

Adaptation of *Arabidopsis* Plants to Tropical Aeroponics Using Cool Root Zone Temperatures

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How to cite this paper: Srikanth, S., Choong, T.W., Chu, J.S., He, J. and Chen, Z. (2017) Adaptation of *Arabidopsis* Plants to Tropical Aeroponics Using Cool Root Zone Temperatures. *American Journal of Plant Sciences*, 8, 3295-3312.
<https://doi.org/10.4236/ajps.2017.813222>

Received: September 23, 2017

Accepted: December 2, 2017

Published: December 5, 2017

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Abstract

Arabidopsis thaliana (L.) Heyhn. is a well known model plant in plant research. However, its growth conditions and diminutive stature associated with low biomass at maturity make it a challenging species for physiological studies. While in the tropical countries, it can only be grown either by tissue cultures or in growth chambers under controlled conditions. An aeroponic technique with $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ root-zone temperatures (RZT) was used to grow *Arabidopsis* (Columbia ecotype) in a tropical greenhouse with natural irradiance and high ambient temperature ($38^{\circ}\text{C}/28^{\circ}\text{C}$ day/night). Seedlings germinated in growth chambers at 20°C or 30°C . At 6 to 8 leaf stage, they were transferred to the aeroponic troughs with their roots exposed to constant temperature of $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ while their aerial parts were subjected to fluctuating ambient temperature from 28°C to 38°C . After a week, plants have acclimatised to both RZTs and started developing normal rosettes, bolted and yielded viable seeds. However, $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ RZT allowed them to recover from turgor pressure despite of wilting, and significantly increased biomass. Mature plants grown in each RZTs were compared morphologically and physiologically to the plants grown in growth chamber (GC) at 20°C (root and shoot) temperature with 60% relative humidity. Aeroponically grown plants did not experience photoinhibition, and also exhibited higher photosynthetic light usage efficiency and higher capacities of heat dissipation, compared to GC plants. This aeroponics with cool RZTs can allow the use of *Arabidopsis* as a model plant even under tropical climate.

Keywords

Aeroponics, *Arabidopsis*, Biomass, Seeds, Root Zone Temperature, Tropical

1. Introduction

Arabidopsis thaliana (L.) Heynh. has been widely adopted as a model plant of choice in many laboratories owing to its unique physiological features. It is a member of the largest flowering family, Brassicaceae, to which all radishes, mustard, cabbages and cauliflowers also belong. It grows as an ephemeral coloniser of temperate regions across the northern hemisphere. As a model plant of agricultural biotechnology with completed genome sequence, *Arabidopsis* presents the opportunity to unravel the molecular mechanisms involved in plant growth and development, biochemical pathways, cell biology, physiology, and pathogenic interactions. It also provides key insights into the large-scale determination of gene function that can affect commercial crop production. Despite of its extensive usage in genetic and molecular studies worldwide, its small size is also hindering the physiological analyses [1]. Standardization of growth conditions is an essential factor for this temperate model plant to obtain high reproducibility and significance and limits its application in tropical region. Therefore, it is necessary to increase the plant size by using the different plant growth techniques to facilitate the effortless identification of proper phenotypes associated with specific gene function.

Growing *Arabidopsis* in many plant research labs relies on aseptic plant culture. Seedlings are grown in vertically placed petri plates and hence the roots and shoots develop along the medium surface. Nonetheless, several disadvantages are essentially linked to such cultures. So far, many protocols have been reported on problems associated with media composition, also obtaining real nutrient-deficient solid media for several macro and micronutrients, and careful usage of gelling agents based on earlier chemical characterization [2] [3]. Moreover, the plant growth is restricted to petri plates which can be easily influenced by intensity of the light exposed and last only for two to three weeks [4]. Secondly, growth chambers with mechanistically controllable abiotic factors are effective to produce large shoot biomass but this lavish growth system with limited space does not allow the large scale production. While lighting, humidity, air flow, and temperature are factors that can be effectively controlled by using plant growth chambers, dealing with all these processes contributes to the high energy consumption by the whole system [5]. Also their maintenance tends to be expensive and laborious. Furthermore, discrepancy in growth chamber conditions may cause stress in plants which results in phenotypic variations [6]. In addition, traditional soil grow plants have limitation for any root analysis as they cannot be detached completely from soil particles. Therefore, these two kinds of growing options provide limited opportunities for growth related experiments.

Researchers and technicians are searching for a better way for *Arabidopsis* growing. In the last few years, several hydroponic systems have been developed for *Arabidopsis* to improve the growing conditions and biomass. However, they also suffer from various shortcomings such as flooding, rotting, poor aeration, loss of root material, root tangling, overcrowding, excess manipulation, and

less-than- favourable environmental conditions, algal growth etc. Especially all previous designs were indoor systems with controlled conditions like low light intensity, $\leq 22^{\circ}\text{C}$ shoot and root temperatures and 60% humidity [1] [7]-[16]. Despite years of research, these systems were all used on a reduced scale due to their complexity while most physiological analyses or mutant screens require large populations of synchronously growing individuals. Hence, optimisation of growth conditions and systems is essential factors for *Arabidopsis* to elude all problems associated with its growth and analysis.

