Physiological responses to alternative flooding and drought stress episodes in two willow (Salix spp.) clones.

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Physiological responses to alternative flooding and drought stress episodes in two willow (*Salix* spp.) clones.

Guillermo N. Doffo¹, Silvia E. Monteoliva², María E. Rodríguez³, Virginia M.C. Luquez ⁴.

Instituto de Fisiología Vegetal (INFIVE), UNLP - CONICET, FCAyF UNLP, CC 327, 1900 La Plata, Argentina.

¹: guilledoffo@gmail.com
²: smonteoliva@yahoo.com.ar
³: emiliar21@gmail.com
⁴: Author for correspondence. vluquez@agro.unlp.edu.ar. Tel: +54-221-423-6618.

FAX: +54-221-423-3698.
Abstract

Climate change will increase the occurrence of flash floods as a consequence of extreme rain events, creating alternate periods of drought and flooding during the growing season. We analyzed the responses of two willow clones with contrasting responses to flooding (clone B: *Salix matsudana* x *Salix alba* hybrid; clone Y: *Salix alba*) to different combinations of stress treatments: continuous flooding or drought for six weeks, or cyclic treatments of two weeks of stress separated by two weeks of watering at field capacity. Drought reduced growth, stomatal conductance and total leaf area in both clones, but flooding did not. Flooding reduced the root/shoot ratio in both clones. The hydraulic conductivity of the main stem was significantly reduced by drought only in clone Y. The area of the vessels was decreased by both drought and flooding, but the number was increased only by drought. The occurrence of drought before flooding reduced the vessel area, but the opposite treatment did not. An episode of drought after one of flooding is more stressful than the opposite situation, especially for clone Y that could not adjust its water transport capacity during the drought period.

**Key words:** water stress, hydraulic conductivity, vessels, stomatal conductance, root to shoot ratio
Introduction

Climate change will increase the occurrence of flooding episodes in several areas of the world (Kreuswieser and Rennenberg 2014, Cavalcanti et al. 2015). The riparian zones are particularly susceptible to an increased risk of flooding and drought under climate change, causing modifications in plant community composition and species richness (Garssen et al. 2014, Garssen et al. 2015). Willows (*Salix* spp.) can colonize and grow successfully on areas periodically disturbed by flooding, like floodplains (Karrenberg et al. 2002) and tidal wetlands (Markus-Michalczzyk et al. 2016 a). In consequence, willows are natural candidates for developing forest plantations in flood prone areas that are marginal for agriculture. There is an increased demand for forest-derived products for different uses, including the supply of biomass for energy production. To fulfill this demand, willow plantations are being developed in areas that can experience alternate short periods of drought and flash flooding during the growing season. To improve the success of plantations in disturbed areas, it is necessary to gain a better understanding of the physiological responses of willows growing under these particular combinations of environmental stresses.

Willow responses to drought and flooding have been previously analyzed separately, and the responses are different according to the genotype and the duration of the stress episode (Li et al 2004, Wikberg and Ögren 2004). It has been shown that willow responses to continuous drought are different from those under cyclic drought (Bonosi et al. 2010). Meanwhile, the responses of different tree species to a flooding event may vary if they suffered from a previous episode of flooding. In poplars, the occurrence of a pre-conditioning flooding period improved waterlogging tolerance (Bejaoui et al. 2012). In willows, the combination of drought and flooding has been addressed to a lesser extent than both stresses separately (Nakai et al. 2010, Nakai and Kisanuki 2011). Some morphological responses to drought are the opposite of those to flooding. For instance, drought increases the root/shoot ratio while flooding reduces it (Kozlowski 1997, Markus-Michalczzyk et al. 2016 b). In this context, a previous episode of flooding that reduced root biomass could be detrimental for a plant experiencing drought later. It has been shown that drought
susceptibility in willows is related to their vulnerability to cavitation (Wikberg and Ögren 2007, Savage and Cavender-Bares 2011, Ogasa et al. 2013). Flooding can alter water transport in tropical trees (Herrera et al. 2008), and reduce vessel size and hydraulic conductivity in Quercus robur (Copini et al. 2016). But nothing is known about the effects of a combination of both stresses on the hydraulic conductivity of willows.

