

Salinity and Water Stress Effects on Biomass Production in Different *Arundo donax* L. Clones

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Abstract Perennial rhizomatous grasses are regarded as leading energy crops due to their environmental benefits and their suitability to regions with adverse conditions. In this paper, two different experiments were carried out in order to study the salinity (*S*) and water stress (*WS*) effects on biomass production in giant reed (*Arundo donax* L.). In Experiment 1, eight clones of giant reed were subjected to four salinity (*S*) and water stress (*WS*) treatments: (1) well watered with non-saline solution, (2) water stress with non-saline solution, (3) well watered with saline solution and 4) water stress with saline solution. In Experiment 2, five clones of giant reed were subjected to increasing *S* levels in two locations: University of Catania (UNICT-Italy) (1) well watered with non-saline solution and (2) well watered with mild saline solution; and University of Barcelona (UB-Spain) (3) well watered with non-saline solution and (4) well watered with severe saline solution. Photosynthetic and physiological parameters as well as biomass production were measured in these plants. According to our data, giant reed seems to be more tolerant to *S* than *WS*. Both stresses mainly affected stomatal closure to prevent dehydration of the plant, eventually decreasing the photosynthetic rate. The differential performance of the giant reed clones was ranked according to their tolerance to *S* and *WS* by using the Stress Susceptibility Index. ‘Agrigento’

was the most *WS* resistant clone and ‘Martinensis’ was the most *S* resistant. ‘Martinensis’ and ‘Piccoplant’ were found to be the most suitable clones for growing under both stress conditions. Moreover, ‘Fondachello’, ‘Cefalú’ and ‘Licata’ were the most resistant clones to increasing *S* levels.

Keywords *Arundo donax* L · Biomass · Water stress · Salinity stress · Photosynthesis · Stress susceptibility index

Abbreviations

A_{sat}	light saturated net CO ₂ assimilation rate (μmol m ⁻² s ⁻¹)
DLP	complete dry leaves percentage (%)
DM	dry matter (g)
FC	field capacity
g_s	stomatal conductance (mol m ⁻² s ⁻¹)
gLA	green leaf area (m ²)
GLP	complete green leaves percentage (%)
H	height (cm)
LAR	leaf area ratio (m ² Kg ⁻¹)
LWR	leaf weight ratio (Kg Kg ⁻¹)
NL	number of leaves
NS	number of stems
PPFD	photosynthetic photon flux density
PRG	perennial rhizomatous grasses
RWC	relative water content (%)
<i>S</i>	salinity
SA	stem area (m ²)
SLA	specific leaf area (m ² Kg ⁻¹)
S/R	shoot/root ratio (g g ⁻¹)
SSI	stress susceptibility index
TDW	total dry weight (g)
<i>WS</i>	water stress
YLP	complete yellow leaves percentage (%)

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Introduction

Salinization is considered by the United Nations Environmental Programme (UNEP) to be the second largest cause of land loss [1]. According to the Food and Agriculture Organization (FAO) of the United Nations, it is estimated that 34 million hectares (11 % of the irrigated area) are affected by some level of salinization [1]. Therefore, salinity is one of the most important challenges facing the supply of food to the world's population in the future [2]. On the other hand, increases in the intensity and/or duration of water stress may occur across the globe due to recent observed changes in climate, especially in the Mediterranean region and West Africa [3, 4]. According to the FAO [5], in 2011, 38.47 % of the world's total land area was dedicated to agriculture and only 6.48 % of this agricultural area was equipped for irrigation. Moreover, it is very likely that arid and semi-arid regions, where evapotranspiration dominates, are becoming more saline [3]. Both salinity [6] and water stress [7] are among the most important environmental limitation affecting plant growth, development and crop yield in Mediterranean environments. According to Munns [8], physiological plant responses to water stress and salt stress have much in common; however, the mechanisms are extremely complex and vary with plant species as well as with the degree and time of exposure to stress.

Photosynthesis, together with cell growth, has been reported among the primary processes affected by salinity or water stress [9]. Decreases in the photosynthetic rate under both stresses may be directly associated with a decrease in CO₂ availability related to stomatal closure [10], or be due to alterations of photosynthetic metabolism [11]. Therefore, photosynthetic performance involves a highly complex mechanism, where limitations are taking place at different sites of the cells and leaves are interacting on different time scales. At the same time, a greater control of transpiration water loss is achieved by reducing the leaf expansion rate, preventing dehydration [12] and acting as the first step in the process of stress acclimatisation [9]. Changes in plant morphological components have also been reported; for example, a decrease in the leaf area ratio (LAR) and specific leaf area (SLA) [13]. Stress tolerance of a plant species is usually determined by the plant's genes and also by morphological, phenological, physiological and biochemical traits [14]. Therefore, measurements of different physiological processes during the plant's response to stress provide important information about the mechanism of the plant that are intended to remove or to reduce the harmful effects of stress in the plant tissues.

Nowadays, there is a need to develop new energy sources that will be able to assume the increasing global energy consumption, which is set to increase 20.2 % from 2010 to 2020 and 30.2 % from 2020 to 2040 [15], due to the significant rise in population, which is projected to reach 9.6 billion by 2050 [16]. Moreover, there is a tendency for these new energy

sources to be respectful of the environment as a way to slow the progression of negative effects of climate change (e.g., drought, sea level rise, global warming). Within these new energy sources, the European Union is interested in developing sustainable agricultural systems to produce non-food energy crops using marginal lands and low input cultivation techniques [4].

At present, plants that are able to grow in degraded areas, including those experiencing water stress, heat, cold and soil salinity, are being extensively investigated as bioenergy feedstocks [4, 17–24]. Indeed, the cultivation of energy crops on arable land has raised a number of concerns regarding land use change (both direct and indirect land use change), because these crops should be grown on the so-called 'marginal lands' to ensure biomass production without encroaching on agricultural lands [19].

Nowadays, research has been increasingly directed towards the use of giant reed as a source of lignocellulosic biomass in warm-temperate and semi-arid environments [17, 19, 25]. Giant reed is a C₃ perennial rhizomatous grass (PRG) belonging to the *Poaceae* family. It has been reported that giant reed has a high photosynthetic potential [26] and a relatively high transpiration rate; therefore, it requires more water to produce the same biomass as many C₄ biomass crops [27, 28]. Related to yield, the greater biomass productivity of giant reed is also achieved under low input conditions. In a semi-arid Mediterranean environment, giant reed increased production from 11.0 Mg DM ha⁻¹ under rainfed and unfertilised conditions to 28.9 Mg DM ha⁻¹ with 100 % maximum evapotranspiration restitution and 120 kg N ha⁻¹ [19]. Moreover, high yields under high salinity conditions have been reported in giant reed and it have been classified as a halophyte [29]. In addition, due to its high hemicellulose, cellulose and lignin contents, this crop can be used for the production of heat and electricity by direct combustion or the production of biogas and second generation bioethanol [30–33], making this crop a promising source for biomass production.

Therefore, our study seeks to address the effects of two of the most important abiotic stresses, salinity (*S*) and water stress (*WS*), in *Arundo donax* L. (giant reed), and we hypothesised that this species is one of the most promising bioenergy crops for feedstock production in the Mediterranean area [17]. This hypothesis is based on previous studies, where it has been classified as a salt tolerant species [29], well adapted to semi-arid environments [17, 19]. Given the aforementioned advantages, we consider it important to classify giant reed according to its tolerance or sensitivity to the two main constraints that often occur in Mediterranean marginal areas: salinity and water stress. The use of a stress susceptibility index (SSI) has been considered as a good tool to perform this task [34].

The aim of the present work was to study the effect of salinity and water stress on plant performance in different

giant reed clones. To this end, two separate experiments were carried out. Firstly, a study with both stresses was performed (Experiment 1). This experiment aimed to determine the effects of salinity and water stress on physiological performance (e.g., photosynthetic capacity, chlorophyll content, relative water content and biomass yield) on eight different giant reed clones. Moreover, a stress index was proposed in order to classify clones according to their response to salinity and water stress in plants grown in controlled environmental conditions (greenhouse). Secondly, we focused on the effect of salinity on the physiological performance of five different clones of giant reed, so different salinity levels were compared in two different locations (Experiment 2) in order to corroborate the salinity tolerance for these clones at increasing salinity levels.

Materials and Methods

Plant Material

The giant reed (*Arundo donax* L.) clones used in Experiments 1 and 2 are shown in Table 1. Five of the clones used in Experiments 1 and 2 (namely ‘Fondachello’, ‘Tortorici’, ‘Cefalú’, ‘Agrigento’ and ‘Licata’) were collected from the Experimental Fields at University of Catania (Italy) (hereinafter referred as UNICT) (10 m a.s.l., 37°24’N, 15°03’E), and their sites of origin are reported in Cosentino et al. [35]. Two clones (namely ‘Martinensis’ and ‘Granadensis’) used in Experiment 1 were collected in different riparian areas of Spain, and a commercial clone (‘Piccoplant’) used in Experiment 1 was provided by a private company (Piccoplant, Oldenburg, Germany).

In order to reduce heterogeneity at the beginning, careful multiplication of rhizomes was done to a similar fresh weight (i.e., the initial fresh weight ranged from 18.3±1.4 g in Piccoplant to 54.6±6.5 g in Agrigento in Experiment 1, and from 25.4±3.6 g in Tortorici to 52.8±2.2 g in Licata in Experiment 2). The initial weight difference was imposed by the dimension and position of the single visible bud per rhizome.

Experimental Design

Experiment 1: Screening for Salinity and Water Stress

Plants were obtained from multiplication of rhizomes during January 2013. Plantlets were grown in a greenhouse at the Experimental Field Service of University of Barcelona (Barcelona, Spain) in 5 L plastic pots containing peat:perlite:vermiculite (3:1:1), and irrigated with a complete Hoagland solution [36]. The average temperature and vapor pressure deficit (VPD) during the growth period was 25/15 °C 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. The experiment was carried out from April to June 2013.

Four months after planting, plants were separated into four treatments. Half of the plants were well watered at 100 % field capacity (FC) and half of the plants (randomly selected) were exposed to water stress (*WS*) conditions (with water withholding) maintaining field capacity at 25 %. Then, the well watered and *WS* plants were equally divided between salinity (*S*; 16 mS cm⁻¹ Hoagland solution) and non-saline conditions (1 mS cm⁻¹ Hoagland solution). Saline solution was prepared by adding NaCl (PANREAC, 99 % sodium chloride) to a complete Hoagland solution until it reached the appropriate saline concentration, as checked by a conductivity meter (Hanna Instruments, Romania). Consequently, a total of three plants per clone were subjected to the following treatments: (1) well watered with non-saline solution (*WW S*-), (2) *WS* with non-saline solution (*WS S*-), (3) well watered with saline solution (*WW S*+) and (4) *WS* with saline solution (*WS S*+). At the beginning of the experiment (*T*₀), plants had two stems, a plant height from 30 to 60 cm and between 6 and 8 fully collared leaves.