Unlike these hydroponic systems, the successful reports of cool root zone temperature (RZT) aeroponics to prevent premature bolting of lettuce [17] [18] [19] under tropical greenhouse conditions paved the way for the current *Arabidopsis* growth on same aeroponic systems in Singapore. Even though these aeroponics with manipulated RZTs used efficiently with the big plants like lettuce, they couldn't succeed in growing the *Arabidopsis* with big plant stature under normal germination and low RZTs conditions. As a tiny temperate plant species, *Arabidopsis* requires a special care during the germination and transfer to the tropical growth conditions. And the current research focussed on the initial germination and final growing RZTs that are appropriate for better growth and higher biomass. Moreover, *Arabidopsis* growth on aeroponics with different RZTs has not been evaluated previously under tropical aerial conditions. Hence, it is well suited as a research tool for imposing and modulating environmental stress at the whole plant level with manipulated RZT. In this paper, we intended to establish simple, inexpensive, and low maintenance big aeroponic growth systems for large scale production of *Arabidopsis* plants. This method is convenient for several purposes like: 1) plant growth can be optimized and harmonized; 2) RZT, nutrient solution concentration and pH can be easily monitored and manipulated; 3) roots can be observed and harvested without damage; and 4) easy accessibility to all plant parts to conduct any experimentation.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Seeds of *A. thaliana* (L.) Heynh. ecotype Columbia-0 (Col-0) were surface sterilized and verbalized at 4°C for 48 h. Then seeds were germinated and grown vertically on Petri dishes containing Murashige and Skoog (MS) medium, pH 5.8, supplemented with 1% sucrose (Sigma-Aldrich), and 2.5 Mm MES (2-N-Morpholinoethanesulfonic acid; Sigma-Aldrich) was used and solidified with 0.8% agar. Seed germination was carried out in climate controlled growth chambers under a 16/8 light/dark cycle at 20°C and 30°C with 50% relative humidity and light intensity $75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. 16 days old seedlings at 4 to 6 leaf stage were transferred by drawing the roots in to slot cut of soft polyurethane foam cubes soaked with nutrient solution (Netherlands Standard Composition-NSC). Plant trays were covered with a clear plastic wrap taped to the trays to minimize the

transpiration rate.

After 3 - 5 days' germination, seedlings were directly planted on to the lid of the large aeroponic troughs, once the roots were visible at the base of the foam cube. The aeroponic troughs with controlled root zone temperature (RZT) systems were located in a large non air conditioned green house in Singapore. Seedlings germinated at 30°C were planted on troughs at 20°C ± 2°C (30°C/20°C) (germination temperature/root zone temperature (GT/RZT) and 30°C ± 2°C (30°C/30°C) RZTs, whereas the seedlings germinated 20°C were planted on ambient troughs at 20°C ± 2°C (20°C/20°C) and 30°C ± 2°C (20°C/30°C) RZTs. Cool RZ trough and ambient RZ troughs were programmed to mist the full strength NSC solution for 1 minute for every 5 minutes (pH 6.8, EC 2.2 mS). The shoot temperatures varied daily between 29°C to 35°C (±2°C). The maximal PPFD during midday in the greenhouse was 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

2.2. Morphological Measurements

Matured plants were measured for different morphological traits such as root length, rosette size, fresh weight, dry weight, leaf shape and size, chlorophyll fluorescence, trichome variation, raceme height, flowering time and pattern, silique size and shape, seeds size. All parameters were compared to growth chamber (GC) plants (20°C) as a control. Images were taken for all the morphological observations.

2.3. Determinations of Shoot and Root Fresh Weight (FW), Dry Weight (DW) and Plant Water Content (WC)

After floral transition, the inflorescence was separated from the rosette. The FW and DW of the 45 days old plants were determined by harvesting and weighing the rosettes and roots of 5 plants from each RZTs and GC. Prior to weighting, the roots were blotted on a tissue to remove the excess of water. DW was determined after drying the same samples at 100°C for 72 h. WC of plants was estimated using the formula $[(\text{FW}-\text{DW})/\text{FW}] \times 100$.

2.4. Imaging and Analysis for Leaf Area Measurements

The mean total leaf area of five plants (45 days) per treatment was obtained by imaging all rosette leaves spread on to a graph sheet and pictures were analysed by comparing with five plant samples from 20°C growth chamber. Images were analysed by using the digital image analysis software Image J (version 1.49; <http://imagej.nih.gov/ij/download.html>) to measure the leaf area. Images were processed using the "threshold" feature of ImageJ. The specific leaf area was calculated as area divided by rosette FW (mm^2/mg).

2.5. Measurement of Chlorophyll (Chl) and Carotenoids (Car) Pigments

Fresh material of 0.05 g was weighted and soaked in 5 ml of N,N-dimethylformamide (N,N-DMF, sigma chemical co.) in the dark for 48 h at 4°C. The ab-

sorption of chlorophyll (Chl) a, Chl b and carotenoids (Car) were measured at 647 nm, 664 nm and 480 nm respectively using spectrophotometer (Du 650, Beckman, USA). Chlorophylls and carotenoids contents were calculated according to the method of [20].

2.6. Measurement of Midday Chl Fluorescence F_v/F_m Ratio

Measurements of midday F_v/F_m ratio were made with the Plant Efficiency Analyser, PEA, (Hansatech Instruments Ltd., England). The readings were carried out 1300 h and 1400 h. Attached leaves were pre-darkened with clips for 15 minutes prior to measurements. Dark-adapted leaves were placed under the light pipe and irradiated with the pulsed lower intensity-measuring beam to measure F_0 , initial Chl fluorescence. F_m , maximum Chl fluorescence was assessed by 0.8 s of saturated pulse ($>6000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The variable fluorescence yield, F_v , was determined by $F_m - F_0$. The efficiency of excitation energy captured by open PSII reaction centres in dark-adapted plant samples was estimated by the fluorescence (F_v/F_m ratio).

