Irrigation frequency transiently alters whole plant gas exchange, water and hormone status, but irrigation volume determines cumulative growth in two herbaceous crops

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Irrigation frequency transiently alters whole plant gas exchange, water and hormone status, but irrigation volume determines cumulative growth in two herbaceous crops

Running title: Irrigation volume and not frequency determines growth

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Highlights

- Irrigation volume, but not frequency, determined biomass growth.
- High deficit irrigation frequency increased plant water status and gas exchange.
- Attenuated growth responses to water deficit explained lack of differences in basil
- High deficit irrigation frequency changed foliar hormone levels of tomato.
- Low deficit irrigation frequency caused compensatory growth after rewatering tomato

Abstract

Physiological effects of irrigation frequency, at the same irrigation volume, have received little attention but might determine crop yield and water use efficiency. Potted plants of two species, tomato and basil, received two irrigation treatments that both supplied the same irrigation volume (75% of that received by a well-watered treatment - WW), but either frequently (once or twice per day-FDI) or infrequently (every three days-IDI). Stem diameter variations, whole-plant gas exchange, root and leaf water potential, and foliar hormones were monitored for 11 days after applying the treatments, and whole-plant biomass accumulation determined at the end of that period. Treatments showed temporal and spatial differences in soil moisture, with FDI resulting in a wet upper layer and dry lower layer. In both species, water stress integral in IDI was three-fold higher than in FDI, and gas exchange lower than FDI plants. Despite these differences, both treatments accumulated biomass and stem diameter growth similarly. In tomato, IDI induced compensatory stem growth (higher than WW plants) after re-watering, and attenuated hormone accumulation (lower jasmonic, gibberellic, and salicylic acid concentrations than FDI plants) that maintained growth. In basil, stem growth of IDI plants only recovered to WW and FDI levels upon re-watering, but lower sensitivity of stem growth to water deficits explained similar final biomass accumulation to FDI plants. Although both deficit irrigation treatments showed similar cumulative growth, temporal differences in physiological responses suggest that irrigation frequency could be tailored to specific crop species depending on their sensitivity to soil water deficits and re-hydration.

Keywords: Whole-plant photosynthesis, stem diameter variations, abscisic acid, jasmonic acid, gibberellic acid, salicylic acid

Introduction

Agricultural water use accounts for 70% of water withdrawals, 92% of global freshwater consumption (Hoekstra and Mekonnen, 2012) and the irrigated agricultural land has doubled in the past 50 years to reach about 24% of the total arable land (Siebert et al., 2010), which illustrates the importance of increasing crop water use efficiency. Applying deficit irrigation (DI) by deliberately

reducing the irrigation volume below the crop evapotranspiration requirement can result in mild soil water deficits with minimal or no yield losses (Fereres and Soriano, 2007; Geerts and Raes, 2009). Crop physiological responses to water deficits include limitation of growth and gas exchange (Bradford and Hsiao, 1982). Soil water deficit is firstly perceived by the roots, causing chemical (xylem-borne phytohormones such as ABA) and/or hydraulic (decreased water potential) signals that are transmitted to the rest of the plant (Tardieu, 2016). Therefore, optimal application of DI requires an understanding of how plants integrate multiple signals and mechanisms.

Irrigation scheduling comprises decisions on volume, placement and timing. Irrigation volume has received the greatest attention, since the difference between applied water and evapotranspiration determines soil water depletion, and hence the degree of water stress experienced by the crop. Thus, determining the soil moisture depletion that reduces crop water use without compromising growth is an essential research aim of deficit irrigation scheduling (Fereres and Soriano, 2007). However, for the same overall water content across the root-zone, plants growing under heterogeneous soil drying show different physiological responses compared to those in homogenously dry soil (Boyle et al., 2016; Puértolas et al., 2017). Thus, DI techniques that impose soil moisture heterogeneity by applying irrigation to only one part of the root-zone (partial rootzone drying - PRD), can improve crop water use efficiency compared to watering the entire rootzone with the same volume (Dodd, 2009). Nevertheless, effects of PRD can vary with irrigation volume and timing of application (Pérez-Pérez et al., 2018; Romero et al., 2012), indicating that either placement, volume, and/or timing are important in irrigation scheduling.

Effects of irrigation timing have received less attention than volume and placement, even though minimising soil moisture depletion between consecutive irrigation events can maintain plant growth and photosynthesis. Therefore, increasing irrigation frequency can increase crop yield (Valiente-Banuet and Gutierrez-Ochoa, 2016), but too frequent irrigation can induce soil salinity and/or hypoxia if water fails to infiltrate quickly (Cavero et al., 2018; Fiebig and Dodd, 2016), so

irrigation frequency has a non-linear effect on crop yield (Amin et al., 2015). Irrigation frequency effects also interact with irrigation volume. More frequent application of sub-optimal water volume (lower than evapotranspiration) increases soil moisture heterogeneity compared to more spaced applications, with higher soil moisture in the upper soil layers (Holzapfel et al., 2015; Sebastian et al., 2016). This has similar effects on plant water status and root-to-shoot regulation of plant water use as described above for PRD (Puértolas et al., 2017). However, soil moisture content and distribution varies greatly between irrigation events and re-watering triggers specific plant physiological responses (Dodd et al., 2015). Therefore, frequent (ideally continuous) and integrative plant physiological measurements are needed to understand whether and how irrigation frequency affects plant growth.

Water deficits decrease plant biomass accumulation by reducing cell growth and photosynthesis. Decreased shoot water potential restricts leaf and stem expansion and stomatal conductance to CO_2 diffusion, and inhibits the biochemical reactions of photosynthesis. However, cell growth slows at much higher potentials than those necessary to induce partial stomatal closure (Hsiao et al., 1976; Muller et al., 2011). Therefore, soil drying initially restricts plant growth by restricting tissue expansion rather than carbon gain (Fatichi et al., 2014). While expansive organ growth seems to be controlled exclusively by hydraulic mechanisms (Tardieu et al., 2014), stomatal closure can also be elicited by changes in xylem sap chemical and phytohormonal composition induced by soil drying (Shabala et al., 2016; Sobeih et al., 2004). Since wet soil layers created by high frequency DI can increase leaf water potential (Boyle et al., 2016), while dry roots could trigger root-to shoot signals that induce stomatal closure, DI frequency could decouple tissue growth from carbon gain. Moreover, re-watering greatly affects plant physiology (Dodd et al., 2015) by inducing rapid changes in plant water status associated with recovery of gas exchange (Kirchsbaum, 1987), induces transcription of many genes involved in cell growth (Spiess et al., 2012) and alters the export of xylem-borne chemical signals from the root to the shoot (Gómez-Cadenas et al., 1996; Hansen and Dorffling, 2003). These changes allow the plant to resume growth and physiological functions after a period of water stress. However, the extent of this recovery depends on the duration and intensity of water stress, with

longer more intense drying episodes inducing a slower recovery of gas exchange upon rehydration (Miyashita et al., 2005; Trueba et al., 2019) but sometimes more vigorous growth (Acevedo et al., 1971; Huang et al., 2000). Thus, the effect of DI on growth rates and stomatal conductance (which determines plant water use efficiency) might depend on the frequency of its application.

This study explored the mechanisms controlling dynamic changes in plant growth (stem diameter and leaf expansion) and whole plant gas exchange when applying contrasting deficit irrigation frequencies to tomato (*Solanum lycopersicum*) and basil (*Ocimum basilicum*). In basil, frequent deficit irrigation increased biomass accumulation compared to less frequent application of the same irrigation volume, in an experiment carried out but this response did not occur in two experiments with tomato, suggesting these two species might respond differently to deficit irrigation frequency (Busari et al., 2019). Changes in whole plant gas exchange and stem growth are expected to explain overall biomass accumulation under the different irrigation treatments. In particular, we hypothesised that:

1. Frequent application of deficit irrigation (FDI) maintains higher stem diameter and biomass growth rates than the same water volume applied less frequently (IDI), by enhancing plant water status.
2. Xylem-borne root-to-shoot signals generated during FDI restrict leaf gas exchange compared to well-watered plants (WW) that receive their evapotranspiration requirements.

Materials and methods

Two experiments (with minor variations) were carried out, each with one of the two species. Seeds of *Solanum lycopersicum* L. 'Ailsa Craig' and *Ocimum basilicum* L.. 'Genovese Gigante' were sown in germination trays and 60 seedlings were transplanted seven days after germination to 0.8 L cylindrical pots as described in Puértolas et al. (2017). Briefly, the pots (21 cm in height, 18 cm soil column, 6.5 cm in diameter) filled with an organic loam (John Innes N2, Westland, UK), are designed

to fit in a pressure chamber to determine root water potential by applying pressure to the roots. Pots were watered to saturation and pot weight at saturation recorded (PW_{sat}). PW_{sat}-40 g was set as the target weight to replace daily water losses at full irrigation, to avoid full saturation to prevent hypoxic conditions at the bottom of the pot (Passioura, 2006). Plant weight changes were calculated negligible compared to total water content in the pot (<5%). Plants were placed in a walk-in controlled environment chamber (400 µmol m^{-2} s^{-1} PAR provided by halogen lamps, HQI-BT 400W/D Osram GmbH, Munich, Germany, 24/16ºC day/night temperature, 40% air humidity, and 14 h photoperiod from 0600 to 2000). They were fully irrigated for three weeks by daily replacing evapotranspiration losses before the irrigation treatments were imposed during 11 days:

WW: plants were watered daily (6-7 hours since the start of the photoperiod) to PW_{sat}-40g. The volume of water applied to each pot was recorded and the average of the 20 plants was calculated. In both species, daily water uptake increased during the experiments from 50-60 g to more than 120 g per day. Therefore, on day 7, irrigation frequency was increased to twice a day (1-2 and 11-12 hours since the start of the photoperiod) to avoid maximum soil moisture depletion of WW plants exceeding θ_v=0.35 cm^3cm^{-3}, corresponding to soil water potential (Ψ_{soil}) = -0.15 kPa, according to a soil moisture release curve for this substrate (Puértolas et al., 2013). ***FDI***: frequent deficit irrigation, plants were watered with the same frequency as in WW (including the change at Day 7) with 75% of the average volume applied to that treatment. ***IDI***: infrequent deficit irrigation; plants were watered every three days with 75% of the average accumulated water volume applied to WW over those three days since the last watering. After three drying/re-watering cycles (Day 9), plants were watered as in FDI (twice a day with 75% of WW volume) until the final harvest (for two days).

