

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

3

4 Pérez-Pérez, J.G.^{1*}, Navarro, J.M.¹, Robles, J.M.¹, Dodd, I.C.²

5 ¹Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA).

6 Departamento de Recursos Naturales, Equipo de Riego y Fisiología del Estrés. c/Mayor

7 s/n 30150 La Alberca (Murcia), Spain. (juang.perez@carm.es)

8 ²The Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, United

9 Kingdom.

10 *Corresponding author: juang.perez@carm.es

11 Tel.: +34 968 357876

12 Fax: +34 968 366792

13 E-mail: juang.perez@carm.es

14

15 **Running title:**

16 Partial rootzone drying effects on citrus

17

18 **Keywords:** plant water relations, gas exchange, biomass partition, ABA, vegetative
19 development.

20

21

22

23

24

25 **Abstract**

26 Partial rootzone drying (PRD) establishes discrete wet and dry parts of the
27 rootzone (for example using parallel drip lines on either side of the crop row), and
28 alternates them to stimulate root growth and root-to-shoot ABA signalling. To assess
29 whether alternation frequency affects plant physiological responses, *Citrus macrophylla*
30 Wester seedlings were grown with the root system split between two pots and 5 irrigation
31 treatments applied: Control, PRD-Fixed (where wet and dry parts of the rootzone were
32 not alternated) and three alternate PRD treatments where the wet and dry parts were
33 swapped at 3 (PRD1), 6 (PRD2) and 12 (PRD3) day intervals, to dry the soil to different
34 degrees before alternating the irrigation. Water was equally distributed between both pots
35 in Control plants, whereas only one pot was watered and the other allowed to dry in PRD
36 plants, with all plants receiving the same irrigation volume. After 24 days, soil water
37 content (θ_v), leaf water potential (Ψ_{leaf}), root water potential (Ψ_{root}), abscisic acid (ABA)
38 concentration in roots ($[\text{ABA}]_{\text{root}}$), leaves ($[\text{ABA}]_{\text{leaf}}$) and shoot xylem sap ($[\text{X-}$
39 $\text{ABA}]_{\text{shoot}}$), biomass allocation and leaf area were measured. Higher soil water availability
40 of the dry side (PRD1 and PRD2) had no significant effects on leaf water relations, ABA
41 status and plant biomass allocation. However, increasing the duration of exposure of part
42 of the root system to dry soil (PRD3 and PRD-Fixed) further decreased Ψ_{root} and
43 stimulated root ABA accumulation, while decreasing Ψ_{leaf} and increasing $[\text{ABA}]_{\text{leaf}}$ of
44 PRD3 plants compared to the other treatments. Differences in physiological response
45 between PRD3 and PRD-Fixed plants were attributed to differences in the proportion of
46 root mass exposed to drying soil: PRD3 plants had a lower Ψ_{leaf} and a higher $[\text{ABA}]_{\text{leaf}}$
47 with a smaller proportion of their root mass in wet soil. Since long drying cycles were
48 required to alter plant biomass allocation and physiological responses in PRD plants,
49 these should be implemented in designing suitable PRD strategies for field application.

50

51

52

53 **1. Introduction**

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

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

76 The agronomic benefits of PRD have been widely demonstrated in several citrus
77 species, such as mandarin (Kirda et al., 2007), sweet orange (Hutton and Loveys, 2011;
78 Consoli et al., 2014; Mossad et al., 2018), lemon (Pérez-Pérez et al., 2012) and recently
79 in grapefruit (Kusakabe et al., 2016). In these studies, PRD principally increased crop
80 water use efficiency (WUE) without detrimentally affecting marketable yields or apparent
81 tree health. However, in navel orange trees PRD reduced yield and fruit size compared
82 with conventional deficit irrigation (Faber and Lovatt, 2014). Improved WUE of PRD
83 lemon trees was not attributed to changes in the root-to-shoot ABA signalling (Pérez-
84 Pérez et al., 2012), suggesting that other mechanisms were involved in this response. The
85 characteristic soil moisture heterogeneity of PRD influences not only root-to-shoot ABA
86 signalling but other plant responses like root growth (Sharp and LeNoble, 2002) or root

87 hydraulic conductivity (Hose et al., 2000). Thus in pot-grown *Citrus* seedlings of the
88 rootstock Swingle citrumelo, PRD increased root-shoot ratio, but did not affect plant
89 WUE (Melgar et al., 2010). It has been argued that an improved understanding of the
90 physiological responses induced by PRD maximises the likelihood of achieving
91 agronomic benefits with this strategy (Dodd et al., 2015).

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

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

117

118 **2. Material and methods**

119 *2.1. Experimental design*

120 The experiment was carried out in a walk-in controlled environment room (3×6.5
121 m) at the IMIDA under a 16 h photoperiod (07.00-23.00 h). Day-night variation caused
122 fluctuations in temperature (20-24 °C) and relative humidity (65-85%). Seeds of *Citrus*
123 *macrophylla* Wester were germinated in vermiculite for 40 days. Then, the main root was
124 cut to stimulate the development of lateral roots, and seedlings were transferred to 10 L
125 containers (20 seedlings per container) filled with modified Hoagland solution (MHS) for
126 hydroponic culture. The nutrient solution composition was: 6 mM KNO₃, 4 mM
127 Ca(NO₃)₂·4H₂O, 2 mM NH₄H₂PO₄, 1 mM MgSO₄·7H₂O, 42.7 μM EDDHA-Fe, and trace
128 elements as prescribed by Hoagland and Arnon (1950). Nutrient solution pH was adjusted
129 to 6.0-6.5 with 1 M NaOH or 1 M HCl.

130 After 8 weeks, roots were pruned to maintain only two main roots, and thirty-five
131 seedlings were transplanted to 2×0.55 L pots (90×90×95 mm), with each main root placed
132 in a different soil compartment. The pots were filled with silica filtration sand (0.4-0.8
133 mm of particle size) with a bulk density of 1.39 g DW cm⁻³. The soil had a volumetric
134 soil water content of 21% at full pot holding capacity and 0.25% at permanent wilting
135 point. Seedlings were grown for 2 more months before 5 different irrigation treatments
136 were applied: Control, PRD-Fixed (dry and wet sides of the rootzone were maintained
137 throughout the experiment) and three alternated PRD with different alternation timing: at
138 3 (PRD1), 6 (PRD2) and 12 days (PRD3). These timings of irrigation alternation ensured
139 the soil dried to different degrees in the non-irrigated rootzone, based on a preliminary
140 experiment. The experimental design consisted of seven replicates per treatment (one
141 seedling per replicate). The irrigation treatments were maintained for 24 days, watering
142 both Control and PRD plants with the same irrigation volume to ensure that all PRD
143 plants received sufficient irrigation to maintain soil moisture in the irrigated side near
144 field capacity, thereby avoiding any salt accumulation. Each soil compartment of Control
145 plants was watered every 72 hours (at 9:00 h) with 80 mL of MHS, while the irrigated
146 side of PRD plants received 80 mL of MHS every 36 hours (at 9.00 and/or 21.00 h).

