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The Response of Maize Seedlings of Different Ages to Hypoxic and Anoxic Stress[1]

Changes in Induction of *Adh1* mRNA, ADH Activity, and Survival of Anoxia

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Previously we showed that there is only a transient induction of *alcohol dehydrogenase 1* (*Adh1*) transcripts and only a small induction of alcohol dehydrogenase (ADH) enzyme activity in root tips of maize (*Zea mays* L.) seedlings subjected to strict anaerobiosis without prior acclimation by exposure to low O_2 (D.L. Andrews, B.G. Cobb, J.R. Johnson, M.C. Drew [1993] Plant Physiol 101: 403–414). Acclimation of root tips of seedlings by low O_2 before anoxia appeared to be necessary for full induction of ADH. Here we have examined the effect of seedling age on changes in the protein content, induction of *Adh1* transcripts, and ADH enzyme activity in 5-mm root tips, root axes, and shoots of maize (cv TX5855). Their ability to survive anoxia was also recorded. Some seedlings were sparged with 4% O_2 for 6 or 18 h (a hypoxic pretreatment) followed by anoxia (sparged with N_2) for up to 48 h. Other seedlings were not acclimated before anoxia. In general, younger seedlings had higher initial (aerobic) levels of total protein, *Adh1* transcripts, and ADH activity than did seedlings that were 2 d older. For younger seedlings, anoxia alone induced *Adh1* transcripts, which reached a peak within 6 to 12 h, whereas ADH activity increased throughout the 48-h treatment. For older seedlings, anoxia caused only a small, transient induction of *Adh1* transcripts or ADH activity. For seedlings of either age, hypoxia induced *Adh1* transcripts and ADH activity, both of which were increased further by subsequent anoxia in the younger seedlings but to a lesser extent in the older seedlings. Despite differences in ADH activity, roots of seedlings of either age showed a similar resistance to anoxia. Thus, acclimation of maize seedlings to survive anoxia does not appear to be related to induction of high levels of ADH activity.

The response of maize (*Zea mays* L.) roots to a restricted O_2 supply (known as the "anaerobic response") has been extensively examined using both classical genetics and molecular approaches (for a review, see Bailey-Serres et al., 1988). Schwartz (1969) showed that mutant maize kernels lacking wild-type *Adh1* gene activity did not survive submersion, thus demonstrating that *Adh1* is essential in conditions of restricted O_2 supply. Maize roots respond to conditions of anoxia by arresting normal protein synthesis and preferentially synthesizing a set of polypeptides that are collectively known as anaerobic proteins (Sachs et al., 1980). These anaerobic proteins are either previously synthesized proteins that are expressed at an elevated level in response to the lack of O_2, or are synthesized only under conditions of restricted O_2 supply. Several anaerobic proteins have been identified, including Suc synthase (Springer et al., 1986), pyruvate decarboxylase (Laszlo and Lawrence, 1983; Kelly, 1989), aldolase (Kelley and Freeling, 1984), and ADH1 and ADH2 (Sachs and Freeling, 1978; Ferl et al., 1980; Dennis et al., 1985; Rowland and Strommer, 1986). It is not surprising that all of these proteins are enzymes involved in glycolysis and fermentation, since this pathway must provide the majority of ATP production when O_2 is limited and oxidative phosphorylation is arrested. The importance of this pathway in the anaerobic response of maize roots has been well documented (Roberts et al., 1989, 1992; Hole et al., 1992; Xia and Saglio, 1992).

Of particular interest is the importance of hypoxia in the maize anaerobic response. When maize seedlings are made hypoxic by exposure to a subambient O_2 concentration before the imposition of anoxia, root tips show a greater energy metabolism and viability during at least 4 d of anoxia compared with root tips not acclimated by HPT, which succumb within 18 to 24 h (Saglio et al., 1988; Johnson et al., 1989). The length of time and O_2 concentration used for the HPT also affected the extent to which ADH was induced (Andrews et al., 1993). For example, the highest level of *Adh1* transcripts was induced in root tips at 6 h of hypoxia, whereas by 18 h of hypoxia transcript levels had declined but enzyme activity was maximal (Andrews et al., 1993). Conversely, when seedlings were anaerobically shocked by transfer from fully aerated conditions to anoxia, there was little increase in *Adh1* transcripts or ADH activity in root tips, and their survival was limited to less than 24 h (Johnson et al., 1989; Andrews et al., 1993). From these data it is apparent that HPT is required for maize root tips to continue metabolism past 24 h when they are subjected to strictly anaerobic conditions.

In our earlier work, we cast doubt on the extent to which ADH was inducible by anaerobic shock in seedling root tips (Johnson et al., 1989). This appeared to be at variance with other investigations that had characterized induction of both

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Abbreviations: ADH, alcohol dehydrogenase; HPT, hypoxic pretreatment; L, long; NHPT, not hypoxically pretreated; S, short.