2.7. Measurements of Photochemical (qP) and Non-Photochemical Quenching (qN) of Chl Fluorescence and Electron Transport Rate (ETR)

Leaves were harvested at 1000 h for Chl fluorescence analysis. The qP and qN of Chl fluorescence and ETR were determined using the Imaging-PAM ChlFluorometer (Walz, Effeltrich, Germany) at 25°C [21]. Leaf samples were pre-darkened for 15 minutes prior to measurements. Via the Imaging-PAM Chlorophyll Fluorometer, images of fluorescence emission were digitized within the camera and transmitted via a Firewire interface (400 megabits/s) (Firewire-1394, Austin, TX, USA) to a personal computer for storage and analysis of qP, qN and ETR.

2.8. Pollen and Stigma Viability

Flower samples were collected at the anthesis stage from each treatment and compared with the GC plants. Stigmas and anthers were stained with Alexander's stain [22] for 48 hrs and images were taken under microscope. Alexander's stain was used to count abortive and non-abortive pollen grains to check pollen fertility. Malachite green, a component of Alexander stain, stains the pollen walls green. Acid fuchsin, another component of Alexander stain, stains the protoplasm red and hence it colors the non-aborted pollens from red to deep red. As the aborted pollen grains were devoid of contents, they were stained green.

2.9. Collection and Measurement F_2 Seed Size

First formed siliques were harvested once they had turned completely brown but before they had dropped seeds. Siliques were allowed to dry in open microcentrifuge tubes for at least three days in 40°C hot air over before measurement. Dried silique materials were sieved and seeds were spread inside 1 cm² and im-

ages were taken under stereo microscope. ImageJ software was used to measure the seed area in μm^2 by adjusting the scale bar.

2.10. F₂ Seed Germination

Completely dried and matured seeds from each treatment (30°C/20°C, 20°C/20°C, 30°C/30°C and 20°C/30°C) were germinated on petri dishes containing 1/2 MS at 20°C GC to check the viability and percentage of germination. Seeds collected from the GC plants were used as the control.

3. Results

3.1. Seedling Establishment

Among four RZTs, 20°C/20°C (GT/RZT) plants which germinated at 20°C and grew at 20°C \pm 2°C RZT showed 97% survival rate, which is the highest among all the tested conditions. Whereas, 20/30°C (GT/RZT) plants were germinated at 20°C and grew at 30°C \pm 2°C RZT showed only 56% survival rate. Furthermore, 30°C/20°C (GT/RZT) and 30°C/30°C (GT/RZT) plants germinated at 30°C grew under 20°C \pm 2°C and 30°C \pm 2°C RZT, exhibited 80% and 88% moderate survival rate respectively. The highest mortality rate (44%) of the 30°C/30°C plants revealed that the plants experienced a severe high temperature attack. Both RZTs (20°C \pm 2°C and 30°C \pm 2°C) allowed normal shoot, root and reproductive developments (**Figure 1**), as long as plants had a root that reached down through the foam into the trough when transferred on the aeroponics. The primary root growth is another key factor of early seedling establishment.

3.2. Growth Measurements and Plant Analysis

A number of growth measurements were made to ascertain the physiological state of plants grown in different RZTs. Screening of mature plants in each treatment revealed that acclimation of a Columbia ecotype to different RZTs with ambient aerial temperature leads to many phenotypic changes such as rosette size, specific leaf area (SLA), root length, fresh weight (FW), dry weight (DW), WC, silique size and seed size.

3.2.1. Rosette Analysis

Rosettes of the plants under 20°C \pm 2°C RZTs were healthy and strong without any signs of stress (**Figure 2(a)**), although some marginal necrosis caused burning of few leaves that are in contact with the surface of the aeroponic lid. Especially 20°C/20°C plants showed wider rosettes compared to growth chamber (GC) plants. Whereas rosettes of 30°C \pm 2°C RZT (ambient temperature) were reduced in size and area, reflecting that high RZTs can cause thick and smaller leaves and reduced relative exposed leaf area (**Figure 2(a)**). The number of leaves varied in both RZT treatments compared to GC plants. Among all, 20°C/20°C plants had more number of leaves (50.2 \pm 12.70) and 20°C/30°C plants showed lower number of leaves (22.6 \pm 3.613) (**Figure 1** and **Figure 2(a)**).

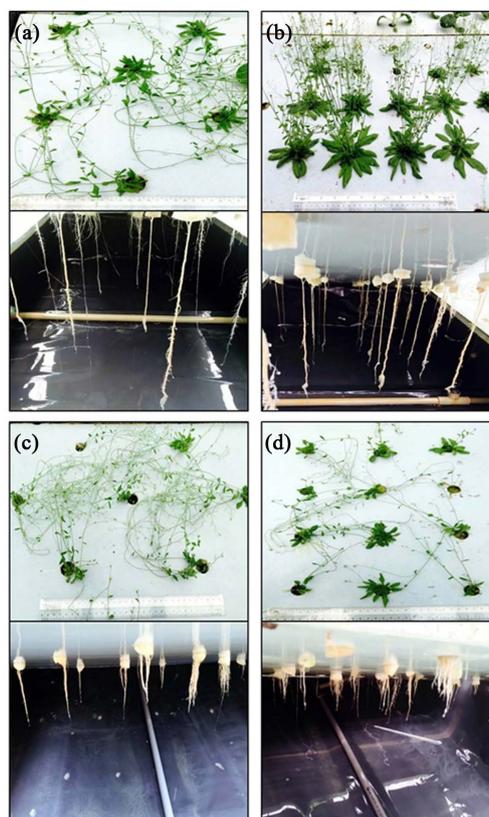


Figure 1. *Arabidopsis thaliana* growing in an aeroponic system in a tropical greenhouse. Conditions of temperature for germination/root zone temperature (RZT) for growth in aeroponic systems are (a) 30°C/20°C, (b) 20°C/20°C, (c) 30°C/30°C and (d) 20°C/30°C.