147 2.2. Measurements

148 Volumetric soil water content was monitored throughout the experiment by
149 inserting a theta probe (Model ML2X, Delta-T Devices) into the top of the pot before
150 each irrigation event. At the end of the experiment, the soil water content of each pot of
151 an individual plant was also determined by the gravimetric method. The volumetric water
152 content (θ_v) was calculated by dividing the measured water loss by the pot volume filled
153 with sand (412 cm³). Dielectric soil moisture sensor readings were calibrated by

154 comparing soil moisture of each pot with the gravimetric calculation. The excellent
155 correlation ($R = 0.99$) confirmed that sensor readings adequately represented volumetric
156 water content throughout the experiment. For estimating soil water potential (Ψ_{soil}) from
157 the volumetric soil water content values, a soil-water retention curve of the substrate used
158 in the experiment was made ($\Psi_{\text{soil}} \text{ (kPa)} = 1597.5e^{-24\theta_v}$, $R=0.90$, $P<0.0001$). Soil water
159 potential was measured with a dew point potential meter (WP4C, Decagon Devices,
160 USA).

161 Physiological measurements were made at the end of the experiment, just before
162 the wet and dry rootzones of the alternate PRD treatments were due to be swapped. Leaf
163 gas exchange was measured in a single youngest fully expanded leaf per plant using a
164 portable photosynthesis system (Li-6400, Li-Cor, Lincoln, Nebraska, USA) equipped
165 with a broad leaf chamber (6.0 cm^2). The air flow rate inside the leaf chamber was 300
166 $\mu\text{mol s}^{-1}$ and the temperature of the block of the leaf chamber was fixed at $24 \text{ }^\circ\text{C}$. Portable
167 12-g cartridges of high-pressure, liquefied, pure CO_2 were attached to the console by an
168 external CO_2 source assembly and were controlled automatically by a CO_2 injector system
169 (6400-01 Li-Cor, Lincoln, Nebraska, USA). The reference CO_2 concentration was fixed
170 at $450 \mu\text{mol CO}_2 \text{ mol}^{-1}$. All the measurements were made using a red-blue light source
171 (6400-02B light emitting diode; Li-Cor, Lincoln, Nebraska, USA) attached to the leaf
172 chamber and the PPFD was fixed at $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

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

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

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

192

193 2.3. Statistical analysis

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

203

204 3. Results

205 The volumetric soil water content (θ_v) (measured by the soil moisture sensor) was
206 maintained above $0.16 \text{ cm}^3 \text{ cm}^{-3}$ in both pots of Control plants, corresponding with soil
207 water potential values (Ψ_{soil}) of -35 kPa (Fig. 1). In all PRD treatments, watering the wet
208 pot (side A or B, depending of the treatment) of the plant every 36 hours maintained θ_v
209 above $0.18 \text{ cm}^3 \text{ cm}^{-3}$ ($\Psi_{\text{soil}} \approx -21 \text{ kPa}$). On the other hand, the θ_v of the dry pot decreased
210 to different levels depending on the frequency with which wet and dry sides were
211 alternated. Plants of the PRD1 treatment were exposed to 4 complete drying/re-wetting
212 cycles (alternated every 3 days) and the θ_v just before each change of the irrigated side
213 ranged between 0.11 - $0.17 \text{ cm}^3 \text{ cm}^{-3}$ (Ψ_{soil} between -27 and -114 kPa) (Fig. 1). Plants of
214 the PRD2 treatment were exposed to 2 complete drying/re-wetting cycles (alternated
215 every 6 days), and the θ_v reached at the end of the each drying cycle ranged between 0.05 -
216 $0.07 \text{ cm}^3 \text{ cm}^{-3}$ (Ψ_{soil} between -298 and -482 kPa). Plants of the PRD3 treatment were
217 exposed to one complete drying/re-wetting cycle (alternated every 12 days). The θ_v was
218 extremely low (ranging between 0.003 and $0.01 \text{ cm}^3 \text{ cm}^{-3}$; $-1,486$ and $-1,556 \text{ kPa}$) towards
219 the end of the drying cycle (day 10 – 36 hours before the alternation). In PRD-Fixed
220 plants, the irrigated pot was maintained throughout the experiment. In dry pot of PRD-

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

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

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

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

246 At the end of the experiment, only PRD3 plants had a significantly higher leaf
247 ABA concentration ($[\text{ABA}]_{\text{leaf}}$), by 44% compared to the other treatments (Fig. 3A).
248 Shoot xylem ABA concentration ($[\text{X-ABA}]_{\text{shoot}}$) was similar in all irrigation treatments
249 (Fig. 3B). For roots in the irrigated pot (Side B), root ABA concentration ($[\text{ABA}]_{\text{root}}$) was
250 similar in all irrigation treatments (Fig. 3C). For roots in the drying pot (Side A),
251 $[\text{ABA}]_{\text{root}}$ was only significantly increased (by 6-fold) in PRD3 and PRD-Fixed plants
252 compared to the remaining treatments (Fig. 3C). Nevertheless, even PRD2 plants showed
253 differential ABA accumulation between the irrigated and drying pots, with $[\text{ABA}]_{\text{root}}$
254 slightly but significantly ($P=0.014$) higher (62%) in the drying pot of this treatment. Thus

255 a threshold Ψ_{soil} or Ψ_{root} was needed to trigger root ABA accumulation in the drying pot
256 (cf. Figs. 2A, B, 3C).

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

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

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

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

283

284 **4. Discussion**

285 While most studies of PRD regularly swap irrigation between wet and dry parts
286 of the rootzone (eg. Hutton and Loveys, 2011) since these changes increase yield
287 compared to maintaining irrigation to only one part (reviewed in Dodd et al. 2015), there
288 has been little systematic investigation of how the time between alternation events affects

289 soil moisture status, and thence physiological responses. Applying the same irrigation
290 volume, but varying the duration of the drying/re-wetting cycles during PRD, generated
291 differences in soil drying and soil moisture distribution (Figs. 1 and 2A). While the short
292 duration (24 days) of the different treatments did not significantly alter total shoot and
293 root biomass, those that exposed plants to more intense soil drying inhibited leaf area
294 expansion and increased the root/shoot ratio (Table 2). Thus root growth was stimulated
295 in the irrigated pot of PRD-Fixed plants (Wang et al., 2005) and in response to alternating
296 wetting and drying parts of the rootzone in PRD-3 plants (Mingo et al., 2004). Although
297 understanding the physiological mechanisms determining root growth dynamics
298 following soil moisture fluctuations was beyond the scope of this study, changes in root
299 phytohormone (auxin, cytokinin) concentrations have been implicated (Han et al., 2015).
300 Moreover, these changes in biomass partitioning were accompanied by physiological
301 changes such as increased intrinsic water use efficiency (Table 1), ostensibly due to
302 differences in plant ABA and water status (Figs. 2 and 3). Although establishing causality
303 between these physiological and biomass changes is difficult, it is important to understand
304 their regulation during PRD.