Plants were subjected to these treatments for 2 months and parameters were measured in each plant every 15 days (*T*₁₅, *T*₃₀, *T*₄₅) until the end of the experiment (i.e., *T*₆₀).

Experiment 2: Screening for Increasing Salinity Levels

The screening of mild *S* stress was performed at UNICT (Italy) from May to December 2012 in pots with a diameter

Table 1 Name, place of collection and additional information for the eight clones of *Arundo donax* L. used in both experiments (m a.s.l. = meters above sea level)

Ecotype	Place of collection	Additional information	Experiment
Piccoplant	Oldenburg (Germany)	Commercial ecotype	1
Martinensis	San Martí Sarroca (Catalonia, Spain)	291 m a.s.l.	1
Granadensis	Granada (Andalusia, Spain)	773 m a.s.l.	1
Fondachello	East Sicily (Italy)	1 m a.s.l.	1 and 2
Tortorici	North of Sicily (Italy)	450 m a.s.l.	1 and 2
Cefalú	North of Sicily (Italy)	16 m a.s.l.	1 and 2
Agrigento	South of Sicily (Italy)	230 m a.s.l.	1 and 2
Licata	South of Sicily (Italy)	8 m a.s.l.	1 and 2

of 24 cm and with a capacity of 9.5 L, arranged in a randomised block experimental design with three replications. The pots arranged in an open space without any control of temperature, humidity, VPD or other environmental variables. During the experimental period, monthly maximum and minimum temperatures increased from May (25.9 and 10.7 °C, respectively) to June (32.2 and 14.7 °C) and peaked in July and August (35.7 and 19.1 °C, in the average) to decrease in September (31.9 and 17.2 °C), October (27.1 and 14.7 °C), November (22.3 and 10.8 °C) and the first 10 days of December (15.1 and 5.1 °C), when the harvest was carried out.

The substrate used was sphagnum peat with the following characteristics: 34 % C_{org} , N_{org} 0.2 %, dry substance 68 %, pH 5.0 to 6.5. Two treatments were studied: (1) Control (UNICT S0), where plants were watered with tap water (no NaCl added); and (2) mild *S* stress (UNICT S4) where plants were watered with a saline solution of 4 mS cm^{-1} . At each irrigation time, NaCl was added to 17 L tap water for a total amount of 21 ± 1.5 g of NaCl for S4 treatment, and the values were checked by means of a Eutech Cond 6+ conductivity meter (Thermo Fisher Scientific Inc.). All pots were maintained in well-watered condition with irrigation supplied up to FC twice a week.

The screening of severe *S* stress was performed at the greenhouse of the Experimental Fields of the University of Barcelona (Spain) (hereinafter referred as UB) from April to June 2013. Temperature was controlled daily at the greenhouse (25/18 °C day/night). Two treatments were studied: (1) Control (UB S0), where plants were watered with 1 mS cm^{-1} Hoagland solution (no salt added); and (2) severe *S* stress (UB S16), where plants were watered with a saline solution of 16 mS cm^{-1} . Growing conditions are the same as reported above for Experiment 1.

Measurements

Gas Exchange

Measurements of carbon assimilation (A_{sat} ; $\mu mol CO_2 m^{-2} s^{-1}$) and stomatal conductance (g_s ; $mol H_2O m^{-2} s^{-1}$) were carried out using a portable photosynthesis system Li6400 (Li6400, Li-Cor Inc., Lincoln, NE, USA) provided with a Leaf Chamber Fluorometer (6400–40) of 2 cm^2 and a 10 % blue light source. Measurements were carried out at 25 °C with a light saturated rate of 1200 $\mu mol m^{-2} s^{-1}$ of PPFD, a flow rate of 500 $\mu mol s^{-1}$ and at a $[CO_2]$ level of 400 ppm ($\mu mol mol^{-1}$). In Experiment 1, measurements were carried out in each clone at treatment ($n=3$) at the beginning of the experiment (T0) and every 15 days (T15, T30, T45, T60) between 10:00 and 18:00 h, and the VPD average was 2.37 (kPa) at T0, 2.93 (kPa) at T15, 2.39 (kPa) at T30, 2.71 (kPa) at T45 and 2.62 (kPa) at T60. The experiment began in late March 2013. T0 was done the previous days and

measurements at regular time intervals were made on 12–13 April (T15) and 27–28 April (T30), 13–14 May (T45) and 2–3 June (T60).

In Experiment 2, UB measurements were carried out after 120 days (Ti DAT) and 180 days (Tf DAT) in the conditions described above. Gas exchange was measured on the third fully collared leaf at the moment of maximum intensity of solar radiation from 12:00 to 14:00 and after 63 and 116 days after transplanting (referred as Ti and Tf DAT) at UNICT. Maximum air temperature at UNICT (as average of measurements) was 33.8 °C at Ti and 32.9 °C at Tf; average PAR_i and average VPD were 1664.2 $\mu mol m^{-2} s^{-1}$ of PPFD and 3.68 (kPa) at Ti and 1564.8 $\mu mol m^{-2} s^{-1}$ of PPFD and 3.46 (kPa) at Tf, respectively and 2.37 (kPa) at Ti and 2.62 (kPa) at Tf at UB.

Chlorophyll Content

Chlorophyll content was measured in the last or second last fully collared leaf of all plants assayed using a portable meter (Minolta SPAD 502 Meter, Plainfield, IL, USA) every 15 days in Experiment 1 and at Ti and Tf in both locations in Experiment 2. Five measurements were performed per leaf, from the middle of the leaf (each measurement is the mean value of six determinations per treatment).

Relative Water Content

Relative water content (RWC, %) of leaves was determined as $(FW-DM)/(TW-DM) \times 100$, where *FW* is the fresh weight, *DM* is the dry matter after being dried in an oven at 60 °C until constant weight was reached, and *TW* is the turgid weight of the leaf after equilibration in distilled water for 24 h at 4 °C. RWC was calculated as the mean of three leaves of each clone and treatment every 15 days.

Biomass Parameters

At the end of the Experiment 1 (T60) and Experiment 2 (Tf), whole plants were harvested, weighed and oven dried at 60 °C until constant weight was reached. Subsequently, the dry biomass of shoots and roots was determined. Total fresh weight and plant leaf area were estimated prior to drying using a flat-bed scanner (Hewlett-Packard ScanJet model licx, San Diego, USA) and analysed with an image processing program (Image, University of Sheffield, 2003). Parameters such as height along the longest stem from the base to the latest totally expanded leaf (H; cm), number of leaves (NL) and stems per plant (NS), green leaf area (gLA; m^2) and stems area (SA; m^2) were measured. Stem area was calculated from the formula for calculating the area of a cylinder, where the height was the stem height and an average ($n=3$) of the diameter was measured with a caliper. The percentage of complete green, complete yellow and complete dry leaves (GLP, YLP, DLP,

respectively; %), total dry weight (TDW; g), the shoot/root ratio (S/R, g g⁻¹), specific leaf area (SLA; m² Kg dry leaves⁻¹), leaf area ratio (LAR; m² Kg total dry weight⁻¹) and leaf weight ratio (LWR; Kg dry leaves Kg⁻¹ total dry weight) were calculated.

Statistical Analysis

All studied parameters were analysed by means of the general lineal model (GLM) procedure by two factors and interactions, considering treatment and clone as “fixed factors”. Statistical analysis was conducted with the SPSS 21.0 software package. The means±standard errors (SE) of each replicates were calculated for each measured parameter. When a particular F-test was significant, we compared the means using a Tukey multiple comparison. The results were accepted as significant at $P<0.05$.

Experiment 1 and 2: Stress Susceptibility Index

In order to identify the most important parameters to be used in the index, a factorial analysis (principal component analysis, PCA) was carried out. Once the most important and common parameters in Experiment 1 and Experiment 2 (A_{sat} , g_s , GLP, TDW, S/R, SLA) were selected, SSI was calculated using the following relationship [37] for each treatment and clone:

$$\text{SSI} = [(1 - (Y_s/Y_p))] / [(1 - (X_s/X_p))]$$

where Y_s , Y_p , X_s and X_p represent parameter under stress, parameter under non-stress conditions for each clone, parameter mean under stress and parameter mean under non-stress conditions for all clones, respectively. An average of the results was done in order to classify clones for their tolerance or sensitivity to different stresses.

Results

Experiment 1: Screening for Salinity and Water Stress

Gas Exchange

Significant differences were found between clones for A_{sat} ($P=0.014$) and g_s ($P=0.010$) at the beginning of the experiment (T0). Granadensis, Tortorici, Agrigento, Fondachello and Cefalú showed the highest values of A_{sat} (Fig. 1), whereas Piccoplant and Martinensis showed the lowest values. In general, A_{sat} and g_s decreased slightly in the control treatment (WW S-) for almost every clone during the experiment (Fig. 1). Significant differences in A_{sat} and g_s were found between measuring periods, treatment, clone and interactions. A highly positive and significant correlation was found

