

# Growth and water deficit resistance of a Peruvian ecotype of *Arundo donax* L.

## Crecimiento y resistencia al déficit hídrico de un ecotipo peruano de *Arundo donax* L.

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**Cite as:** Sáez, M. (2020). «Growth and water deficit resistance of a Peruvian ecotype of *Arundo donax* L.». *South Sustainability*, 1(1), e010  
DOI: 10.21142/SS-0101-2020-010

Article received: 29/5/2020  
Peer reviewed  
Article approved: 18/6/2020



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### ABSTRACT

The aim of the study was to assess the behavior of a Peruvian ecotype of *Arundo donax* L., when subjected to water stress under greenhouse conditions, and to analyze the plant's physiological and growth parameters. For a period of 30 days, in 9.5 L pots, the species was submitted to drought conditions: daily water of 200 ml for the control (100 % of the pot capacity); 100 ml for moderate drought (SM; 50 % of the pot capacity); and 50 ml for severe drought (SS; 25 % of the pot capacity). A slight reduction in the relative water content (RWC), a reduction in plant leaf area (LA) and a favorable increase in the dry weight of both the root and aerial sections of the plant were identified. Although a reduction in chlorophyll concentration was observed, in general terms the species responded favorably to severe drought conditions. The results of the study offer a promising assessment of the tolerance displayed by *A. donax* to severe water stress and its potential biomass production under such conditions.

**Keywords:** Energy crops, Peru, drought, water stress.

### RESUMEN

El comportamiento de un ecotipo peruano de *Arundo donax* L. fue evaluado en condiciones de estrés hídrico en invernadero, para analizar parámetros fisiológicos y de crecimiento. La especie se sometió a condiciones de sequía correspondientes a un riego diario de 200 ml para el control (100 % de capacidad de maceta), 100 ml para sequía moderada (SM; 50 % de capacidad de maceta) y 50 ml para sequía severa (SS; 25 % capacidad de la maceta) en macetas de 9,5 L durante un periodo de 30 días. Se identificó una ligera reducción del contenido relativo de agua (RWC), una reducción en el área foliar (LA) y un aumento favorable en el peso seco de la sección de raíz y aérea de la planta. Aunque se observó una reducción de la concentración de clorofila, en términos generales la especie respondió favorablemente a las severas condiciones de sequía. De esta manera, los resultados permiten observar de forma promisoría la tolerancia presentada por *A. donax* frente al estrés hídrico severo y su potencial producción de biomasa en estas condiciones.

**Palabras clave:** Cultivos energéticos, Perú, sequía, estrés hídrico



## Introduction

Climate change is bringing changes throughout the world. Drought conditions are affecting crops and plants with industrial applications, particularly in agriculture (IPCC, 2014). Another looming crisis reported by the FAO is population growth and consequent increased food demand over the next decades (Alexandratos and Bruinsma, 2012a). Furthermore, energy demand is rising in what is an increasingly technology-based world, and alternative fuel sources to replace oil, an anthropogenic CO<sub>2</sub> contributor, are required. Candidates include bioenergy crops such as *Arundo donax* L. (the giant reed) (Fu *et al.* 2016; Lewandowski *et al.*, 2003; Pilu *et al.*, 2012).

Dubois (2011) proposes five conditions that must be met by a crop destined for the production of biofuels. One is that agricultural production systems must be both efficient and sustainable. While it is accepted that in the development of biofuels the sustainability of the system is closely associated with cultivation of marginal lands, their definition is not always clear. In this regard, in order to arrive at an objective definition, Jiang, Jacobson and Langholtz (2018) propose a frame of reference that defines marginal land as the convergence of four fundamental aspects:

- Biophysics → (soil quality).
- Socioeconomic → (production costs, land rental cost).
- Qualitative → (focus group discussion, interviews).
- Quantitative → (data and mathematical models).

The four remaining conditions proposed by Dubois (2011) are associated with attributes specific to the species or energy crop, and which also contribute to economic and environmental sustainability. Thus, rapid growth (1) and high biomass yield per hectare (2) favour economic sustainability, while an ability to develop in adverse climates and soils (3) and an inherent ability to resist disease, pests and predators (4) contributes to environmental sustainability.

