Prolonged drying cycles stimulate ABA accumulation in *Citrus macrophylla* seedlings exposed to partial rootzone drying

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Partial rootzone drying effects on citrus

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Abstract

Partial rootzone drying (PRD) establishes discrete wet and dry parts of the rootzone (for example using parallel drip lines on either side of the crop row), and alternates them to stimulate root growth and root-to-shoot ABA signalling. To assess whether alternation frequency affects plant physiological responses, *Citrus macrophylla* Wester seedlings were grown with the root system split between two pots and 5 irrigation treatments applied: Control, PRD-Fixed (where wet and dry parts of the rootzone were not alternated) and three alternate PRD treatments where the wet and dry parts were swapped at 3 (PRD1), 6 (PRD2) and 12 (PRD3) day intervals, to dry the soil to different degrees before alternating the irrigation. Water was equally distributed between both pots in Control plants, whereas only one pot was watered and the other allowed to dry in PRD plants, with all plants receiving the same irrigation volume. After 24 days, soil water content (θ_v), leaf water potential (Ψ_{leaf}), root water potential (Ψ_{root}), abscisic acid (ABA) concentration in roots ($[ABA]_{root}$), leaves ($[ABA]_{leaf}$) and shoot xylem sap ($[X\text{-}ABA]_{shoot}$), biomass allocation and leaf area were measured. Higher soil water availability of the dry side (PRD1 and PRD2) had no significant effects on leaf water relations, ABA status and plant biomass allocation. However, increasing the duration of exposure of part of the root system to dry soil (PRD3 and PRD-Fixed) further decreased Ψ_{root} and stimulated root ABA accumulation, while decreasing Ψ_{leaf} and increasing $[ABA]_{leaf}$ of PRD3 plants compared to the other treatments. Differences in physiological response between PRD3 and PRD-Fixed plants were attributed to differences in the proportion of root mass exposed to drying soil: PRD3 plants had a lower Ψ_{leaf} and a higher $[ABA]_{leaf}$ with a smaller proportion of their root mass in wet soil. Since long drying cycles were required to alter plant biomass allocation and physiological responses in PRD plants, these should be implemented in designing suitable PRD strategies for field application.

1. Introduction

Citrus species are one of the most important tree crops grown in the Mediterranean basin. In this area, the predominant climate is characterized by high evaporative demand and scarcity of rainfall during summer, which has been aggravated in recent years by climate change. Thus availability of water is a major limiting factor for irrigated agriculture. For that reason, it is necessary to develop more efficient strategies that optimize the scarce water resources available.

Alternate partial rootzone drying (PRD) is an irrigation strategy that was initially designed to exploit putative root-to-shoot chemical signalling to limit excessive vegetative vigour and luxury transpiration, thereby improving crop water use efficiency (WUE) (Dry et al., 2000). Theoretically, PRD aims to establish heterogeneous soil moisture by keeping part of the root system irrigated (to ensure adequate plant water status), while the other part is exposed to drying soil. Soil drying alters root metabolism to produce chemical signals, while maintaining water uptake from those roots ensures delivery of those signals to the shoot (Dodd et al., 2008). Since prolonged soil drying also decreases sap flow from those roots in drying soil (Dodd et al., 2008), a practical solution to this problem has been to regularly alternate the wet and dry parts of the rootzone. This ensures that some roots remain in drying (not completely dry) soil, allowing continued signal production and transport to the shoot (Dodd et al., 2015) to induce partial stomatal closure thereby enhancing leaf-level WUE by restricting transpiration while maintaining photosynthesis. PRD can also initiate other long-term adaptive responses that maintain water status such as decreased canopy area (Santos et al., 2003; Colak and Yazar, 2017) and increased root biomass (Mingo et al., 2004).

The agronomic benefits of PRD have been widely demonstrated in several citrus species, such as mandarin (Kirda et al., 2007), sweet orange (Hutton and Loveys, 2011; Consoli et al., 2014; Mossad et al., 2018), lemon (Pérez-Pérez et al., 2012) and recently in grapefruit (Kusakabe et al., 2016). In these studies, PRD principally increased crop water use efficiency (WUE) without detrimentally affecting marketable yields or apparent tree health. However, in navel orange trees PRD reduced yield and fruit size compared with conventional deficit irrigation (Faber and Lovatt, 2014). Improved WUE of PRD lemon trees was not attributed to changes in the root-to-shoot ABA signalling (Pérez-Pérez et al., 2012), suggesting that other mechanisms were involved in this response. The characteristic soil moisture heterogeneity of PRD influences not only root-to-shoot ABA signalling but other plant responses like root growth (Sharp and LeNoble, 2002) or root hydraulic conductivity (Hose et al., 2000). Thus in pot-grown *Citrus* seedlings of the rootstock Swingle citrumelo, PRD increased root-shoot ratio, but did not affect plant WUE (Melgar et al., 2010). It has been argued that an improved understanding of the physiological responses induced by PRD maximises the likelihood of achieving agronomic benefits with this strategy (Dodd et al., 2015).

Optimal management of PRD irrigation needs to consider irrigation timing (full crop season - Pérez-Pérez et al., 2012, or only in a specific phenological period - Kusakabe et al., 2016), volume (the percentage of crop evapotranspiration applied - Romero-Conde et al., 2014) and the frequency with which drying and irrigated rootzones are alternated (Affi et al 2013). Empirical studies have established physiological and agronomic impacts of all these variables, with responses often arbitrarily related to the duration of exposure of roots to drying soil. Nevertheless, it is necessary to quantify the changes in soil/root water potential required to enhance root ABA accumulation and its export to the shoot. Prolonged exposure of part of the root system to drying soil was needed to ensure that re-watering promoted new root biomass accumulation (Mingo et al., 2004) and transiently stimulated root-to-shoot ABA signalling to further suppress stomatal conductance (Dodd et al., 2006). In contrast, alternating the wet and dry sides of the rootzone had no impact on leaf xylem ABA concentration irrespective of the degree of the soil drying (Pérez-Pérez and Dodd, 2015). In greenhouse-grown tomato, decreasing substrate water storage (from 80% to 60%) in the non-irrigated side during PRD enhanced yield, leaf area and WUE, but decreased biomass and fruit quality (Affi et al., 2013). Thus further work is needed to understand how the frequency of alternation during PRD affects plant physiological and agronomic responses.

Since the impacts of PRD irrigation on field-grown citrus crops have been variable (as discussed above), it is necessary to know how different degrees of soil drying (prior to alternating the wet and dry parts of the root system) affects plant physiological responses. Split-rooted *Citrus macrophylla* seedlings were established in pots to measure soil water content, root water potential and root ABA concentrations of the different parts of the rootzone, which were related to shoot physiological responses (leaf water potential, gas exchange and ABA concentrations) in response to different alternation frequencies.

2. Material and methods

2.1. Experimental design

The experiment was carried out in a walk-in controlled environment room (3×6.5 m) at the IMIDA under a 16 h photoperiod (07.00-23.00 h). Day-night variation caused fluctuations in temperature (20-24 ºC) and relative humidity (65-85%). Seeds of *Citrus macrophylla* Wester were germinated in vermiculite for 40 days. Then, the main root was cut to stimulate the development of lateral roots, and seedlings were transferred to 10 L containers (20 seedlings per container) filled with modified Hoagland solution (MHS) for hydroponic culture. The nutrient solution composition was: 6 mM KNO_3, 4 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2 mM $NH_4H_2PO_4$, 1 mM $MgSO_4 \cdot 7H_2O$, 42.7 µM EDDHA-Fe, and trace elements as prescribed by Hoagland and Arnon (1950). Nutrient solution pH was adjusted to 6.0-6.5 with 1 M NaOH or 1 M HCl.

After 8 weeks, roots were pruned to maintain only two main roots, and thirty-five seedlings were transplanted to 2×0.55 L pots (90×90×95 mm), with each main root placed in a different soil compartment. The pots were filled with silica filtration sand (0.4-0.8 mm of particle size) with a bulk density of 1.39 g DW cm^{-3}. The soil had a volumetric soil water content of 21% at full pot holding capacity and 0.25% at permanent wilting point. Seedlings were grown for 2 more months before 5 different irrigation treatments were applied: Control, PRD-Fixed (dry and wet sides of the rootzone were maintained throughout the experiment) and three alternated PRD with different alternation timing: at 3 (PRD1), 6 (PRD2) and 12 days (PRD3). These timings of irrigation alternation ensured the soil dried to different degrees in the non-irrigated rootzone, based on a preliminary experiment. The experimental design consisted of seven replicates per treatment (one seedling per replicate). The irrigation treatments were maintained for 24 days, watering both Control and PRD plants with the same irrigation volume to ensure that all PRD plants received sufficient irrigation to maintain soil moisture in the irrigated side near field capacity, thereby avoiding any salt accumulation. Each soil compartment of Control plants was watered every 72 hours (at 9:00 h) with 80 mL of MHS, while the irrigated side of PRD plants received 80 mL of MHS every 36 hours (at 9.00 and/or 21.00 h).