The aim of this work is to analyze the responses of two willow clones to drought, flooding and the alternation of these stresses. In a previous work, we identified willow genotypes with contrasting tolerance to flooding (Cerrillo et al. 2013). We expect that these clones will modify their physiology in different ways to acclimate to drought, flooding and the alternative occurrence of these stresses.

We hypothesize that: 1 – The clones will differ in their tolerance to the alternation of drought and flooding stress; and 2 – The order of occurrence of drought and flooding episodes will modify the growth, water transport capacity, xylem anatomy, gas exchange and leaf area dynamics of willows in different ways.

Materials and Methods

Plant material and growth conditions

The clones used in this work were obtained in a breeding program from INTA (Instituto Nacional de Tecnología Agropecuaria, Argentina), they were a hybrid Salix matsudana x Salix alba “Barrett 13-44 INTA” (clone B); and an open pollinated S. alba clone, “Yaguareté INTA CIEF” (clone Y). Clone B showed susceptibility to long periods of flooding in the field (Cerrillo et al. 2013), while clone Y was more tolerant to flooding in such conditions (T. Cerrillo, personal communication). Both clones have a similar sprouting date, making growth comparisons straightforward.

One-year-old cuttings of 20 cm length were planted in 4.5 L pots filled with a 1:1 sand/soil mixture in a greenhouse in the city of La Plata, Argentina (34° 54′ S). The
maximum irradiance at midday was 1600 µmoles photons m\(^{-2}\) s\(^{-1}\). There was no supplemental artificial light added to increase the irradiance or alter the natural photoperiod.

The planting date was August 13\(^{th}\), 2012, and one cutting per pot was planted. The pots were placed in a randomized design with 6 replicates for each clone and treatment level. The plants were surrounded by a border row that was not used for measurements. Plants were watered to keep the soil at field capacity (FC) until the beginning of the stress treatment. Bud flush occurred in both clones between August 30\(^{th}\) and September 4\(^{th}\). After sprouting and before starting the treatment, 50 ml of complete Hoagland solution was added weekly to the pots to ensure an adequate nutrient availability. To avoid pests, the trees were treated every two weeks with an insecticide (o,s dimethyl-acethyl phosphoroamydotiathe). Before the beginning of the treatment, cuttings were pruned and only one shoot was kept, in order to minimize the variability induced by different number of shoots per tree. Flooding was induced by placing the pots with the trees into a sealed 7 L pot filled with tap water up to approximately 10 cm above soil level; water was added when necessary to keep this level. Drought was induced by watering the plants with 50 ml of water every other day.

The stress treatment started on October 31\(^{th}\), 2012, and the different combinations were applied as follows: 1 - watered to field capacity (FC); 2 - six weeks of continuous drought (Drought); 3 - two weeks of drought, two weeks watered to field capacity, two weeks of drought (D-FC-D); 4 - two weeks of drought, two weeks watered to field capacity, two weeks of flooding (D-FC-F); 5 - two weeks of flooding, two weeks watered to field capacity, two weeks of drought (F-FC-D); 6 - two weeks of flooding, two weeks watered to field capacity, two weeks of flooding (F-FC-F); 7 - six weeks of continuous flooding (Flooding). The period of watering at field capacity was included because it is possible to go from drought to flooding immediately, but not the other way around, because after flooding the soil will be saturated for several days, even without any watering.

Growth, water consumption and gas exchange measurements
Total shoot height (cm) was measured once a week with a graduate stick. At the beginning of the experiment, the last completely expanded leaf was tagged with a color wire, and all leaves below this mark were counted every week, providing a measurement of the abscission of basal leaves in each treatment.

Stomatal conductance was measured on seven different dates with a Decagon SC1 porometer on the abaxial side of the latest fully expanded leaf. The measurements were carried out on cloudless days without any artificial light supplements, between 11.30 am and 01.30 pm, and the average irradiance during those measurements was 1150 µmoles m⁻² s⁻¹. Six repetitions were measured for each clone and treatment.

