Postprint of: Plant Science (227) 37-44 (2014)

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

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Abstract

To investigate effects of soil moisture heterogeneity on shoot physiology and root and foliar gene expression, 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). After 3 days, evapotranspiration was similar in C and DI, but 20% less in PRD, concomitant with decreased leaf water potential (Ψ_{leaf}) and increased leaf xylem ABA concentration. Six water-stress responsive genes were highly induced in roots of PRD plants exposed to drying soil, but not in roots of DI plants exposed to higher soil moisture content. In leaves of PRD plants, gene expression was correlated with increased xylem ABA concentration and decreased Ψ_{leaf}. PRD triggered stronger physiological and molecular responses than DI in roots and leaves suggesting a more intense and systemic stress response due to local dehydration of the dry compartment. Physiological *vs.* molecular correlation studies in PRD/DI plants provide insights into the severity and location of water deficit and may enable a better understanding of long-distance signalling mechanisms.

Key-words: deficit irrigation, partial rootzone drying, ABA, gene expression.

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 responses. Partial rootzone drying (PRD) applies water to only half the root zone (eg. 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 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 signaling 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 [19-22]. 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 (Ouvrard *et al.* 1996; Cellier *et al.* 1998). ACCO (1-aminocyclopropane-1-carboxylic acid oxidase) is a key regulatory enzyme in ethylene synthesis. The *HaACCO2* transcript is preferentially accumulated in sunflower leaves (Liu *et al.* 1997), where this gene is induced in response to drought and exogenous ABA application (Ouvrard *et al.* 1996). ABI5-Interacting Proteins (AFPs) are potentially involved in regulating stress responses mediated by ABA (Garcia *et al.* 2008). 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 upregulated by drought and exogenous ABA application (Liu *et al.* 2004). 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 (Colmenero-Flores *et al*, 1997). The sunflower *HaLTP* transcript is accumulated in response to drought and ABA treatment (Ouvrard *et al.* 1996). The thylakoid early light-inducible proteins (ELIPs) protect plants from photooxidative damage when exposed to high light intensities or abiotic stress (Hutin *et al*, 2003). The sunflower *HaELIP1* gene is induced in leaves by water stress, but not by exogenous ABA application (Ouvrard *et al.* 1996).

Physiological and hormonal responses of sunflower plants to partial rootzone drying were studied in previous work (Masia *et al*. 1994; Dodd *et al*. 2008a,b; Dodd *et al*. 2010). 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 was 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 x 4 m) at the Lancaster Environment Centre under the environmental conditions described previously (Kudoyarova *et al*., 2007). 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 minutes 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 72h 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 –20ºC prior to determination of ABA concentration by radioimmunoassay (Quarrie *et al*., 1988), using the monoclonal antibody AFRC MAC 252. To minimize 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, 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 9,00 AM and 16,30 PM in different pools (each pool contained two control, two DI and two PRD samples) until harvesting was complete. Root samples

were combined from both compartments in control and DI plants, but roots from each compartment were treated separately in PRD plants. Total RNA was extracted from each individual plant using 1g (fresh weight) of roots or pooled leaves as described by Bekesiova *et al.* (1999). Genomic DNA was removed by DNase I treatment.

2.4 Retrotranscription (RT) real-time PCR

The expression profile of six water stress-responsive genes *HaTIP7, HaDHN1, HaACCO2, HaABRC5, HaLTP* and *HaELIP1* was analysed in roots and leaves of PRD, DI and control plants by retrotranscription real-time PCR. Primers were designed with the Primer Express 2.0 program using sequences reported in the GenBank database (Table 1). Retrotranscription reactions were performed using 1 µg of DNA-free RNA with the QuantiTect^{TM} Reverse Transcription kit (Qiagen) following the manufacturer's instructions.

Real time PCR was performed in a Chromo 4, Biorad thermocycler using the Sensimix*Plus* SYBR kit (Bioline). The reaction mixture contained 0.5 µg of synthesised cDNA, 1x master mix, 0.6 µM of forward and reverse primers, respectively for *HaTIP7, HaDHN1* and *HaACCO2* genes, and 0.3 µM of forward and reverse primers for *HaABRC5, HaLTP* and *HaELIP1* genes. PCR reactions were run with the following program: 95ºC for 10 min, and 40 cycles of 95ºC for 15 s and 60ºC for 45 s. A melting curve was applied for checking the specificity of the amplification. The dissociation program consisted of 95ºC for 15 s, 60ºC for 15 s followed by 20 min of slow ramp from 60 to 95ºC. Three technical replicates (3 wells/ PCR reaction) were included per sample.