Pot were weighed daily before irrigation to monitor plant water uptake. In three plants of each deficit irrigation treatment (FDI and IDI), two soil moisture sensors (ML3 Thetaprobe, Delta-T Devices, Cambridge, UK) were inserted through holes made on the pot wall. The central rod of the lower sensor was inserted 4.5 cm from the base of the pot, while the upper sensor was at 13.5 cm (4.5 cm from the

top of the soil column). For both sensors, the other three rods, which are arranged as an equilateral triangle, were inserted so one of the vertices laid on the longitudinal axis of the pot wall and above the central rod. The dielectric constant of the soil was recorded every 30 minutes by a datalogger (DL2e, Delta-T Devices, Cambridge, UK) and transformed into volumetric soil moisture (θ_v) using a calibration factor for organic soils. A linear regression between the last θ_v value logged immediately before the harvest of basil plants and their actual θ_g at harvest was performed and used to transform θ_v into θ_g (Supplementary Fig. 1)

Stem diameter was continuously measured in five plants per treatment with a linear variable displacement transducer (LVDT) connected to a datalogger (CR1000, Campbell Scientific, Logan, UT, USA) provided with a 16-channel relay multiplexer (AM414, Campbell Scientific), and mounted on a sensor holder made of invar alloy to minimise thermal influence on measurements. Data was logged every 30 seconds.

Whole-plant gas exchange was measured nine times during the tomato experiment and six times during the basil experiment (Fig. 1). Measurement took place within a 6 hour interval, either in the morning of the afternoon. Measurement dates and time of the day were selected to represent both deficit treatments at different points of their respective drying cycles (before and after irrigation in FDI, immediately after and before irrigation and at the middle of the cycle in IDI). The number of replicates was limited by time needed for gas exchange to stabilise in the whole-plant chamber, allowing a maximum of 9 plants (3 replicates per treatment and measurement date) to be measured within 6 hours.

Whole plant gas exchange was measured with the open system described in (Jáuregui et al., 2018) with shoots sealed inside a 15 L (25 cm x 20 cm x 30 cm) chamber made of Perspex and placed beneath two sodium lamps providing 400 µmol m^{-2} s^{-1} PAR. Air relative humidity (RH) inside the chamber was controlled by diverting air through a water bath and mixing it with dry air (5% RH) to maintain 40% RH for all measurements (24°C). Sample tubes connected both chamber inlet and outlet

pipes to the head of a gas exchange infrared analyser (Li-6400XT, LI-COR Inc., Lincoln, NE, USA), which measured water vapour and CO_2 concentrations of the air entering and exiting the chamber to calculate transpiration and photosynthesis rates per plant. Gas exchange stabilised after 30-40 minutes. Data was then logged every 15 seconds for 5 minutes, and the average value was recorded. Photosynthesis (*A*) and transpiration (*E*) rates per unit area were calculated using the leaf area measured after the third measurement (see below). Leaf area growth between the two previous measurements and the third (max 36 hours) was considered negligible. For each measurement date, *A* and *E* values of FDI and IDI plants were normalised by dividing them by the average of WW plants.

Immediately before the last gas exchange measurement of each group of measurements, each pot was sealed with duct tape and weighed (W). Right after the gas exchange measurement, water potential (Ψ_{leaf}) was measured in the uppermost fully expanded leaf with a pressure chamber. Fresh weight and leaf area of the excised leaf were measured (Li-3100C, LICOR, Lincoln, NE, USA), and the leaf was immediately collected was collected in a 1.5 ml Eppendorf tube, snap-frozen in liquid nitrogen, stored at -80°C, and then freeze-dried to determine phytohormone concentrations. Pot weight was measured and added to the fresh weight of the excised leaves to calculate water uptake rate as the difference with the initial pot weight divided by time. The shoot was then de-topped and the pot inserted in a pressure chamber. Root water potential (Ψ_{root}) was determined in FDI and IDI plants. It could not be measured for WW, the third group of measurements in IDI plants and the second group in FDI plants, as positive root pressure caused roots to exude sap after de-topping. Continuous estimation of Ψ_{root} was derived from the non-linear regression between this variable and θ_g, using the soil water content in the upper half of the pot (since the correlation was stronger than for the whole-pot θ_g) logged from the soil moisture sensors. An exponential decay function was fitted for both species (Ψ_{root}=-25.75*$e^{(-11.59*\theta g)}$ and -12.21*$e^{(-9.35*\theta g)}$ for tomato and basil, respectively). The water stress integral ($\Delta\Psi$) was calculated from this data as the sum of Ψ_{root} estimated from each θ_g multiplied by the time interval (in days) between each measurement (1/48 day=30 min). Additionally, for each species (no differences between treatments were observed), linear regressions between

actual Ψ_{root} and normalised *A* and *E* were performed (Supplementary Fig. 2). The regression equation was used to estimate diurnal values (at 10:00 and 15:00) from the previously described estimation of instantaneous Ψ_{root}.

After determining root water potential, shoot leaf area (LA) and fresh weight (W_{shoot}) were measured. Soil columns were divided into two 9 cm sections. Roots in each section were extracted, washed and oven dried (48 h at 70°C). A subsample of soil from each section was weighed and oven dried (24 h at 105°C) to calculate gravimetric soil water content (θ_g) in each section. Finally, empty pot weight (W_{pot}) was measured and total pot soil fresh weight was calculated as (W_{soil}= W-W_{shoot}-W_{pot}) (root fresh dry weight was considered negligible) to estimate total dry soil, which was used to calculate θ_g at the previous gas exchange measurements from whole pot weight (shoot fresh weight gain between gas exchange measurements was considered negligible).

At the end of each experiment, the remaining four plants not used in the gas exchange measurements were also harvested to measure dry biomass.

Different phytohormone classes, including three related to growth —indoleacetic acid (IAA), the cytokinins *trans*-zeatin (*t*Z), zeatin riboside (ZR) and isopentenyl adenine (iP), and the gibberellins GA1, GA3 and GA4— and four related to stress —the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA)—, were analyzed in freeze-dried leaves according to the protocol described by (Albacete et al., 2008) with some modifications. Freeze-dried leaf samples of tomato and basil were ground to a fine powder in a ball mill. Homogenized freeze-dried material (50 mg) was dropped in 1 ml of cold (-20°C) extraction mixture of methanol/water (80/20, v/v). Solids were separated by centrifugation (20 000 g, 15 min) and re-extracted for 30 min at 4ºC in an additional 1 ml of the same extraction solution. Pooled supernatants were passed through Sep-Pak Plus †C18 cartridge (SepPak Plus, Waters, USA) to remove interfering lipids and part of plant pigments and evaporated at 40ºC under vacuum either to near dryness or until

organic solvent was removed. The residue was dissolved in 1 ml methanol/water (20/80, v/v) solution using an ultrasonic bath. The dissolved samples were filtered through 13 mm diameter Millex filters with 0.22 µm pore size nylon membrane (Millipore, Bedford, MA, USA). Ten µl of filtrated extract were injected in a U-HPLC-MS system consisting of an Accela Series U-HPLC (ThermoFisher Scientific, Waltham, MA, USA) coupled to an Exactive mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA) using a heated electrospray ionization (HESI) interface. Mass spectra were obtained using the Xcalibur software version 2.2 (ThermoFisher Scientific, Waltham, MA, USA). To quantify the plant hormones, calibration curves were constructed for each analysed component (1, 10, 50, and 100 µg l^{-1}) and corrected for 10 µg l^{-1} deuterated internal standards.

Statistical analyses

For each species, the different variables except those derived from gas exchange measurements were analysed by two-factor ANOVA, with treatment and time of measurement as factors. Whenever measurement x treatment interaction was significant, a simple ANOVA was performed for each measurement date. Differences between treatments were assessed with a post-hoc test (Tukey, $P<0.05$). Gas exchange variables were analysed by repeated measures ANOVA for each of the three groups of consecutive measurements. Linear regression analyses determined relationships between soil water content and stem diameter growth, photosynthesis and transpiration, while an exponential decay function was fitted to the relationship between root water potential and soil gravimetric water content. Bivariate correlation analyses were performed to assess the existence of relationships between gas exchange per unit leaf area (*A, E*) and foliar concentrations of the different hormones. All the analyses were performed with SPSS 26 (IBM, Armonk, NY, USA).

Results

Deficit irrigation decreased soil water content compared to WW plants, but the temporal evolution and the spatial distribution of soil moisture varied with the frequency of application (Fig. 2). When tomatoes received FDI, θ_V in the lower half of the column decreased steadily during the first

seven days, remained stable at 0.2g g^{-1} (~ --600 kPa) for three days and increased again at the end of the experiment when the irrigation frequency increased to twice per day (Fig. 2a). In the upper half of the column, soil moisture decreased rapidly after each irrigation. After irrigation, maximum θ_v generally had values close to pot saturation (around 0.9-1.0 g g^{-1}). In IDI, soil moisture was more homogenous across the two layers, with soil in both halves of the column decreasing similarly between irrigation events down to around 0.2 g g^{-1} at the end of the three drying cycles and recovering to near-saturation values after irrigation (Fig. 2b). Soil moisture evolution was similar in basil (Fig. 2c,d), but soil moisture of the lower half of FDI pots decreased less than in tomato (to 0.4 g g^{-1}). Thus, differences in irrigation frequency caused differences in the temporal and spatial distribution of soil moisture, despite the same irrigation volumes.

