Photosynthetic Assessment of Small Plants Using Red, Green, Blue LED Light Source and New Whole Plant Arabidopsis Chamber

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INTRODUCTION

- In the past, photosynthesis measurements on *Arabidopsis thaliana* have been difficult because plants and leaves are small.
- A chamber and light source for use with the LI-6400/LI-6400XT Portable Photosynthesis System have been designed to solve this issue.
- The new Whole Plant Arabidopsis Chamber overcomes past issues with gas exchange systems by enclosing whole plants, maximizing leaf area and eliminating the need for narrow approach angles.
- Light response studies with different colors were accomplished by using a new light source with red (460 ± 5 nm), green (522 ± 5 nm), and blue (635 ± 5 nm) LEDs.
- Photosynthetic measurements now can augment genetic and molecular toolboxes for Arabidopsis.

CHAMBER DESIGN

- The entire rosette is enclosed in a 7 cm diameter chamber.
- The chamber bottom seals to the growth container and can be used with either 38 mm Cone-tainers™ or 65 mm round pots (Figure 1).
- Chamber top uses an O-ring to quickly create an airtight seal.
- Short stabilization times for chamber CO_2 (< 40 s) and H_2O (< 60 s) speed throughput.
- Adjustable exhaust tube assembly allows adjustment of chamber exhaust flow rate (Figure 2).

Blocking Soil CO_2 Flux

- Containers of plant media, hereafter soil, were used in all experiments to validate chamber performance (Miracle-Gro Potting Mix™ forest compost, peat moss, perlite, wetting agent and 0.21 – 0.07 – 0.14 N-P-K).
- Measurement errors can result from CO_2 fluxes from containers of soil.
- Different methods of blocking soil CO_2 flux were tested by injecting pure CO_2 through a hole in the side of the container 1 – 1.5 cm below the soil surface.
  - Exhaust restrictions forced mass flow through the soil, partially suppressing CO_2 flux (Table 1).
  - A pottery clay cap decreased CO_2 flux into the chamber by 99.8%.
  - Suppression increased when an alternate exhaust path was provided by punching small vent holes (approx. 0.5 mm diameter) in the containers (Table 2).

Figure 1. Whole Plant Arabidopsis Chamber mounts to LI-6400/LI-6400XT Portable Photosynthesis System. Plant and container are sealed inside with an airtight O-ring or flange to facilitate rapid assessment of individual plants.

Table 1. Pottery clay formed an effective cap to block CO_2 flux from the soil. The pottery clay cap consisted of a 3 – 5 mm thick clay cap with a 4 mm hole in the center for a plant to grow through. Covering the soil with pottery clay altered the ΔCO_2 (maximum CO_2 concentration during spike – average CO_2 concentration prior to injection) following injection of 250 ml CO_2 through the side of the container just below the soil surface. The exhaust valve was partially restricted (approx. 25%), diverting 100 – 150 μmol s⁻¹ of flow through the soil and causing a small overpressure (0.15 kPa) in the chamber, which is slightly higher than the normal chamber pressure (about 0.02 kPa). The exhaust valve restriction had little effect on increasing the effectiveness of the clay cap. The values are the average ΔCO_2 ± standard error of the measurement for the number of samples indicated (n).

Injected gas	Hole Closed ΔCO_2	n	Partially Open ΔCO_2	n	Hole Open ΔCO_2	n
Pure CO_2	195.3 ± 21.8	5	60.9 ± 22.1	3	0.14 ± 0.06	5
CO_2-free Air	-0.07 ± 0.29	2			0.11 ±0.05	2

Table 2. Small vent holes in the side of the plant container created an exhaust path for mass flow to suppress CO_2 influx to the chamber. Different vent hole restrictions in the side of 38 mm Cone-tainers™ were measured by injecting pure CO_2 as above. The small vent holes in the container (~0.5 mm, 1 – 1.5 cm from container top) were either sealed, partially occluded or completely open. Mass flow from the injections was insufficient to cause a change in ΔCO_2 based on ambient air (not shown) and CO_2-free air injections. The values are the average ΔCO_2 ± standard error of the measurement for the number of samples indicated (n).

