THE RESPONSE OF *ARUNDO DONAX* L. (C_3) AND *PANICUM VIRGATUM* (C_4) TO DIFFERENT STRESSES

Sanchez E, Gil S, Azcón-Bieto J, Nogués S.

Departament de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona. Avda. Diagonal 643, 28028 Barcelona, Spain.

*To whom correspondence should be addressed. Phone: (+34) 93 4033714; E-mail: elenasanchezub@gmail.com

ABSTRACT

In this work, two perennial rhizomatous grasses (*Arundo donax* L. (giant reed) (C_3) and *Panicum virgatum* L. (switchgrass) (C_4)) considered as promising energy crops have been subjected to four different types of stress in two experiments: (i) both species were subjected to four salinity and water stress treatments [well-watered with non-saline solution (WW S-), low-watered with non-saline solution (WS S-), well-watered with saline solution (WW S+) and low-watered with saline solution (WS S+)]; and (ii) both species were subjected to three temperature and light treatments [ambient temperature and light (C), ambient temperature and darkness (AD) and cold temperature and darkness (CD)]. Photosynthetic and physiological parameters as well as biomass production were measured in these plants. It can be hypothesized that a higher photosynthesis rate (A_{sat}) has to be observed in switchgrass as a consequence of its C_4 metabolic pathway. However, our results indicated a similar A_{sat} at the beginning of the experiment for both species. This could be due to switchgrass being an NAD-ME C_4 type whereas giant reed has been reported as a C_3 species with a high photosynthetic rate. We showed that switchgrass seems to be more resistant to stresses such as water stress, salinity and cold than giant reed in our greenhouse conditions.

Key-words: Arundo donax L., *Panicum virgatum* L., water stress, salinity, cold, continuous darkness.

ABBREVIATIONS

A_{sat}, light saturated net CO_2 assimilation rate; DLP, dry leaves percentage; FC, field capacity; F_v/F_m, maximum quantum efficiency of PSII; F_v'/F_m', photochemical efficiency of PSII; GLP, green leaves percentage; g_s, stomatal conductance; H, height; J_{max}, maximum rate of electron

transport contributing to RuBP regeneration; *l*, stomatal limitation to A_{sat}; LA, leaf area; LAI, leaf area index; LAR, leaf area ratio; LMA, leaf mass area; LWR, leaf weight ratio; NL, number of leaves; NS, number of stems; NPQ, non-photochemical quenching; PPFD, photosynthetic photon flux density; PSII, photosystem II; q_p, photochemical quenching; *R*, recovery; RQ, respiratory quotient; RWC, relative water content; SA, stem area; SLA, specific leaf area; S/R, shoot/root ratio; *T*, transpiration; TDW, total dry weight; $V_{c,max}$, maximum velocity of Rubisco carboxylation; WS, water stress; WUE_{inst}, instantaneous water use efficiency; YLP, yellow leaves percentage; ϕ_{PSII}, relative quantum efficiency of PSII.

1. Introduction

Nowadays, climatic patterns in many regions of the world are being influenced by climate change [1], so many agricultural production areas will be affected by abiotic stresses such as water stress (WS), salinity or flooding. WS [2] and salinity [3] have been described as two of the most important environmental phenomena affecting plant growth, development and crop yield. Photosynthesis, together with cell growth, has been reported among the primary processes affected by WS [4]. Decreases in photosynthesis due to WS have been well studied and reviewed in C_3 plants [5-8] and may be directly associated with decreases in stomatal conductance in the early phase of stress [8, 9], whereas the response of C_4 photosynthesis to WS has been less studied. Ghannoum [10] suggested that although the C_4 CO_2-concentrating mechanism offers C_4 photosynthesis a greater buffering capacity against CO_2 shortages brought about by partial stomatal closure under WS, the biochemistry of C_4 photosynthesis is as - or even more - sensitive to WS than C_3 photosynthesis. According to Munns [11], early physiological plant responses to water and salt stress have much in common, and cell growth and photosynthesis would be affected

by salinity in a similar way to WS [8, 11, 12]. Shoot growth is affected in the first phase of salinity stress, denominated “osmotic phase”, and a reduction in leaf expansion, emergence of new leaves and lateral bud development is observed [3]. The second phase (“ion-specific phase”) begins when salt accumulates to toxic concentrations in the old leaves, and a decrease in the photosynthetic capacity of the plant is noticed due to a greater rate of death of old leaves than production of new leaves.

Other factors limiting photosynthesis in C_3 and C_4 plants are temperature [13, 14] and light [15]. Hurry et al. [16] have described how photosynthesis at low temperature is inhibited due to the low synthesis of sucrose, reaching the limits of inorganic phosphous (Pi) recycling. Tcherkez et al. [17] suggest that the plant reduces the rate of reserve consumption (primarily starch) under chilling conditions, but, on the other hand, an increase in temperature increases mitochondrial respiration with a faster degradation of reserves as a consequence. Light regulates the activity of the complex photosynthetic enzymes in both the Calvin cycle and electron transport. Therefore, lack of light causes the Calvin cycle to stop capturing atmospheric CO_2 because many enzymes, such as Rubisco and PEPC, are activated by red light via phytochrome. Some adaptation responses under low light or darkness are fast and reversible, such as chloroplast movements, but other functional and anatomical changes that occur in leaves are slow and irreversible [18]. The adaptation responses produce changes in leaf anatomy and enzyme activation cycles. Two important parameters for evaluating structural and biochemical acclimation are hydration and activity of Rubisco, respectively. In addition, the content of Rubisco decreases considerably after a period of darkness in C_3 plants [19, 20], whereas the activity of Rubisco is not limited to the dark-acclimated C_4 leaves. In relation to continuous darkness, Nogués et al. [21] suggest that the respiratory substrate after a light period is a mixture in which the current photoassimilates are not the main

component in physiological conditions. In addition, continuous darkness could be used as a tool to decrease the carbon pool of the plant.

Regarding the species used, giant reed and switchgrass are generating much interest in Europe as new renewable sources of biomass for energy production [22-24]. Several benefits are expected from the production and use of perennial grasses, as the important contribution in reducing of anthropogenic CO_2 emissions because the quantity of CO_2 released by combusting biomass does not exceed the amount that has been fixed previously by photosynthesis while the plants were growing [23]. Moreover, perennial grasses have other ecological advantages as a low requiring soil management, reducing the risk of soil erosion [25] and low demands for nutrient inputs due to the recycling of nutrients by their rhizome system [22]. On one hand, giant reed is a plant belonging to the *Poaceae* family. Recent studies [23, 26] have proposed an Asiatic origin and a subsequent spread throughout the Middle East to southern Europe and Africa, being found widely in warm temperate regions all over the world. On the other hand, switchgrass is a native perennial warm-season grass from North America belonging to the *Gramineae* family [22], and is broadly adapted to the central and eastern United States [27]. Giant reed is not able to produce viable seeds due to failure of the megaspore mother cell to divide [26, 28], whereas switchgrass can be established by seeding [22]. Therefore, several endemic species of switchgrass with wide ranges of adaptation can be found, but low genetic variability is found in giant reed [22]. However, spontaneous propagation can occur by rhizome fragmentation in both species [22, 29]. High biomass productivity has been observed in giant reed [23] and switchgrass [22, 30] even with low levels of crop inputs like irrigation, fertilization and plant density. The high yield of giant reed has been reported as being stable in long-term experiments [23, 31].

Both species uses different photosynthetic pathway: Giant reed is a C_3 plant [22, 32], whereas switchgrass is a NAD malic enzyme type C_4 grass [22, 33]. The key feature of C_4 photosynthesis is the operation of a CO_2-concentrating mechanism in the bundle sheath that leads to the suppression of apparent photorespiration in air as well as the saturation of C_4 photosynthesis at a lower ambient [CO_2] than for C_3 plants [10]. Therefore, a higher efficiency of radiation, nutrient and water use is expected in a C_4 species grown in an appropriate climate than a C_3 species [34]. Nevertheless, giant reed has been classified as a C_3 plant but it has been compared with C_4 plants due to its high photosynthetic potential [32], although the latest studies have suggested that giant reed has a relatively high transpiration rate and will therefore use more water than many C_3 and C_4 species used for biomass feedstock [35].

The aim of this paper is to study the physiological response of these two bioenergy species to four stresses (*i.e.* water stress, salinity, cold and continuous darkness) to determine which specie is more tolerant to stress.

2. Materials and methods

2.1. Plant material

Arundo donax L. plants collected in Sant Martí Sarroca (291m asl, 41º23'14" N 1º36'43" E, Catalonia, Spain), named *Arundo donax* clone Martinensis (giant reed), were obtained from multiplication of rhizomes on January 2013. Seeds of *Panicum virgatum* L. cv. Alamo (switchgrass) were donated by the Instituto Nacional de Tecnología Agropecuaria Anguil (INTA, Argentina) and germinated on moist filter paper in Petri dishes, placed in a long day chamber (16h of photoperiod) at a temperature of 22/18ºC day/night respectively and 70% HR.

Giant reed and switchgrass rhizomes, with an initial rhizome fresh weight of 33.2 g ±4.4 and 16.3 g ±2.8, were grown in a greenhouse at the Experimental Field Service of Barcelona University (Barcelona, Spain) in plastic pots containing 5 L of peat: perlite: vermiculite (3:1:1) and were irrigated with a complete Hoagland solution [36]. The average temperature and vapour pressure deficit (VPD) during growth was 25/15ºC day/night and 0.75 kPa, respectively. Relative humidity ranged from 40 to 65% and the maximum PPFD was ~1000 μmol m^{-2} s^{-1}.

In order to study the response of these two species to different stresses, plants were subjected to two different experiments.

2.2. Experiment 1: Water stress and salinity

2.2.1. Plant material and experimental design

Three months after planting, plants were separated into four treatments. Half the plants were subjected to WS by withholding water until 25% of field capacity (FC). Then, well-watered (WW, maintained at FC) and WS plants were equally divided between salinity (e.g. 16 mS·cm^{-1} Hoagland solution) and non-salinity (e.g. 1 mS cm^{-1} Hoagland solution). Saline solution was prepared by adding NaCl (PANREAC, 99% Sodium Chloride) to a complete Hoagland solution until the appropriate saline concentration was reached. Consequently, a total of three plants per ecotype were subjected to the following treatments: (i) well-watered with non-saline solution (WW S-), (ii) low-watered with non-saline solution (WS S-), (iii) well-watered with saline solution (WW S+) and, iv) low-watered with saline solution (WS S+).

Plants were subjected to these four treatments during two months. Measurements were carried out every fifteen days (*i.e.* T15, T30, T45) until the end of the experiment (i.e. T60).

2.2.2. Measurements

2.2.2.1. Gas exchange

Leaf-level gas exchange was measured using a portable photosynthesis system (Li6400, Li-Cor Inc., Lincoln, NE, USA) provided with a Leaf Chamber Fluorometer (6400-40) of 0.0002 m^2 and a 10% blue light source.