In both species, WW and FDI plants maintained a similar daily evapotranspiration (ET) during the first three days after applying the treatments. Then FDI decreased ET of tomato from Days 3 to 6, which stabilised at ca. 70% of WW values during the rest of the experiment (Fig. 3a). In basil, FDI had less effect with ET reaching 80% of WW values with later recovery towards the end of the experiment when plants were irrigated twice a day (Fig. 3b). IDI plants showed greater fluctuations in ET, with ET of IDI tomato decreasing to less than 40% of WW values at the end of the first and second drying cycles, and to 20% at the end of the last. Basil showed similar responses to IDI, but with a more moderate reduction in ET (ca. 55%). In both species, re-watering recovered ET to similar values to FDI plants after 2-3 days, before decreasing again. The percentage of reduction of accumulated ET over the treatment duration was similar for both species, with FDI and IDI decreasing ET by 80% and 70% compared to WW plants. This indicates higher irrigation efficiency (ET/irrigation volume) in IDI, since the same amount of water (75% of WW) was applied to both treatments. Thus, intermittent irrigation (the IDI treatment) decreased water use to a greater extent despite similar irrigation volumes.

Root water potential (Ψ_{root}) was better correlated with soil water content of the upper half of the pot than with whole pot water content in tomato (Fig. 4a, b), while in basil both were similarly correlated reflecting less variability in soil water content between soil layers. For both species, Ψ_{root} estimated from soil moisture monitoring sharply decreased down to ca.-0.5 MPa in IDI during the drying cycles and recovered after irrigation to values close to 0 (Fig. 5). In general, estimated Ψ_{root} in FDI was higher than in IDI, especially in basil, except in the last days of the experiment after IDI irrigation frequency was switched from every three days to twice daily. Actual Ψ_{root} was similar to the estimated values. Ψ_{root} was correlated to Ψ_{leaf} in both species, with a similar relationship across the three different treatments (Supplementary Fig. 3). Water stress integral ($\Delta\Psi$) was higher for IDI than FDI for both species (P=0.005; P=0.04 for tomato and basil, respectively), with very similar values in both species (1.58±0.67, 0.48±0.04) than basil (1.52±0.47, 0.56±0.16 for IDI and FDI, respectively).

At the end of the experiments, accumulated stem diameter growth (SDG) of WW plants was 10-15% greater than in the deficit irrigation treatments in tomato and 35-50% in basil, with no statistical differences between the deficit irrigation treatments (Fig. 6a, b). FDI plants maintained a steady SDG throughout the experiment (Fig. 6 c, d) in both species. In IDI plants of both species, daily SDG decreased during the drying cycles, but the effects were greater in tomato. After re-watering, SDG of IDI plants was restored to (basil), or even exceeded (tomato), the WW values. In both species, total biomass per plant was similar between the three irrigation treatments during most of the experiment. Only at the end did WW plants achieve significantly more (20-30%) biomass, with no significant differences between the two deficit irrigation treatments (Fig. 7). Although the temporal evolution of stem growth differed with irrigation frequency, reflecting changes in soil moisture content and distribution, final accumulated growth was the same.

In general, estimated photosynthesis (*A*) and transpiration (E) of FDI plants maintained similar values as WW plants in both species, as *A* and *E* relative to WW were always close to 1 (Fig. 8,). Only E slightly decreased in tomato before watering. Intermittent irrigation (IDI) greatly decreased gas exchange, with *A* only 60-70 % of WW plants in both species (Fig. 8a, b). Transpiration declined more than photosynthesis, with minimum *E* down to 40-50% (Fig. 8c, d). Photosynthesis was weakly (but significantly) related to soil water content in tomato (Fig. 9a) but not basil (Fig. 9b), whereas transpiration rate was highly correlated with soil water content in both species (Fig. 9c, d). Thus, IDI reduced whole plant gas exchange (especially transpiration) which partially reflected reduced leaf area.

In basil, treatment was only significant for ABA (Supplementary Table 1), with largest (and significant) differences between deficit and WW treatments in at the second measurement (WW = 3.7±0.8 a; FDI = 40.0±19.8 b; IDI = 96.1±49.9 b ng g^{-1} DW). In tomato, leaf concentrations of the cytokinin isopentenyl adenine (iP) and jasmonic acid (JA) were two-fold higher in FDI plants than the other treatments at the first (pre-watering) measurement, but declined during the experiment so no differences were observed at the last measurement (Fig. 10a, d). Both deficit irrigation treatments approximately doubled gibberellin 3 (GA3) and abscisic acid (ABA) concentrations on the first measurement occasion, but then declined throughout the experiment with no differences detected at the last measurement (Fig. 10b, c). After re-watering to terminate the second drying cycle, salicylic acid (SA) concentrations were 5-fold higher in the FDI treatment than the other two treatments. No statistically significant differences between treatments were found for the other hormones studied (Supplementary Table 1). Thus, treatment differences in foliar hormone concentrations were magnified shortly after imposing the treatments, and declined over time.

Discussion

Irrigation frequency altered soil moisture distribution, plant water and hormone status, daily growth, and whole plant gas exchange in the two deficit irrigation treatments, but accumulated growth was similar between them. Thus irrigation volume, rather than how it was distributed over time, determined growth, suggesting a remarkable integration of the mechanisms by which soil moisture deficits regulate plant growth. This homeostatic response occurred in both species, regardless of the magnitude of variation of the abovementioned factors, with the faster growing tomato taking up more water thereby causing larger differences in soil moisture distribution, plant water status and daily growth. While tissue water status regulated the diel distribution of leaf growth when plants were exposed to root-zone salinity and soil water deficit, total growth (over 24 hours) was independent of plant water status suggesting that hormone signals regulated integrated growth (Munns et al., 2000). The results presented here suggest a longer duration of integration, despite of transient differences in hormone status, likely due to varying sensitivity of different processes (e.g. cell expansion, gas exchange) to water deficits.

Plant physiological responses to deficit irrigation frequency agreed with previous studies, as plant water status and water uptake increased under high frequency irrigation relative to infrequent irrigation (Boyle et al., 2016; Puértolas et al., 2017). However, re-watering IDI plants rapidly recovered water status and uptake to the same levels as in WW plants. Plant water status determines cell expansion and photosynthesis, and under varying soil moisture content, the cumulative integral of water potential (water stress integral) has been related to long-term biomass growth (Myers, 1988). Thus, higher leaf water potential of FDI plants (Fig. 5c, d) should have resulted in greater biomass accumulation. How did IDI plants compensate for transient decreases in water potential, allowing them to grow as much as FDI plants?

In tomato, higher growth rate of IDI plants after re-watering could explain similar biomass of IDI and FDI plants. However, it is difficult to ascertain the reasons explaining greater stem diameter

growth, since there were no differences in leaf hormone concentrations in response to re-watering (day 9 in Fig. 10). Other than the hormones measured in our study, re-watering can increase ethylene evolution (Beltrano et al., 1997) and promote growth (Eisinger, 1983), but levels of the ethylene precursor ACC did not show any change. Nevertheless, ACC and ethylene levels can be decoupled as ACC to ethylene conversion is controlled by ACC oxidase activity (Houben and Van de Poel, 2019), so the role of ethylene in regulating growth cannot be discarded.

Alternatively, osmolytes accumulated during drying such as water-soluble carbohydrates (Turner et al., 2012) might increase osmotic potential (osmotic adjustment), which would increase turgor pressure to higher values than cells not subjected to water stress previously (WW and FDI) (Jones and Turner, 1980). Since cell expansion is related to turgor pressure (Cosgrove, 1986), putatively higher turgor pressure of IDI plants would enhance cell growth rates. In addition, adjusting cell wall yielding properties during soil drying might contribute to the increase in growth after re-watering (Serpe and Matthews, 1994). Thus, homeostasis of growth under varying soil water content would rely on the capacity of cells to 'store' growth during drying. This suggests that less frequent irrigation could optimise water use efficiency, as decreased growth when soil is drier would be compensated by higher growth rates after re-watering. The optimal frequency of sub-optimal irrigation would then be determined by the water potential threshold limiting the capacity of the cells to 'store' growth.

While enhanced growth after re-watering can explain a similar overall response between IDI and FDI tomato plants, basil showed minimal differences in stem diameter growth without any compensatory growth after re-watering IDI plants, despite similar soil and plant water status to tomato. This could partially explain previous results showing a beneficial effect of frequent deficit irrigation in greenhouse-grown basil, in contrast with tomato (Busari et al., 2019). The lack of compensatory growth in basil IDI plants might have resulted in slower growth compared to FDI under more stressful conditions, since VPDs were presumably lower in controlled environment rooms in this

experiment compared to the greenhouse. While the lack of compensatory growth of basil might suggest it is unsuited to deficit irrigation techniques, these may be commercially adopted (Montesano et al., 2018) as they enhance quality by promoting phenolic and antioxidant accumulation (Bekhradi et al., 2015; Pirbalouti et al., 2017).

Consistently, photosynthesis per plant did not show any compensatory enhancement upon re-watering IDI plants of either species (Fig. 9), unlike observations in other species like maize or cotton (Avramova et al., 2015; Wang et al., 2018). However, these compensatory increments (above WW levels) were measured at the leaf level, which might overestimate the response. Photosynthetic recovery after re-watering decreases with leaf age (David et al., 1998), and since gas exchange measurements usually select recently fully expanded leaves, the occurrence of these responses are likely overestimated. Similarly, leaf ageing decreases sensitivity of gas exchange to water stress (Andrianasolo et al., 2016; Chen et al., 2013), which might overestimate leaf-level responses to water stress compared to whole-plant measurements.

