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Deep root growth, ABA adjustments and root water uptake response to soil water deficit in giant reed

Walter Zegada-Lizarazu* and Andrea Monti

Department of Agricultural and Food Sciences, University of Bologna, Viale G. Fanin 44, 40127 Bologna, Italy
*For correspondence. E-mail walter.zegadalizarazu@unibo.it

• **Background and Aims** Giant reed (*Arundo donax* L.) is a deep-rooted crop that can survive prolonged dry periods probably as a result of its capacity to uptake water from below ground, but specific information on the functioning of deep/shallow roots is missing. The objective of this study was to understand the dynamic interrelationships of root water acquisition, canopy water conservation and abscisic acid (ABA) signals from both shallow and deep roots.
• **Methods** In transparent split top–bottom rhizotron systems (1-m-high columns), where hydraulically isolated and independently watered layers were created with the aid of calibrated soil moisture sensors, water uptake trends were monitored. Rooting patterns were traced on the walls of the rhizotrons. Leaf gas exchange was determined using a portable infrared gas analyser. Leaf and root ABA concentrations were monitored.
• **Key Results** Under well-watered conditions, water uptake from both upper and deeper soil layers was similar. Water uptake from deeper soil layers increased gradually by up to 2.2-fold when drought stress was imposed to upper layers compared to the control conditions. Despite the significant increase in water uptake from deeper layers, surface root length density of drought-treated plants remained unchanged, suggesting increased root water uptake efficiency by these roots. However, these adjustments were not sufficient to sustain photosynthesis and therefore biomass accumulation, which was reduced by 42 %. The ABA content in shallower drought-treated roots increased 2.6-fold. This increase closely and positively correlated with foliar ABA concentration, increased intrinsic water use efficiency and leaf water potential (LWP).
• **Conclusions** Giant reed is able to change its water sources depending on water availability and to maximize water uptake efficiency to satisfy canopy evapotranspirative demands. The regulation of deep root functioning and distribution, adjustment of canopy size, and root/foliar synthesized ABA play a central role in controlling LWP and leaf transpiration efficiency.

Key words: ABA, *Arundo donax*, biomass, deep roots, drought, leaf water potential, Mediterranean-type climate, photosynthetic capacity, split-root rhizotron systems, stomatal conductance, underground water.

INTRODUCTION

In regions with Mediterranean-type climates, where water reserves are largely variable in time and space, deep soil water sources constitute an important and more reliable resource for plant survival, growth and development than erratic and scarce rainfall surface water sources. Overall, about 300 billion m³ of groundwater is available each year in Mediterranean countries (Mediterranean Groundwater Working Group, 2007). These sources become even more important in the context of a climate change scenario that foresees a reduction of precipitation and increasing temperatures (Christensen *et al.*, 2007), which in turn will result in increased risks of more severe and frequent drought events. Such risks are even higher in Mediterranean regions where soils are usually shallow and have low water retention capacity. However, deep water sources are available to crop species that have evolved to have a deep rooting habit (a common trait among a wide range of annual and perennial crop species and under a variety of climates) as a strategy to overcome drought (Pierret *et al.*, 2016). Among such species, giant reed (*Arundo donax*) is a deep-rooted perennial rhizomatous

crop that is well adapted to Mediterranean conditions and is of emerging industrial interest as a bioenergy feedstock (Lewandowski *et al.*, 2003; Zegada-Lizarazu *et al.*, 2018). It is characterized by high photosynthetic and carbon accumulation capacity and therefore high biomass yields and positive energy balance (Zegada-Lizarazu *et al.*, 2013, 2018; Cosentino *et al.*, 2016). It also has a low demand for mineral nutrients, making it suitable for marginal lands, and thus avoiding competition for land with food crops (Zegada-Lizarazu *et al.*, 2013).

Although research on the drought tolerance of giant reed has increased recently (Mann *et al.*, 2013; Nackley *et al.*, 2014; Sanchez *et al.*, 2015; Pompeiano *et al.*, 2016; Romero-Munar *et al.*, 2017; Ahrar *et al.*, 2017), its physiological adaptation mechanisms remain poorly documented, especially regarding the functional significance of deep roots. Most of the aforementioned studies focused mainly on canopy responses, whereas the capacity of giant reed to adjust its water uptake strategies through deep root distribution and hormonal signals (indicating a continuum of water flow and adjustment of the behaviour of stomata as a function of below-ground water availability) has so far not been evaluated. Although deep root functioning is

not yet fully understood, recent studies suggest that deep roots could play a central role in ameliorating the effects of water stress in many crop species (Sharp and Davies, 1985; Gewin, 2010; Pierret *et al.*, 2016; Hodgkinson *et al.*, 2017). Hodge (2010) described two main possible strategies adopted by the root systems to supply water to cope with the evapotranspirative demands of the plants under patchy soil moisture conditions: (1) through root proliferation in wetter soil profiles in order to increase the absorption surface capacity (structural response), and (2) through increased water uptake rate by the roots already present in soil layers of higher moisture (functional response). Such details are not known for giant reed, so understanding how root architecture, structure and functioning are modulated in response to different moisture distribution patterns along the soil profile would be useful to gain new insights on its mechanisms related to water stress tolerance and water uptake patterns. It is possible that giant reed has the capacity either to adjust its water uptake strategies and root distribution in relation to water availability, and/or to adjust its stomatal conductance to maintain its physiological functions. However, information on the independent or coordinated functioning of the two mechanism is lacking.

Recent studies have demonstrated that rapid biosynthesis of abscisic acid (ABA) occurs in the leaves in order to respond to changes in leaf-to-air vapour pressure deficits and that leaf-derived ABA can sustain ABA accumulation in roots after long-term water stress (Manzi *et al.*, 2015; McAdam and Brodribb, 2018; Zhang *et al.*, 2018). Moreover, ABA is known to alter the osmotic potential of stomatal guard cells, leading to stomatal closure (Zhang *et al.*, 2001), but to what degree such hormonal signals can alter root architecture and functioning is less well understood. Some studies have indicated that ABA influences the root architecture by favouring the growth of primary roots while at the same time inhibiting the formation of lateral roots; such effects are mainly seen at the meristem and elongation zones (Hodge, 2010; Boursiac *et al.*, 2013). In maize roots it was established that ABA facilitates water uptake as the soil dries out, especially under non-transpiring conditions when the apoplastic transport of water is largely excluded (Hose *et al.*, 2000). However, establishing a direct relationship between the location and timing of ABA biosynthesis is difficult, especially in deep-rooted crops. Therefore, determining whether the roots present in dry topsoil layers can accumulate ABA when deep roots can readily access water, or vice versa, could help to develop more efficient water use strategies or improved drought resistance in giant reed and therefore help in the design of more efficient and sustainable cropping systems. In addition, this provides us with a better understanding of the importance of deep roots for survival of the plant.

