fig. 2). In contrast, complete survival was observed in seedlings of 12–20 days when the submergence treatment was initiated at 1800 h (Fig. 2). Soluble sugar and starch analysis of shoots indicated higher concentrations at 1800 h (after approx. 12 h light) than at 0600 h (after approx. 12 h darkness). Similar results were also observed with a doubled haploid population and the parents, FR13A and CT6241, i.e. there was poor survival in the morning and better survival during submergence in the evening when plant carbohydrate concentrations would be high (Boamfa et al., 2002, in press).

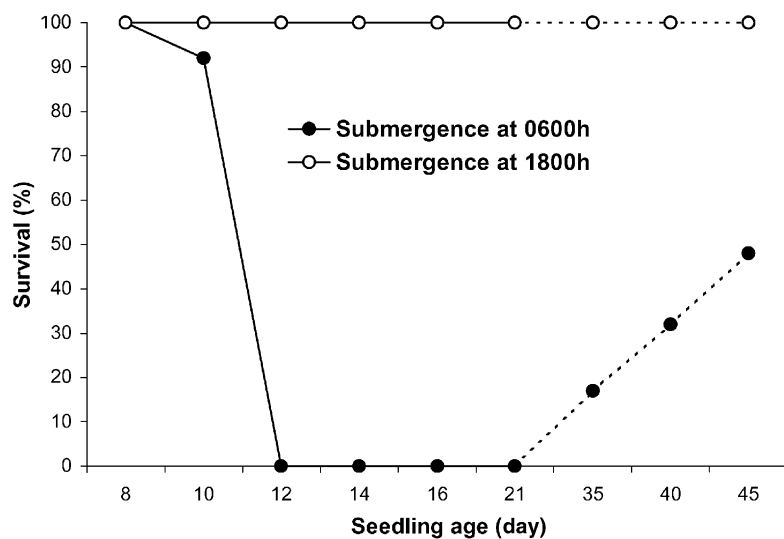


Fig. 2. Effect of seedling age and time of submergence for 12 h in the dark on survival of rice (cv. IR42). Data are combined from two experiments. IR42 plants were grown in a phytotron at 30 °C, and at 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR with 12 h day/night cycles (solid lines), or in the field under natural conditions at Kumarganj, India (dashed lines). Each point is the mean of five replicates and standard errors of the means are less than 5% of individual values.

The relationship of carbohydrate contents to submergence tolerance of plants at different ages was confirmed by experiments in which carbohydrates were manipulated by dark treatments. Complete darkness during submergence reduced survival more than under natural irradiance (Fig. 2), revealing the importance of underwater photosynthesis as one of the components affecting survival of rainfed lowland rice. Alternatively, when plants are submerged in the dark, the O_2 concentration is lower than for plants in the light, in turn affecting growth and survival. Mazaredo and Vergara (1982) emphasized that differences in photosynthesis underwater were not a major factor in distinguishing 12 submergence-tolerant and intolerant cultivars. In the same study, however, they observed a twofold greater photosynthesis under water in FR13A relative to IR42 at low irradiance (approx. $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR).

The genetic diversity in carbohydrate concentrations of plants prior to submergence and its implication on plant survival has been emphasized (Mallik et al., 1995; and see Fig. 2A and B of Setter et al., 1989). This physiological marker for submergence tolerance could be effectively exploited by breeders for screening large number of germplasm without submergence testing in the field. However, environmental factors such as light, temperature, turbidity and nutrients also affect carbohydrate concentrations prior to submergence, and these factors may alter the tolerance ranking of the cultivars and should be given consideration. A strong relationship of initial shoot soluble carbohydrate ($r^2 = 0.78$) and starch content ($r^2 = 0.79$) with survival was observed during 7 days complete submergence for rainfed lowland rice tested in eastern India (Fig. 3A and B).

In a recent study, Ella and Setter (1999) reported the use of specific gravity of seed as selection criterion for high carbohydrate, which putatively helps seedling establishment under anoxia in the field. Glucose feeding of half seeds during anoxia increased survival and plant height and demonstrated that reduced survival was largely due to low carbohydrate supply and not to seed wounding. The authors also emphasized the benefits of producing seeds of high grain density in a non-stressed environment for planting in environments subject to flooding. Seed specific gravity and weight may not, however, always be reliable traits for selection for submergence tolerance. In experiments at

