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In vivo measurement of plant respiration

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Abstract

Respiration is vital; it is the essence of life. Respiration is the mechanism by which energy obtained during the photosynthesis process is transformed into biochemical energy, in the form of ATP. This transformation of energy keeps all cells in all organisms alive. While energy conversion is the main function of respiration in animals, respiration has several other functions in plants. Among them, interactions with photosynthesis such as photorespiration and the production of carbon skeletons for the many compounds synthesized in plants (e.g., pigments, proteins and secondary metabolites). Therefore, it comes as no surprise that such a key role of respiration in plants promoted intense effort to investigate its regulation. Nevertheless, the interactions with other simultaneous processes make its measurement in plants very challenging. In animals, respiration can be simply measured as CO2 or O2 exchange with the atmosphere since there are no other processes performing similar gas exchange. In contrast, in plants, respiration produces CO2 and consumes O2 simultaneously with photorespiration.

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Essay 11.9

In Vivo Measurement of Plant Respiration

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Introduction

"Absentis est quos non anhelo"
"Dead is who does not respire"

Respiration is vital; it is the essence of life. Respiration is the mechanism by which energy obtained during the photosynthesis process is transformed into biochemical energy, in the form of ATP. This transformation of energy keeps all cells in all organisms alive. While energy conversion is the main function of respiration in animals, respiration has several other functions in plants. Among them, interactions with photosynthesis such as photorespiration and the production of carbon skeletons for the many compounds synthesized in plants (e.g., pigments, proteins and secondary metabolites). Therefore, it comes as no surprise that such a key role of respiration in plants promoted intense effort to investigate its regulation. Nevertheless, the interactions with other simultaneous processes make its measurement in plants very challenging. In animals, respiration can be simply measured as CO_2 or O_2 exchange with the atmosphere since there are no other processes performing similar gas exchange. In contrast, in plants, respiration produces CO_2 and consumes O_2 simultaneously with photorespiration. Moreover, photosynthesis performs the exact reverse process (Figure 1). The combined reaction can be described as:

$$(CH_2O) + O_2 \rightleftharpoons CO_2 + H_2O$$

(Photo) Respiration

Photosynthesis

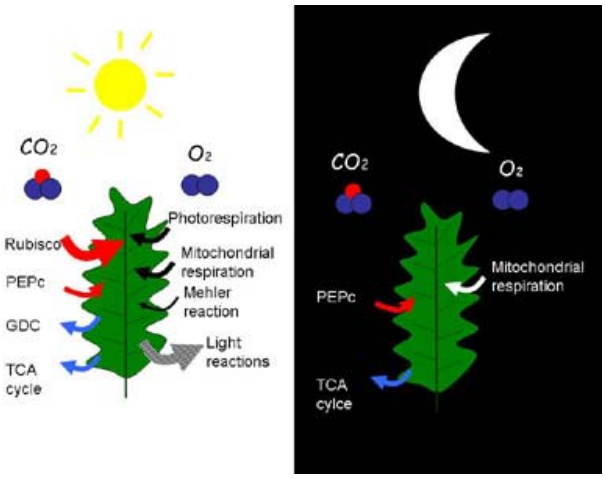

Figure 1 Oxygen and carbon dioxide gas exchanges between the atmosphere and a leaf, in the light and in the dark (Modified from Hurry et al., 2005). PEPc, phosphoenolpyruvate carboxylase; GDC, glycine decarboxylase; TCA cycle, tricarboxylic acid cycle. **(Click image to enlarge.)**

General Aspects on How To Measure Respiration

Biochemically, respiration can be divided in three major parts: 1) glycolysis, 2) tricarboxylic acid (TCA) cycle, and 3) mitochondrial electron transport. The first series of reactions occur in the cellular cytosol, while the other two take place inside the mitochondria. Usual respiration measurements involve gas exchange, that is, O_2 consumption and CO_2 evolution rates. Gas exchange methods use either an open (comparison of inlet air of known composition with outlet air enriched in CO_2 or depleted in O_2) or a closed system (in which the CO_2 raises or O_2 decreases with time). Such a principle applies to dark-respiration. Other methods (e.g., isotopic

measurements) are used in the light to distinguish CO_2 and O_2 fluxes caused by respiration from those due to photosynthesis or photorespiration.