2.2. Measurements

Volumetric soil water content was monitored throughout the experiment by inserting a theta probe (Model ML2X, Delta-T Devices) into the top of the pot before each irrigation event. At the end of the experiment, the soil water content of each pot of an individual plant was also determined by the gravimetric method. The volumetric water content (θ_v) was calculated by dividing the measured water loss by the pot volume filled with sand (412 cm^3). Dielectric soil moisture sensor readings were calibrated by comparing soil moisture of each pot with the gravimetric calculation. The excellent correlation (R = 0.99) confirmed that sensor readings adequately represented volumetric water content throughout the experiment. For estimating soil water potential (Ψ_{soil}) from the volumetric soil water content values, a soil-water retention curve of the substrate used in the experiment was made (Ψ_{soil} (kPa) = $1597.5e^{-240\theta v}$, R=0.90, P<0.0001). Soil water potential was measured with a dew point potential meter (WP4C, Decagon Devices, USA).

Physiological measurements were made at the end of the experiment, just before the wet and dry rootzones of the alternate PRD treatments were due to be swapped. Leaf gas exchange was measured in a single youngest fully expanded leaf per plant using a portable photosynthesis system (Li-6400, Li-Cor, Lincoln, Nebraska, USA) equipped with a broad leaf chamber (6.0 cm^2). The air flow rate inside the leaf chamber was 300 µmol s^{-1} and the temperature of the block of the leaf chamber was fixed at 24 ºC. Portable 12-g cartridges of high-pressure, liquefied, pure CO_2 were attached to the console by an external CO_2 source assembly and were controlled automatically by a CO_2 injector system (6400-01 Li-Cor, Lincoln, Nebraska, USA). The reference CO_2 concentration was fixed at 450 µmol CO_2 mol^{-1}. All the measurements were made using a red-blue light source (6400-02B light emitting diode; Li-Cor, Lincoln, Nebraska, USA) attached to the leaf chamber and the PPFD was fixed at 400 µmol m^{-2} s^{-1}.

At the end of the experiment and following gas exchange measurements, leaf water potential was measured in the same leaf using a Schölander type pressure chamber (model 3000; Soil Moisture Equipment. Corp., California, USA). Then the upper part of the shoot (≈ 15 cm length) was removed and placed in the pressure chamber. Following measurement of shoot water potential, an overpressure (0.5 MPa) was applied to the shoot for 90-120 seconds to express xylem sap, which was collected for later determination of ABA concentration. Root water potential was measured individually in each main root from each soil compartment, by placing each in the Schölander type pressure chamber.

At harvest, roots were separated carefully from the soil and washed with distilled water. Then 200 mg of fine fresh roots and two young actively growing leaves were collected for ABA determination and stored in liquid nitrogen. Tissue samples for ABA determination were freeze-dried, ground and extracted with deionized water at 1:50 ratio. ABA concentration in shoot xylem sap, leaf and root samples were analysed by a radioimmunoassay (Quarrie et al., 1988), using a monoclonal antibody AFRC MAC 252 (provided by Dr. Geoff Butcher, Babraham Institute, Cambridge, UK).

At harvest, leaf area of new leaves that appeared during the experiment was measured using a leaf area meter (model LI-3100, Li-Cor, Lincoln, NE, USA). All leaves, stem and roots from each soil compartment were independently oven-dried for each plant for 48 h to determine dry weights (DW).

2.3. Statistical analysis

Whole plant data were subjected to one-way analysis of variance (ANOVA) (Statsgraphics Centurion XV statistical package; Statpoint Technologies Inc., Warrenton, Virginia, USA), with the five irrigation treatments. When there was a significant difference (P-value < 0.05), means were separated using Tukey's multiple range test. When different parts of the root system were compared, two-way analysis of variance (ANOVA) compared the impacts of treatment, part of the rootzone and their interaction. Relationships between soil and plant variables were fitted to non-linear regressions by combining the data of all treatments, and to linear and non-linear regressions using only the data of PRD3 and PRD-Fixed treatments.

3. Results

The volumetric soil water content (θ_v) (measured by the soil moisture sensor) was maintained above 0.16 $cm^3\ cm^{-3}$ in both pots of Control plants, corresponding with soil water potential values (Ψ_{soil}) of -35 kPa (Fig. 1). In all PRD treatments, watering the wet pot (side A or B, depending of the treatment) of the plant every 36 hours maintained θ_v above 0.18 $cm^3\ cm^{-3}$ ($\Psi_{soil} \approx$ -21 kPa). On the other hand, the θ_v of the dry pot decreased to different levels depending on the frequency with which wet and dry sides were alternated. Plants of the PRD1 treatment were exposed to 4 complete drying/re-wetting cycles (alternated every 3 days) and the θ_v just before each change of the irrigated side ranged between 0.11-0.17 $cm^3\ cm^{-3}$ (Ψ_{soil} between -27 and -114 kPa) (Fig. 1). Plants of the PRD2 treatment were exposed to 2 complete drying/re-wetting cycles (alternated every 6 days), and the θ_v reached at the end of the each drying cycle ranged between 0.05-0.07 $cm^3\ cm^{-3}$ (Ψ_{soil} between -298 and -482 kPa). Plants of the PRD3 treatment were exposed to one complete drying/re-wetting cycle (alternated every 12 days). The θ_v was extremely low (ranging between 0.003 and 0.01 $cm^3\ cm^{-3}$; -1,486 and -1,556 kPa) towards the end of the drying cycle (day 10 – 36 hours before the alternation). In PRD-Fixed plants, the irrigated pot was maintained throughout the experiment. In dry pot of PRD-

Fixed plants, the θ_v decreased parallel to the first drying cycle of plants of PRD3, and extreme soil dryness was maintained until the end of the experiment.

At the end of the experiment (when soil drying was maximal), the different alternation frequencies of PRD plants clearly established differences in the soil moisture distribution. The Ψ_{soil} of the irrigated rootzone (Side B) was maintained low (above -17 kPa) in all PRD plants (Fig. 2B). In the non-irrigated part (Side A), the Ψ_{soil} was significantly increased as the duration of the drying cycle increased, reaching the highest Ψ_{soil} ($\approx$ -1,500 kPa) in PRD3 and PRD-Fixed treatments (Fig. 2A).

At the end of the experiment, differences in Ψ_{soil} were reflected in root water potential (Ψ_{root}) values. Roots growing in the irrigated pot (Side B) had similar Ψ_{root} in all treatments (Fig. 2B), but for roots growing in the drying pot (Side A), Ψ_{root} significantly decreased along with Ψ_{soil}. Although PRD3 and PRD-Fixed plants had similar values of Ψ_{soil} in the drying pot, the Ψ_{root} of the dry part of the rootzone was significantly lower in PRD3 than in PRD-Fixed plants (Fig. 2B). All treatments had a similar leaf water potential (Ψ_{leaf}) exception for PRD3 plants, in which Ψ_{leaf} was $\approx$ 0.3 MPa lower than the other treatments (Fig. 2C).

Leaf CO_2 assimilation rate (A_{CO2}), leaf transpiration rate (E) and stomatal conductance (g_s) measured at the end of the experiment, were similar in all irrigation treatments (Table 1). However, the instantaneous water use efficiency (A/E) was higher in PRD3 plants (by 28%) and lower in PRD-Fixed plants (by 6%) than the remaining treatments (Table 1). The intrinsic water use efficiency (A/g_s) was slightly increased in PRD2 and PRD3 plants (by 28 and 27%) and decreased in PRD-Fixed plants (by 10%), compared with Control and PRD1 plants (Table 1). Thus alternating the irrigated and drying pots enhanced leaf-level water use efficiency, in comparison to the PRD-Fixed treatment.

At the end of the experiment, only PRD3 plants had a significantly higher leaf ABA concentration ($[ABA]_{leaf}$), by 44% compared to the other treatments (Fig. 3A). Shoot xylem ABA concentration ($[X\text{-}ABA]_{shoot}$) was similar in all irrigation treatments (Fig. 3B). For roots in the irrigated pot (Side B), root ABA concentration ($[ABA]_{root}$) was similar in all irrigation treatments (Fig. 3C). For roots in the drying pot (Side A), $[ABA]_{root}$ was only significantly increased (by 6-fold) in PRD3 and PRD-Fixed plants compared to the remaining treatments (Fig. 3C). Nevertheless, even PRD2 plants showed differential ABA accumulation between the irrigated and drying pots, with $[ABA]_{root}$ slightly but significantly ($P=0.014$) higher (62%) in the drying pot of this treatment. Thus a threshold Ψ_{soil} or Ψ_{root} was needed to trigger root ABA accumulation in the drying pot (cf. Figs. 2A, B, 3C).

Root distribution was significantly altered in the PRD1, PRD3 and PRD-Fixed treatments (Fig. 4) at the end of the experiment. In PRD-Fixed plants, the irrigated rootzone (Side B) had 38% more root biomass than did the drying part (Side A) (Fig. 4). In contrast, in PRD3 plants, root biomass from the drying pot (Side A) was 62% higher than the irrigated pot. Root biomass was slightly higher (15% and 11%) and shoot biomass slightly lower (6% and 10%) in the PRD3 and PRD-Fixed treatments respectively, compared to the control, but the differences were not statistically significant (Table 2). Although shoot mass was statistically similar in all treatments, the area of new leaves grown during the experiment decreased (19%) in PRD3 plants compared to PRD1 plants (Table 2). These changes in root and shoot biomass significantly increased root to shoot ratio of plants exposed to prolonged drying cycles, namely the PRD3 and PRD-Fixed treatments (Table 2).

In the drying pot at the end of the experiment, Ψ_{root} decreased linearly with Ψ_{soil} (Fig. 5A). $[ABA]_{root}$ increased exponentially as Ψ_{soil} declined below -17 kPa (Fig. 5B) and as Ψ_{root} in the drying side decreased below -0.6 MPa (Fig. 5C).