Relatively stable, although lower growth rates (compared to WW) of FDI plants likely reflect less variable water status than in IDI and greater changes in plant hormone status than in WW. In tomato, FDI plants had higher leaf levels of the cytokinin isopentenyl adenine (iP), jasmonic acid (JA) and salicylic acid (SA) than both WW and IDI plants (Fig. 10), especially at the first two measurement occasions. These changes could not be attributed to decreased leaf water potential, and likely inhibited growth at the beginning of the experiment. Moderate, but not severe soil drying and re-watering increased plant cytokinin levels (and leaf photosynthesis rates) in rice compared to control (flooded) plants (Zhang et al., 2009). This suggests that high cytokinin (iP) levels can partially explain growth maintenance of FDI tomato plants under soil drying. Alternatively, jasmonic acid usually accumulates transiently as the soil dries, and seems to be required for ABA synthesis (de Ollas et al., 2013), while re-watering does not further change JA levels (Correia et al., 2014; Mahouachi et al., 2007). Frequent deficit irrigation maintained high JA levels, which may act to inhibit growth compared

to WW and IDI plants (Fig. 10d) and be required to allow high foliar ABA accumulation (Fig. 10c), despite higher leaf water potential. Foliar SA accumulation in FDI plants on the second measurement occasion coincided with appreciable soil drying before re-watering (0.2 cm^3cm^{-3}; ~ -33 kPa) which was absent at the third (day 9; 0.3 cm^3cm^{-3}, ~ -1 kPa) (Fig. 1a) occasion. The physiological significance of transient SA accumulation in FDI plants is obscure, but foliar sprays with SA increased growth of greenhouse tomato (Hayat et al., 2008). Overall, changes in JA, SA and iP concentrations in FDI plants were likely involved in growth regulation.

In contrast, foliar ABA and GA3 concentrations were higher in both FDI and IDI than WW plants at the two first measurement dates (Fig. 10 c, d).However, their levels could not be attributed directly either to plant or soil water status, which was higher in FDI plants, in particular for the second measurement when samples were taken immediately after FDI re-watering. This suggests that the mild stress experienced by FDI plants (Ψ_{root}~-0.2 MPa for both first and second measurement dates, Fig. 5a), was sufficient to induce foliar ABA and GA3 accumulation, which remained after re-watering. Lower intensity of water stress in FDI at the end of the experiment would explain the absence of differences in ABA and GA3 at the last measurement date. Nevertheless, while fluctuating ABA levels can explain the reduced transpiration of IDI plants during soil drying (first and second measurement) and its recovery upon re-watering (third measurement), transpiration of FDI plants was not decreased despite similar ABA levels as IDI plants. This suggests that foliar ABA levels can be decoupled from transpiration under rapid and frequent changes of plant water status. High GA3 levels are more difficult to interpret. Gibberellic acid concentrations usually decrease with abiotic stress, which has been linked to the increase of DELLA proteins that restrain growth (Colebrook et al., 2014) and promote ABA-dependant stomatal closure (Nir et al., 2017). Higher concentrations of GA3 (that should promote growth) in the deficit irrigated plants seems counterintuitive, suggesting other growth regulators have a predominant effect.

In conclusion, plant biomass accumulation was independent of irrigation frequency at the same (deficit) irrigation volume, despite the large differences in plant water status and gas exchange created by the contrasting frequencies. In tomato, compensatory growth after re-watering of infrequently irrigated plants, and differences in hormone-mediated growth regulation in plants exposed to frequent deficit irrigation, resulted in similar growth between these treatments. In basil, even though this compensatory growth was not observed, growth was maintained as soil drying had less impact on gas exchange and growth than in tomato. These results might contribute to design more water-efficient irrigation scheduling alternatives in herbaceous crops, allowing an optimal irrigation frequency depending on the crop sensitivity to soil water deficits and re-hydration.

Author statement

JP designed and executed the experiment, and led data analysis and manuscript writing. AA carried out phytohormone analyses and contributed to the analysis and discussion of the phytohormonal data. ICD contributed to the design of the experiment and manuscript writing.

Conflict of interests

We declare no conflict of interests

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References

Acevedo, E., Hsiao, T.C., Henderson, H.D., 1971. Immediate and subsequent growth responses of maize leaves to changes in water status. Plant Physiology, 48, 631-636.

Albacete, A., Ghanem, M.E., Martinez-Andujar, C., Acosta, M., Sanchez-Bravo, J., Martinez, V., Lutts, S., Dodd, I.C., Perez-Alfocea, F., 2008. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. Journal of Experimental Botany 59, 4119-4131.

Amin, M.T., Anjum, L., Alazba, A.A., Rizwan, M., 2015. Effect of the irrigation frequency and quality on yield, growth and water productivity of maize crops. Quality Assurance and Safety of Crops and Foods 7, 721-730.

Andrianasolo, F.N., Casadebaig, P., Langlade, N., Debaeke, P., Maury, P., 2016. Effects of plant growth stage and leaf aging on the response of transpiration and photosynthesis to water deficit in sunflower. Functional Plant Biology 43, 797-805.

Avramova, V., AbdElgawad, H., Zhang, Z.F., Fotschki, B., Casadevall, R., Vergauwen, L., Knapen, D., Taleisnik, E., Guisez, Y., Asard, H., Beemster, G.T.S., 2015. Drought induces distinct growth response, protection, and recovery mechanisms in the maize leaf growth zone. Plant Physiology 169, 1382-+.

Bekhradi, F., Luna, M.C., Delshad, M., Jordan, M.J., Sotomayor, J.A., Martinez-Conesa, C., Gil, M.I., 2015. Effect of deficit irrigation on the postharvest quality of different genotypes of basil including purple and green Iranian cultivars and a Genovese variety. Postharvest Biology and Technology 100, 127-135.

Beltrano, J., Montaldi, E., Bartoli, C., Carbone, A., 1997. Emission of water stress ethylene in wheat (*Triticum aestivum* L) ears: Effects of rewatering. Plant Growth Regulation 21, 121-126.

Boyle, R.K.A., McAinsh, M., Dodd, I.C., 2016. Daily irrigation attenuates xylem abscisic acid concentration and increases leaf water potential of *Pelargonium* × *hortorum* compared with infrequent irrigation. Physiologia Plantarum, 23-33.

Bradford, K.J., Hsiao, T.C., 1982. Physiological Responses to Moderate Water Stress, in: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (Eds.), Physiological Plant Ecology II: Water Relations and Carbon Assimilation. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 263-324.

Busari, M.A., Gao, P., Boyle, R.K.A., Dodd, I.C., 2019. Reconciling physiological and agronomic effects of irrigation frequency. Acta Horticulturae 1253: 253-260.

Cavero, J., Medina, E.T., Montoya, F., 2018. Sprinkler irrigation frequency affects maize yield depending on irrigation time. Agronomy Journal 110, 1862-1873.

Chen, L., Dodd, I.C., Davies, W.J., Wilkinson, S., 2013. Ethylene limits abscisic acid- or soil drying-induced stomatal closure in aged wheat leaves. Plant, Cell, and Environment 36, 1850-1859.

Colebrook, E.H., Thomas, S.G., Phillips, A.L., Hedden, P., 2014. The role of gibberellin signalling in plant responses to abiotic stress. Journal of Experimental Biology 217, 67-75.

Correia, B., Pinto-Marijuan, M., Castro, B.B., Brossa, R., Lopez-Carbonell, M., Pinto, G., 2014. Hormonal dynamics during recovery from drought in two Eucalyptus globulus genotypes: From root to leaf. Plant Physiology and Biochemistry 82, 151-160.

Cosgrove, D., 1986. Biophysical control of plant-cell growth Annual Review of Plant Physiology and Plant Molecular Biology 37, 377-405.

David, M.M., Coelho, D., Barrote, I., Correia, M.J., 1998. Leaf age effects on photosynthetic activity and sugar accumulation in droughted and rewatered *Lupinus albus* plants. Australian Journal of Plant Physiology 25, 299-306.

de Ollas, C., Hernando, B., Arbona, V., Gomez-Cadenas, A., 2013. Jasmonic acid transient accumulation is needed for abscisic acid increase in citrus roots under drought stress conditions. Physiologia Plantarum 147, 296-306.

Dodd, I.C., 2009. Rhizosphere manipulations to maximize 'crop per drop' during deficit irrigation. Journal of Experimental Botany 60, 2454-2459.

Dodd, I.C., Puertolas, J., Huber, K., Perez-Perez, J.G., Wright, H.R., Blackwell, M.S.A., 2015. The importance of soil drying and re-wetting in crop phytohormonal and nutritional responses to deficit irrigation. Journal of Experimental Botany 66, 2239-2252.

Eisinger, W., 1983. Regulation of pea internode expansion by ethylene. Annual Review of Plant Physiology and Plant Molecular Biology 34, 225-240.

Fatichi, S., Leuzinger, S., Korner, C., 2014. Moving beyond photosynthesis: from carbon source to sink-driven vegetation modeling. New Phytologist 201, 1086-1095.

Fereres, E., Soriano, M.A., 2007. Deficit irrigation for reducing agricultural water use. Journal of Experimental Botany 58, 147-159.

Fiebig, A., Dodd, I.C., 2016. Inhibition of tomato shoot growth by over-irrigation is linked to nitrogen deficiency and ethylene. Physiologia Plantarum 156, 70-83.

Geerts, S., Raes, D., 2009. Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. Agricultural Water Management 96, 1275-1284.

Gómez-Cadenas, A., Tadeo, F.R., Talón, M., Primo-Millo, E., 1996. Leaf abscission induced by ethylene in water-stressed intact seedlings of cleopatra mandarin requires previous abscisic acid accumulation in roots. Plant Physiology 112, 401-408.

Hansen, H., Dorffling, K., 2003. Root-derived trans-zeatin riboside and abscisic acid in drought-stressed and rewatered sunflower plants: interaction in the control of leaf diffusive resistance? Functional Plant Biology 30, 365-375.

Hayat, S., Hasan, S.A., Fariduddin, Q., Ahmad, A., 2008. Growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress. Journal of Plant Interactions 3, 297-304.

Hoekstra, A.Y., Mekonnen, M.M., 2012. The water footprint of humanity. Proccedings of the National Academy of Sciences 109, 3232-3237.