Firstly, before the experiment (T0), A/C_i curves with chlorophyll fluorescence determinations were conducted in fully expanded leaves from each species (n=3) at 25ºC with a light rate saturated at 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of PPFD and an airflow rate of 0.008 l s^{-1}. The response of A to the intercellular CO_2 concentration (C_i) was constructed by measuring these values at a range of CO_2 concentrations from 70 to 1500 μmol_{CO2} mol_{air}^{-1}. Net CO_2 assimilation rate (A_{sat}, μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) were measured directly by the Li-Cor, whereas the maximum carboxylation velocity of Rubisco ($V_{c,max}$, μmol CO_2 m^{-2} s^{-1}), the maximum electron transport rate contributing to RuBP regeneration (J_{max}, μmol CO_2 m^{-2} s^{-1}), and stomatal limitation (*l, %*), which is the proportionate decrease in light-saturated net CO_2 assimilation attributable to stomata, were calculated by fitting a maximum likelihood regression below and above the inflexion of the A/C_i response using the method of McMurtrie and Wang [37]. Transpiration (*T,* mmol H_2O m^{-2} s^{-1}) was also measured and instantaneous water use efficiency was calculated as $WUE_{inst} = A_{sat}/ T$. Modulated chlorophyll fluorescence measurements were determined at the same time with the Fluorometer to estimate the maximum quantum yield of PSII (F_v/F_m), the efficiency of excitation energy capture by open PSII reaction centres (F'_v/F'_m), the relative quantum yield of photosystem II (ϕ_{PSII}), as well as the photochemical quenching (q_p) and the non-photochemical quenching (NPQ) determined in a totally expanded leaf after 30 min of dark adaptation [38].

Secondly, during the experiment, instantaneous measurements of assimilation A_{sat} and g_s were done in each species and treatment (n=3) every fifteen days using also a portable photosynthesis

system Li6400 at the same conditions of temperature, light and air flow as mentioned above and at a CO_2 concentration of 400 μmol_{CO2} mol_{air}^{-1}. WUE_{inst} was also calculated for each species and treatment every 15 days as was detailed above.

All gas exchange and fluorescence measurements were made from 10:00 to 18:00h. Stress treatments started on the 1st of April 2013. However, measurements in control conditions were done before the experiment started (T0) and, once stress was implanted every 15 days: April 12-13 (T15) and 27-28 (T30) and on May 13-14 (T45) and June 2-3 (T60).

2.2.2.2. Relative water content

The relative water content (RWC, %) of the leaves was determined as (*FW-DW*)/(*TW-DW*) x 100, where *FW* is the fresh weight, *DW* is the dry weight after being dried in an oven at 60ºC until constant weight and *TW* is the turgid weight of the leaf after equilibration in distilled water for 24 h at 4ºC. RWC value is the main of 3 leaves of each ecotype and treatment every 15 days.

2.2.2.3.Chlorophyll content

Chlorophyll content was measured in the first and second last fully expanded leaf of all the plants assayed using a portable meter (Minolta SPAD 502 Meter, Plainfield, IL, USA) every 15 days. Five measurements were performed per leaf from the middle of the leaf (each measurement is the mean of 6 leaves per treatment).

2.2.2.4. Biomass parameters

At T60, plants were harvested and oven dried at 60ºC up to constant weight, and analyses of biomass of shoots and roots were carried out. Total fresh weight and plant leaf area was estimated prior to drying using a flat-bed scanner (Hewlett-Packard ScanJet model Iicx, San Diego, USA) and analysed with an image processing program (Image, University of Sheffield, 2003).

Parameters such as height (H, m), number of leaves and stems per plant (NL, NS), leaf area (LA, m^2) and stem area (SA, m^2), percentage of green, yellow and dry leaves (GLP, YLP, DLP, %), total dry weight (TDW, g), shoot/root ratio (S/R), leaf area index (LAI), leaf mass area (LMA; kg m^{-2}), specific leaf area (SLA; m^2 kg^{-1}), leaf area ratio (LAR; m^2 kg^{-1}) and leaf weight ratio (LWR; kg kg^{-1}) were determined.

2.2.3. Statistical analysis

The effect of WS and salinity on plant physiology and development in each specie was performed using a two factor analysis of variance (ANOVA) and interactions (treatment, specie and treatment*specie). Statistical analysis was conducted with the SPSS 21.0 software package. The means ± standard errors (SE) were calculated for each parameter. When a particular *F*-test was significant, we compared the means using a Tukey multiple comparison test. The results were accepted as significant at $P < 0.05$.

2.3. Experiment 2: Temperature and continuous dark stress

2.3.1. Plant material and experimental design

Twenty five plants of giant reed and switchgrass, which were grown in the same conditions as described in Experiment 1, where chosen and divided into three treatments: (i) control in greenhouse conditions (25/18 °C day / night, VPD 0.75 kPa, 65% - 70% HR, maximum light ~ 1000 PPFD mol $m^{-2}s^{-1}$) and, (ii) ambient temperature (24-26 °C) and continuous darkness (AD), and (iii) cold temperature (5-6 °C) and continuous darkness (CD). Each treatment was subdivided into three experimental conditions, which corresponded to 3, 6 and 9 days (d) after the start of the treatment. After each experimental condition, the plants were returned to the greenhouse at the

Experimental Field Service of Barcelona University (Barcelona, Spain) for *ca*. 24 h to study their recovery after the the stress treatment (*R*).

2.3.2. Measurements

2.3.2.1. Gas exchange and fluorescence parameters

A portable photosynthesis system Li6400 (Li6400, Li-Cor Inc., Lincoln, NE, USA) provided with a Leaf Chamber Fluorometer (6400-40) of 0.0002 m^2 was also used to carry out measurements of gas exchange and fluorescence as described in Exp. 1. Photosynthesis activation time depended on the treatment duration: 30, 45 and 60 minutes for treatments whose durations were 3, 6 and 9 d, respectively.

A_{sat}, $V_{c,max}$, J_{max} and l were calculated according to the method of McMurtrie and Wang [37]. Chlorophyll fluorescence parameters such as F_v/F_m, F_v'/F_m', ϕ_{PSII} and q_p were measured as described in Exp. 1. All measurements were conducted from 10:00 to 18:00h during the daily photoperiod of the plants.

2.3.2.2. O_2 consumption, CO_2 production and respiratory quotient (RQ)

O_2 consumption (µmol O_2 m^{-2} s^{-1}) was measured with a complete system with a liquid-phase oxygen electrode and dissolved oxygen measurement (Qubit Systems, Kingston, Canada, with data recording with LoggerPro 3.7 software).

0.001 m^2 of tissue of the middle part of the leaf were sliced into small fragments in a $CaCl_2$ solution (0.2mM) over 10-15 minutes at room temperature and in darkness in order to stabilize the walls damaged by the wound. Afterwards, the leaf material was placed in the electrode cuvette with a volume of 5 ml of TES buffer (20mM TES + 2mM $CaCl_2$, pH 7.2), and the whole setup was ambient-air equilibrated under dark conditions. The reaction was carried out at 25 °C with constant

stirring. CO_2 production (μmol CO_2 m^{-2} s^{-1}) was measured with NaOH and phenolphthalein indicator (protocol modified and adapted from [39]).

The nature of the respiratory substrate was determined by the respiratory quotient (RQ), which is the ratio of CO_2 production to O_2 consumption: $RQ = CO_{2\ production} / O_{2\ consumption}$.

2.3.3. Statistical analysis

Measurements of different treatments were performed randomly during the course of the experiment in order to reduce experimental error. Data averages of each treatment were plotted and were subjected to a three factor ANOVA (plant, treatment and day) with a significance level of 5% (IBM SPSS Statistics 20). A Duncan test was used to find the differences between experimental conditions.

3. Results

3.1. Experiment 1: Water stress and salinity

3.1.1. Gas exchange

Significant differences were found in almost every photosynthetic parameter ($V_{c,max}$, J_{max}, l, g_s, T and WUE_{inst}) between species at the beginning of the experiment (T0) except in A_{sat} (P = 0.908) (SM 1) where non-significant differences were found between species. A lower value of $V_{c,max}$ and a higher of J_{max} were found in giant reed, while switchgrass showed lower values of l, g_s and T. Therefore, a higher WUE_{inst} was observed in the second species. Significant differences were found also in the majority of the fluorescence parameters (F_v/F_m, F_v'/F_m', ϕ_{PSII}, NPQ) between species at T0 except in q_p (P = 0.297) (SM 1). The highest values of Fv/Fm, Fv'/ Fm' and ϕ_{PSII} were observed in giant reed, whereas the highest value of NPQ was observed in switchgrass.

Similar values of A_{sat} were found in giant reed and switchgrass at T0 (Fig. 1a and SM 1). Giant reed showed a significant decrease in A_{sat} at T15, particularly in WS S- where it decreased by 50% with respect to the control at the same time, followed by the salinity treatment (WW S+) and the double stress treatment (WS S+) with decreases of 27% and 8%, respectively. Similar values of A_{sat} in the WS S- (9.9 ±0.9) were found at T30, whereas salinity and double stress values of A_{sat} decreased until 37% and 24% compared to the control at the same time, reaching values of 12.4 ±0.6 and 15.1 ±0.6, respectively. At T45, the decrease in A_{sat} remained until T60, when non-significant differences were found between treatments that reached values of 2.15 ±0.3, 2.0 ±0.2 and 1.9 ±0.3 for WS S-, WW S+ and WS S+, respectively. In general, at T60 a decrease in A_{sat} of *ca*. 91% was observed in giant reed for every treatment with respect to the control at T0. Nevertheless, switchgrass did not show significant differences between treatments at T15 or even at T30, although a decrease in A_{sat} was observed in every treatment, particularly in the WS S+ (12.5 ±1.3), which showed a decrease of 27% at T30 relative to the control (17.1 ±0.6) (Fig. 1a). At T45, a decrease in A_{sat} was observed and significant differences were found in all treatments in comparison to the control (16.2 ±1.5), but not between the stress treatments, which had values of 10.2 ±0.4, 10.9 ±0.8 and 6.6 ±1.6 for WS S-, WW S+ and WS S+, respectively. The lowest values of A_{sat} in switchgrass were found in WS S+ at T60 (1.1 ±0.3), representing a decrease of 95% in the A_{sat} observed at T0. In relation to g_s, significant differences were found in the control treatment between species during the experiment, with switchgrass having a difference in g_s values of ca 55% from T0 to T45 and 33% at T60 relative to giant reed (Fig. 1b). However, a similar pattern of decrease in A_{sat} was observed in both species, indicating a high correlation between the two parameters for both species in each treatment (SM 2). In relation to WUE_{inst}, significant differences were found in the control treatment between species (P = 0.000) at T0 where giant reed showed

lower WUE_{inst} than switchgrass (3.3 ±0.2 and 7.2 ±0.2, respectively). An increase in WW S- was observed in giant reed, while switchgrass showed similar values during most of the experiment (Fig. 1c). The highest WUE_{inst} was found in WS S- until T45, whereas similar values were found in all treatments at T60. Switchgrass showed similar values between treatments at T15 followed by an increase of WUE_{inst} in WS S- at T30 and T45. However, the same values were observed at T60 in all treatments (Fig. 1c). In general, switchgrass showed greater values of WUE_{inst} than giant reed for each treatment at each time.

3.1.2. Relative water content (RWC)

A slight decrease in RWC was observed between T0 and T60 in the control treatment (WW S-), although no significant differences were found over time or between species (Fig. 2). The RWC of giant reed decreased by ca 18% in stress treatments (WS S-, WW S+ and WS S+) relative to the control at T60, and no significant differences were found between treatments. Otherwise, switchgrass showed a similar decrease in WS S- and WS S+ (21%) with respect to the control at T60, whereas WW S+ decreased by only 10%. However, no significant differences were found between species for any treatment at any time (T0 and T60).