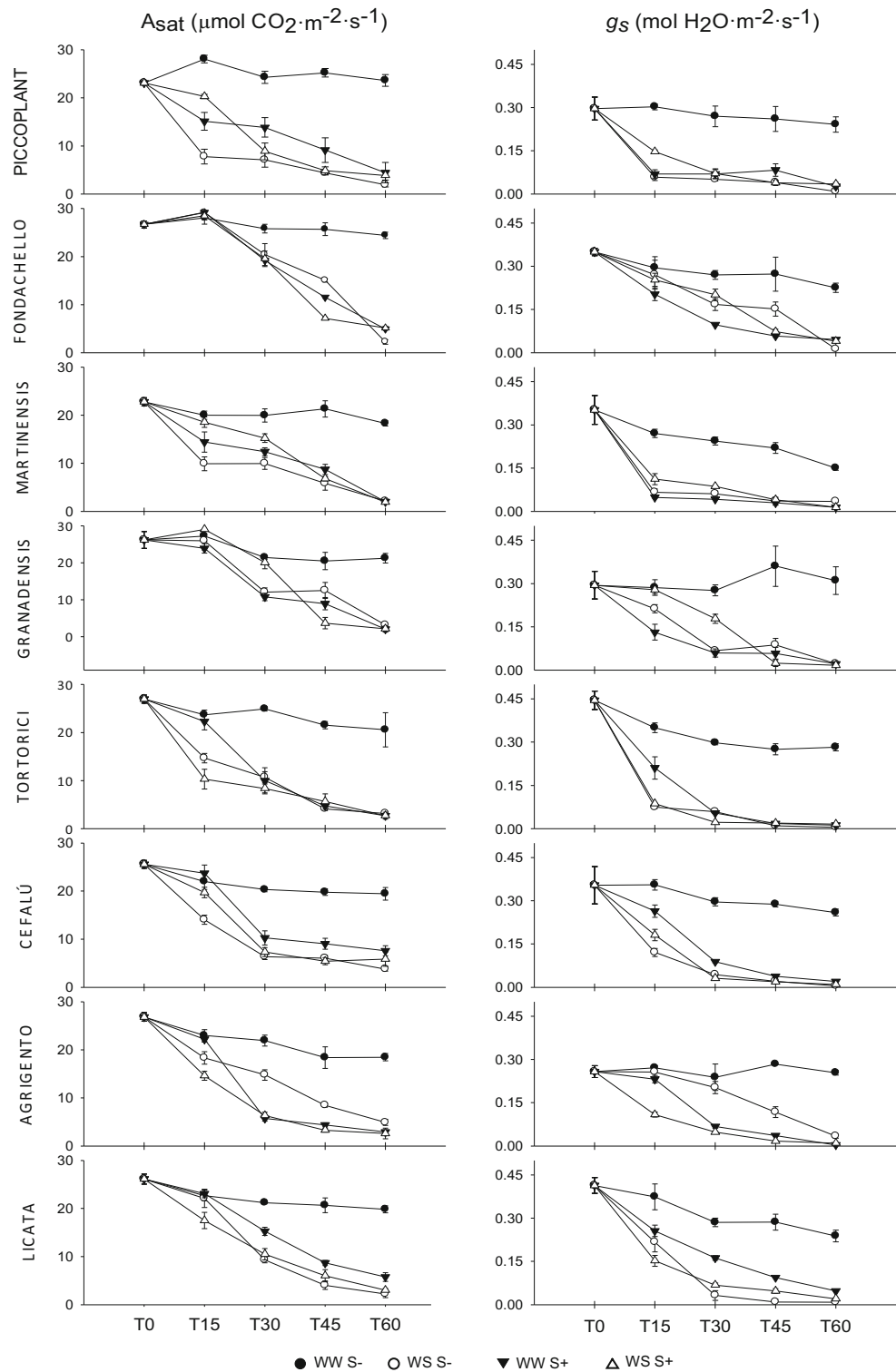
between A_{sat} and g_s during the experiment ($R^2=0.886^{**}$; correlation was significant at $p<0.01$). For each of the four treatments and across the set of eight clones, we studied the linear correlation of g_s against A_{sat} and time. A_{sat} and g_s were strongly and positively correlated ($P=0.001$) in the WS treatments (WS S-; $R^2=0.909$ and WS S+; $R^2=0.954$), and remained positively correlated in the S treatment (WW S+; $R^2=0.844$). Both parameters were negatively correlated with time in each treatment, with the correlation being slightly stronger for A_{sat} than for g_s . In the control treatment, the correlations were less significant (ESM 1).

A_{sat} and g_s markedly decreased at T60 (between 70.3 and 91.3 %) in every stress treatment for each clone ($P=0.001$) in relation with T0 (Fig. 1). In general, all clones showed lower values of A_{sat} and g_s at T15 in all stress treatments apart from Fondachello and Granadensis, where no significant differences were found in any treatments with respect to the control. At T30, Fondachello showed higher values of A_{sat} in each stress treatment in comparison to the other clones. In contrast, Cefalú showed the lowest values in WS S- and Agrigento in both WW S+ and WS S+. As a whole, similar decreases were observed in each clone at T45. In the WS S+ treatment, a large decrease was observed in Fondachello and Granadensis between T30 and T45.

Interestingly, some clones (Piccoplant, Martinensis, Cefalú, Licata) seemed to be more affected by WS (WS S-) than by S treatments (WW S+ and WS S+) during the first month of the experiment, according to their low A_{sat} values (Fig. 1). However, no differences in A_{sat} were found between treatments at the end of the experiment (T60) ($P=0.325$). Some clones (Martinensis, Granadensis and Tortorici) showed similar values, whereas others were more affected by one of the stresses. Piccoplant, Licata and Fondachello underwent the greatest decrease in A_{sat} with respect to the value at T0 in WS S- (91.7, 91.5 and 91.5 %, respectively), while Agrigento decreased by only 81.7 %. WW S+ had less effect on Cefalú, Licata and Piccoplant (70.3, 77.9 and 80.7 %, respectively), whereas Granadensis was substantially affected (92.1 %). In relation to WS S+, Granadensis was the most affected clone (91.8 %), while Cefalú, Fondachello and Piccoplant were the least affected, decreasing by only 77.1, 80.7 and 83.2 % with respect to the T0 values.

However, different clones had different performances under stress over time. Whereas some ecotypes (Granadensis, Fondachello and Licata) showed negligible stress effects with respect to the control until T30, other clones (Piccoplant, Martinensis, Tortorici and Agrigento) were most affected with short-term stress, especially by WS treatments. Over the short term (T15), no S effect was found in the clones except for Piccoplant and Martinensis. As expected, after a month (T30) the stress effect in plants was higher, although no clear pattern was found between treatments: Piccoplant, Cefalú and Licata were the less affected by S, Martinensis and

Fig. 1 Changes in A_{sat} and g_s during T0, T15, T30, T45 and T60 in each clone, respectively, for each treatment (WW S-, WS S-, WW S+ and WS S+). Data are the means of three replicates and the standard errors (SE) are shown when larger than the symbols

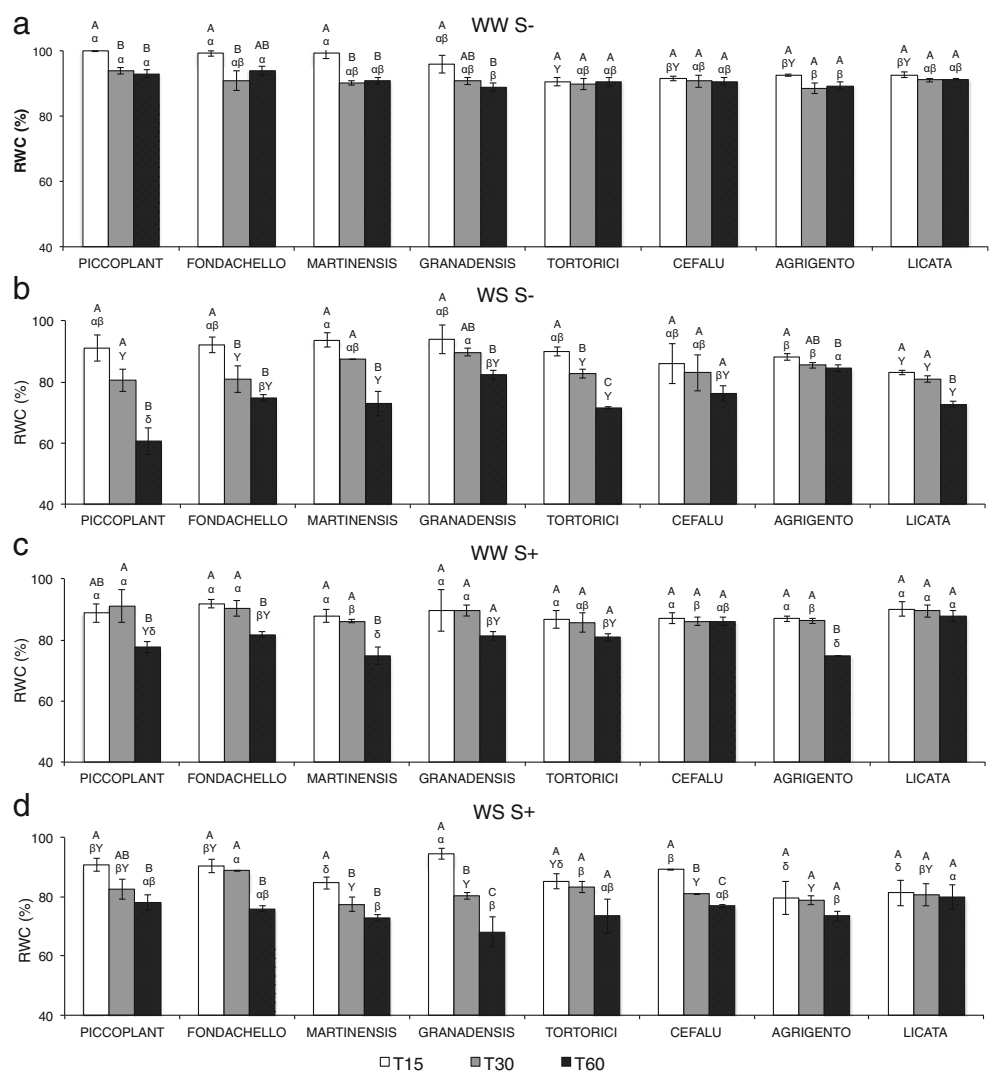


Granadensis were less affected by both stresses and Fondachello was equally affected by *S*, *WS* and both stresses. The effect of both stresses was notable at T45 in some clones. Although clones seemed to be more affected by *WS* than by *S* at T60, no significant differences between treatments were found in Martinensis, Granadensis and Tortorici.

Relative Water Content (RWC)

Significant differences at T0 ($P=0.004$) were found between clones, with Fondachello and Granadensis having the highest and lowest values of RWC, respectively (Fig. 2). A trend of decreasing RWC was found in most of the eight clones of

Fig. 2 Changes in relative water content (RWC) during T15, T30 and T60 in each clone for each treatment [WW S- (a); WS S- (b); WW S+ (c) and WS S+ (d)]. Data are the means of three replicates and the standard errors (SE) are shown when larger than the symbols. Data were analysed with an ANOVA Tukey analysis. Different capital letters indicate significant differences ($P < 0.05$) between time for the same clone and treatment. Different Greek letters indicate significant differences ($P < 0.05$) between clones for the same time and treatment



giant reed subjected to stress over the experiment, although the differences were not significant in some clones. Significant differences were found between treatments and time ($P < 0.0001$ in each case). The decrease in RWC in *WS* treatments was greater than under *WW S+*. *WW S-* resulted in a higher RWC than *WW S+*, but the RWC under *WS S-* and *WS S+* were not significantly different. Moreover, a significant decrease was observed for each time (T15, T30 and T60) in relation to T0.

Each clone sustained a decrease in RWC under *WS S-* (Fig. 2b) except Cefalú, where no significant differences were found ($P = 0.469$). At T60, the RWC of Piccoplant decreased by 37.9 %, while in Agrigento and Granadensis it decreased by only 13.5 and 15.7 % with respect to the values at T0. Some clones (Granadensis, Tortorici, Cefalú and Licata) did not show a significant decrease in RWC throughout the *WW S+* treatment (Fig. 2c), while the decrease in other clones (Piccoplant, Fondachello, Martinensis and Agrigento) was significant only at T60. Agrigento and Martinensis showed a

greater decrease at T60 with respect to T0 (with 23.4 % for both), whereas Licata and Cefalú only decreased by 10.2 and 11.8 %, respectively. A similar pattern was observed for the *WS S+* treatment in some clones (Tortorici, Agrigento and Licata), where no significant differences were found over time (Fig. 2d). Others, such as Piccoplant, Fondachello, Martinensis, Granadensis and Cefalú, showed a decrease in RWC to some extent. Granadensis was the clone with the greatest decrease relative to T0, whereas Licata had the smallest decrease (30.3 and 18.2 %, respectively). Significant and positive linear correlations were found between A_{sat} and g_s and RWC throughout the experiment ($R^2 = 0.688^{**}$ and $R^2 = 0.636^{**}$, respectively).