During respiration, oxygen is consumed by terminal oxidases of the mitochondrial electron transport chain. In contrast to vertebrate animals, which only contain a single oxidase (the cytochrome oxidase), plants possess two terminal oxidases (the cytochrome oxidase and the alternative oxidase; McDonald 2008). Unlike the cytochrome oxidase, the alternative oxidase is resistant to cyanide (hence cyanide-resistant respiration) but is inhibited by salicylhydroxamic acid (SHAM). These two terminal oxidases compete to draw electrons from the ubiquinone pool to reduce oxygen to water. Unlike the cytochrome oxidase, the alternative oxidase is not linked to ATP synthesis (see Web topic 11.3) and the energy is released as heat, for example, to warm the floral ovens of thermogenic plants (see Web essay 11.6 and Watling et al 2006). The role played by the alternative oxidase in non thermogenic plants is an active research topic and specific methodologies (see below) are required to differentiate respiration via the two pathways.

Measuring Respiration in The Dark (*Night Respiration*)

In the absence of light, most of the CO_2 production and O_2 consumption is due to respiration. While CO_2 is produced in the decarboxylation reactions (pyruvate decarboxylase and TCA cycle), oxygen is mostly consumed by the terminal oxidases of the mitochondrial electron transport chain. In order to avoid transient metabolic activities following darkening, known as Light Enhanced Dark Respiration, measurements of night respiration must be performed after 20-30 minutes acclimation to darkness. Carbon dioxide production can be measured with an Infra-Red Gas Analyzer (IRGA) in both open and closed systems. The advantage of this method is that the organ measured can remain attached to the whole plant. The main drawbacks of this method, especially for open systems, are its low sensitivity, as the CO_2 gradient between the inlet and outlet air can fall within the noise/signal ratio of the measuring system (1-2 ppm), and possible leakage around the gaskets of the cuvettes (Hurry et al., 2005).

Measurements of oxygen consumption with oxygen electrodes (Clark et al., 1953) are good indicators of the total respiratory rate. This method is performed in a closed cuvette, either in liquid or gas phase and the main disadvantage is that it must be performed on detached tissues.

Since oxygen can be consumed by either the cytochrome or alternative oxidases, specific methodologies are required when we wish to measure the specific contribution of either pathway. Whilst the capacity of each of these two oxidases can be determined with the oxygen electrode after poisoning tissue with SHAM (cytochrome oxidase capacity) or KCN (alternative oxidase capacity), the actual contribution of each oxidase to total respiration can only be measured with the oxygen isotope fractionation technique (Guy et al., 1989).

The Oxygen Isotope Fractionation Technique

This oxygen isotope fractionation technique, based on Isotope Ratio Mass Spectrometry (IRMS), measures changes in the isotopic composition of oxygen molecules ($^{18}O/^{16}O$) during respiration in a closed system. Both oxidases favor ^{16}O over ^{18}O because it requires less energy to break the $^{16}O=^{16}O$ bond than the $^{18}O=^{16}O$ bond. Therefore, the proportion of ^{18}O ($^{18}O/^{16}O$ ratio) in the air remaining in a closed cuvette that contains a respiring tissue increases as oxygen is decreased due to respiration. The respiratory isotope fractionation is obtained from measurements of the oxygen isotope ratio ($R=^{18}O/^{16}O$) and the fraction of unreacted oxygen remaining in the respiration cuvette at different times during the course of the reaction.

A complete description of the calculations of the isotope fractionation (D) can be found in Guy et al. (1989). It results in the overall equation as follows:

$$D = \frac{\ln (R/R_o)}{\ln ([O_2]/[O_2]_o)}$$

Although both oxidases prefer to use ^{16}O over ^{18}O, their "degree of preference" differs, allowing the calculation of their relative contribution to the total mitochondrial oxygen consumption and electron transport. The two mitochondrial terminal oxidases have a different $^{18}O/^{16}O$ fractionation because they have different catalytic mechanisms to break the double bond of O_2 (Hoefs, 1987). The alternative oxidase fractionates more against ^{18}O than the cytochrome oxidase (Guy et al., 1989). Figure 2 shows a classical experiment in which fractionation by both cytochrome (D_c) and alternative oxidases (D_a) is measured in the presence of the specific inhibitors SHAM and KCN, respectively. Once fractionation values for each respiratory pathway have been obtained, measurements of the fractionation in the absence of any inhibitors (D_n) give the electron partitioning through the alternative pathway (τ_a).