3.1.3. Chlorophyll content

Similar chlorophyll content values (SPAD units) were observed in giant reed between T0 and T60 in WW S-, however, a slight increase in chlorophyll content was noticed in switchgrass, from 33.9 ±0.6 to 38.5 ±0.7 (SM 3). The highest values of chlorophyll content in giant reed at T60 were found in those treatments where WS was not included (WW S- and WW S+) with values of 49.2 ±0.4 and 47.5 ±0.9 respectively, followed by WS S+ (44.4 ±0.4) and WS S- (38.1 ±0.5). No significant differences were found between treatments in switchgrass at T60. Giant reed had higher values of chlorophyll content than switchgrass in WW S- and WW S+, with a difference of 22%

and 19%, whereas no significant differences were found in treatments where WS was included (WS S- and WS S+).

3.1.4. Biomass parameters

No significant differences were found in H in giant reed between treatments (Table 1), even though an increase of 22% in WS S- and a decrease of 24% in WS S+ were recorded. Nevertheless, switchgrass showed a decrease in every treatment relative to the control, with the most substantial decrease (33%) seen in WS S+. A decrease in the NL was observed in every stress treatment and in both species, being more noticeable in treatments related to WS. Giant reed showed a decrease of 76% in WS S-, but NL decreased in switchgrass by 78% under WS S+. Giant reed LA (Table 1) decreased considerably (81%) in WS S- followed by WS S+ (66%) and WW S+ (48%) with respect to WW S-. Switchgrass was more affected by WS treatments, decreasing by 86% and by 91% in WS S- and WS S+ treatments, respectively, whereas WW S+ had a decrease of only 64% compared to the control. No significant differences were found between species in any treatment except in WW S- where switchgrass had a greater LA than giant reed. GLP decreased in both species relative to the control as a consequence of stress, and was more pronounced in giant reed in which the greatest decrease occurred under WS S- (67%). In contrast, the greatest decrease in switchgrass was only of 31% in the same treatment (Table 1). However, leaf curling was noticed in switchgrass leaves as a consequence of water scarcity. TDW also decreased in both species compared to the control (Table 1), being more evident in treatments where WS was included. As in the case with LA, no significant differences were found between species in any treatment except in WW S-, where switchgrass had a greater TDW than giant reed. The highest value of the shoot/root index (S/R) (Table 1) was found in WW S+ in giant reed, which was even higher than the control, whereas the lowest value for the same species was found in WS S- with a decrease by

35% in comparison to the control followed by WS S+ with a decrease of 13%. On the other hand, switchgrass decreased to a similar degree for WS S- and WS S+ (43% and 46%, respectively), while WW S+ remained almost unchanged compared to the control. LMA increased under stress treatments in both species (Table 1), with a remarkable increase in switchgrass under WS S+ (163%). SLA decreased in all treatments with respect to the control. SLA decreased in giant reed by 33% under WS S- and by 51% switchgrass under WS S+. No significant differences were found in these two parameters between species in any of the treatments except for the double stress (WS S+).

3.2. Experiment 2: Continuous darkness and temperature

3.2.1. Gas exchange and fluorescence parameters

A different response to stress was found in both species during the experiment. A_{sat} was less affected by the cold treatment (CD) than by the ambient temperature treatment (AD). The decrease in A_{sat} in the AD treatment was 49% and 50% at 3d in giant reed and switchgrass respectively, increasing over time (Table 2). Declines of *ca*. 30% under the CD at 3d was shown in both species and remained throughout the experiment except for giant reed, which decreased by another 14% from 6d (Table 2). In relation to recovery (*R*), an increase in A_{sat} was observed in both treatments (except for giant reed in the CD treatment at 3d) and some complete *R* was found in the CD treatment, especially in switchgrass (for 3 and 9d; $P < 0.05$) (Table 2).

$V_{c,max}$ was less effect of cold temperature than ambient. Decreases of 65% and 43% were observed in giant reed after 3d in AD and CD respectively, with a constant pattern over time (Table 2). Increases in $V_{c,max}$ were observed under AD after *R*. However, *R* was not observed at 6d and 9d. Significant decreases were noticed in switchgrass for AD and CD at 3d (75% and 66%, respectively). $V_{c,max}$ values decreased by almost 94% at 9d in AD relative to the control, although

in CD the $V_{c,max}$ value was equal to the control value ($P < 0.05$). *R* was observed at 3d in CD conditions as well as in all AD conditions (Table 2). J_{max} was the parameter that showed the most differences between the two species. Decreases by 56% and by 61% were observed in giant reed after 3d in AD and CD, respectively. An additional 15% decrease was observed in AD at 6d, whereas value of J_{max} remained constant in CD ($P < 0.05$). However, after 9d a ubiquitous response was found in both treatments (90% reduction). Despite these differences, both treatments had complete *R* in all experimental conditions (Table 2). On the other hand, a different response was found by switchgrass due to a greater reduction in CD than in AD (81% and 53%, respectively) after 3d. J_{max} value increased in AD (110%) but remained constant in CD conditions after 6d (Table 2). A different situation was found after 9d due to an 81% decrease in AD and a 52% increase in CD with respect to the control. A complete *R* was found in CD during the experiment except at 6d ($P < 0.05$), however a complete *R* was only found at 6d in AD (Table 2). In relation to *l*, significant differences were found in giant reed in CD than in AD conditions (Table 2). An increase in *l* was observed at 3d (39%) and 9d (68%) in the AD treatment, but at 6d *l* remained similar to C. Moreover, *l* increased more than double in CD after 3d, decreasing again after 6d and 9d compared to the control. A progressive increase was observed in switchgrass in AD conditions at 3d and 6d followed by a decrease at 9d to a lower value than C. A similar pattern was observed in CD conditions where an increase at 6d was followed by a decrease to C values (Table 2). *R* was complete in all conditions in AD but in CD it was not observed under any condition.

F_v/F_m decreased in giant reed in both treatments although no significant differences were found in the CD treatment until 6d (Table 3). A complete *R* of F_v/F_m was observed at 3d in AD and CD and at 6d (only in CD conditions). The most significant decrease in F_v/F_m was found in switchgrass under CD conditions (Table 3), where decreases by 7% at 3d and 16% at 6d were observed,

whereas F_v/F_m decreased by only 3% and 5%, respectively, under AD conditions compared to C. An initial decrease by 35% and 15% at 3d in F_v'/F_m' values in giant reed in AD and CD conditions was observed, increasing by 12% and 13% more at 6d in each treatment respectively and remained constant after that time (Table 3). *R* was observed in all conditions except for 9d under AD. A similar decrease by 20-23% was found in AD and CD conditions at 3d in switchgrass. However, a greater decrease was observed at 6d (51%) under AD conditions while a slight *R* was seen at 9d (Table 3). *R* was observed in all experimental conditions although it was only at 3d (under AD and CD) and 9d under CD conditions were *R* was completed. The decrease in ϕ_{PSII} became more pronounced over time until 6d, after which there was a stabilization or slight *R* by 9d in both treatments. In contrast to giant reed, which was more affected by AD (a decrease by 42%) than CD (18%) at 3d, switchgrass was more affected by CD (52%) than by AD (30%, Table 3). Decreases at 6d reached values of 61% and 36% in AD and CD conditions in giant reed while in switchgrass the decreases were almost by 78% and 70%, respectively. In relation to q_p, a greater decrease was observed in CD than in AD conditions in giant reed (Table 3). Moreover, switchgrass showed a greater decrease in CD (38%) than in AD (12%) at 3d but at 6d a similar decrease was observed in both conditions (51% and 59%, respectively; Table 3).

3.2.2. O_2 consumption, CO_2 production and the respiratory quotient (RQ)

In relation to mitochondrial respiration, a more large effect was observed in AD than in CD in both species (SM 4). A decrease by 32% and by 33% in O_2 consumption in AD conditions was observed at 3d in giant reed and switchgrass, respectively, increasing to ca 70% at 6d and 9d (SM 4a, c). Nevertheless, the decrease in O_2 consumption in CD conditions was significantly lower, with a decrease by 53% in giant reed and only by 11% in switchgrass at 9d (SM 4 b,d). CO_2 production after 9d decreased in relation to the control in both species, being more marked in AD than in CD

(Table 4). Giant reed and switchgrass decreased CO_2 production by 90% and by 81%, respectively, whereas under CD conditions CO_2 production decreased by 74% and by 33%, respectively. Greater RQ values were observed in C than in AD or CD conditions in both species (Table 4). A similar decrease was observed in RQ values in AD and CD in giant reed (54% and 43%, respectively) and in switchgrass (29% and 25%, respectively) in relation to C (Table 4), although switchgrass values were larger than giant reed.

4. Discussion

4.1. Experiment 1: Water stress and salinity

Values for A_{sat} in giant reed under control conditions at the beginning of the experiment (T0; SM 1) were similar to those reported by Papazoglou et al. [40] but lower than those reported by Rossa et al. [32] or Nackley et al. [35].The g_s value was also lower than expected according to Nackley et al. [35]. Moreover, switchgrass values of A_{sat} and g_s are similar to the values reported by Albaugh et al. [41]. Although a higher photosynthetic rate is expected in C_4 species due to the operation of a CO_2-concentrating mechanism [34, 42, 43], in this study we have found similar values of A_{sat} in both species andit confirm the high photosynthetic potential of the C_3 grass specie [32]. However, although the WUE_{inst} of giant reed in control conditions was similar to values reported by Mann et al. [44], the greater values in giant reed in relation to switchgrass would confirm a greater use of water than C_4 switchgrass under stress (SM 1) [35]. Giant reed *l* was similar to values reported by Rossa et al. [32] and significantly higher than switchgrass, indicating a higher stomatal closure (SM 1). The high F_v/F_m values in both species at T0 (SM 1), similar to values expected in this species in control conditions [32], were a good indicator of photosynthetic efficiency. A better fluorescence efficiency was observed in giant reed in relation to switchgrass due to a higher ϕ_{PSII}

and a lower NPQ, as would be expected from a higher F_v/F_m value [45]. This fact would confirm that C_3 species need lower energy requeriments to assimilate the CO_2 [46, 47].

Although both species have been described as WS resistant [22, 33, 48], our results indicate a decrease in A_{sat} and g_s due to WS and salinity in both species (Fig. 1), as expected by other studies [49]. The decrease in g_s has been proposed as one of the earliest responses to water and salinity stress [3, 8, 50], reducing internal CO_2 partial pressure and, consequently, reducing photosynthetic rates. Although in our experiment the decrease in A_{sat} was a consequence of decreases in g_s due to a high correlation between both parameters (SM 2), other studies in C_4 grasses have proposed that inhibition of A_{sat} under WS is dependent mainly on biochemical limitations [51]. However, a different performance was observed in the species subjected to WS due to an incipient and faster decrease in g_s and A_{sat} in giant reed than in switchgrass (Fig. 1a, b). Moreover, giant reed seems to be equally affected by different stresses at the end of the experiment, contrasting with switchgrass, which is more affected by both stresses (water and salinity stress) according to the A_{sat} and g_s values (Fig. 1a, b). High values of A_{sat} at T60 in switchgrass might indicate a higher tolerance to salinity than giant reed, as expected according to the results of Long et al. [52] and Sage [53]. These authors have suggested that the C_4 photosynthesis pathway could be advantageous to plants living in habitats where water supply is limiting or salinity is present due to a higher ratio (often twice than C_3 species) of CO_2 assimilated to water transpired. C_4 plants would have an adaptive advantage over C_3 plants due to higher WUE [53], as was observed in control conditions in our experiment and in most of the measurements over time (Fig. 1c). Moreover, a higher effect of salinity in C_3 plants than in C_4 plant has also been described previously [54].

