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Towards Developing Transgenic Rice Plants Tolerant to Flooding Stress

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Rice is a staple food for a large proportion of the world's population. Flooding stress is one of the important abiotic stress factors which adversely affect grain yield of rice crop. In the recent past, genetically transformed rice plants have been produced for varied applications including resistance to insects, viruses, fungal pathogens, herbicides, water stress, salt stress and low temperature stress. This development signifies that the methods for introducing useful genes in this important crop are fairly well-established. Flooding stress leads to an encounter of plants with partial (hypoxic) or complete (anoxic) depletion of oxygen. There are several indications that up-regulation of ethanolic fermentation pathway in rice would result in increased flooding tolerance. A battery of genes encoding pyruvate decarboxylase and alcohol dehydrogenase enzymes has been cloned in rice. The present attempts are focussed on altering the capacity of rice cells for alcoholic fermentation through over-expression of these genes. More basic research work is needed in future course to delineate other genes which may have a positive role in imparting flooding tolerance in rice.

Key Words: Anaerobic conditions, Ethanolic metabolism, Genetic engineering, Flooding tolerance, Rice, Transgenic plants

Introduction

Rice is a major food crop for the entire human race as the diet of nearly 2.7 billion people has rice in it. It is grown and consumed in all continents except in Antarctica. Importantly, 92% of the rice is grown and consumed in Asia; and within Asia, India has the largest area under rice production (Khush & Baenziger 1998). Since rice production oscillates over a wide range particularly in the less favourable environments, incessant rice research is the need of the hour.

The onset of flooding (or submergence/ waterlogging; for the sake of simplicity, the terms 'flooding', 'submergence' and 'waterlogging' have been interchangeably used in the present discussion) leads to the condition of anaerobiosis or oxygen deprivation (partial or complete) as gas diffusion from the atmosphere to water is nearly 10^4 times slower in water as compared to diffusion in air (Armstrong 1979). This effect is accentuated due to (i) the respiratory activities in the roots and the water-borne microorganisms, and (ii) reduced photosynthesis of the submerged portions due to cut-off of light supply. Such a condition is lethal to most land plants. Rice is relatively a flooding-tolerant crop (Perata & Alpi 1993). Alpi and Beevers (1983) compared the ability of rice, wheat and oat seedlings to germinate and grow as the O_2 concentration was lowered to zero. Their study showed that germination in rice was unaffected by O_2 supply, whereas that of oats and wheat was strongly retarded at O_2 levels below 5%. Rice coleoptile was found to grow in low levels of O_2 which was not the case with the other two

cereals. Anaerobiosis-treated rice seedlings are shown to contain cell organelles inherent to a normal aerobic cell including intact mitochondria (Vartapetian et al. 1976).

However, distinctive injury symptoms associated with effects such as reduced growth and yield are noted in rice plants when these are subjected to long intervals of flooding at a stretch. According to surveys recording perceptions of experienced farmers and extension workers, loss in yield of rice due to temporary submergence stress is as much as 110 kg ha^{-1} (Hossain 1996). Grover et al. (1995) have shown that anoxically-grown seedlings of high-yielding IR54 cultivar show lesser shoot extension growth and reduced root branching as against seedlings grown in aerobic conditions. On the whole, improved submergence tolerance is an important trait for rice growing in rainfed lowland regimes when flash-flooding does not give enough time for elongation of the plant through internodal growth and for the deep-water ecosystems when plants cannot keep pace with the rapid increase of water table and are subjected to short-term submergence. Twenty-five percent of world's rice is planted in the rainfed lowland ecosystem (38 million hectares), the produce from which accounts for only 17% of the global rice supply. India has the largest area (i.e. 17.2 million hectares) under rainfed lowland amongst the Southeast Asian countries.

Further, flooding tolerance trait is desirable for evolving rices in which seedlings can be direct-seeded. Direct-seeding is a desirable agronomic trait to save labour-intensive conventional practice of transplanting. It is also important as uneven water depths encountered during transplantation cause some degree of submergence of the germinating seedlings. The sum total of advantages of direct seeding include (i) reduced labour costs, (ii) better seedling vigour and lodging tolerance, and (iii) fewer weeds and pest problems (Yamauchi et al. 1993).

There is a great wealth of literature on physiological/ biochemical changes which take place in rice plants upon submergence. Our objective in this review paper is to present information on utility of transgenic approach for producing flooding-tolerant rices.Therefore, we are including only a limited account of physiological/biochemical details in this paper. The readers can refer several recent reviews/ book chapters for more information in this context (Kennedy et al. 1992, Perata & Alpi 1993, Kundu et al. 1995, Setter et al.1997, Vertapetian & Jackson 1997, Drew 1997). The foremost important requirement for submerged rice plants is to ensure that some levels of O_2 reach the root tissues. The shoot portion above water plays a critical role in transporting O_2 to the submerged portions. This is evidenced by direct measurements of O_2 using O_2 electrode as well as by indirect analysis wherein it has been shown that molecular changes in totally-submerged rice seedlings are different from seedlings which are partially-submerged (Waters et al. 1989, Umeda & Uchimiya 1994). In the partially submerged plants, O_2 is shown to first enter the aerial parts of the leaves via the stomata and subsequently it is diffused to the under-water parts via aerenchyma channels (Barber et al. 1962, Thomson et al. 1990). The aerenchyma is richly-developed in the roots, internodes and leaf sheaths of rice (Barber et al. 1962, Armstrong 1971, Justin & Armstrong 1991). In deepwater rice, leaf is corrugated and is covered with waxes. This feature imparts hydrophobicity to the leaf and aids in the formation of air layers between the leaf surface and the surrounding water. These air layers serve as pipelines for allowing the movement of gases. This gill-like function of air-layers is essential for promoting survival during flash-flooding (Raskin & Kende 1983).

