PhenoVation
Life Sciences

Photosynthetic recovery after drought stress
Evaluating chlorophyll fluorescence-based parameters

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Owner	**PhenoVation B.V.** **Agro Business Park 65A** **6708 PV Wageningen** **The Netherlands** **www.phenovation.com**
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Objective

This experiment aimed to investigate how plants regulate absorbed light energy during drought stress, and how these energy-partitioning strategies change following rewatering. Specifically, we addressed two main research questions:

1. What are the temporal dynamics of photosynthetic recovery in plants after rewatering following water deficit?
2. Which parameters measured using PhenoVation chlorophyll fluorescence imaging systems are most sensitive for detecting drought stress, and at which stage of stress progression are they most responsive?

Materials and methods

Plant material

Arabidopsis thaliana plants were grown from seed in 7 cm pots containing a 9:1 mixture of potting soil and perlite ('Lightmix'; Bertels B.V., Ospel, The Netherlands). Plants were maintained in a climate chamber under controlled conditions (24 °C/18 °C day/night, 90 % relative humidity, 450 ppm CO_2) with a 14 h photoperiod and a light intensity of 150 µmol m^{-2} s^{-1}.

At the full rosette stage, plants were transferred to a PlantExplorer PRO+ system (PhenoVation, Wageningen, The Netherlands) for repeated measurements (Figure 1). Illumination was provided by two white LED strings at a maximum of 150 µmol m^{-2} s^{-1}, following a parabolic diurnal light profile (Figure 2). Temperature and relative humidity in the measurement chamber were ambient, approximately 20 °C and 60 % RH.
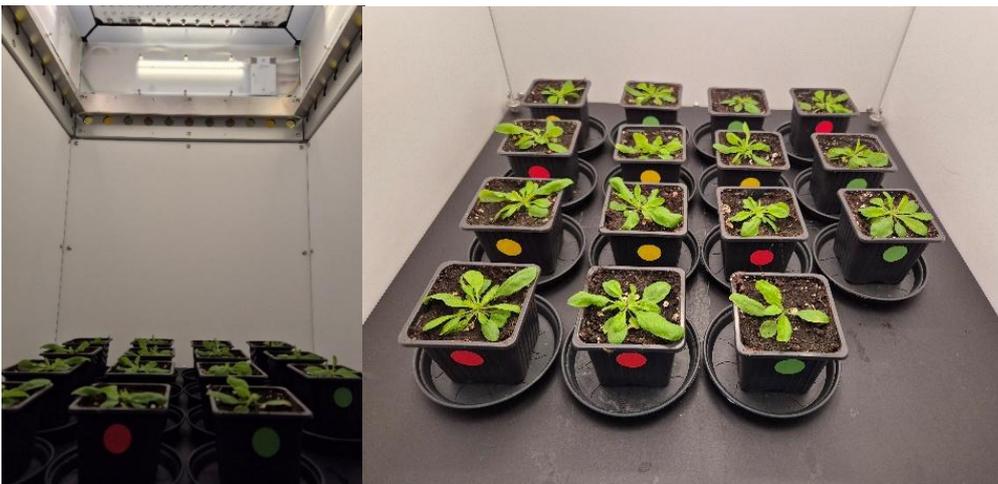