Stress caused a decrease in RWC in both species as expected [8, 10, 11, 35, 49], being lower in switchgrass under saline conditions, which could confirm a certain tolerance to salinity. Chlorophyll content values of giant reed at the beginning of the experiment were similar to those measured by Spencer et al. [55]. WS and salinity have been shown to reduce leaf chlorophyll content in species such as maize [56], rice [57], pumpkin [58], sunflower [59] and sorghum [60], and so do in giant reed as a consequence of stress, being more affected by WS than by salinity (SM 3).

Decreases in plant H, NL and LA (Tale 1) were observed as a consequence of stress as expected [3, 61-63] in order to decrease the water used by the plant and allowing to conserve soil moisture and prevent dehydration. This would confirm that WS and salinity effects on plants is the inhibition of growth [11, 64, 65], which would end up affecting the total production of biomass (TDW, Table 1). Higher TDW in switchgrass than in giant reed in control conditions may have been a consequence of higher photosynthetic efficiency during the experiment, and even at T60, this was probably due to the C_4 CO_2-concentrating mechanism. The greater decrease in TDW in switchgrass than in giant reed under WS and salinity maintaining high photosynthetic rates (Table 1 and Fig. 1) would indicate a certain mechanism to decrease water lost and, consequently, the damage produced by stress [12], contrarily to those reported by Ghannoum [10]. The greater decrease in shoot mass due to stress [3, 63] carried out a decrease in S/R in both species, being more important in those treatments where WS was present (Table 1). Moreover, stress conditions usually result in a reduction in leaf dimensions and an increase in thickness, making leaves more efficient at controlling water losses [66], as was observed with the decrease in SLA (Table 1).

4.2. Experiment 2: Continuous darkness and temperature

Björkman [67] and Lambers *et al.* [47] described the effect of low temperatures as a cryoprotectant of enzymes. Our results showed how, according to these authors, the low temperatures and darkness (CD) caused significant less reduction in photosynthetic parameters than ambient temperature and darkness (AD) (Table 2). However, smaller decreases in A_{sat} under CD treatment in switchgrass than in giant reed would indicate differences in cold tolerance between both species (Table 2). In this sense, Pompeiano et al. [68] showed that the lethal temperature at which 50% of two different giant reed ecotypes were killed (LT_{50}) ranged between -16.4 and -12.8ºC, whereas switchgrass tolerance plateaued at -19 to -20ºC [69].

Irreversible damage observed in the $V_{c,max}$ of giant reed from 6d in CD (Table 2) would indicate that the content or the activation of Rubisco would be affected by CD. This observation would be consistent with those reported by Sage and McKnown [70], who claim that the content or Rubisco activity in many C_3 species may be reduced up to 55% in low light situations compared to normal conditions. However, higher values in terms of A_{sat} in CD, where even plants were fully recovered after 9 days, would indicate that low temperatures, in addition to functioning as a cryoprotectant enzyme could act by delaying slightly the degradative effect of darkness.

It is worth noting that in switchgrass although the C_4 pathway has been commonly associated with warm or tropical climates plants [10, 17, 71], several studies have observed certain cold tolerance in some C_4 species [71] and particularly in switchgrass [69, 72], similar to our study. According to Gutiérrez et al. [73] and Hatch and Osmond [74], the most sensitive to cold C_4 species would belong to the group of NADP-ME, whereas the C_4 species of NAD-ME type, such as switchgrass or *Atriplex*, would be more tolerant to cold [73]. Caldwell et al. [71] showed how the low temperature modified the carbon assimilation kinetics even in cold tolerant C_4 species, without affecting the metabolism of the same as seen in the C_4 species sensitive to cold. The complete

recovery of the machinery of carbon fixation at 9 days in CD (Table 2), would imply that the characteristics of the C_4 pathway enzymes, such as PEPC enzyme and PPDK were not damaged or its quantity were not reduced, as mentioned by Sage and McKnown [70] in C_4 cold tolerant species. Chinthapalli et al. [75] described how tolerance to low temperatures is mainly due to the wide range of conformational changes affecting PEPC enzyme (which is dissociated from its normal tetrameric form a dimeric form, but equally functional active low) as well as increased expression of the enzyme PPDK.

The decrease in fluorescence parameters under the two treatments in both species (Table 3) would indicate a photoinhibitory effect. In giant reed, the decrease in the fluorescence parameters occurred before in CD than in AD, indicating that the low temperature would reduce the photoinhibitory effect of darkness [76], although this is not observed in switchgrass.

Although there were no differences between C_3 and C_4 species in respiration rates (SM 4), according to Byrd et al. [77], mithocondrial respiration was more affected by AD than by CD (SM 4). According to Tcherkez et al. [17], temperature directly affects the rate of carbon consumption, i.e. increasing temperature increases the consumption rate of carbon reserves. This also correlates with the CO_2 production, where the lowest values were found in AD (Table 4) due to the presumably low reserves content in plants. One consequence of the absence of light is that, in the absence of carbon assimilation, the energy demands should be cover from the carbon reserves. Therefore, continuous darkness induces a significant management resources in plants, i.e. prioritizing the consumption of energy resources, as indicated Byrd et al. [77]. Starch is the product consumed mainly at the start of the dark period, and once it was consumed, proteins and lipids are consumed [17]. RQ provides information about which substrate feeds respiratory metabolism [17]. The initial values slightly above 1 in both species (Table 4) indicated a consumption of a mixture

of organic acids and carbohydrates. However, the similar values observed in switchgrass in both treatments would indicate that carbohydrates and proteins were feeding respiration, whereas the different values in giant reed meant that carbohydrates and proteins in CD and lipids in AD were feeding respiration (Table 4). According to Tcherkez et al. [17] and Nogués et al. [21], a slower metabolism produced by lower temperatures indicates lower energy requirements and, therefore, lower consumption of reserves. The slightly greater RQ value in switchgrass under low temperature conditions would indicate a slower metabolism as a result of cold tolerance and therefore greater carbohydrate content [22].

5. Conclusions

Our results indicate similar A_{sat} values between both species at the beginning of the experiment, despite the comparison between a C_3 and a C_4 species. This could be explained because switchgrass is a NAD-ME C_4 type, which would imply a lower photosynthetic efficiency. In addition, giant reed has been reported as a C_3 species with a significant photosynthetic rate.

In relation to WS and salinity, switchgrass seems to have a great tolerance to both stresses separately due to the high photosynthetic rates observed at the end of the experiment, whereas it seems to be very affected by both stresses together at advanced stages of stress. On the other hand, giant reed seems to be very affected by WS from the early stages of the experiment and less affected by treatments where salinity is included. However, photosynthetic rates at the end of the experiment indicate a significant effect from all types of stresses. The high correlation between A_{sat} and g_s in both species and in each treatment indicate an apparent stomatal limitation.

The combination of low temperature and darkness leads to a less significant reduction in the photosynthesis parameters, A_{sat} and $V_{c,max}$, than in ambient temperature and darkness. Moreover, switchgrass seems to be more cold tolerant than giant reed due to a lower decrease in A_{sat}, although this is not correlated with F_v/F_m, which may suggests an increase in photoinhibition. Both treatments resulted in substantial effects on plant performance because permanent changes in chlorophyll fluorescence parameters were produced after 24h. In addition, ambient temperature has a larger effect than low temperature due to a greater reduction in the respiration rate and CO_2 production in the AD treatment (i.e. C pools were consumed quickly).

Our results clearly indicate that switchgrass is a perennial rhizomatous grass with higher tolerance to different stresses (*i.e.* water stress, salinity, cold temperature and continuous darkness) than giant reed in our greenhouse conditions.

6. Acknowledgements

We acknowledge the support of OPTIMA project (Optimization of perennial grasses for biomass production in the Mediterranean area, Grant Agreement n. 289642) and CONICYT BecasChile 72120250 grant for financial support to ES.

REFERENCES

[1] IPCC. Summary for Policymakers. In: Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley, editors. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press; 2013.

[2] Araus JL, Bort J, Steduto P, Villegas D, Royo C. Breeding cereals for Mediterranean conditions: ecophysiological clues for biotechnology application. Ann Appl Biol. 2003;142(2): 129–41.

[3] Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008;59:651–81.

[4] Cornic G, Massaci A. Leaf photosynthesis under drought stress. In: Baker NR, editor. Photosynthesis and the Environment. Netherlands: Kluwer academic publishers; 1996. p. 347-66.

[5] Cornic G, Ghashghaie J, Genty B, Briantais JM. Leaf photosynthesis is resistant to a mild drought. Photosynthetica. 1992;27(3):295-309.

[6] Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature. 1999;401:914-7.

[7] Lawlor DW, Cornic G. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ. 2002;25(2):275–94.

[8] Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. Plant Biol. 2004;6(3):269–79.

[9] Flexas J, Diaz-Espejo A, Galmés J, Kaldenhoff R, Medrano H, Ribas-Carbo M. Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves. Plant Cell Environ. 2007;30(10):1284–98.

[10] Ghannoum O. C_4 photosynthesis and water stress. Ann Bot. 2009;103(4):635-44.

[11] Munns R. Comparative physiology of salt and water stress. Plant Cell Environ. 2002;25(2):239–50.

[12] Chaves MM, Flexas J, Pinheiro C. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot. 2009;103(4):551–60.

[13] Bernacchi CJ, Portis AR, Nakano H, von Caemmerer S, Long SP. Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis in vivo. Plant Physiol. 2002;130(4):1992-8.

[14] Long SP. C_4 photosynthesis at low temperatures. Plant Cell Environ. 1983;6(4):345-63.

[15] Long SP, Humphries S, Falkowski PG. Photoinhibition of photosynthesis in nature. Annu Rev Plant Physiol Plant Mol Biol. 1994;45:633-62.

[16] Hurry V, Strand, Furbank R, Stitt M. The role of inorganic phosphate in the development of freezing tolerance and the acclimatization of photosynthesis to low temperature is revealed by the pho mutants of *Arabidopsis thaliana*. Plant J. 2000;24(3):383-96.

[17] Tcherkez G, Nogués S, Bleton J, Cornic G, Badeck F, Ghashghaie J. Metabolic origin of carbon isotope composition of leaf dark-respired CO_2 in French bean. Plant Physiol. 2003;131(1):237-44.

[18] Sharkey TD, Seemann JR, Pearcy RW. Contribution of metabolites of photosynthesis to post-illumination CO_2 assimilation in response to lightflecks. Plant Physiol. 1986;82:1063-8.

[19] Evans JR. Acclimation by the thylakoid membranes to growth irradiances and the partitioning of nitrogen between soluble and thylakoid proteins. Aust J Plant Phyisiol. 1988;15(2):93-106.

[20] Evans JR, Seeman JR. The allocation of protein nitrogen in the photosynthetic apparatus: cost, consequences and control. In: Briggs WR, editors. Photosynthesis. New York: AR Liss Inc; 1989. p. 183-205.

[21] Nogués S, Tcherkez G, Cornic G, Ghashghaie J. Respiratory carbon metabolism following illumination in intact French bean leaves using $^{13}C/^{12}C$ isotope labelling. Plant physiol. 2004;136(2):3245-54.

