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Ethylene Biosynthesis during Aerenchyma Formation in Roots of Maize Subjected to Mechanical Impedance and Hypoxia[1]

Chuan-jiu He, Scott A. Finlayson, Malcolm C. Drew, Wayne R. Jordan, and Page W. Morgan*

Department of Horticultural Sciences (C.-j.H., M.C.D.) and Department of Soil and Crop Sciences (S.A.F., W.R.J., P.W.M.), Texas A&M University, College Station, Texas 77843

Germinated maize (*Zea mays* L.) seedlings were enclosed in modified triaxial cells in an artificial substrate and exposed to oxygen deficiency stress (4% oxygen, hypoxia) or to mechanical resistance to elongation growth (mechanical impedance) achieved by external pressure on the artificial substrate, or to both hypoxia and impedance simultaneously. Compared with controls, seedlings that received either hypoxia or mechanical impedance exhibited increased rates of ethylene evolution, greater activities of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, ACC oxidase, and cellulase, and more cell death and aerenchyma formation in the root cortex. Effects of hypoxia plus mechanical impedance were strongly synergistic on ethylene evolution and ACC synthase activity; cellulase activity, ACC oxidase activity, or aerenchyma formation did not exhibit this synergism. In addition, the lag between the onset of stress and increases in both ACC synthase activity and ethylene production was shortened by 2 to 3 h when mechanical impedance or impedance plus hypoxia was applied compared with hypoxia alone. The synergistic effects of hypoxia and mechanical impedance and the earlier responses to mechanical impedance than to hypoxia suggest that different mechanisms are involved in the promotive effects of these stresses on maize root ethylene biosynthesis.

Growth and development of plant roots are altered by a number of stress factors that are commonly encountered by roots in the soil environment. The responses of roots to several individual stresses, such as transient nutrient deficiencies, O_2 deficiency, and mechanical impedance, have been studied with maize (*Zea mays* L.) seedlings (Kays et al., 1974; Drew et al., 1979; Konings and Verschuren, 1980; Whalen, 1988). Ethylene biosynthesis and systems modified by ethylene have been shown to be involved closely with the stress responses (Drew et al., 1979, 1989; Konings, 1982; Atwell et al., 1988; Sarquis et al., 1991; He et al., 1992). The promotion of ethylene biosynthesis by several different stress factors suggests a common mechanism. Hypoxia (partial O_2 deficiency) promotes ethylene biosynthesis and the formation of ethylene-dependent aerenchyma in maize roots (Drew et al., 1979; Jackson et al., 1985; Atwell et al., 1988). Exposure of well-aerated roots to 1 μL L^{-1} ethylene in air stimulates aerenchyma formation, and inhibitors of ethylene action (Ag^+) or biosynthesis (aminoethoxyvinyl Gly) effectively block the formation of aerenchyma (Drew et al., 1981; Konings, 1982; Jackson et al., 1985). Another stress factor, mechanical impedance, also alters maize root growth, inhibiting elongation and promoting swelling while stimulating ethylene biosynthesis (Sarquis et al., 1991). The primary effect of mechanical impedance appears to be on ACC synthase activity (Sarquis et al., 1992), and inhibitors of ethylene action (Ag^+) and biosynthesis (aminoethoxyvinyl Gly) partially reverse the effect of mechanical impedance on elongation and swelling (Sarquis et al., 1991).

The triaxial cell, originally designed to test soil strength (Collis-George and Yoganathan, 1985), was modified by Sarquis et al. (1991) for studies of mechanical impedance. Each triaxial cell consists of a Plexiglas container that can be pressurized with compressed N_2 and within which seedlings grow in a soil-like substrate that is contained in a flexible membrane (figure 1, Sarquis et al., 1991). A separate source of breathing air flows under minimal pressure through the plant-containing substrate and out into an ethylene collection and assay system. While plants grow at normal atmospheric pressure, pressure within the triaxial cell compresses the membrane-enclosed plant growth medium and thereby increases its rigidity and resistance to displacement by root tips during elongation growth.

