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Uncoupling differential water usage from drought resistance in a dwarf Arabidopsis mutant

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Abstract

Understanding the molecular and physiological mechanisms of how plants respond to drought is paramount to breeding more drought resistant crops. Certain mutations or allelic variations result in plants with altered water-use requirements. To correctly identify genetic differences which confer a drought phenotype, plants with different genotypes must therefore be subjected to equal levels of drought stress. Many reports of advantageous mutations conferring drought resistance do not control for soil water content variations across genotypes and may therefore need to be re-examined. Here, we reassessed the drought phenotype of the *Arabidopsis thaliana* dwarf mutant, *chiquita1-1* (also called *cost1*), by growing mutant seedlings together with the wild type to ensure uniform soil water availability across genotypes. Our results demonstrate that the dwarf phenotype conferred by loss of CHIQ1 function results in constitutively lower water usage, but not increased drought resistance.

Main Text

Introduction

Among the various stresses plants endure in both natural and cultivated environments, drought stress has the greatest impact on plant productivity (1). From an agricultural context, drought can be defined as the state of insufficient water availability to sustain maximum plant growth (2). The impact of drought on global crop yields has intensified recently and is projected to intensify even more so in the future (3, 4). Identifying and engineering more drought resistant crops is therefore necessary to provide sufficient food to a growing population (5).

Plants employ various mechanisms in response to drought. The specific responses to drought are influenced by the degree of stress, plant species and genotype, and developmental stage (1). Some species respond by hastening the completion of their life cycle before the onset of more severe stress ('drought escape') (6). Other species respond by conserving or acquiring more water ('drought avoidance'), or by maintaining metabolic homeostasis to prevent or repair damaged cells and tissues ('drought tolerance') (6). The many terms used throughout the literature to describe plant responses to water deficit (e.g. drought resistance, drought tolerance, drought avoidance) are often used interchangeably, resulting in ambiguity and a deviation from established terminology (6, 7). This problem is compounded by results which could imply one or more forms of drought resistance (which encompasses escape, tolerance, and avoidance (6)) depending on the available data. For example, in response to reduced soil water availability, a plant could respond by increasing root growth (a drought avoidance response; (8)) or via osmotic adjustment to maintain cell turgor (a drought tolerance response; (9)). Without establishing which of these mechanisms is involved, we cannot ascertain which specific drought resistance response is responsible for an observed phenotype.

Despite the well-reasoned need to evaluate drought responses of mutant lines at equal levels of desiccation stress as controls (6), there are many claims of increased drought resistance that do not include this essential comparison (for example 10–12). In all such cases, mutant seedlings which survived longer and/or had greater rates of recovery after drought were not grown in pots shared with control plants and thus were evaluated at potentially unequal levels of drought stress. This situation is particularly problematic for plants that may use water at different rates, such as dwarf plants.

Using a bioinformatic pipeline to identify novel transcriptional regulators, we previously identified *(CHIQUITA 1) CHIQ1*, a gene of unknown function involved in organ size control in *Arabidopsis thaliana* (Arabidopsis) (13). Bao and colleagues recently implicated *CHIQ1* (which they named as *COST1*) in drought tolerance when grown in pots separate from the wild type (11). Here, we reassessed the drought phenotype associated with loss of *CHIQ1* function when *chiq1-1* seedlings were grown together with the wild type. Contrary to the previous report (11), we found that *chiq1-1* plants do not exhibit increased resistance to drought, despite constitutive lower water usage, compared to the wild type.

