Effects of Submersion, pH, Time, and Certain Inhibitors on Maize and Rice Root Respiration

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Introduction

This study is a continuation of experiments on the respiration of cereal root-tip segments (2). In the previous study marked contrasts were found between the respiratory responses of maize and rice to increases in external oxygen concentrations with the maize roots exhibiting the greater stimulation in both oxygen input and carbon-dioxide output. Factors in the present study include submersion, pH, time, and certain chemical treatments. This study further compares maize and rice root-tip respiration responding to inhibitors which have been shown to influence respiratory pathways (3).

Materials and Methods

One-centimeter root-tip segments of maize (Zea mays) were supplemented by similar segments of rice (Oryza sativa) roots. Oxygen input and carbon-dioxide output were measured by standard methods with Warburg manometers (6). Gas-exchange rates were determined for previously soaked root segments transferred to moist filter paper in Warburg flasks. Differential treatments included concurrently submerged segments, contrasted pH, duration of exposure, and the addition of various test reagents. All rate measurements were made at 25° C and expressed as "Q" values (μl/hr x mg dry wt.).

Results and Discussion

Effect of Submersion

The rate of oxygen input by root-tip segments directly exposed to moist air was 20% higher than with those submerged. This was true for both the maize and rice roots (Figure 1). This relates to the limited solubility of oxygen in warm aqueous solutions (6) restricting the rate of oxygen supply to the submerged roots. Also, the rate of oxygen input by the rice roots was 40% higher than by the maize. This is believed to be related to the finer, more filamentous texture of the rice roots which permitted more rapid penetration of oxygen to the respiratory centers.

Effect of pH and Time

Oxygen-input rates of the control root tips were essentially equal at pHs 4.4, 6.5, and 9.3 during hour-1 (Figure 2). The range of QO_2 values was from 5.9 to 6.1. However, hour-4 measurements of the controls indicated a decrease in rate with the higher pHs so that the measured rate of oxygen input at pH 9.3 was only 75% of that at pH 4.4, and the rate at near neutrality was intermediate. In addition, it should be noted that the oxygen-input rates of the controls at hour-4 were substantially lower at all pHs then during hour-1 owing to a depletion of limited respirable substrate in the excised root-tip segments.