The advantage of using the triaxial cell in studies of mechanical impedance is that it allows quantitative regulation of impedance to growth while allowing near real-time measurements of ethylene production of intact seedlings by sampling air continuously flushed through the porous growth medium (Sarquis et al., 1991). The apparatus also permits regulation of the content of the gas flushed through the seedling chamber, allowing us to compare the detailed time course of responses to both hypoxia and mechanical impedance and the combinations of both stresses. Use of the triaxial cells requires that the experimental tissue be the primary roots of seedlings. In our experience with maize seedlings, nodal roots respond to hypoxia and N_2 starvation (Drew et al., 1989; He et al., 1992, 1994) via effects on ethylene biosynthesis or sensitivity, as do primary roots to physical impedance (Sarquis et al., 1991, 1992). Brailsford et al. (1993) also found similar effects of hypoxia on primary maize roots, as previously

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* Corresponding author; e-mail p-morgan@tamu.edu; fax 1-409-845-0456.

noted for nodal roots (Drew et al., 1979; Jackson et al., 1985; Atwell et al., 1988).

The object of this research was to compare responses of the ethylene biosynthetic system to hypoxia and mechanical impedance as related to modifications of root development. We determined the effects of both stresses on ethylene biosynthesis, cell death, aerenchyma formation, and increases in cellulase activity that are closely associated with cell degradation during aerenchyma formation. We report here that mechanical impedance modifies ethylene biosynthesis more rapidly than does hypoxia and that the two stress treatments together cause a striking synergistic promotion of ACC synthase activity and the rate of ethylene biosynthesis.

MATERIALS AND METHODS

Most of the experimental procedures have been described in detail previously (Sarquis et al., 1991; He et al., 1992, 1994) and for them only a brief description is given.

Plant Growth Conditions

Caryopses of maize (*Zea mays* L. cv TX 5855) were surface-sterilized in commercial bleach that was diluted to 0.53% (w/v) sodium hypochlorite, washed in tap water for 4 h, and allowed to imbibe and germinate on moist germination paper in a 2-L container at room temperature (21°C). Seedlings were generally 3 or 4 d old when experiments were started. A group of eight seedlings with uniform root lengths (approximately 30 mm) was selected and transferred to a volume of screened, fritted clay (previously saturated with aqueous 10^{-4} M $CaCl_2$ and drained) that was held in a cylindrical shape by a tube-shaped membrane, and enclosed within a modified triaxial cell (Soil Test Corp., Evenston, IL) (figure 1, Sarquis et al., 1991). The applied pressure was 100 kPa (1 bar). Ethylene-free air was flushed through the system at 60 mL min^{-1}. For hypoxia treatments 4% (v/v) O_2 and 96% (v/v) N_2 were flushed through the system at the same flow rate. The porosity of the fritted clay was 0.73 (volume fraction) and the total volume of fritted clay enclosing the eight seedlings was 100 cm^3 (Sarquis-Rameriz, 1991), so that the flow rate of 60 mL min^{-1} yielded a calculated eight air exchanges in 10 min.

Ethylene Collection and Assay

Ethylene was collected with a continuous flow system (Morgan et al., 1990), based on one developed by De Greef and De Proft (1978). Ethylene evolved from the seedlings was carried by a stream of air or 4% O_2 through a Poropak R column (50–80 mesh, Alltech Associates, Deerfield, IL) immersed in acetone frozen by liquid N_2. After a 10-min trapping period, the column was immersed in boiling water to release collected ethylene to a second Poropak R column that was immersed in frozen acetone. Ethylene was concentrated on the second column, which was then heated with boiling water, and the sample of ethylene was injected by carrier N_2 gas to a GC (no. 72–5, Beckman) equipped with an activated alumina column, which separated ethylene from ethane and other low-molecular-weight hydrocarbon gases, and a flame ionization detector (Morgan et al., 1990). The GC will detect 50 nL L^{-1} ethylene in a 1-mL injection, but, since the ethylene to be assayed was scrubbed from 600 mL of air, the lower limit of sensitivity for ethylene in air from the triaxial cells was 0.08 nL L^{-1}. Because each determination involved the ethylene that was scrubbed from 600 mL of air and produced by eight maize seedlings, which were not handled after planting, variability between replicate samples was small.