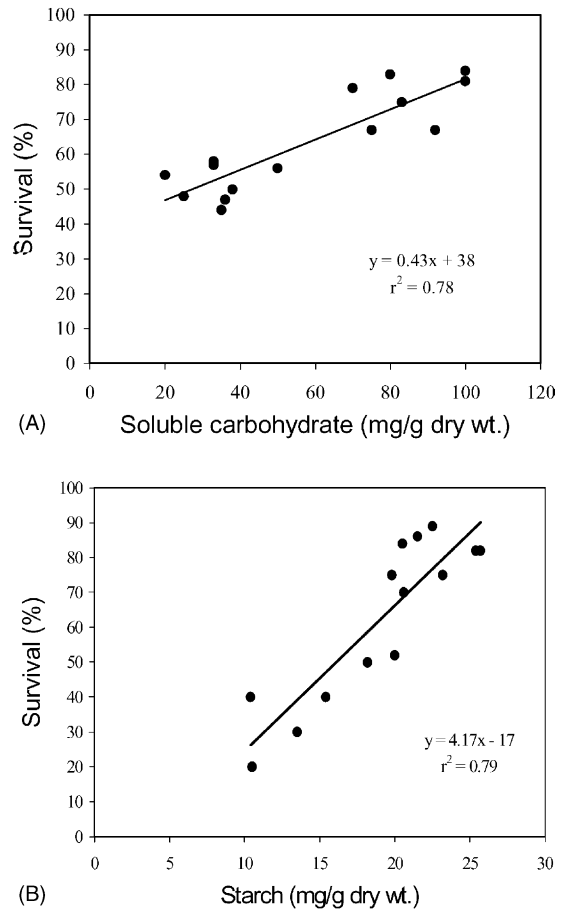


Fig. 3. Relationship between survival (%) of rice during submergence: (A) shoot soluble carbohydrate concentration and (B) shoot starch concentration. Each symbol is a different cultivar or doubled haploid line. Seedlings 14-day-old were submerged for 7 days under natural day/night cycle. Survival was recorded 7 days after post-submergence.

NDUAT, a number of large-seeded rices did not show better submergence tolerance than cultivars with medium seed size. Furthermore, field screening of a DH population derived from a cross between FR13A and CT6241 did not support the proposal that larger seeds are necessarily associated with better submergence tolerance (data not presented).

3.2.2. Alcoholic fermentation

Submergence can shift aerobic respiration to the less efficient anaerobic fermentation pathway as the main source of energy production. The efficiency of

this pathway depends on continued supply of substrate (glucose) and the two key enzymes, alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC). Alcoholic fermentation is the key catalytic pathway for recycling NAD to maintain glycolysis and substrate level phosphorylation in the absence of oxygen (Davies, 1980; Ap Rees et al., 1987). Increased alcoholic fermentation is thus one way to alleviate the adverse effect of anoxia on reduced production of energy for growth and maintenance processes during submergence. The importance of alcoholic fermentation in growth and survival of rice under anoxia has been well emphasized (Taylor, 1942; Avadhani et al., 1978; Alpi and Beevers, 1983; Setter et al., 1994; Gibbs et al., 1999). Support for the importance of the beneficial effects of augmented rates of alcoholic fermentation under anoxia has been demonstrated by a number of experiments:

- (a) Enzymes of alcoholic fermentation often increase (Drew et al., 1994),
- (b) Hypoxic pretreatment increased anoxia tolerance in submerged rice seedlings (Ellis and Setter, 1999),
- (c) High sugar supply improves survival,
- (d) Mutants of maize (Schwartz, 1969; Roberts et al., 1989), barley (Harberd and Edwards, 1982) and *Arabidopsis* (Jacobs et al., 1988) with the ADH enzyme die more quickly under anoxia,
- (e) Alcoholic fermentation is related to waterlogging or anoxia tolerance in several species (Raymond et al., 1985; Rivoal et al., 1989).

Energy shortage due to hypoxia/anoxia may lead to nutrient imbalance and pronounced root injury during submergence. Whether the end products of fermentation (mainly ethanol) are toxic to plants and responsible for cell injuries during anoxia and submergence has been under debate since the hypothesis of metabolic injury was postulated by Crawford (1978). Subsequent work has suggested that low ethanol accumulation is not, however, the reason why rice germinates and coleoptiles grow under anoxia (Avadhani et al., 1978; Alpi and Beevers, 1983), rather it is higher ethanol production of rice under anaerobic condition in comparison to wheat and oat (Alpi and Beevers, 1983).

In addition, ethanol is not highly phytotoxic at concentrations likely to accumulate in flooded plants