*A. donax* L. is a perennial, high biomass crop that grows throughout the world and is used for making musical instruments as well as biofuel. It is also drought tolerant, and its crop breeding is based on ecotype selection (Cosentino *et al.*, 2006; Pilu *et al.*, 2013). As a crop, it constitutes a promising alternative for ethanol production, due to its capacity for rapid biomass storage and ability to grow in a variety of environments (Cosentino *et al.*, 2006; Haworth *et al.*, 2017b; Pilu *et al.*, 2013). This perennial grass lives for about 12-15 years, it does not have a high nutrient requirement, its nitrogen storage rate is high, its water needs are low, and its reproduction is clonal (Angelini *et al.*, 2009; Cosentino *et al.*, 2014; Haworth *et al.*, 2017b).

Previous studies have reported that the water deficit response of *A. donax* is good, and that as a crop it exhibits

the same physiological response and biomass production under conditions of long-term water deficit and well-watered conditions (Mann *et al.*, 2013). In order to grow under conditions of water scarcity, it is likely that this plant employs protective mechanisms to synthesize specific metabolites such as dimethylsulfoniopropionate, which mitigates oxidative stress and functions as an adaptive component (Haworth *et al.*, 2017a), together with a stable photosynthetic apparatus (Ahrar *et al.*, 2017).

In recent decades, in response to food security concerns, research in Peru in the field of dietary crops has improved (Alexandratos and Bruinsma, 2012b). Over the coming years, it will be necessary to identify more Peruvian energy crops capable of thriving in dry conditions, given the exposure of Peru's extensive arid coastal strip to drought conditions influenced by climate change, and to recurrent El Niño events (Mortensen *et al.*, 2018). In this context, the aim of this study is to report upon the behavior of a Peruvian ecotype of *A. donax* and its water deficit response and growth under controlled conditions.

## Materials and methods

### Plant material

The sections of *A. donax* cane collected possessed a node every 20 cm, as indicated by Pilu *et al.* (2013). Individual samples were collected from a low rainfall and moderate temperature ecosystem, located between the Pacific coast and the natural cliff lowlands of the district of Chorrillos (Lima, Peru, 5 meters above sea level; 12°09'38.9"S, 77°01'29.7"W).

The acclimation phase for the *A. donax* samples was conducted in the greenhouse of the environmental engineering faculty of *Universidad Científica del Sur* (Lima, Peru). The period of acclimation in coarse-grained seedbeds was approximately four months (March to July 2016), during which time climatic parameters were monitored by thermometer.

In order to reduce heterogeneity, at the start of the study the canes were carefully selected and manipulated to achieve a similar height, and each cutting was transplanted vertically into a 9.5 liter plastic pot (Ø 27 cm surface area), fitted with drainage holes and filled with a mixture of peat, perlite, and vermiculite (3:1:1). The pots were well watered with Hoagland nutrient solution (Hoagland and Arnon, 1950) from July to September 2016. Finally, the plants were subjected to a stress period (for 30 days during October 2016), and watered only with potable water.

In order to study the response of *A. donax* to different water stress levels six months after planting, the plants were subjected to three water treatments for 30 days: 200 ml of water daily per pot for the Control group (CC, 100 % of pot capacity; n=30), 100 ml per pot for the Mild Stress group (MS, 50 % of pot capacity; n=20); and 50 ml per

pot for the Severe Stress group (SS, 25 % of pot capacity; n=20). T0 (Day 1) did not reveal any indications of water stress. Therefore, the water stress treatments (mild and severe) were assessed from T1 (Day 15) to T2 (Day 30). At the start of the study period, each pot, which contained just one transplanted cutting, exhibited an average of four plants, produced from both nodes and rhizomes from nodes. Evaluations were conducted on Day 1, Day 15 and Day 30 (T0, T1 and T2), following the commencement of treatment. The greenhouse temperature range during the study period was 20 to 26 °C, while relative humidity varied from 50 to 75 %.

