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Growth of *Arabidopsis thaliana* and *Eutrema salsugineum* in a closed growing system designed for quantification of plant water use

Jhon F. Sandoval[a], Chan Yul Yoo[b,1], Michael J. Gosney[a,b], Michael V. Mickelbart[a,b,*]

[a] Department of Botany & Plant Pathology, Purdue University, 915 W. State St., West Lafayette, IN 47907-2054, United States
[b] Department of Horticulture & Landscape Architecture, Purdue University, 625 Agriculture Mall Drive, West Lafayette, IN 47907-2010, United States

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ABSTRACT

The identification of genetic determinants for water-use efficiency (WUE) and their incorporation into crop plants is critical as world water resources are predicted to become less stable over the coming decades. However, quantification of WUE in small model species such as *Arabidopsis* is difficult because of low plant water loss relative to root zone evaporation. Furthermore, measurements of long-term WUE are labor-intensive and time-consuming. A novel high-throughput closed-container growing system for measuring plant WUE is described. The system eliminates nearly all water loss from the media and does not require irrigation throughout the duration of a typical experiment. Using the model species *Arabidopsis thaliana* and *Eutrema salsugineum*, it was confirmed that under growth chamber conditions, this system: (1) eliminates the need for irrigation for as much as 30 days with media water content remaining above 80% full capacity; (2) allows for quantification of WUE in plants with a leaf area as small as *ca.* 20 cm^2; (3) does not inhibit plant growth; and (4) does not alter media conditions outside of an acceptable range for these species. The growing system provides an efficient high-throughput system for quantifying plant water loss and WUE.

1. Introduction

Global environmental change has highlighted the need to develop crops with higher water-use efficiency (WUE) to maintain crop productivity in the face of an increasingly unstable global water supply (Cai et al., 2015). Genetic mechanisms underlying abiotic stress tolerance have been identified and, in some cases, exploited to improve field performance (Mickelbart et al., 2015). There are likely many genetic determinants that can positively impact WUE, so the ability to efficiently screen genetically diverse plant materials is critical. The ability to screen crop plants is improving rapidly (Araus and Cairns, 2014), but the contribution of model systems such as *Arabidopsis* has led to the discovery of important genes and will continue to contribute to our understanding of plant adaptation and crop improvement (Liu, 2010).

The efficiency with which plants use water is an important trait, especially in areas with limited rainfall, since high WUE is often correlated with drought tolerance (Juenger, 2013). Plant WUE can be expressed on an instantaneous basis as carbon assimilation over transpiration (WUE_i), or on a longer-term basis as the amount of water used to accumulate a given amount of biomass over a period of time (WUE_b) (Yoo et al., 2009). The former term does not incorporate temporal plant processes and differential response among genotypes to environmental perturbations (*e.g.*, Seversike et al., 2013), and therefore may be misleading as a screen for WUE.

The ability to screen large populations of *Arabidopsis* for WUE_b has been hampered partly because of the low signal (plant water loss) to noise (root zone water loss) ratio. To deal with this, the surrogate measurement of carbon isotope discrimination ($\delta^{13}C$ or $\Delta^{13}C$) has been used to estimate plant WUE. Plant $\delta^{13}C$ varies by genotype (*e.g.*, Barbour et al., 2010) and plants with high WUE tend to have low $\delta^{13}C$ because responsive stomata close during high vapor pressure deficit periods, and therefore fix more of the total carbon pool available in the substomatal cavity (Farquhar et al., 1982; Farquhar and Cernusak, 2012). Therefore, variation for this trait has been used as the primary surrogate for direct measurements of WUE (Rebetzke et al., 2002). However, although correlations between these traits are often high in C3 species, a large proportion of the variation remains unexplained (Farquhar

* Corresponding author at: Department of Botany & Plant Pathology, Purdue University, 915 W. State St., West Lafayette, IN 47907-2054, United States.
E-mail addresses: jfsandovalp@unal.edu.co (J.F. Sandoval), chanyul.yoo@ucr.edu (C.Y. Yoo), mjgosney@purdue.edu (M.J. Gosney), mickelbart@purdue.edu (M.V. Mickelbart).
[1] Current address: Department of Botany & Plant Sciences, University of California, Riverside, 4412 Boyce Hall, Riverside, CA 92507, United States.

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and Richards, 1984; Martin and Thorstenson, 1988; Nienhuis et al., 1994), suggesting that to identify genetic determinants of WUE, there is a need to directly and accurately assess genotypic differences in WUE_b.

When direct estimates of water loss of containerized plants are quantified, they are typically conducted in one of two ways: (1) water loss from control containers (without plants) is subtracted from total water loss of containerized plants, or (2) the container is sealed to prevent water loss from the root zone. Plants with large leaf areas that lose quantities of water much greater than that of media evaporation, such as tomato, provide a sufficient signal such that plant water loss can be calculated using control containers (Martin and Thorstenson, 1988). Conversely, small plants such as *Arabidopsis* with a small leaf area and typically low transpiration rates per unit leaf area due to the low light conditions of growth chambers have low transpiration relative to evaporation. The elimination of water loss from the root zone negates this signal to noise problem, but presents its own difficulties. Containers are often wrapped with a water-impermeable substance, such as plastic film. This process is time-consuming and may not completely seal the root zone. Also, if plants need to be irrigated during the course of the experiment the time needed for the removal and re-application of the water-impermeable barrier limits the number of experimental units that can be screened. Finally, it is possible that modifying the root zone in this way may lead to conditions that inhibit plant growth.

