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Hypoxic Induction of Anoxia Tolerance in Roots of *Adh1* Null *Zea mays* L.[1]

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Seedlings of *alcohol dehydrogenase 1* null mutants (*Adh1*$^-$) of *Zea mays* L., which fail to synthesize alcohol dehydrogenase 1 (ADH1) isozymes, were hypoxically acclimated by 18 h of exposure to an atmosphere of 4% (v/v) O_2 in N_2 at 25°C. Their ability to tolerate subsequent anoxia by exposure to anaerobic (O_2-free) conditions was compared with that of unacclimated seedlings that were transferred immediately from an atmosphere of 40% (v/v) O_2 to anaerobic conditions. Only 10% of the root tips of unacclimated seminal roots survived 6 h of anoxia, whereas 70% of the hypoxically acclimated root tips were viable at 24 h. During anoxia, acclimated root tips had enhanced ADH activity compared with unacclimated root tips, through induction of *Adh2*. Despite this, enzyme activity was still only about 5% that of acclimated, wild-type root tips and about half that of unacclimated, wild-type root tips. During anoxia, acclimated *Adh1*$^-$ root tips showed a higher rate of anaerobic respiration and ethanol production, greater concentrations of ATP and total adenylates, and a greater adenylate energy charge compared with unacclimated root tips. These results suggest that although enhanced ADH activity may have raised fermentation rates in acclimated *Adh1*$^-$ tissues and thereby contributed to energy metabolism and viability, the high levels of ADH activity inducible in acclimated, wild-type maize root tips appear to be in excess of that required to increase rates of fermentation.

Vegetative growth and yield of maize (*Zea mays* L.), as well as other flood-sensitive species, are depressed by low concentrations of O_2 in the rooting zone caused by saturation or near saturation of the soil with water (Kozlowski, 1984; Drew, 1992). Previous studies have shown that when maize seedlings experience a period of hypoxia by exposure to an atmosphere of 3 to 4% O_2 (v/v, balance N_2), their ability to resist a subsequent period of anoxia is increased (Saglio et al., 1988; Johnson et al., 1989; Andrews et al., 1994). Following acclimation to hypoxia, the tips of the seminal roots in particular have increased viability, greater ATP and total adenine nucleotide levels, a higher degree of ADH activity, and faster ethanolic fermentation than anoxically shocked plants (Saglio et al., 1988; Johnson et al., 1989; Hole et al., 1992; Xia and Saglio, 1992). In maize, two genes (*Adh1* and *Adh2*) are expressed in the roots and encode ADH enzymes; ADH1 has a 10- to 20-fold greater specific activity than ADH2 and it is the predominant enzyme involved in ethanolic fermentation in wild-type maize roots (Freeling and Bennett, 1985; Sachs et al., 1985). *Adh1*$^-$ mutants of maize lack an enzymically active ADH1 protein and are very sensitive to anoxia (Johns et al., 1983; Roberts et al., 1985), although ADH2 enzyme activity remains. However, it is not known if acclimation to hypoxia can improve the subsequent ability of these mutants to survive anoxia as it does in wild-type seedlings.

Here we examine the ability of *Adh1*$^-$ root tips to resist anoxia and compare the response with that of wild-type maize root tips, previously published by Johnson et al. (1989), Hole et al. (1992), and Andrews et al. (1993). Specifically, we examine the effect of hypoxic acclimation on the ability of root tips to survive anoxia and associated changes in energy metabolism, anaerobic respiration, and ADH activity.