Analysis of the hormonal and physiological interactions of giant reed under alternating optimum–drought conditions, however, requires a system set-up that allows us to control independently water sources, water levels, and root distribution through the soil profile, and to monitor canopy growth. Although recent technological advancements now permit the study of deep roots (Sartoni *et al.*, 2015), they remain costly, especially under field conditions in the face of numerous uncontrolled factors. Most current technologies used for the study of roots in the laboratory were developed for small soil volumes and shallow layers

(e.g. transparent window interfaces), and therefore they need to be adapted to the specific needs of studying deep roots. The objective of this study was to understand the dynamic interrelationships between root water acquisition, canopy water conservation and hormonal signals from both shallow and deep roots in a top–bottom split root system of relatively large dimensions (1-m soil columns).

MATERIAL AND METHODS

Experimental set-up

Two independent soil compartments (50 cm heigh) were created in cylindrical split root rhizotrons of 100 cm height and 30 cm diameter (Sartoni *et al.*, 2015) by use of a petroleum jelly/paraffin (ratio 97: 3) waterproof layer (1 cm thick), permeable to roots but not to water, which prevented water movement between upper and deeper soil compartments. To enhance the tensile strength of the impermeable layer a plastic mesh with 5×5-mm openings was incorporated within the waterproof layer. Pressure-compensated drippers (flow rate 2.3 L h^{-1}) were placed about 4 cm below the soil surface and below the waterproof layer in the upper and deeper compartments (see fig. 1 in Sartoni *et al.*, 2015). Drippers in the upper and deeper compartments were connected to independent water supply sources, so that the soil moisture content could be manipulated in each compartment. The water volumes required per treatment and compartment were calculated based on the continuous readings of calibrated soil moisture sensors (TDR; Spectrum waterscout SM-100) that were installed in the centre of each compartment. The sensors were connected to automatic data loggers, which recorded the readings as 6-h averages. Irrigation was applied twice a week (every 3 or 4 d). To allow normal root growth in the dark, the 12 rhizotrons were enclosed in an aluminium structure covered by insulating polycarbonate sheets. The whole system was then placed in a glasshouse under controlled temperature and relative humidity. The average maximum and minimum air temperatures for the experimental period were 32 (±3.6) and 21 (±3.9)°C; and average relative humidity was 55 (±7.8) %. Each rhizotron was filled with sandy loam soil that was homogeneously packed to reach a bulk density of about 1.3 g cm^{-3} in both compartments. The characteristics of the packed soil were: pH 7.7, 0.64 ‰ total N, 23 and 60 mg kg^{-1} assimilable and exchangeable P$_2$O$_5$ and K$_2$O, respectively; and organic matter 1.0 %.

Plant material and treatments

Giant reed (*Arundo donax* L.) rhizomes of the local genotype from northern Italy were transplanted within the rhizotrons on 26 June 2015. To reduce the heterogeneity of the initial planting material, the rhizomes had on average five viable buds and weighed about 70 g. The experiment was arranged in a completely randomized design, with six replicates for each of the two water level treatments imposed: (1) in the drought treatment (DR), soil moisture was adjusted twice a week to 8 % ($\psi_{soil} = -533$ kPa) and 22 % (field capacity; $\psi_{soil} = -12$ kPa) in the upper and deeper compartments, respectively. The drought

stress treatment was started 49 d after transplanting, i.e. when the visible roots had colonized 50 % of the deeper compartment. Before that all plants were grown under non-limiting water (ψ_{soil} = −12 kPa) conditions. (2) In the control treatment (WW), soil moisture was always kept close to field capacity in both upper and deeper compartments.

Biometric, ABA and other physiological measurements

All tillers were tagged in alphabetical order according to their emergence time. At monthly intervals the height, basal diameter and number of green/dry leaves in each tiller per plant were determined. Above-ground biomass was destructively determined at the end of the trial when the plant stems had stopped elongating. Dry mass was determined by oven drying to a constant weight at 105 °C. At harvest, leaf area was measured with a leaf area meter (Li-3000; LI-COR, NB, USA). Water use efficiency (WUE) was defined as the ratio of above-ground dry mass produced to total water used over the growing period.

Midday gas exchange was determined on the youngest fully developed leaves of the median tiller by a portable infrared gas analyser (CIRAS-2; PP-Systems, USA) throughout the experiment. Photosynthesis (P_n), stomatal conductance (g_s), leaf internal CO_2 concentration (C_i) and transpiration (E) were measured under a saturating light of 2000 µmol m^{-2} s^{-1} photosynthetic photon flux density (Rossa *et al.*, 1997) and 390 µmol mol^{-1} CO_2. Photosynthetic response curves (A/c_i curve) between ~50 and 2000 µmol CO_2 mol^{-1} were determined at two late growth stages [days after transplanting (DAT) 111 and 133] when the drought stress plants are well established in the upper compartments. These A/c_i curves were determined at a standard leaf temperature of 25 °C. The Farquhar *et al.* (1980) model of C_3 photosynthesis was used to calculate values of V_{cmax} and J_{max} following Sharkey *et al.* (2007). The effect of patchy stomata was neglected, as suggested by several authors (Buckley *et al.*, 1997; Flexas *et al.*, 2004; Lawlor and Tezara, 2009). These authors indicated that the effect of patchy stomata on A/c_i response analysis is less important than initially thought, especially if g_s is not lower than 30–40 mmol m^{-2} s^{-1} and the plants are not subjected to a fast dehydration, as in our set-up.

Chlorophyll *a* (Chl *a*) fluorescence emissions were measured in dark-adapted leaves for at least 20 min. Chlorophyll fluorescence was measured with a high-time-resolution fluorimeter (Handy PEA, Hansatech, UK) in alternate selected fully developed leaves from the top to the bottom of the plant. Relative leaf water content (RWC) was determined on leaf discs (2 cm^2) taken from the youngest fully expanded leaves. These leaf discs were also used to determine specific leaf area (SLA, leaf area per unit dry mass). Predawn leaf water potential (LWP) was determined on the second fully developed leaf from the top by using a pressure chamber (PMS-670, PMS Instruments CO, USA). A portion of the leaf blade was cut 25 cm below the tip. A 3-cm region of the basipetal portion of the midrib was then exposed by cutting away the blade to make an artificial petiole, which was placed in a single pressure chamber designed to accommodate leaf petioles.

Leaf and root samples were collected for quantification of ABA at the middle and at the end of the drought stress experiment. Leaf ABA content was evaluated on the youngest fully developed and the oldest green leaves of the median tiller at each sampling date (DAT 94 and 137); these values were pooled for the analysis. The DAT 94 root samples were taken with the aid of a hand-held auger through two side openings created for this purpose at approximately the half-height of each compartment. A sample from the whole root biomass sampling was eventually taken at the end of the experiment. Root and leaf samples were immediately freeze dried and finely ground. ABA extraction was done following Puértolas *et al.* (2015) and analysed by a radioimmunoassay (Quarrie *et al.*, 1988).

