Allocation pattern, ion partitioning, and chlorophyll *a* fluorescence in *Arundo donax* L. in responses to salinity stress

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Abstract

Biometric and physiological analyses of salt stress responses were performed in two time-course experiments on giant reed (*Arundo donax* L). Experiment I evaluated biomass production in plants exposed to 128, 256, 512 mM NaCl for 84 days. For Experiment II, plants grown under 256 mM NaCl were further assessed for chlorophyll *a* fluorescence, ionic partitioning and proline content at 14 and 49 days after treatment (DAT). Biomass allocation was affected with all the concentrations of NaCl used from 28 DAT onward. Proline biosynthesis in leaves was more stimulated than that in roots after salt stress. Photosynthetic efficiency of photosystem II (PSII) was not affected by salt stress up to 42 DAT, whilst 49 DAT plants exhibited a significant reduction of both potential (Φ_{PSII}) and maximal (F_v/F_m) PSII quantum yield. *A. donax* resulted a moderately sensitive species in response to 256 and 512 mM NaCl, concentrations that are however higher than that commonly found in most marginal lands (such as 128 mM or lower), where the biomass yield is appreciable especially in short-term cultivation (56 DAT here). Altogether, this study indicates that *A. donax* can be considered as a promising and valuable energy crop for exploiting Mediterranean marginal land.

Keywords: *Biomass allocation, Giant reed, Photoinhibition, Proline, Salt stress*

Introduction

A significant proportion of agricultural land has become saline owing to land clearance or irrigation, both of which cause that water tables rise and concentrate the salts in the root zone. Salt toxicity occurs when Na^+ and Cl^- accumulate above the concentrations required at whole plant level (Shabala & Munns 2012). A hypersaline environment results in reduced plant water availability which is decreased primarily due to the salt-induced osmotic stress (Chaves et al. 2009; Munns 1993). In addition, accumulation of Na^+ can compete with the uptake of other major cations, such as K^+ and Ca^{2+} (Niu et al. 1995). For example, in barley the maintenance of efficient CO_2 assimilation was mainly attributable to the maintenance of high K^+, low Na^+ homeostasis, and the resulting high K^+/Na^+ ratio in the cytoplasm of mesophyll cells (Munns et al. 2006). In addition to the toxic effect of Na^+, some species exposed to high Cl^- are also negatively affected since Cl^- accumulation significantly impairs the photosynthetic process (Tavakkoli et al. 2010).

Photosynthesis alteration under salt stress can be firstly a consequence of stomatal closure induced by the effect of osmotic stress perceived at root level due to the accumulation of salt outside the roots (Shabala & Munns 2012). In these conditions the CO_2 supply into the leaf is limited as well as the utilization of ATP and NADH leading to an excess of excitation energy (Megdiche et al. 2008; Takahashi & Badger 2011). The final consequence of this process is the increase of reactive oxygen species (ROS) production (Takahashi & Badger 2011) which can trigger oxidative damage to the chloroplasts (Niyogi 1999; Triantaphylidès & Havaux 2009). Metabolic limitations of photosynthesis in plants affected by salinity can also result from harmful concentrations of Na^+ and Cl^- in the leaf tissue (Munns et al. 2006). Ion excess (Na^+ and Cl^-) induces a reduction of leaf expansion as a consequence of reduced leaf transpiration rate; this, in turn, results into a buildup of photosynthates surplus in growing tissues (Munns et al. 2000). Excess of photosynthates induces a feedback signal which down-regulates photosynthesis to compensate the lower sugar request due to plant growth inhibition (Paul & Foyer 2001). Accumulation of salt at cytosolic or chloroplastic level inhibits the activity of photosynthesis-related enzymes, thereby further reducing CO_2 photoassimilation (Munns 1993).

Salt tolerance is achieved by plants through different strategies, such as the selective accumulation or exclusion of ions, the control of ion uptake by roots and transport into leaves, the compartmentalization of ions at the cellular and whole-plant levels, the change in photosynthetic pathway, the alteration in membrane structure, the induction of antioxidant systems and plant hormones, and the synthesis of compatible solutes (Parida & Das 2005), including proline. In plants grown under high salinity conditions, proline plays a key role in the stabilization of proteins and protein complexes in the chloroplast and cytosol, and in the protection of the photosynthetic apparatus (as shown in the salt-hypersensitive *p5cs1* Arabidopsis mutant) (Szabados & Savouré 2010).