305 By maintaining Ψ_{soil} of the irrigated pot above -35 kPa in all PRD plants (Figs. 1
306 and 2A), local Ψ_{root} did not vary among treatments (Fig. 2B) and there was no ABA
307 accumulation in the irrigated roots *except* in the PRD3 plants (Fig. 3C). Compared to the
308 other irrigated roots, $[\text{ABA}]_{\text{root}}$ within the irrigated pot of PRD3 plants almost doubled,
309 coincident with decreased Ψ_{leaf} (Fig. 2C) and increased $[\text{ABA}]_{\text{leaf}}$ (Fig. 3A). Increased
310 $[\text{ABA}]_{\text{root}}$ of irrigated roots in the absence of any decrease in Ψ_{root} (the putative stimulus
311 for root ABA synthesis – Simonneau et al., 1998) apparently supports the hypothesis of
312 foliar ABA synthesis (in response to decreased Ψ_{leaf} and turgor) and subsequent basipetal
313 phloem transport of ABA to the roots (Wolf et al., 1990; Jiang and Hartung, 2008). While
314 stimulating root ABA accumulation via this mechanism is less direct than localised soil
315 drying upregulating ABA biosynthesis genes in the roots (Speirs et al., 2013), supplying
316 radioactive ABA to the shoots causes label accumulation in the roots (McAdam et al.,
317 2016). Although it is unknown how basipetally transported ABA is distributed to different
318 roots when soil moisture is heterogeneous, preferential root ABA accumulation in the dry
319 rootzone of PRD plants (Khalil and Grace, 1993; Puértolas et al., 2015) would require
320 those roots to act as stronger sinks for ABA.

321 Should shoot ABA status be the primary regulator of root ABA accumulation,
322 $[\text{ABA}]_{\text{leaf}}$ should be correlated with $[\text{ABA}]_{\text{root}}$. While Control, PRD1, PRD2 and PRD-

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

335 Irrespective of the cause(s) of root ABA accumulation, it is important to determine
336 whether its transport to the shoots induces stomatal closure. In all treatments, irrespective
337 of the magnitude of root ABA accumulation (Fig. 3C), xylem ABA concentration was
338 constant (Fig. 3B) as in citrus plants exposed to PRD in the field (Pérez-Pérez et al.,
339 2012). Similarly, in potato plants exposed to both horizontal and vertical soil moisture
340 gradients that stimulated root ABA accumulation, xylem ABA concentration did not
341 increase (Puértolas et al., 2015). However, root ABA accumulation was accompanied by
342 a substantial decrease in sap flow from roots in drying soil such that xylem ABA
343 concentration was determined by sap flow from roots in irrigated soil (Puértolas et al.,
344 2015). These observations are consistent with a model that explains xylem ABA
345 concentration of PRD plants as a function of xylem ABA concentrations emanating from
346 the irrigated and drying parts of the root system and the relative sap flow from each (Dodd
347 et al., 2008; Pérez-Pérez and Dodd, 2015). Thus local root ABA accumulation need not
348 result in root-to-shoot ABA signalling.

349 Instead, the main role of root ABA accumulation in response to PRD was likely
350 root growth regulation. Plants that accumulated high root ABA concentrations (PRD3,
351 PRD-Fixed treatments) greatly altered their root biomass distribution between soil
352 compartments (Fig. 4), and thus their root-shoot ratio. The altered root biomass
353 distribution in PRD-Fixed plants likely assisted in maintaining leaf water status (Fig. 2C)
354 as previously reported (Martín-Vertedor and Dodd, 2011), since PRD-Fixed plants
355 generally had > 50% of their root biomass in irrigated soil (Fig. 6A). This maintenance
356 of leaf water status was associated with foliar ABA homeostasis such that PRD-Fixed

357 plants had the same $[ABA]_{\text{leaf}}$ as Control plants (Fig. 3A). Thus changes in root biomass
358 distribution can maintain homeostasis of leaf water and ABA relations.

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

367 Regular (every 3 days) swapping of the irrigated and drying pots (PRD1)
368 maintained local Ψ_{soil} of the drying side above -114 kPa, with similar physiological
369 responses as the Control plants. Limited soil drying (Fig. 1) had a minimal effect on Ψ_{root}
370 (Fig. 2A), which was insufficient to affect root ABA concentration (Fig. 3C). Moreover,
371 shoot responses (Ψ_{leaf} , leaf gas exchange) and biomass (Table 2) were similar to Control
372 plants. When local Ψ_{soil} decreased to -482 kPa (PRD2), additional physiological responses
373 were observed. Since Ψ_{root} further decreased, $[ABA]_{\text{root}}$ increased slightly (62%), but
374 significantly ($P = 0.023$), compared to well irrigated roots (Fig. 3C). Nevertheless, $[X-$
375 $ABA]_{\text{shoot}}$ and $[ABA]_{\text{leaf}}$ were not altered (Fig. 3), suggesting that ABA was not
376 transported from the roots to the shoot, perhaps because of diminished water transport
377 from the roots in drying soil (Dodd et al., 2008). Although Ψ_{leaf} , A_{CO_2} and g_s were
378 statistically similar to Control plants, leaf water use efficiency (A/g_s) increased via
379 mechanisms unrelated to changes in root-to-shoot ABA signalling and/or leaf water status
380 (Perez-Perez et al., 2012; Rodrigues et al., 2008). Recent studies indicate that soil drying
381 can increase the transport of other antitranspirants such as sulphate (Machelska et al.,
382 2017) and jasmonates (de Ollas et al., 2018) from the roots, although their impact on leaf
383 WUE has not been investigated in detail.

384 Further soil drying to complete soil moisture depletion (allowing a single
385 complete drying/re-wetting cycle, PRD3) induced further physiological and biomass
386 changes. Leaf and root ABA accumulation (Fig. 3A, C) in response to decreased water
387 status of both tissues (Figs. 2B, C) was not accompanied by any change root-to-shoot
388 ABA signalling (Fig. 3B). Root/shoot ratio was increased and leaf area decreased
389 compared to PRD1 plants, indicating altered resource allocation. While local root ABA
390 accumulation may enhance sink strength (Chen et al., 2003), decreased foliar cytokinin

391 status of plants exposed to PRD (Kudoyarova et al., 2007) is also likely to have decreased
392 biomass allocation to the shoot. Reductions in transpiring area concomitant with
393 potentially increased root surface area may have minimised changes in Ψ_{leaf} , as in
394 grapevines exposed to PRD in the field (Romero et al., 2014).

395 Although PRD3 and PRD-Fixed plants experienced similar degrees of soil drying,
396 greater root growth in the irrigated pot of the latter prevented any change in Ψ_{leaf}
397 compared to Control plants, as in grapefruit grown in the field with PRD (Romero-Conde
398 et al., 2014). Moreover, these irrigated roots likely had higher hydraulic conductance than
399 roots of Control plants (Hu et al., 2011). Nevertheless, changes in root hydraulic
400 conductivity (L_p) are likely of lesser importance in Ψ_{leaf} homeostasis than stomatal
401 regulation, since higher ABA concentrations in the irrigated roots of PRD3 plants (Fig.
402 3C) should stimulate L_p (reviewed in Dodd, 2013), yet Ψ_{leaf} of these plants still declined.
403 In PRD-Fixed plants, the greater fraction of the root biomass exposed to irrigated soil
404 likely facilitated redistribution of water along Ψ gradients from irrigated to drying pots
405 via the roots (Stoll et al., 2000), thereby attenuating the decrease in Ψ_{root} within the dry
406 soil compartment (Fig. 2B). These adjustments in root morphology and plant water
407 relations were not accompanied by any change in leaf water use efficiency (A/g_s), which
408 may diminish the value of applying PRD. Although alternating the irrigated and drying
409 parts of the rootzone during PRD enhances crop yields per unit of irrigation compared to
410 fixed PRD (Dodd et al., 2015), understanding the integration and relative importance of
411 physiological and morphological adjustments during PRD remains a key knowledge gap
412 (Romero et al., 2014) that may limit the application of this technique.