$$\tau_a = \frac{D_n - D_c}{D_a - D_c}$$

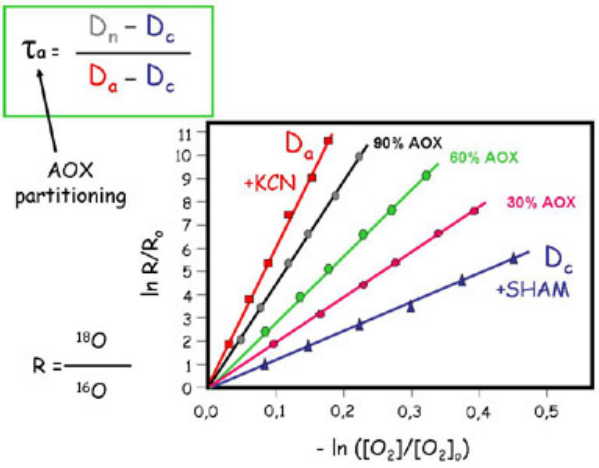

Figure 2 Calculation of the electron partitioning through the alternative oxidase(τ_a) using the oxygen isotope fractionation technique. This is an example of the lines expected in different situations where the participation of the alternative pathway (τ_a) would be approximately 90%, 60% and 30%. This could be the case for the floral receptacle of Nelumbo nucifera (Watling et al., 2006), cotyledons and leaves of *Glycine max* (Ribas-Carbo et al., 2005), respectively **(Click image to enlarge.)**

Measuring Respiration In The Light (Day Respiration)

Measurement of respiration in the light is much more challenging than in the dark, because photorespiration and photosynthesis also occur, thereby masking the O_2 and CO_2 exchanges that are due to mitochondrial respiration. Moreover, there is evidence that the rate of mitochondrial O_2/CO_2 exchange is depressed in the presence of light as compared to the rate in darkness. Several methods have been proposed to estimate the rate of respiration in the light, some of which are briefly explained here but for detailed reviews of all methods see Hurry et al. (2005) and Tcherkez et al. (2005). For a proper understanding of these methods, it should be kept in mind that in the presence of light, leaf gas exchange can be described as:

$$A_N = A_G - PR - R_d,$$

where A_N is the "net" photosynthesis (determined with a standard open gas exchange system), A_G is gross CO_2 assimilation, PR is photorespiration and R_d is respiratory CO_2 evolution in the light. The first published method employed to measure respiration in the light was the so-called Kok method (Kok, 1948). It is based on the inhibitory effect of light on the rate of respiration, and it only requires the use of a commercial CO_2 gas exchange system. It is based on a light-response curve performed at very low light intensity. Generally, the net CO_2 assimilation rate shows an abrupt reduction in slope as light levels increase (Figure 3). The linear extrapolation to zero light from the gradual, "upper" slope indicates the rate of respiration in the light or "day" respiration (R_d) while the steeper part reaches the *y* axis (zero light) at the night respiration rate (R_n) (See Textbook Chapter 9 for more information on light and CO_2 compensation points).

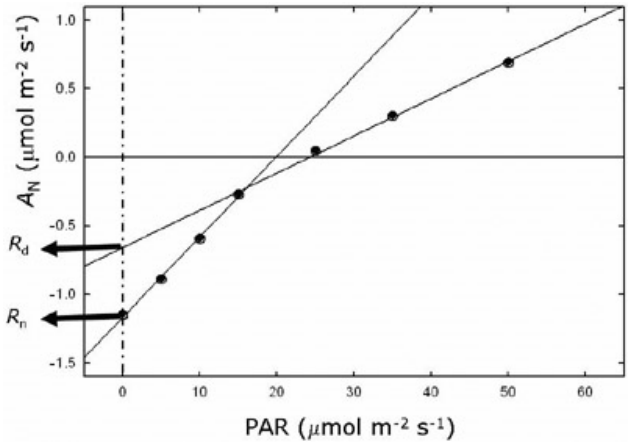

Figure 3 Kok method applied to detached leaves at 21°C in 21% O_2 (typical graph redrawn from the dataset of Tcherkez *et al.* 2008). A_N, Net photosynthesis; PAR, Photosynthetically active radiation; R_d, day respiration; R_n, night respiration. **(Click image to enlarge.)**