Arundo donax L. (giant reed) is a perennial rhizomatous grass widely distributed from the Mediterranean basin to subtropical wetlands, and is considered as an invasive species in many Mediterranean countries including Italy (Mascia et al. 2013). Due to its high vigor, *A. donax* has been recently identified as a leading candidate crop for lignocellulosic feedstock, thanks to its positive energy balance, productivity in terms of yield potential, biomass characteristics, and low ecological/agro-management requirements (Lewandowski et al. 2003; Pompeiano et al. 2013) or under adverse environmental constrains (Jones et al. 2014), including salinity (Nackley & Kim 2015; Williams et al. 2009). Despite the general interest for *A. donax*, few and controversy studies have investigated the effect of salinity on biomass production and plant physiology.

Since the production of nonfood species (such as *A. donax*) has been promoted in marginal lands (Testa et al. 2014), such species should be selected based on their performance under less than favorable conditions found in those areas. The aim of the present research was thus to (*i*) determine response curves and species-specific threshold values for aboveground biomass of *A. donax* under salt-induced stress, and (*ii*) characterize the physiological response of *A. donax* to prolonged salinity exposure, by assessing the allocation of ions mainly perturbed by salinity stress, the accumulation of proline, and the spatial variation in photosystem II (PSII) efficiency within the leaf lamina of giant reed under salinity stress.

Material and methods

Plant material and growth conditions

Two independent studies, addressing each of the objectives stated, were performed, during the 2013 growing season at the research facilities of the Department of Agriculture, Food and Environment of the University of Pisa (Pisa, Italy). Four-week-old *A. donax in vitro* propagated plants were placed into 96-hole seed trays filled with a peat-based mix, and kept in growth chambers for four weeks at 22±1.0 °C with a 12-h photoperiod (light intensity: 200 µmol $m^{-2}s^{-1}$). Plants were watered daily to prevent wilting, and fertilizer was applied weekly with a half-strength Hoagland's solution (pH 6.5±0.1, EC 1.1 dS m^{-1}). Uniform plants (approximately 15 cm in height with a minimum of two stems) were transplanted into 0.7-L plastic pots filled with a mixture of 3:2:1 volume ratio of sphagnum moss peat, silica sand, and perlite. From this point on, the plants were grown under controlled climate greenhouse conditions. Plants were daily watered with tap water for the first week, and the next three weeks with daily tap water and half-strength Hoagland's solution (20 mL per pot) once a week.

To avoid salinity shock, NaCl levels were gradually increased by 64 mM per day until the final concentrations were reached. Plants were placed in pots suspended in nutrient tanks which contain 2 L of a continuously aerated, half-strength modified Hoagland's solution supplemented with the appropriate salinity concentration in deionized water (Epstein & Bloom 2005). The solutions were changed three times a week in order to avoid salt accumulation. Salinity concentrations were monitored by measuring electrical conductivity (EC) of the solution at 22 °C with a Crison conductivity meter (Pt-100, Barcelona, E). Throughout all the experiment period, the pots were rotated within their blocks at one-week intervals to minimize the confounding effects of other external factors. Greenhouse air temperature ranged between 22 and 32 °C during the day, and remained above 13 °C during night, RH between 65 and 80%, with daily maximum photosynthetically active radiation (PAR) levels ranging from 850 to 1530 µmol $m^{-2}s^{-1}$, provided by sunlight.

