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Short Communication

Further Evidence that Cytoplasmic Acidosis Is a Determinant of Flooding Intolerance in Plants[1]

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ABSTRACT

We present two pieces of evidence that regulation of cytoplasmic pH near neutrality is a prerequisite for survival of root tips during hypoxia. First, blackeye peas and navy beans show earlier cytoplasmic acidosis under hypoxia than soybeans or pumpkin or maize, and die earlier. Second, when cytoplasmic acidosis in maize root tips is greatly retarded by treatment with 25 millimolar $Ca(NO_3)_2$, they remain viable under hypoxia for a much longer period of time than untreated hypoxic root tips. We also show that viability of maize root tips is unaffected by the supply of exogenous sugar (and so on the rate of ethanolic fermentation) for at least 16 hours of hypoxia.

We have presented several lines of evidence that cytoplasmic pH is an important factor determining the survival of plant root tips under hypoxia (7). Specifically, the length of time a plant root tip can remain viable (*i.e.* recover its normal functions when returned to an aerobic environment) is greatly reduced when extreme cytoplasmic acidosis occurs. Cytoplasmic acidosis occurs in hypoxic root tips at least in part because H^+ leaks from the acidic vacuoles into the cytoplasm (7). The greater intolerance of pea root tips to hypoxia, relative to maize root tips, can be attributed to leakage of acid from across the tonoplast earlier during hypoxia (6, 7). Cytoplasmic acidosis in maize root tips occurs earlier, and to a greater extent, under one of two circumstances. One condition is found in root tips with a limited capacity for ethanolic fermentation, due to a lack of alcohol dehydrogenase-1. Such root tips undergo significant lactic fermentation, and so cytoplasmic acidosis (6, 7). A second condition occurs in normal maize root tips in which escape from the tissue of CO_2, produced during ethanolic fermentation, is prevented (5, 7)—this weak acid consequently causing cytoplasmic acidosis. Both conditions significantly decrease the amount of time that the root tips can survive under hypoxia (7). With respect to these results, in this paper we answer two questions. First, in crop plants other than maize and peas, can the length of time that root tips survive under hypoxia also be accounted for by their ability to regulate cytoplasmic pH during hypoxia? And second, is there some way by which cytoplasmic acidosis can be delayed in hypoxic root tips and, if so, does prevention of cytoplasmic acidosis enhance the ability of root tips to remain viable under hypoxia?

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MATERIALS AND METHODS

Two mm root tips were used throughout from 2-d-old maize (hybrid Funk 4323; Germain's Seeds, Los Angeles, CA), 3-d-old California blackeye peas, 3-d-old pumpkin seeds (Jack-O'-Lantern; Ferry-Morse, Mountain View, CA), 3-d-old Navy beans (California bean growers association, Oxnard, CA), or 3-d-old soybeans (Prize; Burpee, Riverside, CA). Experiments were conducted at 23 ± 2°C, unless otherwise indicated in "Results and Discussion." Root tip samples were harvested and perfused as described previously (5, 6); all perfusion solutions included 0.1 mM $CaSO_4$. Cytoplasmic pH was determined by ^{31}P nuclear magnetic resonance as described previously (5). Tolerance of root tips to hypoxia was measured as the ability of root tips to recover and grow for 48 h in air (with glucose) after various periods of time under hypoxia; when the root tips were unable to grow, they were considered dead (7). The rate of ethanol production was measured enzymically as described previously (6).

RESULTS AND DISCUSSION

Cytoplasmic pH Regulation and Tolerance of Hypoxia in Root Tips of Three Species of Bean. The cytoplasmic pH and viability of root tips of blackeye peas, navy beans, and soybeans during hypoxia is shown in Figure 1. Both blackeye peas and navy beans exhibit very poor cytoplasmic pH regulation, cytoplasmic pH decreasing throughout hypoxia. These results contrast sharply with the behavior of maize root tips (7) and even pea root tips (7), where cytoplasmic pH stabilizes near neutrality for at least a few hours (after the rapid initial pH decrease due to a transient lactic fermentation). Not only is cytoplasmic pH regulation poorer in these beans, but they survive for a much shorter period of time under hypoxia (~9 h) than maize (~25 h) and pea (~15 h) root tips. Soybeans exhibit better cytoplasmic pH regulation than navy beans or blackeye peas (Fig. 1), and survive approximately twice as long under hypoxia (Fig. 1). However, when soybean root tips are made hypoxic at 30°C, instead of the usual experimental temperature (23°C), they lose their ability to regulate cytoplasmic pH, and they die much earlier under hypoxia (Fig. 1). Root tips of pumpkin show tight pH regulation during at least 24 h of hypoxia, and they remain viable after this treatment (data not shown).