### Chlorophyll content

Chlorophyll content was measured in the last or second from last fully collared leaf of all the plants studied, using a portable meter (Minolta SPAD 502 Meter, Plainfield, IL, USA). Three measurements per leaf were taken from the middle of the leaf (with each measurement recording the mean value of three measurements per pot).

### Relative water content

The relative water content (RWC, %) of the leaves was determined as  $(FW-DW)/(TW-DW) \times 100$ , where *FW* is the fresh weight, *TW* is the turgid weight of the leaf after equilibration in distilled water for 24 hours at 4°C, and *DW* is the dry weight after being dried in an oven at 60°C until constant weight was reached. RWC was calculated as the mean of three leaves from the same plant, one per treatment.

### Biomass parameters

Whole plants were harvested, weighed and oven dried at 60°C until constant weight was reached. Subsequently, the dry biomass of shoots (leaves and stems) and roots (roots and rhizomes) was determined. Total fresh weight and plant leaf area were estimated prior to drying using a flat-bed scanner (Hewlett-Packard ScanJet IIcx, San Diego, USA) and analyzed using an image processing program (Image, University of Sheffield, UK, 2003). Parameters such as height along the longest stem from the base to the most recent fully expanded leaf (*H*; cm), shoot dry weight (SDW; g) and plant leaf area (LA) were calculated. Parameters related to weight, both dry and fresh, were calculated as the average value for each treatment (n=10) for each time period and did not include initial cutting weight.

### Statistical analysis

All studied parameters were tested for analysis of variance (ANOVA) and the means were compared using the Tukey pairwise multiple comparison test. The fixed factors were treatment and time. Statistical analysis was conducted using the SPSS 24.0 software package. The mean  $\pm$  standard error (SE) of each replicate was

calculated for each measured parameter. The results were accepted as significant at  $P < 0.05$ .

## Results

### Plant height

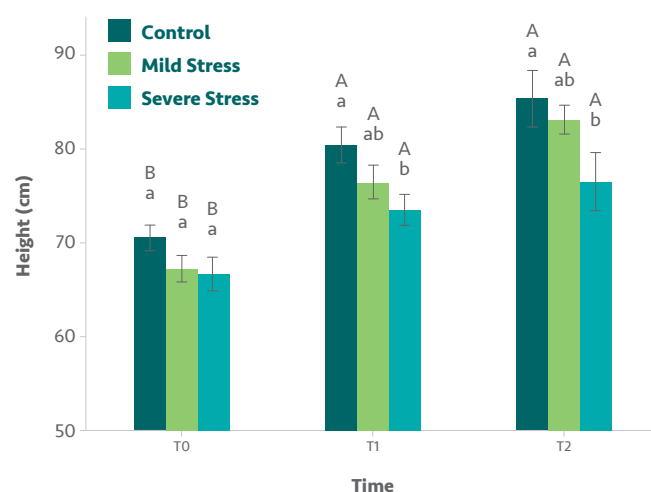
The plants in pots grew during the stress period. Plant height was significantly different for T0, T1 and T2 (Fig. 1) in all water treatments ( $p < 0.01$ ). Also, plant height was significantly different among the treatments. At the beginning of water restriction (T0), there was no significant difference between the control group and the mild and severe stress groups, while at T1 and T2 the height of the control plants was significantly greater than that of those subjected to severe stress (T1,  $p$  value: 0.017 and T2,  $p$  value: 0.028) (Fig. 1).

### Root development

The root growth of the plants was significantly different at T0, T1 and T2 (Fig. 2) ( $p < 0.001$ ) in fresh and dry weight. In both dry and fresh weight there were no significant differences between the water treatments.