Experiment I: Salinity tolerance

Giant reed plants were irrigated with half-strength modified Hoagland's nutrient solution [control, with electrical conductivity (EC) = 1.1 dS m^{-1}], and half-strength modified Hoagland's nutrient solution with 128 mM NaCl (EC = 11.7 dS m^{-1}), 256 mM NaCl solution (EC = 23.4 dS m^{-1}), and 512 mM NaCl (EC = 42.2 dS m^{-1}) for 12 weeks. These concentrations of salt represented moderate to severe salinity stress for the species based on preliminary observations. All the treatments were performed in parallel. For each treatment, ten plants were removed at each time point (0, 1, 2, 4, 8 and 12 weeks after treatment) to assess biometric parameters. Plants were harvested and separated into leaf blades, stems, roots and the remaining rhizome fraction. Each fraction was washed twice in deionized water to remove the bulk of dust and debris, rapidly dried with paper, and the fresh weight (FW) was measured. Each fraction was then dried in a ventilated oven at 60 °C until constant weight in order to assess the dry weight (DW) of the sample.

Experiment II: Physiological responses to salt stress

Based on results from Experiment I, the intermediate salinity treatment of 256 mM NaCl solution (EC = 23.4 dS m^{-1}) was selected for physiological analysis. That salinity level was selected as the concentration limit for which *A. donax* plants do not have any reduction on biomass until 14 DAT. In addition, we selected two data points: 14 and 49 days after treatment (DAT), which were expected to span the range from a very early stage of stress to 75% of aboveground dry matter of the intermediate salinity treatment, based on Experiment I. At each data point, leaves, stems, and roots were collected from plants exposed to all treatments. Samples were immediately processed or ground in liquid nitrogen and stored at –80 °C for mineral and proline analysis.

Dried samples of roots, leaves and stems were finely grounded. Cations (Na^+, Ca^{2+}, Mg^{2+} and K^+) were extracted with concentrated HNO_3 and determined by atomic absorption spectrophotometer. Cl^- content was determined on water extract of lyophilized material. Briefly, 200 mg of sample was incubated in 5 mL of double distilled water for 30 min at 60 °C and the supernatant was collected after centrifugation. This procedure was

repeated twice. The supernatants were pooled, evaporated to dryness and the residues were resuspended in 2 mL of water. Cl^- contents were determined by using ion chromatograph (D-X-100 ion chromatograph, Dionex).

Chlorophyll fluorescence imaging was performed using an IMAGING-PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany). Details of the capture of chlorophyll fluorescence imaging are reported by Guidi et al. (2007). Images of the fluorescence parameters were displayed by means of a false colour code ranging from 0.00 (black) to 1.00 (purple). To determine the light response curve of apparent electron transport rate (ETR), the leaves were adapted to the desired irradiance for 5 min. For the estimation of ETR, the absorption coefficient of leaves over the 400–700 nm wavebands was estimated with an Ocean Optics USB2000 spectrometer (Ocean Optics Inc., Dunedin, FL, USA). Absorption coefficient averaged 0.84 for leaves from control and salt-treated plants.

Proline was extracted from 200 mg of fresh leaf tissue in 4 mL of 3% (w/v) 5-sulfosalicylic acid and measured using the acid-ninhydrin procedure, as reported in Pompeiano et al. (2015). Free proline concentrations were measured using $_L$-proline as a standard and calculated on a FW basis.

Statistical analysis

Pots were arranged in a randomized complete-block design with ten replicates for each time point. Scatter plots of the partitioning of aboveground biomass in relation to the whole plant biomass (percentage of aboveground DW) data versus DAT showed a nonlinear relationship. The data were fitted to a logistic regression model: aboveground DW (g) = 100 / {1 + 10^ [(BR_{50} – DAT) Slope]} where BR_{50} (50% of biomass reduction at a certain DAT) and Slope are estimated model parameters. Parameter estimates were used to calculate confidence intervals (95%) for the number of DAT withheld until giant reed in each treatment reached 75, 50, and 25% aboveground DW (BR_{75}, BR_{50}, and BR_{25}, respectively) for each treatment (Motulsky & Christopoulos 2003). At each aboveground DW yield percentage, the treatments were considered significantly different if their

confidence intervals did not overlap. Following Bartlett's test for homogeneity of variance, data were subjected to one-way analyses of variance (ANOVA), and means separation of treatment effects was accomplished using the least significant difference (LSD) test. For pair mean comparisons of control *vs* salt-treated plants within each time point (Experiment II), Student's *t*-test was applied. Percentage values were angularly transformed. All computations were performed with R 3.1.1 (R Core Team 2014), and the R package *agricolae* (de Mendiburu 2014) was used.