These results provide correlative evidence for the hypothesis that cytoplasmic acidosis is a determinant of the length of time a root tip can survive under hypoxia. Root tips exhibiting tighter

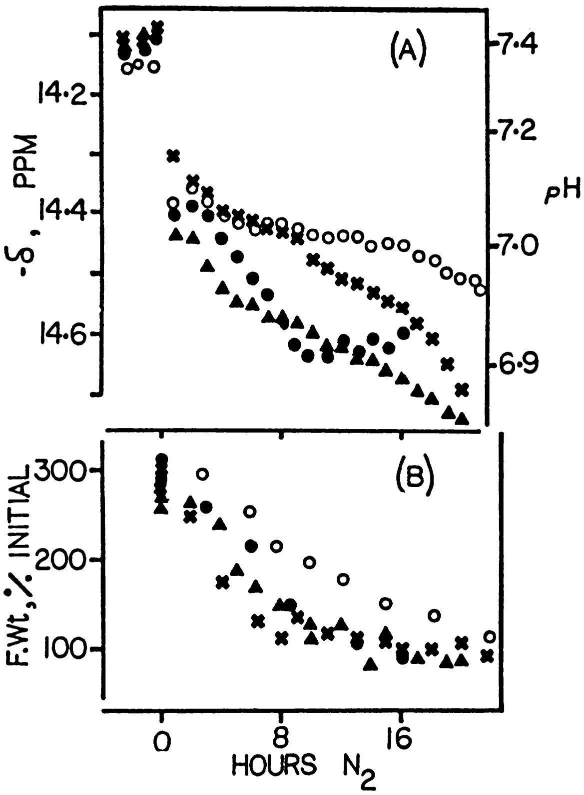

FIG. 1. Cytoplasmic pH in hypoxic bean root tips (A), and their subsequent increase in fresh weight during 48 h in air after the indicated period of hypoxia (B). (×), Blackeye peas, 23°C; (▲), navy beans, 23°C; (O), soybeans, 23°C; (●), soybeans, 30°C.

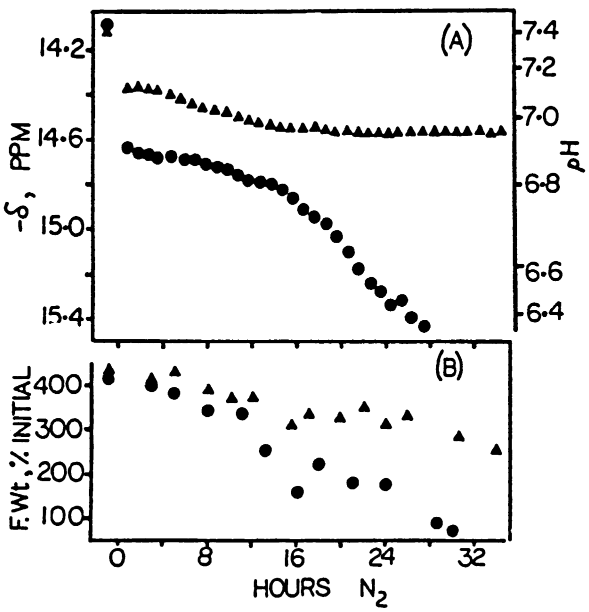

FIG. 2. Effect of 25 mM $Ca(NO_3)_2$ on cytoplasmic pH (A) and growth of maize root tips in air for 48 h after the indicated period of hypoxia (B). (▲), Root tips pretreated with O_2-saturated 50 mM glucose for 2 h followed by O_2-saturated 50 mM glucose plus 25 mM $Ca(NO_3)_2$ for 6 h, then treated with N_2-saturated 50 mM glucose plus 25 mM $Ca(NO_3)_2$. (●), Root tips pretreated with O_2-saturated 50 mM glucose for 8 h, then treated with N_2-saturated 50 mM glucose.