The dry weight of leaves, stems and roots was determined at the end of the experiment, after drying them at 65°C to constant weight. The total leaf area (cm²) was measured at the end of the experiment by scanning the leaves and determining their area using the software IMAGE J (http://rsbweb.nih.gov/ij/, Schneider et al. 2012).

The water consumption (WC) of the whole plant was estimated as follows: Two days before the end of the experiment, the pots were sealed with a double plastic bag, and weighted. The last day, they were weighted again and the difference in weight gave an estimation of the water consumed by the plant.

Hydraulic conductivity measurements

Hydraulic conductivity was measured in four plants of each clone and treatment at the end of the experiment. Measurements were taken on the stem segment immediately above the latest expanded leaf at the beginning of the experiment. This part of the stem was selected because it was still growing, and would eventually reflect changes due to the stresses imposed. In the early morning, shoots were cut under water and taken to the laboratory in water buckets and kept in water until measurements were performed that same day. The stem segment to be measured was re-cut under water, and connected to the hydraulic head. Degassed and deionized water was perfused through one of the ends of the segments. The pressure gradient was 0.011 MPa, and in this situation, the embolisms (if
present) were not removed. When the flux achieved a steady state, the water extruding from the segments was collected in a pre-weighted tube. The time spent on water collection was also measured (minimum 13 minutes, maximum 58 minutes). The water flux was estimated by weighing the tubes on a digital balance. For the measurements, the segments were decorticated. The total length of the segments and the diameter of both extremes were measured with a digital caliper, and the xylem area was calculated with the mean of both diameters; the pith area was not subtracted because the whole decorticated area was conductive. The values of the hydraulic conductivity per unit stem length ($k_h$), the specific hydraulic conductivity per unit of xylem area ($k_s$) and the specific hydraulic conductivity per unit leaf area ($k_l$) were calculated according to the modified Poiseuille’s law (Cruiziat et al. 2002).

**Anatomical analysis**

The anatomical analysis was carried out on the same stem segment used to measure the hydraulic conductivity. To determine wood anatomy, the entire cross-sections (20 µm) of stem segments were cut using a sliding microtome, then stained in safranin (1%), dehydrated, and mounted in Entellan® for microscopic analysis. Images were captured with a digital camera (Olympus DP71) mounted on a research microscope (Olympus BX50, Japan). The captured images were analyzed for the following parameters, using the image analysis software to count/size and measure objects ImagePro Plus v. 6.3, Media Cybernetics USA: vessel lumen diameter (µm), vessel area ($A_V$, µm$^2$) and vessel number ($N_V$, n°/mm$^2$).

**Statistical Analysis**

Most data did not meet the ANOVA assumptions of normality and equality of variance, and this could not be improved by data transformation. In consequence, the Kruskal-Wallis rank sum test was used for the analysis. The statistical analysis was carried
out with the R software 3.2.2 (R Core Team, 2015) using the package *agricolae* version 1.2-3 for the Kruskal-Wallis test.

For the Principal Components Analysis (PCA), the data were centered and standardized. The variables included in the PCA were: RSR (root to shoot ratio); NV (number of vessels); AV (area of vessels); kh (hydraulic conductivity per unit stem length), ks (hydraulic conductivity per unit of xylem area); kl (hydraulic conductivity per unit leaf area), NBL (number of basal leaves); WC (water consumption); TLA (total leaf area); TDW (total dry weight); Hf (final height, day 41); gs (stomatal conductance measured on day 41).

Results

Gas exchange measurements

During the flooding periods, there were no significant differences in stomatal conductance (gs, Fig.1) between control and flooded plants of clone Y. In some dates, gs was significantly reduced in flooded plants of clone B compared to control treatment. However, gs was reduced during the drought periods in all treatments including drought in both clones. The reduction was more pronounced in the permanent drought treatment, whereas in the cyclic drought treatments, gs recovered to the levels of controls when plants were watered to field capacity. These results show that the treatments were effective at inducing drought stress in the willow clones. Growth in height showed the same pattern, being reduced only in the drought treatments (Supplementary Fig.1).

