Critical Oxygen Pressure for Growth and Respiration of Excised and Intact Roots

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ABSTRACT

A method based on the measurement of ATP/ADP ratios is described. It permits the determination of the critical respiratory oxygen pressure of any organ, or part of any organ, of an intact plant. The data obtained by this method with intact maize (Zea mays L. INRA 508) root tips are compared with polarographic determinations on similar excised tissues.

When internal O₂ transport from the aerial part was prevented, the critical oxygen pressure found for the respiration of intact tips was similar to that found with excised tips. It was close to 10 kilopascals in a humid atmosphere and about 30 kilopascals in a liquid medium. Flooding of the gas spaces by vacuum infiltration did not modify these results. When internal O₂ transport from the aerial parts of the plant occurred, significantly lower values were obtained in liquid medium for the critical oxygen pressure, which shifted from more than 21 to 6 kilopascals. The higher values observed with excised root tips, compared to those obtained with intact tissues, can be explained by the lack of internal O₂ transport, rather than by the flooding of gas spaces.

Data are presented which show that root growth started to be limited at a significantly higher pressure than the respiration. These results are attributed to nonrespiratory oxidative processes with a low affinity for O₂ involved in root elongation.

Subterranean organs of higher plants are frequently exposed to large fluctuations of the pO₂¹ in their local environment. These fluctuations can range from 21 kPa, which is the value of the pO₂ in air, and represents an absolute maximum probably never encountered even in very well aerated soils, to values close to zero in flooded soils. In contrast to marsh plants, internal transport in mesophytes plays only a limited role in the O₂ supply of nonadapted plants (12, 13) and cannot meet the respiratory requirements of buried organs which draw most of their O₂ from the rhizosphere. The harmful effects of low O₂ tensions will greatly depend on the restrictions imposed on the respiration which provides most of the energy required by these nonchlorophyllous tissues. Consequently, the precise determination of the COP, which corresponds to the lowest value of the pO₂ that saturates the respiration, appears of particular interest for defining the sensitivity of a tissue to decreases in O₂ tension in the surrounding medium. The value of the COP depends very much on the diffusion barriers acting on the diffusion of O₂ from the external medium to its target: the mitochondrial Cyt oxidase. Whereas the COP of Cyt oxidases in aroid spadices (14) is about

10 Pa, values higher than 10 kPa are usually reported for excised root tips (2, 4, 6). Armstrong and Gaynard (1) have developed an indirect method for measuring the internal O₂ concentration in intact roots which gives significantly lower COP values, of about 2 kPa, for several root tips. Using an electrical analog which mimics root respiration, they concluded that the actual value of the COP of intact tissues should be very much lower than that of excised ones. They impute this discrepancy to a large increase of diffusional impedance induced by the flooding of gas spaces in excised tissues.

The differences between the results observed by the different authors arise mainly from the fact that great difficulties are encountered in the measurement of the respiratory rate of nonexcised tissues.

We have established the existence of a close correlation between the value of ATP/ADP ratios and the respiratory rate under conditions of limiting O₂ tension (12). This correlation permits a quantitative assessment of the relative respiratory rate of any organ, or part of organ, in intact plants (12). Using this relationship, we have compared the COP of excised and intact maize root tips and the results are discussed in relation to the O₂ transport and root growth under conditions of limiting O₂ tension.

MATERIALS AND METHODS

Maize seeds (Zea mays L. INRA 508) were vacuum infiltrated with a mineral nutrient solution (11) and germinated for 3 d in the dark at 25°C between sheets of filter paper soaked with the same solution and inclined at an angle of 45°. In the subsequent experiments, 1-cm-long excised primary root tips or intact seedlings were used. The temperature was 25°C. In some experiments, the seedlings were infiltrated three times under vacuum in order to fill the gas spaces with the incubation medium.

