PROJECT TITLE

Long-term responses to atmospheric CO2 and drought: from stomata to whole plant water use

CONSORTIUM

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SUMMARY OF THE REPORT

Climate change is a reality. Rising CO_2 levels, increasing temperature and altered precipitation patterns are leading to severe episodes of drought across wide areas. Crop productivity and thus global food security is threatened. Whilst research has led an understanding of plant responses to each environmental cue separately (drought, temperature, CO_2), much less is understood about the effects of their interaction on plant physiology and development. Exposure of C_3 plants to elevated CO_2 alters the balance between carbon gain through greater photosynthesis and leaf water loss through lower stomatal conductance, which has been predicted to ameliorate the impacts of drought. However, longer-term responses to CO_2 may result in greater leaf growth, hence greater water use and increased risk of drought stress. The likelihood of positive or negative outcomes will most probably depend on the timing and intensity of drought during the development of the crop.

In STOCOdrought, we aimed at exploring how plant responses to long-term exposure to elevated CO_2 are altered by different scenarios of drought. We specifically questioned how the *timing* of drought during the plant cycle might counteract the positive effects of CO_2. We aimed at understanding the role of specific genes, known to control stomatal regulation or development, in these responses. Thanks to an EPPN^{2020} transnational access, we were able to use the PHENOPSIS platform in Montpellier, and we were supported by a whole team at the facility during the experiment. We studied 14 mutants of *Arabidopsis*, submitted to 4 scenarios of drought (early, late, sustained or followed by re-watering) and 2 atmospheric CO_2 levels. With 9 replicates per entry, this yielded a total of >1000 plants. We achieved high-throughput measurements of a battery of morpho-physiological traits at the leaf and plant level. These included traits related to growth and development (e.g. growth rate, final biomass, SLA), water-use (whole-plant transpiration, rosette temperature), stomatal patterning. This was achieved using cutting-edge tools including visible and thermal imaging and fine gravimetry. The project generated high quality data, providing an ideal basis for analysing the genetic and environmental effects in the control of plant responses to CO_2 depending on the timing of drought. In the ongoing analyses, we are using original modelling tools such as those developed in EPPN^{2020} (e.g. statgenHTP) to account for spatial trends and ensure a precise estimation of genetic and treatment effects. The project revealed a wide range of responses between mutants, depending on the scenario, revealing that the relative importance of the signalling pathways depends on the timing of drought during the growth cycle. We expect this work to shed new light on genes playing a major role in growth maintenance and water saving in the face of combined environmental stimuli.

Figure 1. Overview of the STOCOdrought experiment in the PHENOPSIS platform (here, 504 plants grown at 400 ppm CO_2). The robot with the RGB and thermal cameras is at the back of the platform. Certain pots are sealed for transpiration measurement by gravimetry.