Root development

A transparent film was placed on a glass window of known surface area in the rhizotrons (2375 cm^2) and the visible roots were drawn on the film with different colours (one for each date). Root drawings were repeated about every third week, starting from the beginning of the drought stress treatment. The drawings were scanned and processed with ImageJ (W. S. Rasband, NIH, Bethesda, MD, USA) to calculate surface root length density (SRLD), defined here as the ratio between traced root length and total sampling area. At harvest, root dry weight in the upper and lower compartments was determined by oven drying at 105 °C to constant weight. Cumulated root water uptake was calculated from daily water balances (logged by the TDR sensors) from planting to harvesting. Then, within specific irrigation intervals (subsurface drip irrigation was applied every 3–4 d throughout the trial) root water uptake efficiency for each compartment was calculated as the ratio between total water uptake in the specific time interval around the corresponding sampling date (DAT 66, 87 and 129) and the total surface root length visible at that moment in the respective soil depth. Whole season water uptake efficiency was defined as the ratio between the total root biomass produced and the total water used in a determined soil compartment.

Statistical analysis

The experiment had a complete randomized design with six replicates. Homoscedasticity of the data was ensured by Bartlett's test prior to analysis of variance (ANOVA). A one-way ANOVA was used to evaluate the effects of drought in the upper compartments on above- and below-ground biomass, V_{cmax} and J_{max}, and ABA. A repeated-measures multifactor ANOVA was used to test the effects of drought over time on the evaluated non-destructive parameters (i.e. plant biometry, gas exchange, water status, rooting patterns and water uptake). When ANOVA revealed significant differences ($P \leq 0.05$), Fisher's least significant difference (LSD) test separated means into statistically different groups. The relationships between ABA and selected morpho-physiological parameters were evaluated using Pearson's correlation test.

RESULTS

Biomass yield and biometric components

The drought stress imposed in upper compartments significantly altered the growth and development patterns of the giant

reed plants. Above-ground dry biomass was significantly ($P \leq$ 0.05) reduced in the drought treatment; dry upper soil layers resulted in about 42 % less biomass than in the well-watered plants (Fig. 1). Likewise the leaf area of the drought-treated plants was significantly reduced by ~40 % ($P \leq 0.05$). However, WUE remained similar between well-watered and drought treatments (~9.4 mm kg^{-1}). Even though SLA and root:shoot ratio were also statistically similar between treatments, drought in the upper compartment resulted in an increase of 25 % in SLA and 34 % in root:shoot ratio. Moreover, root biomass in the upper compartment was reduced by 33 % (Fig. 1). Root biomass was maintained at 17 and 19 g per plant in deeper compartments in the drought and well-watered treatments, respectively (data not shown). In general, the aforementioned biomass accumulation differences were reflected in the plant biometry. The well-watered plants were taller and had higher stem elongation rates than the drought-treated plants; elongation rates were significantly higher in the well-watered plants, especially during the tiller fast elongation phase between 66 and 90 DAT (Fig. 2, $P \leq 0.05$).

Leaf photosynthetic parameters and leaf water potential

Photosynthetic differences between the drought and well-watered plants were evident only towards the end of the experimental period (Fig. 3). Between 111 and 132 DAT, the drought stress treatment resulted in an average reduction of ~21 % and 10 % in P_n, 33 % and 25 % in g_s, and 9 % and 10 % in C_i. On the other hand, intrinsic water use efficiency (i.e. WUE$_i = P_n/g_s$) increased between 26 % and 29 % as the upper soil layer dried out (Fig. 3). In contrast to the gas exchange parameters, the maximum quantum yield of photosystem II (PSII) and predawn leaf water potential of the DR treatment closely followed the observed patterns of the well-water plants; F_v/F_m values (maximum quantum yield of PS II) were constant throughout the growing season with a pooled average across treatments and dates of 0.83. Moreover, LWP values were highest at 111 DAT and lowest at DAT 132, while intermediate values were found earlier (DAT 94; Fig. 4). RWC followed a similar pattern with time but without significant differences between treatments and dates; on average the RWC ranged between 96 and 98 % in the
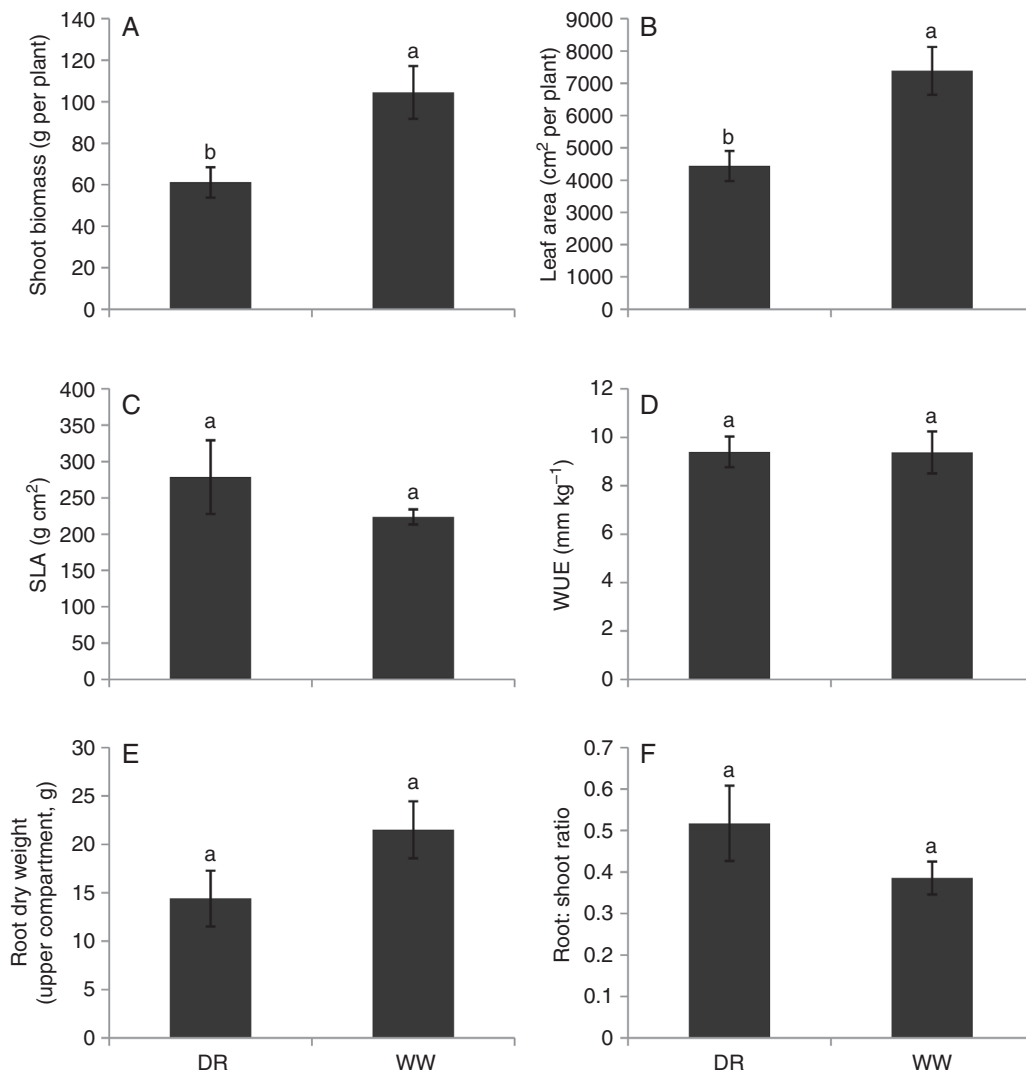