Effect of Cyanide

Figure 2 also shows a marked inhibition in oxygen input in hour-1 by preim-

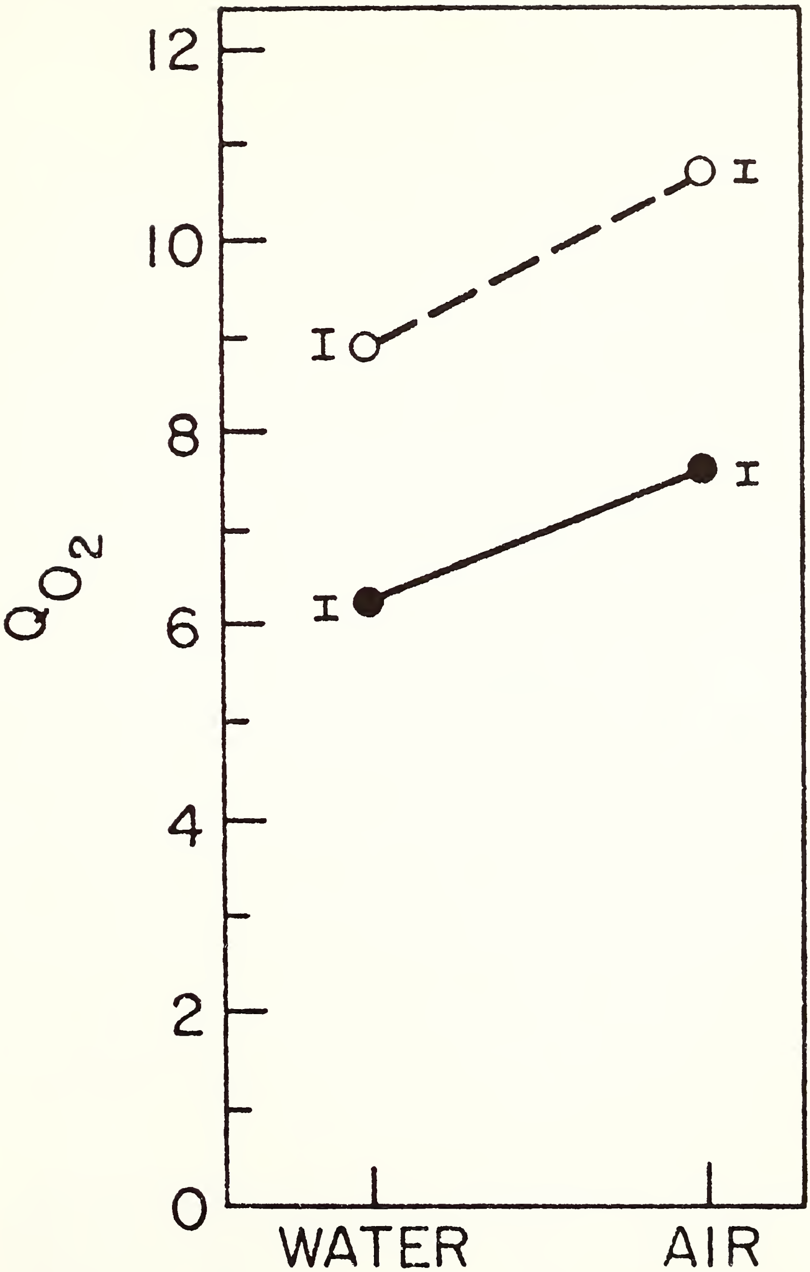

FIGURE 1. *O_2-input rates in water and in air, ○— — rice, ●— — maize; bars represent standard errors of triplicates.*

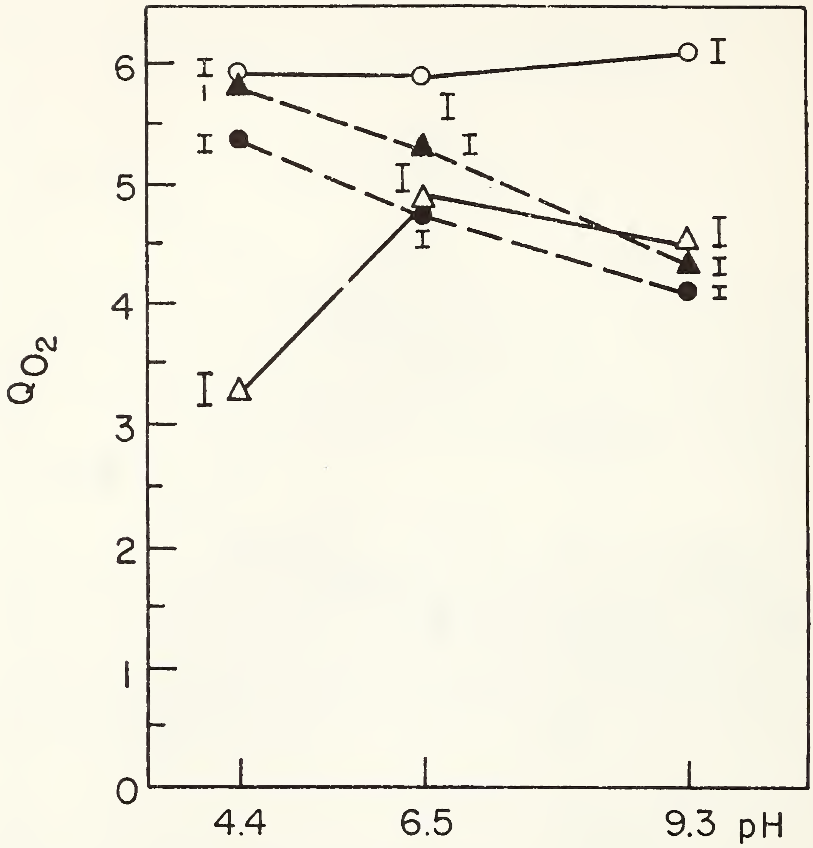

FIGURE 2. *O_2-input rates by maize root-tip segments influenced by pH, time, and cyanide.* ○— — *controls, hour-1;* ●— — *controls, hour-4;* △— —cyanide treated, hour-1; ▲— —cyanide treated, hour-4.

mersion of the root-tip segments in buffered 1 x 10^{-3} M KCN solution. This inhibition was roughly 50% and occurred at pH 4.4. On the other hand, hour-4 oxygen input exhibited recovery at pH 4.4 and some stimulation at all pHs. This loss of inhibition is attributed to volatilization of the cyanide. Cyanide inhibition of respiration is ascribed to a blocking of the respiratory oxidation chain involving cytochromes (1, 3).