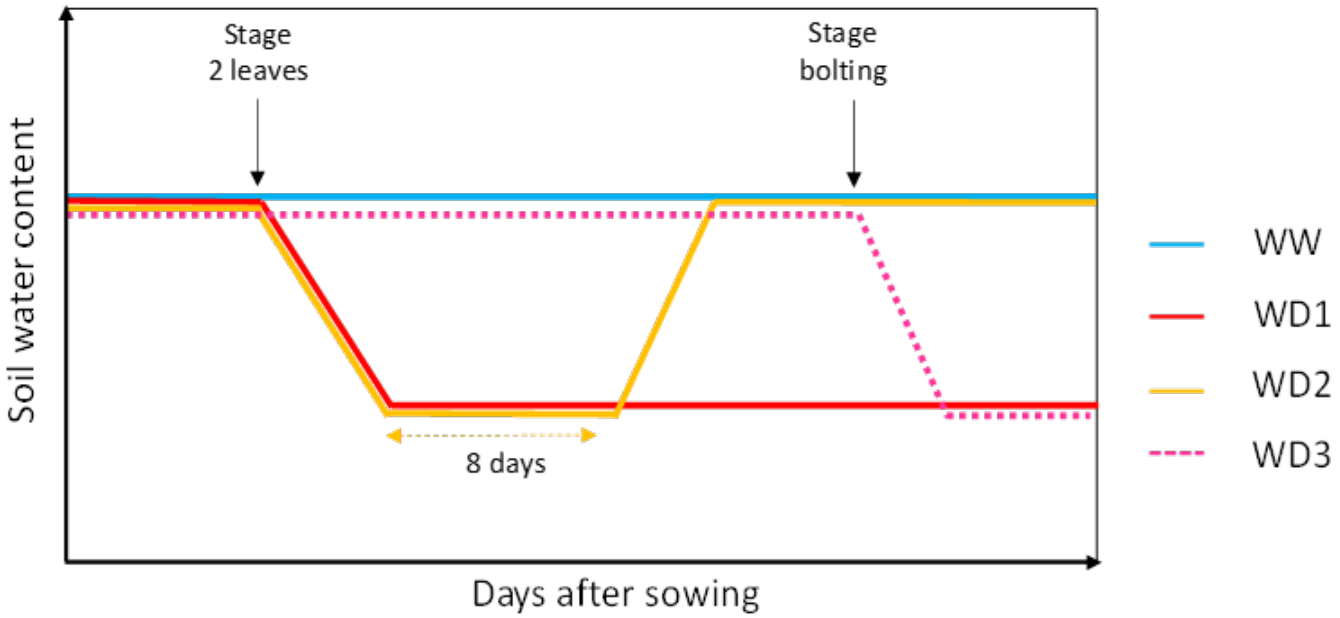

Figure 2. Schematic representation of the watering scenarios in the STOCOdrought experiment. In each CO_2 condition, all 14 genotypes were submitted to 4 different watering scenarios, with one well-watered treatment and three water deficit treatments differing in their timing during the growth cycle. WW: well-watered; WD1: early (starting from stage 2 leaves) and sustained (until the end of the experiment) water deficit; WD2: early water deficit followed by rewatering; WD3: late water deficit (starting at stage bolting).