Figure 1: Photos of the experimental setup inside the PlantExplorer PRO+ with *Arabidopsis thaliana* plants. Pots with red sticks never received water, pots with orange stickers were rewatered after a 25% drop in photosynthetic efficiency, and pots with green stickers were well-watered throughout the experiment.
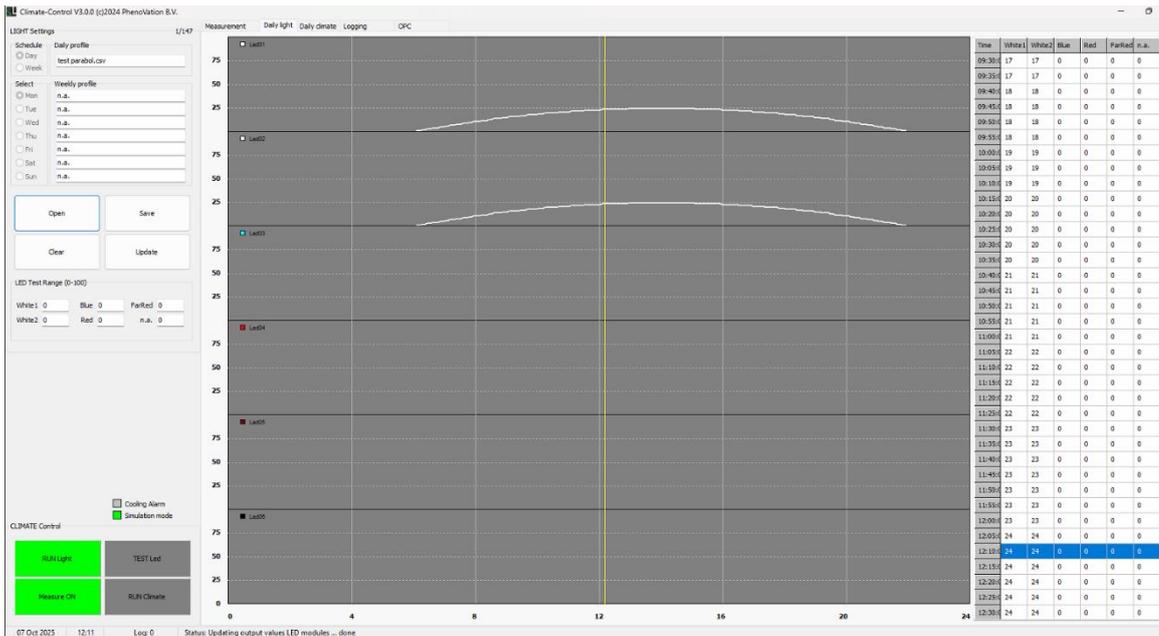
Figure 2: Climate Control Software of PhenoVation used in the PlantExplorer MAX EDEN (full climate control) and PlantExplorer PRO+. Two white LED strings followed a parabolic day, with peak intensity set at 150 µmol m⁻² s⁻¹.

Watering treatments

From seed till the full rosette stage, all the *Arabidopsis thaliana* plants were well-watered. When transferred to the PlantExplorer PRO+, the plants were arranged in a fully randomized design with three treatment groups: (A) well-watered controls, (B) re-watered plants, and (C) continuously non-watered plants. Re-watering was applied when photosynthetic efficiency under light had declined by 25 % relative to the well-watered controls.

Chlorophyll fluorescence measurements

Plants were monitored by measuring photosynthetic efficiency every hour during the day and every two hours at night. From day 7 onward, a PAM quenching protocol was performed at the end of the night (after 10 h of darkness to ensure complete relaxation of all quenching processes and that all PSII reaction centers were in an open state). Ten parameters related to photosynthetic performance and energy partitioning in the plants were calculated (Table 1).

Table 1: Different calculated parameters from the PAM quenching protocol

Parameter	Symbol	Formula	Description
Minimal fluorescence dark adapted	Fo		
Maximal fluorescence dark adapted	Fm		
Steady-state fluorescence light adapted	Fs'		
Maximal fluorescence light adapted	Fm'		
Minimal fluorescence light adapted state	Fo'		
Maximal fluorescence dark recovery phase	Fm''		
Efficiency of photosynthesis dark adapted (potential maximum)	Fv/Fm or ϕPo	(Fm - F0) / Fm	Maximum quantum efficiency of PSII when all reaction centers are open
Efficiency of photosynthesis light adapted (actual)	Fq'/Fm' or ϕPSII	(Fm' - Fs') / Fm'	Operating quantum efficiency of PSII under actinic light
Non-Photochemical Quenching (controlled heat dissipation)	NPQ	(Fm - Fm') / Fm'	Non-Photochemical Quenching; quantifies photoprotective heat dissipation of excess light energy in PSII via regulated non-photochemical processes
Photochemical quenching coefficient	qP	(Fm'- Fs) / (Fm'- F0')	Estimates the fraction of open PSII reaction centers (ready for photochemistry), assuming assuming independent units (no antenna connectivity)
Photochemical quenching coefficient based on lake model	qL	qP * F0' / Fs	Estimates the fraction of open PSII reaction centers (ready for photochemistry), accounting for PSII connectivity (lake model)
Non-photochemical quenching coefficient	qN	1 – (Fm'- F0') / (Fm – F0)	Normalized coefficient describing the fraction of non-photochemical quenching relative to the maximum achievable quenching, bounded between 0 and 1
Energy-dependent non-photochemical quenching	qE	Fm * (Fm'' - Fm') / (Fm'' * Fm')	Fast-relaxing component of NPQ representing energy-dependent thermal dissipation (ΔpH-driven)
Photoinhibitory quenching	qI	(Fm - Fm'') / Fm''	Slow-relaxing NPQ associated with sustained PSII photoinactivation or damage