Holzapfel, E., Jara, J., Coronata, A.M., 2015. Number of drip laterals and irrigation frequency on yield and exportable fruit size of highbush blueberry grown in a sandy soil. Agricultural Water Management 148, 207-212.

Houben, M., Van de Poel, B., 2019. 1-Aminocyclopropane-1-Carboxylic Acid Oxidase (ACO): The enzyme that makes the plant hormone ethylene. Frontiers in Plant Science 10, 695.

Hsiao, T.C., Acevedo, E., Fereres, E., Henderson, D.W., 1976. Stress metabolism - Water stress, growth, and osmotic adjustment. Philosophical Transactions of the Royal Society 273, 479-500.

Huang, X.-M., Huang, H.-B., Gao, F.-F., 2000. The growth potential generated in citrus fruit under water stress and its relevant mechanisms. Scientia Horticulturae, 83, 227-240.

Jáuregui, I., Rothwell, S.A., Taylor, S.H., Parry, M.A.J., Carmo-Silva, E., Dodd, I.C., 2018. Whole plant chamber to examine sensitivity of cereal gas exchange to changes in evaporative demand. Plant Methods 14.

Jones, M., Turner, N., 1980. Osmotic Adjustment in Expanding and Fully Expanded Leaves of Sunflower in Response to Water Deficits. Functional Plant Biology 7, 181-192.

Kirschbaum, M.U.F., 1987. Water stress in *Eucalyptus pauciflora*. Comparisons of effects on stomatal conductance with effects on the mesophyll capacity for photosynthesis and investigation of a possible involvement of photoinhibition. Planta, 171, 466-473.

Mahouachi, J., Arbona, V., Gomez-Cadenas, A., 2007. Hormonal changes in papaya seedlings subjected to progressive water stress and re-watering. Plant Growth Regulation 53, 43-51.

Miyashita, K., Tanakamaru, S., Maitani, T., Kimura, K.., 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environmental and Experimental Botany, 53, 205-214.

Montesano, F.F., van Iersel, M.W., Boari, F., Cantore, V., D'Amato, G., Parente, A., 2018. Sensor-based irrigation management of soilless basil using a new smart irrigation system: Effects of set-point on plant physiological responses and crop performance. Agricultural Water Management 203, 20-29.

Muller, B., Pantin, F., Genard, M., Turc, O., Freixes, S., Piques, M., Gibon, Y., 2011. Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. Journal of Experimental Botany 62, 1715-1729.

Munns, R., Passioura, J.B., Guo, J.M., Chazen, O., Cramer, G.R., 2000. Water relations and leaf expansion: importance of time scale. Journal of Experimental Botany 51, 1495-1504.

Myers, B.J., 1988. Water-stress integral: a link between short-term stress and long-term growth. Tree Physiology 4, 315-323.

Nir, I., Shohat, H., Panizel, I., Olszewski, N., Aharoni, A., Weiss, D., 2017. The tomato DELLA protein PROCERA acts in guard cells to promote stomatal closure. Plant Cell 29, 3186-3197.

Passioura, J.B., 2006. The perils of pot experiments. Functional Plant Biology 33, 1075-1079.

Pérez-Pérez, J.G., Navarro, J.M., Robles, J.M., Dodd, I.C., 2018. Prolonged drying cycles stimulate ABA accumulation in *Citrus macrophylla* seedlings exposed to partial rootzone drying. Agricultural Water Management 210, 271-278.

Pirbalouti, A.G., Malekpoor, F., Salimi, A., Golparvar, A., 2017. Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum*) under reduced irrigation. Scientia Horticulturae 217, 114-122.

Puértolas, J., Alcobendas, R., Alarcón, J.J., Dodd, I.C., 2013. Long-distance abscisic acid signalling under different vertical soil moisture gradients depends on bulk root water potential and average soil water content in the root zone. Plant, Cell, and Environment 36, 1465-1475.

Puértolas, J., Larsen, E.K., Davies, W.J., Dodd, I.C., 2017. Applying 'drought' to potted plants by maintaining suboptimal soil moisture improves plant water relations. Journal of Experimental Botany 68, 2413-2424.

Romero, P., Dodd, I.C., Martinez-Cutillas, A., 2012. Contrasting physiological effects of partial root zone drying in field-grown grapevine (*Vitis vinifera* L. cv. Monastrell) according to total soil water availability. Journal of Experimental Botany 63, 4071-4083.

Sebastian, B., Lissarrague, J.R., Santesteban, L.G., Linares, R., Junquera, P., Baeza, P., 2016. Effect of irrigation frequency and water distribution pattern on leaf gas exchange of cv. 'Syrah' grown on a clay soil at two levels of water availability. Agricultural Water Management 177, 410-418.

Serpe, M.D., Matthews, M.A., 1994. Changes in cell-wall yielding and stored growth in *Begonia-argenteo-guttata L* leaves during the development of water deficits. Plant Cell Physiology 35, 619-626.

Shabala, S., White, R.G., Djordjevic, M.A., Ruan, Y.L., Mathesius, U., 2016. Root-to-shoot signalling: integration of diverse molecules, pathways and functions. Functional Plant Biology 43, 87-104.

Siebert, S., Burke, J., Faures, J.M., Frenken, K., Hoogeveen, J., Doll, P., Portmann, F.T., 2010. Groundwater use for irrigation - a global inventory. Hydrology and Earth System Sciences 14, 1863-1880.

Sobeih, W.Y., Dodd, I.C., Bacon, M.A., Grierson, D., Davies, W.J., 2004. Long-distance signals regulating stomatal conductance and leaf growth in tomato (*Lycopersicon esculentum*) plants subjected to partial root-zone drying. Journal of Experimental Botany 55, 2353-2363.

Spiess, N., Oufir, M., Matusikova, I., Stierschneider, M., Kopecky, D., Homolka, A., Burg, K., Fluch, S., Hausman, J.F., Wilhelm, E., 2012. Ecophysiological and transcriptomic responses of oak (*Quercus robur*) to long-term drought exposure and rewatering. Environmental and Experimental Botany 77, 117-126.

Tardieu, F., 2016. Too many partners in root shoot signals. Does hydraulics qualify as the only signal that feeds back over time for reliable stomatal control? New Phytologist 212, 802-804.

Tardieu, F., Parent, B., Caldeira, C.F., Welcker, C., 2014. Genetic and physiological controls of growth under water deficit. Plant Physiology 164, 1628-1635.

Trueba, S., Pan, R., Scoffoni, C., John, G.P., Davis, S.D., Sack, L., 2019. Thresholds for leaf damage due to dehydration: declines of hydraulic function, stomatal conductance and cellular integrity precede those for photochemistry. New Phytologist, 223, 134-149.

Turner, L.R., Holloway-Phillips, M.M., Rawnsley, R.P., Donaghy, D.J., Pembleton, K.G., 2012. The morphological and physiological responses of perennial ryegrass (Lolium perenne L.), cocksfoot (Dactylis glomerata L.) and tall fescue (Festuca arundinacea Schreb.; syn. Schedonorus phoenix Scop.) to variable water availability. Grass and Forage Science 67, 507-518.

Valiente-Banuet, J.I., Gutierrez-Ochoa, A., 2016. Effect of irrigation frequency and shade levels on vegetative growth, yield, and fruit quality of piquin pepper (*Capsicum annuum* L. var. glabriusculum). Hortscience 51, 573-579.

Wang, X.L., Qin, R.R., Sun, R.H., Hou, X.G., Qi, L., Shi, J., 2018. Effects of plant population density and root-induced cytokinin on the corn compensatory growth during post-drought rewatering. PLoS One 13, 16.

Zhang, H., Xue, Y.G., Wang, Z.Q., Yang, J.C., Zhang, J.H., 2009. An alternate wetting and moderate soil drying regime improves root and shoot growth in rice. Crop Science 49, 2246-2260.

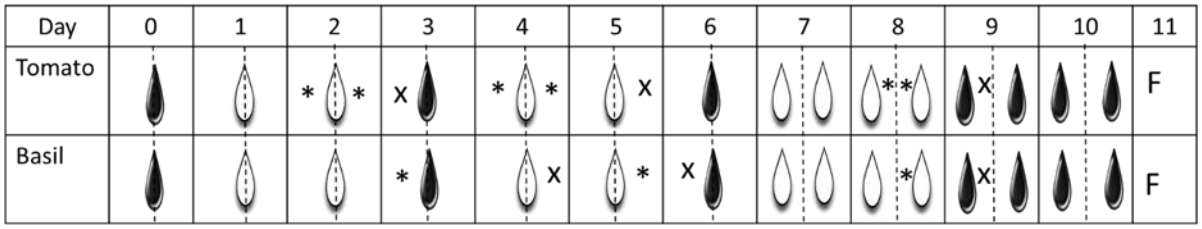

Figure 1. Timeline of the treatment application and measurements in Experiments 1 and 2. Each column represents a day, with the dashed line representing midday. Black drops represent the time when all treatments were watered, while white drops are for irrigation of FDI and WW only. Asterisks represents measurements of whole-plant gas exchange only. Crosses indicate additional measurements (whole-plant gas exchange, water potential measurements and biomass harvest) on the plants measured in the previous set of whole-plant gas exchange measurements. F indicates the final harvest of the remaining plants.

