Anoxia Tolerance of Rice Seedlings

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Rice has the ability to germinate and grow in extremely low oxygen conditions, although most agricultural crop plants fail to germinate and quickly lose their viability in these conditions. The evidence has illustrated that rice seedlings are able to take advantage of the starchy reserves from their endosperm even in anoxia and to accelerate ethanolic fermentation quickly after being transferred to anoxia, which can allow the continuation of active glycolysis leading to ATP production. In addition, rice possesses the great ability to avoid the ethanol-induced injuries, which may contribute its anoxia tolerance.

Keywords: anoxia tolerance, ATP, ethanol toxicity, ethanolic fermentation, glycolysis, *Oryza sativa*.

INTRODUCTION

Soil flooding or total submergence subjects plant roots to considerable periods of anoxic conditions which are a major limitation to plant growth and productivity (Crawford and Braendle, 1996 ; Vartapetian and Jackson, 1997). Many plants respond to this stress through metabolic and morphological changes that enhance their ability to survive (Ricard et al., 1994 ; Vartapetian and Jackson, 1997).

Morphological changes, e.g. formation of lysigenous cortical aerenchyma, stem hypertrophy and adventitious roots, have been well characterized. Those changes are non-reversible, slow (response in days) and induced by ethylene (Crawford and Braendle, 1996 ; Vartapetian and Jackson, 1997). On the other hand, metabolic changes are fast (response in hours) and this response involves the induction of anaerobic polypeptides. Many of these polypeptides have been identified as enzymes of the glycolytic and fermentation pathways (Sachs et al., 1996 ; Drew, 1997).

Rice (*Oryza sativa* L.) has the ability to germinate and grow under extremely low oxygen conditions, even under vacuum (Alpi and Beevers, 1983 ; Cobb and Kennedy, 1987), although most agricultural plants, e.g. maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and sorghum (*Sorghum vulgare* L.) fail to germinate and quickly lose their viability in these conditions (Perata et al., 1997 ; Setter et al., 1997 ; Vartapetian and Jackson, 1997). Rice seedlings survived more than 21 days in N₂ atmosphere and its metabolic activity was high enough to allow protein synthesis and growth (Mocquot et al., 1981 ; Aspart et al., 1983 ; Ricard et al., 1991 ; Mujer et al., 1993). However, the metabolic adaptation underlining the tolerance of rice to anoxia is not clearly illustrated.

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This paper briefly summarizes recent progress in the research on the metabolic adaptation of rice.

CARBOHYDRATE SUPPLY TO GLYCOLYSIS

Plants respond to anoxic stress by switching from oxidative respiration to fermentative glycolysis for production of metabolic energy, which enables their survival in low oxygen tensions (e.g., Kennedy et al., 1992; Ricard et al., 1994; Drew, 1997; Tadege et al., 1999). One determinant in anoxic-induced cell damage in the plants is cytoplasmic acidification due to a net influx of protons into the cytoplasm from the vacuole (Saint-Ges et al., 1991; Fox et al., 1995; Xia and Roberts, 1996). Increased availability of ATP could contribute to reducing this acidification (Roberts et al., 1984; Felle, 1996). Against the free energy gradients, H^+ -translocating ATPases in the tonoplast can drive H^+ ions to the vacuole from the cytoplasm (Johnson et al., 1994; Felle, 1996). Thus, the greater level of ATP is considered to be a significant contributory factor to the subsequent plant survival under anoxic conditions (Johnson et al., 1989; Bouny and Saglio, 1996; Drew, 1997).

The availability of readily glycolytic carbohydrates is essential for the operation of the glycolytic pathway leading to ATP production under conditions of oxygen deficiency where glycolysis replaces the Krebs cycle as the main source of energy, but the amount of soluble sugars in cereal seeds is usually very limited, with starch being the main reserve carbohydrate (Perata et al., 1997; Saglio et al., 1999; Tadege et al., 1999; Gibbs et al., 2000). Among cereals, only rice endosperm was found to possess the complete set of enzymes for starch breakdown to sucrose under conditions of oxygen deficiency (Perata et al. 1992; Guglielminetti et al. 1995a).