413

414 **5. Conclusions**

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

424

425 **Acknowledgements**

426 This research was funded by the INIA through the project RTA2012-00102-00-
427 00. Juan G. Pérez-Pérez gratefully acknowledges the postdoctoral contracts in the INIA-
428 CCAA program, supplied by the INIA and co-financed with the European Social Fund,
429 and ‘Ramón y Cajal’ program, supplied by the Spanish Ministry of Economy, Industry
430 and Competitiveness (MINECO) and the ‘Fundación Séneca’ (19629/EE/14) for the
431 fellowship to support his research at Lancaster.

432

433

434 **References**

- 435 Affi, N., Fadl, A. El, Otmani, M. El, Benismail, M.C., Idrissi, L.M., Rahhaoui, Z., 2013.
436 Partial root zone drying alternation frequency effect on yield, biomass production and
437 fruit quality of tomato crop. *Eur. J. Appl. Eng. Sci. Res.* 2, 12–19.
- 438 Chen, G., Fu, X., Lips, H., Sagi, M., 2003. Control of plant growth resides in the shoot,
439 and not in the root, in reciprocal grafts of *flacca* and wild-type tomato (*Lycopersicon*
440 *esculentum*), in the presence and absence of salinity stress. *Plant and Soil* 256, 205-215.
- 441 Colak, Y.B., Yazar, A., 2017. Evaluation of crop water stress index on Royal table grape
442 variety under partial root drying and conventional deficit irrigation regimes in the
443 Mediterranean Region. *Sci. Hort.*, 224: 384-394. doi: 10.1016/j.scienta.2017.06.032.
- 444 Consoli, S., Stagno, F., Rocuzzo, G., Cirelli, G.L., Intrigliolo, F., 2014. Sustainable
445 management of limited water resources in a young orange orchard. *Agric. Water Manag.*
446 132, 60–68. doi:10.1016/j.agwat.2013.10.006.
- 447 de Ollas, C., Arbona, V., Gomez-Cadenas, A., Dodd, I.C., 2018. Attenuated accumulation
448 of jasmonates modifies stomatal responses to water deficit. *J. Exp. Bot.* 69, 2103-2116.
- 449 Dodd, I.C., 2013. Abscisic acid and stomatal closure: a hydraulic conductance
450 conundrum? *Commentary New Phytol.* 197, 6-8.
- 451 Dodd, I.C., Egea, G., Davies, W.J. 2008. ABA signalling when soil moisture is
452 heterogeneous: decreased photoperiod sap flow from drying roots limit ABA export to
453 the shoot. *Plant, Cell Environ.* 31, 1263-1274.
- 454 Dodd, I.C., Puértolas, J., Huber, K., Pérez-Pérez, J.G., Wright, H.R., Blackwell, M.S.A.,
455 2015. The importance of soil drying and re-watering in crop phytohormonal and
456 nutritional responses to deficit irrigation. *J. Exp. Bot.* 66, 2239-2252.
- 457 Dodd, I.C., Theobald, J.C., Bacon, M.A., Davies, W.J., 2006. Alternation of wet and dry
458 sides during partial rootzone drying irrigation alters root-to-shoot signalling of abscisic
459 acid. *Funct. Plant Biol.* 33, 1081-1089. doi:10.1071/FP06203.
- 460 Dry, P.R., Loveys, B.R., Düring, H., 2000. Partial drying of the rootzone of grape. I.
461 Transient changes in shoot growth and gas exchange. *Vitis* 39, 3-7.
- 462 Faber, B.A., Lovatt, C.J., 2014. Effects of applying less water by partial root zone drying
463 versus conventional irrigation on navel orange yield. *Acta Hort.* 1038, 523-530.
- 464 Han, H.M, Tian, Z.W, Fan, Y.H., Cui, Y.K., Cai, J., Jiang, D., Cao, W.X., Dai, T.B.,
465 2015. Water-deficit treatment followed by re-watering stimulates seminal root growth
466 associated with hormonal balance and photosynthesis in wheat (*Triticum aestivum* L.)
467 seedlings. *Plant Growth Regulation* 77, 201-210.
- 468 Hose, E., Steudle, E., Hartung, W., 2000. Abscisic acid and hydraulic conductivity of
469 maize roots: a study using cell- and root- pressure probes. *Planta* 211, 874–882.

- 470 Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without
471 soils. University of California, Agricultural Experiment Station, Berkeley, CA, Circular
472 347, 1-39 pp.
- 473 Hu, T., Kang, S., Li, F., Zhang, J., 2011. Effects of partial root-zone irrigation on
474 hydraulic conductivity in the soil-root system of maize plants. *J. Exp. Bot.* 62, 4163-4172.
475 doi:10.1093/jxb/err110
- 476 Hutton, R.J.J., Loveys, B.R.R., 2011. A partial root zone drying irrigation strategy for
477 citrus—Effects on water use efficiency and fruit characteristics. *Agric. Water Manag.* 98,
478 1485–1496. doi:10.1016/j.agwat.2011.04.010.
- 479 Jiang, F., Hartung, W., 2008. Long-distance signalling of abscisic acid (ABA): The
480 factors regulating the intensity of the ABA signal. *J. Exp. Bot.* 59, 37–43.
481 doi:10.1093/jxb/erm127.
- 482 Khalil, A.M., Grace, J., 1993. Does xylem sap ABA control the stomatal behaviour of
483 water stressed Sycamore (*Acer pseudoplatanus* L.) seedlings? *J. Exp. Bot.* 44, 1127–
484 1134. doi: 10.1093/jxb/44.7.1127.
- 485 Kirda, C., Lu, F.T., Topçu, S., Kaman, H., 2007. Mandarin Yield Response to Partial
486 Root Drying and Conventional Deficit Irrigation. *Turkey J. Agric.* 31, 1–10.
- 487 Kudoyarova, G.B. Vysotskaya, L.B., Cherkozyanova, A., Dodd, I.C. 2007. Effect of
488 partial rootzone drying on the concentration of zeatin-type cytokinins in tomato
489 (*Lycopersicon esculentum* Mill.) xylem sap and leaves. *J. Exp. Bot.* 58, 161-168.
- 490 Kusakabe, A., Contreras-Barragan, B.A., Simpson, C.R., Enciso, J.M., Nelson, S.D.,
491 Melgar, J.C., 2016. Application of partial rootzone drying to improve irrigation water use
492 efficiency in grapefruit trees. *Agric. Water Manag.* 178, 66–75.
493 doi:10.1016/j.agwat.2016.09.012.
- 494 Malcheska, F., Ahmad, A., Batool, S., Müller, H.M., Ludwig-Müller, J., Kreuzwieser, J,
495 Randewig., D., Hänsch., R., Mendel, R.R., Hell., R., Wirtz., M., Geiger., D., Ache., P.,
496 Hedrich., R., Herschbach., C., Rennenberg, H., 2017. Drought-enhanced xylem sap
497 sulfate closes stomata by affecting ALMT12 and guard cell ABA Synthesis. *Plant*
498 *Physiol.* 174, 798-914.
- 499 Martín-Vertedor, A.I., Dodd, I.C., 2011. Root-to-shoot signalling when soil moisture is
500 heterogeneous: increasing the proportion of root biomass in drying soil inhibits leaf
501 growth and increases leaf abscisic acid concentration. *Plant Cell Environ.* 34, 1164–75.
502 doi:10.1111/j.1365-3040.2011.02315.x.
- 503 McAdam, S.A.M., Brodribb, T.J., Ross, J.J., 2016. Shoot derived abscisic acid promotes
504 root growth. *Plant Cell Environ.* 39, 652–659. <http://dx.doi.org/10.1111/pce.12669>.
- 505 Melgar, J.C., Dunlop, J.M., Syvertsen, J.P., 2010. Growth and physiological responses of
506 the citrus rootstock Swingle citrumelo seedlings to partial rootzone drying and deficit
507 irrigation. *J. Agric. Sci.* 148, 593–602. doi:10.1017/S0021859610000377.