Adh1 transcript levels and ADH proteins under conditions of anoxia (Sachs et al., 1980; Gerlach et al., 1982; Dennis et al., 1984). However, in earlier experiments a variety of conditions involving O_2 deficiency and age of seedlings were used (see Andrews et al., 1993, for discussion). Previous reports had not always noted the physiological age (as judged by the length of the primary root) of the seedlings at the initiation of experimental treatments. Also, in many cases the entire primary root had been used for extraction of mRNA or proteins, although the root tips and the remainder of the primary root (root axis) showed distinctly different patterns of ADH induction (Andrews et al., 1993) and ability to survive anoxia (Freeling and Bennett, 1985).

The aim of the present investigation was to determine the response of maize seedlings of different ages to hypoxia and anoxia. We show that in the root tips of young seedlings there is a high basal level of ADH synthesis, which is increased further by anoxia. By contrast, in older seedlings, sustained ADH induction was strongly dependent on prior acclimation by hypoxia.

MATERIALS AND METHODS

Plant Material and Anaerobic and Hypoxic Treatment of Maize Seedlings

Maize (*Zea mays* L. cv TX5855) was germinated and gas treatments were imposed using the methods previously described (Andrews et al., 1993). Briefly, caryopses were allowed to imbibe on moistened germination paper at 25°C in the dark and transferred to 2 L of 1.0 mM $CaSO_4$ solution after 3 to 5 d. The seedlings were supported by expanded polystyrene floats so that the coleoptile and emerging first leaf were in the gas phase while the primary seminal root was in the solution. All other seminal roots were excised at the time of transfer. The solution, contained in 3.5-L glass jars with gas-tight lids, was continuously sparged with 40% (v/v) O_2 (balance N_2) for 18 h followed by 6 or 18 h at 4% (v/v) O_2 (HPT). The corresponding dissolved O_2 concentrations were 506 and 50 μM, respectively. All HPT seedlings were then made anoxic by sparging vigorously with prepurified N_2 gas (99.97% N_2). NHPT seedlings were transferred directly from the 40% O_2 treatment into N_2. Above-ambient concentrations of O_2 were used because at 25°C, the critical O_2 pressure for the respiration of submerged root tips just exceeds the O_2 concentration in air-saturated water (Saglio et al., 1984), and it was important to avoid prior accumulation of anaerobic proteins by mild hypoxia in NHPT.

Seedlings with primary root lengths of 3 to 4 cm (termed short [S], 3 d after imbibition) or 10 to 12 cm (long [L], 5 d after imbibition) at the start of the gassing treatments were used for all experiments. Following the gassing treatments, seedlings were dissected into root tips (the terminal 5-mm portion of the primary seminal root), root axes (the remaining seminal root tissue), and shoots (the coleoptile and first leaf). All tissues were frozen in liquid N_2 and stored at −80°C until analyzed.

The ability of seedlings to survive anoxia was followed in a parallel experiment. To check on the extension growth of roots, a spot of charcoal powder slurry was applied with a fine brush 10 mm behind the root tip, just before HPT commenced, which was used as a reference mark for measurement in mm. At the end of anoxia, seedlings were transferred to a dilute nutrient solution comprising 0.1 mM KNO_3, 0.4 mM $Ca(NO_3)_2$, 0.1 mM $NH_4H_2PO_4/(NH_4)_2HPO_4$, 0.05 mM $MgSO_4$, 0.05 mM FeEDTA, together with micronutrients. Seedlings were maintained in a controlled environment room (25°C day, 22°C night; 18-h light period of 600 μmol m^{-2} s^{-1} PPFD) to encourage growth. Viable root tips were defined as those that resumed extension during the 48-h recovery period (Roberts et al., 1984a, 1984b; Johnson et al., 1989). Nonviable root tips were water soaked and translucent. After 96 h of recovery, root axes were scored for whether any lateral roots had emerged, and shoots were also checked for color and regrowth: those that were brown and necrotic and failed to show further extension of the first leaf or emergence of additional leaves were judged to be nonviable.