The Cornic method (Cornic 1973) is performed on an illuminated leaf placed in CO_2-free air, in either N_2 (0% O_2) or 21% O_2, and then darkened. The CO_2-production rate in the light is denoted as L_O (in 21% O_2) or L_N (in N_2). When the leaf is darkened, refixation of (photo) respired CO_2 vanishes and a peak of CO_2 production, represented as *p*, can be seen. Under several simplifying assumptions, it can be shown that $R_d = L_O - L_N - p + R_n$.

Another commonly used technique is the Laisk method (Laisk 1977). This is based on the assumption that whilst light *intensity* alters the rates of photosynthesis and photorespiration, it does not affect the rate of mitochondrial respiration. Using a standard CO_2 gas exchange system, assimilation response curves versus leaf intercellular CO_2 concentration are performed at several different light levels (Figure 4). As light decreases both gross photosynthesis and photorespiration will decrease proportionally and so will the slope of the response of net photosynthesis. Therefore, if R_d does not to change with light intensity, all curves should converge to a single point which will correspond to the CO_2 compensation point in the absence of day respiration (Γ^*) on the X-axis and $-R_d$ on the Y-axis (See Textbook Chapter 9 for more information on light and

CO_2 compensation points).

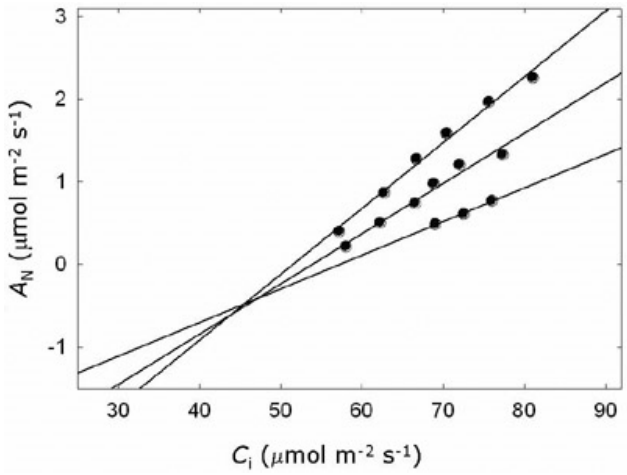

Figure 4 Laisk method. Net photosynthesis response to leaf internal CO_2 (c_i) at 200, 400 and 600 µmol m^{-2} s^{-1} (PAR) at 21°C in 21% O_2 in french bean (*Phaseolus vulgaris*). In this example, the day respiration rate R_d is 0.51 µmol m^{-2} s^{-1}. **(Click image to enlarge.)**

A more recent method uses stable isotopes of carbon to estimate the rate of respiration in the light (Loreto et al., 2001). This method takes advantage of the fact that "day" respiratory substrates turn over more slowly (half time in the order of minutes or more) than photorespiratory substrates (half-time in the order of seconds). That is, plants are grown under natural $^{12}CO_2$-atmosphere and then placed in a $^{13}CO_2$-atmosphere. The production of $^{12}CO_2$ measured in a $^{13}CO_2$-atmosphere indicates the "day" respiration rate R_d (with some corrections required to take into account refixation of respired $^{12}CO_2$).

These methods have contrasting advantages and disadvantages: for example, both the Kok and the Laisk methods are performed at low light intensities and, consequently, plants present small assimilation rates, causing large measurement variability. In addition, the Laisk method requires measurements at several light levels and it is plausible that "day" respiration varies with light.

To summarize, measuring respiration is a very challenging task, especially in the light, when substrates and products are interchanged by different and opposing reactions that operate simultaneously. In the dark, respiration consumes oxygen by two different oxidases that act simultaneously and, consequently, can only be differentiated using stable isotope techniques. Moreover, the recent discovery of the alternative oxidase in other phylogenetic groups means that microbiologists and invertebrate physiologists will also have to reassess their methodologies for measuring respiration and will have much to learn from plant physiologists.

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