Enzyme Assays

ACC synthase was measured by a modified in vitro method based on that described by Spanu et al. (1990). Root tips (0- to 25-mm apical zone) were excised on ice, and 0.5-g fresh weight samples were placed in individual 10-mL glass tubes with 3 mL of permeabilizing buffer consisting of 50 mM potassium phosphate, pH 8.0, 1 mM DTT, 0.5 μM pyridoxal phosphate, and 1% (v/v) Triton X-100. Samples and buffer were frozen at −40°C for more than 1 h and thawed at room temperature. The endogenous ACC was removed by placing the root-tip samples in empty, disposable chromatography columns (no. 731-1550, Bio-Rad) and washing three times with ice-cold permeabilizing buffer and then three times with ice-cold assay buffer (permeabilizing buffer without Triton X-100). Root tips were then transferred to 10-mL glass tubes with 2 mL of assay buffer. Fifty microliters of *S*-adenosyl-L-methionine (1 mM, no. A-4377, Sigma) was added for enzyme assay; the same volume of deionized water was added to tubes for substrate blanks. The glass tubes were kept in a water bath at 30°C for 90 min. The reaction was stopped by adding 150 μL of $HgCl_2$ (10 mM). ACC synthase activity was quantified by measuring newly synthesized ACC by converting it to ethylene as described by Lizada and Yang (1979). One-milliliter samples were assayed for ethylene with a Photovac 10510 GC (Markham, Ontario, CA) with a photoionization detector that will detect 1 pmol ethylene, which in a 1-mL sample would be 2.2 nL L^{-1}. We tested the enzyme activity in subsamples from a population of root tips using different buffer concentrations (5 -1500 mM) and pHs (5.0–10.0). The optimum buffer pH and concentration were 8.0 and 50 mM, respectively, at 30°C during a 90-min assay. We checked that endogenous ACC, present initially in the root samples, was effectively removed by the washing procedure (substrate blank). For example, the ACC content was initially 3.52 nmol g^{-1} fresh weight in roots after 48 h of hypoxia treatment, but only 0.042 nmol ACC g^{-1} fresh weight remained after washing, that is, about 1.2%. Correction for any remaining ACC was made in all assays. For each treatment time point there was a 6-fold replication (three separate experiments with tissue from each treatment divided for duplicate assays, $3 \times 2 = 6n$).

The extraction and assay of ACC oxidase were based on the procedure of Vereridis and John (1991). One 25-mm root tip was placed in a 1.5-mL plastic centrifuge tube, weighed, and frozen in liquid N_2. PVP (10 mg) was added to the tube, and the root tip was homogenized with 200 μL of extraction buffer (100 mM 1,3-bis[tris{hydroxymethyl}-methylamino]propane-HCl, pH 7.0, 10% [w/v] glycerol, and

2 mM DTT) on ice. The tube was centrifuged at 14,000*g* for 10 min at 4°C, and the complete supernatant was poured off and centrifuged again at 14,000*g* for 5 min to precipitate any particles carried over in the supernatant from the first centrifugation. A 50-μL aliquot was injected into a sealed 6.2-mL assay tube containing 500 μL of assay buffer (100 mM 1,3-bis[tris{hydroxymethyl}-methylamino]propane-HCl, pH 7.0, 10% [w/v] glycerol, 20 mM sodium ascorbate, 10 μM $FeSO_4$, and 5 mM ACC), which had been preincubated with 20% CO_2 and 21% O_2 for 20 min at 35°C on a shaking water bath. The reaction mixture was then incubated at 35°C for 20 min on a shaking water bath and a 500-μL gas sample was withdrawn and analyzed for ethylene as in the ACC synthase assays (see above). Control tubes without enzyme extract were also measured. For each treatment time point, a minimum of four individual root tips were extracted and assayed separately in each of two experiments.