Chlorophyll Content

A decrease was observed in all treatments relative to the control at T60, being most important in treatments involving *WS*

(22.9 % in WS S- and 21.9 % in WS S+), whereas *S* treatment decreased only 14.2 % with respect to the control.

Piccoplant, Fondachello and Cefalú underwent a decrease in chlorophyll content under WW S- between T0 and T60 (ESM 2). At T60, significant differences were found between treatments in each clone. A smaller decrease in chlorophyll content was observed under WW S+ than under WS S- and WS S+. Fondachello, Granadensis, Cefalú, Agrigento and Licata showed a similar pattern between WS S- and WS S+, while Piccoplant, Tortorici and Martinensis were more affected by WS S- than by WS S+. Relative to their own chlorophyll content at T0, Cefalú was the most affected by WS S- and WS S+, decreasing by 45.2 and 44.5 %, respectively, while Agrigento was the most affected by *S* (WW S+), decreasing by 34.2 % at T60, and was also the clone with the lowest chlorophyll content values in that treatment (ESM 2). Positive and significant correlations were found between chlorophyll content and A_{sat} (0.459**), SLA (0.541**) and TDW (0.453**) (ESM 3).

Biomass Parameters

In general, NS was affected by the treatments, especially WS S+ relative to WW S-. Only three clones (Tortorici, Cefalú and Agrigento) showed differences between treatments, although no significant differences were observed in the other clones (Table 2). Plant height (H; cm) and stem diameter (SD; cm) were different according to the treatment, being higher under WW S- (73.0 ± 4.0 and 0.7 ± 0.02 , respectively) than under the other treatments. A greater decrease in H and SD was observed in WS S- (54.2 ± 3.5 and 0.6 ± 0.02 , respectively) and WS S+ (48.7 ± 3.5 and 0.6 ± 0.02 , respectively) than in WW S+ (59.2 ± 3.9 and 0.63 ± 0.03 , respectively).

Parameters related to leaf development, such as NL, gLA, GLP, DLP and TDW were more affected by WS S- and WS S+ than by WW S+ (Table 2). gLA was reduced significantly in each treatment with respect to the control, but there were no significant differences between WS S- (84.2 %) and WS S+ (80.8 %). Martinensis and Fondachello showed the lowest values in WW S-. Under WS S- conditions, Cefalú had the largest decline (92.8 %), followed by Tortorici (89.9 %), Licata (86.4 %) and Piccoplant (86.2 %), whereas the smallest decline was observed in Granadensis (78.3 %) and Agrigento (74.9 %). Tortorici and Agrigento were the most affected by WW S+ (70.8 and 68.8 %, respectively), whereas Cefalú and Martinensis were the least affected (59.4 and 47.7 %, respectively). Licata was most affected by both stresses (WS S+), with a reduction of 88.8 %, followed by Cefalú (88.1 %) and Agrigento (86.5 %), whereas Piccoplant, Granadensis and Martinensis were less affected (73.2, 73.0 and 66.1 %, respectively). TDW (Fig. 3) declined by 61.5 and 66.3 % in WS S- and WS S+, respectively, whereas WW S+ decreased by only 45.8 %, relative to the control treatment (Fig. 3).

In general, the shoot/root ratio (S/R, g g^{-1}) decreased in each treatment in comparison to the control, being the lowest in the *WS* treatment compared to the combined stresses or *S* alone (Table 3). Moreover, the decrease in the S/R was due to a differential response to stress between shoots and roots. While shoot growth seemed to be more affected by *WS* relative to the control (69.1 % for WS S-, 49.7 % for WW S+ and 71.3 % for WS S+), root growth seemed to be less affected (34.7 % for WS S-, 43.1 % for WW S+ and 50.1 % for WS S+), although no significant differences were found in a Tukey test between treatments (except for the control) (Table 3). Even so, the S/R data should be considered carefully because our experiment utilised potted plants, where root development is influenced by the size of the pots. Licata and Granadensis had the greatest decrease in the S/R under WS S- (71.4 and 69.1 %) relative to the control and Martinensis the smallest (34.7 %). The S/R was less affected by WW S+, with Piccoplant and Granadensis being the most affected, with 28.6 and 27.4 % reductions, respectively, compared to the control. Both Martinensis and Cefalú showed increases in their S/R under this treatment (WW S+) relative to the control (21.8 and 3.7 %, respectively). WS S+ had similar effects on some clones as WS S- [Piccoplant (52.1 %), Granadensis (64.7 %), Tortorici (41.0 %)], although other clones [Licata (40.6 %), Fondachello (34.6 %), Agrigento (26.3 %) and Martinensis (12.9 %)] were less affected by both stresses than *WS* alone.

WS had an important influence on the LAR and LWR, which decreased 58.4 and 40.9 % with respect to the control under WS S- and by 45.9 and 24.3 % under WS S+ (Table 3). In contrast, WW S+ decreased these parameters by only 32.7 and 13.2 %, respectively (Table 3). Similar to LAR and LWR, the SLA was more affected by treatments involving *WS* (31.6 % in WS S- and 28.2 % in WS S+) than *S* (24.0 %). A strong and positive correlation (ESM 3), was found between SLA and A_{sat} (0.736**) and between SLA and chlorophyll content (0.541**) (Bilateral significance at level $P < 0.01$).

Experiment 2: Screening for Increasing Salinity Levels

Gas Exchange

No significant differences were observed for A_{sat} between UNICT S0 and UNICT S4 at the initial time (Ti) in Fondachello and Agrigento (Fig. 4a). A decrease in the A_{sat} was found in Tortorici and Licata ($P = 0.001$), although a surprising increase was found in Cefalú from 20.5 ± 1.0 to 32.5 ± 1.0 . Moreover, a decrease in g_s was observed in each clone ($P = 0.001$) except Cefalú, which had an increase of 18.9 % in g_s between UNICT S0 and UNICT S4 at Ti. On the other hand, no significant differences were found in A_{sat} and g_s between UB S0 and UB S16 at Ti in any clone studied (Fig. 4b). A decrease in A_{sat} and g_s was found between

Table 2 Biomass parameters in eight clones of *Arundo donax* L. (P, Piccoplant; F, Fondachello; M, Martinensis; G, Granadensis; T, Tortorici; C, Cefalù; A, Agrigento; L, Licata) under four different treatments: (1) well watered with non-saline solution (WW S-), (2) low watered with non-saline solution (WS S-), (3) well watered with saline solution (WW S+) and (4) low watered with saline solution (WS S+) at T60. Means and standard error (SE) are shown. Data were analysed with an ANOVA Tukey test between treatments (capital letters) and between clones (small letters), Number of leaves (NL), number of stems (NS), green leaf area (gLA, m²), stem area (SA, m²), green leaf percentage (GLP, %), yellow leaf percentage (YLP, %), dry leaf percentage (DLP, %)

	NL			NS			gLA (m ²)			SA (m ²)			GLP (%)			YLP (%)			DLP (%)			
	Mean	SE	Tukey	Mean	SE	Tukey	Mean	SE	Tukey	Mean	SE	Tukey	Mean	SE	Tukey	Mean	SE	Tukey	Mean	SE	Tukey	
WW S-	P	42.2	4.48	Aa	5.0	0.71	Aa	0.232	0.024	Aab	0.075	0.009	Aa	97.2	0.95	Aa	2.8	0.95	Aa	0.0	0.00	Cd
	F	29.6	2.99	Aab	3.3	0.66	Aab	0.160	0.023	Abc	0.038	0.006	Ab	95.2	4.76	Aa	4.8	4.76	Aa	0.0	0.00	Bd
	M	22.3	0.88	Ab	2.7	0.33	Aab	0.144	0.024	Ac	0.037	0.008	Aab	95.9	2.23	Aa	0.0	0.00	Ba	4.1	2.23	Bc
	G	24.0	3.61	Ab	2.3	0.33	Aab	0.197	0.022	Aabc	0.027	0.005	Ab	98.5	1.45	Aa	1.5	1.45	Aa	0.0	0.00	Bd
	T	32.7	4.67	Aab	4.0	0.00	Aab	0.245	0.017	Aa	0.055	0.002	Aab	84.9	2.74	Aa	2.3	1.24	ABa	12.8	3.66	Bab
	C	28.7	5.00	Ab	3.7	0.33	Aab	0.230	0.021	Aab	0.059	0.002	Aab	87.0	4.76	Aa	4.2	4.17	Aa	8.8	0.67	Cabc
	A	20.7	2.73	Aab	2.0	0.58	Ab	0.170	0.012	Aabc	0.048	0.009	Aab	82.9	3.76	Aa	1.9	1.96	Ba	15.2	2.33	Ba
	L	29.0	2.61	Aab	2.7	0.48	Aab	0.244	0.029	Aa	0.058	0.006	Aab	92.1	3.78	Aa	1.9	1.18	Ba	6.0	2.64	Bbc
WS S-	P	7.7	1.86	Ca	4.0	1.00	Aa	0.032	0.002	Cab	0.039	0.008	Ba	25.8	8.69	Ba	5.1	1.62	Aab	69.1	7.77	Aa
	F	7.7	1.20	Ca	1.7	0.33	Ab	0.027	0.002	Cab	0.017	0.003	Ac	47.6	1.21	Ba	4.2	2.25	Ab	48.2	2.61	Aab
	M	5.3	1.76	Ba	1.2	0.33	Ab	0.027	0.002	Dab	0.020	0.003	ABbc	31.8	9.43	Ba	8.1	4.12	Aab	60.1	8.43	Aa
	G	8.7	2.73	Ba	2.0	0.58	Ab	0.042	0.002	Ca	0.010	0.001	Bd	52.7	17.7	Aa	9.9	1.84	Aab	37.4	15.93	Ab
	T	8.3	1.20	Ba	2.7	0.33	ABab	0.024	0.010	Cab	0.024	0.001	Babc	32.1	1.81	Ca	7.9	0.80	Aab	60.0	1.01	Aa
	C	7.3	1.76	Ca	2.7	0.67	ABab	0.016	0.009	Cb	0.036	0.003	Ba	31.2	7.91	Ba	8.1	1.96	Aab	60.7	6.34	Aa
	A	7.3	0.67	Ba	2.7	0.67	Aab	0.042	0.012	Ba	0.033	0.007	ABab	31.2	5.54	BCa	16.1	4.06	Aa	52.7	3.27	Aab
	L	5.7	2.03	Ca	2.0	0.00	Aab	0.033	0.002	Cab	0.025	0.002	Babc	31.9	10.8	Ca	11.9	3.17	Aab	56.2	7.71	Aab
WW S+	P	19.0	2.31	Ba	3.0	0.58	Aa	0.082	0.009	Bab	0.030	0.009	Ba	72.3	17.30	Aa	5.0	2.50	Aab	22.7	15.12	Ba
	F	14.5	2.50	Bab	2.0	0.00	Aabc	0.085	0.009	Bab	0.033	0.005	Aa	72.5	12.50	ABa	7.5	2.50	Aab	20.0	10.00	Aa
	M	9.0	1.53	Bbc	2.0	0.58	Aabc	0.075	0.004	Bab	0.027	0.007	ABa	47.2	5.30	Ba	11.6	3.49	Aa	41.2	7.42	Aa
	G	10.3	1.45	Bb	2.0	0.58	Aabc	0.082	0.005	Bab	0.015	0.003	ABa	67.8	16.60	Aa	15.2	7.63	Aab	17.0	9.63	ABa
	T	10.7	1.86	Bb	1.7	0.33	Bbc	0.071	0.005	Bab	0.021	0.006	BCa	57.0	4.03	Ba	1.7	1.67	Bb	41.3	5.68	Aa
	C	14.0	1.53	Bab	2.0	0.00	BCab	0.093	0.001	Ba	0.020	0.004	Ca	70.8	8.22	Aa	1.4	1.45	Ab	27.8	7.01	Ba
	A	6.0	1.53	Bc	1.0	0.00	Bc	0.053	0.009	Bc	0.026	0.001	Ba	44.5	9.94	Ba	9.9	2.21	Aa	45.6	12.11	Aa
	L	11.3	0.33	Bb	2.3	0.33	Aab	0.076	0.005	Bab	0.023	0.003	Ba	56.1	6.07	ABa	8.1	1.69	ABab	35.8	6.21	Aa
WS S+	P	15.1	2.85	Ba	3.0	0.00	Aa	0.062	0.003	Ba	0.020	0.002	Ba	58.8	8.83	Aa	3.4	1.92	Aa	37.8	9.30	ABab
	F	8.0	1.00	Cab	2.0	0.00	Aab	0.038	0.002	Cbcd	0.016	0.001	Aab	58.1	16.9	Bab	14.2	2.45	Aa	27.7	19.36	Ab
	M	7.2	2.76	Bb	2.0	0.41	Aab	0.048	0.005	Cab	0.017	0.001	Bab	47.7	8.33	Bab	14.2	2.12	Aa	39.1	4.38	Aab
	G	7.7	1.45	Bb	2.0	0.58	Aab	0.053	0.005	Cab	0.010	0.001	Ba	47.8	9.34	Aab	1.1	7.97	Aa	37.1	1.37	Aab
	T	6.0	1.53	Bb	1.7	0.33	Bb	0.042	0.006	Cbc	0.014	0.003	Cbc	31.8	7.02	Cbc	7.1	1.71	Aa	61.1	5.62	Aa
	C	4.5	1.04	Cbc	1.5	0.29	Cb	0.027	0.004	Ccd	0.021	0.003	Ca	33.0	9.12	Bbc	10.4	3.62	Aa	56.6	5.77	Aa
	A	2.5	0.50	Cc	1.5	0.50	ABb	0.023	0.005	Bd	0.022	0.001	Ba	17.7	2.31	Cc	13.8	6.15	Aa	68.5	8.46	Aa
	L	4.3	0.33	Cbc	1.0	0.33	Ab	0.027	0.004	Ccd	0.020	0.002	Ba	34.9	5.30	BCabc	7.5	4.44	ABa	57.6	3.37	Aa