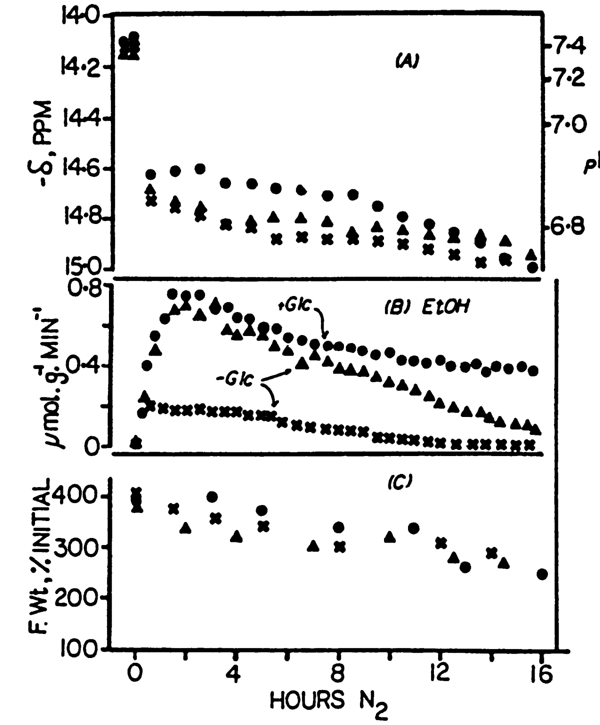

FIG. 3. Effect of exogenous glucose on cytoplasmic pH (A), rate of ethanol (EtOH) production (B), and increase in fresh weight during 48 h in air after the indicated period of hypoxia (C), in maize root tips. (●), 9 h oxygenated 50 mM glucose pretreatment, then N_2-saturated 50 mM glucose; (▲), 9 h oxygenated 50 mM glucose pretreatment, then N_2-saturated water; (×), 9 h oxygenated water pretreatment, then N_2-saturated water.

cytoplasmic pH regulation during hypoxia survive longer.

Retardation of Cytoplasmic Acidosis and Prolongation of Viability in Hypoxic Maize Root Tips by $Ca(NO_3)_2$. The effect of 25 mM $Ca(NO_3)_2$ on cytoplasmic pH and viability in maize root tips is shown in Figure 2. Over ~32 h of hypoxia, cytoplasmic pH in the nitrate-treated root tips is always nearer neutrality than that in control root tips. And nitrate-treated root tips clearly have a greater capacity to grow in air after periods of hypoxia up to ~32 h (Fig. 2). These results again suggest that viability of hypoxic root tips can be determined by cytoplasmic pH. They also offer a possible explanation for the observation of Arnon in 1937 (1; see Ref. 4 for review) that growth of barley in nonaerated solutions is better when the nitrogen source is nitrate, rather than ammonium. We speculate that nitrate results in less severe cytoplasmic acidosis by permitting continued operation of the tricarboxylic acid cycle—nitrate reductase acting as a sink for oxidation of mitochondrial and cytoplasmic NADH. Our experimental conditions should have resulted in considerable induction of nitrate reductase, and accumulation of metabolically available nitrate, prior to hypoxia—thereby facilitating subsequent anaerobic nitrite production (2). Such action would necessarily result in inhibition of lactate dehydrogenase, and so decrease the amount of cytoplasmic acidosis during the first few minutes of hypoxia (Fig. 2) (6).

Exogenous Glucose Has Little Effect on Maize Root Tip Viability during Hypoxia. Webb and Armstrong (8) described experiments in which the supply of exogenous carbohydrate has a dramatic effect on the length of time root tips can survive anoxia. When we compared the viability of maize root tips in solutions with or without 50 mM glucose, no such dramatic effect was apparent (Fig. 3). This was so despite a glycolytic rate in glucose-starved root tips 4 to 20 times lower than that in glucosefed root tips (Fig. 3) (and, incidentally, a rate even lower than that in a maize mutant lacking alcohol dehydrogenase-1 [7]. This discrepancy between our results and those of Webb and Armstrong could be due to one of two reasons. One possibility is species differences, since maize was not a subject of their study. However, we think it more likely that the difference is a result of use of plant material of very different physiological condition. Thus, plants used by Webb and Armstrong were grown under very low light intensities; and, for many experiments, roots were grown in agar such that the O_2 concentration at the root apex was ~5%—an O_2 tension capable of severely limiting respiration in root tips (3). It is possible, then, that their plant roots were under more initial stress than the root tips used in the study described here. This view is supported by the fact that the 'aerobic' roots of Webb and Armstrong showed a considerable lag before growth increased in response to exogenous glucose. This contrasts with our findings with aerobic, excised maize root tips. If depleted of endogenous sugar by exposure to distilled H_2O for 48 h, maize roots increase in fresh weight by only ~16%—compared to a ~310% increase in fresh weight for root tips grown in 50 mM glucose. However, if the glucose-depleted root tips are then supplied with 50 mM glucose, they then increase in fresh weight by ~260%. This indicates that the vigor of the maize root tips was not severely affected by the supply of exogenous sugars, compared to the root tips studied by Webb and Armstrong (8). One may conclude that the particular factors which determine the viability of root tips under hypoxia depends on the physiological condition of the tissue prior to the onset of hypoxia.

CONCLUSIONS

The evidence that cytoplasmic pH is a determinant of viability in plant root tips can be summarized as follows. (a) If severe cytoplasmic acidosis occurs in root tips, viability is reduced (7). (b) Cytoplasmic acidosis, and death, occur eventually in root tips of many crop plants, apparently because of leakage of H^+ from vacuoles into the cytoplasm (7). (c) Plant root tips that can survive for longer periods of hypoxia show better cytoplasmic pH regulation (this paper and Ref. 7). (d) Retardation of cytoplasmic acidosis in hypoxic maize root tips by treatment with $Ca(NO_3)_2$ prolongs their viability.

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