Soil Covering	No Over-pressure	n	Over-pressure	n
Uncovered	192.2 ± 1.0	2	52.7 ± 13.3	2
Clay Cap	0.33 ± 0.2	5	0.23 ± 0.06	5

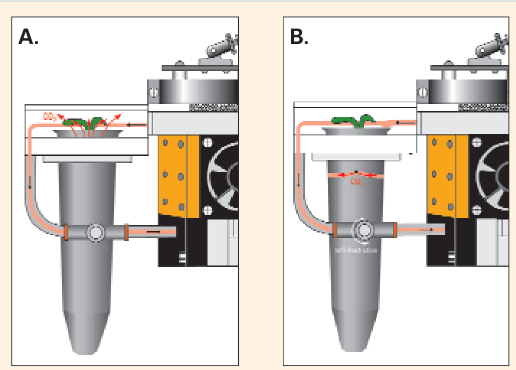
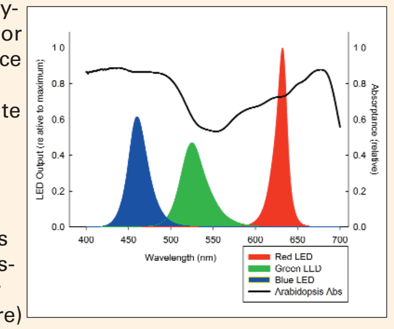


Figure 2. Exhaust path of the Whole Plant Arabidopsis Chamber with the optional Adjustable Exhaust Valve Assembly. The needle valve regulates flow through the exhaust tube and diverts air flow to the small vent holes in the container.
A. An unrestricted (open) exhaust path valve allows CO_2 to flow freely into the chamber from the roots, soil and outside the container.
B. Restriction of the needle valve to 25 – 50% decreases influx by diverting a portion of the flow through the soil and out the small vent hole in the container.

LIGHT SOURCE DESIGN

- 6400-18 RGB (red, green, blue) Light Source mates with the Whole Plant Arabidopsis Chamber top.
- Light intensities are from 0 – 2000 μmol m⁻² s⁻¹ of white light.
- Independently controlled red, green or blue LEDs (wavelength peaks are 460, 522 and 635 ± 5 nm, respectively) allow for color selection.
- Light spatial uniformity is ± 10% over 90% of the output area.

Figure 3. Plant responses to light colors can be measured by mounting the new RGB Light Source to the Whole Plant Arabidopsis Chamber top. Multiple independently-controlled diodes allow for color selection. The RGB Light Source achieves "white" light (equal quanta of each color). The white output spectrum derived from equal quanta of the three LED types shows the bandwidth of each color normalized to the maximum output. Arabidopsis leaf absorption spectrum measured with a spectroradiometer (LI-1800 with integrating sphere) on the adaxial side.



ARABIDOPSIS ROSETTE PHOTOSYNTHESIS

- Wild-type Arabidopsis (Col-0) were germinated on soil and transplanted 5 – 10 days post-germination to individual containers with and without pottery clay caps.
  - They were grown under short days at 175 μmol m⁻² s⁻¹ fluorescent light for 6 – 8 weeks.
- Standard pottery clay (3 – 5 mm thick) was used to cap the soil, with a 5 – 8 mm hole in the center for germination or transplanting seedlings.
- To minimize self-shading and maximize leaf area, Arabidopsis plants with about 10 cm² leaf area typically provided the best results (Figure 4).
- Minimum usable area was 1.5 cm² for healthy plants (Figure 5); mutants may require larger plants.
- Arabidopsis rosette photosynthetic responses to different wavelengths of light were similar despite differences in absorbance (Figure 6).