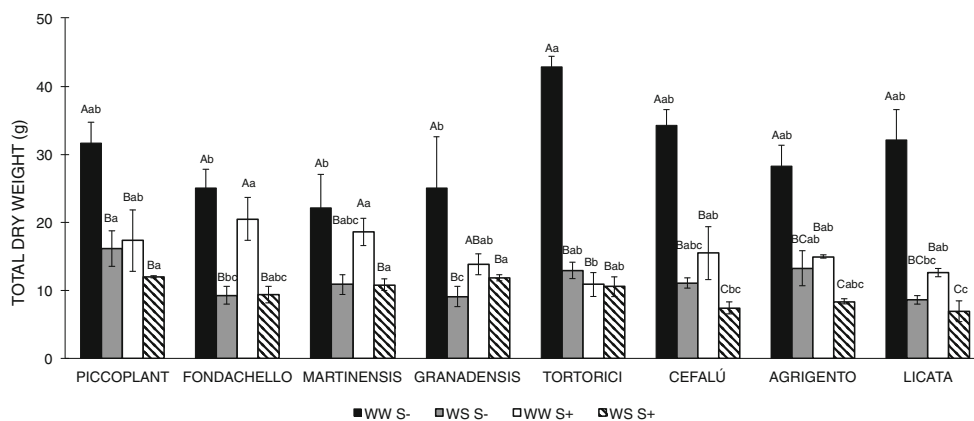


Fig. 3 Changes in total dry weight (TDW, g) at T60 in each clone for each treatment (WW S-, WS S-, WW S+ and WS S+). Data are the means of three replicates and the standard errors (SE) are shown when larger than the symbols. Data were analysed with an ANOVA Tukey analysis.

Different capital letters indicate significant differences ($P < 0.05$) between treatments for a same clone and different small letters indicate significant differences ($P < 0.05$) between clones for the same treatment

UNICT S0 and UNICT S4 at the end of the experiment (Tf) in each clone except in Cefalú, where there was no significant difference found in A_{sat} (Fig. 4a). Significant differences were also found in each clone in both parameters between UB S0 and UB S16 at Tf (Fig. 4b).

S treatment at Tf led to lower values of A_{sat} and g_s at UB than at UNICT. A decrease in A_{sat} and g_s was found in each clone in S treatments (S4 and S16) in both locations (Fig. 4a, b), except for Fondachello at UNICT, where there was no significant difference found in A_{sat} ($P = 0.809$) and an increase of 32.8 % in g_s was observed.

Regarding the differences between clones, significant differences were found between them for A_{sat} and g_s in most of the treatments. At Tf, Fondachello had the highest A_{sat} and g_s values at UNICT S4 (22.7 ± 0.1 and 0.203 ± 0.004 , respectively), followed by Cefalú and Agrigento. Cefalú had the highest A_{sat} value at UB S16 (7.6 ± 1.0), followed by Fondachello (5.0 ± 0.2), with the latter having higher g_s values (0.046 ± 0.002) than Cefalú (0.020 ± 0.001).

Chlorophyll Content

Different behaviour was observed between locations with respect to chlorophyll content (ESM 4). An increase in chlorophyll content was observed in almost every clone at UNICT, except for Tortorici, which had no significant differences between treatments (S0 and S4) ($P = 0.630$). Nevertheless, a decrease in chlorophyll content was observed between UB S0 and UB S16 in each clone. Cefalú had the lowest chlorophyll content at UNICT S0, whereas Fondachello, Tortorici and Licata had the highest chlorophyll content at UB S0 (ESM 4a, b). Under the S treatment, Agrigento and Fondachello had the highest chlorophyll content values at UNICT S4, whereas Fondachello and Cefalú showed the highest values at UB S16. The Agrigento clone

behaved differently at the two locations, showing the greatest increase between UNICT S0 and UNICT S4 (14.8 %) and the greatest decrease between UB S0 and UB S16 (23.9 %).

Biomass Parameters

No significant differences were found in the gLA (Fig. 5a) in most of the clones between UNICT S0 and UNICT S4, except for Agrigento (a decrease of 58.5 %). Surprisingly, an increase in gLA was found in Fondachello (10.6 %) and Licata (25.0 %). In addition, a remarkable decrease in gLA was observed at UB (Fig. 5b), being most noticeable in Tortorici (70.8 %), while Fondachello had the smallest decrease in gLA between UB S0 and UB S16 (46.7 %).

No significant differences were found in TDW (Fig. 5a) between UNICT S0 and UNICT S4 in the majority of clones, apart from Licata, whereas significant differences were found between UB S0 and UB S16 in all clones except Fondachello. The TDW (Fig. 5b) was lower at UB, even in the control treatment (S0). Fondachello had the highest production in both locations.

In relation to the S/R a decrease between control and S treatment was observed although no significant differences were found between treatments or even between clones (Fig. 5a, b). However, lower values of S/R were observed at UNICT for each clone than at UB. Tortorici had the highest S/R value at UNICT S4, while the highest value in UB S16 was found in Fondachello.

The behaviour of SLA was opposite to S/R (Fig. 5a, b), with no significant differences being observed between treatments (except for Licata at UNICT) or between clones in the same location. However, a tendency for SLA to decrease in line with increasing S was observed (except for Licata at UNICT). Moreover, lower SLA values were found at UB than at UNICT.

Table 3 Biomass parameters in eight clones of *Arundo donax* L. (P, Piccoplant; F, Fondachello; M, Martinensis; G, Granadensis; T, Tortorici; C, Cefalù; A, Agrigento; L, Licata) under four different treatments: (1) well watered with non-saline solution (WW S-), (2) low watered with non-saline solution (WS S-), (3) well watered with saline solution (WW S+) and (4) low watered with saline solution (WS S+) at T60. Means and

standard error (SE) are shown. Data were analysed with an ANOVA Tukey test between treatments (capital letters) and between clones (small letters). Shoot/root index (S/R, g g⁻¹), specific leaf area (SLA, m² Kg⁻¹), leaf area ratio (LAR, m² Kg⁻¹) and leaf weight ratio (LWR, Kg Kg⁻¹)