Respiratory Measurements. The effect of pO₂ on the O₂ uptake by excised root tips was studied by polarographic methods essentially as described in Saglio et al. (12), except that NaF was not used. When respiratory measurements were performed in the liquid medium, the chamber of the polarographic cell was filled with the nutrient solution containing 0.1 mM glucose. The solution was pre-equilibrated by bubbling with a gas mixture containing 50% O₂ and 50% N₂. Ten tips were immersed in the solution and bubbles were carefully eliminated when closing the cell. The latter was subjected to a vigorous magnetic stirring while measurements were being performed. The minimum stirring required was determined by the fact that increasing the stirring did not modify the respiratory rate.

Effect of pO₂ on Adenine Nucleotide Ratios. Determinations of adenine nucleotides of root tips placed in a gaseous environment were made either on intact seedlings or on excised tips. The plant material was preincubated for 1 h in the nutrient solution supplemented with 0.1 mM glucose and aerated by air
bubbling. For each determination with excised tissues, five tips were blotted off, placed in 50-ml beakers and treated as described in Saglio et al. (12).

With intact seedlings, the determinations were carried out in either gaseous or liquid environments, on the tip of the main seminal root (about 10–12 cm long) as described in Saglio et al. (12). In some experiments specified in the text, the seed was maintained in the same gaseous environment as the root.

The nucleotides were extracted from the frozen tips and assayed according to Saglio and Pradet (11).

Growth Rate as a Function of \( \frac{\text{PO}_2}{1} \). Intact seedlings were settled on disposable syringes in the dark at 25°C, essentially as described in Saglio et al. (12) for the determination of nucleotide ratios. The main seminal root was maintained under a continuous stream of various gas mixtures (\( \text{O}_2/\text{N}_2 \)) either in a wet gaseous atmosphere or in a nutrient solution. The seeds were always in air, covered by damp cotton wool and aluminum foil to avoid dessication. The root length of 6 to 9 seedlings was measured at the beginning of the experiment and 17 or 24 h later for each \( \text{PO}_2 \) studied. In some experiments carried out at 21% O\(_2\), the growth was also measured after 3 and 7 h.

RESULTS

Results obtained from typical polarographic determinations of \( \text{O}_2 \) uptake, using excised maize root tips at 25°C, are presented in Figure 1. This experiment was repeated several times and always gave similar results. In a gaseous atmosphere, the COP was usually close to 10 kPa (about 10% \( \text{O}_2 \) at atmospheric pressure) and sometimes fluctuated between 8 and 15 kPa, depending on the experiments. In the liquid medium, even with vigorous stirring, the COP of similar root tips usually rose to values close to 30 kPa.

Trying to explain this difference in terms of flooding of air spaces, as postulated by Armstrong and Gaynard (1), we used infiltrated tips. Using a Warburg respirometer, we checked that this treatment did not modify the respiratory rate of the tissues (245 nmol \( \text{O}_2/1 \) cm tip-h), which remained constant for several hours in the presence of 0.1 M glucose. The infiltration did not modify the value of the COP (about 10 kPa) in gaseous environments (Fig. 2).