Northern Blot Analysis

Total RNA for northern analysis was isolated from groups of 25 seedlings using the lithium chloride method (Stiekema et al., 1988). Yield was determined by spectrophotometric absorbance at a wavelength of 260 nm. Nucleic acid blotting was performed using GeneScreen Plus membranes according to the manufacturer's specifications and as previously described (Andrews et al., 1993). An *Adh1* cDNA fragment (approximately 1600 bp) isolated from bacterial plasmid pZML793 (Dennis et al., 1984) was used for random-primed probes (Andrews et al., 1993). The hybridization and washing were done according to standard protocols and the high-stringency final washing conditions have been shown to be specific for *Adh1* (Strommer et al., 1982). After the final wash, the membrane was visualized and quantified on a Betascope 603 Blot Analyzer (Sullivan et al., 1987; Schneider-Gadicke et al., 1989) (Betagen Corp., Waltham, MA). Justification and standardization of this procedure has been presented elsewhere (Andrews et al., 1993). Relative levels of mRNA were expressed on the basis of cpm μg^{-1} total RNA to facilitate comparisons with results obtained in other investigations and on the basis of cpm mg^{-1} fresh weight to allow direct comparisons to be made with the enzyme measurements contained in this study. All northern blots were hybridized with the same probe, allowing for direct comparison of different blots.

Enzyme Assays

Assays for ADH activity were performed using a standard protocol (Cobb and Kennedy, 1987). Although this assay measures total ADH activity, the specific activity of ADH1 is much higher than that of ADH2, and activity represents largely the translation product of *Adh1* (Dennis et al., 1985; Bailey-Serres et al., 1988). Soluble protein concentrations were determined by the method of Bradford (1976). Each sample consisted of 25 seedlings and each was repeated a minimum of three times.

Enzyme activity is expressed in μmol min^{-1} g^{-1} fresh weight and μmol min^{-1} mg^{-1} protein because of the predominance of densely cytoplasmic cells in the apical meristem (5-mm

root tip), which make it instructive to examine enzyme activity in relation to the relative amounts of cytoplasm present in the various organs. Additionally, the alterations in total protein levels and enzymes caused by anoxia/hypoxia warrant the inclusion of both.

RESULTS

Effects of Anoxia on Protein Levels in Seedlings of Different Ages

Regardless of treatment, there was little change in protein levels of organs from S seedlings during 48 h of anoxia (Fig. 1, A–C). In contrast, L seedlings showed a marked difference between the NHPT and the HPT (Fig. 1, D–F). Protein content in NHPT root tips declined throughout 48 h of anoxia, with little protein detectable in root tips at 24 h (Fig. 1D). The protein content of root axes of these seedlings varied little during anoxia, whereas that of shoots of NHPT seedlings declined after 18 h (Fig. 1D). Seedlings subjected to 6 or 18 h of HPT maintained a steady level of protein in the root tips up to 24 h of anoxia and declined thereafter (Fig. 1, E and F). HPT root axes and shoots showed little change in protein during anoxia (Fig. 1, E and F).

ADH Activity during Hypoxia and Anoxia

ADH activity was more strongly induced in S seedlings than in L seedlings (Fig. 2). Induction of ADH activity in root tips of S seedlings was not dependent on HPT (Fig. 2A). Root tips of NHPT S seedlings showed a 6-fold increase in ADH activity through the 48 h of anoxia on a fresh weight basis (Fig. 2A). In contrast, NHPT root tips of L seedlings showed a slight increase in ADH activity at 6 h and declined to initial levels or below by 24 h (Fig. 2D), in agreement with our previous findings (Andrews et al., 1993). Root axes and shoots of NHPT S seedlings also showed a steady elevation of ADH activity throughout anoxia (Fig. 2A), whereas in L seedlings it declined in the shoots to the initial, very low level at 24 h and remained level in root axes (Fig. 2D).

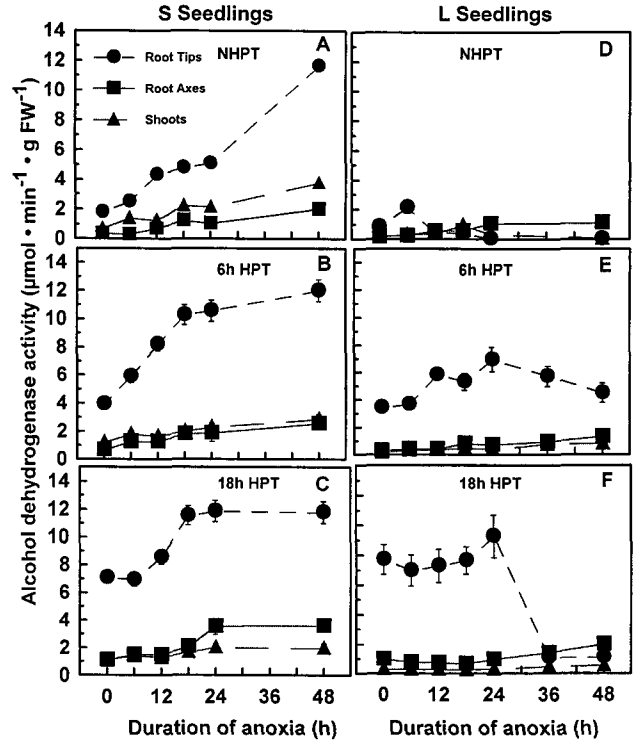

Figure 2. ADH activity (expressed as μmol min^{-1} g^{-1} fresh weight) in primary root tips, root axes, and shoots of seedlings during anoxia. Conditions were as described in Figure 1. Bars represent ± SE, where this exceeds symbol size.