### Aerial development

The aerial biomass (stem and leaf) of the control plants varied significantly at T0, T1 and T2 (Fig. 3 A, B;  $p < 0.001$ ) in both fresh and dry weight. Similar significant differences were found between T0 and T1 in stressed plants (Fig. 3;  $p < 0.001$ ). However, in the stressed plants there were no significant differences in aerial biomass between T1 and T2, with the exception of the dry weight of plants subjected to severe stress (Fig. 3 B). At T1, both the dry and fresh weights of plants subjected to severe water stress were



**Fig. 1. Comparison of plant height.** The height of all plants at time points (T0, T1 and T2) for three water treatments (Control-100 % PC, dark gray; Mild Stress-50 % PC, gray; and Severe Stress-25 % PC, light gray). The significant differences between control and stressed (mild and severe) plants at each time point are represented by the lower case letters, while higher case letters denote the significant differences between the time points for each water treatment ( $p < 0.05$ ).



lower than those of plants subjected to mild stress (Fig. 3 A, B;  $p < 0.05$ ). Comparing the treatments at T2, we found very significant differences between them ( $p < 0.001$ ). The aerial development (fresh weight) of control plants was significantly higher than that of those plants subjected to severe stress ( $p < 0.001$ ), as well as to those subjected to mild stress ( $p < 0.002$ ) (Fig. 3A), while the only significant differences in dry weight were found between the severe stress and control plants (Fig. 3 A, B;  $p < 0.05$ ).

### Leaf relative water content

The leaf relative water content showed no significant differences at T0, T1 and T2 (Fig. 4). In all plants, the measurement was greater than 70 %.

### Leaf chlorophyll content

The leaf chlorophyll content of the plants at T0 was significantly higher than at T1, and subsequently significantly higher than at T2 (Fig. 5;  $p < 0.001$ ). There were no significant differences between the control group or stressed group at T0, T1 and T2 (Fig. 5).

### Plant leaf area

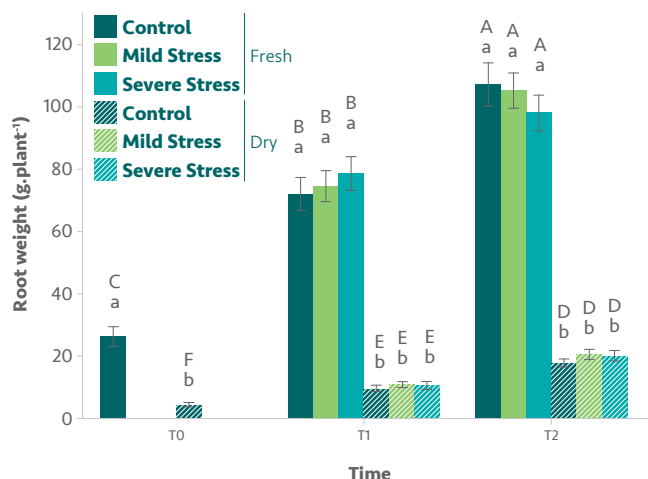
Plant leaf area at T2 displayed a significant difference between the control group and both the stress treatments (Fig. 6,  $p < 0.05$ ).