As explained in a former paper (12), the correlation between the ATP/ADP ratio and the rate of \( \text{O}_2 \) uptake at low respiratory rates was established in the presence of \( \text{NaF} \) in order to render it more precise. In the present study, our interest was focused essentially on the COP corresponding to a range of high respiratory rates. Under these conditions, the use of \( \text{NaF} \) has little effect and was therefore omitted from these experiments. Under these conditions, the ATP/ADP ratio starts to fall at a \( \text{PO}_2 \) similar to that which limits the \( \text{O}_2 \) uptake (Fig. 2). Hence, it seems reasonable to assume that the measurement of ATP/ADP ratios of any organ, or part of that organ, of intact plants as a function of \( \text{PO}_2 \) will permit the determination of their COP. The results of such experiments are shown in Figures 3 and 4. In a gaseous environment (Fig. 3), when the seeds are in air, the ATP/ADP ratio of the root tips starts to fall when the \( \text{PO}_2 \) is close to 10 kPa, which is similar to what was observed for excised tips. This value was not modified when the internal \( \text{O}_2 \) transport was limited by maintaining the seeds at the same \( \text{O}_2 \) tension as the roots, nor when it was stopped by flooding the air spaces by infiltration (12). In the liquid medium (Fig. 4), the observed values were 6 kPa, for the seeds in air, and more than 21 kPa when internal \( \text{O}_2 \) transport was prevented by maintaining the seeds in \( \text{N}_2 \). Here again, the latter value approaches that found for the excised tips. The ATP/ADP ratio may change more or less sharply with the \( \text{PO}_2 \) and its maximum value may differ from one experiment to another (Figs. 2 and 3). These differences were apparently independent of the experimental treatment to which the plant material was submitted. It was more likely linked to the enzymic transformation step of ADP to ATP during the adenine nucleotide assay. The yield of this transformation may vary slightly from experiment to experiment, due to some inhibitory effect of the plant extract. A small variation in the ADP determination can induce a relatively large difference in the value of ATP/ADP ratio, specially for the high values when the amount of ADP is low. In Figure 2, however, a slight effect of filling the gas spaces cannot be completely ruled out. This does not detract from the point that the COP did not change.

In the studies of root elongation as a function of \( \text{PO}_2 \), the
seeds were always in air in order to favor phloem loading, which is an essential step for the supply of sugar to the distal sinks and, hence, for the elongation of root tips. Consequently, in these seedlings, there was some internal O₂ transport from the seed to the root tip. Under these conditions, the growth rate remained constant during the time of the experiment for all the pO₂ studied (Fig. 5), but was limited, both in the liquid medium and in the gaseous environments, for pO₂ lower than 21 kPa. At 10 kPa, the growth rate was only 50% to 60% of its maximum, whereas respiration in both cases was not limited (Figs. 3 and 4). In the absence of O₂, the elongation was nil and, after 24 h, the root tips (0.5–1.5 cm) had died. However, when transferred back to air, the rest of the root was able to initiate some secondary roots. These experiments were repeated three times and gave similar results.

FIG. 4. Values of ATP/ADP ratios of 1-cm root tips of intact plants as a function of pO₂ in the liquid medium at 25°C. (Filled circle), the seed was maintained in N₂; (O), the seed was in air. Each point is the mean of three determinations carried out on three independent samples. Bars represent the range of the measurements.

FIG. 5. Elongation rate of the main seminal maize root at various pO₂ in the liquid medium at 25°C. The seeds were maintained in 21% O₂ (air). Nine seedlings were measured for each pO₂. The bars represent the mean of three independent experiments; the bars represent the SD of the means. Inset: Elongation of the main seminal plant as a function of time at various pO₂ in the liquid medium. The data obtained in gaseous environments are superposable to those obtained in the liquid medium.

DISCUSSION

The values of the COP found with excised maize root tips confirm the results reported by other workers using similar material (4, 6). Values of the same order have been found with onion (2) and rice roots (4, 6), as well as with germinating rice (4) and lettuce seeds (8). These values are high (10 kPa and more) and strongly dependent upon the environment of the root, rising from 10 kPa in a moist gas phase, to 30 kPa in the liquid medium. A similar observation has been made by Yocum and Hackett (14), using aroid spadices. These authors do not attribute this difference of affinity for O₂ in gaseous and liquid environments to the flooding of air spaces, but rather to the increase in resistance to O₂ diffusion created by the layer of static water surrounding the tissues. However, one may argue that, in addition to the external water layer, the flooding of liquid spaces might contribute significantly to the decrease of the apparent O₂ affinity in liquid media (1).