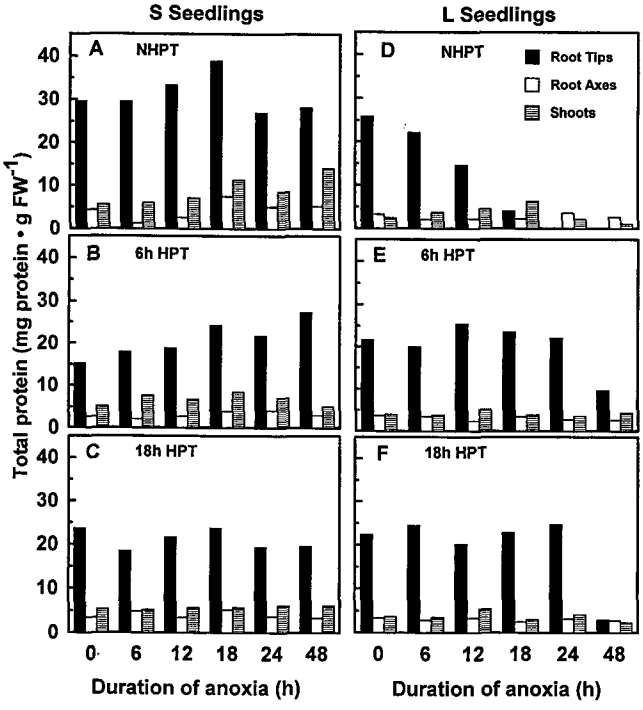

Figure 1. Protein concentration in primary root tips, root axes, and shoots of seedlings during anoxia. A through C, S seedlings; D through F, L seedlings. HPT was for 6 or 18 h prior to anoxia. NHPT seedlings were transferred directly from 40% O_2 to an O_2-free N_2 atmosphere.

Following HPT, S and L seedlings exhibited a differential response to anoxia. Root tips of S seedlings showed an increase in ADH activity mainly in the initial 18 h of anoxia, and there were also increases in the other organs. In root tips, ADH activity reached a similar maximal level more rapidly in HPT than in NHPT seedlings. In L seedlings, ADH activity was generally lower in all organs, and for 18-h HPT root tips, it declined sharply between 24 and 36 h.

Because of the changes in protein content resulting from hypoxia and anoxia (Fig. 1), it is important to consider this when expressing enzyme activity. When ADH activity was expressed relative to protein content (Fig. 3), the prominent activity of the root tip zone was no longer evident. However, the trends associated with hypoxia and anoxia broadly paralleled those seen in Figure 2. For NHPT S seedlings, enzyme activity increased during 48 h of anoxia, whereas this occurred only in the root axes of L seedlings (Fig. 3, A and D).

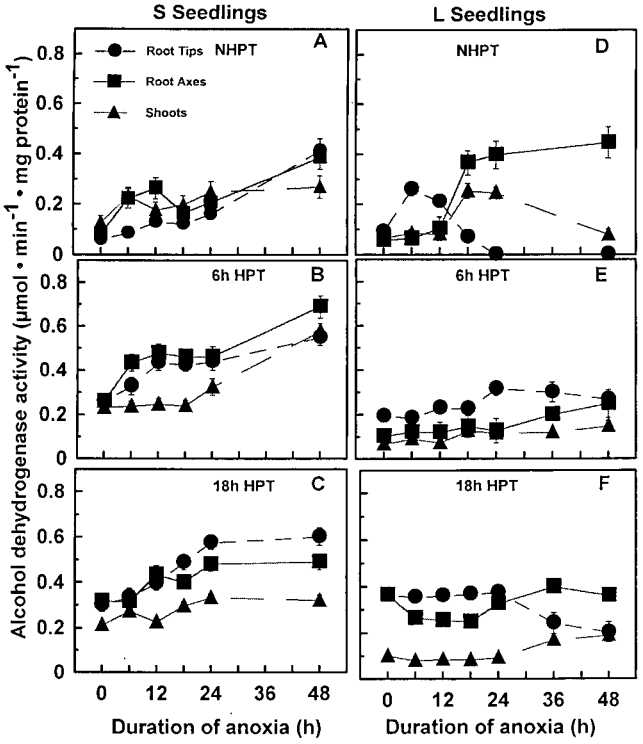

Figure 3. ADH activity (expressed as μmol min^{-1} mg^{-1} protein) in primary root tips, root axes, and shoots of seedlings during anoxia. Conditions were as described in Figure 1. Error bars indicate SE.