Quantum yield of regulated non-photochemical energy dissipation	φNPQ	1 / NPQ + 1 + qL *(Fm/F0)-1)	Fraction of absorbed light energy dissipated as regulated heat
Quantum yield of non-regulated energy dissipation	φNO	1 - Fq' / Fm' - φNO	Fraction of absorbed light energy lost passively via heat or fluorescence, not involved in photoprotection

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Results and discussion

Photosynthesis recovery dynamics

At day 11 of water withholding, the difference in light-adapted photosynthetic efficiency between the well-watered controls and the water-deprived plants exceeded 25% (Figure 3A). At this point, the drought-stressed plants were rewatered. Remarkably, one day after rewatering, their light-adapted photosynthetic efficiency increased to 15.6% above the well-watered controls, and by the following day this difference had further increased to 23.1% (Figure 3A). This suggests a strong compensatory response following rehydration.

The potential maximum photosynthetic efficiency (Fv/Fm) remained unchanged in both the well-watered plants and those rewatered on day 11 (Figure 3B), whereas plants under continuous drought stress showed a decline. Because Fv/Fm is relatively insensitive to short-term stress, this decline indicates more permanent or structural damage to the photosynthetic apparatus, which was clearly prevented by rewatering.
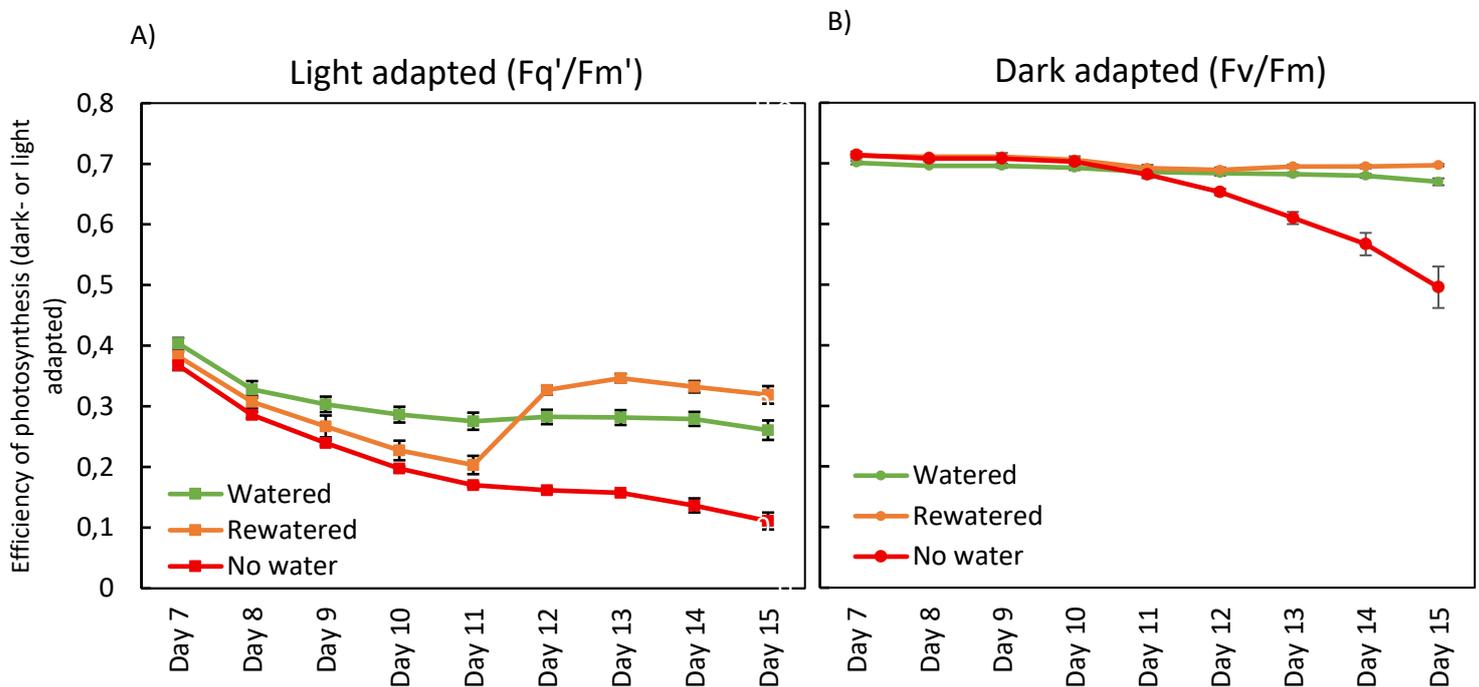