FIG. 1. Above- and below-ground biomass components as affected by a drought period (DR) in the upper compartment of the top–bottom split root rhizotron system in comparison to always well-watered (WW; either in the upper or the deeper compartments) plants. Different letters indicate significant differences ($P \leq$ 0.05) among water treatments. Data are means of six plants per treatment ± s.e.
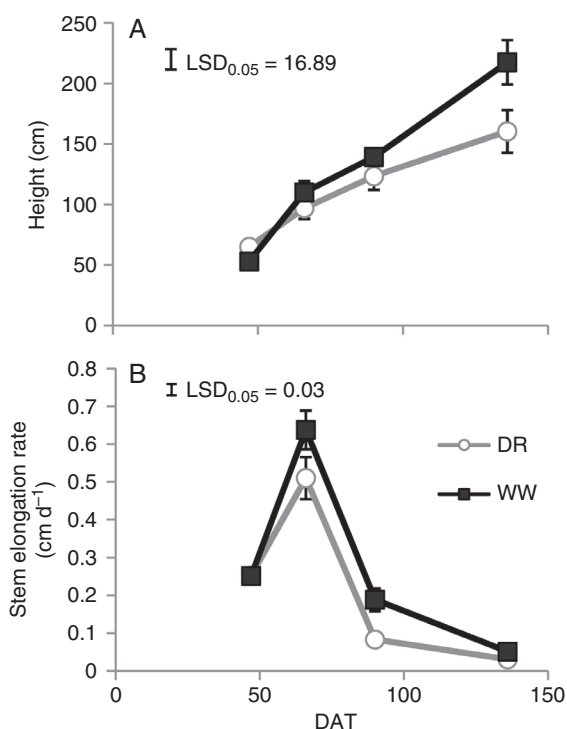
FIG. 2. Plant height and stem elongation rate through a drought period (DR) in the upper compartment of the top–bottom split root rhizotron system in comparison with always well-watered (WW; either in the upper or the deeper compartments) plants. LSD bars indicate the positive and negative critical values for comparisons (*P* ≤ 0.05). Data are means of six plants per treatment ± s.e. DAT, days after transplanting.

DR and WW treatments, respectively (data not shown). In addition, at the time when clear differences between treatments in gas exchange were observed, V_{cmax} and J_{max} were not affected by the drought treatment (DAT 111 and 133; Fig. 5).

Rooting patterns, water uptake and water uptake efficiency

When drought stress was imposed to upper soil layers the main water source for the plant became the deeper compartments, and water uptake from deeper soil layers was on average (across the growing season) 69 % higher than in the control treatment. Such water uptake differences were more noticeable from DAT 67 to DAT 109. When both soil layers (upper and deeper) were well-watered, the water was withdrawn from both compartments with close to 55 % of the water supply coming from the upper compartment (Fig. 6). In close agreement with the water uptake patterns, a gradual increasing difference SRLD was observed between control and drought-treated upper soil layers (~62 % less SRLD at the end of the trial) as the stress period advanced. In deeper compartments (well-watered), however, the SRLD values were always the same in both treatments (Fig. 6).

The significant increase in water uptake from deep layers without an increase in visible SRLD suggests an increased efficiency in root water uptake of plants subjected to drought in upper layers. In fact, in specific irrigation cycles, especially at early developmental stages (DAT 66 and 87), when young roots are more active, root water uptake efficiency was significantly increased from deeper soils due to drought in the upper layers. In both irrigation cycles, maximum water uptake efficiency
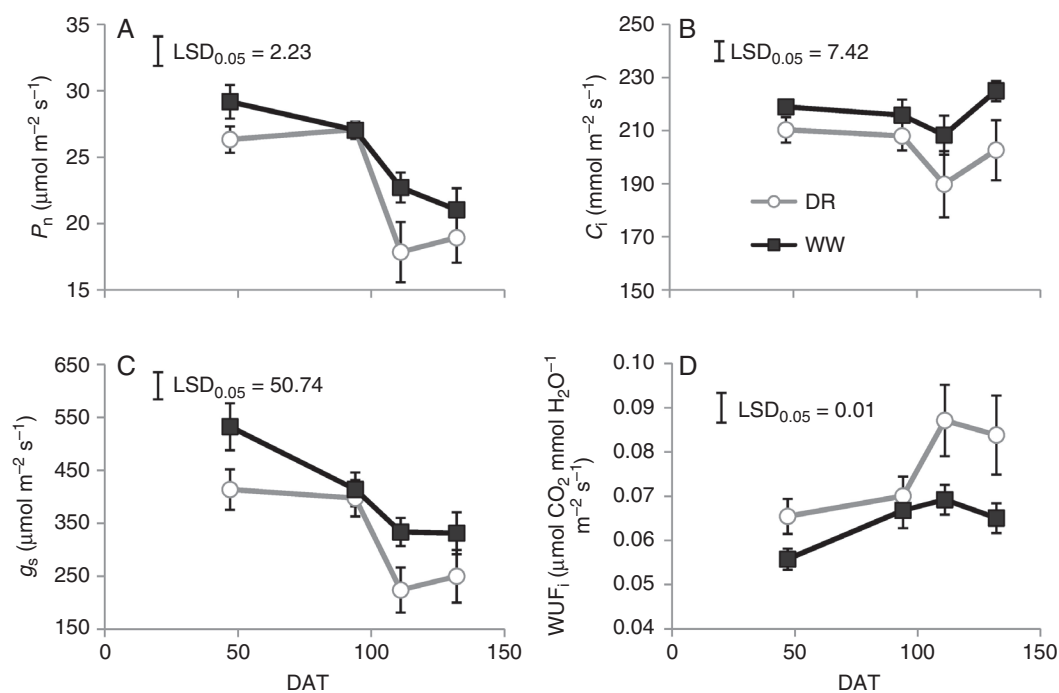