Average across both irrigated and drying pots, $[ABA]_{root}$ exponentially increased as Ψ_{root} decreased (Fig. 6). Similarly, $[ABA]_{root}$ exponentially increased as Ψ_{leaf} decreased although there was much greater scatter (R=0.42) in the relationship compared to that with Ψ_{root} (R=0.85). Thus root ABA accumulation increased as plant water status declined.

In PRD3 and PRD-Fixed plants, which showed similar soil moisture heterogeneity by the end of the experiment (Fig. 2A), Ψ_{leaf} increased as the fraction of roots within the irrigated pot (Side B) was higher (Fig. 7A). Similarly, $[ABA]_{leaf}$ increased as the fraction of roots in irrigated soil declined (Fig. 7B) such that $[ABA]_{leaf}$ decreased as Ψ_{leaf} decreased (Fig. 7C). Thus leaf ABA accumulation depended on both root distribution in a soil with heterogeneous soil moisture, and leaf water status.

4. Discussion

While most studies of PRD regularly swap irrigation between wet and dry parts of the rootzone (eg. Hutton and Loveys, 2011) since these changes increase yield compared to maintaining irrigation to only one part (reviewed in Dodd et al. 2015), there has been little systematic investigation of how the time between alternation events affects soil moisture status, and thence physiological responses. Applying the same irrigation volume, but varying the duration of the drying/re-wetting cycles during PRD, generated differences in soil drying and soil moisture distribution (Figs. 1 and 2A). While the short duration (24 days) of the different treatments did not significantly alter total shoot and root biomass, those that exposed plants to more intense soil drying inhibited leaf area expansion and increased the root/shoot ratio (Table 2). Thus root growth was stimulated in the irrigated pot of PRD-Fixed plants (Wang et al., 2005) and in response to alternating wetting and drying parts of the rootzone in PRD-3 plants (Mingo et al., 2004). Although understanding the physiological mechanisms determining root growth dynamics following soil moisture fluctuations was beyond the scope of this study, changes in root phytohormone (auxin, cytokinin) concentrations have been implicated (Han et al., 2015). Moreover, these changes in biomass partitioning were accompanied by physiological changes such as increased intrinsic water use efficiency (Table 1), ostensibly due to differences in plant ABA and water status (Figs. 2 and 3). Although establishing causality between these physiological and biomass changes is difficult, it is important to understand their regulation during PRD.

By maintaining Ψ_{soil} of the irrigated pot above -35 kPa in all PRD plants (Figs. 1 and 2A), local Ψ_{root} did not vary among treatments (Fig. 2B) and there was no ABA accumulation in the irrigated roots *except* in the PRD3 plants (Fig. 3C). Compared to the other irrigated roots, $[ABA]_{root}$ within the irrigated pot of PRD3 plants almost doubled, coincident with decreased Ψ_{leaf} (Fig. 2C) and increased $[ABA]_{leaf}$ (Fig. 3A). Increased $[ABA]_{root}$ of irrigated roots in the absence of any decrease in Ψ_{root} (the putative stimulus for root ABA synthesis – Simonneau et al., 1998) apparently supports the hypothesis of foliar ABA synthesis (in response to decreased Ψ_{leaf} and turgor) and subsequent basipetal phloem transport of ABA to the roots (Wolf et al., 1990; Jiang and Hartung, 2008). While stimulating root ABA accumulation via this mechanism is less direct than localised soil drying upregulating ABA biosynthesis genes in the roots (Speirs et al., 2013), supplying radioactive ABA to the shoots causes label accumulation in the roots (McAdam et al., 2016). Although it is unknown how basipetally transported ABA is distributed to different roots when soil moisture is heterogeneous, preferential root ABA accumulation in the dry rootzone of PRD plants (Khalil and Grace, 1993; Puértolas et al., 2015) would require those roots to act as stronger sinks for ABA.

Should shoot ABA status be the primary regulator of root ABA accumulation, $[ABA]_{leaf}$ should be correlated with $[ABA]_{root}$. While Control, PRD1, PRD2 and PRD-

Fixed plants had a similar $[ABA]_{leaf}$ and ABA did not accumulate in either irrigated or dried pots of the first 3 treatments, substantial root ABA accumulation occurred in the drying pots of PRD-Fixed plants (Fig. 3C). These changes occurred without any apparent shoot-derived stimulus (decreased Ψ_{leaf} or turgor). In contrast, $[ABA]_{root}$ was highly significantly related to both local soil moisture content (Fig. 5B) and Ψ_{root} (Fig. 5C), with a generally unified response across all treatments. Root ABA concentration depended on Ψ_{root} in detached, air-dried roots (Simonneau et al., 1998), just-germinated seedlings grown at different Ψ_{soil} including treatments where shoot emergence had not occurred (Sharp et al., 1994) and in intact plants grown in drying soil (Puértolas et al., 2013). Taken together, these observations suggest that basipetal phloem transport of ABA from the shoot is not required for root ABA accumulation, and support the hypothesis of root ABA synthesis (in response to decreased Ψ_{root} and turgor).

Irrespective of the cause(s) of root ABA accumulation, it is important to determine whether its transport to the shoots induces stomatal closure. In all treatments, irrespective of the magnitude of root ABA accumulation (Fig. 3C), xylem ABA concentration was constant (Fig. 3B) as in citrus plants exposed to PRD in the field (Pérez-Pérez et al., 2012). Similarly, in potato plants exposed to both horizontal and vertical soil moisture gradients that stimulated root ABA accumulation, xylem ABA concentration did not increase (Puértolas et al., 2015). However, root ABA accumulation was accompanied by a substantial decrease in sap flow from roots in drying soil such that xylem ABA concentration was determined by sap flow from roots in irrigated soil (Puértolas et al., 2015). These observations are consistent with a model that explains xylem ABA concentration of PRD plants as a function of xylem ABA concentrations emanating from the irrigated and drying parts of the root system and the relative sap flow from each (Dodd et al., 2008; Pérez-Pérez and Dodd, 2015). Thus local root ABA accumulation need not result in root-to-shoot ABA signalling.

Instead, the main role of root ABA accumulation in response to PRD was likely root growth regulation. Plants that accumulated high root ABA concentrations (PRD3, PRD-Fixed treatments) greatly altered their root biomass distribution between soil compartments (Fig. 4), and thus their root-shoot ratio. The altered root biomass distribution in PRD-Fixed plants likely assisted in maintaining leaf water status (Fig. 2C) as previously reported (Martín-Vertedor and Dodd, 2011), since PRD-Fixed plants generally had > 50% of their root biomass in irrigated soil (Fig. 6A). This maintenance of leaf water status was associated with foliar ABA homeostasis such that PRD-Fixed plants had the same $[ABA]_{leaf}$ as Control plants (Fig. 3A). Thus changes in root biomass distribution can maintain homeostasis of leaf water and ABA relations.

In contrast, despite changes in root biomass distribution, PRD3 plants had elevated $[ABA]_{leaf}$, since by the end of the experiment, when leaves were sampled, < 50% of their root biomass was in irrigated soil (Fig. 7A) which was associated with decreased Ψ_{leaf} (Fig. 6B). Thus when the root biomass in the irrigated pot was insufficient to keep the leaves well-hydrated, as in PRD3 plants, leaf ABA accumulation occurred (Martín-Vertedor and Dodd, 2011). Thus differential leaf ABA accumulation between PRD3 and PRD-Fixed plants could be explained by differences in their leaf water status, caused by variation in the proportion of root biomass occurring in drying soil.

Regular (every 3 days) swapping of the irrigated and drying pots (PRD1) maintained local Ψ_{soil} of the drying side above -114 kPa, with similar physiological responses as the Control plants. Limited soil drying (Fig. 1) had a minimal effect on Ψ_{root} (Fig. 2A), which was insufficient to affect root ABA concentration (Fig. 3C). Moreover, shoot responses (Ψ_{leaf}, leaf gas exchange) and biomass (Table 2) were similar to Control plants. When local Ψ_{soil} decreased to -482 kPa (PRD2), additional physiological responses were observed. Since Ψ_{root} further decreased, $[ABA]_{root}$ increased slightly (62%), but significantly ($P = 0.023$), compared to well irrigated roots (Fig. 3C). Nevertheless, $[X\text{-}ABA]_{shoot}$ and $[ABA]_{leaf}$ were not altered (Fig. 3), suggesting that ABA was not transported from the roots to the shoot, perhaps because of diminished water transport from the roots in drying soil (Dodd et al., 2008). Although Ψ_{leaf}, A_{CO2} and g_s were statistically similar to Control plants, leaf water use efficiency (A/g_s) increased via mechanisms unrelated to changes in root-to-shoot ABA signalling and/or leaf water status (Perez-Perez et al., 2012; Rodrigues et al., 2008). Recent studies indicate that soil drying can increase the transport of other antitranspirants such as sulphate (Machelska et al., 2017) and jasmonates (de Ollas et al., 2018) from the roots, although their impact on leaf WUE has not been investigated in detail.