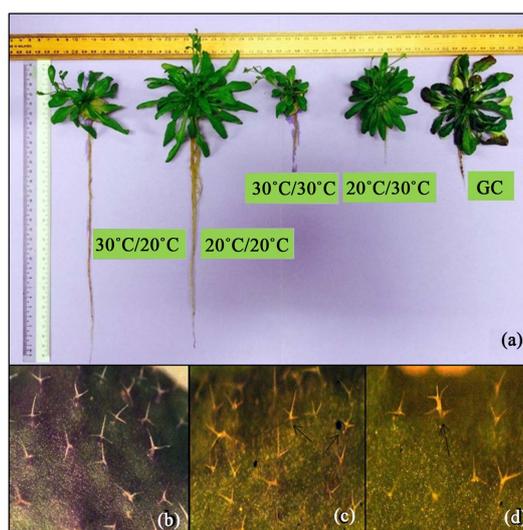


Figure 2. Morphology of mature *Arabidopsis* plants and variation in trichome morphology of 40 days after transplant. (a) Seeds were germinated in a growth chamber at either 20°C or 30°C and grown in the growth chamber (GC) at 20°C shoot and root temperatures, or an aeroponic system at either 20°C-RZT or 30°C-RZT (Conditions of growth mentioned above are germination temperature/RZT); (b) Uniform three-branched trichomes on the leaf surface of growth chamber plants; ((c), (d)) Four- and five-branched trichomes found on the leaf surface of 30°C/30°C (germination temperature/RZT) plants.

Leaf surfaces were normal with regular three dimensional trichomes in all four sets of treatments and control plants, 30°C/30°C plants showed rarely four or five branched trichomes (**Figures 2(b)-(d)**).

Total leaf area of 20°C/20°C (20°C ± 2°C RZT) grown plants measured as 65.7 ± 13.6 cm² which was little higher than 63.0 ± 12.2 cm² of GC plants (20°C shoot and root temperature). While 30°C/20°C and 20°C/30°C plants showed the total leaf area as 26.4 ± 4 cm² and 25.2 ± 7.9 cm², respectively (**Figure 3**). But 30°C/30°C plants that had been germinated and grew at ambient temperature (30°C ± 2°C) were extremely small with lower leaf area (15.5 ± 1.1 cm²). Among four samples from different RZTs, 20°C/20°C plants exhibited 1.8 fold lowest SLA than GC plants (**Figure 3**). Plants samples from 20°C/30°C and 30°C/30°C showed almost similar SLA but 1.6 fold less compared with GC plants. While 30°C/20°C plants exhibited higher SLA among all aeroponic plants and 1.3 fold lower than GC plants.

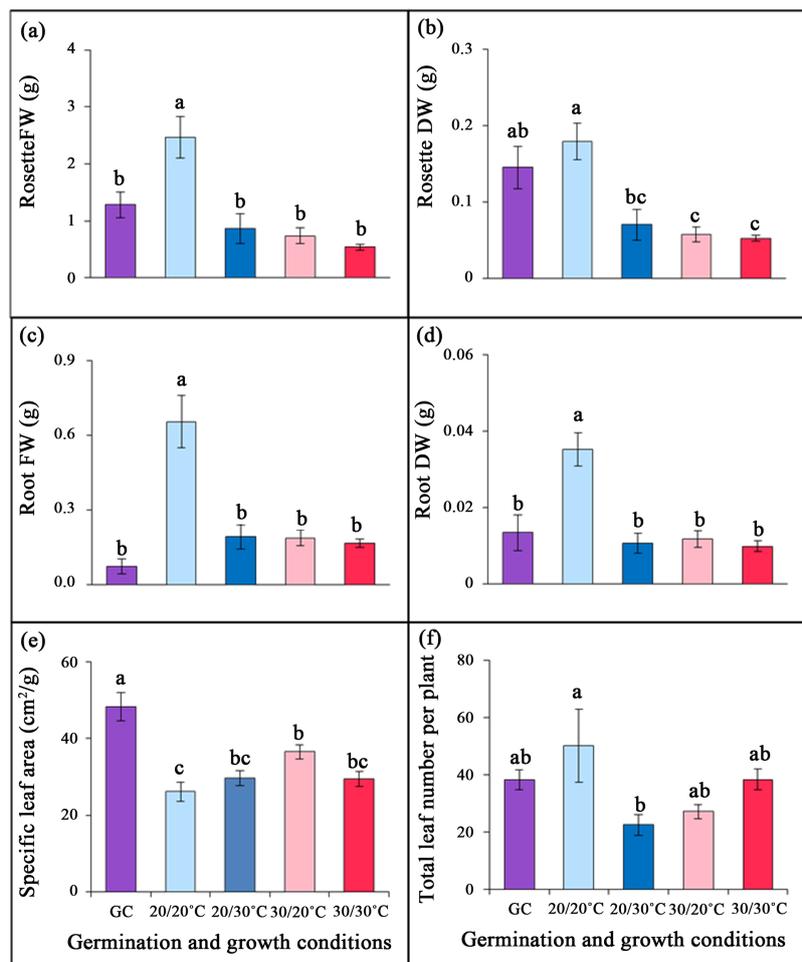


Figure 3. Productivity of *Arabidopsis* 40 days after transplant. Fresh weight (FW) and dry weight (DW) of rosettes ((a), (b)), roots ((c), (d)), specific leaf area (SLA) (e) and leaf number (f) of plants grown in the growth chamber or aeroponic systems under various RZTs. (Vertical bars represent standard error. The different alphabets above the bars represent statistically different means ($p < 0.05$).