- 508 Mingo, D.M., Theobald, J.C., Bacon, M.A., Davies, W.J., Dodd, I.C., 2004. Biomass
509 allocation in tomato (*Lycopersicon esculentum*) plants grown under partial rootzone
510 drying: enhancement of root growth. *Funct. Plant Biol.* 31, 971-978.
- 511 Mossad, A., Scalisi, A., Lo Bianco, R. 2018. Growth and water relations of field-grown
512 'Valencia' orange trees under long-term partial rootzone drying. *Irrigation Sci.* 36, 9-24.
- 513 Pérez-Pérez, J.G., Dodd, I.C., Botía, P., 2012. Partial rootzone drying increases water-use
514 efficiency of lemon Fino 49 trees independently of root-to-shoot ABA signalling. *Funct.*
515 *Plant Biol.* 39, 366–378. doi:10.1071/FP11269.
- 516 Pérez-Pérez, J.G., Dodd, I.C., 2015. Sap fluxes from different parts of the rootzone
517 modulate xylem ABA concentration during partial rootzone drying and re-wetting. *J. Exp.*
518 *Bot.* 66, 2315-2324.
- 519 Puértolas, J., Alcobendas, R., Alarcón, J.J., Dodd, I.C., 2013. Long-distance abscisic acid
520 signalling under different vertical soil moisture gradients depends on bulk root water
521 potential and average soil water content in the root zone. *Plant, Cell Environ.* 36, 1465–
522 1475. doi:10.1111/pce.12076.
- 523 Puértolas, J., Conesa, M.C., Ballester, C., Dodd, I. C., 2015. Local root abscisic acid
524 (ABA) accumulation depends on the spatial distribution of soil moisture in potato:
525 Implications for ABA signalling under heterogeneous soil drying. *J. Exp. Bot.* 66, 2325-
526 2334.
- 527 Quarrie, S.A., Whitford, P.N., Appleford, N.E.J., Wang, T.L., Cook, S.K., Henson, I.E.,
528 Loveys, B.R., 1988. A monoclonal antibody to (S)-abscisic acid: its characterisation and
529 use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and
530 lupin leaves. *Planta* 173, 330–339. doi:10.1007/BF00401020.
- 531 Rodrigues, M.L., Santos, T., Rodrigues, A.P. de Souza, C.R., Lopes, C.M., Maroco, J.P.,
532 Pereira, J.S., Chaves, M.M., 2008. Hydraulic and chemical signalling in the regulation of
533 stomatal conductance and plant water use of field grapevines growing under deficit
534 irrigation. *Funct. Plant Biol.* 35: 565–579.
- 535 Romero, P., Pérez-Pérez, J.G., del Amor, F.M., Martínez-Cutillas, A., Dodd, I.C., Botía,
536 P., 2014. Partial root zone drying exerts different physiological responses on field-grown
537 grapevine (*Vitis vinifera* cv. Monastrell) in comparison to regulated deficit irrigation.
538 *Funct. Plant Biol.* 41, 1087–1106. doi:http://dx.doi.org/10.1071/FP13276.
- 539 Romero-Conde, A., Kusakabe, A., Melgar, J.C., 2014. Physiological responses of citrus
540 to partial rootzone drying irrigation. *Sci. Horticult.* 169, 234–238. http://dx.doi.org/10.
541 1016/j.scienta.2014.02.022.
- 542 Santos, T., Lopes, C., Rodrigues, M.L., Souza, C.R., Silva, J.R., Maroco, J.P., Pereira,
543 J.S., Chaves, M.M., 2003. Partial rootzone drying effects on growth and fruit quality of
544 field-grown grapevines (*Vitis vinifera*). *Funct. Plant Biol.* 30, 663–671. doi: 10.1071/
545 FP02180.

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

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

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

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

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

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

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

566

Figures

567

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

575

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

583

584 Figure 3. Leaf ABA concentration ($[ABA]_{\text{leaf}}$) (A), shoot xylem ABA concentration ($[X-$
585 $ABA]_{\text{shoot}}$) (B) and root ABA concentration ($[ABA]_{\text{root}}$) (C) for irrigation treatments
586 (Control, PRD1, PRD2, PRD3 and PRD-Fixed) at the end of the experiment. Irrigation
587 treatments comprised a Control, a PRD-Fixed treatment (dry and wet sides of the rootzone
588 were maintained throughout the experiment) and three alternate PRD treatments where
589 dry and wet sides of the rootzone were alternated every 3 (PRD1), 6 (PRD2) and 12
590 (PRD3) days. Bars are means \pm SE of 7 replicates. Bars labelled with different letters are
591 significantly different at $P < 0.05$.

