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Lettuce reaction to drought stress: high-throughput automated phenotyping of growth and photosynthetic performance

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Background

Fresh water shortage is a major concern all over the world, not only for human consumption, but also for agriculture. Leafy vegetables, like lettuce, have high leaf water content and can be particularly susceptible to drought stress. In the present study, drought resistance of two different cultivars of *Lactuca sativa* L. var. *Salanova*® has been tested, monitoring their growth and photosynthetic performance with PlantScreen™, a high-throughput non-invasive imaging platform developed at Photon Systems Instruments (PSI, Czech Republic). A range of morpho-physiological traits was monitored every second day to verify and compare the different reactions of the two accessions to a mild drought stress.

Here we present the data for the two cultivars **Aquino** (green butterhead) and **Barlach** (red butterhead), whose growth and photosynthetic performance were measured for a period of 36 days.

Results

Fig.3A-B RGB top images and ChlF images of F_m (maximum fluorescence) in false colors for control and drought stressed plants. Marked difference in size and photosynthetic performance are visible (DoPh = Day of Phenotyping).

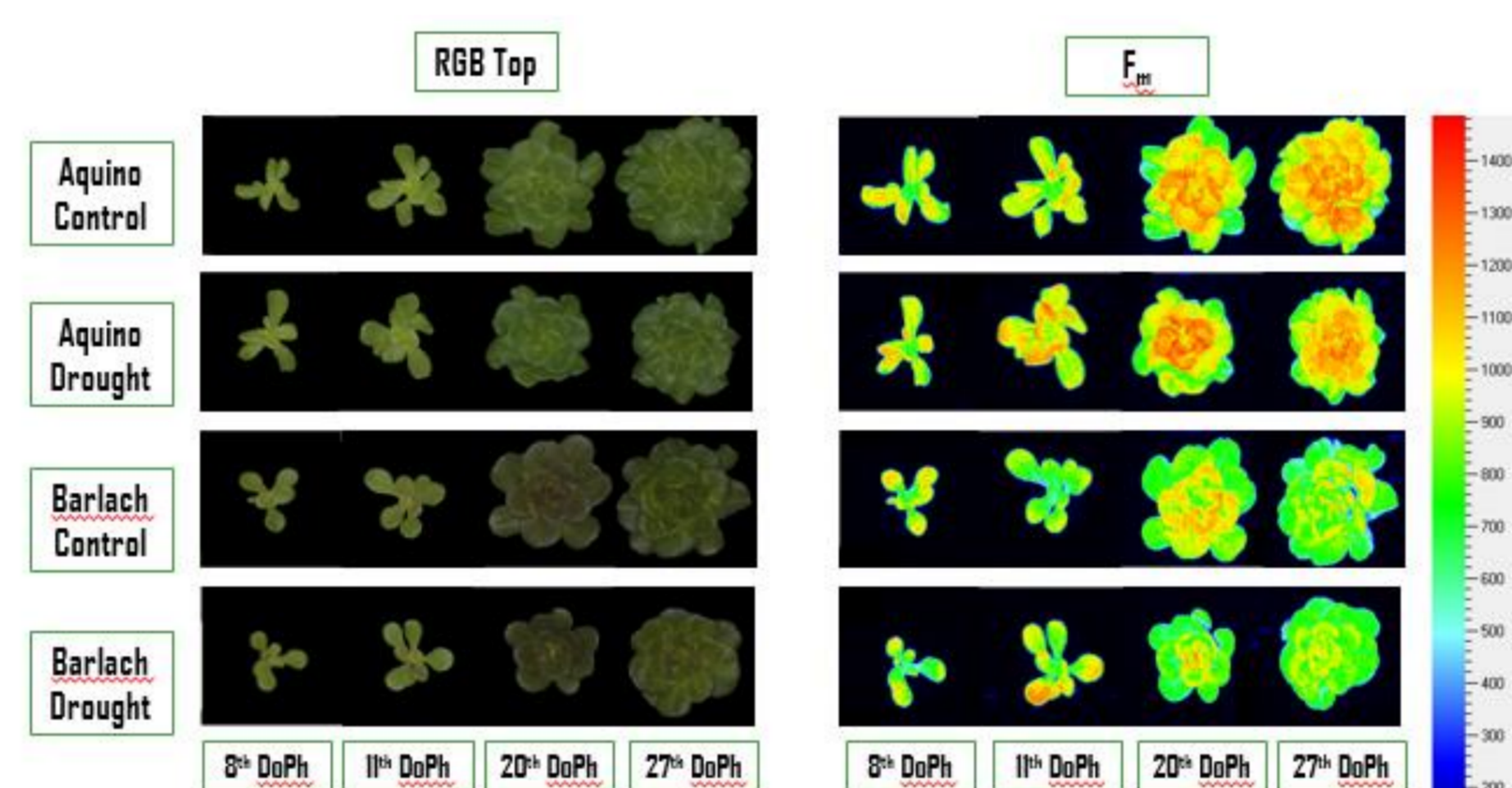


Fig.4 A reduction in projected top area measured in number of green pixels is present in both cultivars as consequence of drought stress.