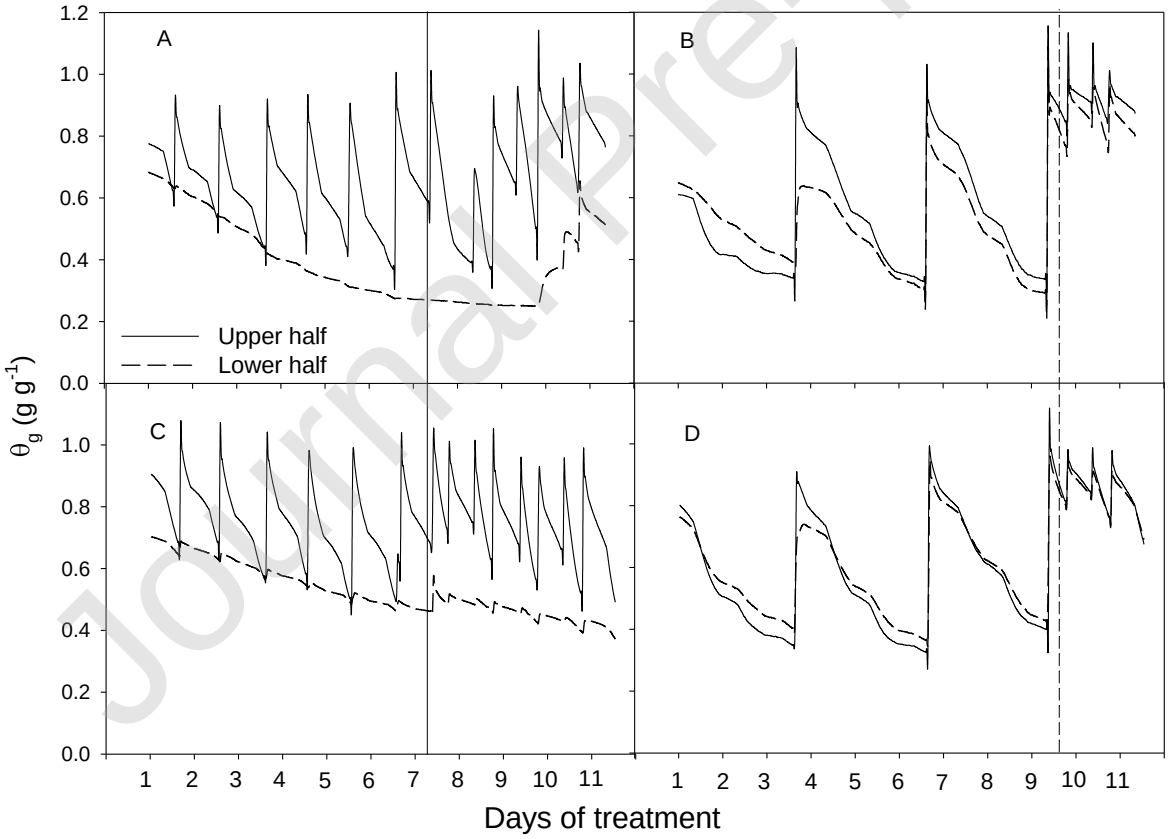

Figure 2. Soil gravimetric water content (θ_g) in the upper (solid line) and lower (dashed line) half of

the pot for the Frequent Deficit Irrigation (A, C) and the Infrequent Deficit Irrigation (B, D) treatments in tomato (A, B) and basil (C,D). Data shown is the average of four plants (error bars not shown for clarity). The vertical solid line marks the change in frequency from once to twice daily in FDI and the dashed line from once every three days to twice daily in IDI. θ_g values were estimated from θ_v measured by soil moisture sensors using the linear regression shown in Supplementary Fig. 1 (θ_g=0.07+1.92*θ_v)

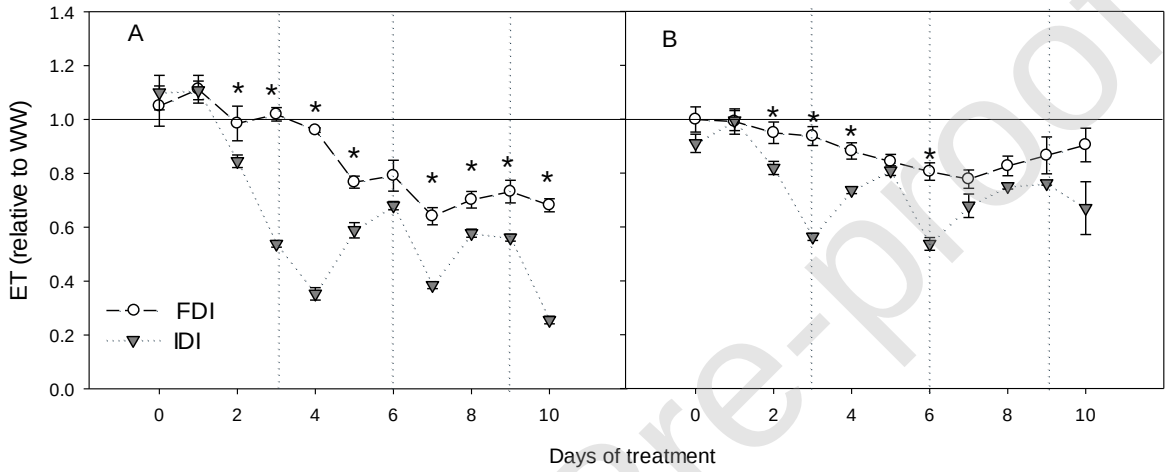

Figure 3. Evolution of daily relative pot evapotranspiration (ET) in tomato (A) and basil (B) during the experiment (n=15± s.e.) in the two deficit irrigation treatments compared to WW (FDI: white circles, dashed line; IDI: grey triangles, dotted line). The solid horizontal line indicates WW plants (relative ET=1). Asterisks denote statistical differences between FDI and IDI treatments within a date (P<0.05). Vertical dotted lines mark re-watering in IDI. Differences with WW were statistically significant for all dates and treatment, except for FDI in tomato on days 0 to 3 and for basil on days 0 to 4 and on day 10, and for IDI in tomato on day 0 and in basil on days 0 and 1).

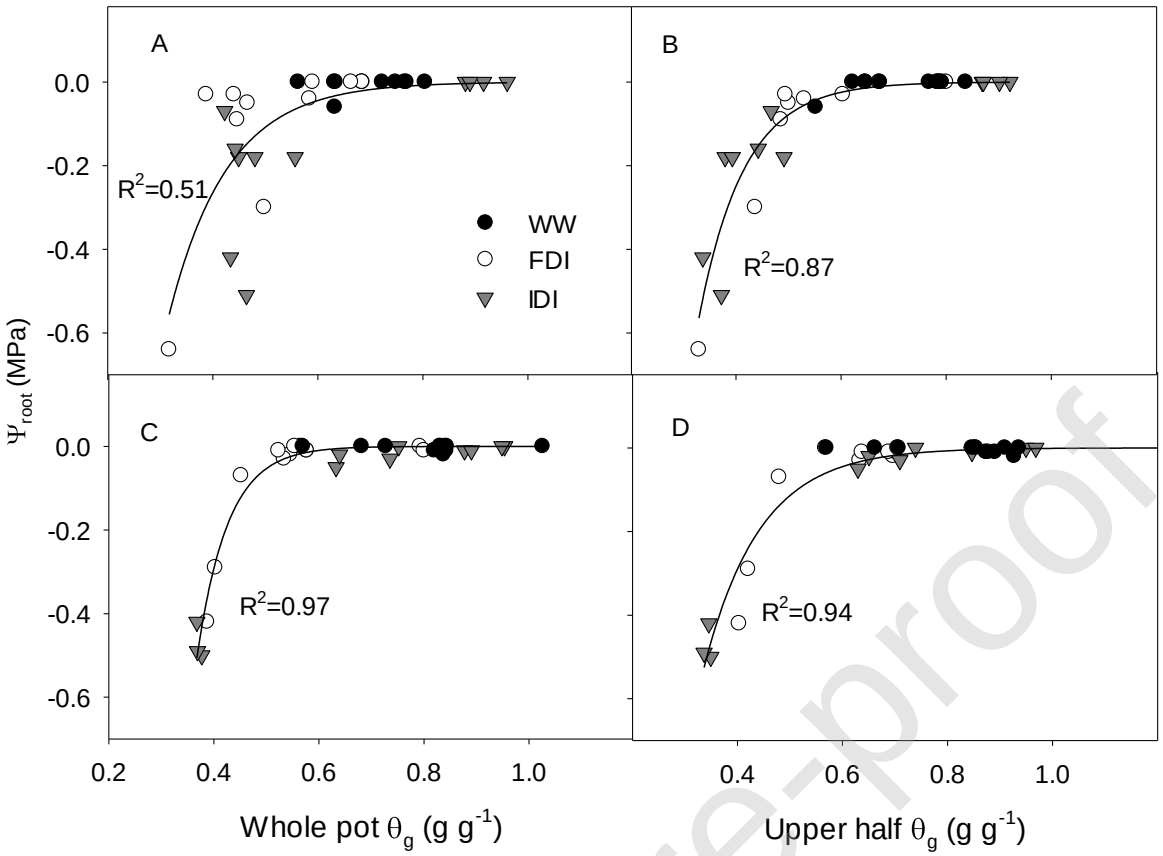

Figure 4. Relationship between whole pot (A, C) and upper half (B, D) gravimetric soil water content (θ_g) and root water potential for tomato (A,B) and basil (C, D) for the three irrigation treatments (WW: black circles; FDI: white circles; IDI: grey triangles). Each point is an individual plant. Exponential decay functions were fitted for the four panels and the R^2 value of the non-linear regression is shown.