A standard curve was constructed using 10-fold serially diluted sunflower genomic DNA from 500 to 0.05 ng. Transcription levels of the studied genes in control plants

were calculated by interpolation of the RT real-time PCR results to the standard curve and calculation of the average and standard error (SE) of 6 biological and 3 technical replicates (3 PCR wells/sample). The expression levels of the studied genes in PRD and DI plants were determined relative to the gene expression level in the control treatment applying the $2^{-\Delta C}{}_{T}$ method as previously described (Livak & Schmittgen, 2001).

2.5 Statistical analysis

Tukey´s Test determined treatment differences at 5% level of significance after analysis of variance (SAS Institute, Inc., Cary, NC, USA). Correlation analyses among soil and plant water parameters, as well as gene expression values, were performed with Pearson´s correlation coefficient at 5% level of significance (SAS Institute, Inc., Cary, NC, USA) on an individual plant basis. Soil and plant variables were soil water content, leaf water potential, xylem sap ABA concentration and root and leaf expression levels of the studied genes (*HaTIP7, HaDHN1, HaACCO2, HaABRC5, HaLTP* and *HaELIP1*).

3. Results

3.1 Physiological traits

Before harvesting the plants, soil water content (Θ) of control plants was significantly higher than that of DI plants and the wet part of PRD plants, whereas Θ of the dry compartment of PRD plants was significantly lower than the other treatments (Table 2). Evapotranspiration rates were similar in control and DI plants throughout the experiment, while it was about 20% less in PRD plants over the last two days (Fig. 1). Leaf water potential (Ψ_{leaf}) was significantly lower in PRD than in control and DI plants ($P < 0.05$) at the end of the experiment, while xylem ABA concentration was

significantly higher in PRD plants than control and DI plants (Table 2). Xylem ABA concentration and Ψ_{leaf} were statistically similar in DI and control plants (Table 2).

Low soil moisture in the dry compartment of PRD plants (PRD-DR) correlated with low leaf water potential (Fig. 2A) and high xylem ABA concentration (Fig. 2B). At the same whole pot soil water content (PRD-AVG), PRD plants had a lower leaf water potential (Fig. 2A) and higher xylem ABA concentration (Fig. 2B) than DI plants. Pearson correlation studies of these parameters found significant (negative) correlations between whole pot and local soil water content and xylem ABA concentration ($P < 0.05$ and $P < 0.01$). These correlations were more negative and significant when local soil water content of dry compartment was used ($P < 0.01$) (Table 3). Thus PRD plants exhibited more severe symptoms of water deficit (higher xylem ABA concentration and lower Ψ_{leaf}) than DI plants, even though both sets of plants were irrigated with 50% of their respective evapotranspiration.

3.2 Expression of water stress responsive genes

All genes studied had higher expression levels in leaves (than roots) of well-watered plants, with the exception of *HaABRC5*, which showed low and similar expression in both plant tissues (Fig. 3). *HaLTP* and *HaTIP7* transcript levels were around 10 to 100-fold higher than the other genes (Fig. 3B).

To study the molecular responses to water deficit in sunflower plants, the differential (treated *vs* control) expression of the genes was quantified (Fig. 4). All studied genes were significantly induced in roots of the dry compartment of PRD plants, and the differential induction was always higher ($P < 0.05$) in this root compartment compared to the wet compartment of PRD plants and roots from DI plants, with the exception of *HaTIP7* gene, in which the differential induction of PRD (DR) was significantly higher

than DI, but not than PRD (WR). In these root compartments (PRD-WR and DI), expression of the *HaABRC5, HaELIP1* and *HaLTP* genes was not induced but *HaTIP7, HaACCO2,* and *HaDHN1* genes were moderately induced (Fig. 4).

Differential induction of *HaACCO2, HaDHN1* and *HaLTP* genes was also significantly stronger ($P < 0.05$) in leaves from PRD plants compared to DI plants (Fig. 4). The *HaELIP1* gene exhibited moderate induction in PRD plants and no induction in DI plants. The *HaTIP7* gene was significantly ($P < 0.05$) down regulated in leaves from the PRD treatment compared to the DI treatment. Finally, gene expression of *HaABRC5* in leaves of DI plants was significantly ($P < 0.05$) less than in PRD plants (Fig. 4). Generally, there was a stronger gene response to water deficit in roots and leaves of PRD plants compared to DI plants (Fig. 4).