Figure 3: Photosynthetic efficiency of *Arabidopsis thaliana* plants for the different treatment groups, starting after seven days of withholding water. (A) Light adapted efficiency of photosynthesis (Fq'/Fm'). (B) Dark adapted efficiency of photosynthesis (potential maximum) (Fq'/Fm'). Plants in the rewatered group were rewatered on day 11. Data represent means ± standard error of five plants (n=5).

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The rapid increase in light-adapted photosynthetic efficiency after rewatering points to reversible regulatory adjustments rather than structural repair, potentially involving enhanced electron transport efficiency, improved stomatal conductance, or increased photoprotective and antioxidant activity. To further investigate this photochemical response, OJIP measurements could provide more insights into specific electron transport processes and energy fluxes within the photosynthetic apparatus. Furthermore, examining how long this response persists after rewatering would indicate whether it reflects short-term regulation or a long-lasting adjustment.

Similar compensatory effects after rewatering have been reported in several crop species. In cotton, short-term drought followed by rewatering enhanced photosynthesis and aboveground biomass within one to three days after rewatering (Luo et al., 2016). Likewise, moderate drought stress has been shown to stimulate root growth and ultimately increase yield (Niu et al., 2018). In potato, short-term drought stress applied at the seedling stage also resulted in enhanced photosynthetic performance and increased yield after rewatering (Lv et al., 2024).

In addition to plant physiological responses, there is evidence that other mechanisms may contribute to these compensatory effects, either independently or alongside plant psychological processes. For example, Voilare et al. (2021, 2023) demonstrated that increased grassland yield following prior drought exposure can be driven by changes in soil nutrient dynamics.

These recovery or enhancement responses in photosynthetic performance and efficiency may form part of a broader mechanism in which plants adjust their physiological status to better cope with future stress. Pre-exposure to controlled stress has been shown to increase plant resistance to subsequent stress during cultivation (Martínez-Andújar et al., 2011; Sintaha et al., 2022; Chen et al., 2025). Vincent et al. (2019) termed this phenomenon **Primed Acclimation (PA)**, defining it as the targeted modification of plant physiological processes through controlled water-stress exposure, and proposed its use as a management strategy alongside traditional breeding approaches.

Historically, primed acclimated has received limited attention, and its underlying mechanisms appear to be highly species- and cultivar specific. These mechanisms may involve modifications in root architecture, water-use efficiency, root-shoot partitioning, osmotic adjustment, antioxidant production, stress memory, or photosynthetic traits (Vincent et al., 2019).

Given this species- and cultivar dependent variation, chlorophyll fluorescence-based measurements provide an important advantage: they are non-destructive, rapid, and suitable for high-throughput screening of large populations. When applied across a range of stress intensities, these measurements also support more precise determination of optimal priming doses, helping to avoid insufficient, excessive, or otherwise counterproductive treatments.