		S/R (g g ⁻¹)			SLA (m ² Kg ⁻¹)			LAR (m ² Kg ⁻¹)			LWR (Kg Kg ⁻¹)		
		Mean	SE	Tukey	Mean	SE	Tukey	Mean	SE	Tukey	Mean	SE	Tukey
WW S-	P	4.26	0.15	Aab	21.9	0.84	Aab	7.3	0.29	Aab	0.34	0.00	Ab
	F	4.57	0.50	Aa	24.6	2.74	Aa	6.4	0.51	Aab	0.26	0.01	Ac
	M	3.26	0.25	Bab	19.8	1.79	Aab	6.8	0.73	Aab	0.34	0.02	Ab
	G	4.05	0.82	Aab	20.8	2.05	Aab	8.7	1.39	Aa	0.41	0.03	Aa
	T	3.17	0.30	Ab	18.7	0.61	Ab	5.8	0.47	Ab	0.31	0.02	Ab
	C	3.78	0.10	Aab	21.9	1.70	Bab	6.7	0.58	Aab	0.31	0.01	Ab
	A	3.34	0.58	Aab	19.9	1.09	Aab	6.2	0.62	Ab	0.31	0.01	Ab
	L	4.34	0.16	Aab	21.8	0.56	Aab	7.6	0.21	Aab	0.35	0.01	Ab
WS S-	P	2.13	0.10	Ca	13.9	2.49	Bab	2.2	0.60	Bb	0.15	0.02	Bb
	F	1.94	0.09	Cab	15.6	3.07	Aab	3.1	0.62	Bb	0.20	0.02	Aab
	M	2.13	0.49	Ca	13.3	1.20	Bab	2.7	0.55	Cb	0.20	0.04	Bab
	G	1.25	0.09	Bb	19.6	0.86	ABa	5.4	0.74	Ba	0.27	0.03	Ba
	T	1.60	0.03	Cab	13.0	4.19	Aab	1.9	0.71	Cb	0.15	0.01	Cb
	C	2.02	0.06	Cab	10.8	3.05	Ab	1.9	0.88	Bb	0.18	0.02	Bb
	A	1.91	0.54	Aab	17.0	1.39	Aab	3.1	0.74	Bb	0.19	0.03	Bb
	L	1.24	0.33	Cb	15.7	0.91	ABab	3.3	0.56	Cb	0.21	0.06	Bab
WW S+	P	3.04	0.15	Bab	17.7	0.64	ABab	5.3	1.04	Aabc	0.30	0.05	Aabc
	F	4.04	0.23	ABa	17.0	3.26	Aab	4.3	1.16	ABbc	0.25	0.02	Abc
	M	3.97	0.05	Aa	15.5	1.10	ABb	4.1	0.28	Bbc	0.27	0.00	ABabc
	G	2.94	0.53	Aab	17.8	1.26	ABab	6.1	0.64	ABab	0.34	0.03	ABab
	T	2.54	0.34	ABb	17.8	0.56	Aab	4.9	0.31	ABabc	0.27	0.01	Aabc
	C	3.92	0.18	Aa	19.3	0.62	Ba	6.8	0.60	Aa	0.35	0.03	Aa
	A	3.12	0.72	Aab	15.0	1.02	Ab	3.6	0.55	Bc	0.24	0.02	ABc
	L	3.60	0.46	Aab	19.5	1.06	ABa	6.1	0.29	Bab	0.31	0.02	Aab
WS S+	P	2.04	0.44	Cab	19.8	0.54	Aa	4.8	0.43	Aa	0.28	0.02	Aab
	F	2.99	0.62	BCba	15.1	0.15	Aa	3.9	0.57	ABab	0.26	0.04	Aab
	M	2.84	0.36	BCa	14.8	1.20	Ba	4.5	0.42	Bab	0.31	0.03	Aa
	G	1.43	0.18	Bb	15.9	0.90	Ba	4.5	0.51	Bab	0.29	0.05	Bab
	T	1.87	0.35	BCab	16.4	0.18	Aa	4.0	0.14	Bab	0.24	0.01	Bab
	C	2.61	0.32	Ba	17.1	1.90	Ba	3.9	0.97	Bab	0.22	0.01	Bab
	A	2.46	0.35	Aab	16.9	7.28	Aa	2.7	0.59	Bb	0.18	0.04	Bb
	L	2.58	0.29	Ba	14.9	2.73	Ba	4.1	0.51	Cab	0.29	0.05	ABab

Experiment 1 and 2: Stress Susceptibility Index (SSI)

After carrying out the principal component analysis (PCA), it was observed that the first two principal components explained 72.04 and 82.60 % of variation. The most significant and positive parameters of component 1 (above 0.750) were selected: gLA, GLP, NL, A_{sat}, g_s, LAR, TDW, RWC, SLA, SPAD and S/R (ESM 5), but only the common parameters in Experiment 1 and Experiment 2 (A_{sat}, g_s, GLP, TDW, S/R, gLA, SLA) were used in the calculation of the SSI.

The eight clones of giant reed in Experiment 1 were classified according to SSI as shown in Fig. 6. Each axis represents the clone tolerance or sensitivity to stress [WS (X axis), S (Y axis) and both stresses (Z axis)]. A higher SSI indicates lower tolerance to stress. Agrigento was the most tolerant to WS (SSI=0.84), whereas its tolerance to S and both stresses was lower (1.08 and 1.00, respectively). Granadensis, Martinensis and Fondachello were also tolerant to WS (0.88, 0.92 and 1.01, respectively). However, Martinensis showed a higher tolerance to S (0.59) and both stresses (0.86) than

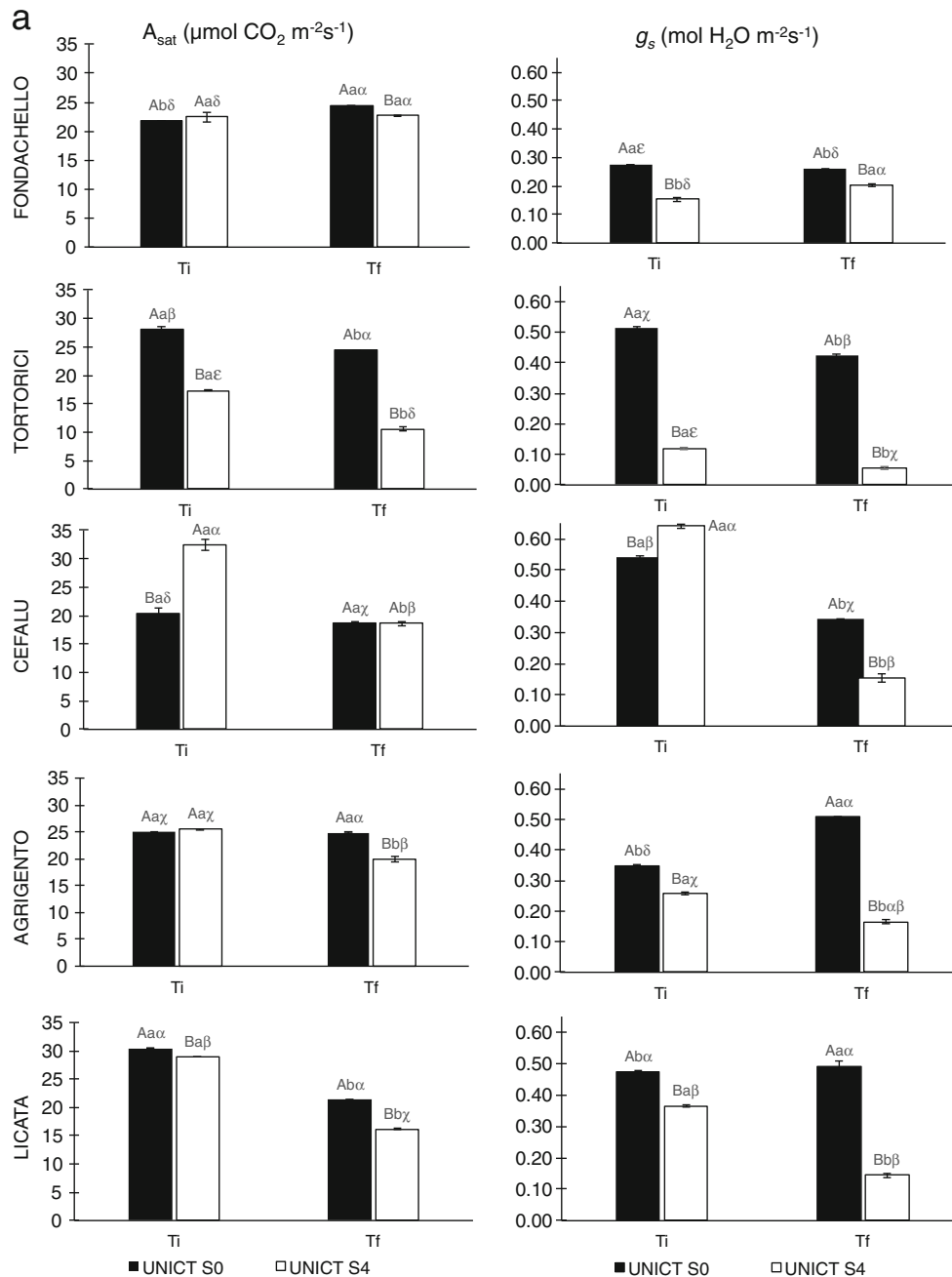


Fig. 4 a Changes in A_{sat} and g_s during Ti and Tf in each clone, respectively, for each treatment (UNICT S0 and UNICT S4). Values are the mean of three replicates \pm standard error (SE). Different capital letters indicate significant differences ($P < 0.05$) between treatments for the same location and time, different small letters indicate significant differences ($P < 0.05$) between time for a same location and treatment, and different Greek letters indicate significant differences ($P < 0.05$) between clones for the same treatment and location. **b** Changes in A_{sat} and g_s at UB during Ti

and Tf in each clone, respectively for each treatment (UB S0 and UB S16). Values are the mean of three replicates \pm standard error (SE). Different capital letters indicate significant differences ($P < 0.05$) between treatments for the same T, different small letters indicate significant differences ($P < 0.05$) between T for the same treatment and different Greek letters indicate significant differences ($P < 0.05$) between clones for the same treatment

Fondachello (0.92 and 1.01) and Granadensis (1.18 and 1.06, respectively). Moreover, Martinensis and Cefalú showed low SSI values in the *S* treatment (0.59 and 0.68, respectively), indicating a high tolerance to this stress. However, Cefalú's tolerance to *WS* and both stresses was significantly lower (1.11 and 1.00, respectively).

Martinensis, Piccoplant and Tortorici showed high tolerance to both stresses (0.86, 0.89 and 0.97, respectively), whereas Piccoplant's tolerance to *WS* was not noticeable (1.04). Martinensis seemed to have performed quite well under *WS*, *S* and both stresses, showing low SSI values in each treatment (0.92, 0.59 and 0.86, respectively). In contrast, the