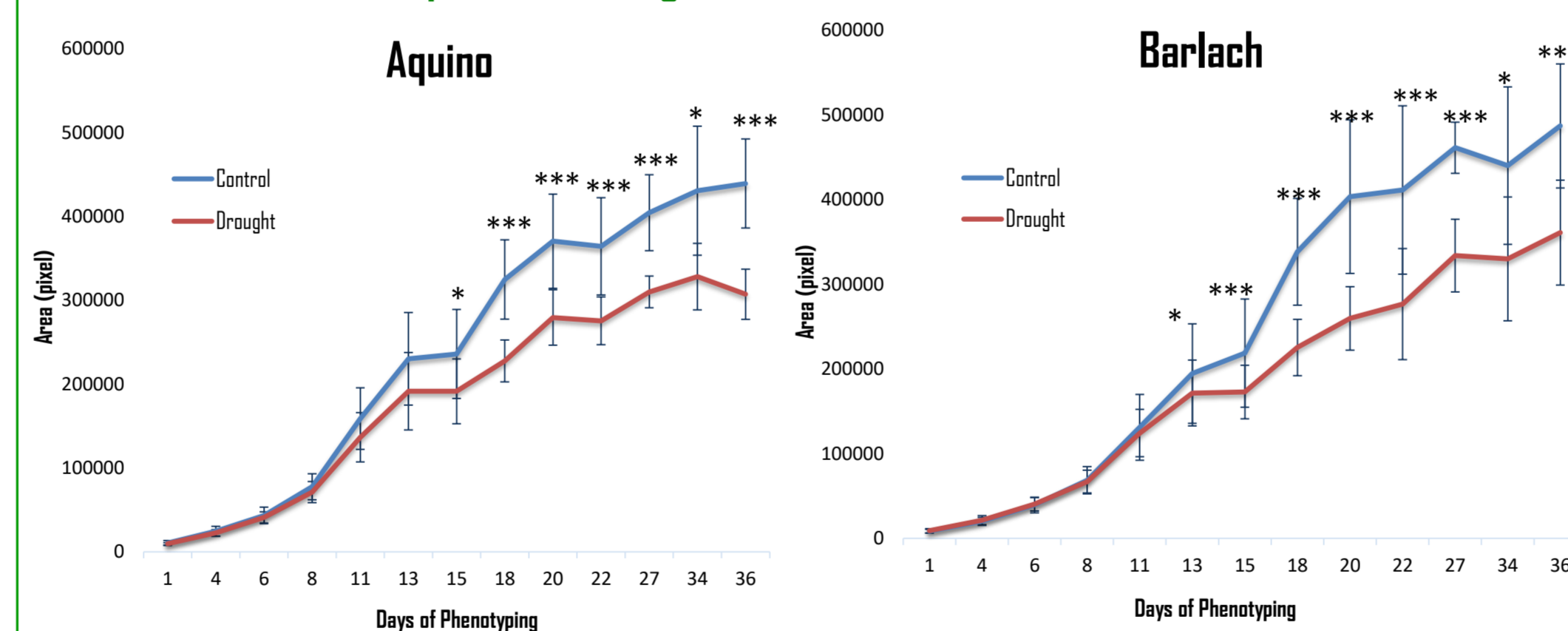


Fig.5 The two cultivars performed similarly in both control and drought conditions.