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Sensitivity parameters

On day 7 of no water, the first full PAM quenching curve revealed two major differences between the water-deprived and well-watered plants, particularly in NPQ (non photochemical quenching, a measure of energy dissipation as heat) and qE (fast reversable component of NPQ), which differed from the control by 82% and 215%, respectively (Figure 4). The plants were visually unaffected, showing no signs of turgor loss or other symptoms related to water stress (Figure 5). On the same day, ΦPSII (actual photosynthetic efficiency in the light) was 'only' 7.1% lower in water-deprived plants. As photosynthetic efficiency (fraction of the light absorbed used to drive photochemistry) declined under mild water stress, the plant dissipated excess absorbed light in a controlled way (i.e. increased NPQ) to prevent damage to the photosynthetic machinery. After rewatering, the NPQ processes returned back to the level of well-watered controls (Figure 6 and 7).

Figure 4 summarizes the deviation of water stressed plants from the well-watered controls plants across multiple measured parameters. Remarkably, Fv/Fm (a commonly used parameter) showed no difference between treatments at day 7, while parameters related with safe energy dissipation showed large differences. This result underscores the importance of evaluating multiple fluorescence parameters, as stress responses may be detectable earlier through other pathways rather than efficiency of photosynthesis alone.

Furthermore, future experiments should evaluate PAM derived parameters alongside OJIP derived parameters. OJIP analysis offers a detailed view of processes around photosystem II that cannot be captured by PAM. Comparing these two approaches under stress conditions would help reveal how sensitive OJIP parameters are to changes in the photosynthetic machinery.
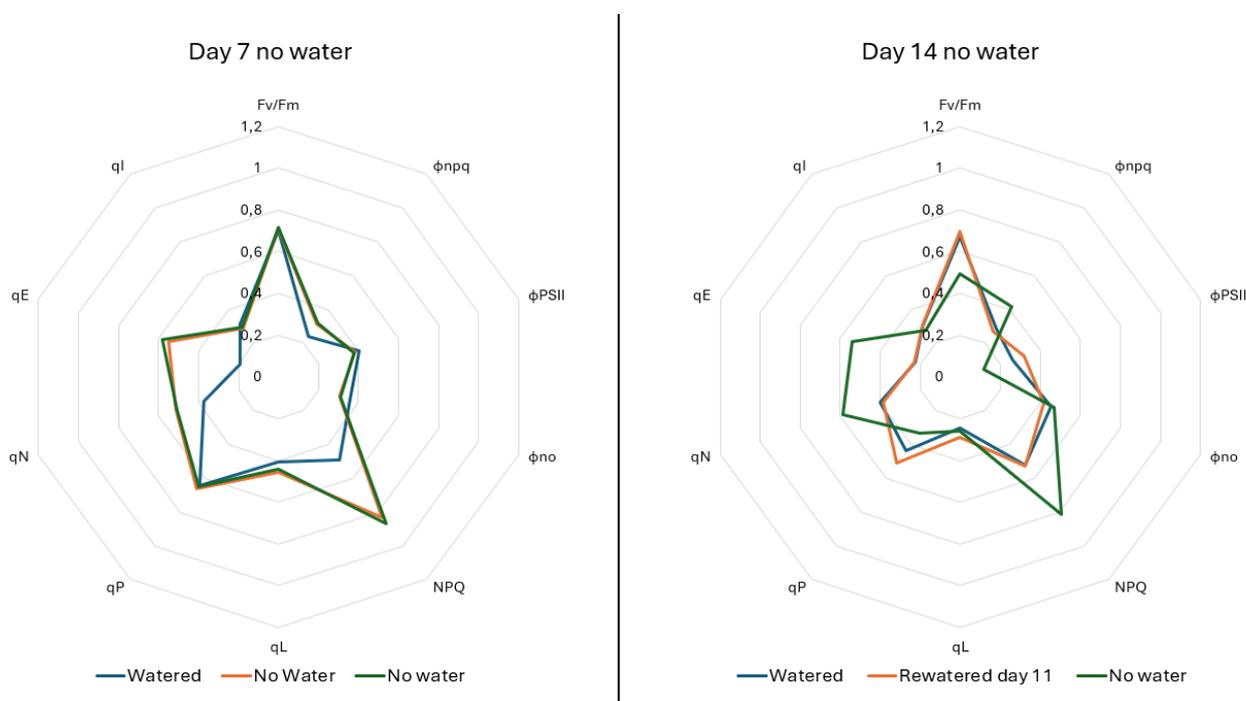

Figure 4: Spider plots of all calculated fluorescence parameters from *Arabidopsis thaliana* plants for the different treatment groups. Left: day 7, when only the control group was well-watered. Right: day 14, with the rewatered group having been rewatered 3 days earlier. Data represent means of five plants per treatment group (n=5).