Growth measurements

The number of basal leaves indicate the occurrence of leaf area adjustment through leaf abscission (Fig. 2). Leaf shedding was scarce on control and flooded plants, while abscission was enhanced by drought in both clones, causing the reduction in total leaf area at the end of the experiment (Supplementary Fig.2). In the F-FC-D and D-FC-F treatment, leaf shedding occurred mainly during the drought periods. The reduction in the number of
basal leaves for the cyclic drought treatments was more pronounced in clone Y than in clone B.

Total dry weight (Fig. 3) was significantly reduced compared to controls under continuous drought, D-FC-D, and F-FC-D. In D-FC-F treatment, the reduction was significant only for clone Y. Continuous flooding and F-FC-F did not reduce the total dry weight in either of the clones.

In spite of the similar value of total dry weight in control plants, the dry matter partitioning was different in both clones. Clone B invested more in roots than clone Y, and consequently had a significantly higher RSR (Fig. 3). Both clones significantly reduced RSR under flooding, applied either in the continuous (Flooding) or cyclic form (F-FC-F). In the other treatments, there were no statistically significant differences in root/shoot ratio compared to control plants.

**Hydraulic conductivity and xylem anatomy**

The hydraulic conductivity measured as $k_h$, $k_s$, or $k_l$ (Fig. 4) was similar in the control plants of clone B and Y. All three parameters were reduced in the D-FC-D and F-FC-D treatments, but these differences were statistically significant only in clone Y.

The $g_s / k_s$ ratio (Table 1) was calculated using the values of $g_s$ at the end of the experiment. This ratio gives an insight of the capacity to maintain water balance under drought stress (Wikberg and Ögren 2007). Clone B significantly reduced this ratio for drought, F-FC-D and D-FC-D treatments, while in clone Y the reduction was only significant in D-FC-D.

The area (AV) and number of vessels per mm$^2$ (NV) were measured in the same stem segment used to determine hydraulic conductivity (Fig. 5). The response of the diameter and area of the vessels to the different treatments was similar, in consequence only the area data is shown. In field capacity plants, clone B had a higher NV and of a smaller size than those of clone Y, but the differences were significant only for the number. In clone B, NV increased significantly only under continuous drought, while there were no differences
in the other treatments. In clone Y, NV increased significantly in drought, D-FC-D, D-FC-F and F-FC-F compared to controls, while there were no changes in F-FC-D and flooding treatments (Fig. 5, and S.Fig.3). AV decreased significantly in treatments drought, D-FC-D, D-FC-F and flooding in clone B, while there were no differences in F-FC-D and F-FC-F. In clone Y, AV was significantly reduced in the drought, D-FC-D, D-FC-F, F-FC-F and flooding treatments, and did not change in F-FC-D (Fig. 5).

We carried out a PCA analysis to explore the relationship between the variables measured in the different combinations of treatments (Fig. 6). For variables measured several times (gs, height and number of leaves), only the last measurement of the experiment was included in the analysis because they could be compared with the other variables measured at the end of the experiment. The first and second components together explained 78% of the total variation. The variables WC, gs, NBL and TLA superposed with each other, lying within the first component. The root to shoot ratio (RSR) had a negative correlation with height (Hf) and total dry weight (TDW). The area and number of vessels (AV and NV) had the opposite tendency: one increased while the other decreased.

Discussion

Drought and flooding caused different degree of stress in willows.

From our results, it is clear that flooding is a less stressful situation than drought for willows, because the flooded plants differ less from the field capacity treatment. This is clearly reflected in the PCA analysis, where the treatments are divided in two main groups along the first component. One group includes the drought treatments (drought, D-FC-D and F-FC-D); and the other group includes the FC, Flooding, F-FC-F and D-FC-F treatments. The reason is that several growth and physiological variables were reduced by drought compared to the field capacity treatment (gs, WC, TLA, TDW, NBL), but this did not happen with flooding. The treatments including both drought and flooding (D-FC-F and F-FC-D) grouped closer to the last treatment than to the first one. The second component of the PCA analysis
mainly reflected differences at clone level. This axis separated the clones because several of
the variables shared were different from the beginning, or because they had different
responses to the stress episodes (NV, AV, height, RSR, kl, ks).