(Jackson et al., 1982; Alpi and Beevers, 1983). Ethanol readily diffuses out in the surrounding water during submergence, leaving a small fraction in the plant tissues (Setter et al., 1994; and our unpublished data). Only high, non-physiological ethanol concentrations exogenously supplied can mimic the injurious effects of anoxia. Exposing aerobically grown tissues to ethanol revealed that the toxic limits of ethanol to plants are 434 mol m⁻³ in willow roots (Chirkova, 1978), and 347 mol m⁻³ in sunflower callus (Hildebrand and Riker, 1955). Cereal seed germination is completely inhibited by ethanol concentrations from 174 to 345 mol m⁻³ (Alpi et al., 1985; Malki et al., 1989). Alpi et al. (1985) observed that 600 mol m⁻³ ethanol inhibits rice seedling growth by 50%, whereas in wheat and oat the injurious levels of ethanol are 150–200 and 80–140 mol m⁻³, respectively. In vitro growth of slices of *Helianthus tuberosus*, the induction of α -amylase by gibberellic acid in barley aleurone layers and somatic embryogenesis in carrot tissue are sensitive to ethanol concentration ranging from 5 to 34 mol m⁻³ (Perata et al., 1986, 1988). Recently Perata and Alpi (1991a-c) provided evidence that inhibitory effects of ethanol on in vitro cultured carrot cells and embryos are attributable to the post-anoxic conversion of ethanol to acetaldehyde and the possible interaction of acetaldehyde with cellular proteins (Perata et al., 1992a). However, a role for acetaldehyde in anoxia-induced injuries has been questioned. Ethanol toxicity could be more likely when tissue is kept in the gas phase where outward leakage is minimized and where ethanol may accumulate to injurious levels on re-entry of oxygen, especially when CO₂ concentration is high (Crawford et al., 1987). Outward diffusion may also be influenced by the morphology of the tissues.

Most of the measurements on ethanol and acetaldehyde production under anoxia were made after accumulation for a definite time period using head space analysis, or as ethanol diffused to the external solution in submergence experiments. Production of acetaldehyde and ethanol can be measured online and with great sensitivity using a flow-through system linked to a CO laser-driven photoacoustic spectrometer (Zuckermann et al., 1997). Similarly CO₂ output is readily monitored online with a commercial infrared analyzer (URAS 14-Hartmann & Braun, Frankfurt, Germany). We found submergence-tolerant

(FR13A) and intolerant (CT6241) rice cultivars produced almost similar rates of fermentation on exposure to 4 h of anoxia. On switching to post-anoxia, however, a sharp peak of acetaldehyde outburst was observed which was twice as large from the tolerant compared to the intolerant cultivar. A corresponding dip in ethanol output on introduction of air indicates the source of this acetaldehyde.

3.2.3. Anaerobic proteins (ANPs)

Plant reaction to oxygen deprivation is expressed as the repression of most aerobic protein synthesis and induction of a number of so called ANPs. Submergence and/or anoxia-tolerant and intolerant species may differ in the number and the level of production of these ANPs. Most studies carried out on the expression of ANPs under hypoxia or anoxia emphasize the importance of energy metabolism, since the majority are enzymes of glycolysis and fermentation. These proteins fall mainly in three functional groups: (i) enzymes mobilizing sucrose (sucrose synthase) or starch hydrolysis (α -amylase), (ii) main glycolytic enzymes (glucose phosphate isomerase, fructose-1,6-bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase) and (iii) enzymes of alcoholic fermentation (PDC and ADH).

In maize, a set of 20 anaerobic polypeptides is selectively expressed in primary roots (Sachs et al., 1980; Bailey-Serres et al., 1988). Similar patterns of gene expression under anoxia have also been shown in barley (Hoffman et al., 1986), rice embryos (Ricard and Pradet, 1989; Ricard et al., 1991), and seeds (Perata et al., 1992b), in tomato (Tanksley and Jones, 1981), cottonwood (Kimmerer, 1987), in peas (Llewellyn et al., 1987) and in soybean (Tihanyi et al., 1989). The level of alanine amino-transferase, an enzyme which generates alanine from pyruvate as an alternative pathway of pyruvate metabolism, is also enhanced by anoxia in barley roots (Good and Crosby, 1989). The rhizomes of the anoxia-tolerant wetlands species *Iris pseudacorus* enhanced superoxide dismutase (SOD) activity in the absence of oxygen known to protect plants against injury caused by superoxide radicals on return to air from anoxia (Monk et al., 1987b). Lactate dehydrogenase is another enzyme expressed at higher levels under anoxia indicating the importance of lactate production in submergence and anoxia tolerance in plants. The

importance of enzymes of alcoholic fermentation in anoxia tolerance was demonstrated by the failure of maize root tips from null mutants lacking *adh1* gene to grow under hypoxia or to survive more than 24 h in anoxia (Schwartz, 1969). Recent studies, however, indicate that only a small amount of ADH is sufficient for acclimation (Johnson et al., 1994). Thus, genes other than ADH may be rate limiting in anoxia tolerance.

Quimio et al. (2000) showed enhancement of submergence tolerance in transgenic rice by overproducing PDC. This indicates that PDC is the enzyme determining the rate of alcoholic fermentation and anoxia/submergence tolerance in rice. Alternatively, this enzyme may only be limiting in the cultivar used here, and other metabolic steps may be limiting in other rice varieties or other crops.