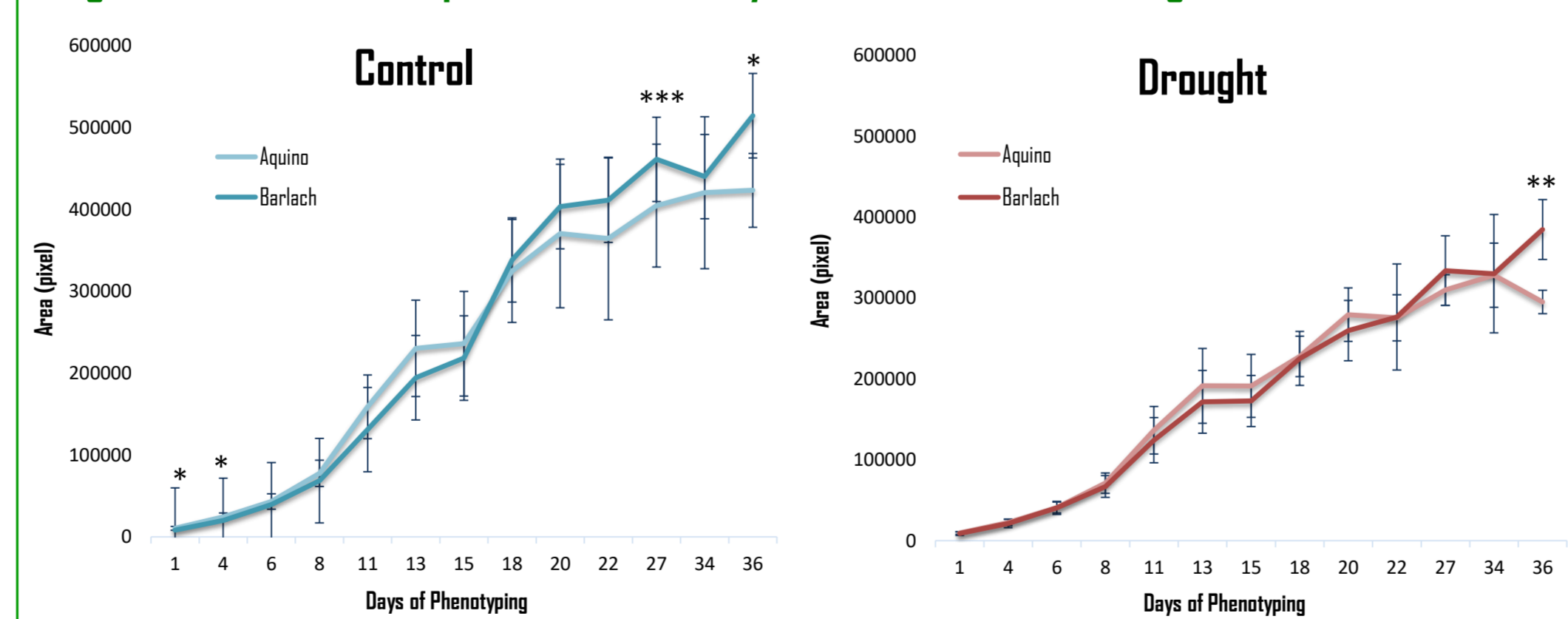
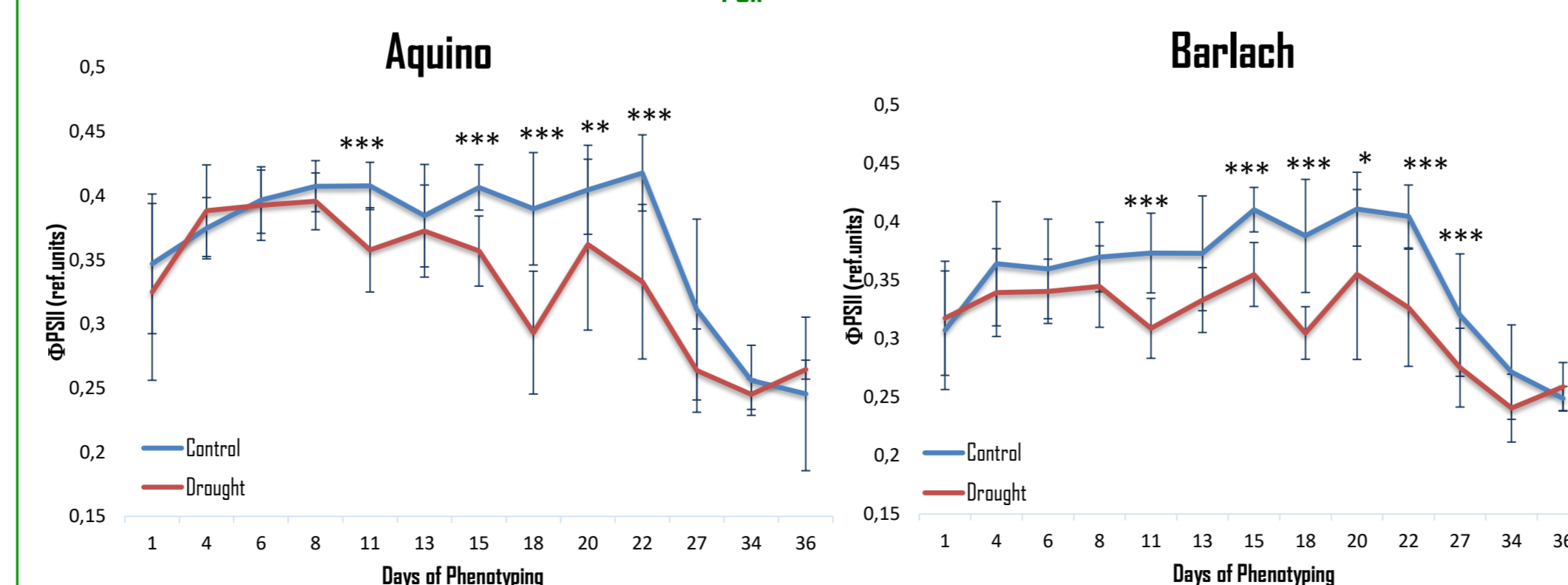