Results

CHIQ1 is not involved in drought resistance

We evaluated *chiq1-1*'s water requirements and survival during drought to determine whether *CHIQ1* is involved in drought resistance or if *chiq1-1* plants simply use less water. When grown in pots with only a single genotype (either all wild type or all *chiq1-1*), *chiq1-1* plants survive longer during drought than wild type plants (Fig. 1A), consistent with the previous study (11). We next asked whether this phenotype was due to increased resistance to drought, or rather due to differences in the rate of water use between genotypes. We found that *chiq1-1* plants take up less water from the soil under both well-watered and drought conditions based on daily soil water content (SWC) levels (Fig. 1 B-C). Reintroducing wild type *CHIQ1* into the mutant background complemented the water-use and survival phenotypes observed in the *chiq1-1* null mutant (Fig. 1A-C). When *chiq1-1* plants were grown in pots together with the wild type such that SWC was always equal for both genotypes, the visual onset of stress symptoms and duration of survival was uniform across genotypes (Fig. 2, Media Files 1-2). Additionally, photosystem II (PSII) quantum efficiency (F_V/F_M), a commonly used metric to quantify plant stress (14), decreased uniformly in both genotypes, when planted together, as a result of withholding water (Fig. 1D). Together, these results indicate that *CHIQ1* is not involved in drought resistance, but rather that *chiq1-1* plants have constitutively lower water needs, resulting in a slower decrease in soil water availability and a delayed onset of stress symptoms when grown separately from the wild type.

Discussion

Plants with reduced size often survive longer in response to water deprivation (6). We previously showed that *chiq1-1* plants have smaller leaves than the wild type (13). In this study, we found that the reduction in plant size as a result of loss of *CHIQ1* function does not confer drought resistance. This is contrary to what was recently published (11), where wild type and *chiq1-1* plants were grown and droughted in different pots with the implicit assumption that SWC was equal in all pots after withholding water. This assumption can dramatically alter the conclusions drawn regarding drought resistance, as illustrated in this study. We showed that *chiq1-1* plants use less water than the wild type and therefore the SWC in pots containing only Col-0 or only *chiq1-1* was different as a function of time after withholding water. When we grew *chiq1-1* plants in the same pot as the wild type, such that both genotypes were always forced to cope with equal levels of SWC, *chiq1-1* plants were qualitatively and quantitatively no more resistant than the wild type to drought stress. This is not to say that *chiq1-1* is not potentially advantageous in an agronomic context (for example in a monoculture environment in which all plants are *chiq1-1*). Indeed, daily water usage in both well-watered and drought conditions demonstrates that the dwarf *chiq1-1* plants constitutively use less water than the wild type. However, when situated in an environment more competitive for water use, *chiq1-1* plants fare no better than their wild type neighbors. Our work highlights the importance of ensuring that comparisons between genotypes are made at equal levels of drought stress by subjecting both genotypes to uniform levels of stress.

Materials and Methods

Plant materials and growth conditions Pots were filled with an equal amount of PRO-MIX HP Mycorrhizae potting soil, (Premier Tech Horticulture, Quakertown, PA) by weight. After stratification in water at 4ºC for 4 days before planting. All seedlings were grown in a growth chamber under a 16:8 hour light:dark cycle at 22ºC, 40% RH, and ~100 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) measured at pot-level.

Single genotype per pot drought experiment For water-use and survival experiments in which each genotype was planted separately, seeds were planted such that each pot contained 12 seedlings of a single genotype (Col-0, *chiq1-1*, *proCHIQ1:CHIQ1-YFP* (in a *chiq1-1* background), or *35Spro:CHIQ1-FLAG* (in a *chiq1-1* background). At 28 days after sowing (DAS), pots were either subjected to drought (total withholding of water) or were maintained at 70% SWC as controls. All pots were weighed daily Monday-Friday to determine water loss in both control and drought conditions.

Multiple genotypes per pot drought experiment For the experiments directly comparing drought resistance between Col-0 and *chiq1-1* plants, one seedling each of Col-0 and *chiq1-1* were planted in individual pots. At 28 DAS, pots were subjected to drought and were weighed daily Monday-Friday to determine SWC as a function of time.

Image capture and timelapse generation Images were taken every 2 hours from directly above pots using a Raspberry Pi Zero W (Raspberry Pi Foundation, Cambridge UK) and an Arducam M12 lens (model B0031; https://arducam.com).