A great deal of research has been carried out on hormonal control of submergence response, particularly on the role of ethylene and GA_3 in enhancing coleoptile extension growth in rice

and molecular cloning of genes which govern ethylene synthesis. Details on this aspect can be referred to in the recent publications of (i) Hans Kende's group from Michigan State University, USA (Cho & Kende 1997 a, b, c), (ii) M. B. Jackson's group from the University of Bristol, UK (Jackson & Pearce 1991, Jackson 1993, Vartapetian & Jackson 1997), and (iii) A. Theologis's group from Plant Gene Expression Center, California, USA (Zarembinski & Theologis 1993, 1997).

Conventional Breeding Approach for Evolving Flooding-Tolerant Rices

At a number of places in India including Central Rice Research Institute (CRRI, Cuttuck, Orissa), Orissa University of Agriculture and Technology (OUAT, Bhubaneshwar, Orissa), Narendra Dev University of Agricultural Technology (NDUAT, Faizabad, U.P.) and Rice Research Station (Chinsurah, W.B.) and elsewhere (International Rice Research Institute, Philippines; Prachinburi Rice Research Centre, Pathum Thani Rice Research Centre and Kasetsart University, Thailand; Institute of Agrobiological Genetics and Physiology, China; Agriculture Western Australia, Australia; Can Tho University, Vietnam), considerable efforts have been made by breeders for producing flood-tolerant rices employing traditional methods of selection of suitable donor genotypes and the introgression of the flooding tolerance trait through sexual hybridization (for details on rice varieties recommended for flooded soils in India, refer Chandra et al. 1990, Nallathambi et al. 1990, Mallik et al. 1991, Sarma & Hazarika 1991, Kundu et al. 1993, Mandal et al. 1993, Mohanty et al. 1994, Singh et al. 1994, Mallik 1995, Mallik et al. 1995a, Mallik et al. 1995b and Mandal & Gupta 1997). At International Rice Research Institute (IRRI), Yamauchi et al. (1993) screened 256 accessions having diverse genetic background and 404 accessions having desirable agronomic characters to establish whether germplasm suitable for direct sowing under flooded soil exists. In this study, several rice types (including a large number from North-East India and Bangladesh such as FR13A, JC148, JC178 and ASD1) were identified for their superior flooding tolerance. FR13A rice from Eastern India needs a special mention here as this rice can withstand complete submergence at the seedling stage for up to two weeks (Mazaredo & Vergara 1982, Mallik et al. 1995b, Setter et al. 1997) and is often employed as a check in national and international programmes aimed at producing flood-tolerant rices.

However, it has been experienced that naturally-existing flooding-tolerant rice types have otherwise poor agronomical traits such as low yield, susceptibility to diseases, poor plant type and grain quality. Obviously, the interest is to find out whether such tolerant rice can lead to production of cultivars with superior submergence tolerance in the background of other desired agronomic traits. Mohanty & Khush (1985) studied the genetics of submergence tolerance in rice employing FR13A, Kurkaruppan and a range of other lowland rice varieties. On the basis of combining ability analysis, this group predicted that hybrids involving FR13A or Kurkaruppan and certain specific nontolerant rices as parents should be promising candidates for incorporating an adequate level of submergence tolerance into lowland rice cultivars. Tests conducted by Saha Ray et al. (1993) indicate that both submergence tolerance and elongation ability characters could be combined in the same genotype, provided strongly submergence tolerance genes like those from FR13A and Kurkaruppan are available in submergence tolerant plants. To sum up, while it should be possible to have all other advantages together with desired submergence tolerance if specific genes for submergence tolerance are transferred into high-yielding, high-grain quality cultivars, the production of such material through conventional breeding methods is yet to be realized.

Transgenic Approach for Improving Flooding Tolerance in Rice

Plant genetic engineering science has taken a firm rooting at the dawn of the 21st century. While the initial attempts in raising transgenic crops were mainly focussed on dicotyledenous plants, particularly tobacco, introduction of genes in cereals (such as wheat, rice, maize and barley) for agronomically-favourable traits has also been largely optimized in recent years (Hiatt 1993, Galun & Breiman 1997). Table 1 provides a list of selected reports in which transfer of agronomically-important genes was successfully carried out in rice. As it appears now, the major limiting factor in the widespread extension of the plant genetic engineering is the availability of the relevant genes (Gibson & Somerville 1993). Genetic engineering for abiotic stresses (such as those caused by high levels of salts in soils, reduced/excess availability of water and sub- and supra-optimal temperature regimes) was once considered to be an arduous task, if not impossible. It is noteworthy in this context that the past five years (1993-1998) have witnessed significant achievements in terms of generating transgenics for enhanced tolerance to stresses such as salt, water, low temperature and high temperature (Grover et al. 1993, Dhaliwal et al. 1998, Grover et al.1998a,b, Khanna-Chopra & Sinha 1998 for reviews on this topic).