3.3 Correlations between plant water status and gene expression

To determine correlations between gene expression and the measured soil and plant water status, Pearson correlation studies were performed (Tables 4 and 5). In the root (Table 4), gene expression of *HaABRC5*, *HaACCO2*, *HaTIP7*, and *HaDHN1* genes was most significantly and negatively correlated with local soil water content, and significantly and positively correlated with xylem ABA concentration (except for *HaABRC5*). Only root expression of the dehydrin gene *HaDHN1* was significantly and positively correlated with leaf water potential.

Foliar gene expression was most significantly correlated with leaf xylem ABA concentration, with significant and positive correlations for expression of *HaDHN1* and *HaLTP*, and a significant but negative correlation for *HaTIP7* expression. Leaf water potential was positively and significantly correlated with *HaDHN1* and *HaELIP1*, whereas whole pot soil water content was only negatively and significantly correlated

with expression of the dehydrin gene *HaDHN1* in the leaf. No significant correlation was found between either root or leaf gene expression and evapotranspiration (data not shown). Interestingly, root and shoot *HaDHN1* gene expression was significantly correlated with all parameters associated with water deprivation (decreased soil water content and Ψ_{leaf}, and increased xylem ABA concentration).

4. Discussion

While previous work has demonstrated that irrigation placement (PRD *versus* DI) can affect leaf water status, stomatal conductance and xylem ABA concentration in plants at the same soil water status (Dodd *et al*., 2008 a, b; Wang *et al*., 2012), relatively little work has aimed to determine whether these irrigation techniques alter gene expression in different organs or parts of the root system. Both ABA-dependent and ABA independent regulatory systems govern drought-inducible gene expression, which can be locally triggered in different plant organs due to tissue dehydration (causing loss of cell turgor or increased osmolarity) and/or ABA accumulation (Bartels and Sunkar, 2005; Yamaguchi-Shinozaki and Shinozaki 2006; Fujii *et al*., 2009; Fujii and Zhu, 2012; Huang *et al*., 2012). In this work, although both PRD and DI plants received 50% of their measured evapotranspiration, the PRD treatment induced stronger physiological (decreased Ψ_{leaf} and increased xylem ABA concentration) and molecular (gene expression) responses compared to the DI treatment, probably due to severe soil drying of the non-irrigated compartment, suggesting the importance of the watering pattern.

These observations seem contrary to the stated aims of PRD, that of increasing xylem ABA concentration to maintain leaf water potential (Dry *et al*. 1996). Whether these changes occur *in planta* depend on irrigation volumes supplied to the crop, and thus total soil water availability (Romero *et al*. 2012). Supplying 50% less irrigation to

containerised plants has usually decreased Ψ_{leaf} (relative to well watered plants) irrespective of irrigation placement (Wakrim *et al*. 2005; Campos *et al*. 2009) although in some cases Ψ_{leaf} was statistically similar (Stoll *et al*., 2000; Sobeih *et al*., 2004) perhaps due to root system proliferation of PRD plants (Mingo *et al*., 2004). At the same irrigation volumes, PRD plants had either statistically similar or lower (Wang *et al*. 2012) Ψ_{leaf} (depending on the day of measurement) than DI plants, although the large difference (0.32 MPa) detected here requires further explanation.

Severe soil drying increases hydraulic resistance at the root/soil interface, especially in sandy soils (Bristow *et al*., 1984) due to poor soil-root contact (Nobel and Nobel 1997). Although alternating wetting and drying parts of the rootzone every 10 days increased whole root system hydraulic conductance (Lp) of maize by increasing Lp of roots in drying soil, maintaining some roots in dry soil (as applied here) for 40 days decreased Lp of these roots by 80-90% compared to those in irrigated soil (Hu *et al*., 2012). Similar decreases in hydraulic conductance of roots in drying soil likely explains the decreased whole plant hydraulic conductance of PRD plants compared to DI plants (data not shown), even though both treatments were at similar total soil water availability. Although PRD decreased evapotranspiration by 20% (Fig. 1), leaf water potential fell by 38% (Table 2; Fig. 2), in contrast to the responses of DI plants. Whether decreased whole plant hydraulic conductance of PRD plants can be attributed directly to increased xylem ABA concentration is less certain. Recent reports suggest that supplying high (μM) exogenous ABA concentrations to detached leaves decreased leaf hydraulic conductance (Shatil-Cohen *et al*., 2011; Pantin *et al*. 2013) unlike ABA's stimulatory effect on root hydraulic conductance (Thompson *et al*. 2007). Further direct measurements of both leaf and root hydraulic conductance of PRD plants seem

necessary, since the direction of response of hydraulic conductance to ABA is highly dose-dependent (Dodd 2013).