Root length under the ambient RZT ($30^{\circ}\text{C} \pm 2^{\circ}\text{C}$) was significantly reduced in size compared to cool RZT ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$). The highest mean root length of the 10 mature plants from $20^{\circ}\text{C}/20^{\circ}\text{C}$ was measured as 25.6 cm, whereas $20^{\circ}\text{C}/30^{\circ}\text{C}$ plants showed lowest root length as 3.85 cm. Remaining batches such as $30^{\circ}\text{C}/20^{\circ}\text{C}$ and $30^{\circ}\text{C}/30^{\circ}\text{C}$ exhibited 20.3 and 6.36 cm mean root length, respectively. However, the roots of GC plants were separated from the peat moss and measured as mean root length 4.47 cm. The results indicating that the cool RZT ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$) was favourable for the healthy and elongated root system.

3.2.2. Biomass and Water Content (WC)

Determination of the biomass of plants revealed major differences between GC and aeroponically grown plant samples. Among four RZTs, $20/20^{\circ}\text{C}$ plants had significantly increased biomass (rosette and root) as compared to GC plants (**Figure 3**). Remaining plants ($30^{\circ}\text{C}/20^{\circ}\text{C}$, $30^{\circ}\text{C}/30^{\circ}\text{C}$ and $20^{\circ}\text{C}/30^{\circ}\text{C}$) showed moderate biomass values (**Figure 3**). From the results it was clear that aeroponic plants had high amount of FW (rosettes and roots) compared with GC plants (**Figure 3**). Nevertheless, dry weights of the aeroponic samples were reduced drastically as they retained high amount of water in their rosettes and roots. Hence, WC of the GC plants was lower than the aeroponically grown plants. Surprisingly, the WC of all aeroponic samples' roots was ranged between 94% and 94.6%, while GC plants retained only 81.9% water. The WC of the well watered rosettes from GC was 88.7%. However, $20^{\circ}\text{C}/20^{\circ}\text{C}$ ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ RZT) and $30^{\circ}\text{C}/30^{\circ}\text{C}$ ($30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ RZT) which were germinated and planted in their respective RZTs showed the lower WC as 92.7% and 90.3% respectively. But $20^{\circ}\text{C}/20^{\circ}\text{C}$ ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ RZT) and $20^{\circ}\text{C}/30^{\circ}\text{C}$ ($30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ RZT) which were germinated and grown in contrary temperatures showed 97.1% and 95.3% WC. Although plant WC was different among different plants, all plants had WC more than 80%, indicating that there was no severe water deficit occurred in any plants.

3.2.3. Chlorophyll (Chl) and Carotenoids (Car) Pigments

Total Chl contents, Chl a/b ratio, total Car contents and Chl/Car ratios in the leaves of GC and aeroponic samples are shown in **Figure 4**. Among all samples, Chl content was higher than Car content. GC plants had significantly higher total Chl and Car content compared to all greenhouse grown plants (**Figure 4(a)** and **Figure 4(b)**). However, Chl content of GC plants was 5.7 fold higher than the Car content. That could be due to the much lower light intensity (PPFD of $75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) compared to the light level (PPFD of $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in the greenhouse, under which GC plants were grown. All aeroponically grown plants in the greenhouse except for $30^{\circ}\text{C}/20^{\circ}\text{C}$ plants had significantly higher Chl a/b ratios than that of GC plants (**Figure 4(c)**). However, all plants had Chl a/b ratios higher than "3", which is the optimum ratio normally found in plants grown under higher light. GC plants and $30^{\circ}\text{C}/20^{\circ}\text{C}$ plants grown in the greenhouse had highest Chl/Car ratio followed by $30^{\circ}\text{C}/30^{\circ}\text{C}$ plants grown in the greenhouse (**Figure 4(d)**). Plants grown under the conditions of $20^{\circ}\text{C}/20^{\circ}\text{C}$ and $20^{\circ}\text{C}/30^{\circ}\text{C}$ had the lowest level of Chl/Car ratios.

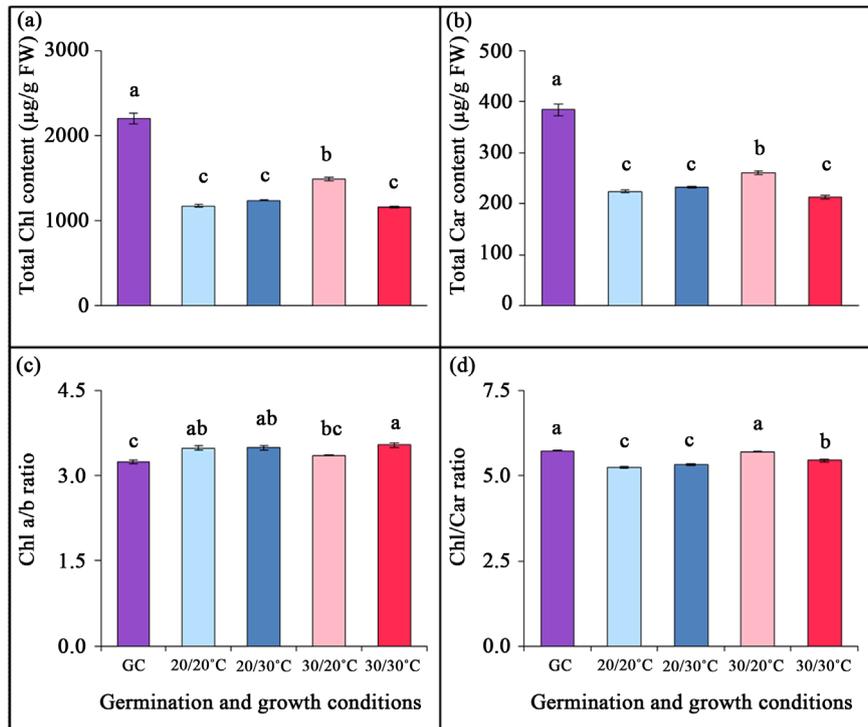


Figure 4. Photosynthetic pigments of *Arabidopsis* leaves measured 30 days after transplant. Each bar graph is a mean of 3 measurements from 3 different plants ($n = 3$). Vertical bars represent standard error. The different alphabets above the bars represent statistically different means ($p < 0.05$), as determined by Tukey's multiple comparison test.