The importance of ANPs in inducing anoxia tolerance has been demonstrated by experiments with ruthenium red, which inhibits anaerobic gene expression, resulting in lowering of anoxia tolerance (Subbaiah et al., 1994). Vartapetian and Poljakova (1994) also demonstrated the inhibition of ANPs synthesis in rice coleoptiles and a consequent decline in anoxia tolerance when treated with cycloheximide, a protein synthesis inhibitor. It is unknown whether such inhibitor treatments themselves have effects, hence the demonstration of reversibility of these treatments is essential.

Anaerobiosis induces an initial decline in protein synthesis due to destabilization of polysomes (Bailey-Serres and Freeling, 1990). The signal transduction pathway that induces transcription of ANP genes is probably mediated through cytosolic calcium (Subbaiah et al., 1994). However, information on this subject is still lacking and it is not yet clear how transcript accumulation patterns differ among anoxia inducible genes over time. Besides the enzymes of energy metabolism, genes encoding for enzyme xyloglucan *endo* transglycosylase (XET), a cell wall loosening enzymes are known to be induced in maize roots (Peschke and Sachs, 1994) as is the ethylene biosynthesis gene 1-aminocyclopropane-1-carboxylic acid synthase (Zarebinsky and Theologis, 1993; Mekhedov and Kende, 1996) and a pyrophosphate-dependent phosphofructokinase (Mertens et al., 1993). Enzymes such as adenylate kinase (Kawai et al., 1998), cytosolic pyruvate orthophosphate dikinase (PPDK)

(Moons et al., 1998), sucrose synthase (Ricard et al., 1991) and α -amylase (Perata et al., 1993) are also known to be stimulated in submerged rice seedlings in response to O₂ depletion.

3.2.4. Hormonal regulation

Submergence-induced shoot elongation and reorientation of leaves are initiated by high concentrations of ethylene (Ku et al., 1970; Voeselek and Blom, 1989). In completely submerged plants, gas exchange between intercellular spaces and the aerial atmosphere is severely impeded. Physical entrapment of high concentrations of ethylene in plant tissues which then acts in concert with gibberellin, promotes elongation (Musgrave et al., 1972; Voeselek et al., 1993). Inhibited gas exchange may influence O₂ and CO₂ concentrations also, which are likely to influence the regulation of ethylene biosynthesis (Yang and Hoffman, 1984). In addition to promoting elongation, high ethylene induces leaf chlorosis during submergence, but not at high CO₂ concentration (Jackson et al., 1987).

Although, the role of ethylene and gibberellin in regulating underwater stem elongation and survival of deepwater rice is well known, their role in regulating underwater leaf elongation of young plants is less certain. Recent studies on ethylene responses (in the gas phase) with rice DH lines showed that differences in ethylene sensitivity between lines might not be the underlying explanation of slow or fast underwater elongation or differences in submergence tolerance. There may be an interplay between gibberellins, ABA and ethylene (Jackson et al., unpublished).

To test if the ability to elongate slowly or quickly underwater is linked to a similar responsiveness to ethylene, DH rice lines were screened for their reaction to ethylene. The ethylene-induced elongation in DHLs was compared with the effects on elongation underwater and against submergence damage. The results showed that distribution of ethylene promoted leaf elongation does not mimic closely the effects of submergence. The studies between increase in plant height and damage score sometimes showed poorer relationship ($r^2 = 0.20$). Furthermore, growth promotion by ethylene in submergence-tolerant lines was greater than that induced by submergence. Elevating the carbon dioxide concentrations and lowering the oxygen availability (to mimic night time conditions

underwater) did not bring ethylene effects more closely into line with those of submergence. An interplay and hitherto unexplored feature of submergence-induced elongation is that underwater elongation of tolerant lines can be inhibited compared with air-grown controls. This was particularly clear, when plants remained longer underwater. One-third of the DH lines were inhibited after 3 days submergence and a half after 6 days submergence (data not presented), with maximum inhibition being 72 mm after 3 days and 145 mm after 6 days submergence. In contrast, ethylene inhibited growth of only one rice line with the inhibition of only 6 and 12 mm after 3 and 6 days treatments, respectively. The lack of inhibition in ethylene-treated plants further emphasizes the difference between submergence and ethylene responsiveness amongst the DHLs.

The involvement of two other hormones, abscisic acid (ABA) and gibberellic acid (GA₃) has also been investigated. Pretreatment with exogenous ABA-reduced underwater elongation and improved survival of intolerant plants but there was little effect on elongation or survival of tolerant lines. This suggests that endogenous levels of ABA may be higher in submergence-tolerant lines. The endogenous ABA level analyzed in 2 cm long shoot section of rice was higher in tolerant parent FR13A (8 ng g⁻¹ FW), whereas levels in CT6241, the intolerant parent and 337 the most intolerant DH line were approximately half this concentration (Figs. 4 and 5). Contrary to expectations, the concentration of ABA in four DH lines of contrasting tolerance fell rapidly to similar values of 2 ng g⁻¹ FW. After just 2 days submergence, and remained low during the subsequent 24 h in the two lines where a third ABA measurement was made (Fig. 5). Indeed the largest drop in endogenous ABA was found in FR13A, the most tolerant line. These results imply differences in underwater elongation cannot be attributed to endogenous ABA since during submergence, internal levels are similar between tolerant and intolerant lines. Although, the submergence-induced fall in endogenous ABA cannot be the discriminatory factor, ABA may still be important in submergence responses. The low endogenous levels open a possibility of another substance to differentially interfere with the growth of submerged plants. The involvement of gibberellins in submergence responses is advocated.

