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# Physiological and gene expression responses of sunflower (*Helianthus annuus* L.) plants differ according to irrigation placement

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ABSTRACT

To investigate effects of soil moisture heterogeneity on plant physiology and gene expression in roots and leaves, three treatments were implemented in sunflower plants growing with roots split between two compartments: a control (C) treatment supplying 100% of plant evapotranspiration, and two treatments receiving 50% of plant evapotranspiration, either evenly distributed to both compartments (deficit irrigation – DI) or unevenly distributed to ensure distinct wet and dry compartments (partial rootzone drying – PRD). Plants receiving the same amount of water responded differently under the two irrigation systems. After 3 days, evapotranspiration was similar in C and DI, but 20% less in PRD, concomitant with decreased leaf water potential ($\Psi_{leaf}$) and increased leaf xylem ABA concentration. Six water-stress responsive genes were highly induced in roots growing in the drying soil compartment of PRD plants, and their expression was best correlated with local soil water content. On the other hand, foliar gene expression differed significantly from that of the root and correlated better with xylem ABA concentration and $\Psi_{leaf}$. While the PRD irrigation strategy triggered stronger physiological and molecular responses, suggesting a more intense and systemic stress reaction due to local dehydration of the dry compartment of PRD plants, the DI strategy resulted in similar water savings without strongly inducing these responses. Correlating physiological and molecular responses in PRD/DI plants may provide insights into the severity and location of water deficits and may enable a better understanding of long-distance signalling mechanisms.



## 1. Introduction

Alterations in rainfall patterns caused by climate change, and increasing competition for water between industrial/domestic and agricultural sectors will mean that less water will be available for irrigated agriculture in the future. While farmers may have traditionally irrigated to satisfy crop water requirements, crops of the future are likely to receive less water than their requirements, termed "deficit irrigation" (DI; [1]). Deliberate application of deficit irrigation can both reduce agricultural water use and modify crop quality and crop water use efficiency; thus considerable research has aimed to determine which deficit irrigation techniques allow water savings with minimal effects on crop yield.

There has been considerable recent interest in whether the spatial distribution of water alters crop physiological responfses. Partial rootzone drying (PRD) applies water to only half the root zone (*e.g.* one side of a row) while the other half is allowed to dry [2,3]. Part of the rootzone may remain irrigated throughout the growing season (fixed PRD) or more commonly the roots are exposed to sequential drying/re-wetting cycles. Meta-analyses have shown that this technique can increase crop yield in 20–40% of experiments, compared with crops receiving the same irrigation volumes *via* conventional deficit irrigation where the entire rootzone is irrigated [4,5]. Thus there has been considerable interest in determining the physiological mechanisms that cause differences in plant response according to irrigation placement.

PRD was originally applied to field-grown grapevines to stimulate root-to-shoot chemical signalling to limit excessive vegetative

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vigour [6]. Subsequent biochemical analyses showed that plant roots exposed to drying soil had increased ABA concentrations and decreased cytokinin concentrations, concurrent with stomatal closure [7]. However, prolonged soil drying of one soil compartment resulted in stomatal re-opening [7], as sap flow (and signalling) from roots in drying soil decreased [8,10]. For this reason, the wet and dry parts of the root system are frequently alternated to ensure root viability and transiently stimulate ABA signalling [11] which increases crop water use efficiency [12].

Although many papers have investigated plant ABA dynamics during different deficit irrigation treatments, PRD has either increased [13,14], decreased [13] or had no effect [15] on xylem ABA concentration compared to DI plants, probably since root-to-shoot ABA signalling may depend on total soil water availability [16]. Other experiments demonstrated that PRD could also enhance foliar ethylene evolution [3] and decrease foliar cytokinin concentrations [17], although it is not clear whether these responses are unique to PRD (or occur more generally in response to soil drying). Despite some evidence of differences in chemical signalling between DI and PRD plants, there has been limited research on whether plants subjected to these different irrigation techniques show differential expression of water-stress responsive genes. Tomato (*Solanum lycopersicum*) plants exposed to DI and PRD showed no consistent changes in the expression of genes related to ABA biosynthesis (*SlTAO1* and *SlNCED*) and ethylene sensitivity (*SlEIL1*; [18]), and these changes were not consistently related to soil or plant water status.