Sucrose is known to be the major form of carbohydrate transported from endosperm to the growing plant parts (Saglio et al., 1999). Therefore, the metabolism of sucrose in the cells of the growing plant parts is required for operation of glycolysis, and the sucrose synthase pathway plays a major role in the process of sucrose degradation under conditions of oxygen deficiency (Guglielminetti et al., 1995b; Perata et al., 1997; Saglio et al., 1999). In anoxia, sucrose synthase activity in rice was increased but the activity in several cereals, showing a low anoxia tolerance, was inhibited (Perata et al., 1997; Ricard et al., 1998). These results indicate that compared with the other cereals, only rice may be able to utilize the set of enzymes involved in the metabolism of soluble sugars, and to supply of glycolytic substrates to glycolysis under anoxic conditions.

GLYCOLYSIS

The anoxic stress results in a drastic alteration in the pattern of gene expression and protein synthesis in the plants (Sachs and Ho, 1986; Sachs et al., 1996). In general, the stress quickly inhibits protein synthesis with dissociation of polysomes (Bailey-Serres and Freeling, 1990). Under the stress condition, however, 20 and 4 anaerobic stress proteins (ANPs) are selectively synthesized in maize and soybean seedlings, respectively (Bailey-Serres et al., 1988; Russell et al., 1990; Sachs et al., 1996). Most of the ANPs in their plants were identified as glycolytic and fermentation enzymes, and the corresponding cDNA have been cloned (Ricard et al., 1994; Sachs et al., 1996). Three major ANPs (36-, 40- and 87-kD protein) were selectively induced in rice seedlings under an anoxic condition, and were identified as glyceraldehyde-3-phosphate dehydrogenase, sucrose synthase and alcohol dehydrogenase (ADH; Kato-Noguchi, 2000b; 2000c). Glyceraldehyde-3-phosphate dehydrogenase was found to play an important regulatory role in glycolysis for the maintenance of high-energy

status in plants under anoxic conditions (Russell et al., 1989; Yang et al., 1993), suggesting that glycolysis may be accelerated by inducing this enzyme under the conditions.

Pyrophosphate: fructose 6-phosphate 1-phosphotransferase (PFP) catalyzes another key reaction in plant glycolysis; the interconversion of fructose 6-phosphate (Fru-6-P) and pyrophosphate (PPi) to fructose 1,6-bisphosphate (Fru-1,6-bisP) and phosphate (Pi; Black et al., 1987). The reaction is fully reversible and PFP is strongly activated by the regulatory metabolite, fructose 2,6-bisphosphate (Fru-2,6-bisP; Stitt, 1990; Plaxton, 1996). Thus, PFP is expected to play an integral function in plant carbohydrate metabolism, and suggested several possible roles in glycolysis, gluconeogenesis, PPi formation or PPi removal (Stitt, 1990; Plaxton, 1996; Theodorou and Kruger, 2001).

It has been reported frequently that Fru-2,6-bisP preferentially enhances the activity of PFP in the forward direction from Fru-6-P to Fru-1,6-bisP more than the reverse direction from Fru-1,6-bisP to Fru-6-P (van Schaftingen et al., 1982; Black et al., 1987; Trípodí and Podestá, 1997). From a theoretical point Nielsen (1995) concluded, however, that Fru-2,6-bisP activates both the forward and the reverse reaction equally. The operating direction of PFP in vivo could depend on substrate concentrations in cytoplasm of the cells (Stitt, 1989; Nielsen, 1995). It was found that Pi is a potent inhibitor of the forward reaction of PFP and PPi is a far more effective inhibitor of the reverse reaction of PFP (Stitt, 1990; Plaxton, 1996).

Anoxic stress increased in vitro PFP activity in whole rice seedlings (Mertens et al., 1990), rice coleoptiles (Gibbs et al., 2000; Kato-Noguchi, 2002b) and cultured rice cells (Mohanty et al., 1993). In addition, PPi concentration in rice coleoptiles in anoxia has been found to be above the K_m for the forward direction of PFP and to be sufficient to inhibit the reverse direction of PFP (Kato-Noguchi, 2002b), which suggests that PFP may play an important role in acceleration of glycolysis under anoxic conditions.