Further soil drying to complete soil moisture depletion (allowing a single complete drying/re-wetting cycle, PRD3) induced further physiological and biomass changes. Leaf and root ABA accumulation (Fig. 3A, C) in response to decreased water status of both tissues (Figs. 2B, C) was not accompanied by any change root-to-shoot ABA signalling (Fig. 3B). Root/shoot ratio was increased and leaf area decreased compared to PRD1 plants, indicating altered resource allocation. While local root ABA accumulation may enhance sink strength (Chen et al., 2003), decreased foliar cytokinin status of plants exposed to PRD (Kudoyarova et al., 2007) is also likely to have decreased biomass allocation to the shoot. Reductions in transpiring area concomitant with potentially increased root surface area may have minimised changes in Ψ_{leaf}, as in grapevines exposed to PRD in the field (Romero et al., 2014).

Although PRD3 and PRD-Fixed plants experienced similar degrees of soil drying, greater root growth in the irrigated pot of the latter prevented any change in Ψ_{leaf} compared to Control plants, as in grapefruit grown in the field with PRD (Romero-Conde et al., 2014). Moreover, these irrigated roots likely had higher hydraulic conductance than roots of Control plants (Hu et al., 2011). Nevertheless, changes in root hydraulic conductivity (Lp) are likely of lesser importance in Ψ_{leaf} homeostasis than stomatal regulation, since higher ABA concentrations in the irrigated roots of PRD3 plants (Fig. 3C) should stimulate Lp (reviewed in Dodd, 2013), yet Ψ_{leaf} of these plants still declined. In PRD-Fixed plants, the greater fraction of the root biomass exposed to irrigated soil likely facilitated redistribution of water along Ψ gradients from irrigated to drying pots via the roots (Stoll et al., 2000), thereby attenuating the decrease in Ψ_{root} within the dry soil compartment (Fig. 2B). These adjustments in root morphology and plant water relations were not accompanied by any change in leaf water use efficiency (A/g_s), which may diminish the value of applying PRD. Although alternating the irrigated and drying parts of the rootzone during PRD enhances crop yields per unit of irrigation compared to fixed PRD (Dodd et al., 2015), understanding the integration and relative importance of physiological and morphological adjustments during PRD remains a key knowledge gap (Romero et al., 2014) that may limit the application of this technique.

5. Conclusions

Changing the frequency of PRD irrigation in citrus seedlings demonstrated that this technique can be better managed by varying the soil moisture the non-irrigated roots are exposed to. Varying the alternation frequency did not enhance root-to-shoot ABA signalling, but altered biomass partitioning between roots and shoots and between roots occupying different soil compartments. Prolonging the drying cycles during alternate PRD exposed more roots to severe soil drying, increasing root and leaf ABA accumulation and enhancing leaf water use efficiency. Whether these findings can be extrapolated to a field-grown citrus crop to enhance crop yields and quality requires further research.

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References

Affi, N., Fadl, A. El, Otmani, M. El, Benismail, M.C., Idrissi, L.M., Rahhaoui, Z., 2013. Partial root zone drying alternation frequency effect on yield, biomass production and fruit quality of tomato crop. Eur. J. Appl. Eng. Sci. Res. 2, 12–19.

Chen, G., Fu, X., Lips, H., Sagi, M., 2003. Control of plant growth resides in the shoot, and not in the root, in reciprocal grafts of *flacca* and wild-type tomato (*Lycopersicon esculentum*), in the presence and absence of salinity stress. Plant and Soil 256, 205-215.

Colak, Y.B., Yazar, A., 2017. Evaluation of crop water stress index on Royal table grape variety under partial root drying and conventional deficit irrigation regimes in the Mediterranean Region. Sci. Hort., 224: 384-394. doi: 10.1016/j.scienta.2017.06.032.

Consoli, S., Stagno, F., Roccuzzo, G., Cirelli, G.L., Intrigliolo, F., 2014. Sustainable management of limited water resources in a young orange orchard. Agric. Water Manag. 132, 60–68. doi:10.1016/j.agwat.2013.10.006.

de Ollas, C., Arbona, V., Gomez-Cadenas, A., Dodd, I.C., 2018. Attenuated accumulation of jasmonates modifies stomatal responses to water deficit. J. Exp. Bot. 69, 2103-2116.

Dodd, I.C., 2013. Abscisic acid and stomatal closure: a hydraulic conductance conundrum? Commentary New Phytol. 197, 6-8.

Dodd, I.C., Egea, G., Davies, W.J. 2008. ABA signalling when soil moisture is heterogeneous: decreased photoperiod sap flow from drying roots limit ABA export to the shoot. Plant, Cell Environ. 31, 1263-1274.

Dodd, I.C., Puértolas, J., Huber, K., Pérez-Pérez, J.G., Wright, H.R., Blackwell, M.S.A., 2015. The importance of soil drying and re-watering in crop phytohormonal and nutritional responses to deficit irrigation. J. Exp. Bot. 66, 2239-2252.

Dodd, I.C., Theobald, J.C., Bacon, M.A., Davies, W.J., 2006. Alternation of wet and dry sides during partial rootzone drying irrigation alters root-to-shoot signalling of abscisic acid. Funct. Plant Biol. 33, 1081-1089. doi:10.1071/FP06203.

Dry, P.R., Loveys, B.R., Düring, H., 2000. Partial drying of the rootzone of grape. I. Transient changes in shoot growth and gas exchange. Vitis 39, 3-7.

Faber, B.A., Lovatt, C.J., 2014. Effects of applying less water by partial root zone drying versus conventional irrigation on navel orange yield. Acta Hort. 1038, 523-530.

Han, H.M, Tian, Z.W, Fan, Y.H., Cui, Y.K., Cai, J., Jiang, D., Cao, W.X., Dai, T.B., 2015. Water-deficit treatment followed by re-watering stimulates seminal root growth associated with hormonal balance and photosynthesis in wheat (*Triticum aestivum* L.) seedlings. Plant Growth Regulation 77, 201-210.

Hose, E., Steudle, E., Hartung, W., 2000. Abscisic acid and hydraulic conductivity of maize roots: a study using cell- and root- pressure probes. Planta 211, 874–882.

Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soils. University of California, Agricultural Experiment Station, Berkeley, CA, Circular 347, 1-39 pp.

Hu, T., Kang, S., Li, F., Zhang, J., 2011. Effects of partial root-zone irrigation on hydraulic conductivity in the soil-root system of maize plants. J. Exp. Bot. 62, 4163-4172. doi:10.1093/jxb/err110

Hutton, R.J.J., Loveys, B.R.R., 2011. A partial root zone drying irrigation strategy for citrus—Effects on water use efficiency and fruit characteristics. Agric. Water Manag. 98, 1485–1496. doi:10.1016/j.agwat.2011.04.010.

Jiang, F., Hartung, W., 2008. Long-distance signalling of abscisic acid (ABA): The factors regulating the intensity of the ABA signal. J. Exp. Bot. 59, 37–43. doi:10.1093/jxb/erm127.

Khalil, A.M., Grace, J., 1993. Does xylem sap ABA control the stomatal behaviour of water stressed Sycamore (*Acer pseudoplatanus* L.) seedlings? J. Exp. Bot. 44, 1127–1134. doi: 10.1093/jxb/44.7.1127.

Kirda, C., Lu, F.T., Topçu, S., Kaman, H., 2007. Mandarin Yield Response to Partial Root Drying and Conventional Deficit Irrigation. Turkey J. Agric. 31, 1–10.

Kudoyarova, G.B. Vysotskaya, L.B., Cherkozyanova, A., Dodd, I.C. 2007. Effect of partial rootzone drying on the concentration of zeatin-type cytokinins in tomato (*Lycopersicon esculentum* Mill.) xylem sap and leaves. J. Exp. Bot. 58, 161-168.

Kusakabe, A., Contreras-Barragan, B.A., Simpson, C.R., Enciso, J.M., Nelson, S.D., Melgar, J.C., 2016. Application of partial rootzone drying to improve irrigation water use efficiency in grapefruit trees. Agric. Water Manag. 178, 66–75. doi:10.1016/j.agwat.2016.09.012.

Malcheska, F., Ahmad, A., Batool, S., Müller, H.M., Ludwig-Müller, ,J., Kreuzwieser, J, Randewig., D., Hänsch., R., Mendel, R.R., Hell., R., Wirtz., M., Geiger., D., Ache., P., Hedrich., R., Herschbach., C., Rennenberg, H., 2017. Drought-enhanced xylem sap sulfate closes stomata by affecting ALMT12 and guard cell ABA Synthesis. Plant Physiol. 174, 798-914.

Martín-Vertedor, A.I., Dodd, I.C., 2011. Root-to-shoot signalling when soil moisture is heterogeneous: increasing the proportion of root biomass in drying soil inhibits leaf growth and increases leaf abscisic acid concentration. Plant Cell Environ. 34, 1164–75. doi:10.1111/j.1365-3040.2011.02315.x.

McAdam, S.A.M., Brodribb, T.J., Ross, J.J., 2016. Shoot derived abscisic acid promotes root growth. Plant Cell Environ. 39, 652–659. http://dx.doi.org/10.1111/pce.12669.

Melgar, J.C., Dunlop, J.M., Syvertsen, J.P., 2010. Growth and physiological responses of the citrus rootstock Swingle citrumelo seedlings to partial rootzone drying and deficit irrigation. J. Agric. Sci. 148, 593–602. doi:10.1017/S0021859610000377.

Mingo, D.M., Theobald, J.C., Bacon, M.A., Davies, W.J., Dodd, I.C., 2004. Biomass allocation in tomato (*Lycopersicon esculentum*) plants grown under partial rootzone drying: enhancement of root growth. Funct. Plant Biol. 31, 971-978.