Although *Arabidopsis* has been the primary model plant species for at least three decades (Somerville and Koornneef, 2002), it is a difficult species on which to conduct physiological measurements. The ability to monitor growth of *Arabidopsis* in a high-throughput system has improved substantially in recent years (*e.g.*, Zhang et al., 2012; Dhont et al., 2013), but its small size makes adequate tissue collection for some measurements difficult and physiological processes such as CO_2 assimilation and transpiration are much lower compared to many crop species. Therefore, specialized protocols specifically for small-stature plants have been used to measure *Arabidopsis* physiological processes such as gas-exchange (Kölling et al., 2015).

Wild relatives of *Arabidopsis* that exhibit stress tolerance are increasingly being utilized as model plants (Orsini et al., 2010; Zhu, 2015). Of these, *Eutrema salsugineum* (formerly *Thellungiella salsugineum* or *T. halophila*) is genetically similar to *Arabidopsis* (Inan et al., 2004), but novel alleles have been identified, such as the *E. salsugineum* HKT1 homolog, that appear to underlie superior adaptive mechanisms, such as salt tolerance (Ali et al., 2012). Given the natural variation in water loss of *Eutrema* genotypes (Mickelbart lab, unpublished results), the screening of available diverse *Eutrema* germplasm may result in the identification of important novel WUE determinants.

A high-throughput closed growing system for quantifying water loss from small species such as *Arabidopsis* and *Eutrema* that eliminates water loss from the root zone and provides a growing environment for the plant that does not adversely affect media characteristics or plant growth and development is described. Data is provided to support its use as an efficient screen for plant water loss.

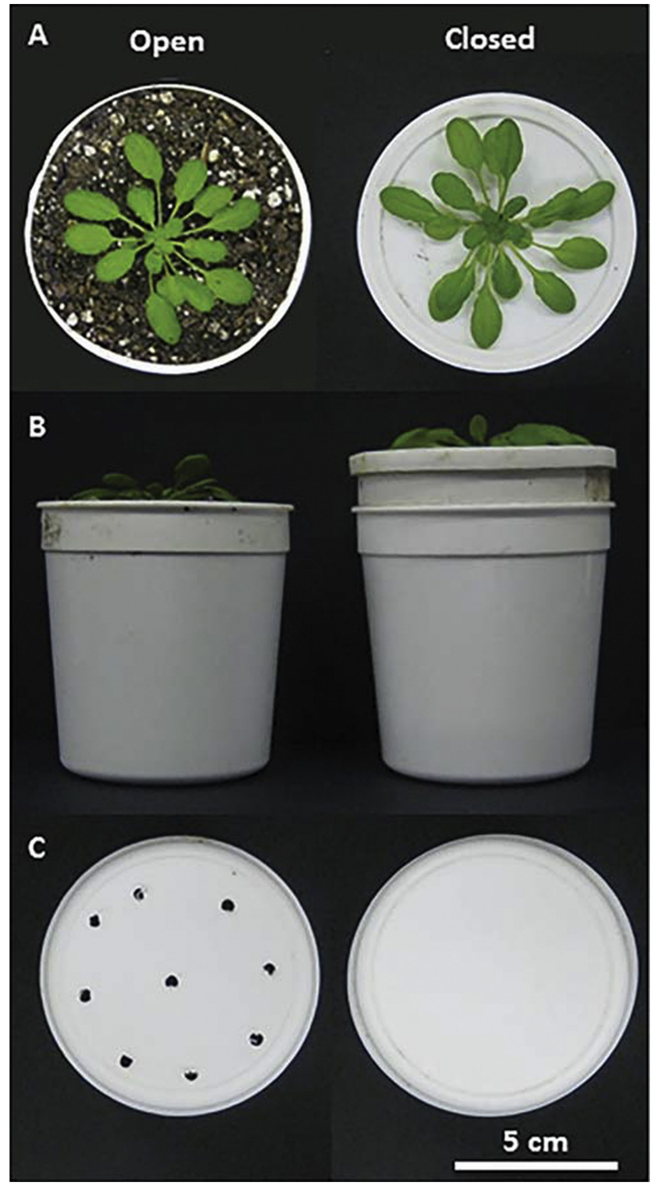

Fig. 1. Top (A), side (B), and bottom (C) views of open (left) and closed (right) containers used for quantifying plant growth and water-use efficiency. A container with drainage holes is filled with media and a lid with a 5 mm hole is placed on top. Seed is sown on the media surface, the container is sub-irrigated and allowed to drain, then placed in a second container without drainage holes.

2. Materials and methods

2.1. Container design and media

Treatments and growth conditions for all experiments are summarized in Table S1. A container system was developed using 465 mL white opaque disposable specimen containers (Berry Plastics Corporation, Evansville, IN, USA) (Fig. 1). Nine 4 mm holes were drilled into the bottom of each container for sub-irrigation and drainage. A 5 mm hole was drilled into the center of a lid that was used to cover the media surface. After irrigation, "closed" containers were placed in a second container without holes. "Open" containers were not placed in a second closed container and no lid was used to cover the media surface. For all experiments, Sungro Sunshine Mix #2 (Sun Gro Horticultural, Agawam, MA, USA) was used. Containers of both types were filled with 135–137 g of media or 205–208 g of media with Turface® calcined clay (Profile Products LLC., Buffalo Grove, IL, USA) with a ratio of 2:1 media:clay.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.jplph.2016.02.010.

2.2. Plant materials and growth conditions

Arabidopsis thaliana Columbia-0 (Col-0) and Shahdara (Sha) seed was stratified for 4 days and *Eutrema salsugineum* Shandong (SH)