MATERIALS AND METHODS

Plant Material

Kernels of homozygous *Adh1*$^-$ maize (*Zea mays* L.) were kindly provided by Dr. R. Ferl (designation *Adh1*$^-$ 5657) and back-crossed and maintained in cv TX5855. This *Adh1*$^-$ mutant has been described in a different genetic background (Freeling and Birchler, 1981). It is cross-reacting material negative, and corresponds to the mutant used by Roberts et al. (1984a). To verify that only *Adh1*$^-$ seedlings were used, a random sample of five kernels from each cob was immersed in water for 48 h to induce ADH activity. The ADH activity as measured by published procedures (Cobb and Kennedy, 1987) was approximately one-tenth that of wild-type maize kernels. Further verification was provided by gel electrophoresis; no ADH1 isozymes were detectable, but following a hypoxic acclimation a faint ADH2 band was observed. Kernels were germinated on moist blotter paper at 25°C, and at 3 d after imbibition the germinated seedlings were transferred to a stainless-steel screen suspended above a 1-mM $CaSO_4$ solution. The roots were inserted through the screen and into a 1-mM $CaSO_4$ solution that was vigorously bubbled with 40% O_2 (v/v, balance N_2). When the roots were 5 to 7 cm long, the seedlings were transferred to expanded polystyrene floats in 3.5-L screw-top glass jars containing 2 L of 1 mM $CaSO_4$, so that the coleoptile and emerging first leaf were in the gas phase and the roots were in solution. All roots, other

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Abbreviations: ADH, alcohol dehydrogenase; *Adh1*$^+$, alcohol dehydrogenase 1 wild-type genotype; *Adh1*$^-$, *Adh1*-deficient mutant or null lacking ADH1 enzyme activity; AEC, adenylate energy charge; HPT, hypoxic pretreatment; NHPT, not hypoxically pretreated.

than the primary seminal one, were excised at this stage. The tops of the jars were fitted with entrance and exit tubes so that the atmosphere within the jars could be controlled, as described by Johnson et al. (1989).

Gas Treatments

At 25°C, the critical O_2 pressure for maize root tips in well-stirred solution is in excess of that in equilibrium with air (Saglio et al., 1984). To avoid the development of hypoxia within the root tissues, solutions were vigorously sparged with 40% O_2 for at least 12 h. Aerobic controls were maintained under these conditions for the duration of the experiment. For HPT, seedlings were gassed with 4% O_2 for 18 h before sparging with O_2-free N_2. For NHPT, seedlings were sparged for 18 h with 40% O_2 before switching to O_2-free N_2. Gases were mixed and flow rates were regulated by electronic mass-flow controllers. All gas treatments were in the dark to avoid contamination from photosynthetically produced O_2.

Viability Determination

A minute amount of charcoal powder was applied with a camel-hair brush to visibly mark the primary seminal root exactly 10 mm behind the tip. Root length was then measured from this mark with a mm rule at the end of each gassing treatment and average extension was calculated for only those roots that elongated. Ten seedlings per jar per treatment were individually monitored for viability. For reaeration, the plants were transferred to a dilute nutrient solution sparged with ambient air and placed in a growth room at 25°C in the light (600 μmol m^{-2} s^{-1} PPFD) to give favorable growing conditions. Root tips were considered viable if they resumed elongation during 48 h of reoxygenation.

Extraction and Estimation of Adenine Nucleotides

Because of the rapidity with which ATP levels can rise as a result of contamination by O_2, special precautions were taken to exclude this possibility during the handling of seedlings before extraction of nucleotides. At the start of each anaerobic treatment, the entrance and exit lines to each jar were clamped off to prevent O_2 contamination from air. The jar was then placed in an anaerobic workbench and continuously purged with a flow of 99.97% prepurified N_2 (5–8 L min^{-1}). Entrance lines into the workbench provided a flow of prepurified N_2 to the jars independently of the purging of the workbench. Similarly, the exit lines from the jars were routed to an independent exit line outside the chamber. Gas flow into the jars was kept at 200 mL min^{-1} jar^{-1}. Absence of O_2 in the workbench was monitored with an O_2 electrode and with germinating rice as a "bioassay" for O_2. Rice germinates anaerobically but it will green if traces of O_2 are present. While still inside the anaerobic workbench, roots were excised 5 mm from the tip and placed in 5-mL serum vials (5 tips/vial). The vials were plunged into liquid N_2, removed from the workbench, placed in a prechilled lyophilizer (−45°C), and lyophilized overnight. Extraction and assays of ATP, ADP, and AMP were by published procedures using luciferin-luciferase (Johnson et al., 1989).