Mossad, A., Scalisi, A., Lo Bianco, R. 2018. Growth and water relations of field-grown 'Valencia' orange trees under long-term partial rootzone drying. Irrigation Sci. 36, 9-24.

Pérez-Pérez, J.G., Dodd, I.C., Botía, P., 2012. Partial rootzone drying increases water-use efficiency of lemon Fino 49 trees independently of root-to-shoot ABA signalling. Funct. Plant Biol. 39, 366–378. doi:10.1071/FP11269.

Pérez-Pérez, J.G., Dodd, I.C., 2015. Sap fluxes from different parts of the rootzone modulate xylem ABA concentration during partial rootzone drying and re-wetting. J. Exp. Bot. 66, 2315-2324.

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 Environ. 36, 1465–1475. doi:10.1111/pce.12076.

Puértolas, J., Conesa, M.C., Ballester, C., Dodd, I. C., 2015. Local root abscisic acid (ABA) accumulation depends on the spatial distribution of soil moisture in potato: Implications for ABA signalling under heterogeneous soil drying. J. Exp. Bot. 66, 2325-2334.

Quarrie, S.A., Whitford, P.N., Appleford, N.E.J., Wang, T.L., Cook, S.K., Henson, I.E., Loveys, B.R., 1988. A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. Planta 173, 330–339. doi:10.1007/BF00401020.

Rodrigues, M.L., Santos, T., Rodrigues, A.P. de Souza, C.R., Lopes, C.M., Maroco, J.P., Pereira, J.S., Chaves, M.M., 2008. Hydraulic and chemical signalling in the regulation of stomatal conductance and plant water use of field grapevines growing under deficit irrigation. Funct. Plant Biol. 35: 565–579.

Romero, P., Pérez-Pérez, J.G., del Amor, F.M., Martínez-Cutillas, A., Dodd, I.C., Botía, P., 2014. Partial root zone drying exerts different physiological responses on field-grown grapevine (*Vitis vinifera* cv . Monastrell) in comparison to regulated deficit irrigation. Funct. Plant Biol. 41, 1087–1106. doi:http://dx.doi.org/10.1071/FP13276.

Romero-Conde, A., Kusakabe, A., Melgar, J.C., 2014. Physiological responses of citrus to partial rootzone drying irrigation. Sci. Horticult. 169, 234–238. http://dx.doi.org/10.1016/j.scienta.2014.02.022.

Santos, T., Lopes, C., Rodrigues, M.L., Souza, C.R., Silva, J.R., Maroco, J.P., Pereira, J.S., Chaves, M.M., 2003. Partial rootzone drying effects on growth and fruit quality of field-grown grapevines (Vitis vinifera). Funct. Plant Biol. 30, 663–671. doi: 10.1071/FP02180.

Sharp, R.E., LeNoble, M.E., 2002. ABA, ethylene and the control of shoot and root growth under water stress. J. Exp. Bot. 53, 33–37.

Sharp, R.E., Wu, Y., Voetberg, G.S., Saab, I.N. LeNoble, M.E., 1994. Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials ABA, ethylene and the control of shoot and root growth under water stress. J. Exp. Bot. 45, 1743–1751.

Simonneau, T., Barrieu, P., Tardieu, F., 1998. Accumulation rate of ABA in detached maize roots correlates with root water potential regardless of age and branching order. Plant, Cell & Environment 21, 1113–1122.

Speirs, J., Binney, A., Collins, M., Loveys, B.R., 2013. Expression of ABA synthesis and metabolism genes under different irrigation strategies and atmospheric VPDs is associated with stomatal conductance in grapevine (*Vitis vinifera* L. cv Cabernet Sauvignon). J. Exp. Bot. 64, 1907-1916.

Stoll, M., Loveys, B., Dry, P., 2000. Hormonal changes induced by partial root-zone drying of irrigated grapevine. J. Exp. Bot. 51, 1627-1634.

Wang, L., de Kroon, H., Bogemann H., Smits, A.J.M., 2005. Partial root drying effects on biomass production in *Brassica napus* and the significance of root responses. Plant and Soil 276, 313- 326.

Wolf., O., Jeschke, W.D., Hartung, W., 1990. Long distance transport of abscisic acid in NaCl-treated intact plants of *Lupinus albus*. J. Exp. Bot. 41, 593-600.

Figures

Figure 1. Evolution of the soil water content (measured with the portable soil moisture sensor) in each side of the pot – side A (A) and side B (B) for irrigation treatments (Control, PRD1, PRD2, PRD3 and PRD-Fixed) throughout the experiment. Irrigation treatments comprised a Control, a PRD-Fixed treatment (dry and wet sides of the rootzone were maintained throughout the experiment) and three alternate PRD treatments where dry and wet sides of the rootzone were alternated every 3 (PRD1), 6 (PRD2) and 12 (PRD3) days. Points are means ± SE of 7 replicates.

Figure 2. Whole pot soil water potential (A), root water potential (B) and leaf water potential (C) for irrigation treatments (Control, PRD1, PRD2, PRD3 and PRD-Fixed) at the end of the experiment. Irrigation treatments comprised a Control, a PRD-Fixed treatment (dry and wet sides of the rootzone were maintained throughout the experiment) and three alternate PRD treatments where dry and wet sides of the rootzone were alternated every 3 (PRD1), 6 (PRD2) and 12 (PRD3) days. Bars are means ± SE of 7 replicates. Bars labelled with different letters are significantly different at $P < 0.05$.

Figure 3. Leaf ABA concentration ($[ABA]_{leaf}$) (A), shoot xylem ABA concentration ($[X\text{-}ABA]_{shoot}$) (B) and root ABA concentration ($[ABA]_{root}$) (C) for irrigation treatments (Control, PRD1, PRD2, PRD3 and PRD-Fixed) at the end of the experiment. Irrigation treatments comprised a Control, a PRD-Fixed treatment (dry and wet sides of the rootzone were maintained throughout the experiment) and three alternate PRD treatments where dry and wet sides of the rootzone were alternated every 3 (PRD1), 6 (PRD2) and 12 (PRD3) days. Bars are means ± SE of 7 replicates. Bars labelled with different letters are significantly different at $P < 0.05$.

Figure 4. Root biomass in each side of the pot of each irrigation treatment (Control, PRD1, PRD2, PRD3 and PRD-Fixed) at the end of the experiment. Irrigation treatments comprised a Control, a PRD-Fixed treatment (dry and wet sides of the rootzone were maintained throughout the experiment) and three alternate PRD treatments where dry and wet sides of the rootzone were alternated every 3 (PRD1), 6 (PRD2) and 12 (PRD3) days. Bars are means ± SE of 7 replicates. Bars labelled with different letters are significantly at $P < 0.05$.

Figure 5. Relationships between root water potential (Ψ_{root}) and whole pot soil water potential (Ψ_{soil}) from the side A (A), root ABA concentration $[ABA]_{root}$ and whole pot soil water potential from the side A (B) and $[ABA]_{root}$ and root water potential from the side A (side not irrigated at the end of the experiment) (C). Irrigation treatments comprised a Control, a PRD-Fixed treatment (dry and wet sides of the rootzone were maintained throughout the experiment) and three alternate PRD treatments where dry and wet sides of the rootzone were alternated every 3 (PRD1), 6 (PRD2) and 12 (PRD3) days. Each point represents an individual plant.

Figure 6. Relationships between average root ABA concentration ($[ABA]_{root}$) and average root water potential (Ψ_{root}) (A) and $[ABA]_{root}$ and leaf water potential (Ψ_{leaf}) (B). Irrigation treatments comprised a Control, a PRD-Fixed treatment (dry and wet sides of the rootzone were maintained throughout the experiment) and three alternate PRD treatments where dry and wet sides of the rootzone were alternated every 3 (PRD1), 6 (PRD2) and 12 (PRD3) days. Each point represents an individual plant.

Figure 7. Relationships between leaf water potential (Ψ_{leaf}) and the fraction of roots placed in the irrigated pot at the end of the experiment (side B) (A), leaf ABA concentration ($[ABA]_{leaf}$) and the fraction of roots placed in the irrigated pot at the end of the experiment (side B) (B) and $[ABA]_{leaf}$ and leaf water potential (C) for each irrigation treatment. Irrigation treatments comprised a Control, a PRD-Fixed treatment (dry and wet sides of the rootzone were maintained throughout the experiment) and three alternate PRD treatments where dry and wet sides of the rootzone were alternated every 3 (PRD1), 6 (PRD2) and 12 (PRD3) days. Each point represents an individual plant.