In most root tips of mesophytes not previously adapted to hypoxic conditions, the gas-filled porosity is very low (6, 7), especially in the meristematic region which shows the greatest respiratory activity. In such tissues, flooding of air spaces should have very little effect on the diffusion of O₂. Consequently, it is unlikely that such flooding could account for the high values of the COP found for excised tissues. These suggestions are strongly supported by the fact that vacuum infiltration of the tissues did not modify their COP in a gaseous atmosphere and by the fact that, in the absence of internal O₂ transport, the COP of intact root tips, estimated by the measurement of ATP/ADP ratios, was similar to that of excised ones. Because of the good agreement between the respiratory rate and the values of the ATP/ADP ratio for limiting O₂ tensions in excised tips, we assume that the pO₂ which induced the decrease of this ratio in intact root tips represents the actual value of the COP. Since the flooding of cortical air spaces cannot be evoked in the case of intact plants, we believe that they represent the true values. They are high and are essentially the same as those found for excised tips.

The differences between the COP values found in gaseous and liquid environments cannot be explained in terms of the flooding of gas spaces, but rather in terms of an O₂ diffusion barrier.
created by the film of static water absorbed onto the root surface, even under conditions of vigorous stirring. Variations of the thickness of this film, due to the presence of variable amounts of hydrated slime, may explain the fluctuations of the COP observed for various batches of root tips submitted to similar experimental conditions.

On the other hand, the results presented here (Fig. 4) clearly show that the internal O₂ transport from the aerial part of the plant can significantly contribute to a decrease of the apparent COP of the tip in liquid media. In gaseous environments, this contribution was apparently negligible (Fig. 3). One reason for this could be that O₂, which tends to flow via the least resistant pathways, namely the gas spaces, escaped all along the transport pathway into the external gaseous medium and did not reach the tip. In the liquid medium, the situation was different. The diffusion coefficient of O₂ in water being 10⁴ times lower than in air, the transported O₂ could not escape through the film of water surrounding the root. This jacketing effect contributes to the efficiency of the internal O₂ transport for the respiration of distal sinks. In our material, not previously adapted to hypoxic conditions, the aerenchymas are not at all developed and this contribution is relatively low (12). However, the internal O₂ transport induced a significant, but limited decrease of the external COP. With plants adapted to internal O₂ transport, the presence of aerenchymas, either constitutive (10) or induced by hypoxic conditions (3), allows a better ventilation of the tissues, especially in media having a strong jacketing effect such as agar (5). From our results, the external COP of intact root tips of such plants should be very much lower than the COP of similar excised tissues, in which no O₂ transport can occur.

The COP values of excised root tips may indeed be higher than that of intact tips and should be considered with some caution, not because of the flooding of gas spaces, as suggested by Armstrong and Gaynard (1), but because the internal O₂ transport from the aerial part is prevented by the excision. These values, however, are representative of those of intact tissues presenting little or no internal O₂ transport.

The results obtained for the growth as a function of the pO₂ are particularly interesting. We expected to find a close correlation of these results with the respiratory rate. Such a correlation would permit the use of the growth rate as an assessment of the affinity of intact root tips for O₂. In fact, it appears that the pO₂ which started to limit the growth is significantly higher than the COP for respiration. Preliminary results (not shown) indicate that neither the number of cells nor the number of mitotic figures in the part of the root which had elongated during the experiment were modified until the pO₂ reached values lower than 1 kPa. These data suggest that it was not the cell division rate which was decreased due to an increased resistance to O₂ flow in the meristematic region, brought about by the interaction of the high respiratory activity of the meristematic cell body and the low porosity of the tissue, but more likely that some nonrespiratory oxidative process with a low affinity for O₂ was implied in root elongation.

The present paper does not allow any conclusion about the nature of these processes to be drawn, but it shows that the pO₂ in the rhizosphere is a major limiting factor for root growth, even if internal transport can partly balance the O₂ deficiency of the external medium. Moreover, it provides another example of the usefulness of adenine nucleotide ratios as indicators in in vivo studies of non-green cells under conditions where the metabolic activity is limited, as emphasized by Pradet and Raymond (9).

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