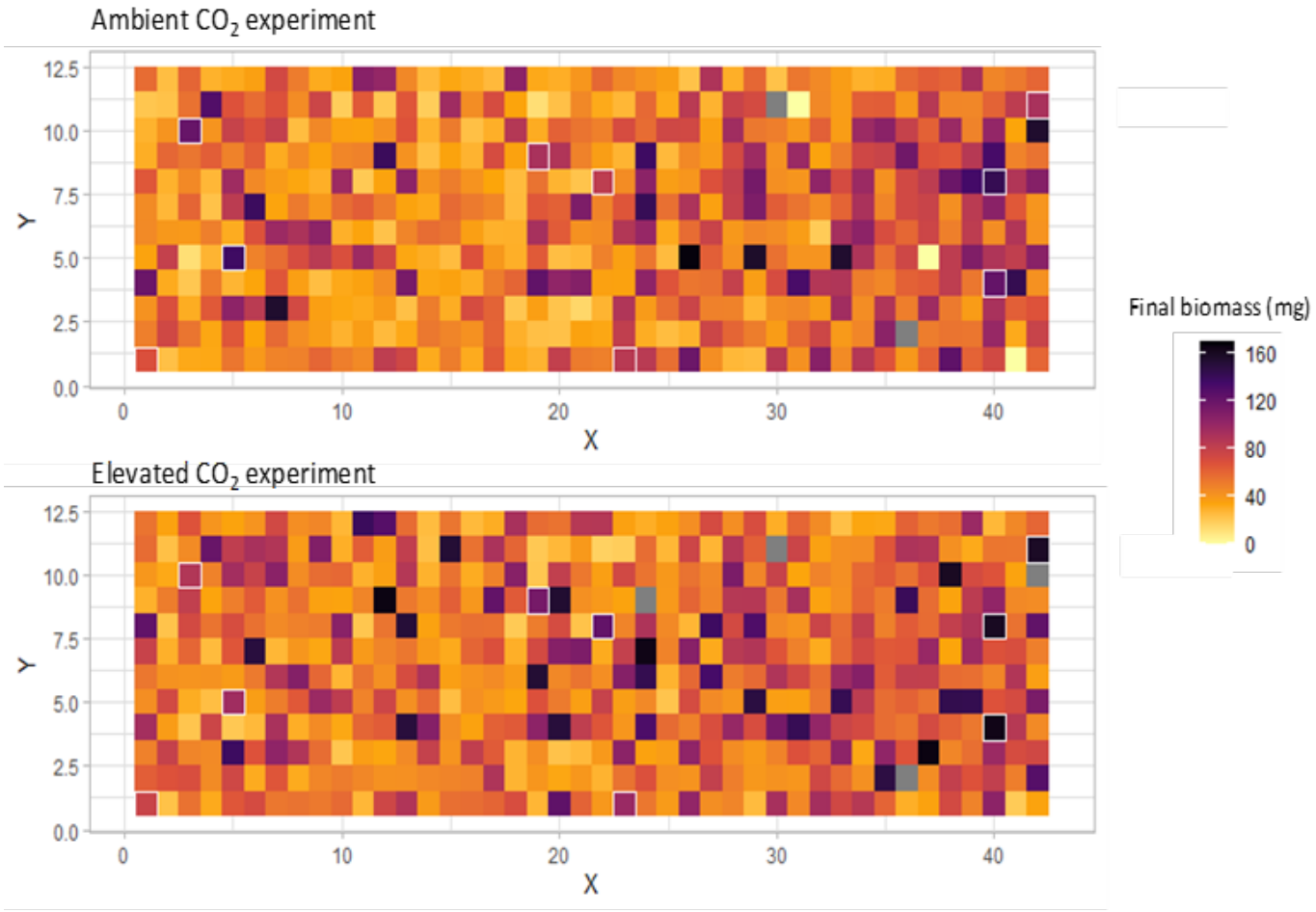

Figure 3. Experimental design and spatial variability for the final biomass in the STOCOdrought experiment. For each CO_2 condition (Ambient, ca. 400ppm ; or Elevated, ca. 800ppm), 14 genotypes x 4 watering scenarios combinations were studied, with 9 replicates per entry. This yielded a total of 504 plants for each CO_2 condition. All genotypes and watering scenarios were randomized within the platform organized as a rectangular layout of 42 rows (X) per 12 columns (Y). The heatmap represents values of final dry biomass measured at flowering stage. Plants of the wild-type Col-0 genotype grown under the well-watered (WW) scenario are highlighted with a white rectangle. A spatial gradient can be observed along the X axis, with a trend for higher biomass values towards the right end of the platform, likely due to spatial heterogeneity in environmental variables (e.g. light or air temperature). In the ongoing analyses on all traits, we are using statistics and spatial modelling tools to account for these spatial trends and ensure a precise estimation of genetic and treatment effects (e.g. those available in the statgenHTP package developed in the frame of EPPN[2020]).

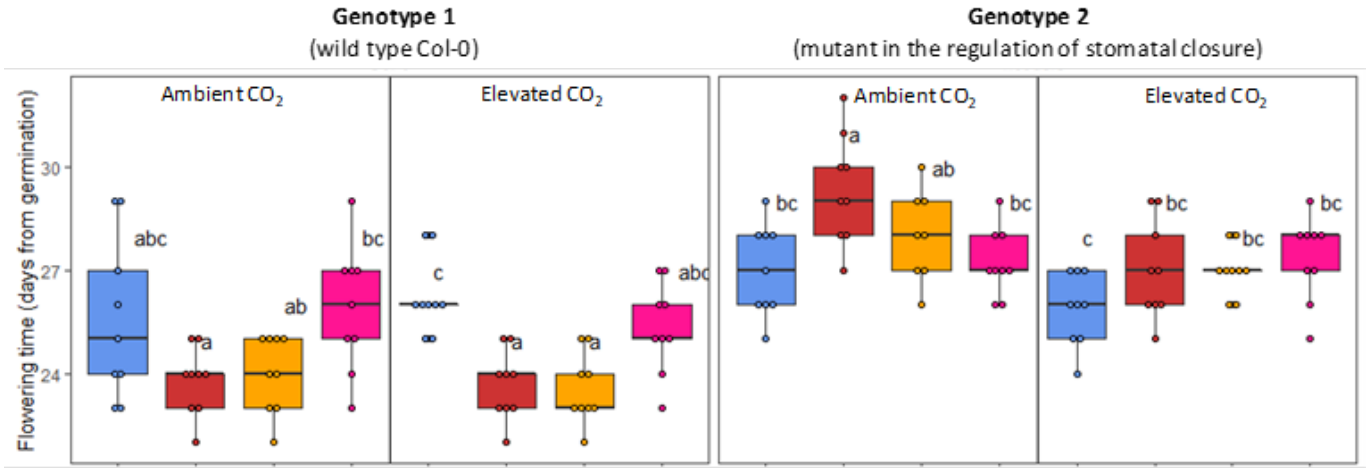

Figure 4. Phenology is differentially affected by the watering scenario depending on the atmospheric CO_2 level and the genotype. Boxplots showing the interaction effects of watering scenario, CO_2 level and genotype on flowering time exemplified for two genotypes in the STOCOdrought experiment.