[22] Lewandowski I, Scurlock JMO, Lindvall E, Christou M. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. Biomass Bioenerg. 2003;25(4):335-61.

[23] Angelini GL, Ceccarini L, Nassi o Di Nasso N, Bonari E. Comparaison of *Arundo donax* L. and *Miscanthus x giganteus* in a long-term field experiment in Central Italy: Analysis of productive characteristics and energy balance. Biomass Bioenerg. 2009;33(4):635-43.

[24] Palmer IE, Gehl RJ, Ranney TG, Touchell D, George N. Biomass yield, nitrogen response and nutrient uptake of perennial bioenergy grasses in North Carolina. Biomass Bioenerg. 2014;63:218-28.

[25] Heaton E, Voigt T, Long SP. A quantitative review comparing the yields of two candidate C_4 perennial biomass crops in relation to nitrogen, temperature and water. Biomass Bioenerg. 2004;27(1):21-30.

[26] Mariani C, Cabrini R, Danin A, Piffanelli P, Fricano A., Gomarasca S, et al. Origin, diffusion and reproduction of the giant reed (*Arundo donax* L.): a promising weedy energy crop. Ann Appl Biol. 2010;157(2):191–202.

[27] Zhang Y, Zapala J, Jakubowski AR, Price DL, Acharya A, Wei Y, et al. Natural hybrids and gene flow between upland and lowland switchgrass. Crop sci. 2011;51(6):2626-41.

[28] Balogh E, Herr JM, Czakó M, Márton L. Defective development of male and female gametophytes in *Arundo donax* L. (Poaceae). Biomass Bioenerg. 2012;45:265–9.

[29] Pilu R, Manca A, Landoni M, Agrarie S. *Arundo donax* L. as an energy crop : pros and cons of the utilization of this perennial plant. Maydica. 2013;58.

[30] McLaughlin SB, Walsh ME. Evaluating environmental consequences of producing herbaceous crops for bioenergy. Biomass Bioenerg. 1998;14(4):317-24.

[31] Angelini GL, Ceccarini L, Bonari E. Biomass yield and energy balance of giant reed (*Arundo donax* L.) cropped in central Italy as related to different management practices. Eur J Agron. 2005;22(4):375-89.

[32] Rossa B, Tuffers AV, Willert DJ Von. *Arundo donax* L. (Poaceae) - a C_3 species with unusually high photosynthetic capacity. Bot Acta. 1998;111(3):216–21.

[33] Barney JN, Mann JJ, Kyser GB, Blumwald E, Van Deynze A, DiTomaso JM. Tolerance of switchgrass to extreme soil moisture stress: Ecological implications. Plant Sci. 2009;177(6):724-32.

[34] Beale CV, Morison JIL, Long SP. Water use efficiency of C_4 perennial grasses in a temperate climate. Agri Forest Meteorol. 1999;96(1-3):103-15.

[35] Nackley LL, Vogt KA, Kim SH. *Arundo donax* water use and photosynthetic responses to drought and elevated CO_2. Agr Water Manage. 2014;136:13–22.

[36] Hoagland DR, Arnon DJ. The water-culture method for growing plants without soil. California Agric Exp Station Circ. 1950;347:1-32.

[37] McMurtrie RE, Wang Y-P. Mathematical models of the photosynthetic responses of tree stands to rising CO_2 concentrations and temperatures. Plant Cell Environ.1993;16(1):1-13.

[38] Nogués S, Baker NR. Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation. J Exp Bot. 2000;51(348):1309-17.

[39] Sinclair WB, Bartholomew ET, Ramsey RC. Analysis of the organic acids of orange juice. Plant Physiol. 1945;20(1):3-18.

[40] Papazoglou EG, Karantounias GA, Vemmos SN, Bouranis DL. Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. Environ Inter. 2005;31(2):243 –9.

[41] Albaugh JM, Domec JC, Maier CA, Sucre EB, Legget ZH, King JS. Gas exchange and stand-level estimates of water use and gross primary productivity in an experimental pine and switchgrass intercrop forestry system on the Lower Costal Plain of North Carolina, USA. Agr Forest Meteorol. 2014;192-193:27-40.

[42] Pinto H, Sharwood RE, Tissue DT, Ghannoum O. Photosynthesis of C_3, C_3-C_4 and C_4 grasses at glacial CO_2. J Exp Bot. 2014;65(13):3669-81.

[43] Osmond B. Functional significance of different pathways of CO_2 fixation in photosynthesis. In: Lange OL, Nobel PS, Osmond B, Ziegler H, editors. Physiological plant ecology II. Encyclopedia of plant physiology New Series. Berlin: Springer-Verlag; 1982. p. 479-549.

[44] Mann JJ, Barney JN, Kyser GB, Di Tomaso JM. *Miscanthus* × *giganteus* and *Arundo donax* shoot and rhizome tolerance of extreme moisture stress. GCB Bioenergy. 2013;5(6):693–700.

[45] Maxwell K, Johnson GN. Chlorophyll fluorescence: A practical guide. J Exp Bot. 2000;51(345):659-68.

[46] Pearcy RW, Ehleringer JR. Comparative ecophysiology of C_3 and C_4 plants. Plant Cell Environ. 1984; 7(1):1-13.

[47] Lambers H, Chapin III FS, Pons TL. Plant physiological ecology. New York: Springer Verlag; 1998.

[48] Mack RN. Evaluating the credits and debits of a proposed biofuel species: giant reed (*Arundo donax*). Weed Sci. 2008;56(6):883-8.

[49] Stroup JA, Sanderson MA, Muir JP, McFarland MJ, Reed RL. Comparaison of growth and performance in upland and lowland switchgrass types to water and nitrogen stress. Bioresource Technol. 2003;86(1):65-72.

[50] Centritto M, Loreto F, Chartzoulakis K. The use of low [CO_2] to estimate diffusional and non-diffusional limitations of photosynthetic capacity of salt-stressed olive saplings. Plant Cell Environ. 2003;26(4):585–94.

[51] Ghannoum O', Conroy JP, Driscoll SP, Paul MJ, Foyer CH, Lawlor DW. Non-stomatal limitations are responsible for drought-induced photosynthetic inhibition in four C_4 grasses. New Phytol. 2003;159(3):599-608.

[52] Long SP, Incoll LD, Woolhouse HW. C_4 photosynthesis in plants from cool temperate regions, with particular reference to *Spartina townsendii*. Nature. 1975;275(5527):622-24.

[53] Sage RF. Environmental and evolutionary preconditions for the origin and diversification of the C_4 photosynthetic syndrome. Plant Biol. 2001;3(3):202-13.

[54] Stepien P, Klobus G. Antioxidant defense in the leaves of C_3 and C_4 plants under salinity stress. Physiol Plantarum. 2005;125(1):31-40.

[55] Spencer D, Sher A, Thornby D, Liow P, Ksander G, Tan W. Non-destructive assessment of *Arundo donax* (Poaceae) leaf quality. J Freshw Ecol. 2007;22(2):277-85.

[56] Sanchez RA, Hall AJ, Trapani N, de Hunau RC. Effects of water stress on the chlorophyll content, nitrogen level and photosynthesis of leaves of two maize genotypes. Photosynth Res. 1983;4(1):35-47.

[57] Chutia J, Borah SP. Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice (*Oryza sativa* Linn.) genotypes of Assam, India. II. Protein and proline status in seedlings under PEG induced water stress. Am J Plant Sci. 2012;3(7):971-80.

[58] Sevengor S, Yasar F, Kusvuran S, Ellialtioglu S. The effect of salt stress on growth, chlorophyll content lipid peroxidation and antioxidative enzymes of pumpkin seedling. Afr J Agri Res. 2001;6(21):4920-4.

[59] Santos CV. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Sci Hort. 2004;103(1):93-9.

[60] Jagtap V, Bhargava S, Streb P, Feierabend J. Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) Moench. J Exp Bot. 1998;327(49):1715-21.

[61] Berg LV, Zeng YJ. Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000. S Afr J Bot. 2006;72(2):284-6.

[62] Liu F, Stützel H. Leaf expansion, stomatal conductance, and transpiration of vegetable Amaranth (Amaranthus sp.) in Response to Soil Drying. J Am Soc Hortic Sci. 2002;127(5):878–83.

[63] Blum A. Drought resistance, water-use efficiency and yield potential — are they compatible, dissonant, or mutually exclusive? Aust J Agr Res. 2005;56(11):1159–68.

[64] Barhanni MJ, Barhami H, Haghighi AAK. Effect of water stress on ten forage grasses native or introduced to Iran. JSGS. 2010;56(1):1-5.

[65] Yousfi S, Serret MD, Araus JL. Shoot $\delta^{15}N$ gives a better indication than ion concentration or $\Delta^{13}C$ of genotypic differences in the response of durum wheat to salinity. Funct Plant Biol. 2009;36(2):144-55.

[66] Erice G, Louahlia S, Irigoyen JJ, Sanchez-Diaz M, Avice JC. Biomass partitioning, morphology and water status of four alfalfa genotypes submitted to progressive drought and subsequent recovery. J Plant Physiol. 2010;167(2):114–20.

[67] Björkman O. Responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, editors. Physiological plant ecology. I. Responses to the physical environment, Vol. 12A. Berlin: Springer-Verlag; 1981. p. 57-107.

[68] Pompeiano A, Vita F, Miele S, Guglielminetti L. Freeze tolerance and physiological changes during cold acclimation of giant reed [*Arundo donax* (L.)]. Grass Forage Sci. 2015;70(1):168-175.

[69] Hope HJ, McElroy A. Low-temperature tolerance of switchgrass (*Panicum virgatum* L.). Can J Plant Sci. 1990;70(4):1091-6.

[70] Sage RF, McKown AD. Is C_4 photosynthesis less phenotypically plastic tan C_3 photosynthesis? J Exp Bot. 2006;57(2):303-17.

[71] Caldwell M, Osmond C, Nott D. C_4 pathway photosynthesis at low temperature in cold-tolerant *Atriplex* species. Plant Physiol. 1977; 60(1):66-71.

[72] Jones MB. C4 species as energy crops. In: Raghavendra AS, Sage RF, editors. C_4 Photosynthesis and related CO_2 concentrating mechanisms. Dordrecht: Springer; 2011. p. 379-397.

[73] Gutiérrez M, Gracen V, Edwards G. Biochemical and cytological relationships in C_4 plants. Planta. 1974;119(4):279-280.

[74] Hatch M, Osmond C. Compartamentation and transport in C_4 photosynthesis. In: Stocking C and Heber U, editors. Encyclopedia of Plant Physiology III. Berlin: Springer Verlag; 1976. p. 144-184.

[75] Chinthapalli B, Murmu J, Raghavendra AS. Dramatic difference in the responses of phosphoenolpyruvate carboxylase to temperature in leaves of C_3 and C_4 plants. J Exp Bot. 2002; 54(383):707-14.

[76] Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI. Photoinhibition of photosystem II under environmental stress. Biochim Biophys Acta. 2007;1767(6):414-21.

[77] Byrd GT, Sage RF, Brown RH. A comparison of dark respiration between C_3 and C_4 plants. Plant Physiol. 1992;100(1):191-8.

TABLES AND FIGURES

Table 1: Biomass parameters in *A. donax* and *P. virgatum* under four different treatments[b] at the end of Exp. 1 (T60).