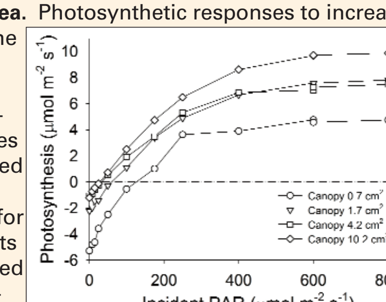


Figure 4. Minimum plant size for photosynthetic measurement is approximately 1.5 cm² leaf area. Photosynthetic responses to increasing light were measured in the Whole Plant Arabidopsis Chamber with the RGB Light Source. Exhaust tube restriction with both small vent holes and pottery clay caps was used to suppress soil CO_2 flux. White light response curves for larger-sized Arabidopsis plants (1.7, 4.2 and 10.2 cm²) produced consistent photosynthetic responses. The light compensation points for the entire rosette were 25 – 50 μmol m⁻² s⁻¹ and dark respiration rates were 1 – 2 μmol m⁻² s⁻¹, which are appropriate for C3 plants. The smallest plants (0.7 cm²) had too little leaf area to produce consistent results. Total plant leaf area was measured by photographing the plant and analyzing the image with ImageJ software (NIH, http://rsbweb.nih.gov/ij/).

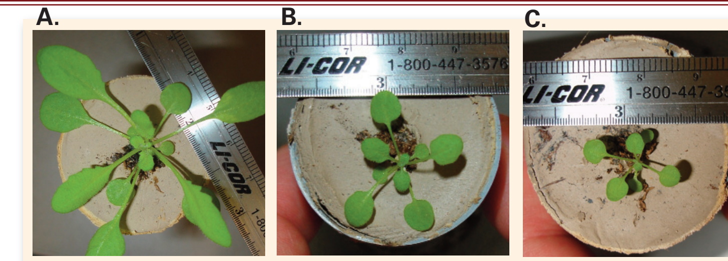


Figure 5. Examples of Arabidopsis leaf area: recommended, minimum and too small for reliable gas exchange measurements. Range of Arabidopsis in 38 mm Cone-tainers™ with pottery clay caps over soil. **A.** Recommended Arabidopsis plant (10 cm²) measured in the Whole Plant Arabidopsis Chamber has a rosette diameter of 5 – 7 cm, which generates a good signal for measuring photosynthesis with limited leaf self-shading. **B.** Minimum recommended size (1.5 cm²) for measurements to generate adequate signal (2.5 μmol mol⁻¹) for accurate measurements. **C.** Total leaf area is approximately 0.7 cm², which is too small to give adequate results.

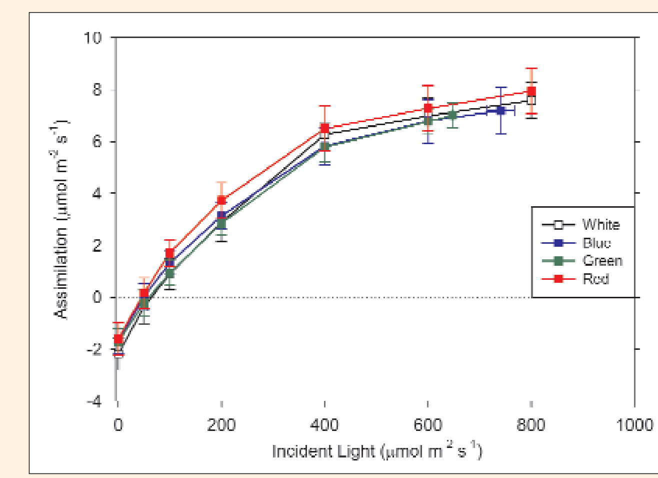


Figure 6. Photosynthetic responses of Arabidopsis to different light colors were similar. Light response of Arabidopsis rosettes under different colored LEDs measured as in Figure 4. The light intensity is incident on the plane of the majority of leaves of the rosette. The values are the average photosynthetic assimilation rate ± standard error.

CONCLUSIONS

- Whole Plant Arabidopsis Chamber and RGB Light Source make rapid gas exchange measurements possible, expanding molecular and genetic studies.
- Pottery clay capping and instrument adjustments suppress CO_2 from the soil and roots.
- Measurements are possible on plants as small as 1.5 cm² leaf area, with 10 cm² recommended.
- Photosynthetic responses to red, green, blue, or white light color were similar for Arabidopsis.

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