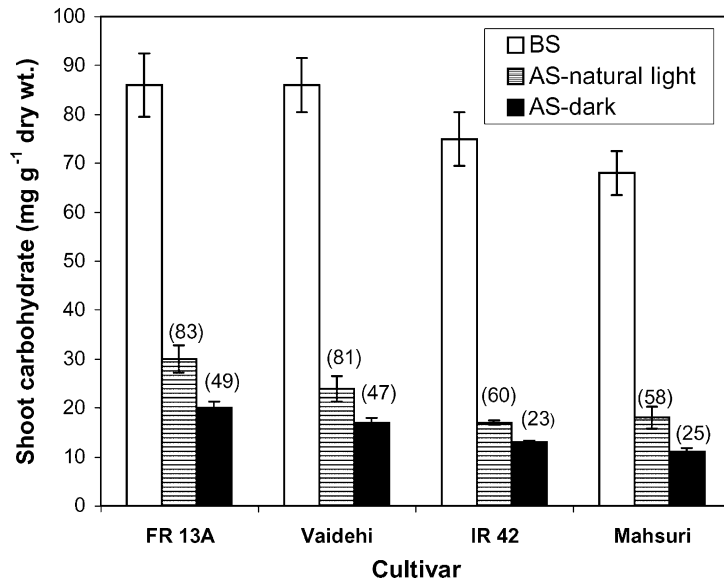


Fig. 4. Effect of darkness during submergence on shoot carbohydrate concentrations (\pm S.E.M.) and survival of rice cultivars. Carbohydrates were measured: immediately before submergence (BS), after submergence (AS-natural light), or after submergence with submergence in continuous darkness (AS-dark). Seedlings 14-day-old were submerged for 7 days. Numbers at the top of the bars are survival (%). Data are means of four replicates with standard errors.

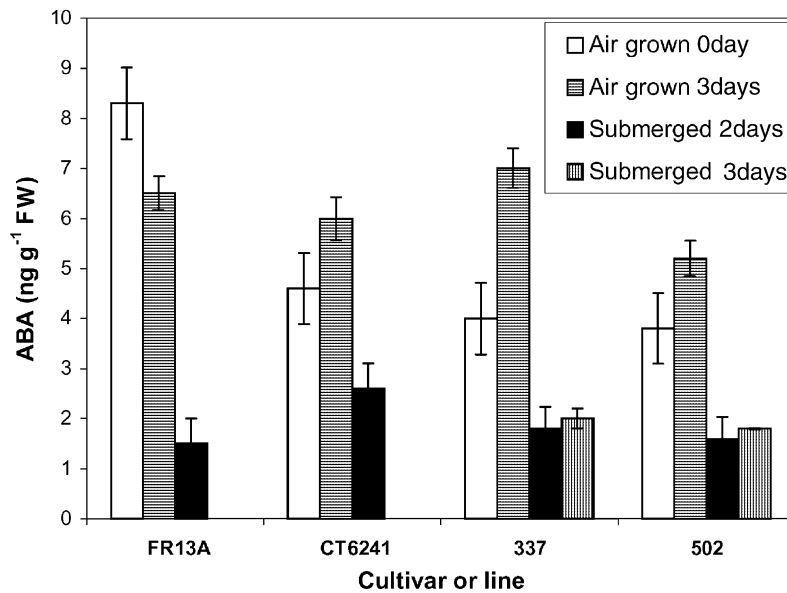


Fig. 5. Concentration of abscisic acid (ABA; ng g^{-1} FW) in 2 cm basal-shoot sections of 14-day-old rice plants. Samples were collected from plants grown in air or submerged for 2 or 3 days. FR13A and 502 are submergence-tolerant cultivars/lines; 337 and CT6241 are intolerant cultivars/lines. Data from Jackson and Summers (unpublished).

The levels of endogenous gibberellins in submerged and air-grown shoots were also analyzed. Gibberellic acid stimulates underwater elongation of normally slowly growing lines to rates similar to intolerant lines and survival is worsened, whereas there is little effect on lines already elongating rapidly underwater (Jackson and Summers, unpublished data). On the contrary, ABA has the opposite effects. These two hormones have a clear, demonstrable and opposing effect on elongation and survival when applied exogenously. It would appear that as the GA response of susceptible lines and the ABA response of tolerant lines is almost fully saturated. Further research on determining the levels of endogenous GA and ABA could provide supportive evidence (as with deepwater rice), whether these two hormones are directly responsible for submergence-induced elongation downstream of ethylene perception.