Dipyridyl Effects

The responses in air of maize and rice root-tip segments to pretreatment with 1 x 10^{-3} M dipyridyl at pH 4.4 are shown in Figure 3. Dipyridyl strongly inhibited the input of oxygen reducing it to approximately 50% of the control rate. This was true for both the maize and rice root-tip segments and is attributed to blockage of aconitase which converts citric acid to isocitric acid in the citric-acid cycle (3). As with oxygen input, carbon-dioxide output was also inhibited by dipyridyl. Here the maize roots exhibited an inhibition of 40%, the rice 30%. These lower values compared with the effect on oxygen input indicate less carbon dioxide-output inhibition

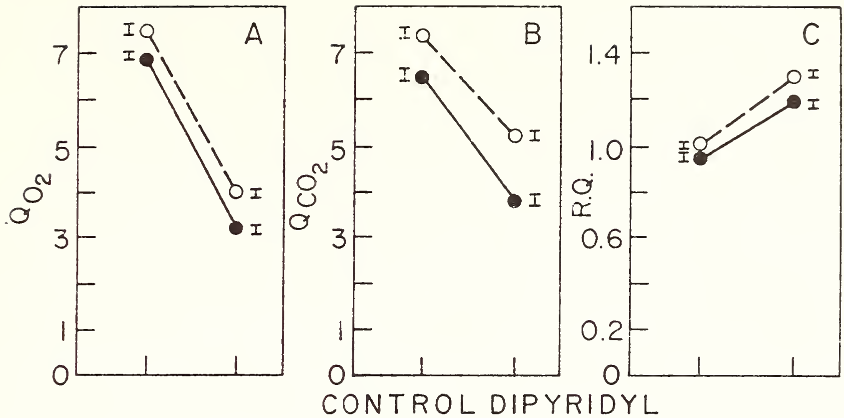

FIGURE 3. *Dipyridyl effects on root-tip segment gas exchanges.* ○— — *rice,* ●— — *maize. A, O_2 input; B, CO_2 output; C, respiratory quotient (R.Q.).*

owing, in part, to anaerobic carbon-dioxide production. The lower inhibition with the rice roots is considered to be due to a greater capacity for fermentation activity.

In consequence of less inhibition of carbon-dioxide output than oxygen input, RQ values were increased. With maize the average controls RQ of 0.95 was increased to 1,2, and increase of 25%. Similarly, rice root tips exhibited an increase from 1.0 to 1.3.

Dipyridyl plus Dieca

In experiments with 5 x 10^{-4} M dieca (diethyldithiocarbamate) it was necessary to renew the dieca treatments every 15 minutes because of its rapid decomposition (4). With the maize-root segments the dieca treatment over a 90 minute period depressed oxygen input 11% (Figure 4), in contrast to dipyridyl's 50%. When the dieca treatment was combined with dipyridyl, the oxygen input was depressed 34%. This, compared with dipyridyl alone, gave 16% less depression in oxygen-input rate.

Somewhat similar relationships by the rice-root segments were evident. Treatment with dieca alone gave an inhibition of 27% compared with the 11% for maize. However, when the dipyridyl and dieca were combined in a single treatment, oxygen input was depressed 10% less than with dipyridyl alone. Thus with both maize and rice, the addition of dieca alleviated the inhibitive action of the dipyridyl. This suggests competition in the penetration and action by the two inhibitors. Also, the greater response by rice than by maize to these inhibitors is thought to be related to the finer texture of the slender rice roots facilitating easier penetration and action. Incidentally, the work of James and Ward (5) with barley and wheat roots showed a species contrast for the dieca-induced inhibition with the barley roots the more sensitive. The difference was thought due to different levels of ascorbic-oxidase activity.