A. donax [a]	WW S-			WS S-			WW S+			WS S+		
H (m)	0.57	± *0.14*	Aα	0.69	± *0.12*	Aα	0.55	± *0.17*	Aα	0.43	± *0.11*	Aα
NL	22.3	± *0.9*	Aβ	5.3	± *1.8*	Bβ	9.0	± *1.55*	Bβ	7.3	± *2.7*	Bβ
NS	2.7	± *0.3*	Aβ	1.2	± *0.3*	Aβ	2.0	± *0.6*	Aβ	2.0	± *0.4*	Aβ
LA (m^2)	0.014	± *0.002*	Aβ	0.003	± *0.000*	Cα	0.008	± *0.000*	Bα	0.005	± *0.001*	BCα
SA (m^2)	0.004	± *0.001*	Aβ	0.002	± *0.000*	ABβ	0.003	± *0.001*	ABβ	0.002	± *0.000*	Bβ
GLP (%)	95.9	± *2.2*	Aα	31.8	± *9.4*	Bβ	47.1	± *5.3*	Bβ	47.7	± *8.3*	Bβ
YLP (%)	0.0	± *0.0*	Bα	8.1	± *4.1*	ABα	11.7	± *3.5*	Aα	14.2	± *2.2*	Aα
DLP (%)	4.1	± *2.2*	Cα	60.1	± *8.4*	Aα	41.2	± *7.4*	ABα	39.1	± *4.4*	Bα
TDW (kg)	0.022	± *0.005*	Aβ	0.011	± *0.001*	Bα	0.019	± *0.002*	ABα	0.011	± *0.001*	Bα
S/R	3.3	± *0.2*	Bα	2.1	± *0.5*	Cα	4.0	± *0.1*	Aα	2.8	± *0.4*	BCα
LAI	12.7	± *2.1*	Aβ	2.4	± *0.2*	Dβ	6.7	± *0.3*	Bα	4.3	± *0.5*	Cα
LMA ($kg\ m^{-2}$)	51.4	± *5.1*	Cα	76.2	± *6.3*	Aα	65.2	± *3.6*	Bα	69.0	± *5.4*	ABβ
SLA ($m^2\ kg^{-1}$)	19.8	± *1.8*	Aα	13.3	± *1.2*	Bα	15.5	± *1.1*	Bα	14.8	± *1.2*	Bα
LAR ($m^2\ kg^{-1}$)	6.8	± *0.7*	Aα	2.7	± *0.5*	Cα	4.1	± *0.3*	Bα	4.5	± *0.4*	Bα
LWR ($kg\ kg^{-1}$)	0.34	± *0.02*	Aα	0.20	± *0.04*	Bα	0.27	± *0.01*	ABβ	0.31	± *0.03*	Aα
P. virgatum [a]	WW S-			WS S-			WW S+			WS S+		
H (m)	0.73	± *0.03*	Aα	0.58	± *0.05*	Bα	0.57	± *0.05*	Bα	0.49	± *0.05*	Bα
NL	68.5	± *5.0*	Aα	7.8	± *0.6*	Cα	30.8	± *6.2*	Bα	14.8	± *0.8*	Cα
NS	19.0	± *1.4*	Aα	10.0	± *1.5*	BCα	11.5	± *1.3*	Bα	6.5	± *0.3*	Cα
LA (m^2)	0.025	± *0.001*	Aα	0.004	± *0.001*	Cα	0.009	± *0.001*	Bα	0.002	± *0.000*	Cβ
SA (m^2)	0.019	± *0.001*	Aα	0.006	± *0.000*	BCα	0.009	± *0.001*	Bα	0.004	± *0.001*	Cα
GLP (%)	96.8	± *3.2*	Aα	67.2	± *5.0*	Cα	80.1	± *2.2*	Bα	68.1	± *3.7*	Cα
YLP (%)	0.0	± *0.0*	Aα	0.0	± *0.0*	Aβ	0.0	± *0.0*	Aβ	0.0	± *0.0*	Aβ
DLP (%)	3.2	± *3.2*	Cα	32.8	± *5.0*	Aβ	19.9	± *2.2*	Bβ	31.9	± *3.7*	Aα
TDW (kg)	0.040	± *0.004*	Aα	0.013	± *0.001*	Cα	0.021	± *0.003*	Bα	0.012	± *0.000*	Cα
S/R	2.5	± *0.4*	Aα	1.4	± *0.2*	Bα	2.5	± *0.1*	Aβ	1.3	± *0.2*	Bβ
LAI	22.0	± *0.9*	Aα	3.6	± *0.6*	Cα	7.9	± *0.9*	Bα	2.0	± *0.3*	Dβ
LMA ($kg\ m^{-2}$)	47.7	± *2.8*	Cα	64.2	± *8.5*	Bα	70.3	± *3.5*	Bα	125.5	± *18.1*	Aα
SLA ($m^2\ kg^{-1}$)	21.2	± *1.2*	Aα	16.1	± *1.9*	Bα	14.3	± *0.7*	Bα	10.4	± *2.3*	Cβ
LAR ($m^2\ kg^{-1}$)	6.5	± *0.6*	Aα	3.2	± *0.7*	Cα	4.2	± *0.1*	Bα	1.9	± *0.3*	Dβ
LWR ($kg\ kg^{-1}$)	0.30	± *0.02*	Aα	0.19	± *0.02*	Bα	0.30	± *0.02*	Aα	0.19	± *0.02*	Bβ

[a] Values are the mean (n=3) ± SE and were analysed with an ANOVA Tukey between treatments (capital letters) and between ecotypes (Greek letters).

[b] Well-watered without salinity (WW S-); low-watered without salinity (WS S-); well-watered with salinity (WW S+) and low-watered with salinity (WS S+).

Table 2: Photosynthesis parameters of *A. donax* and *P. virgatum* under three different treatments[c] of Exp. 2.

A. donax [a]		A_{sat}[a,b]			$V_{c,max}$[a,b]			J_{max}[a,b]			l[a,b]		
Control		28.3	± *1.9*	A	180.5	± *19.4*	A	409.8	± *38.6*	A	12.8	± *1.4*	A
	3d	14.5	± *1.1*	Bbα	63.5	± *5.4*	Bbα	178.7	± *4.9*	Bbα	17.8	± *2.2*	Baα
	R	18.6	± *1.3*	a	126.4	± *4.1*	a	354.5	± *20.7*	a*	13.0	± *1.6*	b*
AD	6d	11.4	± *0.9*	Cbα	65.7	± *8.9*	Bbα	117.8	± *14.0*	Cbα	12.2	± *0.2*	Aaα
	R	18.0	± *0.3*	a	96.0	± *4.9*	a	381.9	± *10.6*	a*	7.7	± *0.7*	b
	9d	10.0	± *1.2*	Cbα	56.3	± *3.4*	Bbα	45.6	± *3.7*	Dbα	21.5	± *1.2*	Baα
	R	17.4	± *0.7*	a	97.7	± *1.6*	a	364.1	± *14.6*	a*	20.4	± *0.2*	a*
	3d	20.1	± *1.5*	Baβ	102.8	± *10.5*	Bbβ	157.3	± *16.5*	Bbα	26.2	± *1.8*	Baβ
	R	18.5	± *1.7*	b	178.5	± *12.4*	a*	411.3	± *33.6*	a*	12.5	± *1.4*	b*
CD	6d	14.7	± *1.3*	Caβ	87.4	± *10.8*	Caβ	175.1	± *12.9*	Bbβ	18.5	± *0.5*	Cbβ
	R	17.6	± *0.4*	a	70.9	± *1.4*	a	313.1	± *28.0*	a*	23.8	± *0.4*	a
	9d	14.5	± *1.2*	Cbβ	100.8	± *17.3*	Baβ	41.1	± *5.3*	Cbα	17.1	± *1.4*	Caβ
	R	23.4	± *1.0*	a*	91.1	± *8.0*	a	394.6	± *12.6*	a*	7.8	± *1.0*	b
P. virgatum [a]		A_{sat}[a,b]			$V_{c,max}$[a,b]			J_{max}[a,b]			l[a,b]		
Control		24.0	± *2.1*	A	187.4	± *18.5*	A	108.4	± *9.3*	A	10.0	± *1.1*	A
	3d	12.1	± *1.0*	Bbα	46.1	± *5.4*	Bbα	50.9	± *1.7*	Baα	21.2	± *1.3*	Baα
	R	20.8	± *0.4*	a	129.2	± *10.0*	a	52.3	± *2.7*	a	11.4	± *0.7*	b*
AD	6d	2.5	± *0.4*	Cbα	21.5	± *2.0*	Cbα	227.4	± *24.9*	Caα	46.5	± *5.8*	Caα
	R	13.5	± *1.5*	a	56.0	± *4.8*	a	209.6	± *4.2*	a*	13.2	± *1.7*	b*
	9d	5.2	± *0.6*	Dbα	12.1	± *0.8*	Dbα	20.3	± *1.2*	Dbα	6.0	± *0.7*	Dbα
	R	16.6	± *0.4*	a	95.6	± *3.9*	a	70.1	± *7.6*	a	13.9	± *1.5*	a*
	3d	16.1	± *0.8*	Bbβ	63.0	± *1.6*	Bbβ	20.9	± *2.3*	Bbβ	12.3	± *1.1*	Aaβ
	R	23.2	± *0.8*	a*	140.9	± *1.3*	a	124.2	± *9.3*	a*	6.2	± *0.7*	b
CD	6d	18.3	± *0.2*	Caβ	64.3	± *1.1*	Baβ	28.9	± *1.3*	Cbβ	24.4	± *1.4*	Baβ
	R	16.5	± *0.9*	a	63.2	± *4.5*	a	75.9	± *0.6*	a	25.9	± *1.3*	a
	9d	18.9	± *1.3*	Caβ	189.7	± *7.1*	Aaβ*	164.8	± *7.4*	Daβ	10.9	± *1.3*	Aaβ
	R	21.7	± *0.9*	a*	164.1	± *11.1*	a*	93.3	± *4.9*	b*	7.6	± *1.1*	b

[a] Values are the mean (n=6-7) ± SE and were analysed with a Duncan test between time for a same treatment (capital letters), between *R* for a same time (small letters) and between treatments (Greek letters). *Complete *R*.

[b] A_{sat} (µmol CO_2 m^{-2} s^{-1}); $V_{c,max}$ (µmol CO_2 m^{-2} s^{-1}); J_{max} (µmol CO_2 m^{-2} s^{-1}); l (%).

[c] Control conditions, darkness and room temperature (AD) and darkness and cold temperature (CD)

Table 3: Fluorescence parameters of *A. donax* and *P. virgatum* under three different treatments[b] of Exp. 2.