592

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

600

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

609

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

616

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

625

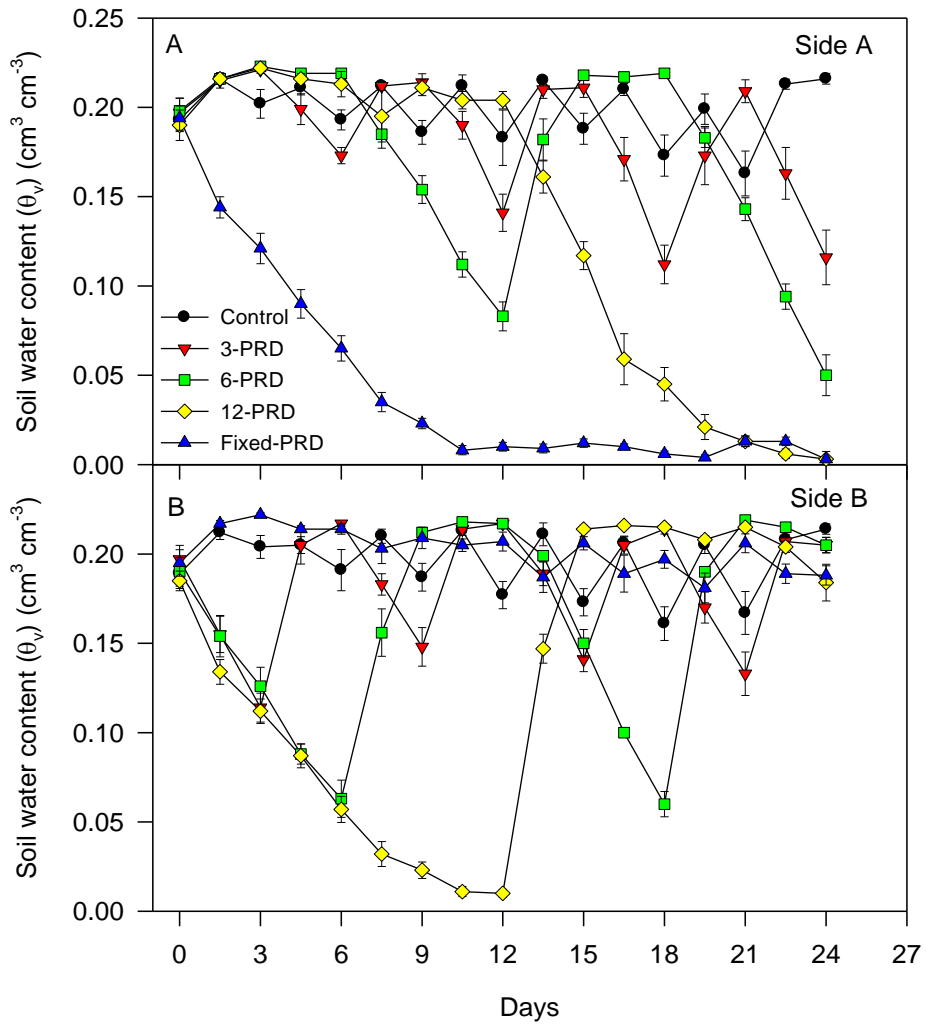


Figure 1

626

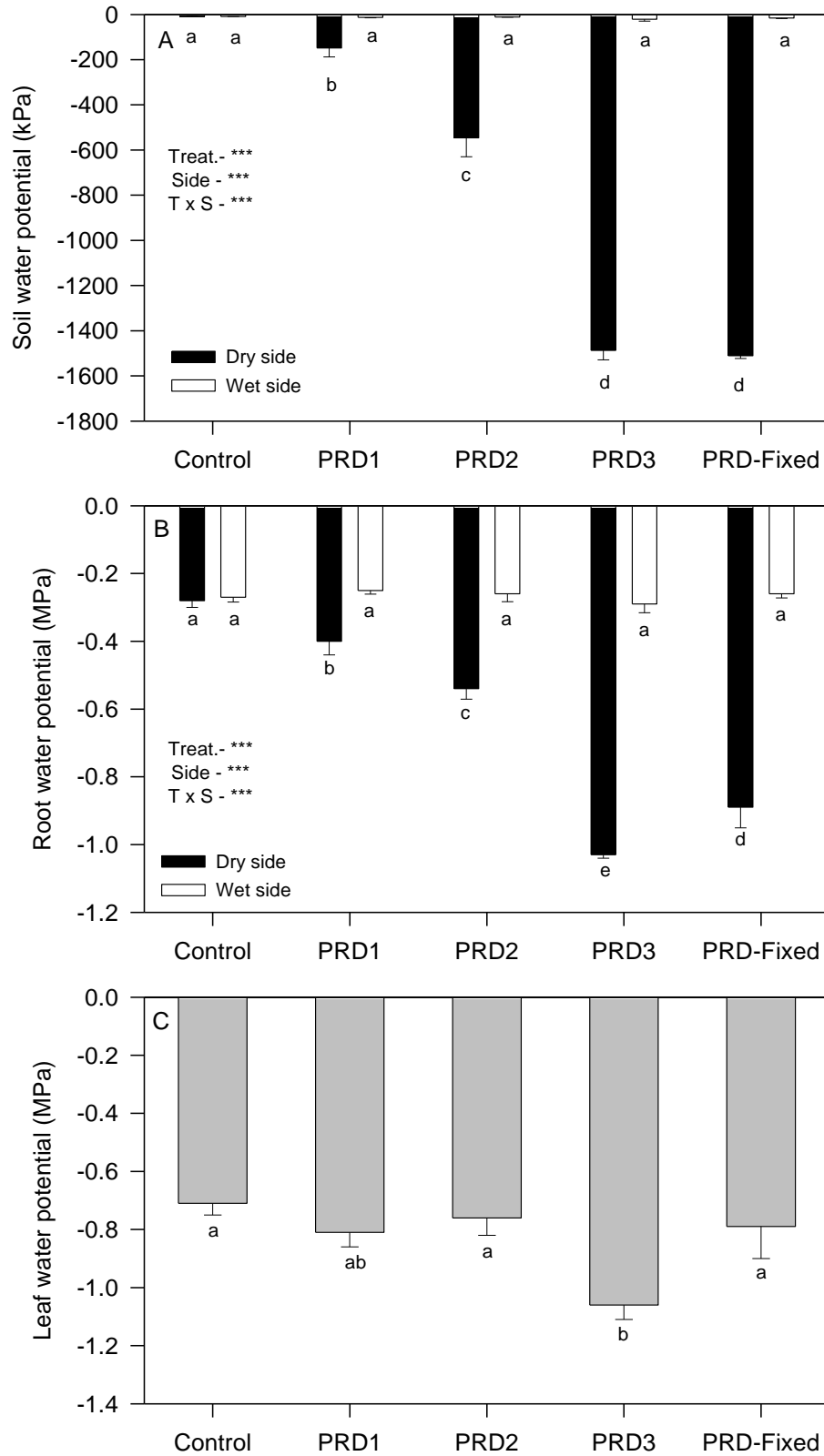
627

628

629

630

631



632
 633
 634

Figure 2

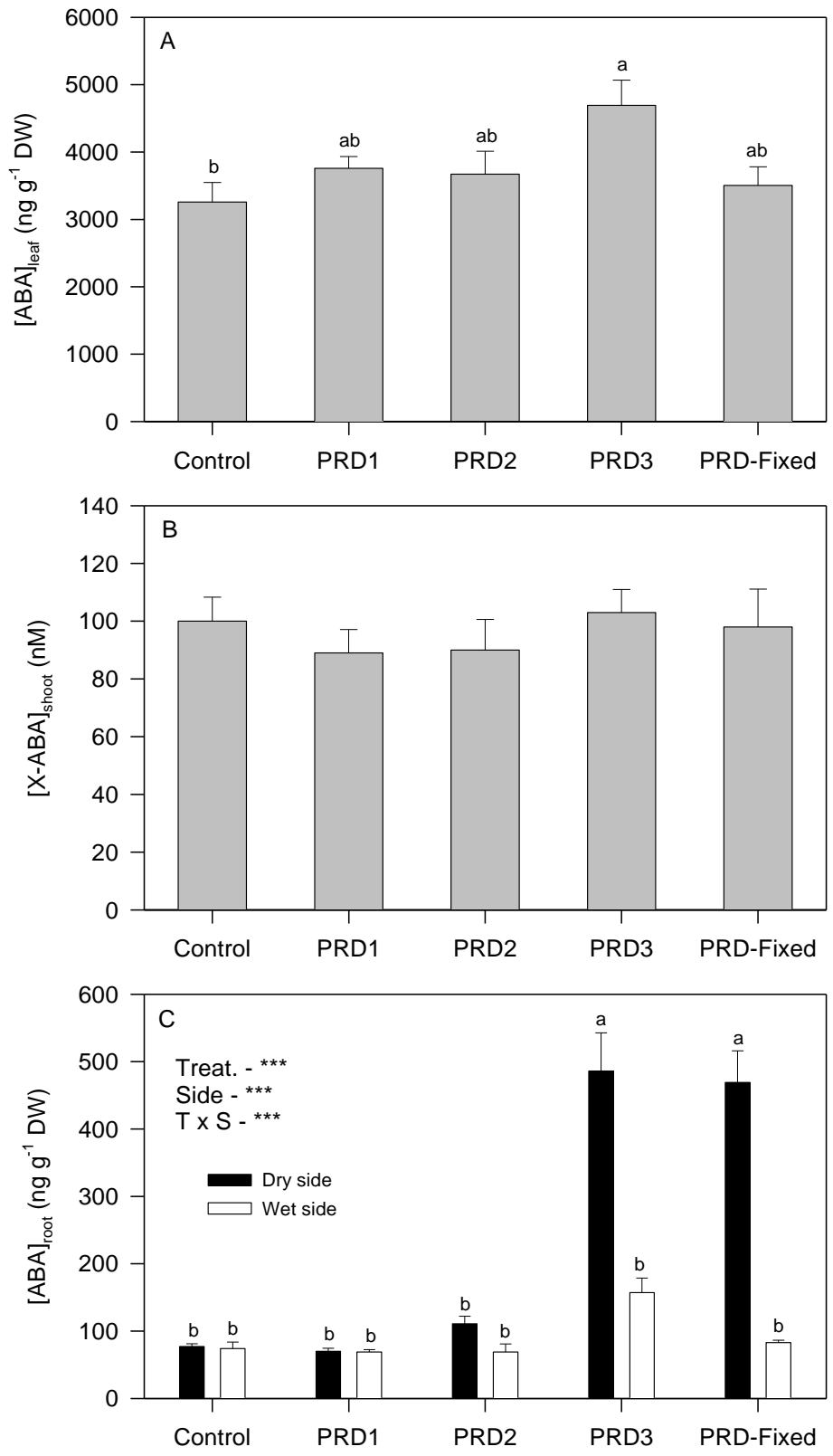


Figure 3

635
636
637

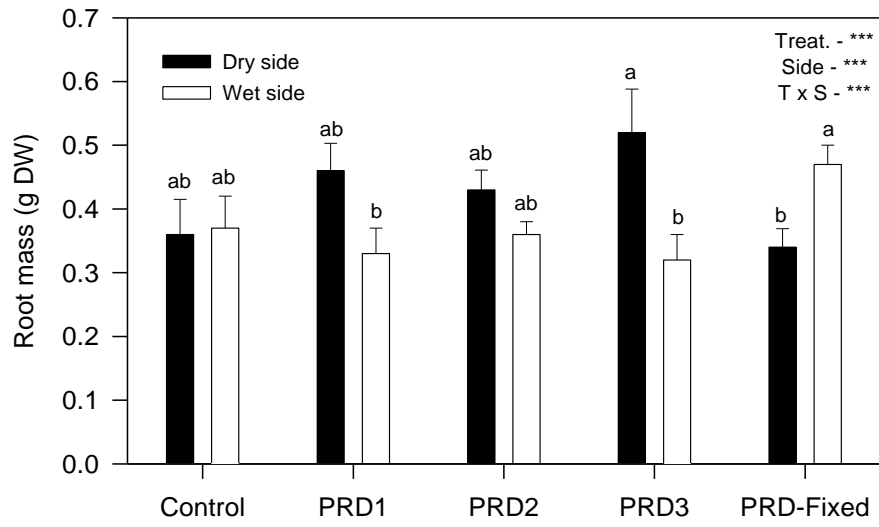


Figure 4

638
639
640

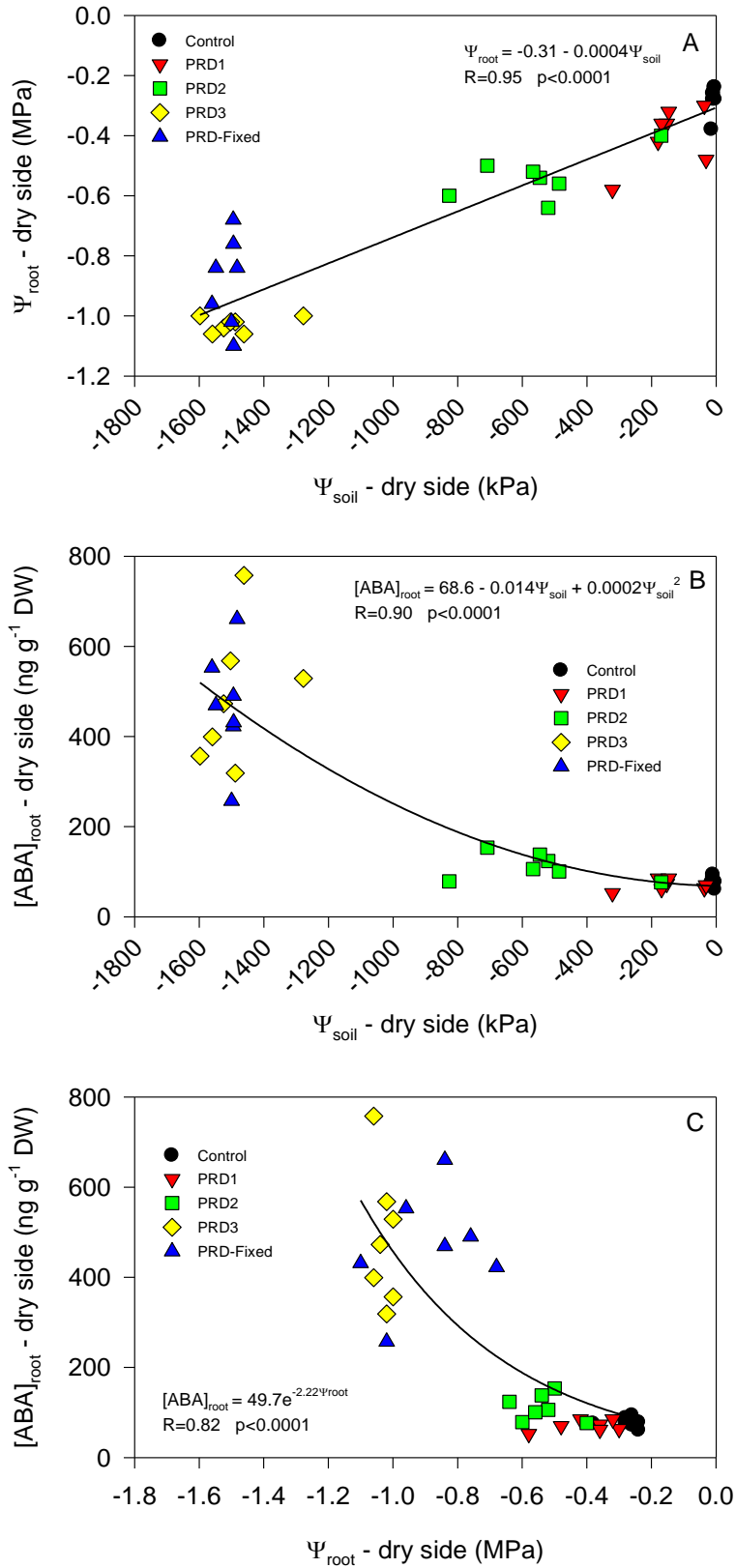


Figure 5

641
642
643

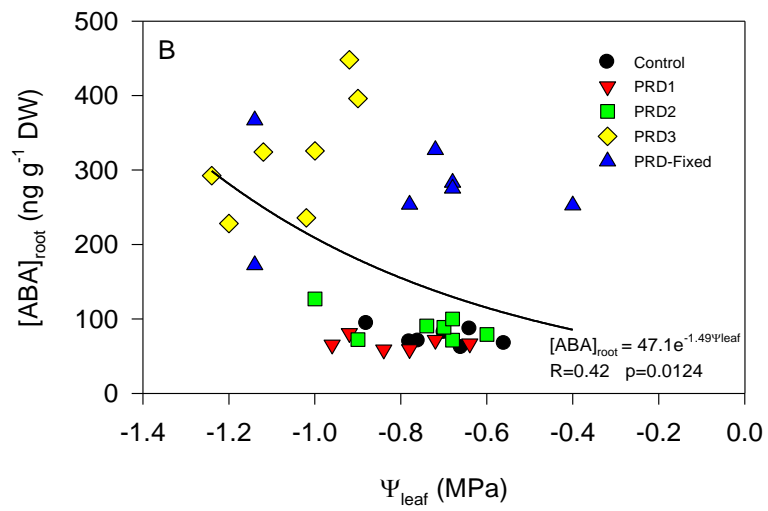
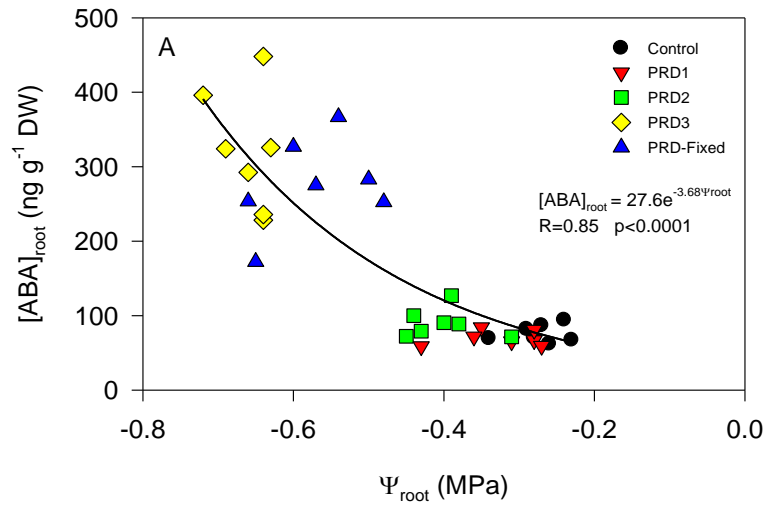
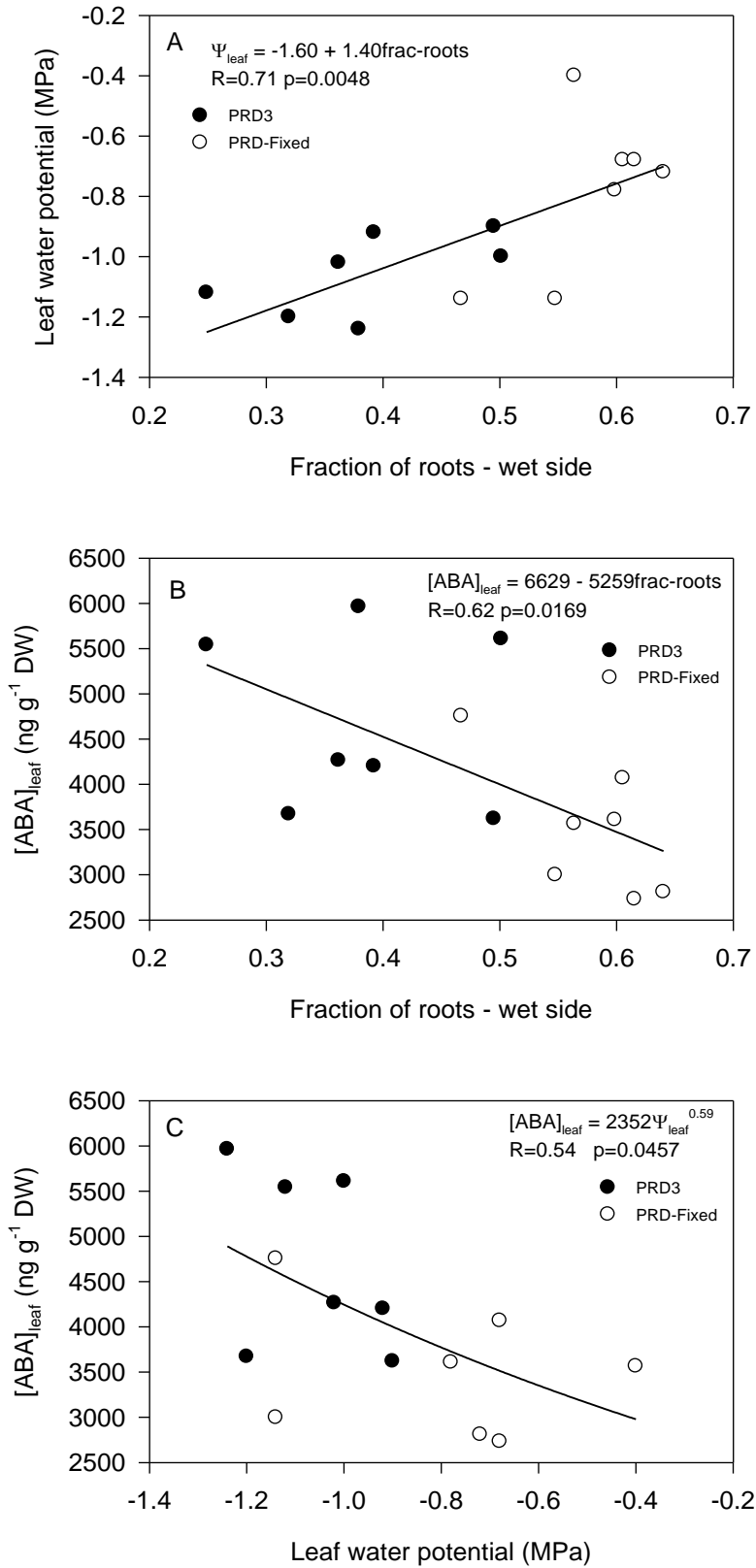


Figure 6

644
645
646
647
648



649
 650
 651
 652
 653

Figure 7

654 Table 1. Leaf gas exchange for each irrigation treatment (Control, PRD1, PRD2, PRD3
 655 and PRD-Fixed) at the end of the experiment. Irrigation treatment consisted in a Control,
 656 and three alternated PRD with different alternation timing: PRD1 (3 days), PRD2 (6
 657 days), PRD3 (12 days) and PRD-Fixed (dry and wet sides of the rootzone were
 658 maintained throughout the experiment). A_{CO_2} CO₂ assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$),
 659 E transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), g_s stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), A/E
 660 instantaneous water use efficiency $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O A/g}_s$ intrinsic water use
 661 efficiency ($\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$).

662

Treatments	A_{CO_2}	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	*	*

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

665

666 Table 2. Root and shoot biomass and leaf area for plants of each irrigation treatment
 667 (Control, PRD1, PRD2, PRD3 and PRD-Fixed) at the end of the experiment Irrigation
 668 treatment consisted in a Control, and three alternated PRD with different alternation
 669 timing: PRD1 (3 days), PRD2 (6 days), PRD3 (12 days) and PRD-Fixed (dry and wet
 670 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	***	*

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

673

674

675