Genetic Engineering for Submergence Stress through Pyruvate Decarboxylase (pdc) and Alcohol Dehydrogenase (adh) Genes

As stated above, application of plant genetic engineering for improving tolerance to abiotic stresses warrants research on identification, isolation and cloning of the stress tolerance genes. For the purpose of defining stress-responsive genes in general, one effective approach is to bank on the lead provided by the physiological/ biochemical studies (see Grover et al. 1993 for a general discussion on approaches for identifying stress-responsive genes). In special reference to flooding stress, bulk of the physiological/ biochemical analysis has been focussed on carbohydrate metabolism for the obvious reason that a reduced O_2 supply directly hampers normal respiration resulting in decreased levels of ATP synthesis and concomitantly, accumulation of NADH (Mayne & Kende 1986, Guglielminetti et al. 1995a, Guglielminetti et al. 1995b, Perata et al. 1997, Setter et al. 1997; figure 1). The pathway of respiration switches-over from oxidative to the fermentative mode during anaerobiosis (figure 1). Bertani et al. (1980) showed that when 3-d-old aerobic rice seedlings are subjected to 48 hr of anaerobiosis, a strong alcoholic fermentation system is induced. Detailed studies on this subject have shown that this transition is important in two ways: (i) to generate low levels of ATP under conditions when normal respiratory ATP generation is not feasible, and (ii) to permit oxidation of reduced NADH, for generating NAD which helps in continuation of glycolysis (Perata & Alpi 1993, Grover et al. 1995, Hossain et al. 1995). Two reactions of pyruvate are significant here: (i) pyruvate is reduced to lactate by the reaction catalyzed by lactate dehydrogenase (LDH) and (ii) pyruvate gives rise to ethanol via a two-step conversion involving decarboxylation of pyruvate through a reaction catalyzed by PDC and reduction of acetaldehyde to ethanol by NADH, in a reaction catalyzed by ADH (Good & Muench 1993).

According to Davies (1980), transient lactate fermentation acidifies the cytoplasm at the start of anaerobiosis thereby triggering the functioning of PDC which swings fermentation away from the lactate to neutral ethanol. Roberts and co-workers (Roberts et al. 1982, Roberts et al. 1984, Roberts et al. 1985) have indicated that those plants which switch-over quickly to ethanol formation (thus, preventing injurious lactate-based cytoplasmic acidosis) are relatively more resistant to anaerobic stress. Menegus et al. (1991) compared intracellular *p*H

Table 1 *Selective reports on transgenic rices produced thus far for varied agronomic applications.*

Trait	Gene	Reference
Herbicide resistance	*bar**	Christou et al. (1991), Datta et al. (1992)
	*csr1-1**	Li et al. (1992)
Insect resistance	*bt**	Fujimoto et al. (1993), Wunn et al. (1996) Ghareyazie et al. (1997), Datta et al. (1997) Nayak et al. (1997), Wu et al. (1997)
	*pin II**	Duan et al. (1996), Xue et al. (1996)
	*cc**	Irie et al. (1996)
	*oc**	Hosoyama et al. (1996)
	*cpTi**	Xu et al. (1996a) Xue et al. (1996)
Virus resistance	*cp- stripe**	Hayakawa et al. (1992)
	*rysy N**	Fang et al. (1996)
	different portions of the genome of brome mosaic virus	Huntley and Hall (1996)
Sheath blight resistance	*bar*	Uchimiya et al. (1993)
	*chi**	Lin et al. (1995)
Bacterial blight resistance	*xa* 21*	Song et al. (1996) Wang et al. (1996), Tu et al. (1997)
Rice blast disease	*bar*	Tada et al. (1996)
Water stress and salt stress tolerance	*hva 1**	Xu et al. (1996b)
Chilling tolerance	*gpat**	Yokoi et al. (1998)
Vitamin A synthesis	*psy**	Burkhardt et al. (1997)
Protein content	*β-phy* *	Zheng et al. (1995)

**bar* encodes phosphinothricin acetyl transferase (PAT) which can convert phosphinothricin (PPT) into acetylphosphinothricin (Ac-PPT); *csr1-1* encodes mutated acetolactate synthase; *bt* refers to the gene for insect toxin protein. Bt toxin is encoded by different *cry* genes; *pinII* encodes proteinase inhibitor proteins; *cc* encodes corn cystatin; *oc* encodes *Oryza* cystatin; *cpTi* encodes cowpea trypsin inhibitor proteins which is a class of proteinase inhibitors; *cp-stripe* encodes coat protein gene; *rysy* N encodes a nucleocapsid protein of the rice yellow stunt virus; *chitinase* refers to the gene for fungal chitin degrading enzyme; *xa 21* gene encodes a receptor-kinase like protein and confers resistance to *Xanthomonas oryzae; hva1* encodes osmotic stress induced late embryogenesis-abundant (LEA) protein; *gpat* encodes glycerol-3-phosphate acyltransferase enzyme; *psy* encodes phytoene synthase enzyme which catalyzes a critical step in proVitamin A synthesis; *β-phy* gene refers to the gene for β-phaseolin, a seed storage protein.

of wheat and rice using *in vitro* ^{31}P NMR and found that wheat acidified much more extensively than rice, in accordance with the greater tolerance of rice to anoxia during the germination process.