Fig. 6 Quantum yield of photosystem II (Φ_{PSII}) rapidly reflected level of drought stress.



Analysis of variance (ANOVA). *P < 0.05; **P < 0.01; ***P < 0.001.

Materials and Methods

250 ml pots were filled with 100 g of Klasmann 2 substrate saturated with water and put into trays. One lettuce seed (Aquino or Barlach) was sown per each pot. The trays were covered with a transparent lid and put into the Growth Chamber (Step-in **FytoScope FS-SI**), where they were kept at 23°C day and 19°C night, 60% relative humidity and 12h light-12 h dark regime (250 μ E white light, 5.5 μ E far-red light). Plants have been watered every second day using the Weighing and Watering station of PlantScreen™ Compact System. 18 DAS (days after sowing), plants have been divided into control plants (well-watered) and stressed plants (that were given a sub-optimal level of water). The watering has been done up to their reference weight:

- **Control plants** (70% of field capacity): 156 g + 15 g (pot weight) + 1 g (blue mat weight) = 172 g
- **Stressed plants** (40% of field capacity): 104 g + 15 g (pot weight) + 1 g (blue mat weight) = 120 g

Phenotyping protocol started on the same day (1 DoPh, Days of Phenotyping) and consisted of RGB measurement from the top of the plants (morphological and growth analysis), chlorophyll fluorescence measurement (photosynthetic performance) and weighing and watering of the pots. The measurement started in the morning, around 8 a.m., and lasted until late afternoon. Trays were manually loaded into the PlantScreen™ Compact System in a random order. Plants were kept for 15 minutes in the dark adaptation chamber, then moved under the FluorCam, under the RGB camera and eventually to the Weighing and Watering system.

Fig.1 Experimental timeline, showing main events of the experiment.