ADH (EC 1.1.1.1)

Following each treatment, 10 root tips (5 mm), the root axis (comprising the remainder of the seminal root), and shoots were excised, frozen in liquid N_2, and assayed for ADH activity as described by Cobb and Kennedy (1987). Determination of protein was according to Bradford (1976).

Respiration

The ability to respire anaerobically following HPT and NHPT was measured using a Gilson differential respirometer at 25°C. The jars were transferred to the anaerobic workbench as described above for the extraction of adenine nucleotides and 5-mm root tips (four replicates of 20 tips each) were excised in the anaerobic workbench and transferred to Gilson sidearm reaction vessels (25 mL). Each vessel contained 1 mL of buffer solution comprising 50 mM Glc, 0.5 mM $CaSO_4$, and 0.5 mM KH_2PO_4 at pH 5.6. The top of the reaction vessels were then covered with Parafilm while they were in the anaerobic atmosphere to exclude O_2 when the vessels were transferred to the respirometer. The vessels were continuously sparged with prepurified N_2 while they were being connected to the respirometer and during thermal equilibration (Hole et al., 1992). Thick-walled tubing was used to avoid any contamination by diffusion of O_2 through the tubing and into the reaction vessels. CO_2 evolution was measured directly by volume change, and measurements were converted to standard temperature and pressure.

Ethanol and Lactate Production

At the end of the respiration measurements, the root tips were removed from the sidearm flasks, blotted, and weighed. The root tips, and separately the buffer solution, were transferred at once to rubber-capped vials that were sealed, immersed in liquid N_2, and stored at −40°C. Ethanol in solution was measured by GC using a Varian model 3400 gas chromatograph equipped with a 6-foot glass column packed with 60/80 Carbopack B/5% Carbowax 20M, at isothermal 110°C, and with a flame ionization detector at 120°C. Standard ethanol concentrations were prepared, internal standards were used throughout, and injection was direct using the Varian model 2000 autosampler. Lactate was measured by enzymic analysis, essentially as described in Rumpho and Kennedy (1981). Internal standards for lactate were included to check on recovery.

RESULTS

Root Tip Viability

Root tips of *Adh1*$^-$ seedlings hypoxically pretreated with 4% O_2 for 18 h maintained 70% viability at 24 h of anoxia (Fig. 1A). By contrast, very few root tips of *Adh1*$^-$ seedlings transferred directly from 40% O_2 to N_2 (NHPT) survived up to 6 h of anoxia and none survived to 12 h. During reaeration, the elongation of HPT root tips decreased with increasing previous duration of anoxia (Fig. 1B). There was no extension of roots during anoxia. Prior to reaeration, NHPT root tips were flaccid and the root axes were flaccid and water soaked

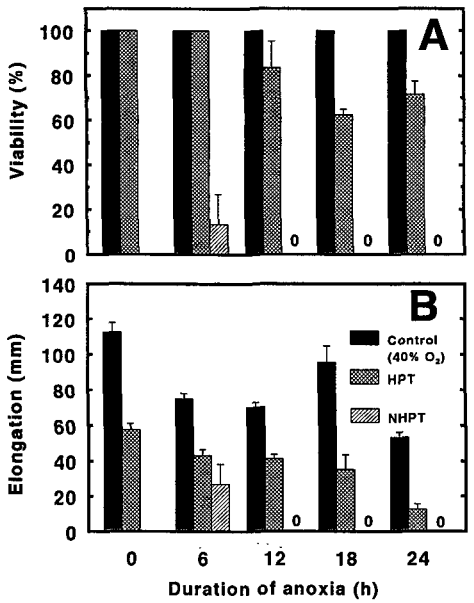

Figure 1. Viability of intact *Adh1*⁻ root tips following different durations of anoxia. A, Viability; B, elongation. At the end of anoxia, seedlings were reaerated for 48 h, and elongation of the seminal root for that 48-h period was recorded. No NHPT root tips survived beyond 6 h. Values are means of three independent experiments. Bars indicate ± SE (n = 30).

to varying extents, from very little at 6 h of anoxia to the entire length at 24 h. This indicates that injury occurred primarily during the period of anaerobic conditions rather than as a consequence of reaeration itself. All HPT root tips and root axes that were later found to be viable remained turgid during anoxia. The root tips of those that did not survive became flaccid usually during reaeration. Shoot survival for the HPT plants was limited to about 12 h of anoxia, whereas NHPT shoots did not survive beyond 6 h (data not shown). During reaeration (48 h), the development of laterals was apparent on the HPT root axes but not on the NHPT ones.