## Discussion

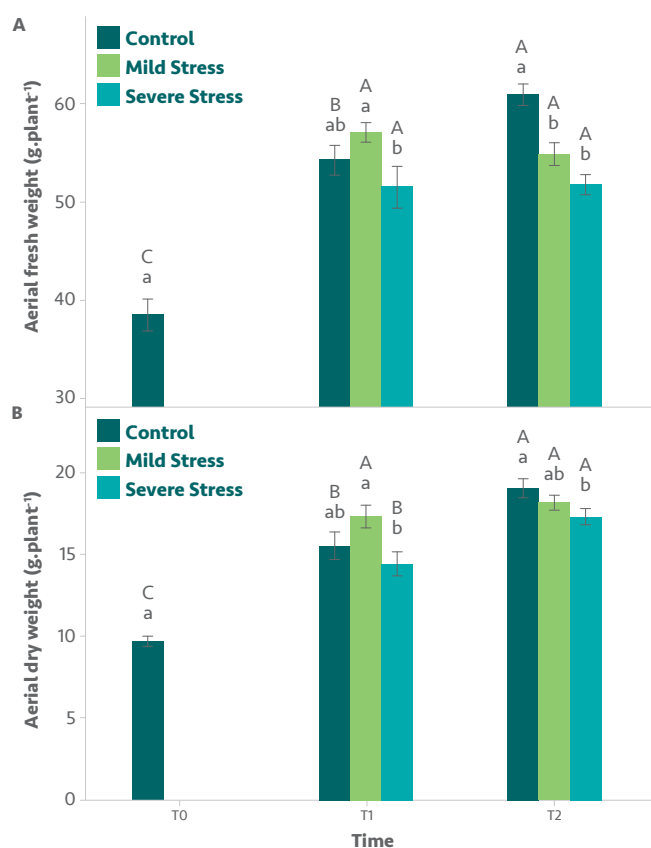
### Plant growth

Results showed how water stress has a considerable effect on biomass production in *A. donax*, with a decrease in the height, aerial fresh and dry weight biomass parameters (Fig. 1 and Fig. 3), while other parameters, namely fresh and dry root weight (Fig. 2) and the number of stems, remained stable.

One of the mechanisms that limits plant water use and reduces damage due to water stress is decreased plant leaf area and development (Blum, 2005). The limiting of water affected the production of aerial biomass due to lower carbon assimilation as a consequence of lower stomatal conductivity (Medrano *et al.*, 2002). A range of dehydration avoiding mechanisms have been reported in plants, including stomatal closure, leaf abscission and leaf rolling (Kramer and Boyer, 1995). These mechanisms lead to decreases in growth parameters such as fresh and dry weight of aerial biomass (Fig. 3). Our results showed a decrease in the dry weight of the aerial part of the plant, but not in the part below ground (Fig. 2 and Fig. 3), mirroring the results obtained by other authors (Maroco, 2000; Blum, 2005; Munns and Tester, 2008; Mann *et al.*, 2013). This would indicate that the maintenance of a good root system is important in conditions of water stress, to enable the plant to access groundwater sources (Lewandowski *et al.*, 2003).



**Fig. 2. Comparison of root growth.** The fresh root weight (solid colors) and dry root weight (hatched colors) of all plants compared at three time points (T0, T1 and T2) for three water treatments (Control-100 % PC, dark gray; Mild Stress-50 % PC, gray; and Severe Stress-25 % PC, light gray) are shown. The significant differences between control and stressed (mild and severe) plants at each time point are represented by lower case letters, while the higher case letters denote the significant differences across time points for each water treatment ( $p < 0.05$ ).



**Fig. 3. Comparison of aerial development.** The fresh (A) and dry (B) weight of all plants compared across three time points (T0, T1 and T2) for three water treatments (Control-100 % PC, dark gray; Mild Stress-50 % PC, gray; and Severe Stress-25 % PC, light gray) are shown. The significant differences between control and stressed (mild and severe) plants at each time point are represented by lower case letters, while the higher case letters denote the significant differences at time points for each water treatment ( $p < 0.05$ ).

The effect of water stress was observed on the height of the *A. donax* plants subjected to the treatments applied during the study (Fig. 1). However, one of the mechanisms employed by *A. donax* to enable it to remain viable under conditions of water limitation is modification of the anatomy of its leaves; they become smaller (Fig. 6), but at the same time thicker (Erice *et al.*, 2010). Leaf size is an important characteristic, contributing to the production of biomass and tolerance to drought, because plants with smaller leaves are able to reduce water evaporation, making them more tolerant of drought conditions (Ray *et al.*, 1998; Medrano *et al.*, 2007).

After 30 days of subjecting *A. donax* to drought-induced stress, the number of stems per pot in both drought treatments (SM and SS) increased from 4 to 5 despite the stressful conditions. However, Sánchez *et al.* (2015), who planted rhizomes rather than cuttings, identified that among eight ecotypes studied, Piccoplant (Germany) and Tortorici (Italy) were those that presented the highest number of stems, compared to the other ecotypes. Respectively, they reduced the number from 5 to 4 and from 4 to 2.7 on average, but with no significant differences for this parameter between the two ecotypes, thereby confirming that the stem number parameter would not be affected by drought.