3.2.4. Midday Chl Fluorescence F_v/F_m Ratio

They were measured from 1300 to 1400 h, when average PPFD inside the greenhouse was about $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. All leaves had midday Chl F_v/F_m ratio higher than 0.8 (data not shown), indicating that no photoinhibition had occurred in any plants and the maximum efficiency of PSII photochemistry was quite good among all the tested plants.

3.2.5. Photochemical (qP) and Non-Photochemical Quenching (qN) of Chl Fluorescence and Electron Transport Rate (ETR)

All greenhouse grown plants had higher qP, qN and ETR especially under higher measured PPFDs, indicating higher photosynthetic light use efficiency and higher capacities of heat dissipation, compared to GC plants. No clear trends were among the greenhouse plants grown under different temperatures (Figure 5 and Figure 6). *A. thaliana* plants grown under higher PPFD in the greenhouse had higher photosynthetic efficiency and higher heat dissipating capacity when they were exposed to higher PPFD compared to those GC plants grown under lower PPFD.

3.2.6. Flowering, Seed Collection and F_2 Seed Germination

Days to flower were recorded as 40 - 45 days after planting in GC plants whereas aeroponic plants flowered few days later in 45 - 50 days after germination. However all aeroponic plants developed normal racemes except the height of

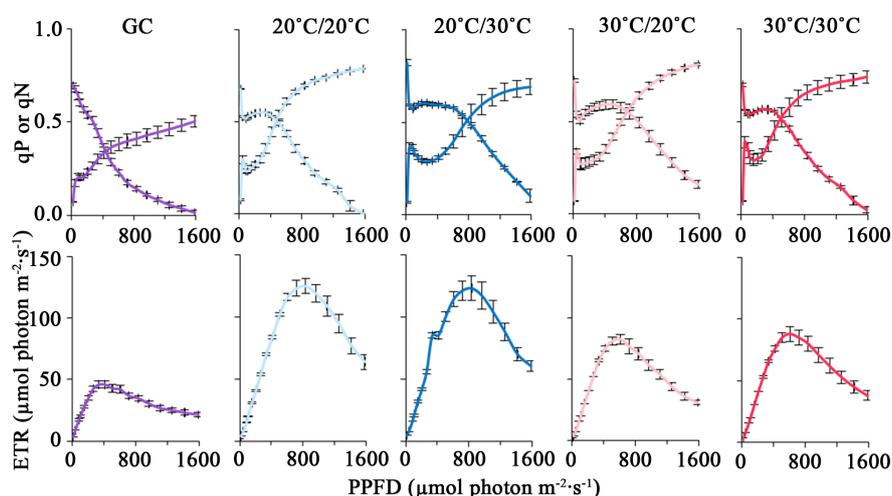


Figure 5. Photochemical (qP) and non-photochemical quenching (qN) and electron transport rate (ETR) of *Arabidopsis* plants measured 30 days after transplant. Each point on the graph is a mean of 3 measurements from 3 different plants ($n = 3$). Vertical bars represent standard error.

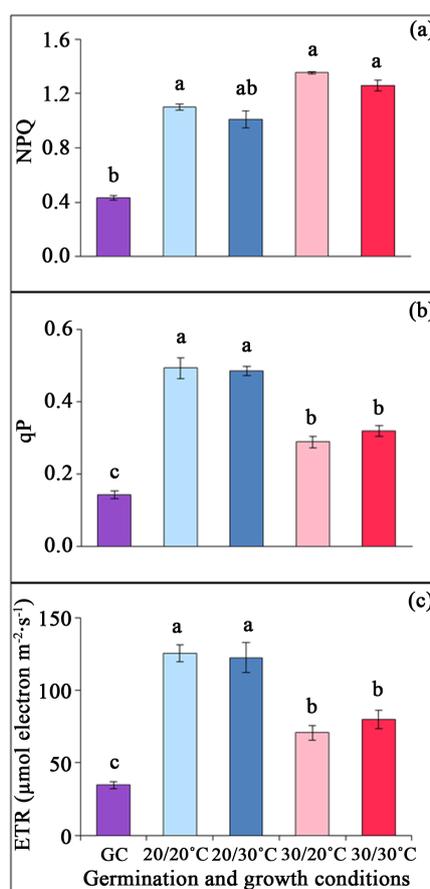


Figure 6. NPQ, qP and ETR of *Arabidopsis* plants, at 835 nm, measured 30 days after transplant. Each bar graph is a mean of 3 measurements from 3 different plants ($n = 3$). Vertical bars represent standard error. The different alphabets above the bars represent statistically different means ($p < 0.05$), as determined by Tukey's multiple comparison test.