Chlorophyll fluorescence measurements Chlorophyll fluorescence parameters were measured between 9:30-10:00am on the 7th true leaf of each sample using a chlorophyll fluorometer (OS30p+, Opti-Sciences, Inc. Hudson, New Hampshire).

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References

1. H. Hu, L. Xiong, Genetic engineering and breeding of drought-resistant crops. *Annu. Rev. Plant Biol.* **65**, 715–741 (2014).
2. J. Deikman, M. Petracek, J. E. Heard, Drought tolerance through biotechnology: improving translation from the laboratory to farmers' fields. *Curr. Opin. Biotechnol.* **23**, 243–250 (2012).
3. G. Leng, J. Hall, Crop yield sensitivity of global major agricultural countries to droughts and the projected changes in the future. *Sci. Total Environ.* **654**, 811–821 (2019).
4. C. Lesk, P. Rowhani, N. Ramankutty, Influence of extreme weather disasters on global crop production. *Nature* **529**, 84–87 (2016).
5. H. C. J. Godfray, *et al.*, Food security: the challenge of feeding 9 billion people. *Science* **327**, 812–818 (2010).
6. D. W. Lawlor, Genetic engineering to improve plant performance under drought: physiological evaluation of achievements, limitations, and possibilities. *J. Exp. Bot.* **64**, 83–108 (2013).
7. A. Blum, Genomics for drought resistance – getting down to earth. *Functional Plant Biology* **41**, 1191 (2014).
8. J. Levitt, Responses of plants to environmental stresses. Volume II. Water, radiation, salt, and other stresses (1980).
9. J. M. Morgan, Osmoregulation and Water Stress in Higher Plants. *Annu. Rev. Plant Physiol.* **35**, 299–319 (1984).
10. S.-W. Zhang, *et al.*, Altered architecture and enhanced drought tolerance in rice via the down-regulation of indole-3-acetic acid by TLD1/OsGH3.13 activation. *Plant Physiol.* **151**, 1889–1901 (2009).
11. Y. Bao, *et al.*, COST1 regulates autophagy to control plant drought tolerance. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 7482–7493 (2020).
12. Y. Hong, *et al.*, Two Chloroplast Proteins Negatively Regulate Plant Drought Resistance Through Separate Pathways. *Plant Physiol.* **182**, 1007–1021 (2020).

13. F. Bossi, *et al.*, Systematic discovery of novel eukaryotic transcriptional regulators using sequence homology independent prediction. *BMC Genomics* **18**, 480 (2017).
14. N. R. Baker, Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* **59**, 89–113 (2008).
15. F. Bossi, *et al.*, *CHIQUITA1* maintains temporal transition between proliferation and differentiation in *Arabidopsis thaliana*. bioRxiv (2021). MS ID#: BIORXIV/2021/469926

Figures

Figure 1.

***chiq1-1* plants use less water than the wild type, but do not display increased drought resistance when grown together.** A) Representative images of Col-0, *chiq1-1*, and the complemented line *CHIQ1pro:CHIQ1-YFP* grown in separate pots in control and drought conditions (12 days since last watering). B) Average daily water loss by genotype in well-watered (control) conditions (n = 31-46; N = 4). Black asterisk indicates statistical significance (p-value < 0.05) using Dunnett's test with Col-0 as control. C) Percent soil water content by genotype during drought. Light-colored bands represent 95% confidence intervals of (n = 4-6; N = 2-3). Representative Col-0 and *chiq1-1* images are shown at 0, 12, and 17 days since the last watering. D) Photosystem II quantum efficiency (F_V/F_M) as a function of drought of Col-0 and *chiq1-1* when grown in shared pots (n = 14-46; N = 2-3). Soil water content % at each time-point is overlaid in the black dashed line (n = 48; N = 3). Letters represent significantly different groups (p-value < 0.05) as determined by two-way analysis of variance followed by Tukey's HSD test. n = number of samples per genotype per condition per experiment. N = number of independent experiments.