Flooding and drought caused different effects on willows growth.

The main differences between treatments occurred in biomass accumulation and
partition between plant organs. Flooding did not reduce TDW in any of the clones, while
drought –continuous or cyclic– decreased it. In spite of having a similar total dry weight under
well-watered conditions, the dry matter partitioning was different in both clones. Clone B
allocated more biomass to the root system, whereas clone Y did so in the stem, resulting in
differences in RSR. Under F and FCF treatments RSR was reduced in both clones compared
to control plants, these results are similar to those of Salix alba where repeated flooding
reduced root biomass (Markus-Michalczyn et al. 2016 b).

In D, D-FC-D, F-FC-D and D-FC-F treatments, the RSR did not differ significantly
compared to field capacity (Fig. 3). This result was similar to the one described previously for
Salix gracilistyla, where RSR was similar in control and drought-stressed plants (Nakai et al.
2010). The lack of a significant increase in RSR could be a factor increasing drought
susceptibility of willows compared with other forest trees. An increased allocation of biomass
to roots allows the exploration of a higher volume of soil, improving water extraction under
drought conditions, but this response did not occur in our clones. In addition, it has been
suggested that in Salix gracilistyla, a period of flooding sensitized plants to subsequent
drought because of root damage (Nakai and Kisanuki 2011).

The growth measurements closely correlate with total leaf area. Leaf area reduction is
a mechanism to diminish the water consumption of the whole plant when the water supply is
limited (Savage et al. 2009, Bonosi et al. 2010). Salix species tend to experience extensive
defoliation under drought, and it has been proposed that it is a mechanism to confine
embolism to petioles and leaves (Savage and Cavender-Bares 2011). The D and FCD
treatments showed the stronger reduction in leaf area due to leaf abscission. The reduction
in leaf area is not the only mechanism to reduce transpiration; stomatal closure is clearly relevant to the control of water loss in plants under drought, as Fig.1 shows. This fact is highlighted by the coincidence of TLA, gs and WC in the first component of the PCA analysis. This can explain why plants of the F-FC-D treatment experienced a sharper decline in gs and leaf abscission (NBL) in the drought period compared with the D-FC-F treatment (Fig. 1 and 2).

The order of occurrence of drought and flooding episodes caused different acclimation responses of water transport capacity in willows.

Drought, flooding and drought followed by flooding caused a significant reduction in vessel size compared to non-stressed plants, but flooding followed by drought did not. The reduction in xylem vessel size and the increase in vessel number are well-documented drought responses in several species, including *Populus* (Fichot et al. 2009). Smaller vessels in general have higher resistance to embolism, increasing drought tolerance. This development is not surprising, since drought resistance correlates with sensitivity to xylem cavitation in willows (Wikberg and Ögren 2004, Ogasa et al. 2013). We found a reduction in AV and an increase in NV in drought and D-FC-D treatments, indicating that both clones can acclimate to drought stress. Vessel size was reduced in the flooding treatment, but without a significant increase in NV (Fig. 5). This fact could explain the lower hydraulic conductivity observed in the same treatment, albeit it is not statistically significant (Fig.4). The smaller vessel area in flooded plants may indicate an adaptation to a reduction in water uptake caused by flooding, as found in *Quercus robur* (Copini et al. 2016). In *Campsiandra laurifolia*, there was a reduction in hydraulic conductivity in the first stages of seasonal flooding, but this fact was reversed later in the season (Herrera et al. 2008).