Results

Experiment I

Under salinity stress, *A. donax* exhibited a reduction in plant DW over time (Figure 1A). The first reduction in DW was observed with 512 mM NaCl at 14 DAT, and from that period the DW reduction was continuous with the severity of treatment. Of note, at the end of the experiment (84 DAT) *A. donax* plants did not survive at 512 mM NaCl (Figure 1A). During plant growth, controls allocated more biomass in leaves and stems than in roots, while under salinity conditions a significant change in biomass allocation was observed. Indeed, in salt-treated plants leaves exhibited a decline from 25 to 18% (of the whole plant biomass) under 512 mM NaCl at 56 DAT as compared to controls. Conversely, stem and belowground fraction increased from 38 to 43% and 37 to 40%, respectively on whole plant bases (Figure 1A).

Under all the salt conditions, *A. donax* exhibited increasing susceptibility along with the time exposure, as testimony by the reduction of aboveground biomass. The sigmoid models used to predict aboveground DW provided a good fit of the aerial biomass data, resulting in average R^2 values of 0.96 during salinity stress (data not shown). No significant difference in the estimated BR_{75}, the predicted number of days until the plant reached 75% aboveground DW, between intermediate and high salinity conditions was observed, averaging 21.1 DAT (Figure 1B). Marked differences were recorded among treatments in the estimated BR_{50} and BR_{25}. The estimated BR_{50} value was 55.0±1.42 d for giant reed in low salinity conditions, whereas it was significantly abrupt ($P < 0.001$) when the species

was subjected to increased salinity levels (36.6±3.12 d and 30.5±1.26 d, respectively for 256 and 512 mM NaCl treatments). The response of the species under saline conditions recorded for BR_{25} was reflected in the patterns observed in the previous estimated parameter (71.1±2.02 d, 50.2±3.57 d, and 41.6±2.02 d, respectively for 128, 256, and 512 mM NaCl treatments).

After 56 DAT, salinity stress caused a significant decrease in FW together with the reduction of plant height and stalk number (Table 1). Conversely, the percentage of dry matter allocated in the shoots increased significantly following salt severity as shown in Table 1 (i.e., 25.6% in controls *vs* 30.5%, 32.7%, 44.5% for 128, 256 and 512 mM NaCl, respectively). Although the most relevant negative effects were induced by the highest NaCl treatment, even 256 mM significantly affected plant performance (e.g., compared to the controls -86, -44, -38% for FW, stalk number, and height, respectively).

Experiment II

At 14 DAT, Na^+ and Cl^- significantly increased by 1.23 and 1.71% respectively under salinity stress, whereas Ca^{2+} and Mg^{2+} were relatively stable (Figure 2). In contrast, K^+ concentrations decreased slightly in response to salinity in all the tissues analyzed, although it was only significant in the roots. As expected, at 49 DAT, ion concentration in plant tissues increased, likely due to ontogenesis in both control and treated plants (*data not shown*). However, in stressed plants, ion accumulation was even higher and particularly pronounced for Ca^{2+} and K^+ (Figure 2).

Chlorophyll fluorescence analysis was carried out weekly starting from 14 to 49 DAT. Since between 14 and 49 DAT no effects on chlorophyll fluorescence parameters were observed (*data not shown*), figure 3 reports data at the beginning (14 DAT) and the end of the experiment (49 DAT). At 14 DAT, no changes in any parameters were observed, confirming the healthy status of PSII. Chlorophyll parameters were homogeneous on leaf lamina in both controls and stressed plants (Figure 3). A significant reduction in the maximum photochemical PSII efficiency (F_v/F_m), 0.70 *vs* 0.82 in salt-treated plants and controls, respectively, was recorded at 49 DAT. The decline in F_v/F_m in salt-stressed plants

was accompanied by a 30.8% decrease in the operational photochemical PSII efficiency (Φ_{PSII}) and a 10.3% decline in the photochemical quenching (q_P) values. No significant changes in non-photochemical quenching (q_{NP}) coefficient were recorded.