Elevated xylem ABA concentration of PRD plants also requires explanation. Previous work with "two-root, one-shoot" grafted plants grown in a range of substrates showed that xylem ABA concentration increased more sensitively in response to decreased soil matric potential in sand (used in this work) than in other substrates such as loam and clay soils (Dodd *et al*., 2010). Sand water content was significantly correlated with xylem ABA concentration (Table 3), likely since roots in drying soil were exposed to a critical soil moisture threshold (Fig. 2). While roots in drying soil accumulate ABA as soil moisture decreases (Zhang and Davies 1989; Puertolas *et al*. 2013), there is less certainty that much of this ABA is actually transported to the shoots since excessive soil drying decreases sap flow (and thus transport of root-to-shoot signals such as ABA) from root systems of severely dehydrated plants (Gomez-Cadenas *et al*, 1996) or from root systems of plants exposed to PRD for prolonged periods of time (Romero *et al*. 2012). An alternative explanation is that the increased xylem ABA concentration of PRD plants was triggered by leaf water deficit (Fig. 2B), even though xylem ABA concentration and leaf water potential were not correlated across all treatments (Table 3). The relatively rapid decline in Ψ_{leaf} of PRD plants (-0.1 MPa day^{-1}) may not have allowed sufficient osmotic adjustment to maintain turgor as evidenced visually from the wilted appearance of these plants, triggering ABA biosynthesis in response to decreased turgor (Pierce and Raschke 1981).

Comparing gene expression in response to DI and PRD treatments in different organs can help elucidate the site of primary responses and how they are transduced. In this work, the largest response at the gene expression level occurred in roots present in

the dry compartment of the PRD treatment (Fig. 4), where all the genes tested were significantly induced, and more intensely, than in other treatments and compartments. Thus gene expression correlated better with local soil water content (Table 4), supporting the concept that soil dehydration is the primary effector leading to secondary responses like ABA accumulation and gene expression. In other root compartments with higher soil water contents (the well-watered compartment of the PRD treatment and the DI compartments), root gene expression was similar to control conditions (differential expression values close to zero in *HaABRC5*, *HaELIP1*, *HaDHN1* and *HaLTP*; Fig. 4) or the induction value was significantly lower than that of the PRD dry compartment (in *HaTIP7* and *HaACCO2*; Fig. 4). Typical drought-responsive genes like those encoding the dehydrin and the lipid transfer proteins (*HaDHN1* and *HaLTP* respectively; Colmenero-Flores *et al* 1997; Bartels *et al*, 2005) were not induced in DI roots under the experimental conditions described here, indicating that root gene expression responded primarily to local dehydration of the dry compartment of PRD plants rather than to total irrigation volume.

In leaves, consistent with the arguments above, the strongest molecular response was also observed in PRD plants, with four genes (*HaLTP*, *HaDHN1*, *HaACCO2*, and *HaTIP7*) significantly responding to PRD and a single gene (*HaABRC5*) barely responsive to DI (Fig. 4). Altered foliar gene expression was better correlated with leaf xylem sap ABA concentration and/or Ψ_{leaf} than soil water content (Table 5), indicating that foliar gene expression is better related to local (rather than systemic) processes. In contrast to the roots, where expression of selected genes was universally upregulated in response to soil drying, foliar gene expression showed differential responses according to the gene of interest. PRD significantly increased the expression of some genes (*HaLTP*, *HaDHN1*, *HaACCO2*) while strongly downregulating the tonoplast aquaporin

HaTIP7. Downregulation of the *HaTIP7* gene in leaves in response to drought was previously described (Poormohammad Kiani *et al*, 2007). It has been broadly illustrated how some gene families can be differentially regulated within different organs. This is usual in membrane transporters, where biological function can vary depending on the location, even when placed in the same cell type (see for instance Brumos *et al*, 2009; Wei-hong *et al*, 2013), supporting the idea that the biological role played by these type of proteins in water stress conditions requires simultaneously increased activity in the root and lower activity in the leaf (Fig. 4).