A. donax [a]		F_v/F_m			F_v'/F_m'			ϕ_{PSII}			q_p		
Control		0.80	± *0.00*	A	0.52	± *0.01*	A	0.33	± *0.01*	A	0.64	± *0.00*	A
	3d	0.74	± *0.01*	Bbα	0.34	± *0.02*	Bbα	0.19	± *0.01*	Bbα	0.54	± *0.01*	Bbα
	R	0.80	± *0.01*	a*	0.45	± *0.01*	a	0.28	± *0.01*	a*	0.64	± *0.01*	a*
AD	6d	0.76	± *0.01*	Baα	0.28	± *0.01*	Cbα	0.13	± *0.01*	Cbα	0.46	± *0.01*	Cbα
	R	0.80	± *0.00*	a	0.39	± *0.01*	a	0.21	± *0.01*	a	0.53	± *0.02*	a
	9d	0.80	± *0.01*	Baα	0.31	± *0.01*	Caα	0.12	± *0.02*	Caα	0.37	± *0.04*	Daα
	R	0.77	± *0.00*	a	0.34	± *0.01*	a	0.16	± *0.02*	a	0.45	± *0.05*	a
	3d	0.78	± *0.01*	Abβ	0.44	± *0.01*	Bbβ	0.27	± *0.01*	Bbβ	0.62	± *0.01*	Aaβ
	R	0.81	± *0.00*	a*	0.51	± *0.01*	a	0.34	± *0.01*	a*	0.62	± *0.01*	a*
CD	6d	0.73	± *0.02*	Bbβ	0.37	± *0.02*	Cbβ	0.21	± *0.02*	Caβ	0.55	± *0.02*	Baβ
	R	0.78	± *0.01*	a*	0.42	± *0.01*	a	0.24	± *0.01*	a	0.58	± *0.01*	a
	9d	0.76	± *0.00*	Baα	0.37	± *0.00*	Cbβ	0.21	± *0.00*	Caβ	0.56	± *0.01*	Baβ
	R	0.77	± *0.00*	a	0.40	± *0.01*	a	0.23	± *0.02*	a	0.56	± *0.03*	a
P. virgatum [a]		F_v/F_m			F_v'/F_m'			ϕ_{PSII}			q_p		
Control		0.75	± *0.00*	A	0.35	± *0.01*	A	0.23	± *0.01*	A	0.66	± *0.02*	A
	3d	0.73	± *0.01*	Abα	0.28	± *0.01*	Bbα	0.16	± *0.01*	Bbα	0.58	± *0.02*	Bbα
	R	0.75	± *0.01*	a*	0.35	± *0.00*	a*	0.23	± *0.01*	a*	0.65	± *0.01*	a*
AD	6d	0.71	± *0.00*	Baα	0.17	± *0.00*	Cbα	0.05	± *0.01*	Cbα	0.27	± *0.03*	Cbα
	R	0.70	± *0.00*	a	0.27	± *0.03*	a	0.12	± *0.01*	a	0.41	± *0.03*	a
	9d	0.72	± *0.00*	Bbα	0.21	± *0.00*	Dbα	0.07	± *0.01*	Cbα	0.34	± *0.01*	Dbα
	R	0.74	± *0.00*	a*	0.29	± *0.01*	a	0.14	± *0.01*	a	0.49	± *0.02*	a
	3d	0.70	± *0.01*	Bbβ	0.27	± *0.00*	Bbα	0.11	± *0.01*	Bbβ	0.41	± *0.04*	Bbβ
	R	0.74	± *0.00*	a*	0.34	± *0.01*	a*	0.22	± *0.01*	a*	0.66	± *0.01*	a*
CD	6d	0.63	± *0.01*	Cbβ	0.22	± *0.01*	Cbβ	0.07	± *0.01*	Cbα	0.32	± *0.03*	Cbα
	R	0.73	± *0.02*	a*	0.27	± *0.01*	a	0.15	± *0.02*	a	0.52	± *0.04*	a
	9d	0.65	± *0.02*	Cbβ	0.28	± *0.00*	Bbβ	0.13	± *0.01*	Bbβ	0.47	± *0.05*	Bbβ
	R	0.75	± *0.00*	a*	0.35	± *0.01*	a*	0.20	± *0.01*	a	0.56	± *0.01*	a

[a] Values are the mean (n=6-7) ± SE and were analysed with a Duncan test between time for a same treatment (capital letters), between *R* for a same time (small letters) and between treatments (Greek letters). * Complete *R*.

[b] Control conditions, darkness and room temperature (AD) and darkness and cold temperature (CD)

Table 4: Representation of O_2 consumption (µmol O_2 m^{-2} s^{-1}), CO_2 production (µmol CO_2 m^{-2} s^{-1}) and respiratory quotient (RQ) of *A. donax* and *P. virgatum* under three different treatments[b] in Exp. 2 at 9d.

		O_2 consumption[a]			CO_2 production[a]			RQ[a]		
A. donax	C	0.59	± *0.05*	Aa	0.80	± *0.08*	Aa	1.34	± *0.17*	Aa
	AD	0.13	± *0.01*	Ca	0.08	± *0.01*	Cb	0.62	± *0.05*	Bb
	CD	0.28	± *0.03*	Bb	0.21	± *0.05*	Bb	0.76	± *0.08*	Ba
P. virgatum	C	0.60	± *0.06*	Aa	0.73	± *0.10*	Aa	1.21	± *0.12*	Aa
	AD	0.16	± *0.02*	Ba	0.14	± *0.02*	Ca	0.86	± *0.09*	Ba
	CD	0.54	± *0.02*	Aa	0.49	± *0.06*	Ba	0.91	± *0.12*	Ba

[a] Values are the mean (n=7) ± SE and were analysed with a Duncan test between treatments (capital letters) for the same specie and between species (small letters) for the same treatment.

[b] Control conditions (C), ambient temperature and darkness (AD) and cold temperature and darkness (CD)

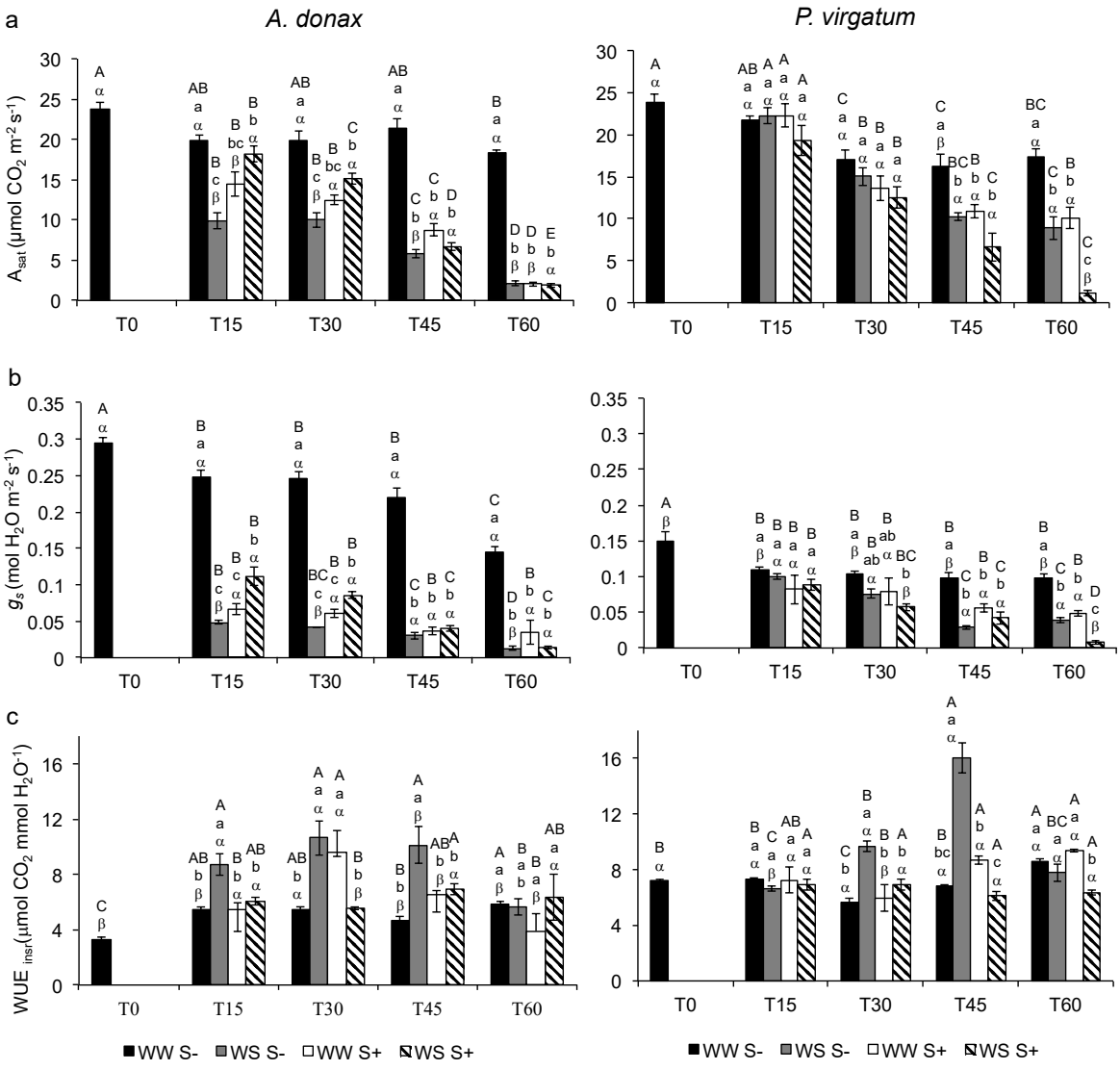


Fig. 1: Representation of A_{sat} (a), g_s (b) and WUE_{inst} (c) values of *A. donax* and *P. Virgatum* during the Exp. 1 (T0, T15, T30, T45, T60) for each treatment: i) well watered without salinity (WW S-); ii) low watered without salinity (WS S-); iii) well watered with salinity (WW S+) and iv) low watered with salinity (WS S+). Values are the mean of four replicates and standard errors (SE) are shown. Data were analysed with an ANOVA Tukey analysis. Different capital letters mean significant differences ($P < 0.05$) between time (T0-T60) for the same species and treatment, different small letters mean significant differences ($P < 0.05$) between treatments for the same species and time and different Greek letters mean significant differences ($P < 0.05$) between species for the same treatment and time.

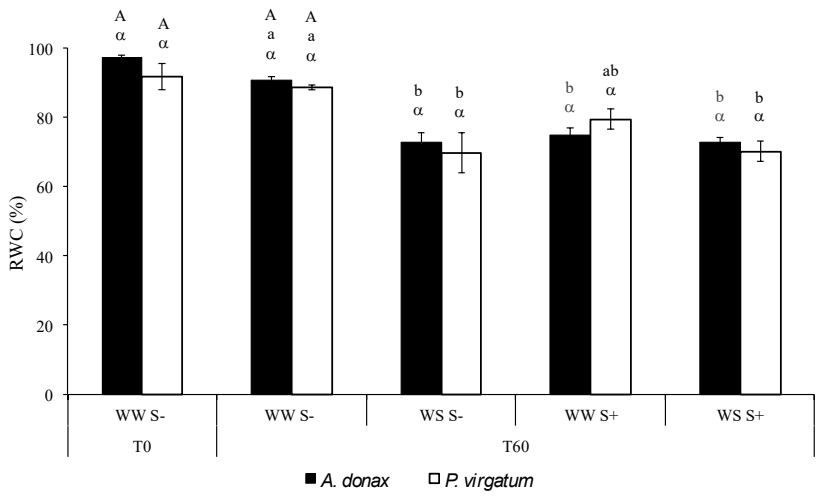


Fig. 2: Changes in relative water content (RWC, %) between the beginning (T0) and the end of the experiment (T60) for both species and treatments (WW S-, WS S-, WW S+ and WS S+). Data are the means of three replicates and the standard errors (SE) are shown. Data were analysed with an ANOVA Tukey analysis. Different capital letters mean significant differences ($P < 0.05$) between time (T0 – T60) for the same species in the control treatment (WW S-), different small letters mean significant differences ($P < 0.05$) between treatments for a same species at T60 and different Greek letters mean significant differences ($P < 0.05$) between species for same treatment and time.