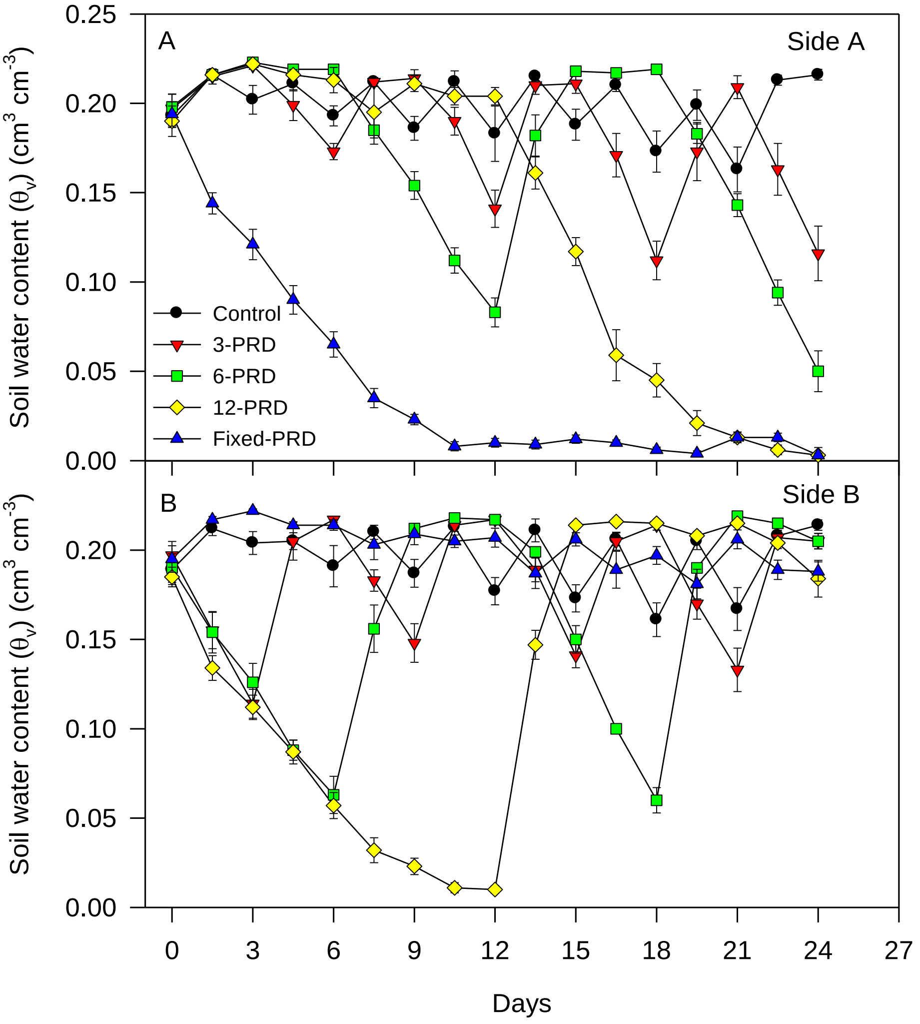

A
Side A
B
Side B
Soil water content (θ_v) (cm^3 cm^{-3})
0.25
0.20
0.15
0.10
0.05
0.00
Control
3-PRD
6-PRD
12-PRD
Fixed-PRD
0
3
6
9
12
15
18
21
24
27
Days

Figure 1

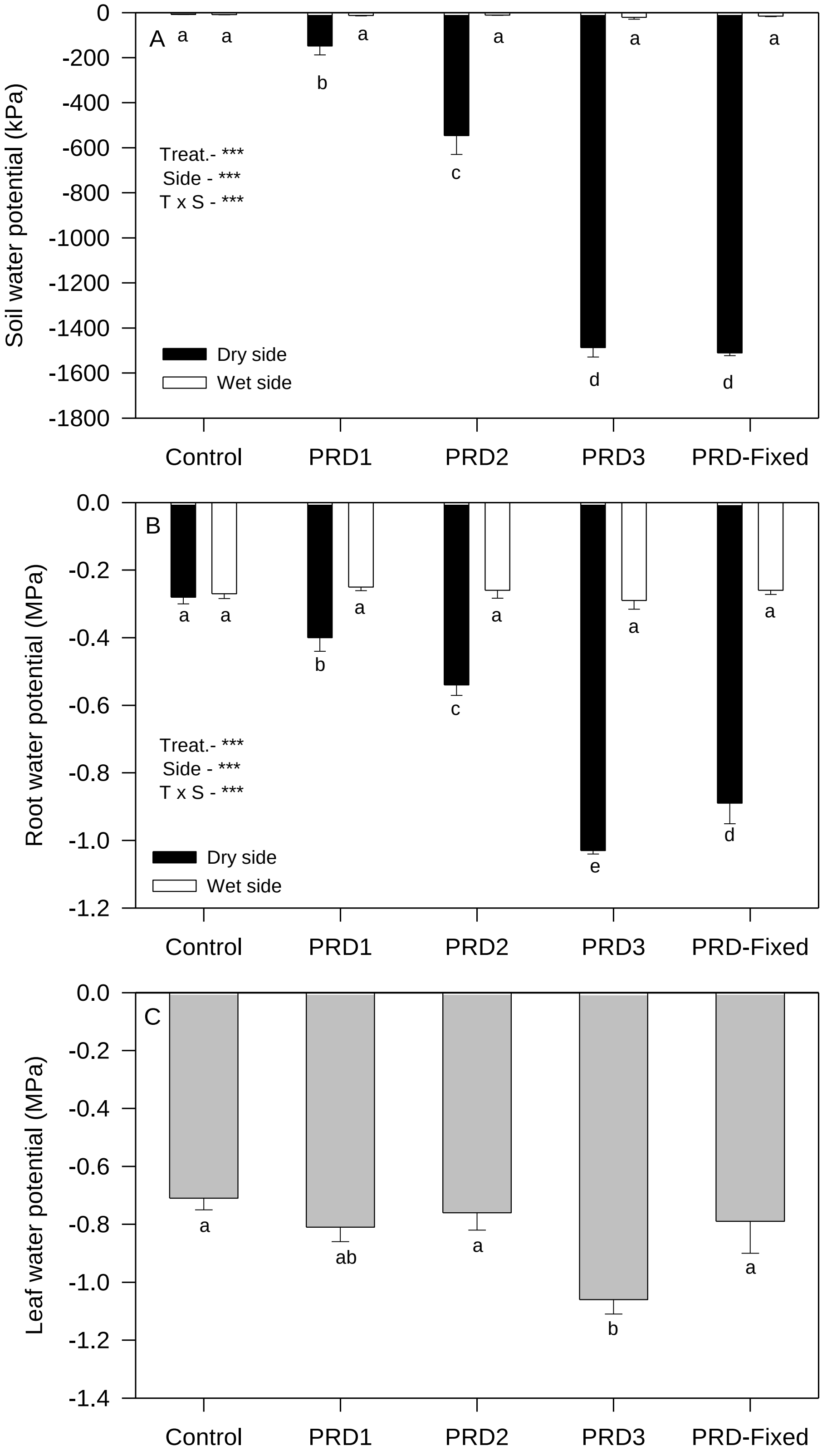

A
Soil water potential (kPa)
0
-200
-400
-600
-800
-1000
-1200
-1400
-1600
-1800
Treat.- ***
Side - ***
T x S - ***
Dry side
Wet side
a a b a c a d a d a
B
Root water potential (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
Treat.- ***
Side - ***
T x S - ***
Dry side
Wet side
a a b a c a e a d a
C
Leaf water potential (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
a ab a b a
Control
PRD1
PRD2
PRD3
PRD-Fixed

Figure 2

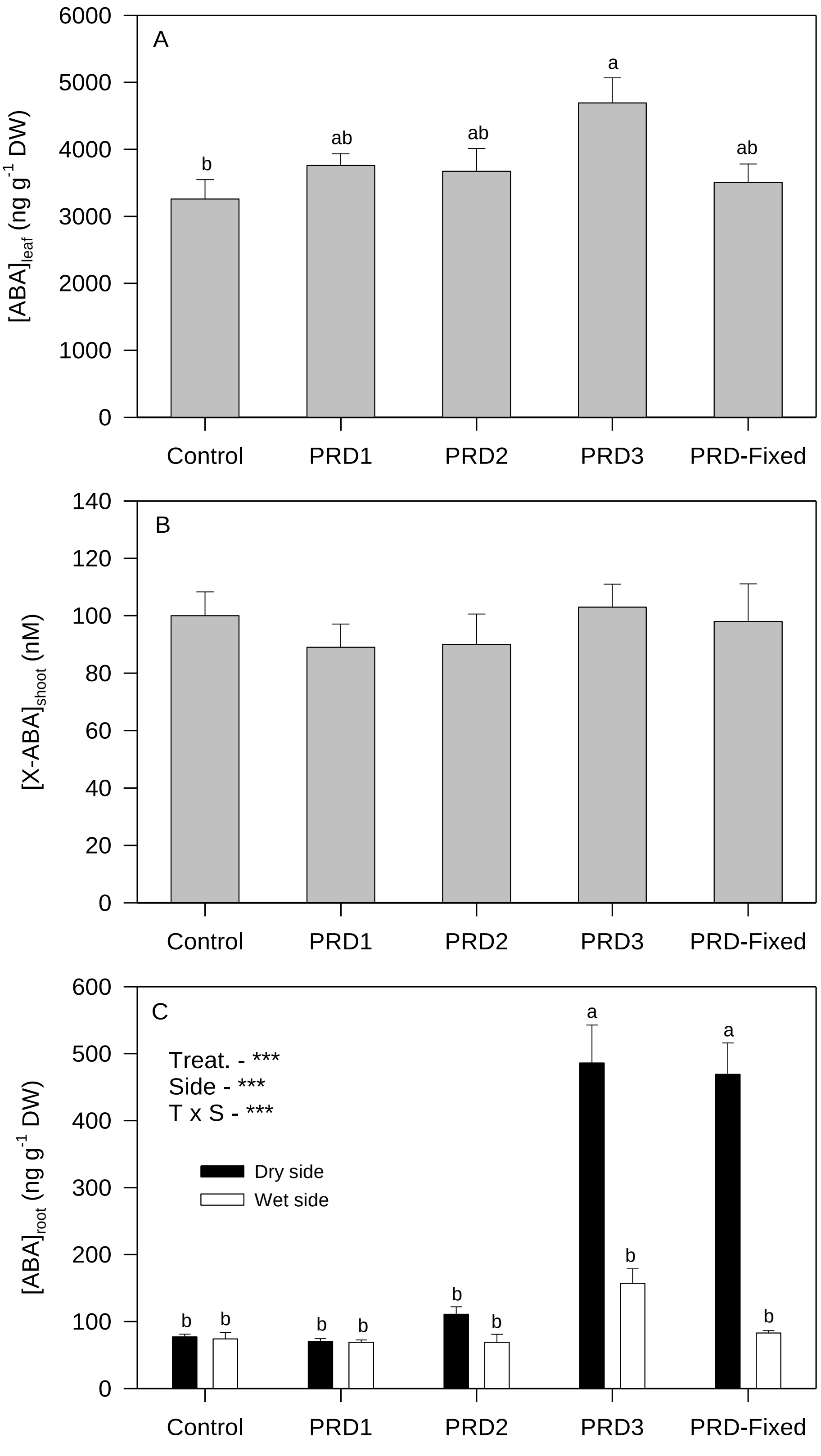
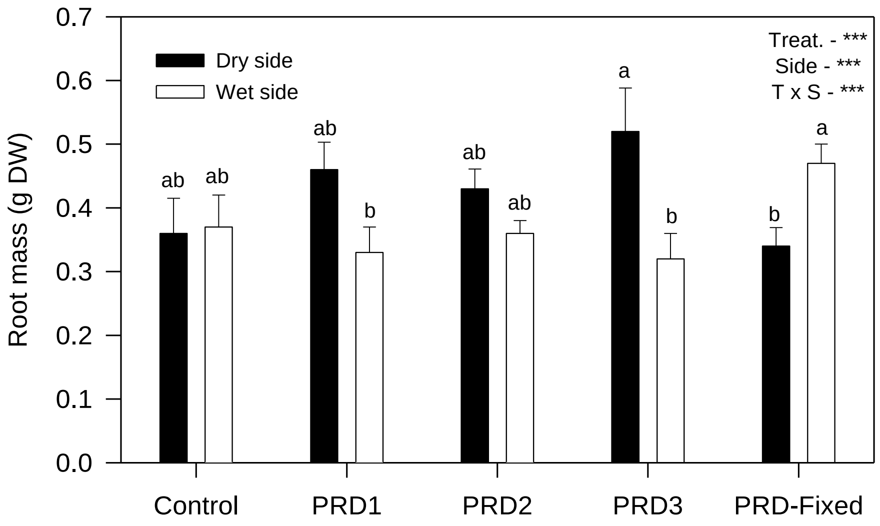