The expression in roots and leaves of genes like *HaLTP, HaDHN1, HaACCO2 HaTIP7*, previously described as ABA-responsive in sunflower and other plant species (Ouvrard *et al.* 1996; Colmenero-Flores *et al*. 1997; Wei-hong *et al*. 2013), was indeed correlated with xylem ABA concentration (Table 4 and 5). This group of ABA-responsive genes was particularly responsive to soil water deficits applied as PRD, in both root and leaf organs (Fig. 4). In contrast to this group of genes, *HaELIP1* was the only drought-inducible gene that did not respond to exogenous ABA application according to Ouvrard *et al.* (1996). Interestingly, *HaELIP1* did not respond to water deficit (PRD) in leaves (Fig. 4), providing an additional line of evidence supporting the correlation between foliar gene expression and shoot ABA accumulation. Therefore, gene expression of *HaELIP1* measured in root and leaves could potentially distinguish the primary dehydration response in the root from secondary responses caused by leaf water deficit.

Although both *HaLTP* and *HaDHN1* genes were similarly induced by PRD in root and leaf organs (Fig. 4), *HaDHN1* expression correlated much better than *HaLTP* with plant and soil variables (Table 4 and 5). This probably occurs because *HaLTP* is

highly expressed in leaves under control conditions (Fig. 3) due to its involvement in housekeeping and defence functions (Carvalho and Gomes 2007) whereas LEA genes (including dehydrins), are almost exclusively expressed under stress conditions in vegetative tissues, showing negligible transcript levels in well-watered plants and strong induction by stress (Close, 1997; Colmenero-Flores *et al*., 1997; Garay-Arroyo *et al*., 2000; Poormohammad Kiani *et al*., 2007). Gene expression of the dehydrin *HaDHN1* was significantly correlated with all soil and plant water status variables measured in both root and leaf organs, making this gene a highly sensitive marker of water deficit.

In conclusion, measuring gene expression may inform our understanding of water stress development, severity and location. Furthermore, the coordinated expression analysis of ABA-responsive (like *HaLTP, HaDHN1, HaACCO2 HaTIP7*) and ABA-insensitive (like *HaELIP1*) genes in leaf tissues provides information on the water status of both leaf and root organs. Further identification of additional drought-responsive and ABA-insensitive genes may enable a better understanding of the role of different long-distance signalling mechanisms regulating gene expression in response to different irrigation regimes.

Acknowledgements

This work was part-supported by the EU SIRRIMED (FP7- KBBE-2009-3-245159) project, which allowed AA to conduct research at the Lancaster Environment Centre, and the Spanish Ministry of Science and Innovation-FEDER grant AGL2009-08339/AGR

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Figure legends

Figure 1. Evapotranspiration rate in sunflower plants under partial root drying (PRD), deficit irrigation (DI) and well-watered (C) irrigation treatments over time. Differences between C and DI plants (Volume) and between DI and PRD plants (Placement), as determined by Student's unpaired *t*-test, are indicated thus: NS, not significant; *$P <$ 0.05. Values are means ± SE of 6 biological replicates.

Figure 2. Correlations between leaf water potential, leaf xylem ABA concentration and soil water content after 3 days in control (C), deficit irrigation (DI) and partial rootzone drying (PRD) treatments. For PRD plants, soil moisture values are given from the dry compartment (DR), and from the average soil water content of both dry and wet compartments (AVG). Paired measurements for each plant allowed data to be expressed as means ± SE of 6 biological replicates.

Figure 3. Expression levels of water-stress responsive genes in roots and leaves of *Helianthus annuus* control plants. A) Genes with lower expression levels: *HaABRC5, HaACO2, HaELIP1* and *HaDHN1*. B) Genes with higher expression levels: *HaLTP* and *HaTIP7*. Values are means ± SE of 6 biological and 3 technical replicates.

Figure 4. Expression levels of the studied genes (*HaABRC5, HaACO2, HaELIP1, HaDHN1, HaLTP* and *HaTIP7*) in root and leaves tissues of *Helianthus annuus* under partial rootzone drying (PRD) and deficit irrigated (DI) treatments relative to the expression of well-watered plants. The relative transcript levels after 3 days treatments were determined by real-time RT PCR. Values are means ± SE of 6 biological and 3 technical replicates.