3.2.5. Post-submergence/post-anoxic events

Plants experience sudden increases in O₂ concentration on the re-entry of air after submergence. Visual symptoms of injury normally are not apparent immediately after short submergence (2–4 days), but these symptoms develop gradually during the post-submergence phase (Crawford, 1992; Gutteridge and Halliwell, 1990; and our unpublished data).

The phenomenon of post-anoxic or post-hypoxic injury is probably brought about by generation of reactive oxygen radicals (Hunter et al., 1983; Monk et al., 1989), and toxic oxidative products such as acetaldehyde (Crawford, 1992). Conditions favoring the formation of reactive oxygen species, such as low energy charge, high level of reducing equivalents and a saturated electron transport chain, usually prevail in plant tissues when oxygen supply is restricted (Van Toai and Bolles, 1991). All aerobic organisms have evolved antioxidative systems of enzymes and free-radical scavengers such as glutathione and ascorbate, which alleviate the toxic effects of free radicals, but the level of protection can vary between species.

Plants exposed to aerobic conditions after hypoxia or anoxia are damaged if generation exceeds elimination of active oxygen species. Monk et al. (1987b) and Van Toai and Bolles (1991) demonstrated that high SOD activity might contribute to flooding tolerance by promoting detoxification of superoxide upon re-admission of oxygen. The antioxidative system is

undeveloped in rice seedlings germinated underwater, but is synthesized (within 24 h) on exposure to air. The activities and protein levels of the six antioxidant defense enzymes (SOD), ascorbate peroxidase (APX), catalase, monodehydroascorbate peroxidase, dehydroascorbate peroxidase and glutathione reductase) were lower in rice seedlings germinated underwater than in aerobically grown controls and the activities increased upon transfer of submerged seedlings to air (Ushimaru et al., 1992, 1997) accompanied by the development of the electron transport system in mitochondria (Shibasaka and Tsuji, 1988a,b). It is still unclear if the regulation of the antioxidative system in rice seedlings is reversible. Activities of associated peroxidase and glutathione reductase are shown to decrease in roots of wheat seedlings during anoxia, but activity approached the untreated controls on exposure to air (Biemelt et al., 1998). On the other hand, drastic increase in activities of catalase and SOD are reported in rhizomes of *Glyceria maxima* and *I. pseudocorus*, respectively, during anoxia (Monk et al., 1987a,b). Ushimaru et al. (1999) studied the reversibility of an antioxidative defence system in aerobically grown rice seedlings after submergence in water for 24 h and observed some decreases in SOD, monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR). The activities of APX and glutathione reductase (GR) showed no marked change after submergence, whereas catalase activity was increased by the shift to hypoxia.

Our own data with aerobically grown lowland rice cultivars submerged for 4 and 7 days, indicate that SOD, catalase and peroxidase activities increased markedly just after post-submergence by 4–6-fold in tolerant and by 2–3-fold in intolerant cultivars. The activity of SOD increased for 24 h and then declined, but it was still higher than for non-submerged plants (Singh et al., unpublished data). Tolerant cultivars, FR13A and TCA-48, had twice the SOD activity of intolerant cultivars, IR42 and Mahsuri, at 24 h post-submergence.

Lipid peroxidation is an important event that occurs when plants are transferred from anaerobic (submerged) to aerobic environment and depends upon the sensitivity of plants to anoxia. Rhizomes of an anoxia intolerant *Iris germanica* are subject to considerable peroxidation (157-fold higher) of lipids after return to air from anaerobic conditions, while no such

damage is observed with rhizomes of an anoxia-tolerant *Iris pseudocorus* (Hunter et al., 1983).

In the case of rice seedlings, little information is available on lipid peroxidation with variation in oxygen environment. In experiments at IRRI, lipid peroxidation was 1.2–1.4-fold higher in submergence intolerant IR42 than in submergence-tolerant FR13A during post-submergence (Kawano et al., unpublished data, cited from Ito et al., 1999). The activities of enzymes APX (except for first 24 h post-submergence), SOD and glutathione reductase and the level of oxidized ascorbic acid (AsA) measured during post-submergence were higher in FR13A than in IR42 (Kawano et al., unpublished data).

We measured ethane (C₂H₆), a product of lipid peroxidation of the cell membrane, in submergence-tolerant and intolerant rice cultivars, in our search for a useful indicator of post-anoxic plant injury during submergence and on exposure to air during post-submergence. A CO overtone laser was used to measure ethane emission after 24–72 h dark anaerobic submergence periods imposed by bubbling nitrogen through the water, and also during a post-submergence aerobic phase. The level of ethane showed a positive relationship with tolerance level. CT6241, an intolerant line, produced twice as much ethane as FR13A (tolerant) in a clear post-anoxic peak. A similar pattern was also observed with IR331 (tolerant) and IR337 (intolerant) DH lines (data not presented), suggesting more membrane breakdown in the intolerant lines. Emission of ethane also closely relates with the extent of visual injury observed in rice plants after various duration of submergence. Plants submerged in the morning experienced greater injury and also produced more ethane than those submerged in the evening (Table 4) irrespective of the level of tolerance of the rice cultivar.