HPT induced a lower ADH activity in L than in S seedlings. It is notable that ADH activity was maintained in the root axes during 48 h of anoxia and appeared to increase under conditions where it decreased in the root tips.

Because seedlings had been grown in the dark in dilute $CaSO_4$, it seemed possible that the NHPT L seedlings, after a prolonged HPT, might have depleted their seed reserves and so failed to maintain ADH activity during anoxia. Therefore, seedlings were grown in the dark in a nutrient solution containing macro- and micronutrients, as described in "Materials and Methods," together with 50 mM Glc. Previous experiments had shown that Glc is readily absorbed and metabolized by maize roots under these conditions (Hole et al., 1992). Following 18 h of HPT, seedlings were made anoxic and sampled for ADH activity as before. The results (data not presented) were indistinguishable from those in Figure 2F; that is, ADH activity declined after 24 h on a fresh weight basis. Furthermore, when L seedlings received an 18-h HPT and were then maintained at 40% O_2, they remained viable for at least a further 96 h and reached lengths in excess of 30 cm in dilute $CaSO_4$ solution (data not presented). From these observations we conclude that the low level of ADH activity during anoxia in the root tips of L seedlings that was seen after 24 h cannot be simply a consequence of a lack of substrates for growth and metabolism.

Adh1 mRNA Induction by Hypoxia and Anoxia with Seedling Age

In view of the marked difference in induction of ADH activity in seedlings of varying ages, we next examined whether there were comparable changes in response at the mRNA level, and we found that the patterns of *Adh1* mRNA level were quite different (Figs. 4 and 5). Expressed on a fresh weight basis, HPT caused little change in the root tips of S seedlings (compare 0 time anoxia in Fig. 4, A–C). The level of mRNA in NHPT root tips was high, even in fully aerobic tissue (Fig. 4A). By contrast, in L seedlings there was a clear accumulation of mRNA at 6 h of HPT, and a decline by 18 h of HPT (Fig. 4, D–F, 0 time points). For the S seedlings during anoxia there was a further rise in transcript levels in all the organs, reaching a peak between 6 and 12 h followed by a gradual decline.

In the L seedlings, transcript levels rose abruptly in the NHPT root tips (Fig. 4D) but this was transient and declined to initial values by 12 h. Following 6 h of HPT, transcript levels in the root tips were high at the beginning of anoxia and declined progressively to almost zero at 48 h. Interestingly, after 18 h of HPT (during which time transcript levels rose and fell), a second phase of induction took place during anoxia. This was less transient than in the NHPT plants, but by 48 h the levels were very low again. For the other organs in L seedlings, transcript levels were continuously low on a fresh weight basis, except for the root axes (Fig. 4D).

When transcript levels were expressed relative to total RNA (Fig. 5), the root tip values no longer showed the highest concentrations. HPT did not increase levels in the S seedlings (Fig. 5, A–C), but for NHPT as well as HPT seedlings anoxia

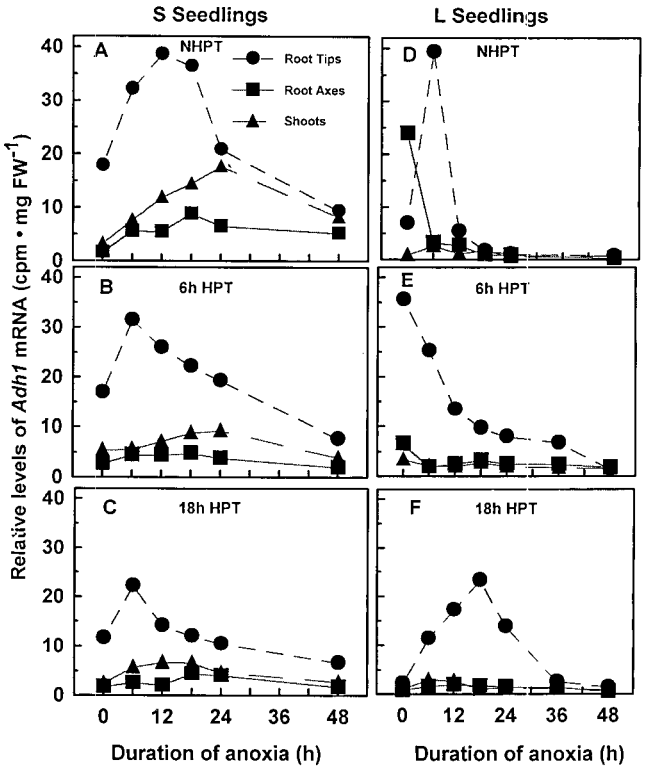

Figure 4. Relative levels of *Adh1* mRNA (expressed as cpm mg^{-1} fresh weight) in primary root tips, root axes, and shoots of seedlings during anoxia. These values represent quantitation of northern blots as described in "Materials and Methods."