FIG. 3. Photosynthesis (P_n), stomatal conductance (g_s), leaf internal CO_2 concentration (C_i) and intrinsic water use efficiency (WUE$_i$ = P_n/g_s) through a drought period (DR) in the upper compartment of the top–bottom split root rhizotron system in comparison with always well-watered (WW; either in the upper or in the deeper compartments) plants. LSD bars indicate the positive and negative critical values for comparisons (*P* ≤ 0.05). Data are means of six plants per treatment ± s.e. DAT, days after transplanting.
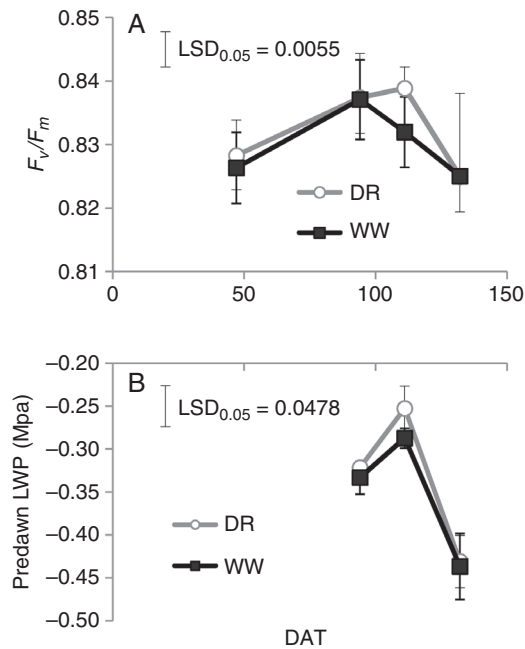
FIG. 4. Photosynthetic vitality (i.e. maximum quantum yield; F_v/F_m) and pre-dawn leaf water potential (LWP) through a drought-period (DR) in the upper compartment of the top–bottom split root rhizotron system in comparison with always well-watered (WW; either in the upper or in the deeper compartments) plants. LSD bars indicate the positive and negative critical values for comparisons ($P \leq 0.05$). Data are means of six plants per treatment ± s.e. DAT, days after transplanting.
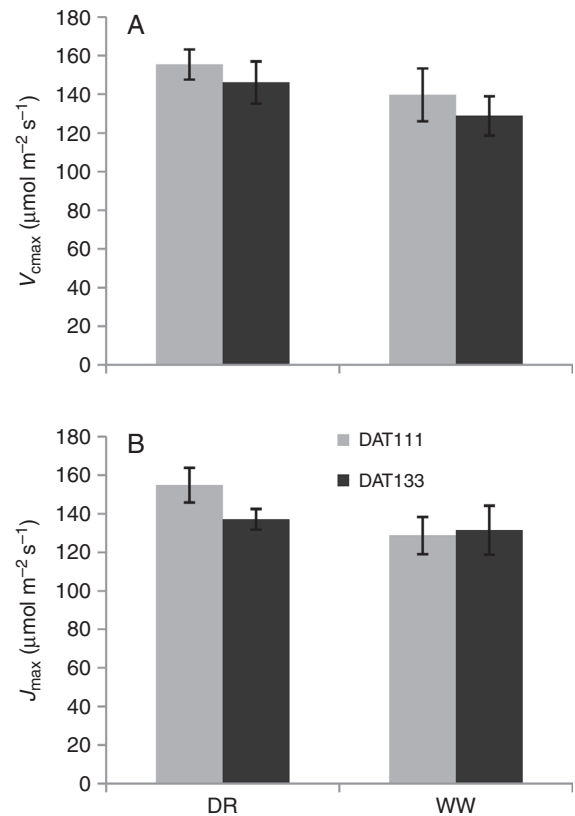

FIG. 5. Maximum rate of carboxylation (V_{cmax}) and electron transport (J_{max}) as affected by a drought period (DR) in the upper compartment of the top–bottom split root rhizotron system in comparison with always well-watered (WW; either in the upper or in the deeper compartments) plants. Data are means of six plants per treatment ± s.e.

from the deeper compartments occurred between 48 and 72 h after irrigation. Water uptake efficiency was almost constant throughout the irrigation cycles in the upper dry soil layers. At DAT 129, however, root water uptake efficiency patterns were less clear, with very similar values between treatments at both upper and lower compartments (Fig. 7). As a confirmation of the aforementioned patterns induced by drought in upper soil layers, root water uptake efficiency from deeper soil layers for the whole growth period (determined in terms of total root biomass produced and volume of water used) was ~50 % higher than in the corresponding layer in the control treatment. The opposite pattern between the drought and well-watered treatments was seen in the upper compartments (Fig. 7).

ABA

Levels of foliar ABA (averaged across the sampling dates) were 13 % higher in the DR treatment than in the control, but this difference was not statistically significant ($P \leq 0.05$). Moreover at earlier growth stages (DAT 94) the biosynthesis of ABA in the roots, at either upper or deeper soil layers, showed an average increase of ~30 % in the DR treatment compared with the control, but these differences were not statistically significant. On the other hand, at 137 DAT the ABA in the roots of the upper drought-treated layers was 2.6 times higher than in the corresponding layer under well-watered conditions (Fig. 8; $P \leq 0.05$). This increased signal was closely and positively correlated with the foliar ABA concentration, WUE_i and pre-dawn

leaf water potential, in correspondence with the overall lower photosynthetic and stomatal conductance values in the drought treatment. At the same time, ABA content from shallower roots was negatively correlated with SRLD, and water uptake efficiency and soil water potential from top soil layers (Table 1).

DISCUSSION

The regulation of water uptake from deep, wet soil layers may help some species, under specific hydrological conditions, to more effectively respond to water deficits in topsoil layers than is possible through the regulation of leaf transpiration and/or the adjustment of canopy size. One of these hydrological conditions is commonly seen in some arid and semi-arid areas with Mediterranean climates where a high water table is usually present.