The D-FC-F treatment caused a reduction in vessel size in both clones, but in the F-FC-D treatment the vessel area was not reduced. Since both drought and flooding alone caused a reduction in vessel area, the results in F-FC-D are surprising. Clearly, the order of occurrence of the stresses caused different responses in this trait. In F-FC-D treatment,
clone B experienced a 50% drop in kl compared to field capacity treatment but in clone Y, kl was decreased to 10% compared with the same treatment. The reduction in hydraulic conductivity can be due to loss of vessel functionality through embolism or the reduction in vessel size or number in the xylem formed during the stress. Clone B demonstrated a reduced gs/ks ratio under the F-FD-D treatment, while clone Y was unaffected (Table 1). This effect occurs only in F-FC-D treatment, while the other drought treatments clone Y can reduce the gs/ks ratio, as other willows species do to acclimate to moderate drought (Wikberg and Ögren 2007). This difference in response may be due to clone Y having an increased sensitivity to embolism. The cause for the greater sensibility to embolism in clone Y is probably the lack of reduction of vessel size, although other reasons cannot be discarded. It has been suggested that the vulnerability to cavitation in poplars is related to the porosity of the vessel pit membrane (Fichot et al. 2015); accordingly this or other xylem traits are responsible for the higher susceptibility of clone Y to embolism.

The clones had contrasting responses to flooding and drought.

In a revision evaluating stress tolerance in 806 tree and shrubs species, a negative correlation has been found between drought and waterlogging tolerance, implying a trade-off between tolerance to these stresses (Niinemets and Valladares 2006). Our results seem to be in line with the hypothesis of a trade-off between drought and flooding tolerance. The flood tolerant clone Y was more drought sensitive, experiencing extensive defoliation and a steep reduction of its water transport capacity under water shortage. On the other hand, flood sensitive clone B (Cerrillo et al. 2013) retained a greater water transport capacity under drought stress. It has been proposed that there are two possible strategies to cope with drought in tree saplings: a low resistance to cavitation combined with lack of osmotic adjustment and high abscission rate (desiccation avoidance), and a higher resistance to cavitation combined with osmotic adjustment and leaf area retention (desiccation tolerance, Yazaki et al. 2010). Willows seem to fit in the first strategy, because they are more sensitive to cavitation than other tree species (Savage and Cavender-Bares 2011, Ogasa et al. 2013).
and experience extensive defoliation under drought (Savage et al. 2009, Bonosi et al. 2010). Both clones can reduce leaf area and gs under drought, but this is not enough to prevent embolism in clone Y. In several temperate species, including Salix, a low cavitation resistance is compensated by a higher recovery capacity through vessel refilling (Ogasa et al. 2013). It is possible that clone Y has a reduced capacity to repair embolism, while clone B is more efficient refilling the vessels and maintaining its water transport capacity under drought. The architecture of clone B, with more biomass allocated to roots than shoot also helps this clone to cope with water shortage. On the other hand, clone Y has a lower root to shoot ratio, and this could explain the higher growth in height of this clone under field capacity or waterlogged conditions.

Conclusion

Our first hypothesis stated that the clones will differ in their tolerance to the alternation of drought and flooding stress. It proved correct for the F-FC-D treatment, since clone B was more tolerant than clone Y under this treatment, while there was no difference between the clones in the D-FC-F treatment. Regarding the second hypothesis, water transport capacity, xylem anatomy and leaf area dynamics were affected in different ways in F-FC-D and D-FC-F treatments. For willows, the occurrence of a drought episode after one of flooding is more stressful than the opposite situation, especially for clone Y that is not able to adjust its water transport capacity during the drought period.

Acknowledgements

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References


Table 1 Ratio between stomatal conductance (gs, mmoles m$^{-2}$ s$^{-1}$) and hydraulic conductivity per unit xylem area (ks, g H$_2$O m MPa$^{-1}$ s$^{-1}$ m$^{-2}$) under Field Capacity, Drought, D-FC-D, D-FC-F, F-FC-D, F-FC-F and Flooding, at the end of the experiment. Mean values: plus minus one standard error of the mean. Treatments followed by the same letter did not differ according to the Kruskal-Wallis test ($p< 0.05$).

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<td>Drought (D)</td>
<td>$0.07 \pm 0.03$ ef</td>
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Legends to the figures

**Fig. 1** Stomatal conductance (gs) of plants of two willow clones growing under different water regimes and their combinations. FC: field capacity. Vertical bars: standard error of the mean. Means marked with an asterisk differ significantly from the field capacity treatment on the same date according to the Kruskal-Wallis test ($p < 0.05$).