Figure 2.

***chiq1-1* plants display visual symptoms of drought stress at the same time as the wild type when grown together.** Representative images of pots containing one Col-0 and *chiq1-1* seedling over the course of drought. Day numbers represent days since last watering. Arrows point towards the *chiq1-1* seedling.

Supplementary Information

Media Files 1 and 2.

***chiq1-1* plants display equal drought resistance to the wild type when grown in shared pots.** Timelapse videos of pots containing one Col-0 and *chiq1-1* seedling. Orange (File 1) and blue (File 2) arrows point towards the *chiq1-1* seedling. Video begins on the last day of watering (28 DAS) and end 20 days later.

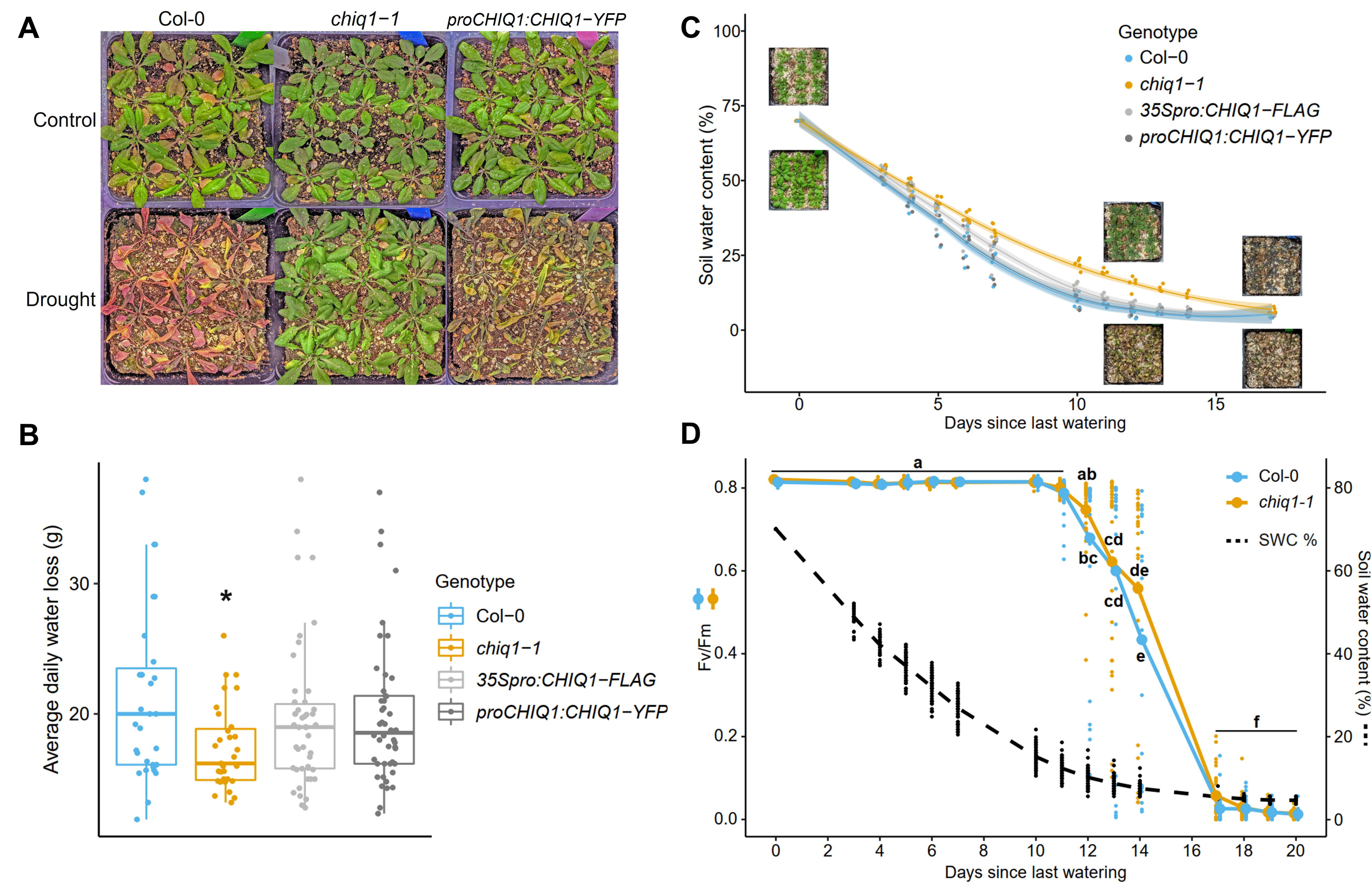
Extended Methods:

Plant materials and growth conditions Wild type *Arabidopsis thaliana* accession Columbia-0 (Col-0) and *chiq1-1* mutant (SALK_064001) seeds were obtained from the Arabidopsis Biological Resource Center (ABRC). *CHIQ1* complementation lines were obtained as described in (15). Water content of fresh PRO-MIX HP Mycorrhizae potting soil was determined by drying 3 samples of fresh soil at 45ºC for 1 week. Average water content of fresh soil was calculated as dry weight/fresh weight. To determine soil water holding capacity (100% SWC), 8 pots were filled with fresh soil, weighed, saturated with water, covered, and then left to drip until pots reached pot capacity (cessation of dripping). They were then weighed again to determine the average water-holding capacity of the soil.

To obtain 12 seedlings per pot for the single-genotype per pot experiments, 3-4 seeds were planted in each of 12 locations within a pot. After seeding, pots were put into flats and were covered for 1 week, after which covers were removed and each pot was thinned to contain 12 seedlings. Flats were rotated daily Monday-Friday to avoid positional effects. Statistical differences in weekday water usage per day across genotypes was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test ($P < 0.05$) setting Col-0 as control and using the DunnettTest() function within the DescTools package in R version 3.6.3.

Image capture and timelapse generation Images were captured using the camera.capture() Python function and were taken at 2-hour intervals using the command-line job scheduler, crontab (Unix). To remove lens distortion, images were corrected in Adobe Photoshop CS6 (Adobe Systems, Inc., San Jose, CA, USA) using the "Lens correction" feature. All images were then stitched together into a time series video using Davinci Resolve 17 (Blackmagic Design, Port Melbourne, Victoria, Australia).

Chlorophyll fluorescence measurements After dark-adapting leaves for 30 minutes, a weak modulated light (0.1 µmol m^{-2} s^{-1} PPFD) was applied to measure minimum fluorescence (F_0). Maximum fluorescence (F_M) was measured after applying a saturating light pulse (6000 µmol m^{-2} s^{-1} PPFD) of 1 second to the sampled region. Photosystem II quantum efficiency (F_V/F_M) was calculated as $(F_M - F_0)/F_M$. Statistical differences in F_V/F_M values between genotypes as a function of time were determined by two-way ANOVA followed by Tukey's honestly significant difference test ($P < 0.05$) using the lsmeans() function within the lsmeans package in R version 3.6.3.