A
[ABA]$_{leaf}$ (ng g^{-1} DW)
6000
5000
4000
3000
2000
1000
0
b
ab
ab
a
ab
Control
PRD1
PRD2
PRD3
PRD-Fixed
B
[X-ABA]$_{shoot}$ (nM)
140
120
100
80
60
40
20
0
Control
PRD1
PRD2
PRD3
PRD-Fixed
C
[ABA]$_{root}$ (ng g^{-1} DW)
600
500
400
300
200
100
0
Treat. - ***
Side - ***
T x S - ***
Dry side
Wet side
a
a
b
b
b
b
b
b
b
b
Control
PRD1
PRD2
PRD3
PRD-Fixed

Figure 3



0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Root mass (g DW)
Dry side
Wet side
Treat. - ***
Side - ***
T x S - ***
ab
ab
ab
b
ab
ab
a
b
b
a
Control
PRD1
PRD2
PRD3
PRD-Fixed

Figure 4

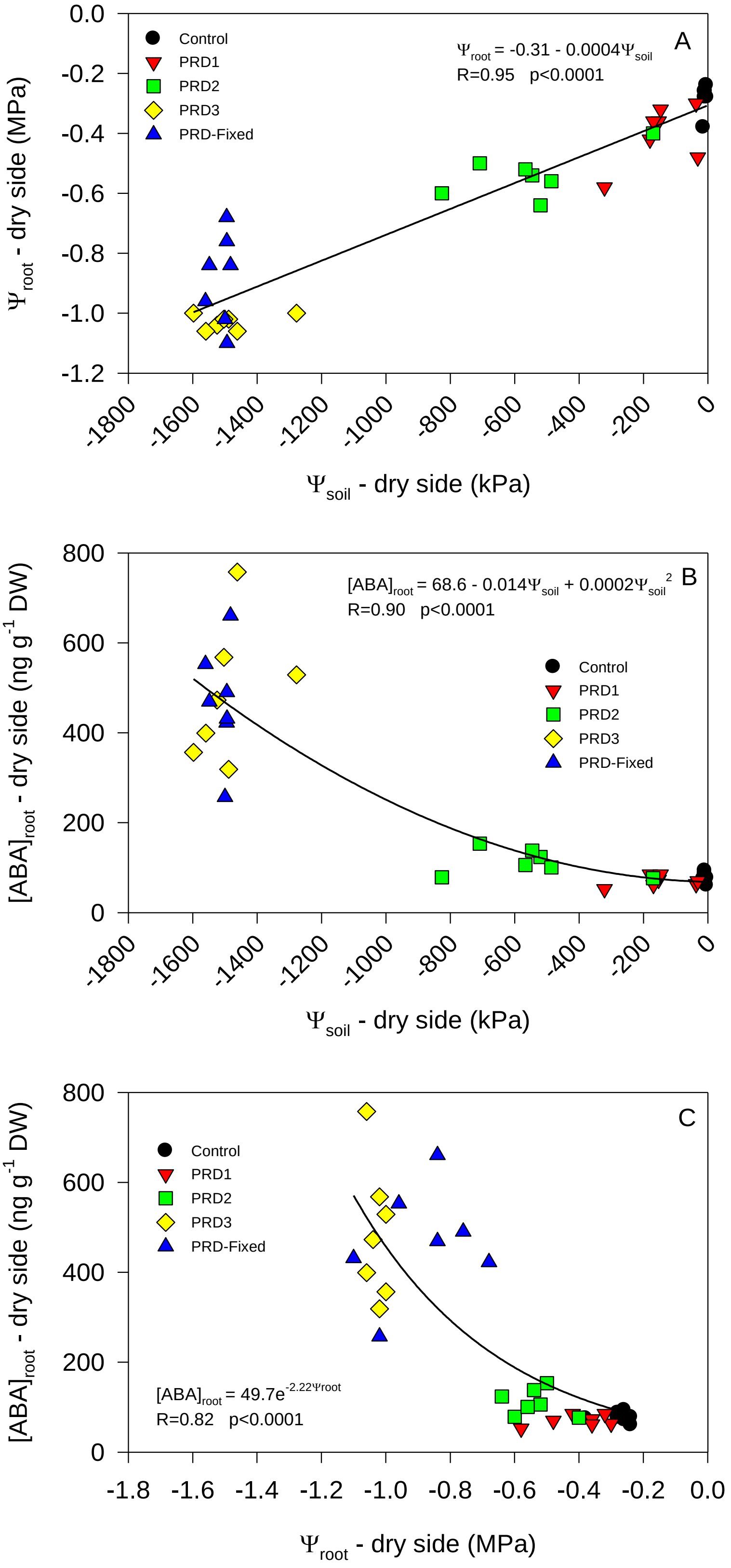

A
Control
PRD1
PRD2
PRD3
PRD-Fixed
Ψroot = -0.31 - 0.0004Ψsoil
R=0.95 p<0.0001
Ψroot - dry side (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1800
-1600
-1400
-1200
-1000
-800
-600
-400
-200
0
Ψsoil - dry side (kPa)
B
[ABA]root = 68.6 - 0.014Ψsoil + 0.0002Ψsoil 2
R=0.90 p<0.0001
Control
PRD1
PRD2
PRD3
PRD-Fixed
[ABA]root - dry side (ng g-1 DW)
800
600
400
200
0
-1800
-1600
-1400
-1200
-1000
-800
-600
-400
-200
0
Ψsoil - dry side (kPa)
C
Control
PRD1
PRD2
PRD3
PRD-Fixed
[ABA]root = 49.7e-2.22Ψroot
R=0.82 p<0.0001
[ABA]root - dry side (ng g-1 DW)
800
600
400
200
0
-1.8
-1.6
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
Ψroot - dry side (MPa)