Tables

Table 1. PCR amplification primers of studied genes

Gene name	GenBank Accession No.	Primer sequence (5'-3´)
HaTIP7	X95950	Forw: CTCCAGCTCCATCAAGGCC
		Rev: GGTGGATCTAGGGCAGCATCT
HaDHN1	X92647	Forw: GAACCTTCCAAAACCAACCCA
		Rev: GAACGATGCAGAATGCCTGTT
HaACCO2	X92651	Forw: AGAAATGGTGGCTGCCAATG
		Rev: GGGAGATGGCGGAGATAGAAAG
HaABRC5	AY346009	Forw: ATAGAAAGCGGTTGCAGTCGC
		Rev: CGATGAAGAAAACCGCACCTT
HaLTP	X92648	Forw: ATTCCATCTCCGGCGTCAA
		Rev: TGCCAAAGCATCCCATATGTC
HaELIP1	X02646	Forw: TGATGACGTCTGATGCAGAGCT
		Rev: TCATACAAGTGGACTGCCGGT

Table 2. Cumulative evapotranspiration, along with soil and plant water status at the end of the experiment.

Treatments	Soil water content at 72 h ($g.\ g^{-1}$) (±SE)	Evapo-transpiration (mL) (±SE)	Ψ_{leaf} (MPa) (±SE)	Xylem [ABA] (nM) (±SE)
Control	0.069 (± 0.015) a	107.41 (±18.79) a	-0.74 (±0.155) a	21.6 (±3.35) a
DI	0.030 (± 0.012) bc	111.12 (±11.03) a	-0.86 (±0.089) a	27.5 (±5.26) a
PRD (Wet Root)	0.046 (± 0.0037) ab	86.15 (±9.38) b	-1.18 (±0.108) b	164.9 (±46.81) b
PRD (Dry Root)	0.008 ± (0.0044) c			

Different letters indicate significant differences by Tukey´s Test (P< 0,05)

Table 3. Pearson Correlation coefficient between plant and soil variables of PRD *Helianthus annuus* plants after 3 days of drying treatments

	Xylem [ABA] (nM)	ψ_{leaf} (MPa)	Soil water content ($g \cdot g^{-1}$)
ψ_{leaf}	0.41^{NS}	-	
Local soil water content[1]	-0.77**	-0.40^{NS}	-
Whole pot soil water content[2]	-0.48*	-0.08^{NS}	-
Evapotranspiration[3]	0.19^{NS}	0.24^{NS}	-0.04^{NS}

[1,3] using the local soil water content of the dry compartment of PRD pots

[2] using the average soil water content of dry and wet compartments of PRD pots

*, **, and *NS* indicate significance at 0.05 and 0.01 probability level and non-significance, respectively

Table 4. Pearson correlation coefficient between root gene expression and plant and soil variables after 3 days PRD and DI treatments

	HaABRC5	***HaACCO2***	***HaTIP 7***	***HaDHN1***	***HaELIP1***	***HaLTP***
Local soil water content	-0.85***	-0.85***	-0.61*	-0.69*	-0.31^{NS}	-0.58^{NS}
Xylem sap ABA Concentration	0.60^{NS}	0.67*	0.83***	0.77***	-0.09^{NS}	0.40^{NS}
ψ_{leaf}	0.33^{NS}	0.39^{NS}	0.58^{NS}	0.69*	0.14^{NS}	0.33^{NS}

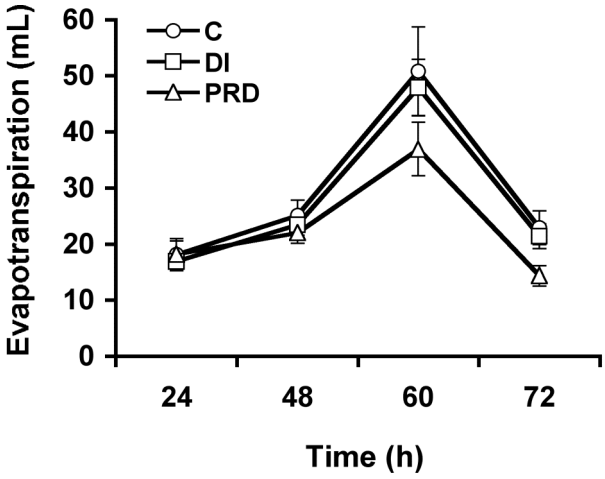
*, **, *** and *NS* indicate significance at 0.05, 0.01, 0.001 probability level and non-significance, respectively