Color

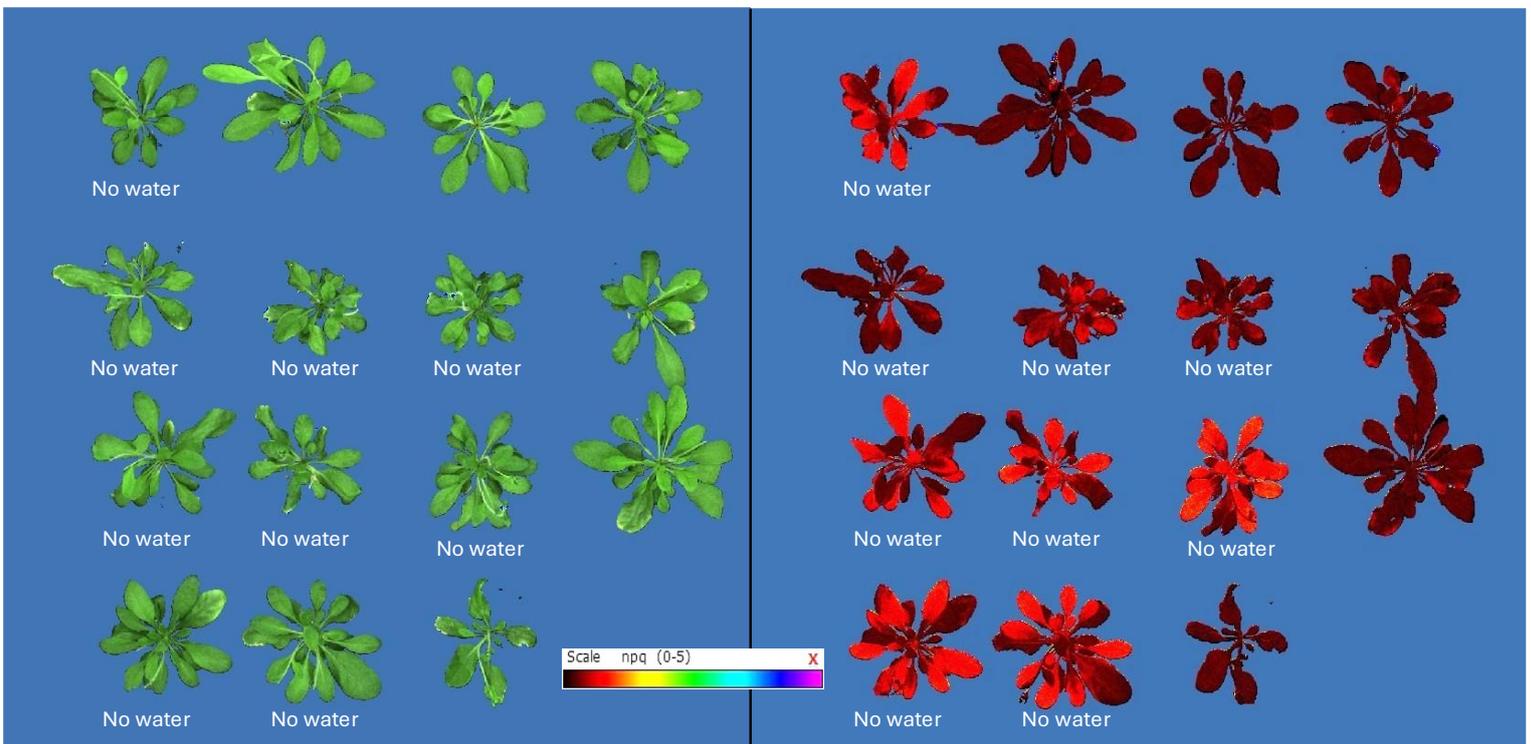**7 days no water**

NPQ

Scale   npq (0-5)   x

Figure 5: Color and Non-Photochemical Quenching (NPQ) images