Several groups have examined the possibility of toxicity caused by excess ethanol to cellular activities. Generally, when plant cells are subjected to anoxia/ hypoxia, ethanol is produced at concentrations of 2 to 50 mM (Perata & Alpi 1993). On the other hand, it has been shown that high, non-physiological concentrations of exogenously-supplied ethanol are required to cause the symptoms of anoxia-related injuries (Jackson et al. 1982). For instance, only when the concentration of ethanol reaches values as high as 600 mM, is the growth of rice seedlings inhibited by 50% (Alpi et al.1985). Further, it is estimated that 98% of the ethanol produced in rice seedlings is dispelled into the surroundings (Bertani et al. 1980).

From the above discussion, it accrues that induction of ethanolic fermentation pathway (and proteins/ genes which govern this process) is an important component of the responses which are elicited in rice (and other plants) against flooding stress. A good correlation between anoxia tolerance and the rate of ethanol formation is further evidenced through analysis of *adh*-null mutants of *Zea mays* and *Arabidopsis thaliana* which are unable to produce ethanol and die more rapidly under anaerobic stress than the wild type plants (Schwartz 1969, Jacobs et al. 1988).

If rice plants naturally shift to ethanolic fermentation when subjected to flooding, will high-level induction of this pathway by plant genetic engineering methods be of help? Fortunately, operation of ethanolic fermentation (from pyruvate to ethanol) is a relatively simpler trait involving only two enzymes (i.e. PDC and ADH). PDC is a crucial protein in this respect since (i) it is the first enzyme channeling carbon towards this pathway, (ii) this enzyme step is considered to rate-limit ethanol formation and (iii) acetaldehyde, the end product of its reaction, is an extremly toxic metabolite. In this relationship, ADH enzyme activity appears to be a significant step to avoid the accumulation of acetaldehyde.

Maize and rice are the most extensively analyzed plant systems for the characterization of ADH and PDC enzymes (largely through the efforts of Hank Greenway, Tim Setter and associates, University of Western Australia, Australia) and their corresponding genes (largely through the efforts of W.J. Peacock, Elizabeth S. Dennis, R. Dolferus and associates, CSIRO Division of Plant Industry, Australia and M.M. Sachs and associates from USDA, Urbana, USA). Anaerobiosis- associated increases in PDC and ADH activities in maize have been shown (Wignarajah & Greenway 1976, Laszlo & Lawrence 1983). Increased activity of maize PDC is through increased transcription (Kelley 1989). Initially, it was documented that maize PDC is governed by one gene (Kelley et al. 1991). Subsequent work has shown that three different genes govern PDC in maize (Peschke & Sachs 1993). In 2-d-old rice seedlings subjected to anoxic stress, PDC activity increased 9 fold during a seven-day period (Rivoal et al. 1997). On Western blot, there was a 5.6 fold increase in PDC protein levels. Rice PDC has been purified and the holozyme is shown to be a tetramer of two types of subunits of 64 and 62 kDa (Rivoal et al. 1990). Recent work has underlined that these two subunits are differentially-regulated in response to anoxia stress in rice (Rivoal et al. 1997). According to Hossain et al. (1996a), a small family of genes govern PDC synthesis in rice. So far, 4 different genes have been isolated for rice PDC (Hossain et al. 1994a, Hossain et al. 1994b, Huq et al. 1995, Hossain et al. 1996a, Hossain et al. 1996b, Rivoal et al. 1997; *see* Table 2 for details on these genes). Northern blotting experiments have shown that rice *pdc1* gene is rapidly up-regulated in response to anoxia stress.

Table 2 *Characteristics of different rice pdc genes*

Gene	Characteristics	Chromosomal location	Reference
pdc1	Both cDNA and genomic clones isolated and characterized, cDNA is 1809 bases, genomic clone has 5 introns, open reading frame has 602 amino acids 95% similar and 90% identical to maize pdc, molecular weight 65 kDa, strong anoxia-inducibility on northerns.	5	Hossain et al. 1996a, Huq et al. 1997
pdc2	Both cDNA and genomic clones isolated and characterized, genomic clone has 5 introns, open reading frame has 603 amino acids, estimated molecular weight is 62 kDa.	3	Huq et al. 1995, Huq et al. 1997
pdc3	Genomic clone has open reading frame of 1755 nucleotides and 585 amino acids, corresponding to estimated molecular weight of 62 kDa, considered to be a psuedogene.	7	Hossain et al. 1994b, Huq et al. 1997
pdc4	Partial cDNA isolated and sequenced, shows 96% and 95% identity to pdc1 in nucleic acid and amino acid sequence, respectively.	Not known	Rivoal et al. 1997

Anaerobiosis- associated increased ADH enzyme activity and corresponding transcript levels have been noted in rice (Ricard et al. 1986, Kadowaki et al. 1988, Xie & Wu 1989). Increased rice ADH enzyme activity is also reflected by a change in isozymic profiles of the ADH protein (Rivoal et al. 1989, Xie & Wu 1989). On the basis of isozyme analysis, Grover & Pental (1992) have indicated that the number of *adh* genes in rice are possibly three. However, two genes (*adh1* and *adh2*) have so far been cloned and characterized in rice (Xie & Wu 1989).