raceme up to first silique. GC plant' racemes were measured as mean of 8 cm height up to first silique. Highest raceme height of 20°C/20°C plants was mean of 11 cm. 30°C/30°C plants showed lowest raceme height as 6.05 cm while 30°C/20°C and 20°C/30°C racemes were similar in height and recorded as 8 cm. However, matured and profusely flowered racemes with dry siliques measured as high as 38 cm in 20°C/20°C plants as compared to GC plants which measured only up to 28 cm. Remaining aeroponic plants measured in the range of 22 - 28 cm in height. Fertility of stigmas and anthers at anthesis stage were examined by staining in Alexander's stain. Except 30°C/30°C plants which showed 3% - 5% of pollen sterility (**Figure 7**), remaining all plants showed 100% fertility which resulted in mature and big sized siliques. Among all, 20°C/20°C siliques were flat and long with maximum length in comparison with GC siliques (**Figure 8**). Dried F₂ seeds of 20°C/20°C were bigger in size as compared to controls and F₂ GC seeds (**Figure 8**). Completely dried seeds were germinated on culture medium in petri plates at 20°C to check the seed viability and germination percentage. Interestingly, aeroponic seed germination percentage ranged between 96% and 99% while F₂ seeds of GC germinated 100%. High water content and inadequate drying periods might be the reason for slightly reduced germination in aeroponic seeds.

4. Discussion

Optimal growth conditions, commonly used for *Arabidopsis* growth in laboratory studies, are generally recorded as 20°C - 25°C temperature, with relatively low light levels of 130 - 150 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiation [23]. Particularly for *Arabidopsis*, cooling conditions are required during seed germination and early stages of seedling development. In contrast, temperatures of those greenhouses in tropical region without cooling can reach up to 40°C, with a peak irradiance of 1500 - 2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ during midday of

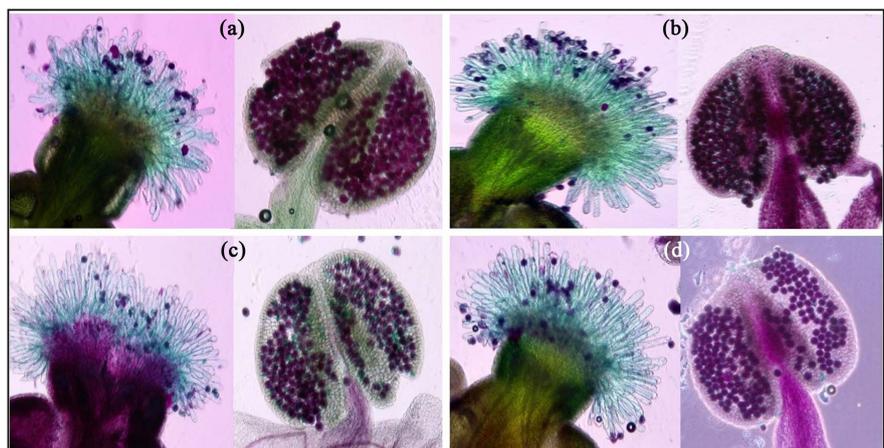


Figure 7. Viability of stigma and anther of *Arabidopsis* flowers. Plants were germinated/grown in various RZTs of (a) 30°C/20°C; (b) 20°C/20°C; (c) 30°C/30°C; and (d) 20°C/30°C.

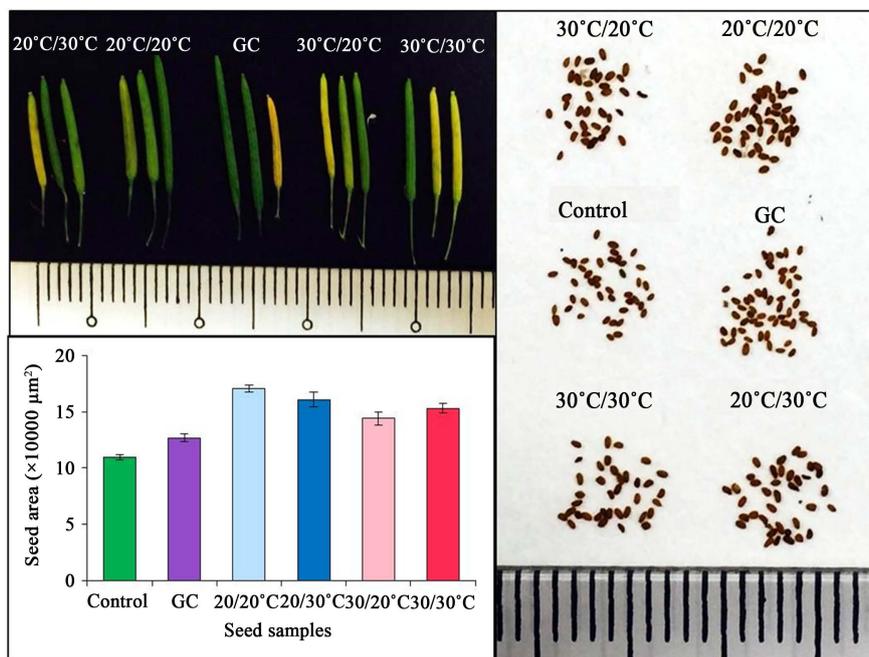


Figure 8. Size and shape of siliques and seeds from *Arabidopsis* plants. Plants were grown in a growth chamber (GC) or aeroponic systems at different RZTs. Seeds of wild-type plants (Control) have also been included for comparison.

sunny days [24]. Hence these differences would not allow *Arabidopsis* growth under tropical greenhouse conditions in a practical proposition. However, the supply of chilled nutrient solution ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$) in cool RZ trough reduced the impact of high shoot temperature stress and allowed them to grow well in early stages of seedling development. But within a week of transplantation, some of the plants died due to stress and severe root damage. It was clear from the results that the germination temperature also plays a major role in seedling establishment other than transplanting and root damage effect. Damage to the main root, especially at initial transplanting into foam, caused a long-term obstacle to rosette development. Nevertheless, these trials also revealed that the cool RZ aeroponic technique allowed the Columbia ecotype of *Arabidopsis* to establish well under the high temperature and light conditions of a tropical greenhouse.