Table 5. Pearson correlation coefficient between leaf gene expression and plant and soil variables after 3 days PRD and DI treatments

	HaABRC5	***HaACCO2***	***HaTIP 7***	***HaDHN1***	***HaELIP1***	***HaLTP***
Whole pot soil water content	-0.22 NS	-0.15^{NS}	0.41^{NS}	-0.60*	-0.23^{NS}	-0.50^{NS}
Xylem sap ABA Concentration	0.44^{NS}	0.07^{NS}	-0.64*	0.73***	0.38^{NS}	0.76***
ψ_{leaf}	0.37^{NS}	0.00^{NS}	-0.22^{NS}	0.78***	0.78***	0.09^{NS}

*, **, *** and *NS* indicate significance at 0.05, 0.01, 0.001 probability level and non-significance, respectively

Figure(s)



Volume	NS	NS	NS	NS	
Placement	NS	NS	NS	*	

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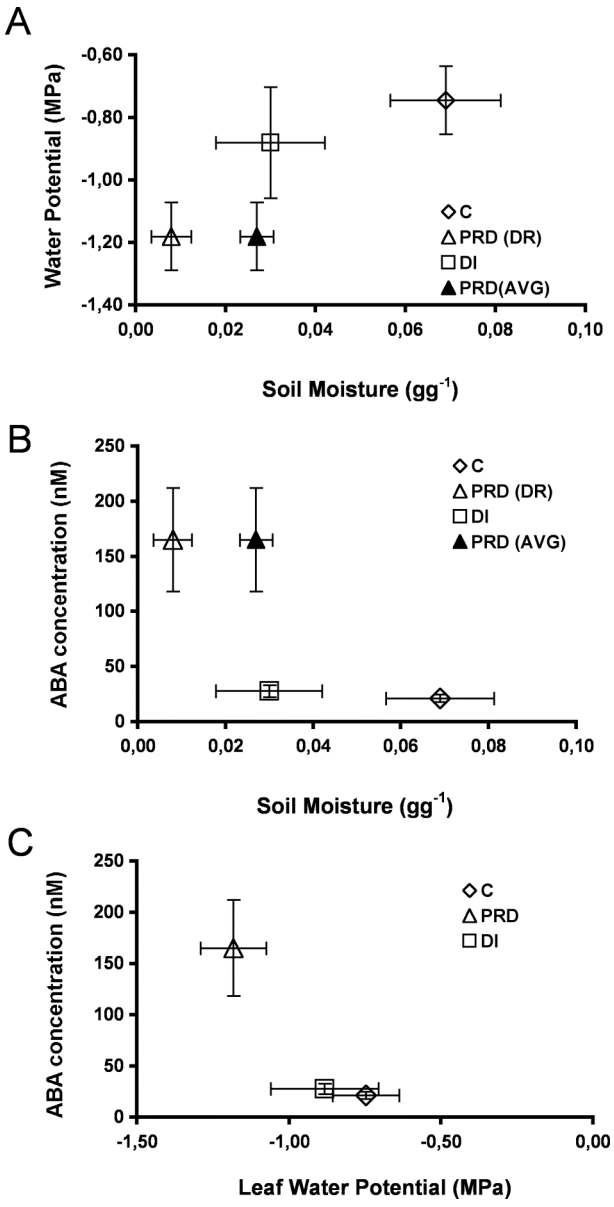