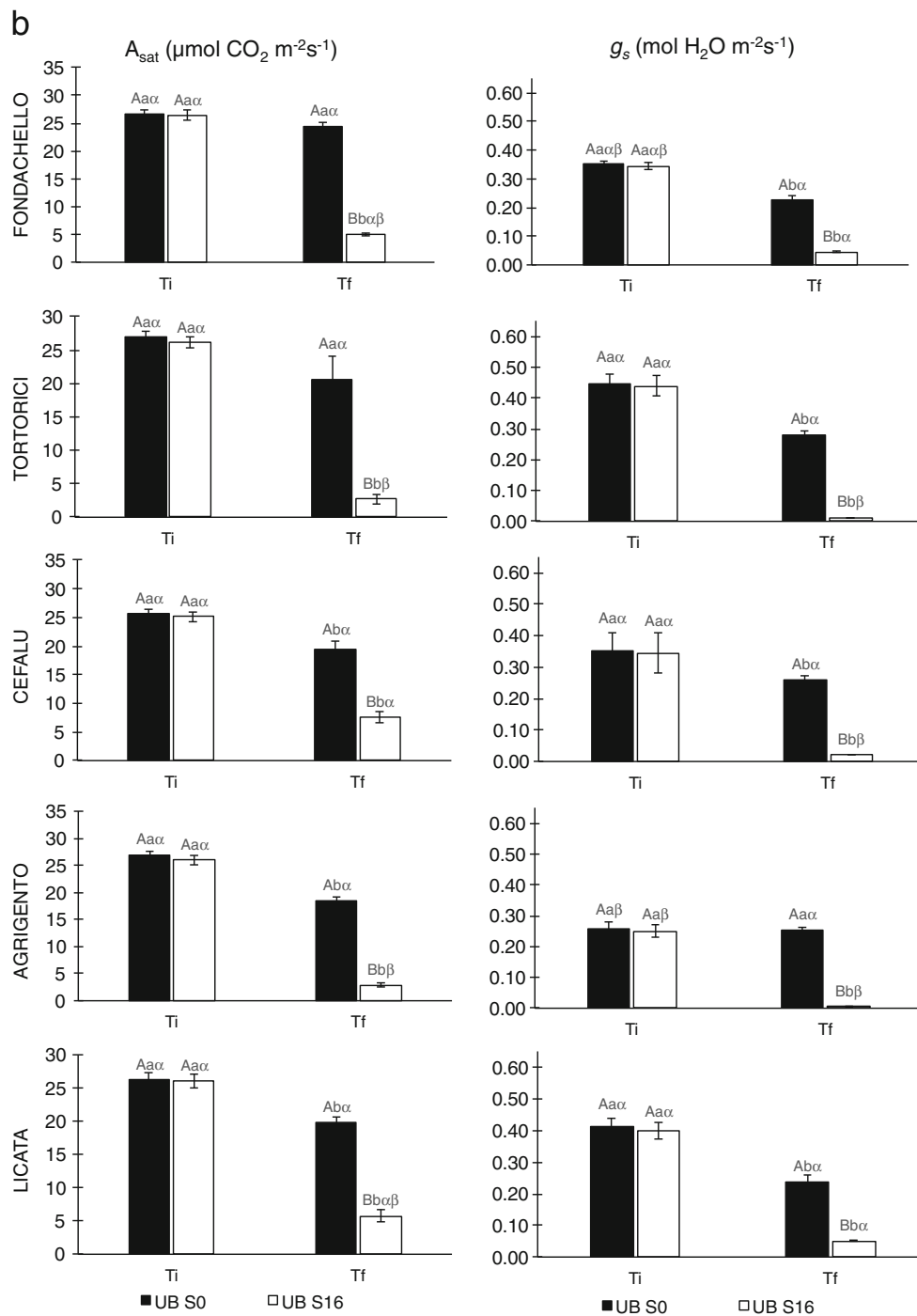


Fig. 4 continued.

results for Licata indicate a poor response to stress with high SSI values for almost every treatment (1.09, 1.05 and 1.12, for WS, S and double stress, respectively).

A linear correlation was calculated in order to check the existence of a relation between stress and the altitude of the area where the clones were collected (ESM 6). No strong relation was found except for S, with a Pearson correlation coefficient of -0.783 , although it was non-significant ($P=$

0.065). In relation to increasing S levels, Fondachello recorded the lowest SSI for UNICT S4 (0.55), followed by Cefalu (1.40) and Licata (1.44), with the same three clones having similar low SSI values for UB S16 (0.99, 0.71 and 0.99, respectively). On the other hand, Agrigento (2.87 and 1.18) and Tortorici (2.01 and 1.04) had the highest SSI values for the UNICT S4 and UB S16 treatments, respectively.

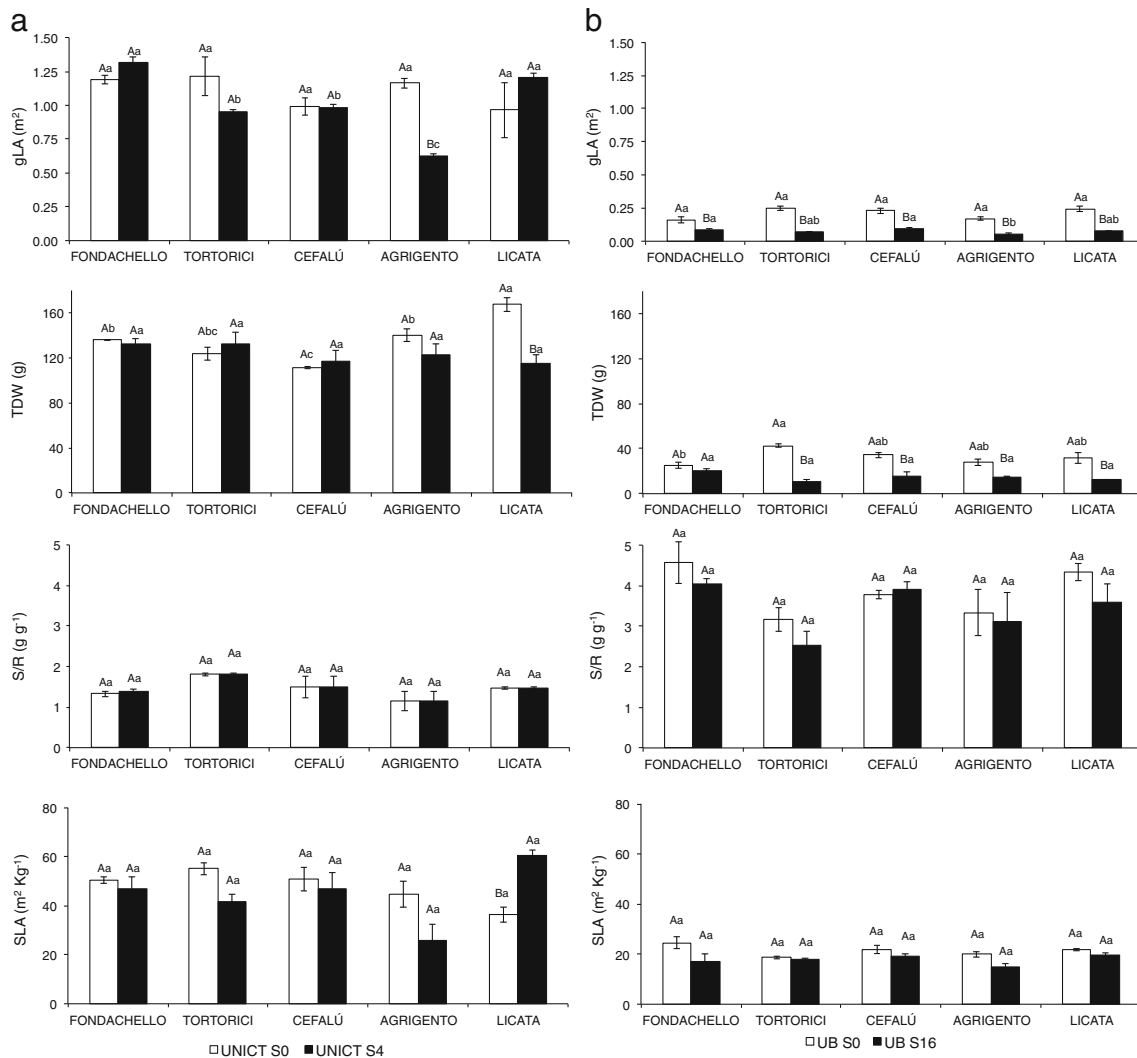


Fig. 5 Differences in gLA (m^2), TDW (g), S/R (g g^{-1}) and SLA ($\text{m}^2 \text{Kg}^{-1}$) at T60 in each clone and location (a; UNICT and b; UB) for each treatment (S0 and S4-S16). Values are the mean of three replicates \pm standard error (SE). Different capital letters indicate significant

differences ($P < 0.05$) between treatments for the same location and clone. Different small letters indicate significant differences ($P < 0.05$) between clones for a same location and treatment

Discussion

Salinity Effect on Plants

It has been reported that one of the major effects of salinity on plant performance is a decrease in photosynthetic rates [38]. In our experiments (1 and 2), decreases in A_{sat} and g_s were reported in almost all clones of giant reed as an effect of saline stress over time (Fig. 1) and at increasing salinity levels (Fig. 4).

The values of A_{sat} and g_s at T0 (Exp. 1) and Ti (Exp. 2) were similar in the control treatments (WW S- and S0, respectively) across the giant reed clones even at different locations, with the A_{sat} values similar to those reported by Papazoglou et al. [39] and Nackley et al. [27].

The earlier decrease in g_s than in A_{sat} that was observed in Experiment 1 (Fig. 1) and Experiment 2 (Fig. 4) in most of the

clones (except Fondachello) is supported by the findings of Centritto et al. [40], Munns and Tester [6] and Yousfi et al. [41], where one of the earliest responses to moderate stress is a decrease in stomatal conductance in order to reduce water loss. This is reflected in our study with the strong and positive correlation between both parameters (A_{sat} and g_s) and the negative correlation of both parameters over time (ESM 1). Moreover, as shown by Cornic and Massacci [42], the positive relation between A_{sat} , g_s and RWC indicates a decrease in RWC over time due to S (Fig. 2c). Even though there are few studies on the effects of salinity on photosynthesis in giant reed, similar observations of decreasing g_s after a stress period have been observed in other C_3 plants exposed to salinity, such as wheat [41] and olive [40].

The fact that the chlorophyll content decreased in one location (UB) under salinity and increased in the other location

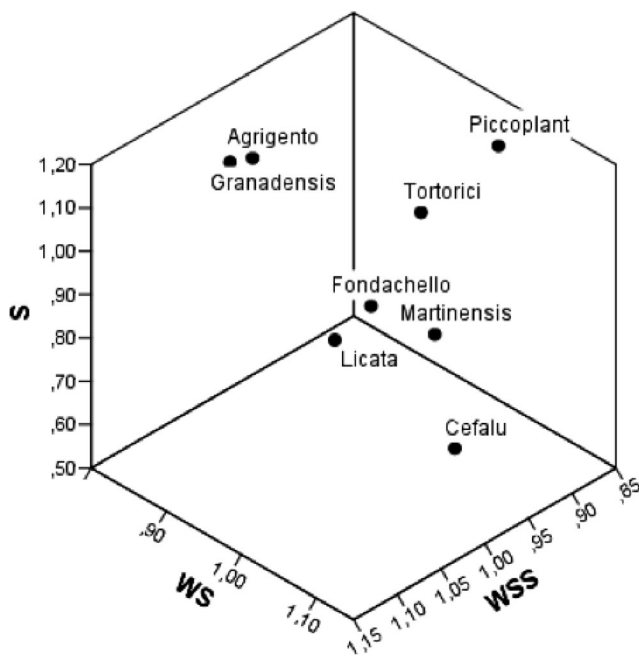


Fig. 6 3-D graphic representing stress susceptibility index (SSI) of each clone according to water stress (*WS*; X axis), salinity (*S*; Y axis) and both stresses (*WSS*; Z axis). Higher SSI values indicate lower tolerance to stress

(UNICT) (ESM 4) does not help to clarify the effect of salt on chlorophyll content. Santos [43] reported that saline stress decreased chlorophyll content even at low concentrations. Although our data from UNICT do not support that finding (ESM 4), the high levels of A_{sat} and TDW in the salinity treatment (UNICT S4 similar to UNICT S0 at Tf) (Figs. 4 and 5) would suggest a small amount of damage to the photosynthetic capacity at mild salinity level. However, a higher salinity dose resulted in a decrease in chlorophyll content in giant reed plants, as observed at UB in Experiment 1 (ESM 2) and Experiment 2 (ESM 4).