The response of ETR in increasing light intensities was comparable in controls and salt-treated plants at 14 DAT, while a pronounced decline in ETR values was found at 49 DAT especially at a PAR higher than 175 µmol m^{-2} s^{-1} (Figure 4). Levels of ETR at saturating light conditions were 27.9% lower in treated plants compared to the controls. Values of ETR were higher in young control plants (14 DAT) compared to 49 DAT plants.

Leaf and root proline content increased dramatically during salt stress (Figure 5). In control plants, proline was below the threshold of detectable values, both in leaves and roots. At 14 DAT, proline concentrations ranged from 4.8 to 2.4 µmol g^{-1} FW in leaves and roots of stressed plants, respectively. A more pronounced increase in proline was recorded in plants exposed for 49 DAT to salinity stress (about 4-fold higher than that at 14 DAT).

Discussion

Giant reed performance under salinity was measured in terms of changes in biometric traits and time-dependent alterations in physiological attributes. It is well known the detrimental effect of high salinity on plants which can be observed at whole-plant level as the death of plants and/or decrease in productivity (Parida & Das 2005). The reduction of plant growth is the result of the deleterious effects of root zone salinity that induce low osmotic potential, nutritional imbalance, specific ion effect and/or combination of these factors. Each salinity level used in our experiments negatively impacted the biomass production even though in a short period (56 DAT) when the biomass yield can still be considered a valuable amount for marginal lands, at least under 128 and 256 mM NaCl. Indeed, on the bases of the economic analyses of *A. donax* cultivation *vs* other food crops made by Testa et al. (2016), a reduction of DW of about the 70% (as recorder in this work with 256 mM at 56 DAT) still allows an annual gross margin not far from the profitability of some crops cultivated in arable areas (such as tomato). When the salinity stress is prolonged (84 DAT), plants

showed a strong reduction in biomass production and even mortality at the highest NaCl concentration (512 mM). Even the lowest salt concentration (128 mM NaCl) was sufficient to cause severe reduction of biomass after prolonged salt exposure (84 DAT). Nackley & Kim (2015) reported an interesting experiment in which *A. donax* plants were grown for 60 days under a wide range of salinity (0-42 dS m^{-1}). The authors found effects of salinity treatment only when EC was higher than 6 dS m^{-1}. At level of EC similar to that used in Experiment II (23 dS m^{-1}) the reduction in biomass found by Nackley & Kim was about 70% as compared to 86% found in our experiment. In agreement with Nackley & Kim (2015), mortality of plants did not occur before 56 days of salt exposure, even at the highest solute concentration utilized by the authors (~ 512 mM NaCl).

The maintenance of ion balance is crucial for an optimal plant growth under salinity because of one of the main effect of salinity is the disruption of ion homeostasis (Adams et al. 1992). Despite Cl^- toxicity is considered as a detrimental factor when plants are exposed to excessive NaCl, the perturbation of cation homeostasis (principally K^+, Ca^{2+}, and Mg^{2+}) provoked by high Na^+ uptake can also be considered deleterious (Tavakkoli et al. 2011). It is well known that K^+ is essential for osmoregulation and protein synthesis, maintaining cell turgor and stimulating photosynthesis (Flowers et al. 2015). In addition, both K^+ and Ca^{2+} are required for maintaining the integrity and functionality of cell membranes (Cakmak 2014), while Mg^{2+} is the key component of chlorophylls. In Experiment II, the main significant change was a slight reduction in K^+ (significant only in roots) at 14 DAT, but at the end of the experiment (49 DAT) plants under stress were able to rebalance ion partitioning within tissue. It is also remarkable the strong increment of Ca^{2+} found in each plant tissue at 49 DAT. It has been demonstrated that maintenance of Ca^{2+} acquisition and transport under salt stress is also an important determinant of salinity tolerance (Shabala & Munns 2012), and other authors reported that the main response to salt stress is also a change in Ca^{2+} homeostasis (Rengel 1992). It is well known that abscisic acid (ABA) and Ca^{2+} play a key role in stomata aperture (Blatt 2000; Evans et al. 2001) and that ABA can induce an increase of cytoplasmic Ca^{2+} in guard cells, which in turn results in stomatal closure (McAinsh et al. 2000). In addition, Ca^{2+} has also been reported