Energy Metabolism

In NHPT *Adh1*⁻ root tips, ATP and total adenine nucleotides (ATP + ADP + AMP) declined to low values at 6 h of anoxia, and had almost disappeared at 12 h (Fig. 2, A and B). By contrast, with HPT the levels of ATP and total adenine nucleotides were maintained at about half those of the aerobic controls. The initial concentrations of ATP and adenine nucleotides were virtually identical to those of *Adh1*⁺ tissues (Johnson et al., 1989) and changed to a similar extent during anoxia, but during a much shorter time span.

The AEC ([ATP] + 0.5[ADP]/[ATP] + [ADP] + [AMP]) in HPT root tips was maintained between 0.75 and 0.9 during the first 12 h of anoxia (Fig. 2C). For NHPT root tips, the AEC decreased sharply by 6 h and then remained at a value of 0.45 to 0.5 until 18 h, when it increased. It should be remembered that from 12 h onward, absolute concentrations of all nucleotides in NHPT root tips were very low and that tissues were undoubtedly moribund, so that AEC values become unreliable as sole indices of energy status. In the aerobic controls, [ATP] declined considerably between 12 and 24 h, resembling the decline between 48 and 96 h we noted earlier with wild-type root tips (Johnson et al., 1989).

ADH Activity during Hypoxia and Anoxia

HPT induced ADH activity in *Adh1*⁻ maize root tips (Fig. 3), reaching a maximum (μmol min^{-1} g^{-1} fresh weight) of 0.38, less than 10% of that for wild type (Andrews et al., 1993). In the root axes, ADH activity was about 5-fold less, averaging 0.07 during the initial 18 h of anoxia for HPT axes, but only 0.03 for NHPT axes. However, relative to the protein content, ADH activity in axes was similar to, or in excess of, that in root tips, reaching an activity (μmol min^{-1} mg^{-1} protein) of 0.06 for HPT axes at 12 h of anoxia, and for NHPT axes about one-third of that. No induction of ADH activity was detected in shoots. The ADH activity of the HPT root tips was induced to a level that was 6-fold higher than in the aerobic controls (Fig. 3A). In NHPT root tips, ADH activity was similar to or less than that in aerobic controls. This contrasts with NHPT wild-type root tips, for which anoxia caused a distinct peak in ADH activity between 6 and 12 h (Andrews et al., 1993).

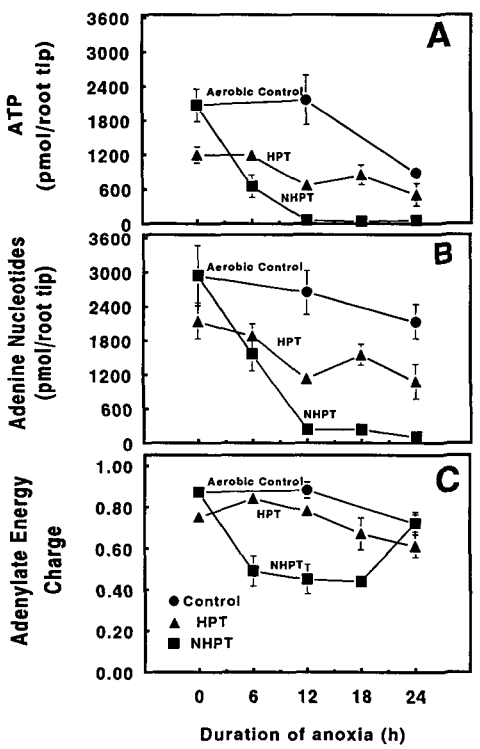

Figure 2. A, Content of ATP in 5-mm root tips of intact *Adh1*⁻ maize seedlings during anoxia. B, Content of total adenine nucleotides (ATP + ADP + AMP). C, AEC. Values are means of two independent experiments. Bars indicate ± SE (n = 9).