In the present study, we evaluated the ability of giant reed to adjust its water uptake strategies through root distribution and hormonal signals and/or adjust its stomatal conductance in response to below-ground water availability. We clarified whether ABA signalling and deep roots play an important role in the response of giant reed to drought. Such responses are not well documented among perennial grasses and are largely variable among annual crop species. In inbred lines of maize with variable drought tolerance, lines with a deeper root system had
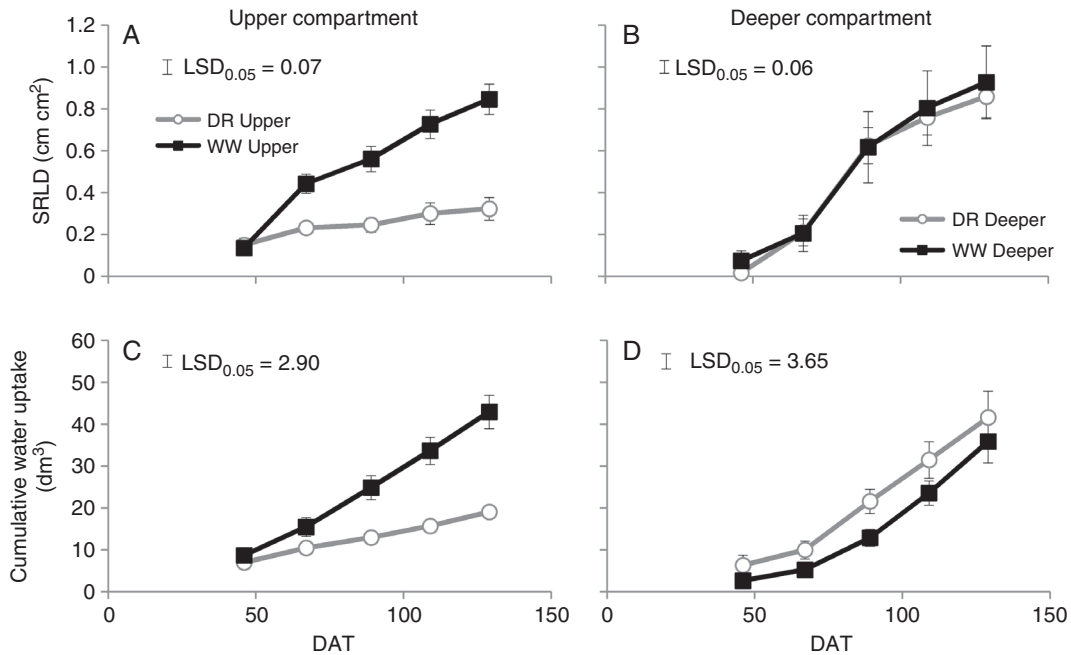
Fig. 6. Surface root length density (SRLD) and cumulative water uptake computed throughout the experimental period from daily water balances (as determined from continuous readings taken by soil moisture sensors). The water level treatments in the top–bottom split root rhizotron system consisted of the following combinations: (i) drought-treated upper (DR Upper) + well-watered deeper (WW Deeper) compartments; and (ii) well-watered upper (WW Upper) and deeper (WW Deeper) compartments. LSD bars indicate the positive and negative critical values for comparisons ($P \leq 0.05$). Data are means of six plants per treatment ± s.e. DAT, days after transplanting.
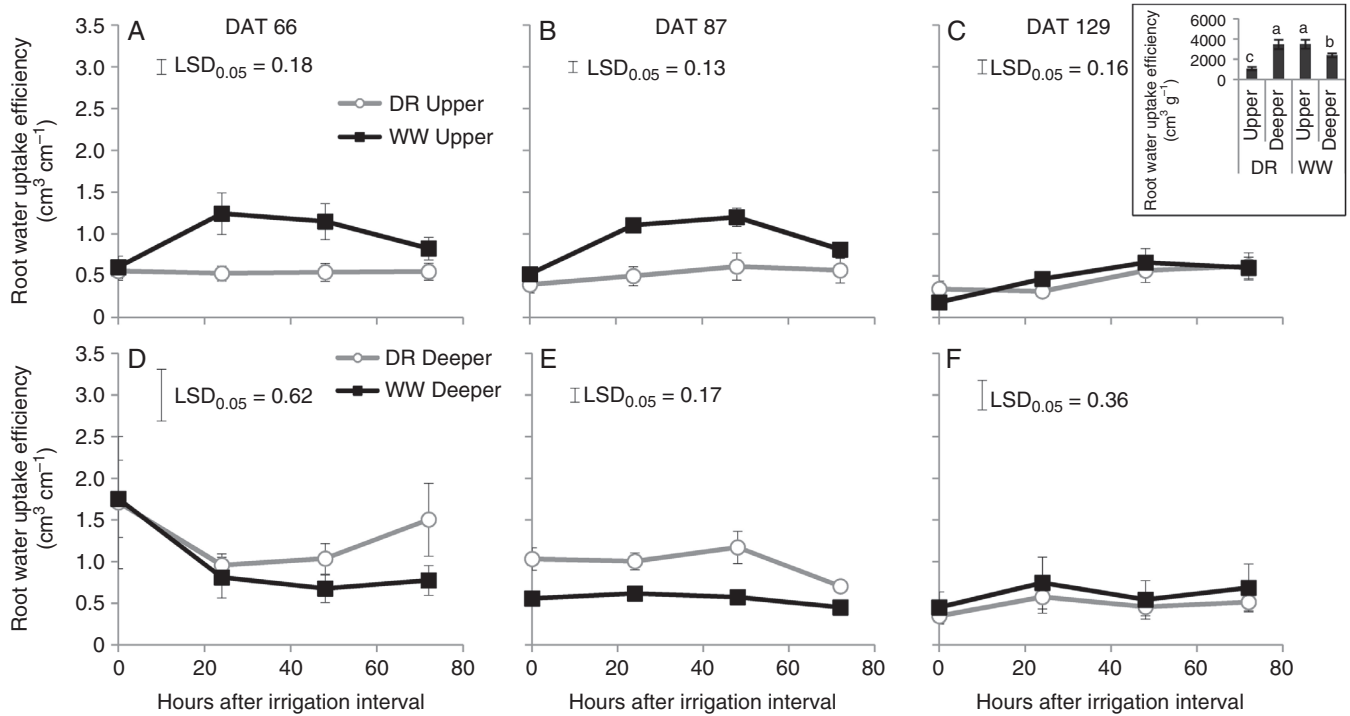

Fig. 7. Root water uptake efficiency during specific 3-d irrigation intervals at DAT 66, 87 and 129 in the upper and deeper compartments of the top–bottom split root rhizotron system: the water level treatments consisted of the following combinations: (i) drought-treated upper (DR Upper) + well-watered deeper (WW Deeper) compartments; and (ii) well-watered upper (WW Upper) and deeper (WW Deeper) compartments. Root water uptake efficiency was calculated as the ratio between total water uptake in the specific time interval and the total surface root length visible at that moment in the respective soil compartment. LSD bars indicate the positive and negative critical values for comparisons ($P \leq 0.05$). Data are means of six plants per treatment ± s.e. DAT, days after transplanting. Inset shows the root water uptake efficiency for the whole growing season based on total root biomass (g) and total water used (cm^3) in the corresponding compartment. Different letters indicate significant differences ($P \leq 0.05$) among water treatments.
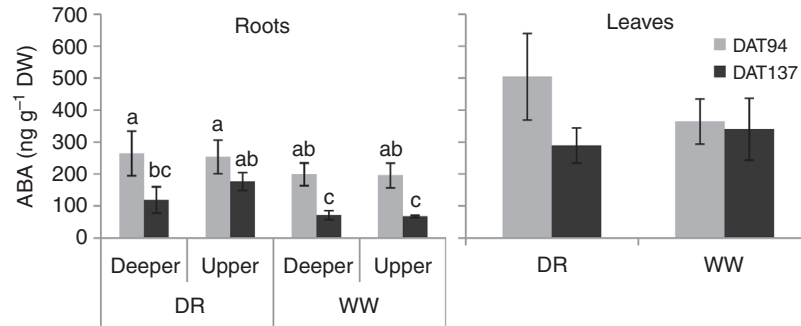
Fig. 8. ABA in the root and leaves as affected by a drought period (DR) in the upper compartment of the top–bottom split root rhizotron system in comparison with always well-watered (WW; either in the upper or in the deeper compartments) plants. Different letters indicate significant differences between water treatments at the corresponding DAT ($P \leq 0.05$) and are shown only where significant differences were found. Data are means of six plants per treatment ± s.e. DAT, days after transplanting.