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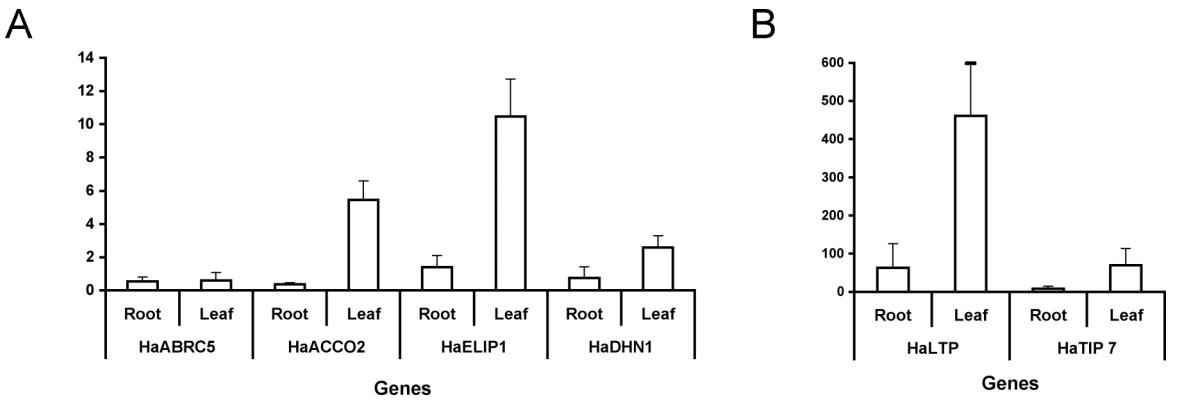

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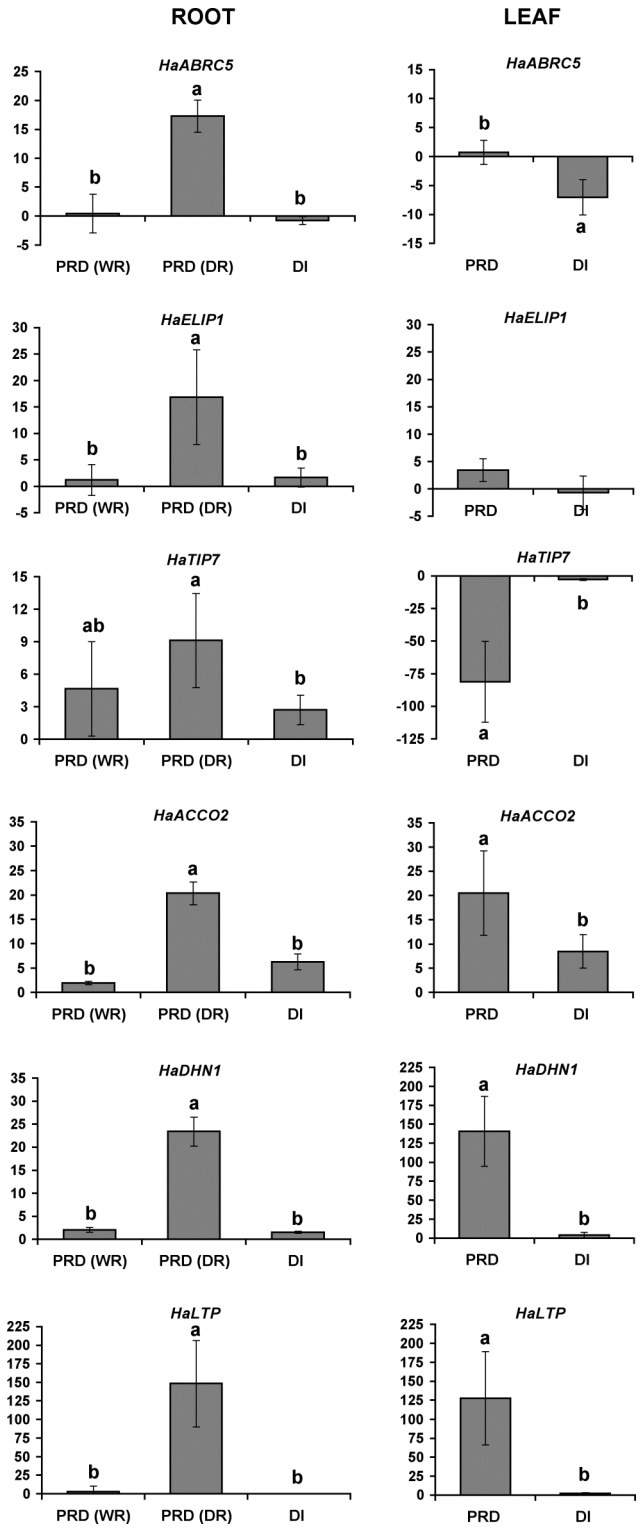

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Highlights

- Deficit irrigation strategies (PRD and DI) induce differential genetic responses.
- Local dehydration in PRD triggers stronger physiological and molecular responses.
- Root gene expression mostly correlates with local soil water content.
- Leaf gene expression correlates with xylem sap ABA and leaf water potential.
- This may enable a better understanding of long-distance signalling mechanisms.