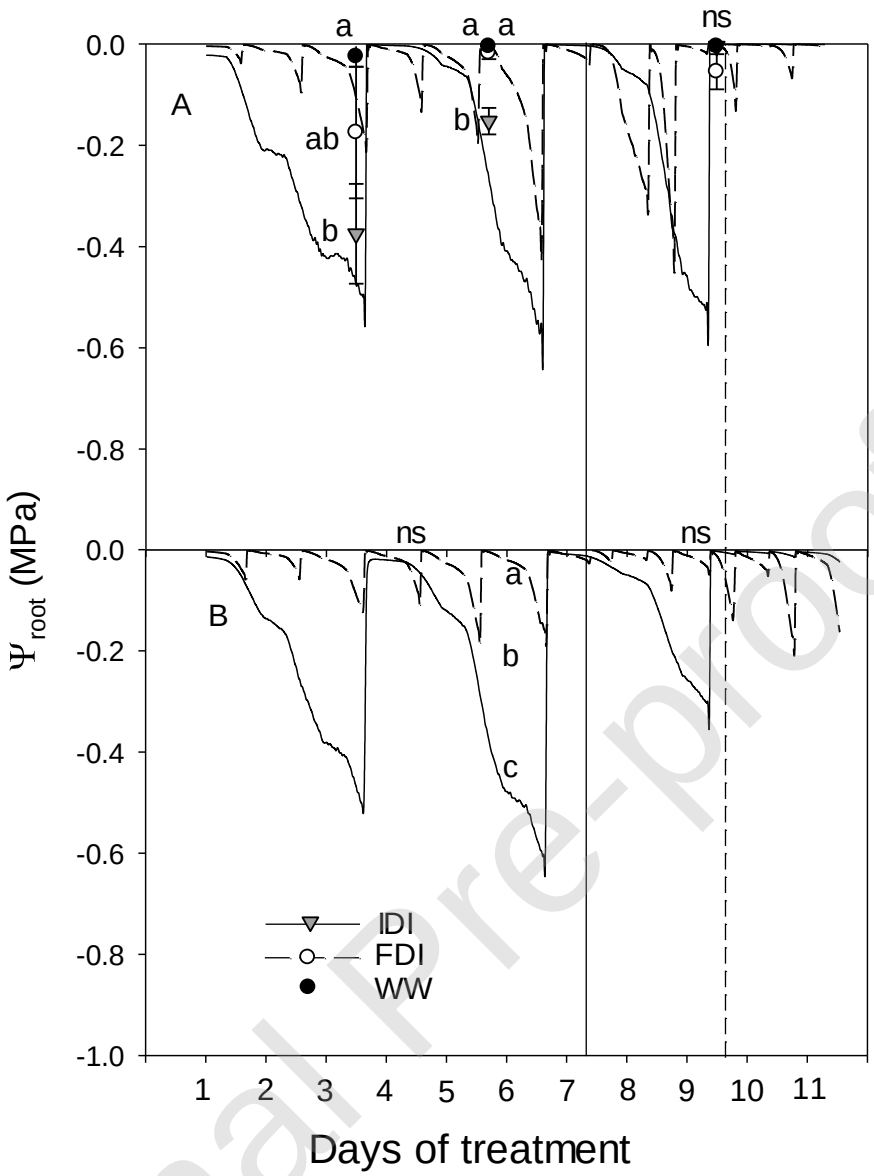

Figure 5. Root water potential (Ψ_{root}) in tomato (A) and basil (B) during the experiment for the three irrigation treatments (WW: black circles; FDI: white circles or dashed lines, IDI: grey triangles or solid line). The symbols represent actual measurements (n=4±s.e.), while lines depicts estimated values derived from soil moisture sensor measurements (n=3, error bars not shown for clarity). The vertical solid line marks the change in frequency from once to twice daily in FDI and the dashed line from once every three days to twice daily in IDI. Different letters denote statistical differences in actual Ψ_{root} between irrigation treatments within each measurement date (ns= non-significant differences).

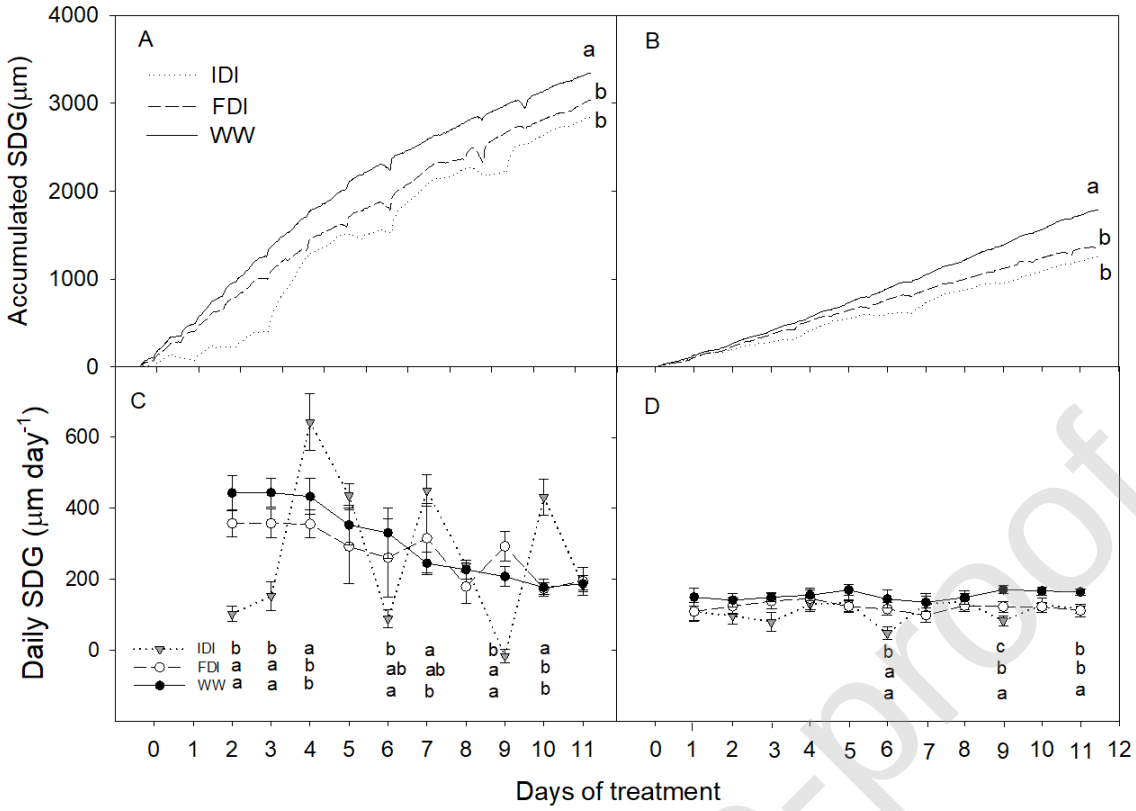

Fig 6. Accumulated (A, B) and daily (C, D) stem diameter growth (SDG) since applying the irrigation treatments to tomato (A, C) and basil (B, D). The three irrigation treatments are indicated as WW: solid line, black circles; FDI: dashed line, white circles; IDI: dotted line, grey triangles. Values for each treatment is the average of five plants. Standard errors not shown for clarity in A and B. Letters denote statistical differences for accumulated SDG on the last day (A,B) and on dates when differences between treatment were significant (C,D). Letters are assigned to each treatment following the vertical order of the legend.

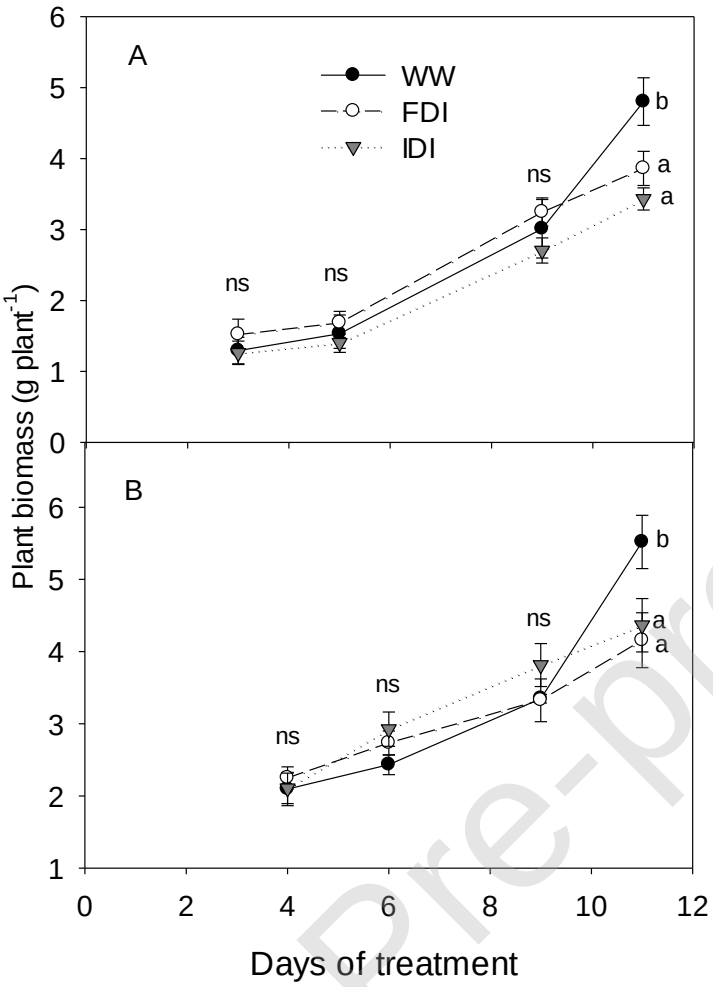

Figure 7. Evolution of total plant biomass in tomato (A) and basil (B) during the experiment (n=3 for intermediate harvests, n=8 in the final harvest on day 11 ± s.e.) in the three irrigation treatments (WW: black circles, solid line; FDI: white circles, dashed line; IDI: grey triangles, dotted line). Letters denote statistical differences between treatments (Tukey, $P<0.05$; ns: non-significant).