Dipyridyl plus Citrate

Maize root-tip segments pretreated with 1 x 10^{-3} M dipyridyl again showed marked depression in oxygen input. The addition of 3.3 x 10^{-2} M sodium citrate, however, decreased the dipyridyl inhibition of oxygen input 10%. This decrease is

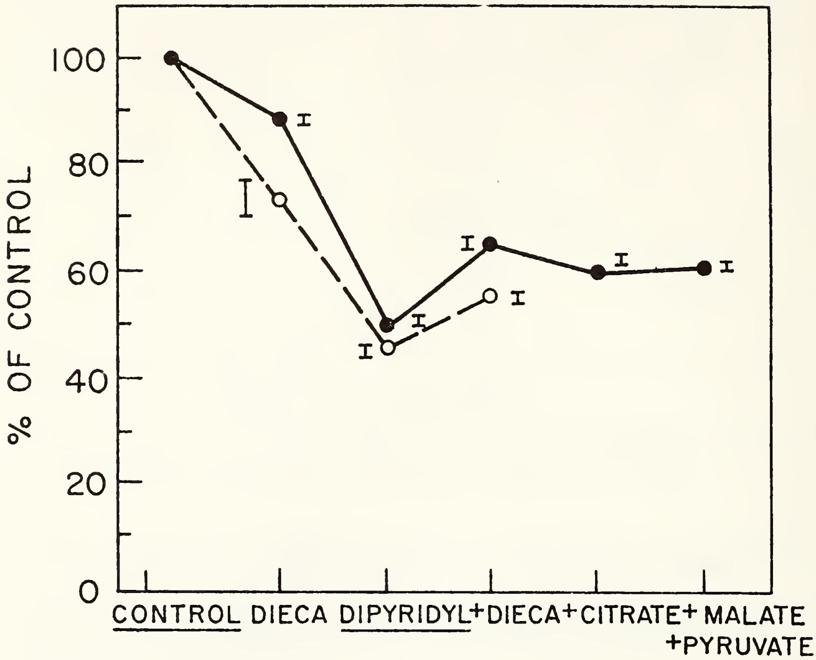

FIGURE 4. *Effects of dieca, dipyridyl, dipyridyl plus dieca, dipyridyl plus citrate, and dipyridyl plus malate and pyruvate.* ○— — *rice,* ●— — *maize.*

ascribed to a promotion of the citric-acid cycle by partially reversing the dipyridyl inhibition at the citric-acid to isocitric stage (3).

Dipyridyl plus Sodium Pyruvate and Malic Acid

The addition of 3.3 x 10^{-2} M sodium pyruvate and 3.3 x 10^{-2} M malic acid to dipyridyl-treated maize root tips decreased the dipyridyl inhibition of oxygen input 11%. This is equivalent to a 22% increase in oxygen input calculated on the basis of the dipyridyl-inhibited rate. This effect may be attributed to a promotion of the acetyl-CoA and succinyl-CoA steps in the respiratory chain (3).

Conclusions

Factors such as submersion and cyanide, during the first hour of exposure, acted to restrict oxygen input owing to the relatively low solubility of oxygen in water, and to interference of the respiratory oxidation chain in the case of cyanide. Dipyridyl, an iron chelating agent, greatly reduced both oxygen input and carbon-dioxide output. Since the decrease was more marked with the oxygen input than with the carbon-dioxide output, an increase resulted in the RQ. All of these effects took place in both species. Dieca, a copper-reacting agent, was found to be less inhibitory, either alone or when combined with dipyridyl, indicating competition between the two inhibitors. A similar effect was observed with citrate and with pyruvate plus malate.

Literature Cited

1 . FOGG, G. E. 1963. The growth of plants. Penguin Books Ltd., Harmondsworth.

2. GIRTON, R. E. 1979. Effects of oxygen concentration on the respiration of excised root-tip segments of maize and rice, and of germinating grains of rice and buckwheat. Physiol. Plant. 46:58-62.

3. JAMES, W. O. 1971. Cell respiration. English Universities Press. London. 138 p.

4. JAMES, W. O. and GARTON, N. 1952. The use of diethyldithiocarbamate as a respiratory inhibitor. J. Exp. Bot. 3:310-318.

5. JAMES, W. O. and WARD, M. M. 1957. The dieca effect in the respiration of barley. Proc. Roy. Soc. B. 309-315.

6. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F. 1957. Manometric techniques. Burgess Pub. Co., Minneapolis. 338 p.

Figures for "Effects of Submersion, pH, Time, and Certain Inhibitors on Maize and Rice Root Respiration" by Raymond E. Girton.

Q_{O_2} and Q_{CO_2} are rate values as ul gas per hour and per mg dry weight. R.Q. values are CO_2/O_2 ratios.