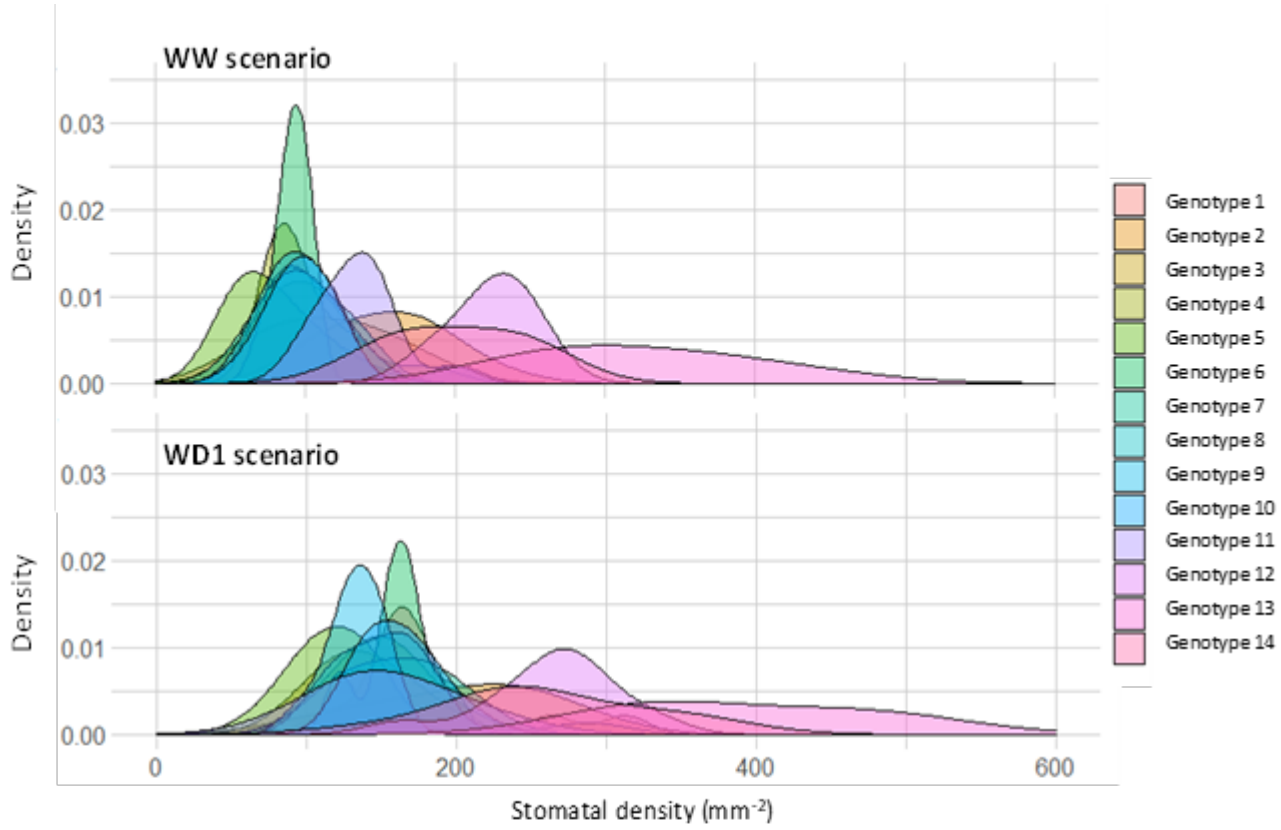

Figure 5. Stomatal density is markedly affected by sustained water deficit, with a strong variability depending on the genotype. Density plots representing the variability in stomatal density for all 14 genotypes, either under the well-watered (WW) or the early and sustained water deficit (WD1) scenario, independent on CO_2 condition. Watering scenario was found to have a stronger effect of stomatal density than CO_2 scenario.