Lipid peroxidation and associated harmful effects of anoxia and submergence can be reduced by substances like α -tocopherol and carotenoids. The level of α -tocopherol was three times higher in submerged rice seedlings than that of aerobically grown controls and remained higher for 24 h after transfer of seedlings to air (Ushimaru et al., 1994). The products of lipid peroxidation were at one-third of the levels found in aerobic controls and their levels increased after transfer to air. The importance of the redox system, especially the ascorbate–glutathione cycle, has been

Table 4

Production of ethane by rice plants (nl (g FW)⁻¹ h⁻¹) during submergence and post-submergence. Submergence-tolerant (FR13A) and intolerant (CT6241) plants were grown in a controlled environment chamber and were 14 days at the start of treatments. A positive relationship between leaf injury and the amount of ethane produced was observed in both the cultivars (Edi et al., unpublished data)

Cultivar	Time of day and duration of submergence			
	Morning (1000–1100 h)		Evening (1600 h)	
	24 h	48 h	24 h	48 h
FR13A				
Submergence	7.84	9.91	3.87	5.38
Post-submergence	1.09	1.09	0.29	0.37
CT6241				
Submergence	12.78	19.26	8.27	11.35
Post-submergence	1.65	4.14	0.43	2.37

advocated by Biemelt et al. (1998) in roots of young wheat seedlings exposed to hypoxia or anoxia. An exogenous supply of ascorbate toward the end of submergence and just before re-exposure to air during post-submergence improved survival and growth of chickpea seedlings (Crawford and Wollenweber-Ratzer, 1992). Albrecht and Wiedenroth (1994) reported an increased oxygen uptake by wheat roots following re-aeration as an indicator of re-activated metabolism. Accelerated mitochondrial electron transport toward its final acceptor, oxygen, and associated increasing production of oxygen radicals and hydrogen peroxide are possible consequences of post-anoxic injury (Elstner, 1990). Therefore, the anti-oxidative defense system and the levels of antioxidants play pivotal role in conferring post-submergence tolerance in plants.

4. Genetics of traits for submergence tolerance

4.1. Gene mapping and marker-assisted selection (MAS)

Rainfed lowland rice copes with excess water by reduced underwater elongation and high initial carbohydrate status, but molecular markers provide a more convenient and possibly a more reliable method for selecting tolerant progeny. Recent studies have revealed that a single locus on chromosome 9,

designated as *SUB1(T)* is responsible for most of the submergence tolerance derived from the tolerant parent FR13A (Xu and Mackill, 1996). This locus had a remarkably high LOD score of about 35, and accounted for approximately 70% of the phenotypic variation for the trait. The presence of a single submergence tolerance gene in the same locus has also been reported in crosses between three of the most submergence-tolerant cultivars (FR13A, Kurkaruppan and Thavalu). Mazaredo and Vergara (1982) did not observe any intolerant genotype in the F₂ generation of the above crosses. However, another submergence-tolerant cultivar Goda Heenati (from Indonesia) did not have the same tolerance gene as demonstrated by a large number of dead plants in the F₂ of the cross of Goda Heenati, with three other tolerant cultivars (Setter et al., 1997). Some earlier published work using survival score from segregating populations of crosses between tolerant and intolerant cultivars has concluded that three submergence tolerance genes exist in FR13A Thavalu, Kurkaruppan and Goda Heenati (Suprianto and Coffman, 1981). FR13A also possess additional minor QTLs, which influence submergence tolerance (Nandi et al., 1997).

The *Sub1* locus maps to a region of chromosome 9 near the centromere that is not very dense in RFLP markers. One RFLP marker R1164 from the Rice Genome Programme of Japan has been measured to be 1 cM from the gene (Xu et al., 2000). Recently, a microsatellite marker, RM219, has been shown to map within 5 cM of the gene (Xu et al., 2000). This marker also appeared in the genetic map of Chen et al. (1997) and seems to be suitable for selection of this gene. Another cultivar, Sudhir, developed from the cross FR13A/Biraj at Chinsurah Rice Research Station, West Bengal, India, has proved to be submergence-tolerant and is presently being used as check (Mallik et al., 1999; Mohanty et al., 2000).

Genes for important traits of rainfed lowland cultivars for submergence tolerance have been tagged with molecular markers, and molecular marker-aided selection is being evaluated (Khush and Sarkarung, 1998). For example, F₂ and F₃ populations segregating for submergence tolerance can be easily classified as tolerant or intolerant by examining for the presence or absence of a linked molecular marker. DH populations developed at IRRI through anther culture from a cross between FR13A × CT6241 and recombinant inbred

lines are presently being used for mapping and QTL analysis for submergence tolerance.