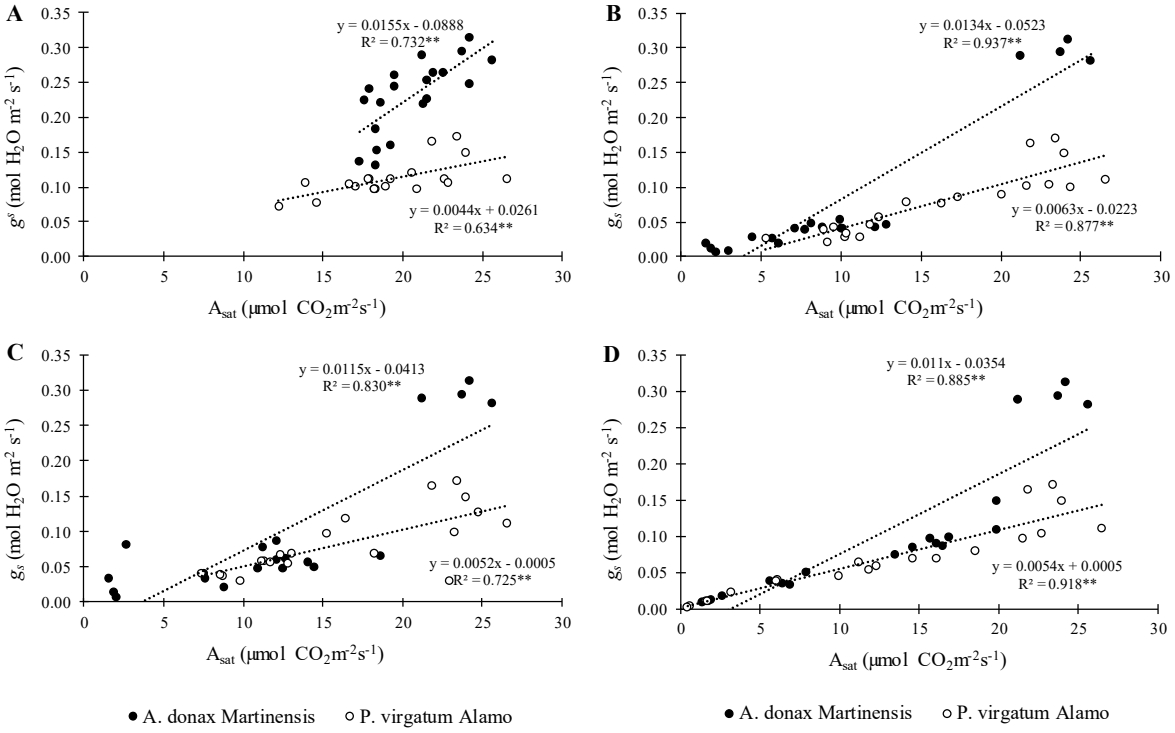
SUPPLEMENTARY MATERIAL

SM 1: Comparison of photosynthesis (a) and fluorescence parameters (b) of *A. donax* and *P. virgatum* at the beginning of Exp. 1 (T0).

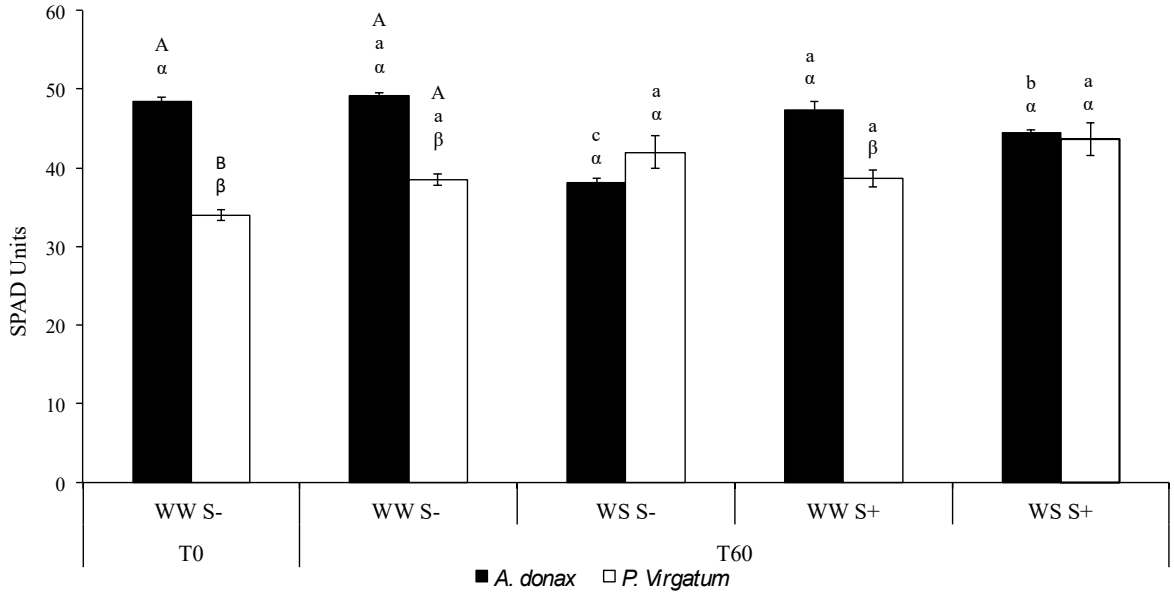
a	A_{sat}[a,b]	g_s[a,b]	$V_{c,max}$[a,b]	J_{max}[a,b]	L[a,b]	T[a,b]	WUE_{inst}
A. donax	23.6	0.295	111.1	263.2	22.6	7.2	3.3
	± 1.3	*± 0.009*	*± 5.5*	*± 32.5*	*± 2.2*	*± 0.3*	*± 0.3*
P. virgatum	23.9	0.149	139.9	113.6	7.7	3.3	7.16
	± 1.4	*± 0.018*	*± 3.0*	*± 3.5*	*± 2.6*	*± 0.1*	*± 0.2*
Sig.	n.s	**	**	**	*	***	***
b	F_v/F_m[a]	F_v'/F_m'[a]	ϕ_{PSII}[a]	q_p[a]	NPQ[a]		
A. donax	0.775	0.499	0.305	0.610	1.4		
	± 0.003	*± 0.002*	*± 0.017*	*± 0.036*	*± 0.02*		
P. virgatum	0.748	0.361	0.241	0.670	1.8		
	± 0.005	*± 0.006*	*± 0.013*	*± 0.033*	*± 0.08*		
Sig.	*	***	*	n.s	*		

[a] Values are the mean (n=3) ± SE and were analysed with a Tukey test. Asterisk represents differences between species (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) and n.s represent non-significant differences ($P > 0.05$).

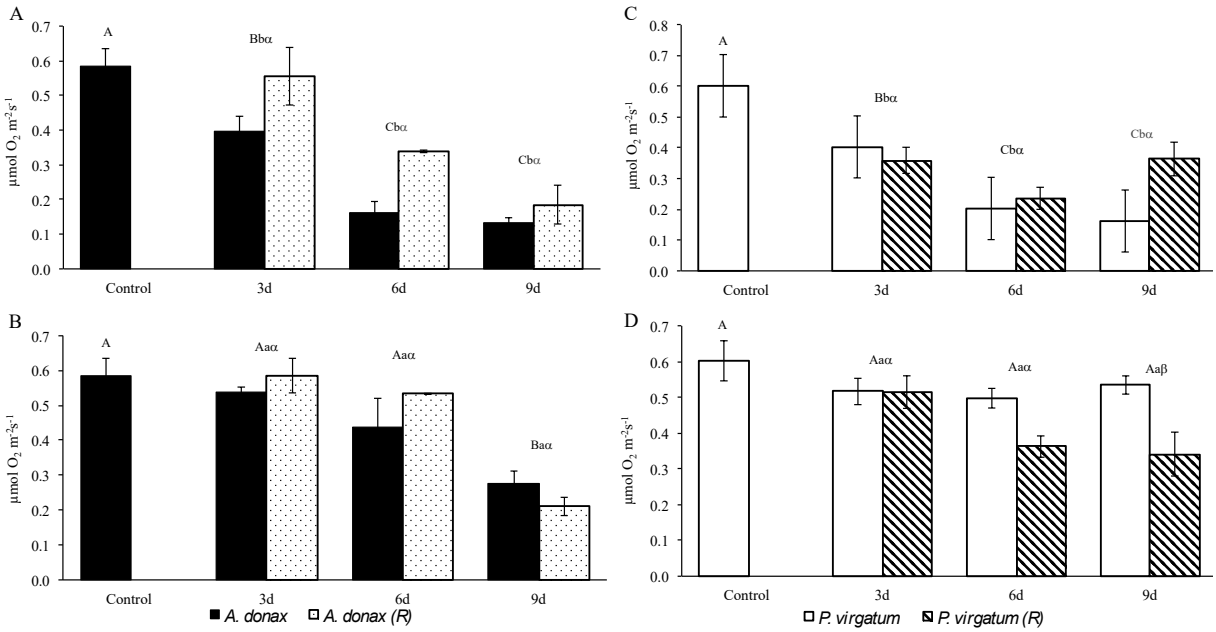
[b] A_{sat} (µmol CO_2 m^{-2} s^{-1}); g_s (mol H_2O m^{-2} s^{-1}); $V_{c,max}$ (µmol CO_2 m^{-2} s^{-1}); J_{max} (µmol CO_2 m^{-2} s^{-1}); l (%), T (mmol H_2O m^{-2} s^{-1}).



SM 2: Bivariate correlations between A_{sat} (µmol CO_2 m^{-2} s^{-1}) and g_s (mol H_2O m^{-2} s^{-1}) for each treatment: (i) well watered with non-saline solution (WW S-; A), (ii) water stress with non-saline solution (WS S-; B), (iii) well watered with saline solution (WW S+; C) and iv) water stress with saline solution (WS S+; D) during the experiment (T0 - T60). Line equation, Pearson correlation coefficient and bilateral signification are shown for both species: *A. donax* (closed circles) and *P. virgatum* (open circles). ** Mean that correlation is significant at level 0.05 (bilateral) and 0.01 (bilateral) respectively.



SM 3: Changes in chlorophyll content (SPAD units) between the beginning (T0) and the end of the experiment (T60) for both species and treatments (WW S-, WS S-, WW S+ and WS S+) in Exp. 1. Data are the means of three replicates and the standard errors (SE) are shown. Data were analysed with an ANOVA Tukey analysis. Different capital letters mean significant differences ($P < 0.05$) between time (T0 – T60) for the same species in the control treatment (WW S-), different small letters mean significant differences ($P < 0.05$) between time for a same species and different Greek letters mean significant differences ($P < 0.05$) between species for same treatment.



SM 4: Mitochondrial respiration rates (µmol O_2 m^{-2} s^{-1}) of *A. donax* and *P. virgatum* under darkness and room temperature (AD; A and C) and darkness and cold temperature (CD, B and D) and recovery (*R*) of Exp. 2. Values are the mean (n=7) ± SE and were analysed with Duncan test between time (capital letters), treatments (small letters) and between species (Greek letters) ($P < 0.05$).