Screening mature plants highlighted variations in physiological and morphological properties occurred by shoot thermal adaptation and cool RZTs. As expected, the morphological properties such as leaf size, number and rosette size, root length and seed size differed depending on acclimation temperature. Leaf area expansion determines the light interception which is an important parameter in determining plant productivity [25]. But when shoot temperature rises to $>33^{\circ}\text{C}$, leaf blades in both the RZT (irrespective of cool RZT) folded back to minimize surface area exposed to sunlight, thereby reducing transpiration rate. However, SLA is a key parameter which contributes to morphological plasticity [26] [27] [28]. SLA has been used as an indirect indicator of leaf thickness [29]. It was reported to be determined by leaf and plant age, genetic and environ-

mental factors. During the present study, measured data indicated that there was a decrease in SLA of aeroponically grown plants; however, under the high light conditions, temperate *Arabidopsis* plants were capable of maintaining a higher plant biomass and thick leaves by lowering the SLA (Figure 3). At higher light levels, there is an advantage to thicker leaves that can take benefit of the extra light to increase leaf area, thereby increasing the FW. Leaf thickening has been attributed to the extra number of cell layers or enlarged palisade cells and hence can increase the capability for area-based photosynthesis [30] [31]. There are long distance signals that might be involved in leaf thickening [32] and it is known that leaf thickness responds to the total number of photons received in a day, not the peak irradiance [33]. It has been reported that the plants grown under shade can produce leaves with a higher SLA or lower leaf mass area [27] [28]. GC plants grown under lower light conditions ($75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) have shown a higher SLA (Figure 3) compared with aeroponic plants due to reduced photosynthetic rates. It was clear from the data that selecting temperate plants with lower SLA or higher leaf mass area may allow selection of plants which can produce a greater harvest index even under higher irradiances.

Wavy root growth pattern is another common problem in *Arabidopsis*, induced by obstacle touching in cultured petri plates oriented in vertical mode. Plants grown in vermiculite or peat moss are very difficult to separate from soil particles without damaging the root system. Well-developed and functional roots are essential to support healthy plant growth and development. However, the study of roots is hampered by their underground growth and characterizing complex root system architecture consequently remains a challenge. But with this aeroponic growth system, roots grew straight downward in the aeroponic RZ provided with easy access to the root system.

Even under tropical greenhouse conditions, *Arabidopsis* plants showed higher survival rate correlated with chlorophyll fluorescence kinetics data. Plants grown under low light normally have higher Chl content but low Car content [34]. Natural variation for photosynthetic traits was studied by determining chlorophyll fluorescence parameters in *Arabidopsis* plants grown under different RZTs and in GC. The variation found among *Arabidopsis* plants of different RZTs for photosynthetic efficiency was very small, although the considerable variation was observed in GC plants compared to green house plants. Photo damage of photosystem generally occurs at high light intensities, in conjunction with other stresses that inhibit carbon metabolism, such as water deficit or high temperature [35]. As discussed earlier, photoinhibition and water deficit did not occur in any greenhouse grown plants regardless of RZT. The physiological parameters were also consistent with the plant health and performance in greenhouse and GC plants. Adjusting the metabolic rate, growth and development to the prevailing RZT provided a fitness advantage in tropical climate.

The results are consistent with the cool RZT to promote normal growth in temperate species *Arabidopsis* under tropical conditions. This system empha-

sized on necessity of minimal root disturbance during plant transfer to aeroponics. Nevertheless, the cool RZT can permit utilization of *A. thaliana* as a model plant even under tropical conditions, as long as a healthy root system is present. The major advantages of the aeroponic growth system have been recognized in the higher biomass production and more uniform plants than those grown in soil pots inside growth chamber. In addition, aeroponics provides an excellent aeration to the root environment in RZ area which is considered to be a major factor that allows superior plant growth compared with soil grown plants. Moreover, the obvious benefits of high biomass, soil less culture system and elimination of stress factors associated with the aeroponic growth systems also allow the easy harvest of root tissues for studies of growth, physiology, biochemistry, plant-soil interactions and plant-soil microbe interactions. Mechanistic control over RZT using an aeroponic technology, along with an exponential decline in their cost compared with the growth chambers, has made them an attractive choice for many applications including that of temperate plant growth systems.

The results indicating that cool RZT plays an important role in the acclimatization and establishment of this temperate model plant in ambient shoot temperature, which results in a plant survival and greater yield. Moreover, the technique paved the way for effortless characterization of the shoot and root systems in small tender plants. We also present this aeroponic growth system as an adaptable system for characterising the entire *Arabidopsis* plant and other plants by a variety of physiological and molecular biological methods. This method is also superior to and more reasonable than many other techniques currently in use as they are all small indoor systems [1] [7]-[16]. However, these results do not either account for possible temperature variations or water resources in aeroponic systems, nor for all possible forms of adaptations. The results show that the adaptation is of first order importance for predicting any future changes in plant architecture due to ambient shoot temperature at tropical weather. Further research should be undertaken concerning the ability to adapt to changing different RZTs and shoot climate, including trials in different regions. In addition, this application is of more agronomic model for crop development of other temperate *Brassica* species with trials employing artificially increased temperature in tropical climate. Moreover, aeroponic cultivation of important model plants may offer solutions to the production and quality control issues in commercial crops.

Acknowledgements

We thank the NIE AcRF Grant (RI 3/13 CZ) and MOE Tier 1 Grand (RP 1/14 CZ).

Conflicts of Interest

The authors declare no competing or financial interests.

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