Quantitative trait locus (QTL) mapping determines the number, genome location and effect of QTLs associated with responsive traits to submergence stress. Map-based QTL, revealed that a major QTL for underwater elongation, stay green and plant recovery during post-submergence was located on chromosome 9. The tolerant parent, FR13A, contributes other favorable alleles for suppressed elongation, stay green, and plant recovery. The other parent CT6241 contributes an unfavorable allele for plant elongation. These QTLs accounted for 61–78% of the phenotypic variance (PVC). The major QTLs were detected on chromosome 9 and followed by two minor ones on chromosomes 7, 2, 3 and 5. Plant height before submergence was influenced by different loci; the effects of QTL chromosome 9 on responsive traits were tightly regulated by submergence stress. The coefficient of determination for QTL chromosome 9 on plant elongation, leaf senescence, recovery score and recovery rate ranged from 9.5 to 63% (Apichart et al., personal communication).

4.2. MAS

MAS is relatively more efficient than selection by phenotype alone (Gimelfarb and Lande, 1994; Whitaker et al., 1995). However, application of the technology is limited by its expense (Mackill, 1999). MAS is a more efficient approach if a sizeable proportion of the variability for the traits is controlled by a few QTL. The effective utilization of MAS requires a marker or flanking markers closely linked to the gene or QTL of interest and a high level of polymorphism for the markers between the parents. A PCR-based marker that can be quickly assayed with low quality DNA from small tissue samples is preferable to increase the number of plants that can be accommodated in the program (Mackill et al., 1999).

Microsatellite markers are highly suitable for MAS in rice (Mackill et al., 1999) compared to RFLP and RAPD markers. These markers are highly polymorphic (Yang et al., 1994) and are abundantly available for all the rice chromosomes (McCouch et al., 1997). An important consideration for applying MAS in submergence tolerance breeding is selection of the appropriate tolerant parent. The parent should be a

good combiner and have QTLs for submergence tolerance, even though it may not possess highly desirable agronomic traits. The other parent should have high yield potential, good grain quality and large panicle size. The initial population will consist of a cross to blend the beneficial alleles into a common background. Single or multiple backcross F_2 population could be appropriate to begin MAS. Once the desirable loci are fixed in the homozygous state, selection can be based on phenotype alone. MAS can be quite useful if the donors of the desirable traits for submergence tolerance are agronomically inferior as in the case of FRI3A. Hospital et al. (1992) and Hospital and Charcosset (1997), using simulation studies, have shown that markers can provide a gain of about two generations over backcrossing based on phenotype alone. However, MAS could not be a replacement for conventional breeding where large populations and rigorous selection pressure are commonly used to fix the other desirable agronomic traits in a genotype.

5. Conclusion and forward look

The progress made so far in developing improved cultivars for the rainfed lowland that combine ample submergence tolerance with good agronomic traits is still far from excellent. This is at least partly due to the diversity of environments and the complexity of factors affecting submergence tolerance of rice. With the identification of physiological traits, DNA markers, and genes associated with submergence tolerance, the prospects for breeding suitable rice cultivars for rainfed lowlands have been improved.

Molecular marker technology offers a tool for dissecting the genetics of complex traits and for tracing transfer of genes into elite lines. Rapid accumulation of data from rice genome mapping and introduction of cost-effective marker technology will improve the relevance of MAS for many breeding applications relevant to the rainfed lowland ecosystem. Cloning of major genes or QTL related to submergence tolerance and transfer of suitable alleles into elite cultivars will provide an additional advantage for rice improvement in the rainfed lowlands. The availability of the entire rice genome sequence through a publicly available database also offers improved opportunities for

isolation and incorporation of desired genes into suitable constructs to obtain rice plants with high performance under abiotic stresses.

Plant sensitivity and tolerance to submergence stress have been difficult to dissect mechanistically in the past because of the many unknown genes involved and a lack of models delineating stress responses. The difficulties are about to recede due to the availability of a superior model system for genes from *Arabidopsis* and yeast. Progress is being made in elucidating the physiological and genetic basis of submergence tolerance in plants such as *Arabidopsis*, maize and rice.

Null mutants of *adh* maize and rice genomes with over-expression of *ADH* and *PDC* are helping to unravel the mechanisms of submergence tolerance. In addition, by studying plant responses to severe stresses, more is learnt about metabolism, its plasticity, limits and diversity. Expression of submergence-responsive genes with specificity to cell, tissue and developmental stages and gene cloning will become invaluable resources. Tools are now available to characterize, map and identify genes for their functions and cellular locations using mutant analysis. Future research needs to focus on biochemical and physiological analyses of cloned genes. Improved strategies for the expression of inducible transgenes with cell and tissue specificity are also highly desirable.

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