Figure 5

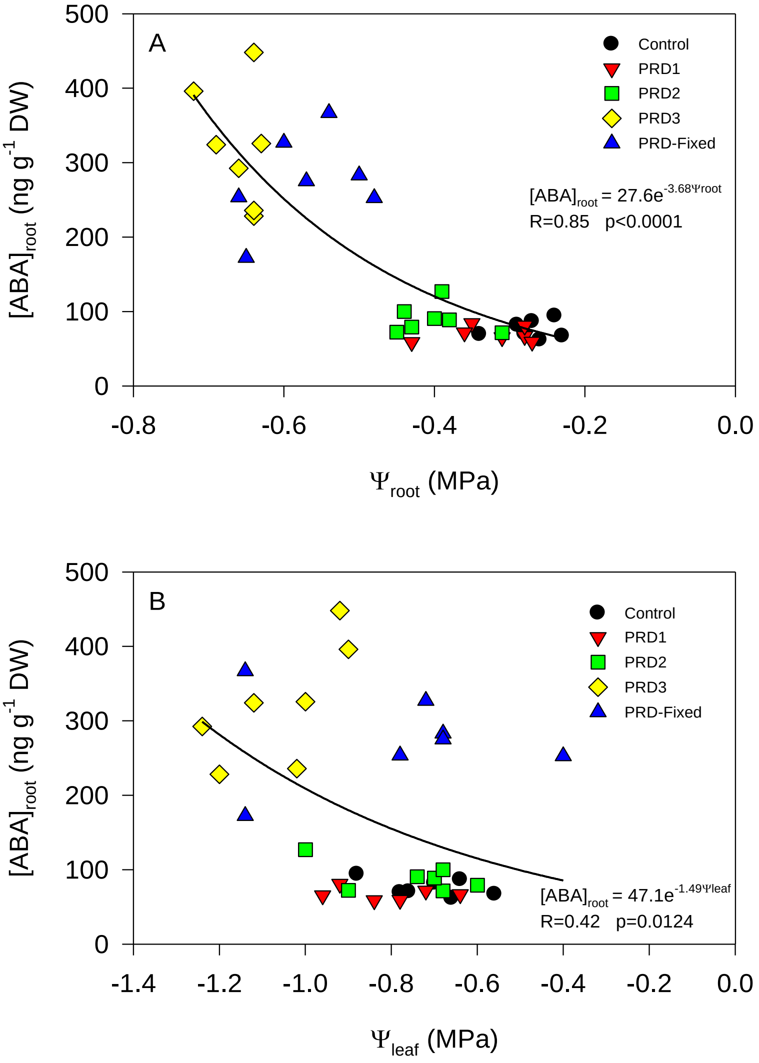

A
500
400
300
200
100
0
[ABA]$_{root}$ (ng g^{-1} DW)
-0.8
-0.6
-0.4
-0.2
0.0
Ψ$_{root}$ (MPa)
Control
PRD1
PRD2
PRD3
PRD-Fixed
[ABA]$_{root}$ = 27.6e$^{-3.68Ψroot}$
R=0.85 p<0.0001
B
500
400
300
200
100
0
[ABA]$_{root}$ (ng g^{-1} DW)
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
Ψ$_{leaf}$ (MPa)
Control
PRD1
PRD2
PRD3
PRD-Fixed
[ABA]$_{root}$ = 47.1e$^{-1.49Ψleaf}$
R=0.42 p=0.0124

Figure 6

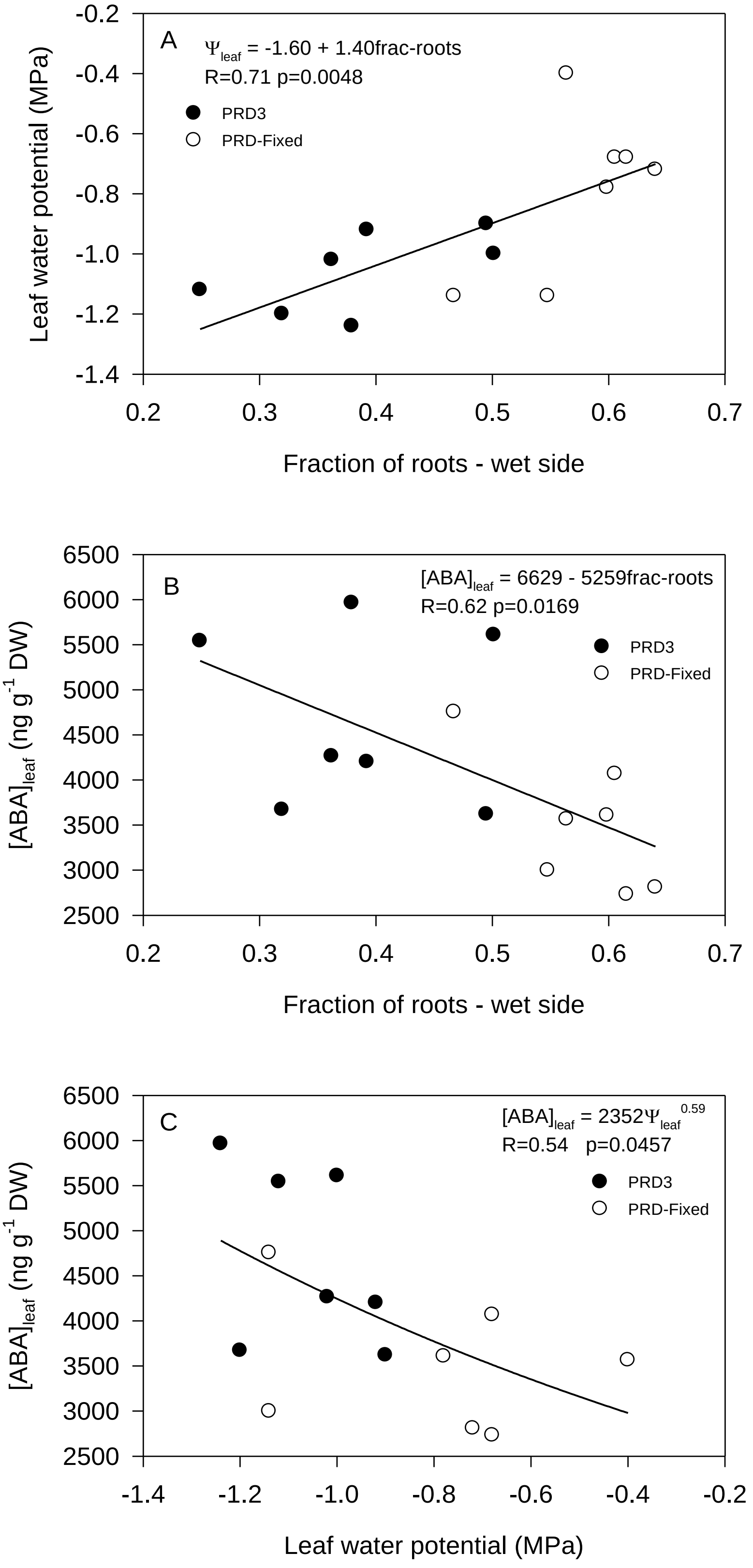

A
Ψleaf = -1.60 + 1.40frac-roots
R=0.71 p=0.0048
PRD3
PRD-Fixed
Leaf water potential (MPa)
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
0.2
0.3
0.4
0.5
0.6
0.7
Fraction of roots - wet side
B
[ABA]leaf = 6629 - 5259frac-roots
R=0.62 p=0.0169
PRD3
PRD-Fixed
[ABA]leaf (ng g-1 DW)
6500
6000
5500
5000
4500
4000
3500
3000
2500
0.2
0.3
0.4
0.5
0.6
0.7
Fraction of roots - wet side
C
[ABA]leaf = 2352Ψleaf 0.59
R=0.54 p=0.0457
PRD3
PRD-Fixed
[ABA]leaf (ng g-1 DW)
6500
6000
5500
5000
4500
4000
3500
3000
2500
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
Leaf water potential (MPa)

Figure 7

Table 1. Leaf gas exchange for each irrigation treatment (Control, PRD1, PRD2, PRD3 and PRD-Fixed) at the end of the experiment. Irrigation treatment consisted in a Control, and three alternated PRD with different alternation timing: PRD1 (3 days), PRD2 (6 days), PRD3 (12 days) and PRD-Fixed (dry and wet sides of the rootzone were maintained throughout the experiment). A_{CO2} CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}), E transpiration rate (mmol H_2O m^{-2} s^{-1}), g_s stomatal conductance (mol H_2O m^{-2} s^{-1}), A/E instantaneous water use efficiency µmol CO_2 $mmol^{-1}$ H_2O A/gs intrinsic water use efficiency (mol CO_2 mol^{-1} H_2O).

Treatments	A_{CO2}	*E*	g_s	*A*/*E*	A/g_s
Control	7.63	1.32	0.066	6.10 ab	130 ab
PRD1	6.61	1.07	0.050	6.20 ab	135 ab
PRD2	7.18	0.99	0.046	7.55 ab	166 a
PRD3	7.52	1.02	0.049	7.83 a	165 a
PRD-Fixed	7.94	1.65	0.089	5.73 b	117 b
ANOVA	ns	ns	ns	*	*

'*ns*' and * indicate not significant and $p<0.05$ respectively (n=7). For each column, different letters indicate significant differences at $p \leq 0.05$, by Tukey's test.

Table 2. Root and shoot biomass and leaf area for plants of each irrigation treatment (Control, PRD1, PRD2, PRD3 and PRD-Fixed) at the end of the experiment Irrigation treatment consisted in a Control, and three alternated PRD with different alternation timing: PRD1 (3 days), PRD2 (6 days), PRD3 (12 days) and PRD-Fixed (dry and wet sides of the rootzone were maintained throughout the experiment).

Treatments	Root mass (g DW)	Shoot mass (g DW)	Root/shoot ratio	Leaf area (cm^2)
Control	0.73	2.59	0.28 b	236 ab
PRD1	0.78	2.79	0.28 b	265 a
PRD2	0.79	2.45	0.32 ab	212 ab
PRD3	0.84	2.43	0.35 a	190 b
PRD-Fixed	0.81	2.33	0.35 a	212 ab
ANOVA	ns	ns	***	*

'*ns*', * and *** indicate not significant, $p<0.05$ and $p<0.001$, respectively (n=7). For each column, different letters indicate significant differences at $p\leq 0.05$, by Tukey's test.