TABLE 1. Pearson correlations between ABA content from dry topsoil layers (upper compartment of the split-root rhizotron system) and selected morpho-physiological parameters

	ABA root (upper compartment)	
ABA leaf	0.59	*
P_n	0.24	n.s.
g_s	0.10	n.s.
F_v/F_m	0.04	n.s.
LWP (pre-dawn)	0.53	*
WUE_i	0.42	*
$SRLD^\dagger$	−0.50	*
Root water uptake efficiency†	−0.58	*
Soil water potential†	−0.42	*
Soil water potential‡	−0.55	*

*Significance of the Pearson correlation coefficients at $P \leq 0.05$.

†Data from the upper compartment.

‡Data from the deeper compartment.

the ability to take up more water from deep layers, and thus to sustain stomatal conductance under water-limited conditions (Hund *et al.*, 2009). Similarly, giant reed is able to change its water sources depending on availability and to maximize water supply in an attempt to compensate for the potential imbalances between root water uptake and leaf transpiration caused by drying upper soil layers (Figs 3, 6 and 7).

The contribution of deep roots to the whole plant water needs was of greater value when the upper soil matrix potential and soil hydraulic conductivity decreased. Some studies using systems in which the entire soil column was allowed to dry also showed that upper soil drying allowed greater water uptake from deep roots, but with a substantial redistribution of root towards deep layers (Sharp and Davies, 1985; Zhang and Davies, 1989). Under the present study conditions (hydraulically isolated/independently watered layers) water uptake from deeper wet layers represented 69 % of the plant water needs but without root redistribution.

Under arid and/or semi-arid conditions, where giant reed is normally grown, topsoil layers may contribute marginally to total root water uptake. Although the exact mechanism was not explained, Lobet *et al.* (2014) suggested that restricted uptake from dry layers may further limit the contribution of wetter areas. In our case, however, this seems not be happening as the

cumulative water uptake from deeper wet layers was higher (specially from DAT 46 to 109) than the corresponding uptake in well-watered plants (Fig. 6), although such increased supply from the deeper layer was not enough to maintain biomass productivity (Fig. 1). Such increased water uptake from deeper layers could be, as proposed by Wasson *et al.* (2012), due to increased radial (mainly apoplastic transport) conductivity in deep roots, resulting in the observed increased water uptake capacity per unit length and/or mass of deep roots (Fig. 7). In fact, maintenance of midday leaf RWC was verified in the present study. Moreover, Haworth *et al.* (2017) found that a giant reed genotype from Morocco grown in small pots showed increased mean stem xylem vessel area as a response to drought and from that they derived an increased theoretical hydraulic conductivity.

Changes in root hydraulic conductivity could be related to increased ABA synthesized in roots subjected to stress conditions (Jeschke *et al.*, 1997; Hose *et al.*, 2000; Steudle, 2000; Aroca *et al.*, 2006; Hodge, 2010) which may at the same time induce transcription factors involved in the gene expression of aquaporins (Shinozaki *et al.*, 1998; Aroca *et al.*, 2006), the water channel proteins that facilitate intense water flow across root tissues as observed in the present study. We found that the relationship between ABA and water uptake efficiency in the upper soil compartments was significant but negative (Table 1) while water uptake and water uptake efficiency from deeper layers was increased. Therefore, we speculate that the deep root system (i.e. basipetal transport through the phloem) is one of the sinks for the ABA synthesized due to drought either in the roots or in the leaves (as suggested by Manzi *et al.*, 2015). This may have led to improved hydraulic conductivity, especially at the apoplastic level, where the effect of ABA is more pronounced (Hose *et al.*, 2000). Moreover, the reduced SRLD in upper soil layers leads to more effective allocation of energy and carbohydrates in deeper layers (in terms that the existing roots receive all transient assimilates), thus allowing deeper roots to function more efficiently (Figs 6 and 7). Wasson *et al.* (2012) suggested reduced root length density in the topsoil as a strategy to improve soil water extraction from deep layers in water-limited environments.

When the aforementioned strategies to improve deep root water uptake and efficiency are not sufficient to meet the potential evapotranspirative demands of the plant, giant reed seems

to activate additional protective mechanisms against dehydration at either root and/or canopy levels. In addition to the seemingly increased hydraulic conductivity of deep roots, the increased ABA synthesized by drought-treated leaves and/or roots may have induced stomatal closure (Hund *et al.*, 2009; Manzi *et al.*, 2015; McAdam and Brodribb, 2018; Zhang *et al.*, 2018) with the consequently lower rates of photosynthesis and therefore decreased biomass productivity. However, the temporal synchrony of stomatal closure and ABA accumulation is not that clear in our study (Figs 3 and 8) probably because as described by some authors (Aroca *et al.*, 2006; McAdam and Brodribb, 2018) guard cell turgor is autonomously regulated and with largely variable responses to a wide range of environmental stimuli with time. However, the significant ABA accumulation in the upper roots in response to soil drying was directly and significantly related to foliar ABA accumulation (Table 1). However, foliar ABA levels under well-watered and drought conditions were statistically similar at either sampling date (Fig. 8), suggesting a close interrelationship between ABA accumulation and root distribution. Moreover, Manzi *et al.* (2015) demonstrated that leaf-derived ABA can sustain ABA accumulation in roots after long periods of water stress. In the present study, however, it is difficult to discern between the cause–effect of root placement and hormonal signals when variable proportions of the total root mass are located in contrasting soil moisture layers (e.g. if the leaf-derived ABA can allow deep rooting). In that regard, Puértolas *et al.* (2015) indicated that drought-induced ABA accumulation in roots depends on their position within the soil profile and on their age. In addition, Martin-Vertedor and Dodd (2011) indicated that leaf ABA concentrations were higher in plants with more roots in drying portions of the soil.

ABA may have also played a central role in controlling the leaf water potential ($r = 0.53$) and WUE$_i$ ($r = 0.42$) (Figs 1, 3, 4 and 8, Table 1). Under stressful conditions, in fact, the main concern is the amount of dry matter produced per unit of water used rather than the total biomass yield (Zegada-Lizarazu and Iijima, 2005). Yang *et al.* (2016) demonstrated in Arabidopsis that the activation of ABA signalling due to drought mediated the plant acclimation responses through reduced transpiration and maintenance of CO_2 assimilation (possibly by means of increased CO_2 gradients across the stomatal pore). The sustained WUE and increased WUE$_i$ (Figs 1 and 3) under drought stress could then be seen as an indication of the adaptability of giant reed to adverse conditions and its capacity to prevent damage due to soil dehydration. Moreover, Tardieu and Davies (1992) suggested that the sensitivity of stomata to ABA is increased under low LWP conditions. The lowest LWP values we found were at DAT 137 (Fig. 4). Moreover at that growth stage ABA concentration in drying roots was the highest (Fig. 8) and highly correlated with ABA accumulation in the leaves ($r = 0.59$; Table 1), and with increased WUE$_i$ ($r = 0.42$; Table 1). Such increased WUE$_i$ was determined by a relatively larger decrease in g_s than in P_n, suggesting that ABA synthesized either in the leaves or in the roots induces guard cell adjustments and stomatal closure. The ABA synthesized in the leaves probably plays a major role in stomatal regulation, as recently suggested to occur in tomato plants by Manzi *et al.* (2015). However, it remains to be determined if ABA produced in the leaves or in the roots is the main