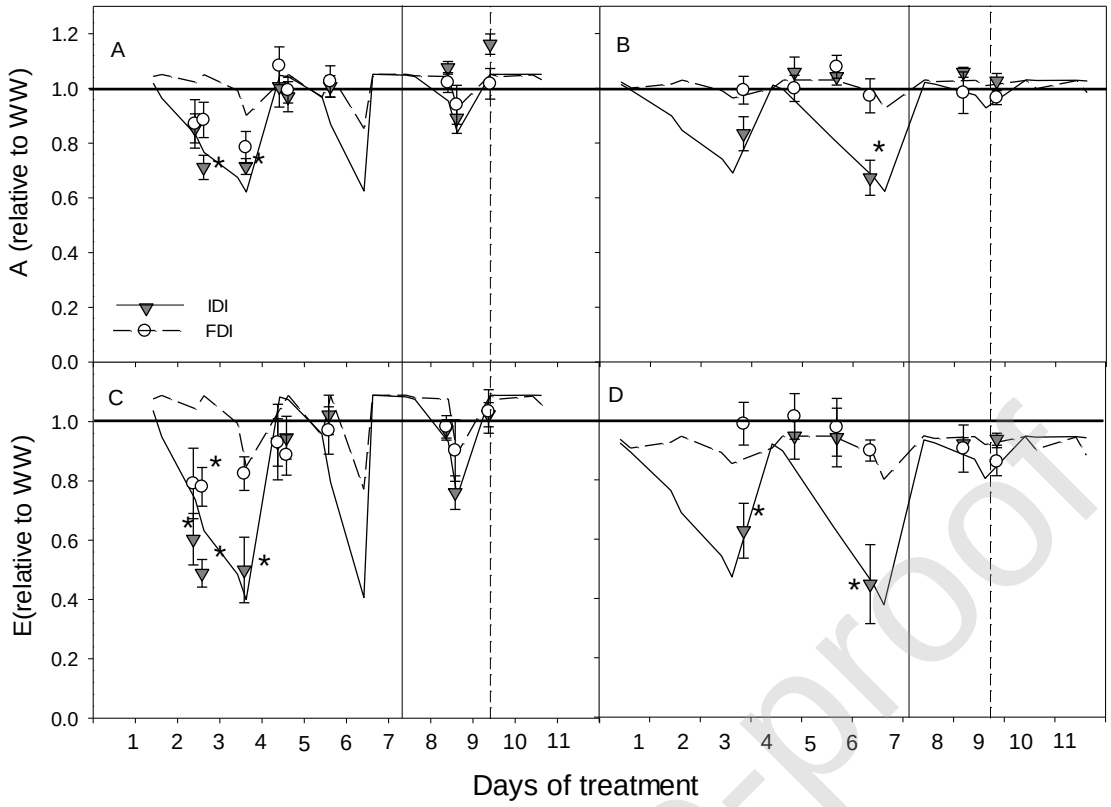

Figure 8. Relative photosynthesis (*A*) (A, B) and transpiration (E) (C, D) rates per leaf area unit estimated from continuous soil moisture measurements compared to average WW values for the two deficit irrigation treatments (FDI: white circles or dashed lines, IDI: grey triangles or solid line). The symbols represent actual measurements (n=4±s.e.), while lines depicts estimated values derived from soil moisture sensor measurements (n=3, error bars not shown for clarity). The solid horizontal line indicates no difference with WW (relative values of 1). The vertical solid line marks the change in frequency from once to twice daily in FDI and the dashed line from once every three days to twice daily in IDI. Asterisks denote statistical significance between WW and a particular treatment on that measurement.

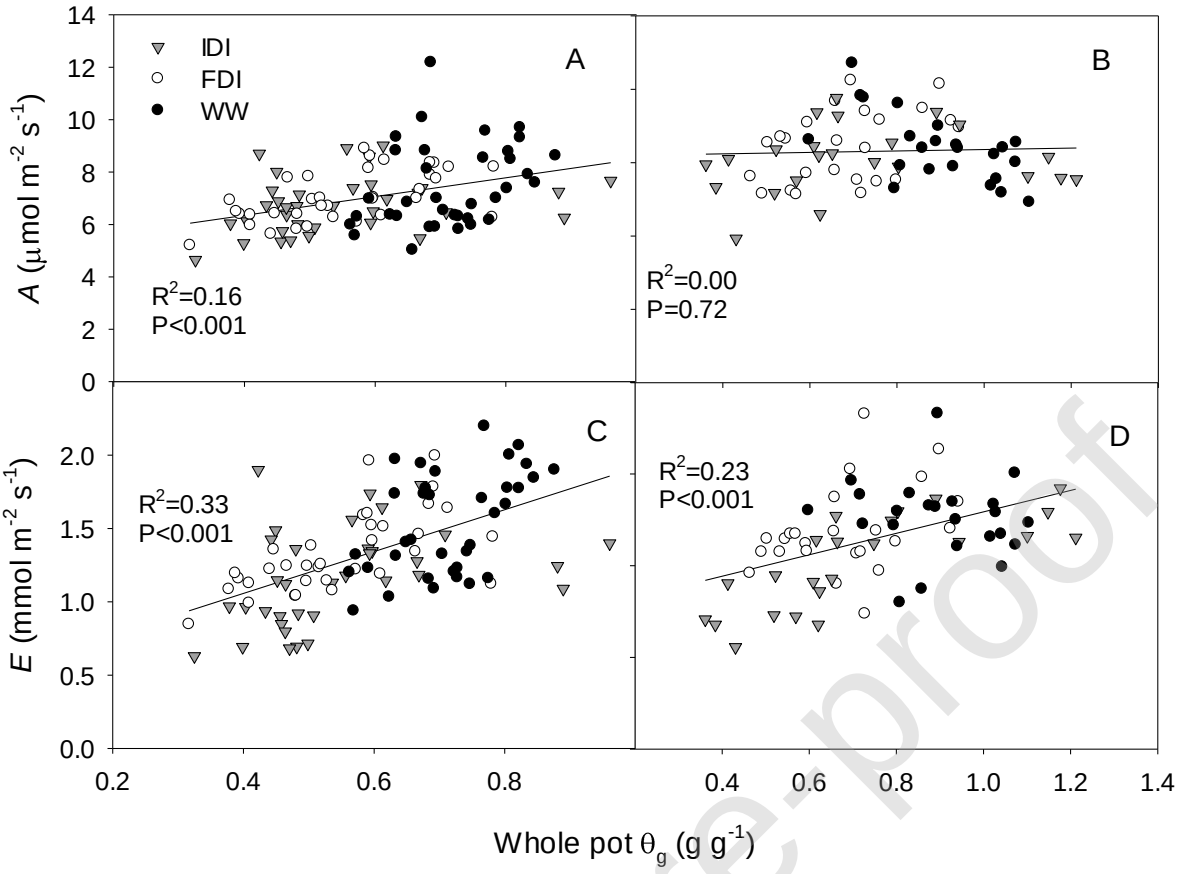

Figure 9. Relationship between whole pot soil gravimetric water content (θ_g) and photosynthesis rate (*A*) (A, B) and transpiration rate (*E*) (C,D) per unit leaf area for tomato (A,C) and basil (B, D) plants subjected to the three different irrigation treatments (WW: black circles; FDI: white circles; IDI: grey triangles). Each point represents one plant. θ Regression lines, r^2 and P-values are shown for the whole dataset in each relationship.

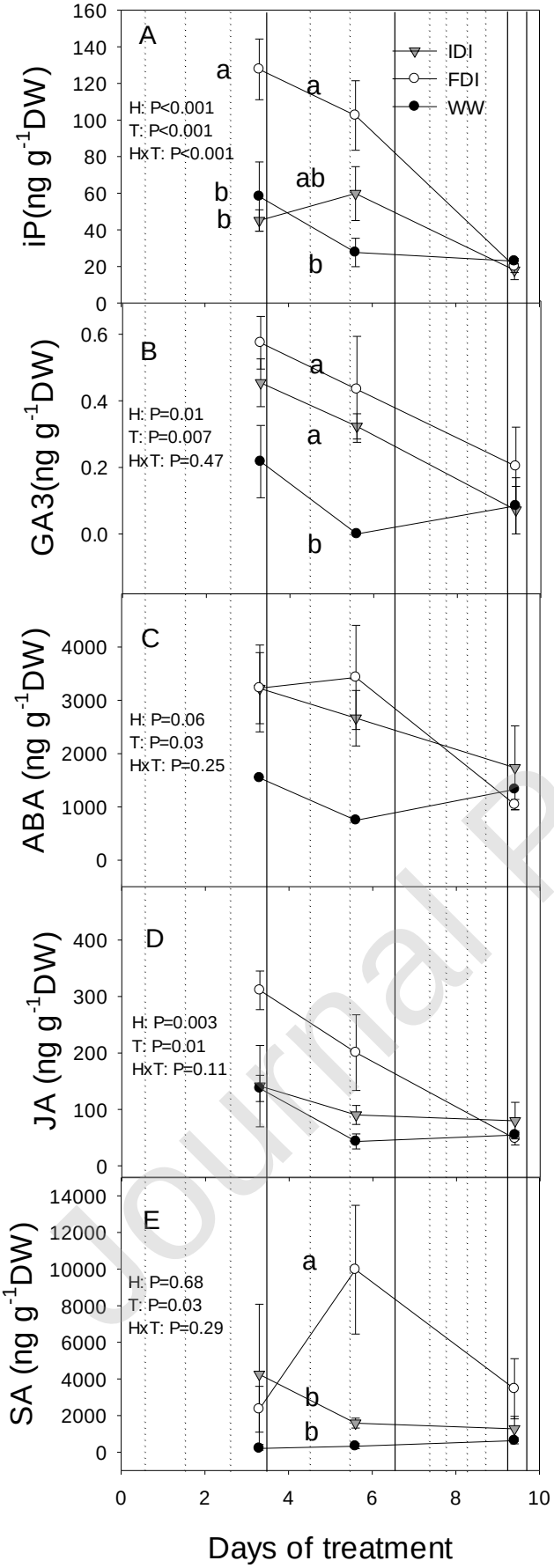

Figure 10. Foliar hormone concentration of isopentenyl adenine, iP (A); Gibberellic acid 3, GA3 (B); abscisic acid, ABA (C); jasmonic acid, JA (D) and salicylic acid, SA (E). Data area means ± SE of the three different irrigation treatments (WW: black circles; FDI: white circles; IDI: grey triangles). Different letters denote differences between treatments within a measurement date. P-values from the two-way ANOVA are shown for each hormone (H=Measurement date; T=Treatment; HxT=interaction). Letters denote statistical differences between treatments (Tukey, $P<0.05$; ns: non-significant). Vertical solid lines indicates irrigation for all treatments, while dotted lines indicate irrigation for FDI and WW only.