Water deficit up- or down-regulates the expression of many genes [19,20,22]. Aquaporins are important in regulating water fluxes through the plant [23]. The sunflower aquaporin gene *HaTIP7* is expressed in guard cells and root phloem, and its transcript accumulation is induced by water deficits in the root [24] and correlates with stomatal closure in the leaf [25]. Hydrophilins and late-embryogenesis abundant (*LEA*) genes, including the subgroup of plant dehydrins, are highly soluble proteins that preserve cellular integrity in drying conditions [26,27], which are typically induced by water deficit in different tissues. The sunflower *HaDHN1* is a drought-responsive dehydrin gene isolated from a tolerant sunflower line [28,29]. ACCO (1-aminocyclopropane-1-carboxylic acid oxidase) is a key regulatory enzyme in ethylene synthesis. The *HaACCO2* transcript is preferentially accumulated in sunflower leaves [30], where this gene is induced in response to drought and exogenous ABA application [28]. ABI5-interacting proteins (AFPs) are potentially involved in regulating stress responses mediated by ABA [31]. The sunflower *HaABRC5* gene is a member of the AFP family that is constitutively expressed at very low levels in leaves, seedling shoots and roots, and is up regulated by drought and exogenous ABA application [32]. Non-specific lipid transfer proteins (LTPs) are epidermal cell wall proteins involved in secretion and deposition of extracellular lipophilic material. *LTP* genes are typically induced by water deficit and ABA application [33]. The sunflower *HaLTP* transcript is accumulated in response to drought and ABA treatment [28]. The thylakoid early light-inducible proteins (ELIPs) protect plants from photooxidative damage when exposed to high light intensities or abiotic stress [34]. The sunflower *HaELIP1* gene is induced in leaves by water stress, but not by exogenous ABA application [28].

Physiological and hormonal responses of sunflower plants to partial rootzone drying were previously studied [8,9,35,36]. To ascertain whether gene expression provides additional insights into the severity and location of water stress in plants subjected to different deficit irrigation strategies, transcript levels of *HaTIP7*, *HaDHN1*, *HaACCO2*, *HaABRC5*, *HaLTP* and *HaELIP1* genes were investigated in sunflower plants subjected to both DI and PRD treatments. Gene expression was compared between DI and PRD treatments and correlated with different water status variables such as soil water content, leaf water potential and xylem ABA concentration.

## 2. Materials and methods

### 2.1. Plant culture and treatments

Sunflower (*Helianthus annuus* cv. tall single yellow) seeds were planted into 0.43 L pots (130 mm height, 65 mm diameter) containing sand (Redhill-T, J Wylie and Sons, UK) and placed in a single walk-in controlled environment room (3 × 4 m) at the Lancaster Environment Centre under the environmental conditions described previously [17]. After 4 weeks, seedlings having 6–8 leaves were carefully transplanted to new 3 L pots (200 mm diameter, 130 mm height) containing the same substrate, and the roots equally divided into two compartments separated by a vertical plastic wall within the pots. Plants were irrigated daily with a commercial nutrient solution (16:10:27, N:P:K ratio, Wonder-Gro, Wilkinson's, UK) until different irrigation treatments began.

Plants were distributed in three blocks with two pots per treatment in each block and treatments randomly arranged in the blocks. Three different irrigation treatments were applied: control (C) (well watered); deficit irrigation (DI); and PRD (partial rootzone drying). The day before initiating treatments, mean evapotranspiration was independently determined for each group of plants (C, DI, and PRD) by weighing. Well watered plants received every day 100% of the calculated mean evapotranspiration applied equally between both soil compartments; DI plants received every day 50% of the calculated mean evapotranspiration applied equally between both soil compartments and PRD plants received every day 50% of the calculated mean evapotranspiration applied to only one of the two soil compartments. Treatments were maintained for 3 days.

### 2.2. Physiological measurements

Moisture status of the upper 6 cm of substrate from both pot compartments was measured immediately before and 20 min after daily irrigation with a theta probe (Model ML2x, Delta-T Devices, Burwell, UK). Readings were recorded in millivolts (mV) and transformed to gravimetric water content based on a substrate-specific calibration. In control and DI plants, values were averaged from both compartments, while both compartments were measured independently in PRD plants.

Evapotranspiration was measured gravimetrically as the difference in pot weight determined 20 min after watering and immediately before the next watering. Measures were taken at 24, 48, 60 and 72 h after the beginning of the assay. Leaf water potential was measured using a Scholander-type pressure chamber (Soil Moisture Inc.), and then leaves were subjected to an overpressure of 0.2–0.4 MPa, to allow xylem sap to be collected into pre-weighed microcentrifuge vials. Sap was immediately frozen in liquid nitrogen and stored at −20 °C prior to determination of ABA concentration by radioimmunoassay [37], using the monoclonal antibody AFRC MAC 252. To minimise the time between leaf abscission and sealing the leaf into the pressure chamber, this was located near to the controlled environment room.

### 2.3. RNA extraction

After 72 h of the different irrigation treatments, plant roots were carefully washed from the pots (which was achieved rapidly, since plants were grown in sand which did not adhere strongly to the roots), and leaf and root samples were immediately frozen in liquid nitrogen. To minimise diurnal changes in gene expression confounding our analysis, plants were harvested between 0900 and