The availability of cloned *pdc* and *adh* genes (from microbial as well as higher plant systems) has but naturally prompted interest of molecular biologists to employ these for transgenic experiments. The group of Cris Kuhlemeier (University of Berne, Switerzerland) has produced transgenic tobacco plants which have the constitutive capacity of ethanolic fermentation by expressing *pdc* gene (derived from the obligate anaerobe *Zymomonas mobilis*) that had been subcloned to cauliflower mosaic 35S (CaMV 35S) promoter. In the tissues of the transgenic tobacco, the PDC protein accumulated to high levels and was active in an *in vitro* assay. The levels of acetaldehyde and ethanol formed in response to anoxia stress were noted to be several-fold higher in the shoots of transgenic plants as compared to the wild types

(Bucher et al. 1994). These tobacco plants have now been more thoroughly analyzed for their response to flooding stress (Tadege et al. 1998). It has been seen that overexpression of the bacterial *pdc* caused only a moderate increase in acetaldehyde and ethanol production in the transgenic roots under anoxia compared to wild type roots under the same conditions. Importantly, it was noted that the increased ethanolic flux in transgenics compared to the wild type did not enhance anoxia tolerance (Tadege et al. 1998). On the contrary, it was observed that the rapid utilization of carbohydrate reserves enhances premature cell death in the transgenics. These results defy the expected notion (as discussed above) that increasing levels of *pdc* should be beneficial against submergence stress. However, it is too early to make conclusions that increased *pdc* (and also *adh*) has little or no role in submergence tolerance because several pertinent questions remain unanswered. For instance, Kuhlemeier's group has employed CaMV 35S promoter for over-expressing *pdc* gene. It should be worthwhile if different promoters (with varying strengths and not only constitutive but also anoxia-induced) are employed to bring differential levels of *pdc* expression and then the response is examined. It should also be important to bring about concomitant expression of *pdc* and *adh* in the host cells. Further, the advantage, if any, of the use of plant *pdc* gene(s) rather than the bacterial *pdc* gene(s) for such experiments is left to be seen. Lastly, it is important to employ rice rather than tobacco as the host system for such work because tobacco and rice may differ in their responses to flooding. Work on genetically altering levels of *pdc* and *adh* in rice is currently in progress at CSIRO, Australia (under the supervision of Drs E S Dennis and W J Peacock) in collaboration with Purdue University, USA (under the supervision of Professor T K Hodges) . This group has subcloned rice *pdc1* cDNA at the 3' end of three different promoters [constitutive promoters such as CaMV 35S, actin1 and anoxia-induced 6X ARE promoter which is a synthetic promoter) in both sense and antisense orientations and introduced it into rice. Further, a large number of transgenic rices have been produced with all of these constructs (*see* Grover et al. 1994, Grover et al. 1995, Hossain et al. 1995, Grover et al. 1996, Hossain et al. 1996a, Hossain et al. 1996b, Grover et al. 1997, Huq et al. 1997, Rahman et al. 1997; also unpublished data of M. Rahman et al. at CSIRO, Canberra). However, this group is yet to analyze the response of the transformed lines to flooding stress. The group at CSIRO (Canberra) has also made considerable progress in altering levels of *adh* gene in transgenic rice (as well as cotton) systems (unpublished data of M. Rahman et al. and M. Ellis et al.).

Genetic Engineering through other Genes of the Respiratory Pathway

Apart from PDC and ADH, several other enzymes play a role in (i) mobilization of carbon from complex carbohydrate forms (i.e. starch, sucrose etc.) to simpler six-carbon forms which readily enter glycolysis (i.e. glucose) and (ii) metabolizing the simpler sugars to pyruvate (see figure 2). Most of these enzymes show an up-regulation (in terms of enzyme activity as well as transcript levels) in response to anoxia stress (Umeda & Uchimiya 1994, Sachs et al. 1996; figure 2). Interestingly, some of these enzyme proteins were initially seen as stress proteins which were expressed under anaerobic stress and were referred to as anaerobic proteins (ANPs) (Sachs et al. 1980). The subsequent detailed analysis has shown that most ANPs represent enzymes of the respiratory pathway (figure 2). In recent years, genes for some of these proteins have been cloned and characterized and their up-regulation has been shown by northern blotting/ western blotting (Sachs et al. 1996). However, till date, no attempts directed at making transgenics over-expressing these genes have been reported.