Cellulase activity was measured by a modification of the method described earlier (He et al., 1994). Seminal root tips 50 mm long were used because aerenchyma development normally proceeds from older to younger cells and cellulase activity declines as aerenchyma formation is complete (He et al., 1994). Root tips from treated or control maize seedlings (0.5 g fresh weight) were excised on ice and macerated with river sand in 3 mL of 30 mM potassium phosphate extract buffer, pH 6.1, containing 1 M NaCl (final concentration). The extract was centrifuged at 3000*g* for 10 min, and then 2.5 mL of supernatant solution was passed through a Sephadex GM-25M desalting column (9-mL bed volume) and eluted by 3 mL of potassium phosphate assay buffer, pH 6.1, which did not contain NaCl. Two milliliters of the eluted solution was added to reaction vials in viscometers with 4 mL of 1% sodium carboxymethyl cellulose of medium viscosity (Sigma). Cellulase activity in the eluted solution was assayed by measuring the change in the viscosity of carboxymethyl cellulose during 2 h of incubation at 25°C using a flow viscometer (20–200-centipoise range). A cellulase preparation from *Aspergillus niger* (no. c7377, Sigma) was used to prepare a standard curve on each occasion to convert changes in viscosity to units of enzyme activity.

Aerenchyma Estimation

Seminal roots were marked with carbon (charcoal slurry) 10 mm behind the root tip. This mark was used as a baseline reference to trace root extension and age during the experiment so that sections from treated and control roots would be comparable. These marked roots were later used to prepare transverse sections with a razor blade. Camera lucida drawings were made of the transverse sections, identifying the areas that comprised intact cells, cells in the process of lysing, and clearly delineated gas-filled spaces. These areas were quantified for each section from the drawings.

Data Analysis

The significance of the differences between means was tested using the Student's *t* test.

RESULTS

Ethylene Biosynthesis

Mechanical impedance stimulated the rate of ethylene production within 1 h (Fig. 1, inset). The rate (nmol g^{-1} fresh weight h^{-1}) for the control at 1 h was 0.044 ± 0.013 (mean ± SD), and that with mechanical impedance was 0.061 ± 0.010 ($P < 0.01$). The statistically significant (Student's *t* test) increase in rate above the control continued throughout the experiment. In contrast, hypoxia required 4 h to significantly increase the rate of ethylene production (Fig. 1, inset); at 4 h the rates for control and hypoxic seedlings were 0.044 ± 0.014 and 0.068 ± 0.001 nmol g^{-1} fresh weight h^{-1}, respectively ($P < 0.01$). The combination of mechanical impedance and hypoxia caused the largest promotion of ethylene production above the control by increasing the rate to 0.071 ± 0.012 nmol g^{-1} fresh weight h^{-1} at 1 h ($P < 0.01$). Moreover, the combination of mechanical impedance and hypoxia amplified the rate of ethylene production synergistically, especially during the first 10 h of the treatments. At 10 h the sum of the increase in rates above the control for the hypoxia and impeded treatments was 0.23 nmol g^{-1} fresh weight h^{-1}, and the combination of both treatments increased the ethylene production rate to 0.75 nmol g^{-1} fresh weight h^{-1} above the control, representing a 3.3-fold increase over the sum of the individual treatments. At 24 h the corresponding synergistic increase was 2.9-fold, and even at 72 h some synergism remained, with the increase amounting to 1.4-fold. The ethylene production rate increased steadily with time in all treatments, whereas no increase in the ethylene production rate occurred with time in the control.

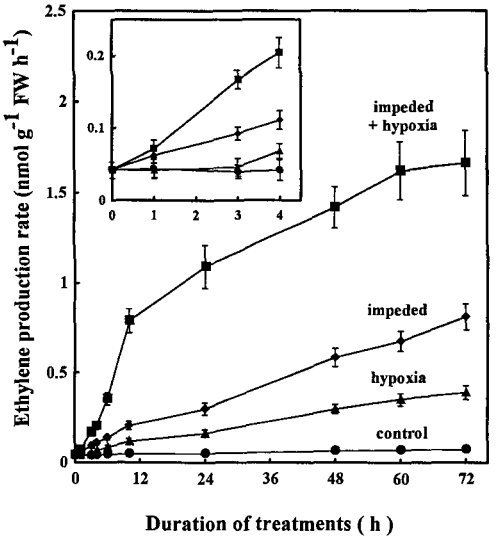

Figure 1. Effects of 4% O_2 (hypoxia), mechanical impedance, and hypoxia plus impedance on the ethylene production rate of maize seedlings. Treatments began at zero time. Control seedlings were in a similar container receiving 20% O_2 and no physical impedance. The inset illustrates data for the first 4 h with an expanded scale. Data are averages of six separate experiments ($n = 6$) and SDs are shown by vertical bars where they exceed symbol size. FW, Fresh weight.