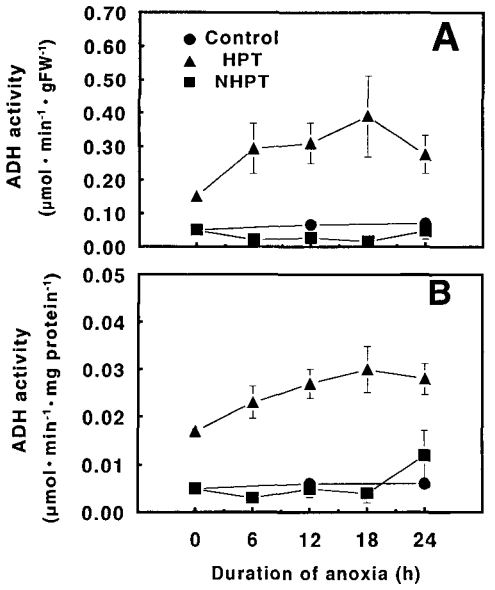

Figure 3. ADH activity of intact 5-mm root tips of *Adh1*⁻ maize seedlings during anoxia. A, Activity expressed per g fresh weight of root tips. B, Activity expressed per mg of protein. Values are means of two independent experiments. Bars indicate ± SE (n = 8).

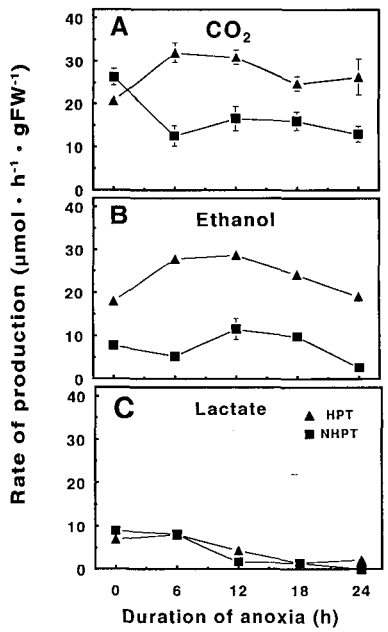

Figure 4. Rates of anaerobic production of CO_2, ethanol, and lactate by excised 5-mm root tips following different durations of anoxia. A, CO_2; B, ethanol; C, lactate. Root tips were excised from intact seedlings after different durations of anoxia and anaerobically incubated for 2.5 h to obtain steady-state rates of production. Values are means of three independent experiments. Bars indicate ± SE (for A and B, n = 8; for C, n = 3).

Anaerobic Respiration and Ethanol Production during Anoxia

HPT root tips had a higher anaerobic respiration rate than NHPT root tips (Fig. 4A). The rate of CO_2 evolution by HPT root tips was almost half that of aerobic control root tips (56 ± 3 μmol h^{-1} g^{-1} fresh weight), but this may represent a stimulation of glycolysis in HPT root tips because CO_2 evolution in ethanolic fermentation is one-third of that in aerobic respiration per mol of hexose respired.

In a separate experiment, we compared the anaerobic respiration rates of 5-mm root tips of *Adh1*⁻ with those of *Adh1*⁺ excised after 6 and 24 h of anoxia (Table I). *Adh1*⁻ HPT root tips respired at about 60% of the rate of *Adh1*⁺ HPT root tips. For NHPT root tips of either genotype, the respiration rate was less than half that of the HPT root tips.

The corresponding ethanol production (Fig. 4B) paralleled the trends in anaerobic respiration. The ethanol production rate of HPT root tips was at least double that of the NHPT root tips. Lactate production was appreciable during the initial 6 h of anoxia but then declined (Fig. 4C). In HPT tissues an average of 87% of the evolution of CO_2 (Fig. 4A) could be accounted for by the production of ethanol (Fig. 4C), noting that mol of CO_2 should equal mol of ethanol in the pathway of glycolysis and fermentation. However, for NHPT root tips, ethanol production accounted for only 36% of CO_2 evolved for the initial 6 h of anoxia, while root tips remained alive. At greater times, when an appreciable fraction of the NHPT root tip cells were dead, fermentation continued but became very low at 24 h. Other decarboxylation reactions presumably contributed to CO_2 production by NHPT root tips.