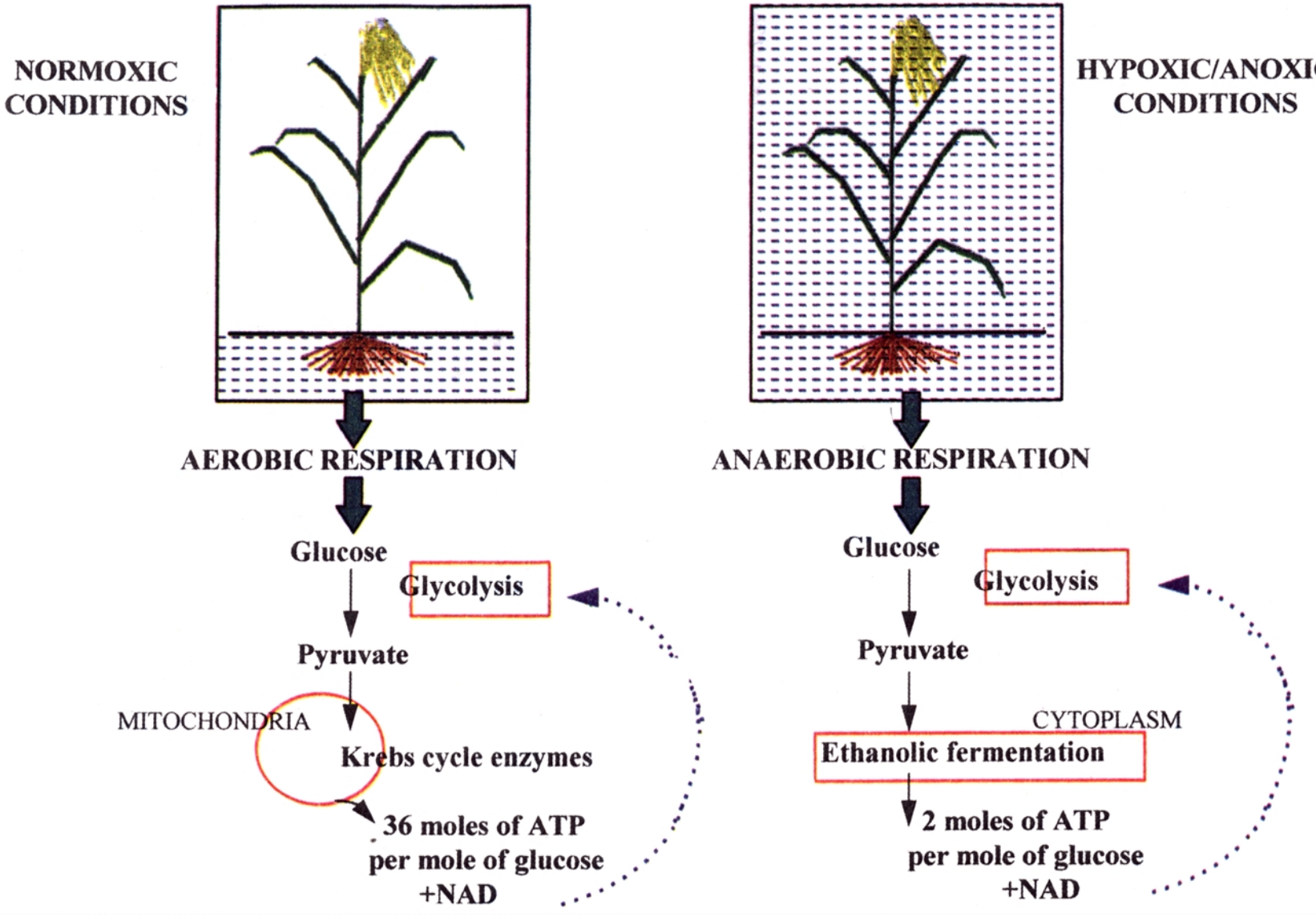

Figure 1 Schematic representation of the comparison between plants subjected to hypoxic/ anoxic (reduced O_2 availability) conditions and plants growing under normoxic (normal O_2 availability) conditions. The plants growing under hypoxic/ anoxic conditions metabolize pyruvate through ethanolic fermentation pathway which enables these plants to generate low levels of ATP as well as leads to regeneration of NAD which is critical for the continuation of glycolysis.