ACC Synthase Activity

In vitro ACC synthase activity was measured with time after imposition of the different stresses (Fig. 2). In seminal roots enzyme activity increased above control levels at 1 h under mechanical impedance or the combination of mechanical impedance with hypoxia ($P < 0.01$). In contrast, ACC synthase activity in hypoxic roots did not increase above the control level until 3 h ($P < 0.01$). ACC synthase activity steadily increased in roots with time in all the treatments. It was also increased synergistically by the combination of O_2 stress and mechanical impedance compared with the two stresses applied individually (Fig. 2). For example, at 10 h the increase in activity above the control of the combination treatment was about 2.3-fold higher than the sum of the increases due to O_2 stress and impedance applied alone. Similar results were obtained for ACC synthase activity in coleoptiles of maize (Fig. 3), in which there were statistically significant increases in activity over the controls at 1 h with impedance or impedance plus hypoxia ($P < 0.01$). However, in this tissue the response to hypoxia was a little slower (Fig. 3). ACC synthase activity under hypoxia treatment was not higher than control activity at 4 h ($P = 0.07$), but was clearly different at 12 h ($P < 0.01$).

ACC Oxidase Activity

The response of ACC oxidase activity (Fig. 4) to the stress treatments was clearly unlike that of ACC synthase. During the initial 3 h ACC oxidase activity values for treatments were not significantly different from the controls. ACC oxidase activity did not increase above control rates until 4 h after the initiation of any treatment (Fig. 4, inset). The effect of simultaneous hypoxia and impedance was not greater than either of the two stresses applied separately until after 12 h of treatment. The combination of hypoxia and impedance did not increase ACC oxidase activity synergistically; the sums of activities in the separate hypoxia and impedance treatments equaled or exceeded the activity of the simultaneous treatment at all observation times. The

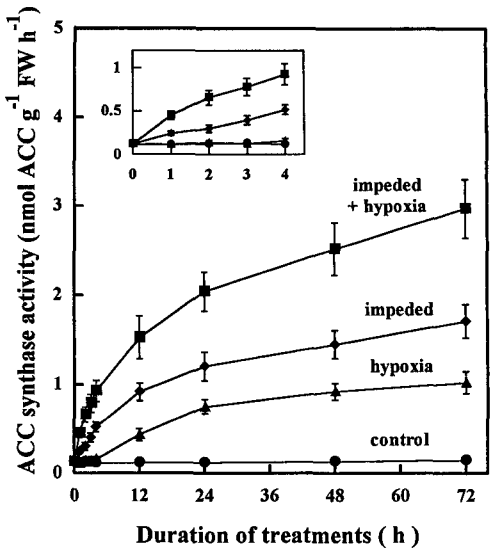

Figure 3. Effects of 4% O_2 (hypoxia), mechanical impedance, and hypoxia plus impedance on the ACC synthase activity of coleoptiles of maize seedlings. The control conditions were the same as in Figure 1. The inset illustrates data for the first 4 h with an expanded scale. The number of experiments, replications, and presentation of SD are the same as in Figure 2. FW, Fresh weight.