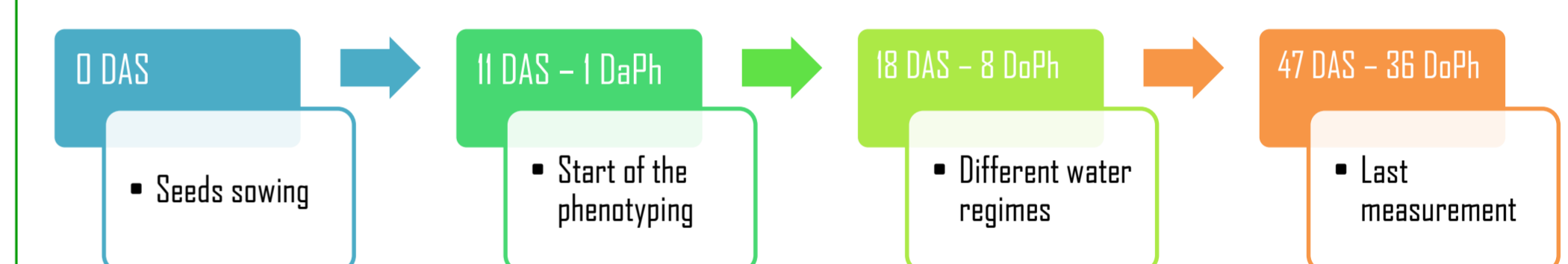
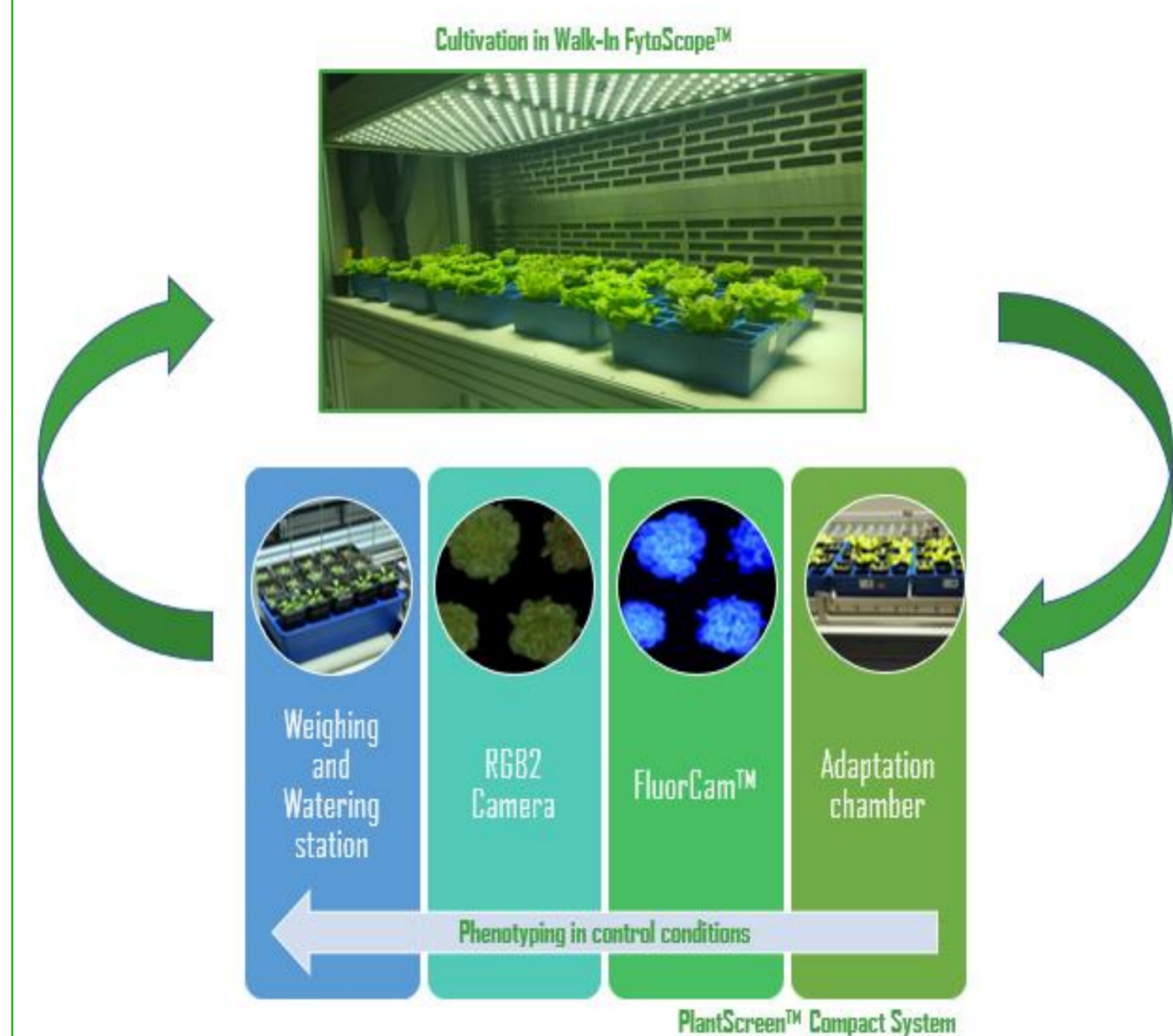


Fig.2 Plants were grown in Walk-in **FytoScope FS-WI** and phenotyped in **PlantScreen™ Compact System**. Phenotyping protocol in **PlantScreen™ Compact System** included RGB analysis for morphological and growth analysis, Chlorophyll Fluorescence (ChlF) measurement for photosynthetic performance analysis and automated watering and weighing of the pots.



Acknowledgements

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Conclusions

Here we present the phenotypic data quantitatively characterising area increase and photosystem II performance in two different cultivars of *Salanova* lettuce measured over a period of 36 days. The analysis of RGB and ChlF images of the plants, taken every second day for the entire duration of the trial, shows that the two varieties perform similarly, both in control and stress conditions. However, some differences between the two cultivars are present:

- at the end of the trial, Barlach plants reached higher area values than Aquino ones, both in control and in stress conditions;
 - significant differences regarding dimensions between control and stressed plants showed earlier in Barlach plants (13th day of phenotyping) than in Aquino ones (15th day of phenotyping).
- Light curve protocol was used to address light use efficiency of the two cultivars. Interestingly, we observed a rapid decline in PS II operating efficiency already 3 days upon mild drought stress initiation. Nevertheless, there was no obvious difference in the performance between the two cultivars.