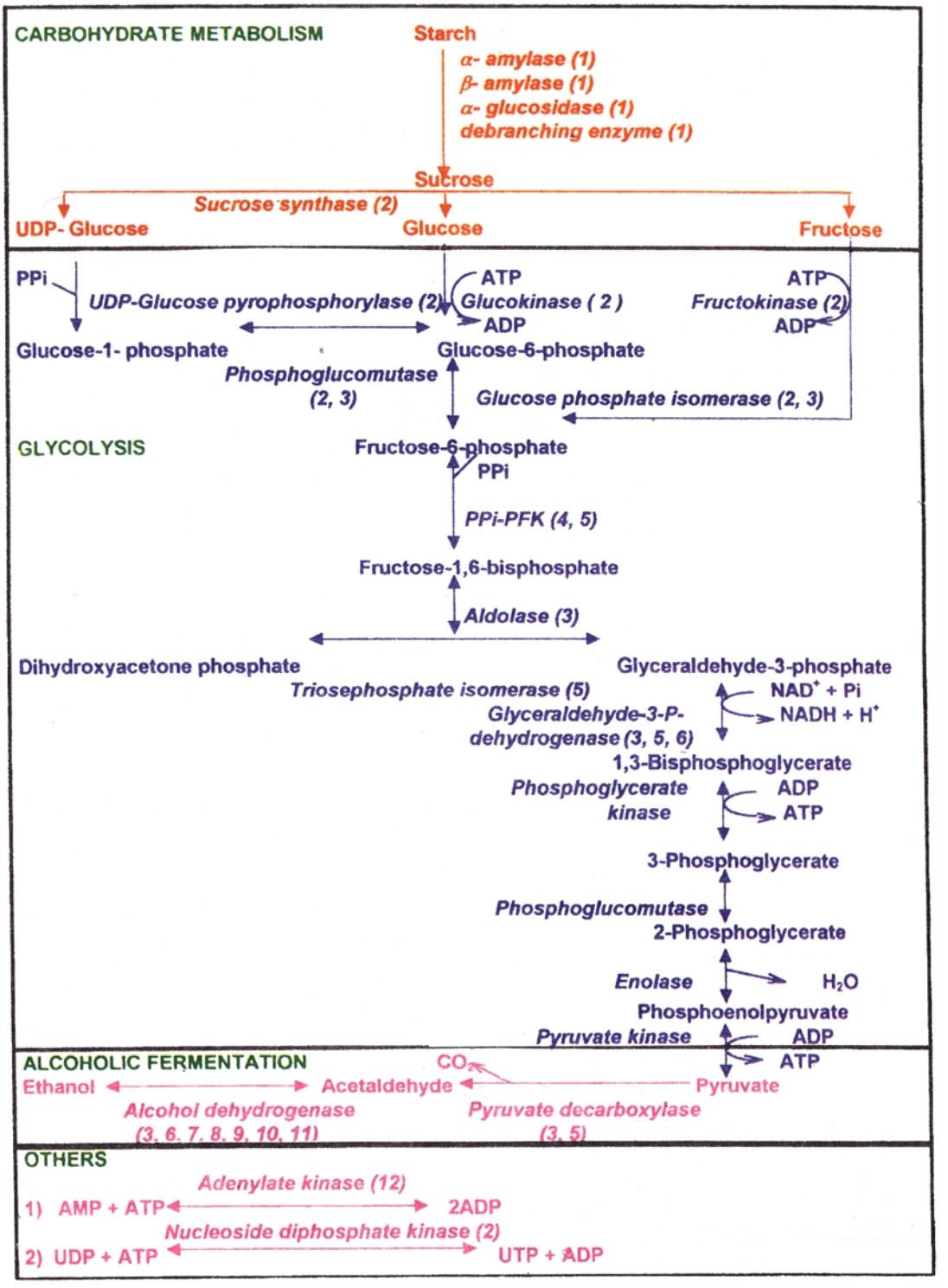

Figure 2 Schematic representation of the major metabolic reactions associated with carbohydrate metabolism, glycolysis and alcoholic fermentation. The activities of most of these enzymes have been shown to be up-regulated in response to submergence stress in rice. Numbers in brackets next to enzymes represent the corresponding reference of the work as detailed below (see 'References' in the text for complete details): 1. Guglielminetti et al. (1995a). 2. Guglielminetti et al. (1995b). 3. Rivoal et al. (1989). 4. Mertens et al (1990). 5. Umeda and Uchimiya (1994). 6. Ricard and Pradet (1989). 7. Wignarajah et al. (1976). 8. John and Greenway (1980). 9. Bertani et al. (1980). 10. Shimomura and Beevers (1983). 11. Xie and Wu (1989). 12. Kawai et al. (1993).

Genetic Engineering through Altering Transcriptional Machinery

A general criticism against genetic engineering for tolerance to abiotic stresses has been that since the response of plants to these stresses is often multigenic, it is not possible to affect the whole cascade of cellular changes when single genes are employed. If so, expression of the entire battery of stress-responsive genes such as genes for different ANPs would have a greater beneficial effect on stress tolerance than the individual genes. Is it possible to achieve this?

Jaglo-Ottosen et al. (1998) have recently produced transgenic *A. thaliana* plants that over-express CBF1. CBF1 (CRT/DRE binding factor1) is a positively-acting transcription factor which binds to CRT [C-repeat/ DRE (drought responsive element)] sequence which is cis-acting sequence that stimulates transcription of the cor genes in response to low temperature. Lee et al. (1995) have followed a similar approach for producing thermotolerant *A. thaliana* plants. These workers were able to de-repress the HSF (heat shock transcription factor) function by experimental means which led to constitutive expression of HSPs (heat shock proteins) at normal temperatures. The above two reports show that by changing the transcription factor genes, it should be possible to alter levels of several target genes at the same time (Grover et al. 1998b).