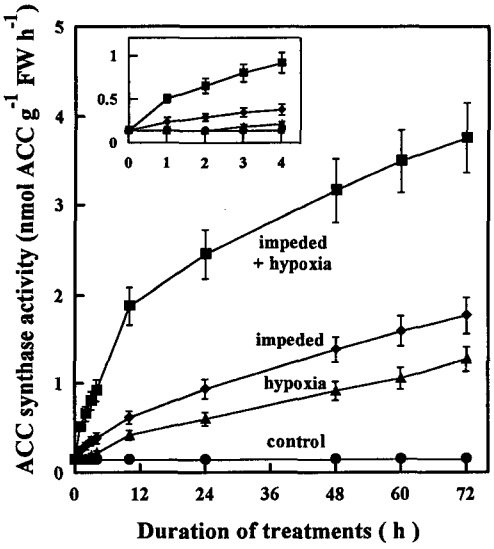

Figure 2. Effects of 4% O_2 (hypoxia), mechanical impedance, and hypoxia plus impedance on the ACC synthase activity of root tips of maize seedlings. The control conditions were the same as in Figure 1. The inset illustrates data for the first 4 h with an expanded scale. Data are averages of three separate experiments with two analyses per point for each treatment ($n = 6$) and SDs are shown as vertical bars where they exceed symbol size. FW, Fresh weight.

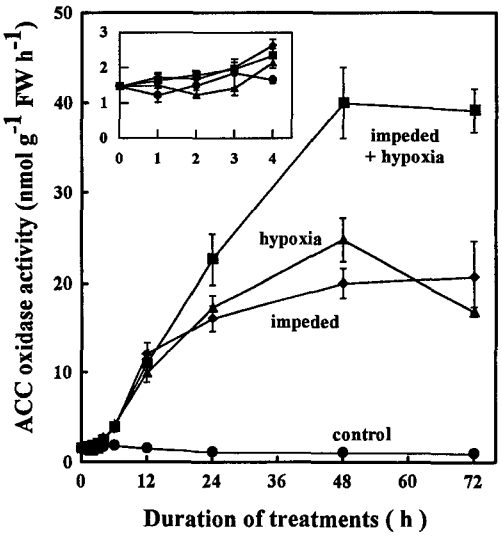

Figure 4. Effects of 4% O_2 (hypoxia), mechanical impedance, and hypoxia plus impedance on the ACC oxidase activity of root tips of maize seedlings. The control conditions were the same as in Figure 1. The inset illustrates data for the first 4 h with an expanded scale. The experiment was repeated twice with four or more root tips analyzed per point. Results of a typical experiment are presented. FW, Fresh weight.

increase in ACC oxidase activity was comparable to that of ACC synthase in stressed roots, reaching 30- to 40-fold above control levels during impedance combined with hypoxia.

Cellulase Activity and Aerenchyma Formation

Promotion of cellulase activity and aerenchyma formation are secondary effects of hypoxia, resulting from elevated levels of ethylene (Atwell et al., 1988; He et al., 1994), and we hypothesized that similar responses might occur with mechanical impedance. In preliminary experiments, induction of cellulase activity and aerenchyma formation were not evident at 24 h of treatment or earlier (C.-j. He, S.A. Finlayson, M.C. Drew, W.R. Jordan, and P.W. Morgan, unpublished data), and, therefore, assays in this study began at 36 h.

At 36 h of treatment, cellulase activity was stimulated by hypoxia alone, impedance alone, and the combination of hypoxia and impedance (Fig. 5), and continuously increased for the duration of the experiment in all treatments. There was a trend for the effect of impedance to exceed that of hypoxia for the first 48 h and for the combination of the two stresses to have a greater effect than either alone at 60 h (Fig. 5). However, these differences were not statistically significant and, generally, cellulase activity was increased above the control similarly by hypoxia, impedance, and hypoxia plus impedance. Aerenchyma formation in roots was clearly stimulated by 48 h of hypoxia or impedance or the combination (Fig. 6). A continuous increase in the amount of aerenchyma present was observed with the duration of treatments. The total area of lysed cells approached 25% of the cortex by hypoxia or the combination of hypoxia with impedance and 20% by impedance only. No aerenchyma formation occurred in the control roots.