as a second messenger capable of enhancing or even switching on genes involved in salt-stress tolerance (Mahajan & Tuteja 2005). Thus, in agreement with previous findings in other species (Cramer et al. 1994; Rengel 1992), the maintenance of adequate K^+ and Ca^{2+} may be a response of *A. donax* to counteract the detrimental effect of high salinity. Na^+ accumulation observed in the stressed leaf tissues during the experiment was lower than that in the roots, especially at 14 DAT (Figure 2), and leaf/root Na^+ decreased under salinity (*data not shown*). This revealed the ability of giant reed to partially restrict Na^+ translocation and accumulation in the shoots. This mechanism is a typical response of glycophytes to salt exposure (Niu et al. 1995), even though *A. donax* is not usually classified as a glycophyte species, but rather as a halophyte (Quinn et al. 2015; Williams et al. 2009). However, in view of the considerable accumulation of Na^+ and Cl^- in the leaves, the compartmentalization mechanism (for instance, vacuole storage) is likely to assist *A. donax* after exposure to salt toxicity once Na^+ and Cl^- have been inevitably absorbed and translocated to the leaf tissues (Zhu 2003).

Beside compartmentation and translocation, ion homeostasis could be also maintained by the reduced stomatal conductance, which reduces transpiration flux, salt transport and accumulation (Everard et al. 1994). Nackley & Kim (2015) reported that stomatal closure is the main protective mechanism in *A. donax* against salinity. In turn, stomatal closure limits the supply of CO_2 and thus a decrease in the demand of energy and reducing power occurred. Therefore, the excess of excitation energy that is not tunneled to electron transport can alter the photochemical activity of photosystems. The result is an increase in the reduction state of PSII reaction centers that is recorded in *A. donax* plants under salinity (decrease in q_P). The maximal PSII activity (estimated by the ratio F_v/F_m) of dark-adapted leaves for control and NaCl stressed plants were similar until 42 DAT (Table S1). The leaf photochemistry, therefore, was rather resistant to salt stress as determined by Brugnoli & Björkman (1992) in salt-stressed cotton grown at different seawater concentrations and by Delfine et al. (1999) in spinach cultivated with saline water (175 mM) for 22 days. However, we found that either F_v/F_m as well as Φ_{PSII} of the salt stressed leaves of *A. donax* decreased at 49 DAT, indicating that prolonged high salt concentrations

affected leaf photochemistry. The significant decrease in Φ_{PSII} was not accompanied by an increase in q_{NP}, thus the decline in F_v/F_m was not attributable to an enhancement in thermal dissipation in the attempt to avoid photodamage. Conversely, the decrease in F_v/F_m can be attributed to the down-regulation of PSII activity and/or impairment of its photochemical activity, suggesting damages to the photosynthetic apparatus (Gallé et al. 2007). The decreased PSII activity observed at 49 DAT may also occurred due to the leaves senescence (Lu & Zhang 1998) exacerbated by salinity. Consequently, even ETR declined at 49 DAT in NaCl-treated plants and the effect was more pronounced at light intensities higher than 300 μmol $m^{-2}s^{-1}$, it is plausible that the accumulation of Na^+ and Cl^- ions could have affected the thylakoid membrane structure by disrupting the lipid bilayers or lipid-protein complexes and impairing the electron transport activity. As a consequence, salt stress could also have triggered higher ROS production in chloroplast due to the impaired electron transport processes (Miller et al. 2010).