ETHANOLIC FERMENTATION

Induction of ADH during low oxygen conditions has been observed in many plant species (Kennedy et al., 1992; Ricard et al., 1994; Tadege et al., 1999). The advantage of ADH induction in the low oxygen conditions is reported to be acceleration of ethanolic fermentation pathway, which allows the continuation of glycolysis owing to pyruvate consumption and recycling of NAD^+ (Kennedy et al., 1992; Ricard et al., 1994; Tadege et al., 1999). Moreover, ADH is necessary for removal of acetaldehyde because of its phytotoxic effect (Perata and Alpi, 1991) and for tight cytoplasmic pH regulation (Roberts et al., 1984). Furthermore, ADH null mutants of maize and *Arabidopsis* seedlings were more sensitive to the anoxic stress than their wild types (Johnson et al., 1989; 1994; Ellis et al., 1999). Therefore, induction of ADH and activation of ethanolic fermentation was considered to be one of the strategies for plants to survive in low oxygen conditions (Kennedy et al., 1992; Ricard et al., 1994; Drew, 1997).

In rice seedlings, ADH was induced soon after the onset of anoxia (Ricard et al., 1986; Xie and Wu, 1989; Gibbs et al., 2000) and ethanolic fermentation occurred without a lag phase (Rivoral et al., 1989; Xie and Wu, 1989; Setter and Ella, 1994). In addition, an ADH null mutant of rice could not germinate under anoxic condition (Matsumura et al., 1995).

The relationship between primary root growth and ethanolic fermentation was investigated in five cultivars of rice seedlings subjected to an anoxic stress (Kato-Noguchi, 2001a). The anoxic stress inhibited the growth of all rice cultivars, however, there were significant differences in the growth among the cultivars. The stress increased ADH activities and ethanol concentrations in the roots of all cultivars, and the activity of ethanolic fermentation

in the roots was closely correlated with the root growth of the corresponding cultivars. Growth of rice coleoptiles was also found to be closely correlated with the activity of ethanolic fermentation in the coleoptiles of several cultivars of rice seedlings subjected to low oxygen stresses (Setter et al., 1994 ; 1997 ; Kato-Noguchi, 2001b).

These findings suggest that rice seedlings display a strong ethanolic fermentation system under anoxic conditions and the growth of rice seedlings subjected to anoxic stresses was closely correlated with the activity of ethanolic fermentation. Thus, the ability to increase ADH and ethanolic fermentation may play an important role in anoxia tolerance of rice seedlings where glycolysis replaces the Krebs cycle as the main source of energy.

ETHANOL SENSITIVITY

It has been under debate for a long time whether the production of ethanol in plants subjected to anoxia can be harmful (Perata and Alpi, 1993 ; Vartapetian and Jackson, 1997). Crawford (1978) hypothesized that the causing injury to anoxia sensitive plant species is self-poisoning by ethanol, the end-product of ethanolic fermentation, and anoxia tolerant plants survive longer without oxygen because they form less ethanol than anoxia sensitive plants. This hypothesis was accepted and supported by a number of researchers (e.g. Levitt, 1980 ; Moore, 1982). However, the idea has proved incompatible with more recent research (Perata and Alpi, 1993 ; Vartapetian and Jackson, 1997).

The penetration of exogenously applied ethanol to cytoplasm of carrot cells where ADH catalyzed reduction of ethanol to acetaldehyde was found (Perata et al., 1988 ; Perata and Alpi, 1991) and the ethanol-induced injuries was attributed to toxicity of acetaldehyde (Jacobs et al., 1988 ; Perata and Alpi, 1991). Thus, the inhibitory effect of exogenously applied ethanol on plant growth may be related to the level of acetaldehyde in the plant tissues.