DISCUSSION

Survival of Anoxia of *Adh1*⁻ Root Tips

The experiments reported here demonstrate that pretreatment of *Adh1*⁻ maize seedlings with 4% O_2 enhances their ability to survive a subsequent period of anoxia. The improved survival of the seminal root tips appears to be related, at least in part, to induction of a faster rate of glycolysis and fermentation and to maintenance of energy metabolism under anoxia. In some respects the overall response is similar to that found earlier with *Adh1*⁺ maize (Saglio et al., 1988; Johnson et al., 1989; Hole et al., 1992; Andrews et al., 1994), although the increased duration of survival of *Adh1*⁻ is for a shorter time than in *Adh1*⁺ seedlings at a comparable stage in development.

Table I. *CO_2 produced during anaerobic respiration of Adh1⁻ and Adh1⁺ Zea mays root tips*

Intact seedlings were made anaerobic for 6 or 24 h, the root tips were excised in an anaerobic atmosphere, and the respiration rate was measured during the subsequent 2.5 h.

Duration of Anoxia	*Adh1*⁻		*Adh1*⁺	
	NHPT	HPT	NHPT	HPT
h	*μmol h^{-1} g^{-1} fresh weight*			
6	14.3 ± 2.3	28.8 ± 5.1	14.6 ± 3.0	40.0 ± 1.0
24	10.0 ± 1.4	21.9 ± 0.9	13.3 ± 1.3	33.0 ± 1.7

Adh1$^-$ maize is known to be highly sensitive to excess water at germination, or at the seedling stage, so that submersion in water for several days, giving rise to O_2 shortage, causes loss of seedling viability (Schwartz, 1969; Lemke-Keyes and Sachs, 1989). The primary seminal roots of *Adh1*$^-$ seedlings are likewise less tolerant of anoxia than are the roots of *Adh1*$^+$ seedlings. Roberts et al. (1984a, 1984b) found that unacclimated *Adh1*$^-$ root tips survived a maximum of about 12 h of anoxia compared with about 24 h in *Adh1*$^+$. We also found unacclimated *Adh1*$^-$ root tips to be intolerant of anoxia, with 88% loss of viability within the initial 6 h (Fig. 1). Following HPT, *Adh1*$^-$ root tips survived at least 30 h of anoxia (data not shown), which exceeds that of NHPT *Adh1*$^+$ root tips, none of which survived 24 h (Johnson et al., 1989).

The response to HPT found in seedling roots is not restricted to maize. In wheat, HPT root tips survived more than 24 h of anoxia, whereas for NHPT roots, only 5% survived 12 h (Waters et al., 1991). The ability to survive anoxia was associated with higher activities of pyruvate decarboxylase and ADH induced during HPT.

Energy Metabolism of Anoxic *Adh1*$^-$ Root Tips

Levels of ATP, total adenylates, and AEC in *Adh1*$^-$ root tips at the start of anoxia were very similar to those previously reported for *Adh1*$^+$ (Johnson et al., 1989), so that the mutant cells do not appear to be at any disadvantage at the start of anoxia compared with wild type. However, once under anoxia, energy metabolism declined much more rapidly in *Adh1*$^-$ NHPT root tips (Fig. 2) than in Adh1$^+$ tips, which is in agreement with earlier in vivo estimations of NTP by ^{31}P NMR (Roberts et al., 1984a, 1984b). For the corresponding HPT seedlings, the decline in energy metabolism over 4 d in *Adh1*$^+$ root tips (Johnson et al., 1989) is compressed into about 24 h in *Adh1*$^-$ tissues (Fig. 2).