of *Arabidopsis thaliana* plants, after 7 days without water. Unlabelled plants represent well-watered controls.
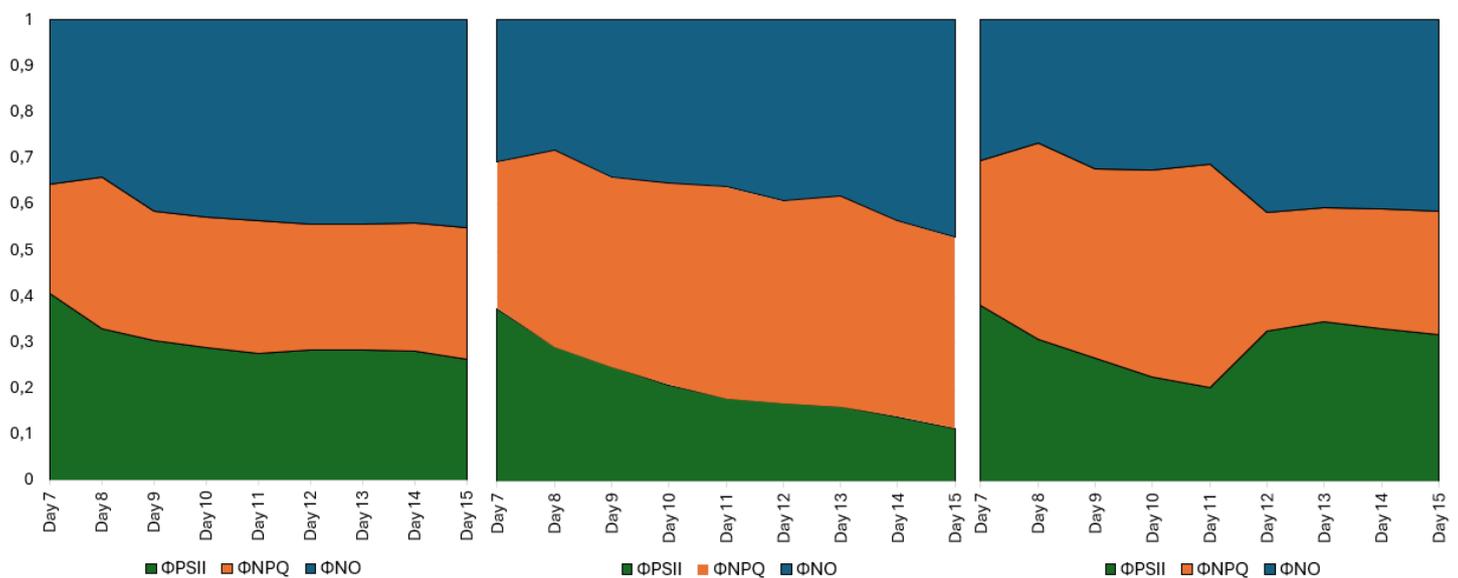