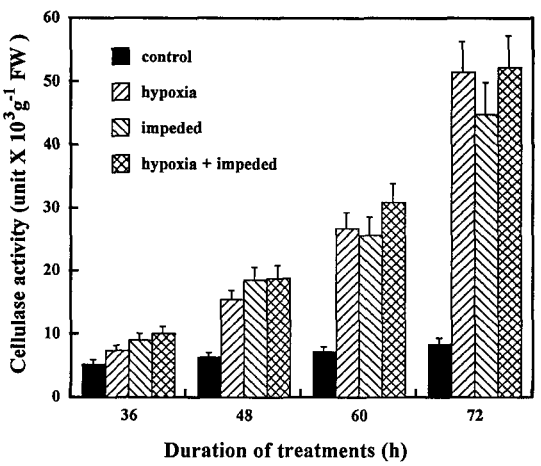

Figure 5. Effects of 4% O_2 (hypoxia), mechanical impedance, and hypoxia plus impedance on cellulase activity of maize seedlings roots. The control conditions were the same as in Figure 1. The number of experiments, replications, and presentation of SDs are the same as in Figure 2. FW, Fresh weight.

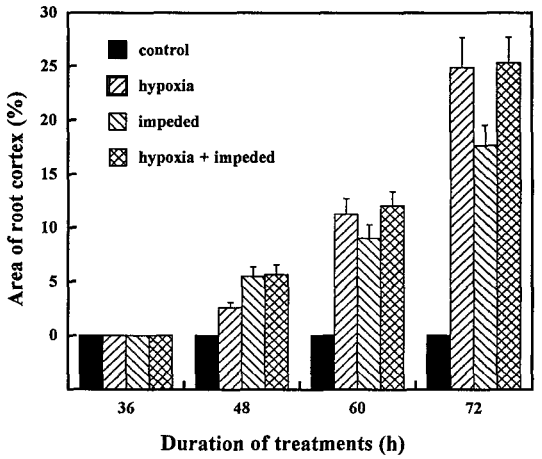

Figure 6. Effects of 4% O_2 (hypoxia), mechanical impedance, and hypoxia plus impedance on aerenchyma formation in maize seedling roots. Data are expressed as the percentages of lysed cortical cells in a cross-section of the root. The control conditions were the same as in Figure 1. Data are averages of three separate experiments with three root segments examined in each and SDs are shown as vertical bars (n = 9).

DISCUSSION

The rate of ethylene production by maize seedlings was shown to be promoted after only 1 h of mechanical impedance by Sarquis et al. (1991), and this promotion was fully consistent with increases in ACC concentrations, malonyl/ACC concentrations, and in vivo ACC synthase activity at 1 h at the level of impedance used in this study (100 kPa) (Sarquis et al., 1992). In the present study, mechanical impedance alone or in combination with hypoxia elevated ACC synthase activity and ethylene biosynthesis rapidly, with statistically significant differences at 1 h. Timing of the response to hypoxia, measured in the same experiments, was clearly different from that of mechanical impedance. The rate of ethylene production by hypoxic seedlings did not increase above control levels until 4 h, which is consistent with the data for ACC synthase activity, which showed that in roots there was a 3-h lag before activity increased, whereas in coleoptiles an increase in activity required more than 4 h (Figs. 2 and 3).

It was possible to distinguish small differences in the ethylene production rates in our experiments because the assay method allows the collection of a large sample of air flowing around intact seedlings. Because seedlings are not handled and air flow is continuous, the method eliminates variability that results from static containers or handling during sampling. The greater time lag with hypoxia cannot be due to the time taken to change the O_2 level from 21 to 4% and achieve equilibrium: considering the air space in the fritted clay medium and the flow rate, there would be eight void volume changes in 10 min, thereby decreasing the average O_2 content to 5.07% in 5 min and 4.07% in 10 min.

A major feature of the time-course data was a clear, synergistic effect of mechanical impedance and hypoxia applied together versus a separate application of these

stress treatments (Figs. 1–3). One interpretation of the data is that mechanical impedance and hypoxia promote ethylene biosynthesis by different mechanisms. This interpretation is supported by the fact that the mechanical impedance effect is faster by 2 to 3 h than the hypoxia effect (Figs. 1–3).