The ability to avoid the ethanol-induced injury was evaluated in rice and oat coleoptiles. The growth of the rice and oat coleoptiles was inhibited by ethanol exogenously applied at concentrations greater than 200 and 30 mM, respectively (Kato-Noguchi and Kugimiya, 2001). At 300 mM ethanol, oat coleoptiles were brown and flaccid but rice coleoptiles did not show any visible symptoms of toxicity (Fig. 1). The acetaldehyde level in rice and oat coleoptiles was increased by exogenously applied ethanol and the increases was greater in oat than in rice

Fig. 1 Effects of ethanol on rice and oat seedlings. Rice and oat seedlings were treated with ethanol in air for 48 h.

coleoptiles under aerobic and anoxic conditions. At 300 mM ethanol, the acetaldehyde concentrations in the rice and oat coleoptiles were 46 and 87 nmol g⁻¹ fresh weight under aerobic condition, respectively, and 52 and 124 nmol g⁻¹ fresh weight under anoxic condition, respectively. The activity of ADH in the direction of ethanol to acetaldehyde was greater in oat than in rice coleoptiles and ADH protein in oat coleoptiles was more induced by exogenously applied ethanol than that in rice coleoptiles (Kato-Noguchi, 2002a). These results suggest that in vivo conversion rate of ethanol to acetaldehyde by ADH is lower in rice than oat coleoptiles, which may be one of the reasons that ethanol sensitivity of rice is much lower than that of oat since acetaldehyde is considered to play critical role in the ethanol-induced injuries (Perata and Alpi, 1991; Perata et al., 1997). The great ability of rice to avoid the ethanol-induced injuries may contribute its anoxia tolerance.

HYPOXIC ACCLIMATION

Under natural environments, plants may unexpectedly exposed suddenly to severe low oxygen conditions. A gradual transition from aerobiosis to anoxia provides an opportunity for acclimation before conditions become lethal (Crawford and Braendle, 1996; Sachs et al., 1996). If seedlings of several plants are exposed to a hypoxic pretreatment (H-PT), their viability during a subsequent period of anoxia is greatly improved (Johnson et al., 1994; Bouny and Saglio, 1996; Germain et al., 1997; Ellis et al., 1999; Kato-Noguchi, 2000a). Studies of the mechanism in the hypoxic acclimation claimed that the induction of ADH and the activation of ethanolic fermentation were necessary for the acclimation (Bouny and Saglio, 1996; Germain et al., 1997). In addition, the ADH-null mutants of maize and *Arabidopsis* could not be acclimated by exposing to H-PT (Johnson et al., 1994; Ellis et al., 1999).

Anoxia tolerance of rice seedlings was improved with increasing duration of H-PT, but H-PT longer than 6 h gave no additional improvement (Kato-Noguchi, 2002c; 2003). Concentrations of ATP and ethanol, and activity of ADH in the seedlings were increased by H-PT, and the times and patterns of increasing in these concentrations and activity were similar to those of increasing in the anoxia tolerance. These results suggest that the H-PT may increase anoxia tolerance of rice seedlings due to maintenance of glycolysis with rapid induction of ethanolic fermentation to generate ATP, and hypoxic acclimation to anoxic stress in rice may occur within 6 h.

CONCLUSION

Much research on anoxia tolerance of rice seedlings has been conducted because of their remarkable ability to withstand these conditions (Setter et al., 1997; Vartapetian and Jackson, 1997). The tolerance of rice seedlings is related to the ability to maintain an active glycolysis leading to ATP production (e.g., Guglielminetti et al. 1995a; Perata et al., 1997). Compared with the other cereals, rice may utilize the set of enzymes involved in the sucrose degradation, and supply glycolytic substrates to glycolysis under anoxic conditions. Rice seedlings were also demonstrated to display a strong ethanolic fermentation system under anoxic conditions (e.g., Setter and Ella, 1994; Gibbs et al., 2000). The induction of ethanolic fermentation is essential in anoxia because the fermentation allows the continuation of glycolysis owing to pyruvate consumption and recycling of NAD⁺ where glycolysis replaces the Krebs cycle as the main source of energy (Kennedy et al., 1992; Ricard et al., 1994; Tadege et al., 1999; Drew, 1997). In addition, rice was found to possess the great ability to avoid the ethanol-induced injuries, which may contribute its anoxia tolerance (Kato-Noguchi, 2002a). As a consequence, rice may have exceptional tolerance to anoxia.

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