Available online at www.sciencedirect.com
ScienceDirect
Plant Physiology and Biochemistry 46 (2008) 212–218
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Research article

Critical evaluation and statistical validation of a hydroponic culture system for *Arabidopsis thaliana*

Karen Smeets [a,*], Joske Ruytinx [b,a], Frank Van Belleghem [c], Brahim Semane [a], Dan Lin [d], Jaco Vangronsveld [a], Ann Cuypers [a]

[a] *Environmental Biology, Centre for Environmental Sciences, Hasselt University, Campus Diepenbeek, Agoralaan – Building D, B-3590 Diepenbeek, Belgium*
[b] *General Botany and Nature Management, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium*
[c] *School of Science, Open University Netherlands, P.O. Box 2960, 6401 DL Heerlen, The Netherlands*
[d] *Centre for Statistics, Hasselt University, Campus Diepenbeek, Agoralaan – Building D, B-3590 Diepenbeek, Belgium*

Received 11 April 2007
Available online 9 October 2007

Abstract

Arabidopsis thaliana is one of the most widely used model organisms in plant sciences. Because of the increasing knowledge in the understanding of its molecular pathways, a reproducible and stable growth set-up for obtaining uniform plants becomes more important. In order to be able to easily harvest and study both roots and shoots, and to allow simple exposure to water-soluble toxic substances, a hydroponic system is the desired cultivation method for controlled plant growth. Based on earlier developed hydroponic cultivation protocols, a hydroponic set-up was optimized and statistically validated using linear mixed-effects models. In order to determine important components that influence the level of variability in a hydroponic set-up, stress-related indicators were examined at the biochemical as well as at the molecular level. It is highly recommended that statistical as well as biological assumptions are carried out before post-analyses are performed. Therefore, we suggest a model where factors that influence variability such as the usage of different pots and harvesting on different times are taken into account in the analyses. Furthermore, in contrast to what has been reported in earlier studies, our findings indicate that continuous aeration of the hydroponic solution is highly important.

Keywords: *Arabidopsis thaliana*; Control growth condition; Gene expression; Aeration; Hydroponics

1. Introduction

Arabidopsis thaliana is one of the most widely used model organisms in plant sciences. Particularly in molecular research, *Arabidopsis* is of great interest and its widely available genetic information makes several molecular techniques less problematic to work with [1–3]. It is a very useful and popular species for studying gene function and regulation and for investigating (new) genetic pathways in normal conditions, but also in comparison with several stress situations. Furthermore, *Arabidopsis* is relatively easy to grow and transform and multiple mutants are available.

Using *Arabidopsis* as a model organism makes it possible to study contemporaneously at physiological, biochemical and molecular levels; however, a sufficient amount of biomass is required. On the other hand, from statistical point of view, the individual plant and/or sample variation has to be minimized. It is highly recommended to prevent variation in the controls induced by external factors. Gene expression is one of the most sensitive parameters and even small external variations can cause high variability in the transcriptome [4–6].

Abbreviations: ADH, alcohol dehydrogenase; CAT, catalase; GPX, guaiacol peroxidase; GR, glutathione reductase; GSSG, glutathione disulfide; LDH, lactate dehydrogenase; PDC, pyruvate decarboxylase; SOD, superoxide dismutase.

* Corresponding author. Tel.: +32 11 268319; fax: +32 11 268301.
E-mail address: karen.smeets@uhasselt.be (K. Smeets).

0981-9428/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.plaphy.2007.09.014

Several protocols have been described for the hydroponic cultivation of *A. thaliana* [7–14]. We combined some of these protocols to obtain a large-scale system with a high uniformity of plant material. In order to determine important components that influence the level of variability in a hydroponic set-up, stress-related indicators such as parameters of the antioxidative defence system were examined at the biochemical as well as at the molecular level. The level of variability and the reproducibility of the set-up were statistically evaluated using linear mixed-effects models. Furthermore, the need for aeration, even when plants are harvested in a quite young stage, was statistically evaluated in this cultivation system.

2. Materials and methods

2.1. Hydroponic cultivation

As a plant material, *A. thaliana* (Columbia ecotype) was used. The seeds were surface sterilized and, in order to synchronize the germination, incubated in dark for three days at 4 °C on a filter paper soaked with tap water.

Based on previous studies [7,8], a hydroponic system was developed to grow *Arabidopsis* plants simultaneously with a small individual variation.