Our data do not reveal the mechanism by which either stress acts, except that both mechanical impedance and mechanical impedance plus hypoxia elevated ACC synthase activity at a time when there was no differential effect on ACC oxidase activity (Fig. 4). Timing of the promotion of ACC synthase by hypoxia alone (Fig. 2) was about the same as for the promotion of ACC oxidase by hypoxia (Fig. 4). In other species there is evidence for the effects of stress treatments on ethylene biosynthesis at both the transcriptional and posttranscriptional levels (Chappell et al., 1984; Felix et al., 1991; Huang et al., 1991; Spanu et al., 1993, 1994; Botella et al., 1995). It will be interesting to determine whether effects of mechanical impedance and hypoxia on ACC synthase activity are at the transcriptional or posttranscriptional level and whether some difference occurs between the two that relates to the observed synergism.

Our working hypothesis for the maize root stress responses has been as follows: stress → perception → signal transduction → promotion of ethylene biosynthesis → signal transduction → cellulase induction → cell-wall dissolution → aerenchyma formation (He et al., 1994). That promotion of ethylene biosynthesis is an early response is indicated by the fact that it is rapid (Sarquis et al., 1991), that ethylene biosynthesis and action inhibitors can block the effects of both impedance (Sarquis et al., 1991) and hypoxia (Drew et al., 1981; He et al., 1994), and that ACC synthase activity increases long before cellulase activity increases (He et al., 1994). In addition, the proposed signal transduction pathways may not be shared; several antagonists of specific steps in a putative signal transduction pathway have been found to block cell death, cellulase activity, and aerenchyma formation, but not reduce ethylene biosynthesis (He et al., 1996). Conversely, other antagonists promoted cell death, cellulase activity, and aerenchyma formation without promoting ethylene biosynthesis (He et al., 1996). The present data support the hypothesis shown above in that ethylene biosynthesis, ACC synthase activity, and ACC oxidase activity are all promoted in a few hours (Figs. 1–4), whereas no effect on cellulase activity was evident at 24 h (data not given). Furthermore, the initial increase in cellulase activity preceded aerenchyma formation by several hours (Figs. 5 and 6). We assume that the dissolution of cell walls, demonstrated in the formation of air spaces, is complex, and we assay cellulase as typical of a number of hydrolases presumed to be involved, with no suggestion that cellulase activation is exclusive.

We previously reported that ethylene signals the formation of aerenchyma in response to hypoxia or in response to short periods of N or P deficiency under normoxia, and that it induces an increase in cell-wall-degrading enzymes such as cellulases (He et al., 1994). Stimulation of cellulase activity or aerenchyma formation in maize roots by mechanical impedance or the combination of mechanical impedance plus hypoxia (Figs. 5 and 6), to our knowledge, has not previously been reported. Although cellulase activity and aerenchyma formation were both promoted by hypoxia and mechanical impedance, differential effects were minimal and there were no synergistic stimulations resulting from the combination of the two treatments (Figs. 5 and 6). This suggests that these responses result from a threshold level of ethylene. Above this threshold, further changes in ethylene concentrations, hypothetically, would have minimal effects on the induction of cellulase activity and aerenchyma formation. This suggestion is consistent with the earlier observations that aerenchyma formation was promoted by N or P treatments in roots, despite the lower ethylene biosynthesis rate (Drew et al., 1989), because of increased sensitivity of root cortical cells to ethylene (He et al., 1992). The role of aerenchyma formation in response to mechanical impedance is obscure unless the metabolites that are released during dissolution of cortex cells translocate to the root apex and provide respiratory substrates and osmolites to cells in the zones of cell division and expansion. Alternatively, the occurrence of the aerenchyma syndrome in mechanically impeded root stress may emphasize the general nature of the root signaling system.

It is widely assumed that ACC synthase regulates ethylene biosynthesis and the similarity of Figures 1 and 2 supports that assumption. However, the increase in ACC oxidase (Fig. 4) is dramatic, and the activity level is much greater than that of ACC synthase and well in excess of biosynthetic rates (Fig. 1). Thus, although ACC oxidase appears not to be involved in the initiation of the response to either hypoxia or mechanical impedance, the large increase in its activity appears to be involved in the large increase in ethylene biosynthesis during the 72-h time course of these experiments (Fig. 1). English et al. (1995) recently showed that ACC oxidase activity regulates ethylene production in response to flooding in tomatoes.

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