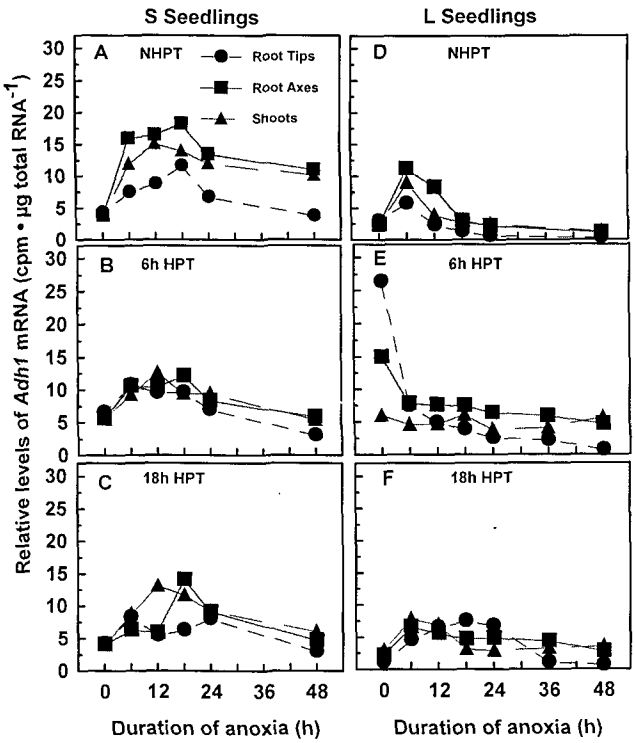

Figure 5. *Adh1* mRNA (expressed as cpm mg^{-1} total RNA) in primary root tips, root axes, and shoots of seedlings during anoxia. Conditions were as described in Figure 4.

did, with peak levels at 12 to 24 h. For L seedlings, 6 h of HPT raised transcript levels in all three organs (Fig. 5E), and by 18 h these had declined again to near the initial levels (Fig. 5F). Anoxia produced a transient rise in transcript levels in NHPT seedlings (Fig. 5D), and the second phase of induction was also apparent following 18 h of HPT (Fig. 5F).

Seedling Survival of Anoxia

Because of the marked differences in ADH activity and mRNA between S and L seedlings, it was of interest to determine whether there were differences in their ability to survive anoxia. At the end of anoxia, seedlings were transferred to nutrient solution, and the growth of root tips, axes, and shoots were scored separately. For root tips, the main difference was the greater susceptibility of NHPT L seedlings, which did not survive to 12 h (Fig. 6). In all other respects, the seedlings were very similar in their response. HPT for 18 h gave almost 100% survival of up to 48 h of anoxia, although growth was slowed. HPT for only 6 h was sufficient to extend the survival of up to 40% of the root tips to 36 h, although none survived to 48 h.

Root axes were less susceptible to anoxia than were the root tips, and almost all survived at least 48 h following 6 or 18 h of HPT (data not presented). With NHPT seedlings, survival was of shorter duration; for S seedlings axis survival declined to 40% at 48 h, and for L seedlings survival declined to 60% at 24 h. The anoxic survival of shoots also varied with the duration of anoxia and with HPT. Nearly all were alive after 24 h of anoxia, but beyond that time only HPT shoots survived. For example, for shoots of S seedlings after 48 h of anoxia, no NHPT or 6-h HPT survived, whereas with 18-h HPT, 70% were alive.

DISCUSSION

The present results show that maize seedlings at two stages during development respond quite differently to O_2 deficiency. Gauging this response using *Adh1* transcripts and ADH enzyme activity as markers, it was possible to see several distinct consequences of the imposition of anaerobic conditions that were dependent on the age of the seedling (S or L) and on previous acclimation by hypoxia.

Induction of ADH Activity

With S seedlings, the basal level of ADH activity was high, even though NHPT seedlings were in solutions sparged with 40% O_2 until sampled. This activity increased manyfold during anoxia in the root tips, and to a lesser extent in root axes and shoots (Fig. 2), in sharp contrast to the older NHPT L seedlings, where induction of ADH activity was small and brief (Fig. 2; also Andrews et al., 1993). At the *Adh1* mRNA level, differences between NHPT seedlings were particularly striking, with a high basal level in S seedling root tips and a sustained further increase during anoxia. This is in contrast with the transient peak in L seedlings (Fig. 4). In these respects, the L seedlings closely resembled those that we have used in previous publications (Johnson et al., 1989; Andrews et al., 1993).