contributor to stomatal closure in this species. Regardless, the ABA accumulation may have a complementary role to LWP in the regulation of stomatal behaviour (Christmann *et al.*, 2007). Our results also suggested that the influence of ABA on photosynthetic characteristics may not have a direct effect on Rubisco activity, as at both sampling dates (DAT 111 and 133) no significant differences were observed in V_{cmax} and J_{max} between drought-treated and control plants (Fig. 5). Similarly, a study looking at carbon assimilation and stomatal function in ABA-deficient mutant tomato plants found no effect of ABA on plant photosynthetic capacity (Bradford *et al.*, 1983). Therefore, the decreased photosynthesis of drought-treated plants can be ascribed to the availability of CO_2 and not to decreased Rubisco activity. Flexas *et al.* (2006) indicated that the Rubisco activity of soybean and tobacco remains unchanged as long as g_s remains above 100 mmol m^{-2} s^{-1}. In our case, the mean stomatal conductance of the drought-treated plants was always above 200 mmol m^{-2} s^{-1}.

The adaptability of giant reed to drying upper soil layers is also reflected in the reduced leaf area and sustained SLA and root to shoot ratio (Fig. 1), which can be seen as important characteristics in reducing leaf water loss to avoid interruption of the water flow continuum, supplied almost exclusively by the deep roots (Fig. 6). Accordingly, we observed a slowing of the stem elongation rate (Fig. 2). Although stem water potential was not measured, these results suggest that water uptake by deep roots could not sustain sufficient root hydraulic conductivity to maintain the water flow continuum for a normal cell elongation rate. Such a plastic response suggests that the root biomass was proportionally reduced accordingly to the reduction in shoot biomass. Maintaining an equilibrium root to shoot ratio (in this case with the deep roots) instead of developing a larger root system (Fig. 6) could be seen as as a means to develop a better water uptake capacity, possibly through improved hydraulic conductivity and therefore sustained plant water status (Hernández *et al.*, 2009; Haworth *et al.*, 2017; Fig. 4). This pattern of response has also been reported in other species (Zegada-Lizarazu and Iijima, 2005). Therefore, the reduction in leaf area together with the sustained SLA and root to shoot ratio may help to maintain WUE, which may render giant reed resistant to drought.

CONCLUSION

The direct and indirect relationships found between water uptake strategies, rooting patterns, hormonal content, stomatal adjustments and photosynthetic capacity could influence the survival, adaptation and growth of giant reed in arid and semi-arid regions with a Mediterranean climate. Given the prevailing water shortage in such areas, it is evident that deep rooting, stomatal control, hormonal signalling either from roots to shots or vice versa play a the major role in giant reed survival and adaptation strategies to such conditions. Giant reed was able to change its water sources depending on availability and to maximize its water supply efficiency in an attempt to compensate for potential imbalances between root water uptake and leaf transpiration caused by drying upper soil layers. In fact, the contribution of deep roots to the whole plant water needs was greater when the topsoil matrix potential and soil hydraulic

conductivity decreased. Moreover, ABA accumulation in the upper roots in response to soil drying was directly and significantly related to foliar ABA accumulation and to increased water uptake efficiency, suggesting that ABA synthesized by drought-treated leaves and/or roots may have played a central role in controlling leaf water potential and WUEi, but may not had have a direct effect on Rubisco activity. The adaptability of giant reed to drying upper soil layers is also reflected in the reduced leaf area and sustained specific leaf area and deep root to shoot ratio (Fig. 9). These plant responses may have significant implications for selection of the areas where giant reed can be cultivated (i.e. arid or semi-arid marginal areas with a shallow water table). They also have implications for the implementation of some agronomic management practices (irrigation, planting density, etc.) aimed to develop more efficient water use strategies, as the main concern in arid and semi-arid

Mediterranean climates is production per unit of applied water rather than absolute production, so more efficient and sustainable cropping systems could be developed.

FIG. 9. Summary figure describing the main findings of the study together with a schematic illustration of how the two root compartments work together.

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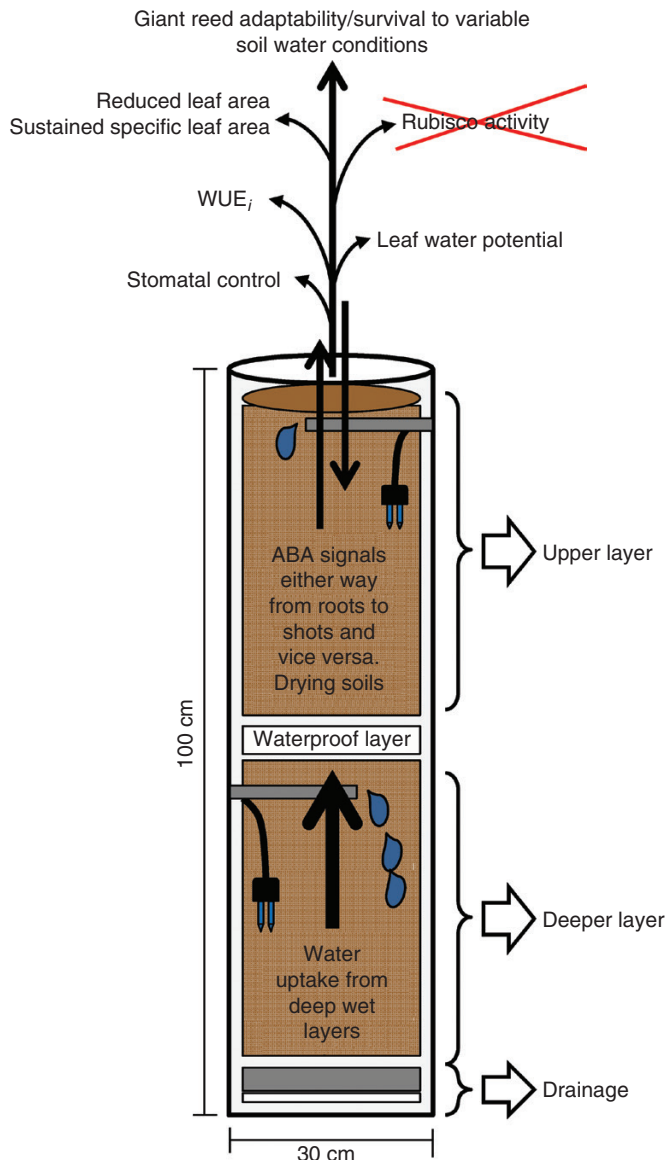
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