How is the transcriptional activation of anaerobic genes brought about? Bulk of the research in this subject has been carried out employing *adh* gene, both with respect to cis-elements and the trans-acting factors that play a role in transcription (Olive et al. 1990, DeLislie & Ferl 1990a, Dolferus et al. 1994, Dolferus et al. 1997, Paul & Ferl 1997). The regions of the *adh1* gene promoter that are required for anaerobic induction of transcription have been assessed by testing the function of the *in vitro* mutagenized promoters in transient assays (Walker et al. 1987) or stably transformed lines (Ellis et al. 1987, Kyozuka et al. 1991, Kyozuka et al. 1994). A string of specific nucleotide bases called the anoxia response element (ARE) with a consensus sequence of its core element as TGGTTT, has been shown to be the underlining basis for anoxia-inducibility of several anaerobic genes.

In vivo footprinting data have suggested that there are several different DNA binding proteins that interact with the *adh1* gene promoter of maize. When grouped together according to the sequence of their binding sites and including data for both maize *adh1* and *Arabidopsis adh*, there appear to be two classes of DNA binding proteins: (i) those that have a 5'-GTGG-3' core within their binding site and (ii) those that have 5'-GCCCC-3' sequence in the same. It is thought that the GTGG- binding protein may represent a group of general transcription factors while the proteins that interact with the 5'-GCCCC-3' sequence are uniquely a part of the ARE (McKendree et al. 1990, DeLislie & Ferl 1990b, Paul & Ferl 1991). Ferl (1990) have shown that a protein complex (termed ARF-B2) specifically binds to part of the anaerobic response element of maize *adh1*. de Vetten & Ferl (1995) have isolated a cDNA encoding a maize G-box binding factor (GBF). Paul & Ferl (1997) have recently cloned some of the protein factors involved in regulating the expression of adh genes which may hopefully lead to the understanding of the possible associations that exist among the gene regulatory proteins and diverse cell signalling pathways.

Coming back to the question of making transgenics for flooding stress tolerance, employing the regulatory mechanisms of gene like *adh* is a distinct possibility in transgenic systems. This work should be facilitated by the availibility of cloned sequences for transcription factors governing anoxia-induced genes. It is hoped that this possibility would be tested using transgenic approach in time to come.

Conclusions and Future Outlook

There is as yet no high-yielding rice variety which can grow vigorously in flooded soils. The rice yield from such ecosystems is staggeringly poor. Flooding is a recurrent phenomenon in many pockets of India, Bangladesh, Thailand, Vietnam and Sri Lanka. Flooding stress has caused a severe damage to the crops in U.P. and the North-East part of India as well as in Dhaka, Bangladesh, this year (1998). Any improvement in flooding tolerance of rice would be a boon to inhabitants of these places. The urgency in employing transgenic technology for producing flooding tolerant rices is therefore warranted.

How many genes govern tolerance to flooding stress? Definitely, the answer is more than one but the exact figure is not yet known. Mackill (1996) has succeeded in localizing a segment on chromosome 9 of rice [named *Sub1(t)*] which reportedly accounts for 70% of the phenotypic variation for submergence tolerance. From this result, it appears that there are some major loci which control submergence tolerance. It would be extremely rewarding if the *Sub1(t)* locus is cloned through map-based cloning technique. Recently, this group has made remarkable progress in development of a high-resolution map around the Sub1 locus through the use of RFLP (restriction fragment length polymorphism) and AFLP (amplified fragment length polymorphism) markers and is presently cloning the tightly-linked AFLP markers for eventual screening of gene libraries (Kenong et al. 1997). This work is facilitated due to recent progress made on the genome analysis of rice. Rice has emerged as a model cereal crop, due to its smaller genome size, availability of RFLP markers, ESTs (expressed sequence tags), YAC (yeast artificial chromosome) and BAC (bacterial artificial chromosome) libraries (*see* Sasaki & Moore 1997 for review on this subject). Further, there is a great deal of upsurge in genome technology in the recent years as evidenced by the rapid progress being made in the human genome project, and in similar projects for several other organisms. Efforts have also been initiated for sequencing the complete genome of rice. The availability of gene sequences through this approach would hopefully bring a major change in isolation of flooding stress- responsive genes.

The current work on raising flooding- tolerant transgenic rice is centered on altering the capacity of ethanol metabolism. Towards this end, considerable advances have been made in (i) unveiling genes which govern synthesis of PDC and ADH proteins in rice, (ii) employing the cloned sequences for designing suitable plasmid constructs which have requisite plant promoters and (iii) stable genetic transformation of such constructs into rice cells. The consequences of the introduction of the exogenous pdc gene into rice cells remain to be seen with special reference to homology-based recombination (between the exogenous and the endogenous counterparts) and gene silencing (Kumpatla et al. 1998). Once the PDC and ADH over-expressing lines are obtained, it should be possible to bring in these desired traits in one cultivar by conventional breeding. It is hoped that the long-standing question as to how critical is the fermentation pathway for inducing flooding tolerance would be answered soon owing to the above developments. Apart from ensuring that high level metabolism of pyruvate to ethanol takes place, there is a need to ensure that more sugar is mobilized to the respiratory pathway and more efficient glycolytic pathways are also designed in the due course of time.

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