Some early studies made with young seedlings (Sachs et al., 1980; Kelley and Freeling, 1984; Roberts et al., 1984a, 1984b) reported a sustained induction of *Adh1* mRNA and ADH activity in roots under anoxia. Our findings that slightly older seedlings (L seedlings) do not respond like young seedlings (S seedlings) to anoxia explain our earlier concern

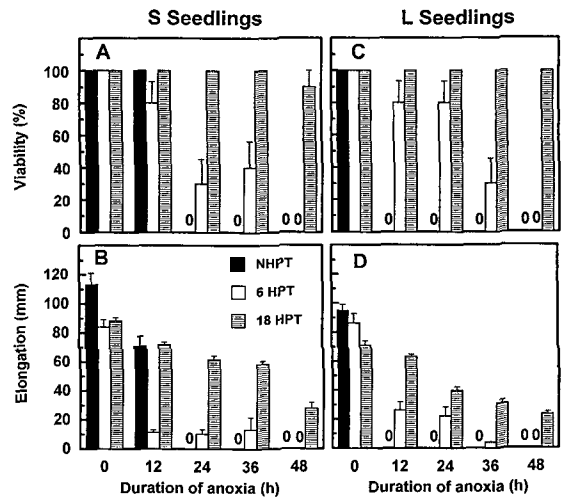

Figure 6. Viability of primary root tips with duration of anoxia. At the end of anoxia, seedlings were reaerated and viability of root tips was estimated. A and C, Percent viability; B and D, elongation per root during 48 h of recovery (n = 10). Error bars indicate SE.

that the "anaerobic response" might occur only after a period of acclimation during hypoxia (Johnson et al., 1989). However, the present results leave no doubt that in young maize seedlings ADH activity is inducible by hypoxia or by anoxia.

The regulation of the levels of *Adh1* transcripts and of ADH enzyme during hypoxia or anoxia is likely to be complex, and there is no reason a priori to expect this regulation to change in parallel. As expected, the transcript levels rose initially, leading to enhanced synthesis of ADH protein, which did not decline as rapidly as the corresponding message (Figs. 2 and 4). Increased levels of *Adh1* transcripts during O_2 deficiency in roots have been reported (Ferl et al., 1980; Gerlach et al., 1982; Dennis et al., 1984; Rowland and Strommer, 1986). The level measured at any point in an experiment, however, is dependent on the rate of transcription and on transcript stability. For *Adh1* it appears that both are enhanced during submergence in buffer solution (Rowland and Strommer, 1986). Translation is highly demanding of energy in the form of ATP and GTP, and energy metabolism in anoxic cells is severely depressed because of the decline in synthesis of high-energy phosphate bonds and the lowering of cytoplasmic pH (Roberts et al., 1984a, 1984b, 1985). Specifically, initiation as well as elongation and termination of protein synthesis are likely to be inhibited (Webster et al., 1991a, 1991b). Because of the progressive decline in pH and energy metabolism with prolonged periods under anoxia, the relationship between transcript levels and the concurrent level of a particular protein is unlikely to remain constant. This is especially evident in the L seedlings, where for NHPT root tips the sharp rise in transcript level was not matched by ADH activity. Likewise, in 18-h HPT root tips, the second phase of transcript induction peaking at 18 h was not paralleled by a change in enzyme activity.

It may be that hypoxia induces *Adh1* transcription by a mechanism that is different from that induced by subsequent anoxia. Alternatively, and more likely, the cells that make up the root constitute a heterogeneous population with respect to their O_2 status. Berry and Norris (1949) recognized that at or below the critical O_2 pressure, cells in the core of the root become O_2 starved and start to ferment, while the outer cells are still aerobic. Induction of *Adh1* by hypoxia may thus reflect the response of cells in the presumed "anaerobic core," and the second phase of induction by anoxia would then be the response of the outer, previously aerobic cells. It would be valuable to examine the radial distribution of ADH in root sections as a function of the O_2 concentration to distinguish between these possibilities.

Hypoxic Induction of Anoxia Tolerance

In our earlier research, we standardized the germination schedule and always selected seedlings with an average primary seminal root length of 6 cm at the start of the gas treatments. If such seedlings were made hypoxic for 18 h prior to anoxia, the entire seedling including the root tip was able to survive anoxia for at least 96 h, whereas those with no HPT died after 18 to 24 h of anoxia (Saglio et al., 1988; Johnson et al., 1989; Andrews et al., 1993). A similar enhancement of root tip viability during anoxia of HPT wheat seedlings has also been reported (Waters et al., 1991). In the present paper we demonstrate that in maize seedlings of contrasting ages, the efficiency of HPT in prolonging anoxia tolerance of root tips, axes, and shoots was maintained, despite marked differences in protein content (Fig. 1) and ADH induction (Figs. 2 and 3).