The enhancement of the biosynthesis of compatible solutes, in particular proline, is another common response induced by salinity (Iqbal et al. 2014; Verbruggen & Hermans 2008). Proline is a multifunctional amino acid as well as a signalling molecule acting as a plant growth regulator by triggering cascade signalling processes (Yang et al. 2009). Proline is preferred as a common osmolyte in plants and up-regulated against different stresses, including salt stress (Szabados & Savouré 2010). On the other hand, some authors reported that the increment in proline content was not correlated with salt tolerance in barley, but rather only a consequence of salt excess in plant tissue (Chen et al. 2007; Szabados & Savouré 2010; Widodo et al. 2009). To confirm the not obvious relation between proline biosynthesis and salt tolerance, Liu & Zhu (1997) reported that a salt-sensitive Arabidopsis mutant had a higher proline content compared to the less sensitive control. Our data are in agreement with the latter hypothesis and suggest that proline accumulation is likely as a consequence of salt stress rather than an indicator of stress tolerance, thus the high proline content in *A. donax* salt-treated plants was not correlated with ameliorative effects on plant physiology (especially at 49 DAT).

Overall, our dataset reveals that *A. donax* is able to grow without any detectable alteration of photosynthetic apparatus during a short/medium period of high salt exposure (256 mM of NaCl for 42 DAT). Certainly, the biomass reduction detected at the early stage of treatment, even at 128 mM NaCl (28 DAT), is indicative of the negative effect of salinity on plant physiology. The reduction in the rate of leaf surface expansion as salt concentration increases represents indeed an immediate response to salinity (Munns & Termaat 1986; Wang & Nii 2000) as a strategy adopted by a wide range of plant species to improve water use efficiency and minimize evapotranspiration loss (Nackley & Kim 2015). To conclude, even though some authors classified this species as a halophyte (Peck 1998; Perdue 1958; Quinn et al. 2015; Williams et al. 2009), we consider *A. donax* a moderately salt-sensitive species in accordance with Nackley & Kim (2015). However, the concentrations of NaCl applied in our experiments (256 and 512 mM) are higher than those normally found in salt-enriched marginal land of Mediterranean areas and, in agreement with Nassi o Di Nasso et al. (2013), *A. donax* could become an important energy crop due to its high yield, low agronomic input requirements, and the capacity to grow in marginal land, therefore not competing with the arable land used for food production.

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509 **Caption of figures**
510

Figure 1. (A) Dry weight of *Arundo donax* plant portion in relation to days of treatment with 0, 128, 256 and 512 mM NaCl (first, second, third and fourth bars, respectively). Black: root and rhizome fraction; Grey: stem fraction; White: leaf fraction. At time 0 only controls were represented. At 84 days after treatment (DAT), the highest NaCl concentration is not shown since plant did not survive. For each sampling data, bars with similar letters are not statistically different from one to another according to Fisher's protected LSD ($\alpha = 0.05$). (B) The 95% confidence intervals for the number of DAT until each treatment reached 75% (square symbol), 50% (triangle symbol), and 25% (circle symbol) aboveground dry yield. Within each aboveground dry yield percentage, treatments with overlapping bars were not significant different ($p \geq 0.05$).

Figure 2. Percentage variation (difference between salt-treated and control plants) of element content in leaf, stem, and root tissues of *Arundo donax* grown with 256 mM NaCl at 14 (gray bars) and 49 (black bars) days after treatment (DAT). For each bar the significance of the differences between control and treated plants means (five replicates) after Student's *t*-test is reported (n.s. = $p \geq 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$).

Figure 3. Maximum (F_v/F_m) and operational (Φ_{PSII}) photochemical PSII efficiency, photochemical quenching (q_P) and non-photochemical quenching coefficient (q_{NP}) in leaves of *Arundo donax* grown with (256 mM) or without NaCl in the nutrient solution at 14 and 49 days after treatment (DAT). For each parameter and for each date, Student's *t*-test was applied for pair mean comparisons of control *vs* salt-treated plants (n.s. = $p \geq 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$).

Figure 4. Light response curves of electron transport rate (ETR) in leaves of *Arundo donax* grown in control condition (open circle) and in a nutrient solution containing 256 mM NaCl (closed circle) at 14 and 49 days after treatment (DAT). Data (mean ± SD) are fitted using a polynomial second-order curves.

Figure 5. Percentage variation (difference between salt-treated and control plants) of proline content in leaves and roots of *Arundo donax* grown with 256 mM NaCl at 14 days after treatment (DAT) (grey bar) and 49 DAT (black bar). For each bar the significance of the differences between control and treated plants means after Student's *t*-test is reported (*** = $p \leq 0.001$).