The effect of salinity on plant performance implicates a decrease of growth parameters such as H, NL, NS, gLA, TDW and indices such as S/R, SLA and LAR, not only in high salinity levels (Tables 2 and 3, Figs. 3 and 5), but also in low salinity levels (Fig. 5) as a consequence of changes in elongation rates due to leaf water status [44]. A decrease of leaf growth after an increase in soil salinity is primarily due to the osmotic effect of the salt around roots, producing a transient loss of water in leaf cells, which becomes more important over time [6] and produces similar effects to drought in salt-tolerant plants [8]. Not only were decreases in shoot mass observed, there also was a reduction of leaf development at the end of the experiment, in accordance with reports by Liu and Stützel [12], Munns [8] and Munns and Tester [6]. A reduction in plant size (gLA) relative to root growth would decrease the water use by plants, thus allowing conservation of soil moisture and preventing an increase in the salt concentration of the soil. Therefore, a decrease in the plant size could

be a major strategy for injury reduction under stress [6, 12, 45]. A possible reason for a lower biomass production (TDW) at UB than at UNICT (Table 3) could be due to the use of smaller pots (5.0 vs 9.5 L) that limited root development, as observed by a lower value of the S/R at UNICT.

On the contrary, in plants with high salt uptake rates, the oldest leaves may start showing symptoms of injury. A higher rate of leaf death than formation of new leaves will mean that the photosynthetic capacity of the plant will no longer be able to supply the carbohydrate requirements of young leaves, reducing their growth rate. Therefore, giant reed seems to be a salt-resistant plant according to the high GLP and S/R values in the salinity treatment, as observed in Experiment 1 (Tables 2 and 3), and it decreases in biomass production (TDW) only at high levels of *S*, as seen in Experiment 2 (Fig. 5). It is worth noting that the salinity level used in Experiment 1 (16 mS cm⁻¹) has been considered as “severe” in other crops such as durum wheat [41], but similar salinity levels have been used in other studies with giant reed and saline wastewater [29, 46].

One of the traits of salt tolerance in crops suitable for large scale evaluation in greenhouses is ion ‘exclusion’ [47], which is defined as the ability to restrict the rate of entry of potentially toxic Na⁺ and Cl⁻ into the shoots combined with the maintenance of K⁺ uptake, even when the Na⁺/K⁺ ratio is very high in the soil solution [48]. Rossa et al. [26] studied concentrations of K⁺, Na⁺ and Cl⁻ (mmol kg⁻¹ DM) in leaves of different ages in giant reed and reported a high content of K⁺ along shoots (from 351.6 to 441.4 mmol kg⁻¹ DM, from bottom to top), and a decreasing trend of Na⁺ and Cl⁻ along the shoot (from 163 to 20.8 and from 370.2 to 163.5 mmol kg⁻¹ DM, from bottom to top, respectively). Ball and Farquar [49] and Ball et al. [50] mentioned that plants in saline environments require a high K⁺ concentration for efficient photosynthesis performance, especially for the operation of PSII. Although more studies are needed to confirm this hypothesis, it is likely that high K⁺ content and low rate of Na⁺ and Cl⁻ transport to leaves [8] with increasing plant height may occur in saline conditions, probably explaining the high tolerance of giant reed to salinity.

Water Stress Effects

It has been widely described that water stress induces a decrease in photosynthetic rates [51, 9]. Decreases in A_{sat} and g_s were reported for all clones of giant reed as an effect of water scarcity (Figs. 1 and 4). Giant reed clones showed a higher rate of assimilation in the control treatment, and Mann et al. [23] found similar significant decreases in CO₂ assimilation in drought treatments in giant reed, reducing CO₂ assimilation to 4–10 % relative to controls. One of the earliest responses to water stress is a decrease in stomatal conductance [52] as a way of decreasing transpiration and water loss from leaves. At the same time, a

decrease in the CO₂ diffusion into leaves is produced, reducing internal CO₂ partial pressure, and consequently, reducing photosynthetic rates, as observed by Li et al. [53] in wheat. In our study, decreases in photosynthesis rates (A_{sat}) are associated with reductions in stomatal conductance (g_s), as shown by the strong and positive correlation between both parameters (ESM 1), as previously observed by Flexas et al. [52].

Decreases in RWC observed in Experiment 1 in giant reed as a consequence of *WS* (Fig. 2b), might indicate a loss of cell turgor. Cornic and Massacci [42] showed that photosynthetic biochemistry is significantly affected only when leaf water deficit reaches 30 %. Therefore, giant reed should not have experienced damage to its photosynthetic machinery. Chlorophyll contents (ESM 2) decreased in water stress treatment relative to the control, due to an increase in chlorophyll degradation or a decrease in chlorophyll synthesis. A positive and significant correlation was found between chlorophyll content and A_{sat} and TDW even though measurements were made in completely mature leaves, although young ones. We believe that a stronger correlation would have been found if chlorophyll content had been measured in the oldest leaves, and this could be used as a screening criterion for plants under water stress and salinity, as proposed for barley by Rong-Hua et al. [54] and for wheat by Munns and James [47].

Giant reed growth was substantially affected by water stress, as indicated by large decreases in parameters such as H, NL, NS, gLA, TDW, S/R, SLA and LAR (Tables 2 and 3, Fig. 3). In addition, a decrease in the SLA (Table 3) was observed with respect to the control, indicating that leaves became smaller and thicker, in order to be more efficient for controlling water losses under water stress conditions [13]. Large decreases in shoot DM compared with root DM were observed under water stress, in accordance with Blum [45] and Munns and Tester [6]. The values of the S/R in control and water stress treatment as well as the S/R reduction (%) under water stress treatment relative to the control (Table 3) were slightly lower than those obtained by Mann et al. [23], reaching 4.34 and 2.13 g g⁻¹ (control and severe drought treatment, respectively) and 49–60 %, respectively.

In general, giant reed has been classified as a perennial rhizomatous grass with a high biomass yield in field conditions [25, 26, 55, 56]. However, the TDW was affected by water stress due to dehydration (Fig. 3). This biomass yield of giant reed under water stress was slightly larger than the results of Mann et al. [23], where only 21–28 % of the biomass of the control plants was produced under drought stress. In semi-arid Mediterranean field conditions, Cosentino et al. [19] concluded that giant reed grown in deficit irrigation (e.g., 50 % ETm restitution) not only conveyed to relevant biomass yield (reduction of up to 13.7–21.9 % with respect to the maximum achieved), but allowed a savings of more than 50 % of irrigation water. In conditions of low soil water availability, giant reed was able to improve its water use efficiency

and to maintain a high level of biomass production. Nevertheless, it is difficult to compare our data with data obtained from other giant reed experiments carried out in field conditions, where the growth of rhizomes and roots is not limited by the pot size.

The Effect of Both Stresses

Both stresses together affected A_{sat} and g_s in the same way that they were affected by water stress or salinity stress separately, although no significant differences were found between stress treatments at the end of the experiment (T60) (Fig. 1). Despite the lack of significant difference between clones at T60, there are significant differences after shorter stress durations, highlighting that the duration of stress is a critical factor.

The significant decrease in the RWC (Fig. 2d) and chlorophyll content (ESM 2) in stresses where water stress was involved indicates that giant reed seems to be more affected by water stress than by salinity. A similar pattern was observed for the growth parameters (H, NL, NS, gLA, TDW, S/R, SLA and LAR), confirming this hypothesis. However, the significant and positive correlation found between A_{sat} and SPAD, SLA and TDW in Experiment 1 (ESM 3) shows how these parameters could be used to determine the degree of stress encountered by the plant.

Stress Susceptibility Index (SSI)

According to our results, Agrigento seems to be a suitable clone for cropping in Mediterranean areas under *WS* conditions, due its smaller decrease in A_{sat} (Fig. 1), RWC (Fig. 2b) and gLA (Table 2). Useful characteristics under *WS* were also observed for parameters such as chlorophyll content and gLA, among others, with this clone having the lowest SSI value (Fig. 6). In contrast, Martinensis seems to be the most salt tolerant clone in Experiment 1, making it suitable for cultivation in marginal lands where salinity may predominate. Its smaller decrease in A_{sat} (Fig. 1) and gLA (Table 2) ensured that this clone had the lowest SSI value against salinity (Fig. 6), followed by Cefalú and Fondachello. In order to have a successful and constant performance and obtain a substantial biomass yield, which is the main objective of growing PRG, we propose Martinensis and Piccoplant as the most suitable clones for growing under both stress conditions for biomass production. This result has important implications for the agronomy of these plants in marginal lands. Fondachello seems to be the most tolerant to increasing salinity levels according to the results of Experiment 2, followed by Cefalú and Licata (ESM 6), and this confirmed the low tolerance of Agrigento and Tortorici to salinity. However, considering the projected future effects of climate change, it is important to focus on giant reed clones that perform well under both stresses. In our case, with plants grown under greenhouse

conditions, the clones with the better results for both salinity and water stress were Martinensis and Piccoplant (Fig. 6).

Summary

Our findings clearly indicate that:

- 1) In general, giant reed might be more tolerant to salinity than to water stress.
- 2) Salinity and water stress mainly affect stomatal conductance (g_s), generating stomatal closure in order to prevent dehydration of the plant, and consequently decreasing photosynthesis.
- 3) There was a differential response to salinity and water stress among the eight ecotypes of giant reed, with the most water stress-resistant ecotype being Agrigento and the most salinity-resistant ecotypes being Martinensis and Cefalú.
- 4) Fondachello, Cefalú and Licata were the most salinity-resistant ecotypes in relation to increasing salinity levels.
- 5) In order to benefit from a constant performance and to obtain a maximised yield of biomass, which is the main objective of growing PRG, we propose Martinensis and Piccoplant as the most suitable ecotypes for growing in both stress conditions for biomass production.

However, it is important to note that this screening against salinity and water stress was performed under greenhouse conditions in pots, where the growth and behaviour of plants might be different than in field conditions. On the other hand, we have imposed a high level of stress, which will be rare under field conditions, but this approach was used to physiologically characterise the behaviour of giant reed under adverse conditions. The gain in knowledge of the behaviour of giant reed under stress conditions (similar to those in marginal lands) will assist in the establishment of this energy crop and the development of this type of renewable energy.

Furthermore, given the tolerance that giant reed seems to have to salinity in general, it would be interesting to study in more depth the behaviour of these species under saline conditions, and to analyse, for example, the content of K^+ and Na^+ in the different plant's organs, in order to understand the mechanism of salt tolerance of giant reed.

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Author Contribution Study conception and design: ES, DS, SC, SN; Acquisition of data: ES, DS, GL, CA; Analysis and interpretation of data: ES, DS, SN; Drafting of manuscript: ES; Critical revision: DS, SC, SN.

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