Relation between Induction of ADH Activity and Anoxia Tolerance

In this research we focused on induction of ADH activity as a marker of the anaerobic response. It is doubtful, however, if induction of high levels of ADH activity in normal maize seedlings during O_2 deficiency is needed for survival of anoxia. For example, high levels of ADH activity were found in the root tips of NHPT S seedlings (Fig. 2), but survival of anoxia was limited to less than 24 h. Conversely, there was low ADH activity in 18-h HPT L seedling root tips that had 100% survival (Fig. 6). Using a range of maize *Adh1* mutants that differed 200-fold in ADH activity, Roberts et al. (1989) found that low levels of enzyme activity, about 0.02 μmol min^{-1} mg^{-1} protein, would be sufficient to maintain maximal fermentation rates in root tips. They concluded that greater ADH activity would not enhance energy production through fermentation. In the present experiments, even the uninduced levels of activity exceeded that value (Fig. 3). With *Adh1* null maize mutants (Johnson et al., 1993), HPT improved anoxia tolerance of the root tips, although the ADH activity failed to exceed that of NHPT normal root tips that were much more susceptible to anoxia. Furthermore, VanToai et al. (1985) found no correlation between ADH activity and flooding tolerance of maize seeds. The significance of induction of high levels of ADH activity in maize root tips is therefore obscure. The regulation of ethanolic fermentation rates, and hence substrate-linked phosphorylations during glycolysis, could, however, be through pyruvate decarboxylase activity, which is lower than that of ADH by a factor of 6 to 9 in hypoxic maize or wheat roots (Wignarajah and Greenway, 1976; Waters et al., 1991). In general, Waters et al. (1991) found that pyruvate decarboxylase activities in extracts of wheat roots were similar to in vivo rates of ethanol production. Transcript levels of the *Pdc* gene were greatly increased by hypoxia or anoxia in maize seedlings (Kelley, 1989; D.L. Andrews, B.G. Cobb, P.M. Kelley, J.R. Johnson, and M.C. Drew, unpublished data) and closely paralleled changes in *Adh1* in the present study (Figs. 4 and 5).

Anoxic cells not only have to generate ATP in the absence of oxidative phosphorylation, but for prolonged survival accumulation of the end products of fermentation must be avoided. Ethanol readily diffuses across lipid bilayers to the external solution, but lactate requires a specific transport system at the plasma membrane. In barley (Hoffman et al., 1986) and in some species of *Limonium* (Rivoal and Hanson, 1993), there is a sustained fermentation under hypoxia or anoxia to lactate, which is transferred to the surrounding solution. Recently, Xia and Saglio (1992) suggested that hypoxic acclimation involves in part the synthesis of lactate transporters, which then remain functional during anoxia. They found greater accumulations of lactic acid in NHPT compared with HPT roots, with corresponding differences in lactic acid in the external solution.

In the present experiments it is possible that the shorter anoxic survival of NHPT root tips and axes is related in some way to a lowered ability to remove lactate from the cells, thus contributing to cytoplasmic acidosis and accelerated cell death. However, the metabolism of anoxic maize shoots has not been characterized, and although it seems reasonable to assume that ethanolic fermentation takes place because of ADH activity in the shoots, it is not clear whether the mechanism of acclimation during HPT resembles that in the roots.

In this research we have assumed that injury is sustained as a result of anoxia, and nearly all root tips were distorted, discolored, or flaccid and water soaked at the end of anoxia. However, reexposure to air might cause injury to axes and shoots through generation of superoxide radicals (Monk et al., 1987, 1989). Under normal aerobic conditions, cells are protected from these highly reactive radicals by superoxide dismutase. Anoxia tolerance, as judged by the ability to return to an aerobic environment, may thus also involve an ability to maintain adequate superoxide dismutase levels to deal with reaeration (VanToai and Bolles, 1991).

In conclusion, our results with maize seedlings at different developmental stages identify distinctly different responses to O_2 deficiency. In the youngest seedlings we have examined (S seedlings), either hypoxia or anoxia induces a marked increase in *Adh1* transcripts and ADH activity. This induction takes place regardless of whether the anaerobic stress is preceded by hypoxia, although prior hypoxia shortened the time needed for maximal enzyme activity to be attained. In L seedlings and in those previously examined (Andrews et al., 1993), induction of *Adh1* and ADH in root tips by anoxia is small and transient. Irrespective of seedling age, hypoxia is an essential prerequisite for the ability of root tips to survive more than 24 h of anoxia. However, it is clear that induction of high levels of ADH is not directly involved in the acclimation process that gives rise to the improved survival of anoxia of normal maize roots.

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LITERATURE CITED

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