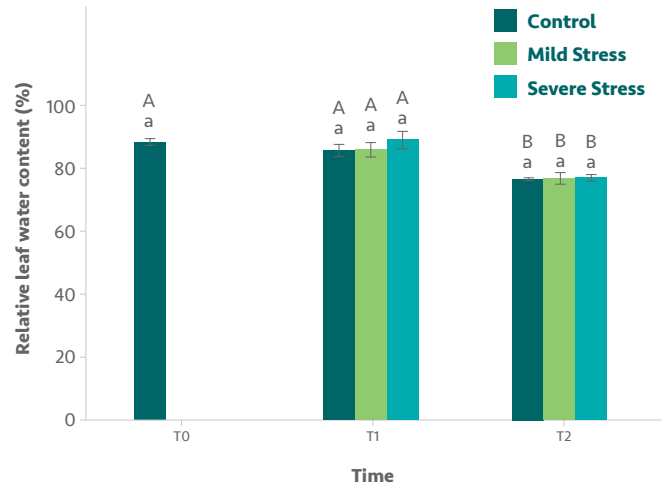
#### Leaf relative water content

Under hydric deficit conditions, the water content of a plant is affected and cells lose turgidity. This loss of turgor inhibits activities such as cell expansion, affecting directly the overall growth of the plant (Arve *et al.*, 2011), as well as stomatal closure, which has negative effects on photosynthesis, transpiration, and water and nutrient absorption (Flexas *et al.*, 2006; Parent *et al.*, 2009).

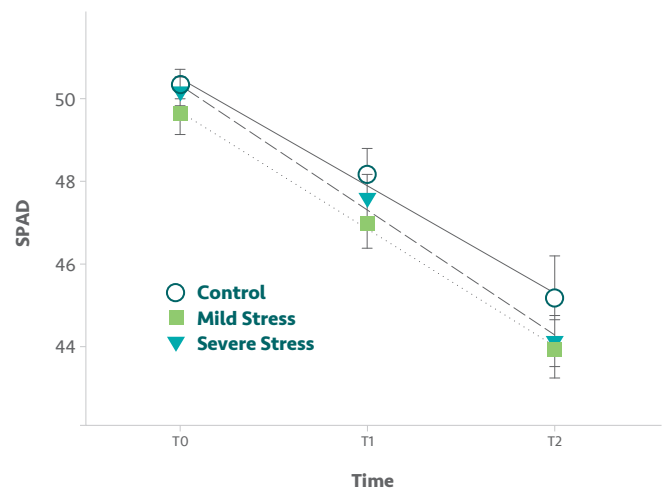
Thirty days after commencement of the stress period (T2), no differences were identified between the C and SS treatments (Fig. 4), mirroring the results reported by Sánchez *et al.* (2018) after twenty-one days at similar water stress levels; however, the research conducted by Sánchez *et al.* (2016) reported differences between the control and water stress treatment following 60 days subjected to stress, in smaller pots (5 l), and using rhizomes rather than cuttings at the beginning of the experimental procedure.

Although there were significant differences in the aerial fresh weight between groups C and SS (Fig. 3 A), this water loss does not result in critical damage to the plant, as the RWC data indicates (Fig. 4).

Abscisic acid is the hormone linked to the responses of plants to stress (Xiong, Schumaker and Zhu, 2002). According to Zhang *et al.* (2006), Haworth *et al.* (2016), and Sánchez *et al.* (2018), reduction in water loss by evaporation is strongly linked to stomata closure, which is the product of an increase in abscisic acid concentration in the leaves. Also, according to Cornic and Massacci (1996),