2.1.1. Set-up

The bottom part (±9 cm) from 15 ml polyethylene centrifuge tubes was removed and rockwool plugs of 2 cm by 1.5 cm were placed in the remaining part of the tubes (Fig. 1). The rockwool-holding plugs were positioned in test tube racks that, for their part, were placed in aluminium-covered pots filled with nutrient solution (Fig. 2). The nutrient solution was based on Hoagland growth medium. Nutrient concentrations were tested and optimized: 0.505 mM KNO_3, 0.15 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.1 mM $NH_4H_2PO_4$, 0.1 mM $MgSO_4 \cdot 7H_2O$, 4.63 μM H_3BO_3, 0.91 μM $MnCl_2 \cdot 4H_2O$, 0.03 μM $CuSO_4 \cdot 5H_2O$, 0.06 μM $H_2MoO_4 \cdot H_2O$, 0.16 μM $ZnSO_4 \cdot 7H_2O$, 1.64 μM $FeSO_4 \cdot 7H_2O$ and 0.81 μM Na_2-EDTA. A few seeds were placed on top of each rockwool plug. The plugs were pre-moistened with the nutrient solution. To simplify the seed placement, seeds were brought in water and pipetted onto the plugs. A 12 h photoperiod at 65% relative humidity and day/night temperatures of 22 °C and 18 °C were used. Light was supplied by cool white fluorescent lamps (L 140W/20SA, Osram, Augsburg) at a photosynthetic photon flux density of 165 μmol m^{-2} s^{-1} at the leaf level above all pots. During the first three days of germination, pots were covered with glass plates to prevent the dehydration of the rockwool and to obtain an even more controllable and stable growth situation.

The nutrient solution was refreshed every three days based on the changes in pH and conductivity. After one week of

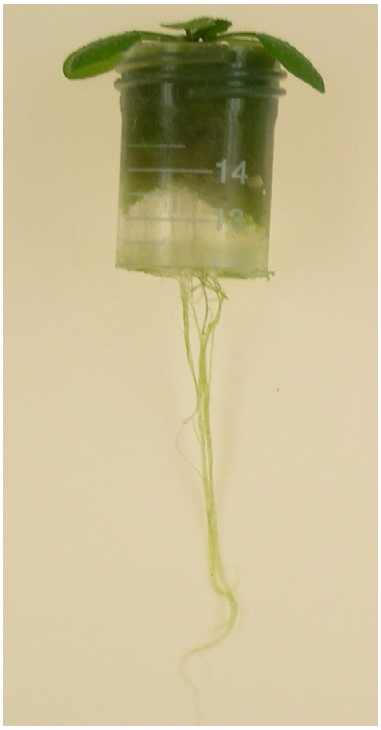

Fig. 1. Set-up of an individual rockwool-holding tube. Rockwool pieces of 2 cm by 1.5 cm are placed in the upper part of a 15-ml polyethylene centrifuge tube, as an inert support for the cultivated plant.

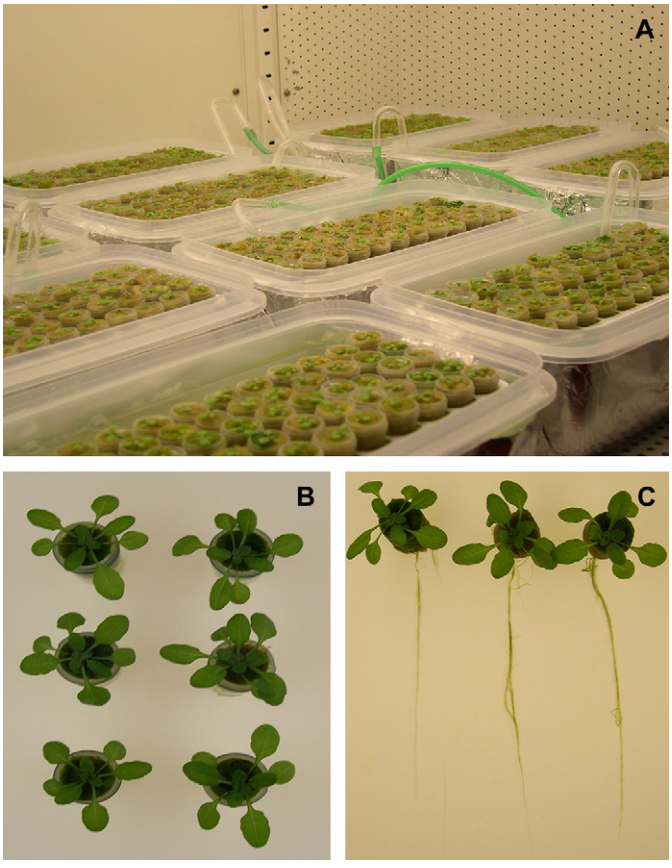

Fig. 2. (A) Set-up of the cultivation system. The rockwool-holding tubes are positioned in test tube racks that are placed in aluminium-covered pots filled with nutrient solution. The pots are continuously aerated. (B, C) *Arabidopsis* plants after three weeks of growth. Morphologically, no visual differences are noticed.