A
Col-0
chiq1-1
proCHIQ1:CHIQ1-YFP
Control
Drought
B
Average daily water loss (g)
20
30
*
Genotype
Col-0
chiq1-1
35Spro:CHIQ1-FLAG
proCHIQ1:CHIQ1-YFP
C
Soil water content (%)
100
75
50
25
0
Days since last watering
0
5
10
15
Genotype
Col-0
chiq1-1
35Spro:CHIQ1-FLAG
proCHIQ1:CHIQ1-YFP
D
Fv/Fm
0.0
0.2
0.4
0.6
0.8
Soil water content (%)
0
20
40
60
80
Days since last watering
0
2
4
6
8
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12
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16
18
20
a
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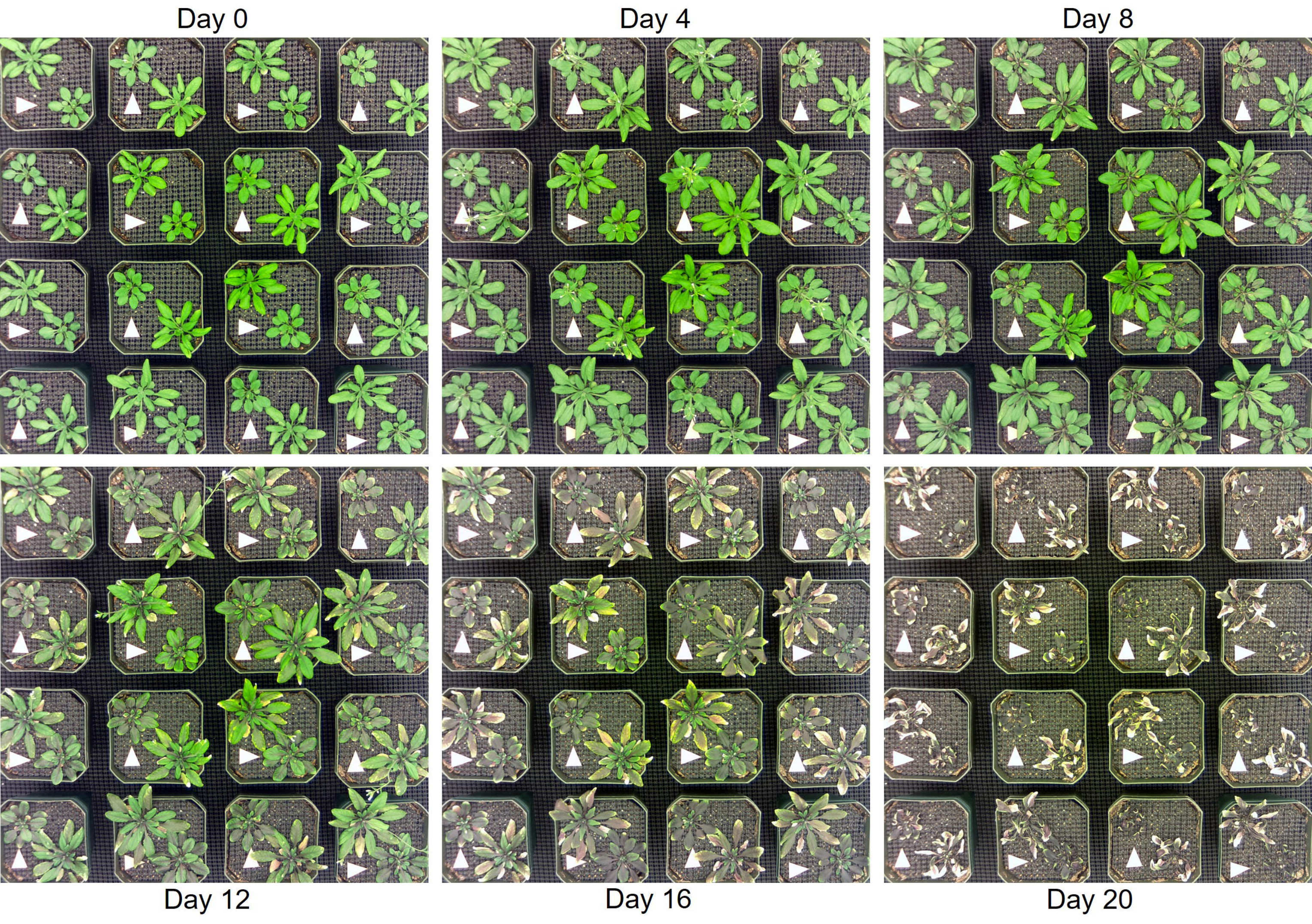
Day 0
Day 4
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Day 12
Day 16
Day 20