**Fig. 2** Number of basal leaves (leaves completely expanded at the beginning of the experiment) of plants of two willow clones growing under different water regimes and their combinations. FC: field capacity. Vertical bars: standard error of the mean. Means marked with an asterisk differ significantly from the field capacity treatment on the same date according to the Kruskal-Wallis test ($p < 0.05$).

**Fig. 3** Dry matter partitioning in plants of two willow clones growing under different water regimes and their combinations: FC (field capacity), F (flooding) and D (drought). Treatments followed by the same letter did not differ according to the Kruskal-Wallis test ($p < 0.05$). The significance indicated is that of each compartment (root, shoot, leaves, and total dry weight). In italics: root/shoot ratios. Those values that differ significantly from controls according to the Kruskal-Wallis test ($p < 0.05$) are marked with asterisks. B: clone B. Y: clone Y.

**Fig. 4** Hydraulic conductivity per unit stem length ($k_l$), hydraulic conductivity per unit xylem area ($k_s$) and hydraulic conductivity per unit leaf area ($k_l$) of two willow clones growing under different water regimes: FC (field capacity); F (flooding) and D (drought). Treatments followed by the same letter did not differ according to the Kruskal-Wallis test ($p < 0.05$).

**Fig. 5** Number and area of vessels in plants of clone B and clone Y under different water regimes: FC (field capacity); F (flooding) and D (drought). Treatments followed by the same letter did not differ according to the Kruskal-Wallis test ($p < 0.05$).
Fig. 6 Principal Components Analysis (PCA) of the different variables measured in the experiment. The variables included in the PCA were: RSR (root to shoot ratio); NV (number of vessels); AV (area of vessels); kh (hydraulic conductivity per unit stem length), ks (hydraulic conductivity per unit of xylem area); kl (hydraulic conductivity per unit leaf area), NBL (number of basal leaves); WC (water consumption); TLA (total leaf area); TDW (total dry weight); Hf (final height, day 41); gs (stomatal conductance measured on day 41).
**FIG. 1**

**Field capacity (FC)**

- Clone B
- Clone Y

**Drought**

- Clone B
- Clone Y

**Drought**

- Clone B
- Clone Y

**Drought**

- Clone B
- Clone Y

**Flooding**

- Clone B
- Clone Y

**Flooding**

- Clone B
- Clone Y

**Flooding**

- Clone B
- Clone Y

**Flooding**

- Clone B
- Clone Y

**Flooding**

- Clone B
- Clone Y

(gs (mmoles m$^{-2}$ s$^{-1}$))

Days of treatment
**Figure 2**

Number of basal leaves over Days of treatment for Clone B and Clone Y under different conditions:

- **Field capacity (FC)**
- **Drought**
- **Flooding**

The graphs show a decrease in the number of basal leaves over time for both clones, with Clone Y generally having fewer basal leaves compared to Clone B. Significant differences are indicated by asterisks (*) and double asterisks (**) for Clone B and Clone Y, respectively.

The significance levels are determined by statistical analysis, with **asterisks** denoting significant differences between treatments and clone groups.
FIG. 5

**Vessel Number**

- **Field Capacity**
- **Drought**
- **D−FC−D**
- **D−FC−F**
- **F−FC−D**
- **F−FC−F**
- **Flooding**

**Vessel area (µm²)**

- **Field Capacity**
- **Drought**
- **D−FC−D**
- **D−FC−F**
- **F−FC−D**
- **F−FC−F**
- **Flooding**

*Note: The figure shows statistical comparisons with letters indicating significant differences among treatments.*
S. Fig. 1 – Height of plants of two willow clones under different water regimes and their combinations: FC (field capacity); F (flooding) and D (drought). Vertical bars: standard error of the mean.
S. FIG.2 – Leaf area and water consumption in two willow clones under different water regimes: C (field capacity); F (flooding) and D (drought). Black: clone B, White: clone Y. Treatments followed by the same letter did not differ according to the Kruskal-Wallis test ($p < 0.05$).
Supplementary Figure 3 – Images of the vessels in the control treatment (Panel A clone B, panel B clone Y) and in the continuous drought (D, panel C clone B, panel D clone Y). Scale bar: 50 µm.