Figure 6: Quantum yields of energy partitioning between photochemical quenching (φPSII), regulated non-photochemical quenching (φNPQ), and non-regulated energy dissipation (φNO) for the three water treatment groups. Data represent means of five plants per treatment group (n=5). Note that φPSII + φNPQ + φNO = 1 for each measurement.
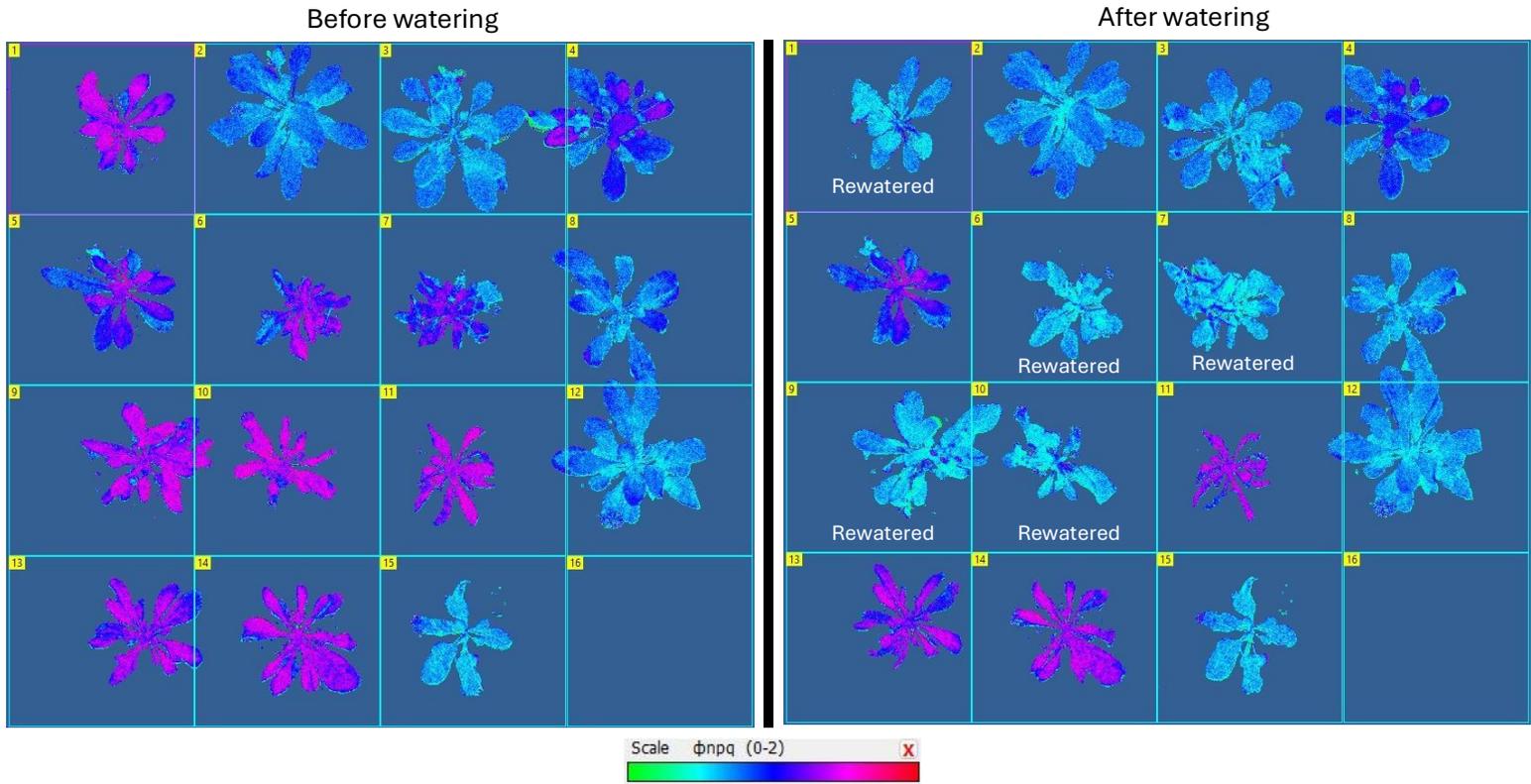
Figure 7: Quantum yield of non-photochemical quenching (φNPQ) before watering and one day after rewatering.

Conclusion

This experiment evaluated the potential of chlorophyll fluorescence parameters for early detection of drought stress and for assessing the effectiveness of timely rewatering. Rewatering *Arabidopsis thaliana* plants after an 11-day period without water restored light-adapted photosynthetic efficiency within two days to levels 23.1% higher than those of well-watered controls, suggesting a compensatory response. Rewatering also prevented declines in the potential maximum efficiency of photosynthesis, indicating that severe stress had not yet developed. After seven days of water withholding, NPQ and qE values were 82% and 215% higher, respectively, in drought-stressed plants compared with well-watered controls, suggesting activation of photoprotective energy-dissipation mechanisms. On the same day, light-adapted photosynthetic efficiency was reduced by only 7.1%. These results demonstrate that NPQ and qE respond earlier and more strongly to drought than efficiency-based parameters such as Fv/Fm or Fq'/Fm'. Therefore, NPQ and qE offer enhanced sensitivity for early drought stress detection, enabling more rapid intervention or faster screening of plant populations.

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