In *Adh1*$^-$ mutants, AEC was not a reliable indicator of cellular energy status once cell death occurred and adenylates were close to zero, as we found earlier for wild type (Saglio et al., 1988; Johnson et al., 1989). For anoxic NHPT root tips at 24 h, concentrations of ATP and total adenylate were only 3% of those of controls, and tips had lost viability, yet AEC values equaled those of controls. Fully aerobic *Adh1*$^-$ root tips showed a decline of ATP and other adenylates (per tip) during the experiment. A similar but less rapid decline was noted earlier in *Adh1*$^+$ root tips, which was accounted for by a decrease in the diameter of the root tips (Johnson et al., 1989). The important feature is that HPT causes energy metabolism to be maintained at an appreciably higher level during anoxia in both *Adh1*$^+$ and *Adh1*$^-$ root tips, but in *Adh1*$^-$ root tips this effect is of shorter duration.

Cytoplasmic Acidosis and ADH Activity of *Adh1*$^-$ Root Tips

A determinant of cell death in anoxic maize root tips is acidification of the cytoplasm. In excised, uninduced *Adh1*$^+$ root tips, cytoplasmic acidosis took place (from pH 7.4–6.8) during the initial 20 min of anoxia, coinciding with a temporary burst of lactic acid production at the onset of anoxia (Roberts et al., 1984a). Thereafter, ethanol production predominated and cytoplasmic pH remained stable for about 10 h. Using uninduced root tips of *Adh1*$^-$ seedlings, Roberts et al. (1984a) found that the pH of the cytoplasm failed to stabilize, but instead continued to decline throughout anoxia, which was attributed to a slow production of lactic acid. In their research, the mutant root tips, virtually lacking ADH activity, made barely detectable amounts of ethanol, and ATP had virtually disappeared within 30 to 60 min.

Our results with NHPT *Adh1*$^-$ root tips of intact seedlings are consistent with the above and reinforce the view that ADH1 enzyme activity is important for survival of anoxia of uninduced root tips. However, whether lactate accumulation adequately accounts for the initial acidification of the cytoplasm has been disputed by Saint-Ges et al. (1991), who found that the sharp decline in pH at the onset of anoxia failed to coincide with a much more gradual accumulation of lactic acid. Additionally, Menegus et al. (1989) proposed that intracellular pH might be regulated during anoxia by enhancing succinic acid production at the expense of lactic acid production and by metabolic proton consumption in the decarboxylation of glutamate to γ-aminobutyric acid. More recent evidence indicates that although these processes take place in anoxic maize roots, they are negligible in relation to lactic acid and ethanol production, and they are unlikely to play a role in cytoplasmic pH regulations (Roberts et al., 1992).

An important question is if the enhanced level of ADH activity of acclimated (HPT) root tips contributes to the improved anoxic survival. It is evident that HPT leads to marked stimulations of the activity of ADH and rates of anaerobic respiration and ethanol production of *Adh1*$^-$ root tips (Fig. 4) to an extent that closely resembles that of *Adh1*$^+$ root tips (Hole et al., 1992). Thus, it seems plausible that a faster rate of ATP production accounts for the improved energy metabolism and survival of anoxia. ADH activity in *Adh1*$^-$ root tips was enhanced by HPT, but only to a level (0.02–0.03 μmol min^{-1} mg^{-1} protein) less than half that of uninduced *Adh1*$^+$ root tips that failed to survive as long as did the induced *Adh1*$^-$. This level of enzyme activity corresponds closely to that identified by Roberts et al. (1989), at which further increases did not serve to raise the fermentation rate or energy metabolism. This indicates that hypoxic induction of ADH could have been a contributor to improved survival of anoxia of *Adh1*$^-$ root tips. However, it calls into question the significance of the even higher ADH activities induced in *Adh1*$^+$ root tips. We found that root tips of *Adh1*$^+$ maize seedlings of different ages have contrasting ADH activities during anoxia, yet their ability to survive anoxia is similar (Andrews et al., 1994). Roberts et al. (1989) also concluded that the uninduced ADH activity of *Adh1*$^+$ root tips was not limiting to energy production in ethanolic fermentation. By studying maize lines differing over a 200-fold range in ADH activities, they found that fermentation rates were limited by ADH activity only at very low levels. The threshold level of ADH activity in unacclimated 2-mm root tips of maize, above which further increases in fermentation did not take place, was 0.02 μmol min^{-1} mg^{-1} protein. However, this threshold might be higher in acclimated tissues because hypoxia induces higher levels of mRNA and enzyme

activity of several other enzymes of glycolysis and fermentation before the imposition of anoxia (D.L. Andrews, B.G. Cobb, J.R. Johnson, and M.C. Drew unpublished data), dependent perhaps on coordinate induction (Bailey-Serres et al., 1988).

The ADH activity of unacclimated *Adh1*$^-$ root tips was about one-tenth that of unacclimated *Adh1*$^+$ tips, in agreement with other published values (Johns et al., 1983; Freeling and Bennett, 1985; Saglio et al., 1988). Following acclimation, ADH activity in *Adh1*$^-$ null mutants was only 5% that of acclimated *Adh1* wild type. Enhanced ADH activity in the root tip and to a lesser extent in the root axis of *Adh1*$^-$ seedlings indicates that there was a small hypoxic induction of *Adh2*. ADH2 activity is likely to go unnoticed in *Adh1*$^+$ seedlings because of the much larger contribution of ADH1, but in the *Adh1*$^-$ seedlings it takes on a special significance.

Freeling (1973) found induction of ADH2, analyzed electrophoretically, in maize primary roots during hypoxia or anoxia. At the mRNA level, both unlinked *Adh* genes are inducible within a similar time frame and range of O_2 concentrations (Paul and Ferl, 1991a, 1991b), although the precise mechanism of regulation at the level of DNA-binding factors within the promoter region appears to have little in common (Paul and Ferl, 1991a).

CONCLUSIONS

This study indicates that hypoxic acclimation enables *Adh1*$^-$ root tips to survive 24 h or more of anoxia. At least part of the mechanism of acclimation is associated with induction of ADH activity (ADH2), enhanced fermentation, and energy metabolism. However, it is unlikely that ADH activity is the sole factor responsible for anoxic survival in either *Adh1*$^-$ or *Adh1*$^+$ roots. Pyruvate decarboxylase is a more likely candidate for regulation of ethanolic fermentation (Morell et al., 1990), and levels of pyruvate decarboxylase mRNA (Kelley, 1989) and enzyme activity (Laszlo and Lawrence, 1983) are increased by O_2 deficiency in *Adh1*$^+$ maize roots. Induction of the other anaerobic polypeptides by anoxia (Sachs et al., 1980; Russell and Sachs, 1991, 1992) also presumably plays a role in maintaining glycolysis and fermentation, even if ADH activity is itself in excess of requirements for ethanol production.

The inability of unacclimated root tips of *Adh1*$^-$ seedlings to survive anoxia, compared with their wild-type counterparts, is readily explained by the slower anaerobic respiration rate and earlier collapse of energy metabolism in the mutants lacking ADH1 isozymes. The difference in survival between acclimated mutants and acclimated or unacclimated wild type is not explained in terms of ADH activity, although it may be significant that improved survival accords with a faster anaerobic respiration rate (Table I). Intracellular accumulation of lactic acid may also be a contributory factor in the death of unacclimated *Adh1*$^-$ and *Adh1*$^+$ root tips during anoxia. In 3-mm root tips of HPT *Adh1*$^+$ maize, there was less production of lactic acid during the initial period of anoxia, and of that lactic acid, a greater proportion was transported to the external medium, compared with NHPT tissues (Xia and Saglio, 1992). Acidification of the cytoplasm was also somewhat less in HPT root tips (estimated at 0.1–0.15 pH units), suggesting that the smaller accumulation of lactate was important in pH regulation. The authors suggest that HPT permits expression of an inducible lactate-transporting system. Therefore, NHPT *Adh1*$^-$ roots are likely to be especially vulnerable to anoxia because of their accumulation of lactate and continuous cytoplasmic acidosis (Roberts et al., 1984a) associated with the very low constitutive level of ADH2 activity.

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