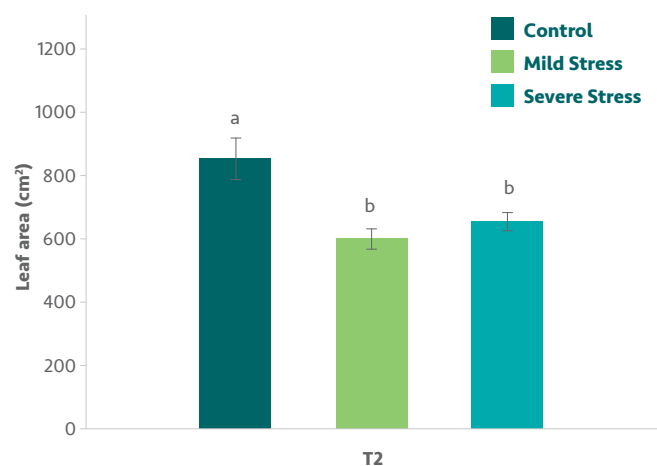
**Fig. 4. Comparison of aerial relative water content.** Relative water leaf content of all plants compared across three time points (T0, T1 and T2) for three water treatments (Control-100 % PC, dark gray; Mild Stress-50 % PC, gray; and Severe Stress-25 % PC, light gray) are shown. The significant differences between control and stressed (mild and severe) plants at each time point are represented by lower case letters, while the higher case letters denote the significant differences at the time points for each water treatment ( $p < 0.05$ ).



**Fig. 5. Comparison of leaf chlorophyll content (SPAD).** The chlorophyll content of the plants compared across three time points (T0, T1 and T2) for three water treatments (Control-100 % PC, white circles; Mild Stress-50 % PC, black square; and Severe Stress-25 % PC, black inverted triangles) are shown.

biochemical damage in the photosynthetic apparatus is significant only when the water deficit in leaves reaches 30 %. Therefore, according to the results obtained, and despite the conditions of severe water stress, the RWC of *A. donax* did not significantly decrease, and nor did those conditions affect the plant's photosynthetic processes, since in both treatments this parameter was higher than 70 %, similar to the results obtained by Sánchez *et al.* (2018) after 21 days of exposure to the same water deficit conditions.





**Fig. 6. Comparison of plant leaf area (cm<sup>2</sup>).** The foliar area of the plants compared at the final time point (T2) for three water treatments (Control-100 % PC, dark gray; Mild Stress-50 % PC, gray; and Severe Stress-25 % PC, light gray) are shown. The significant differences between control and stressed (mild and severe) plants at each time point are represented by lower case letters ( $p < 0.05$ ).

### Leaf chlorophyll content

The chlorophyll content values of *A. donax* at the beginning of the experiment were similar to those measured by Spencer *et al.* (2007) and Sanchez *et al.* (2016). A decrease in chlorophyll content (SPAD units) of 3 % was evidenced. Sánchez *et al.* (2016) identified a reduction in this parameter of 22.6 % under drought conditions (25 % of field capacity) for the same crop. This difference in the reduction percentage could be due to differences in experimental conditions (pot size, temperature, humidity, photoperiod) and to the fact that the duration of the drought period was 60 days.

It is known that water stress decreases chlorophyll content in some crops, including corn (Sánchez *et al.*, 1983), rice (Chutia and Borah, 2012), sunflower (Santos, 2004), sorghum (Jagtap *et al.*, 1998), and this is also the case with *A. donax*, which is more affected by water stress than by salinity (Sanchez *et al.*, 2016); however, the Peruvian ecotype used in this experiment produced good results under conditions of water stress.

*A. donax* has been identified as a drought tolerant plant (Lewandowski *et al.*, 2003) and results obtained under greenhouse conditions indicate a greater tolerance, good performance, and adaptation to water stress (Quinn *et al.*, 2015; Sánchez *et al.*, 2018).

To summarize, the parameters of plant leaf area, chlorophyll content, and aerial biomass production were only slightly affected by drought, confirming high biomass production and acclimation under conditions of water stress. Height and the root system were not affected by drought, indicating that normal root growth is employed as a mechanism of acclimation, as the plant seeks out water sources in deeper soil layers.

### Sustainability

Among the characteristics proposed by Dubois (2011), a crop suitable for the production of biofuels must be fast growing and be able to maintain such rapid growth in severe climates and poor soils. These attributes coincide with the results obtained, where a 75 % reduction in water supply did not reduce the growth of the crop. Similarly, they coincide with the results obtained by Cosentino *et al.* (2014) who reported that *A. donax* can grow under conditions of water deficit, and those reported by Perdue (1958) and Pilu *et al.* (2012), who recorded a growth rate of 5 cm/day under adequate environmental conditions.

Another requirement for the agricultural production of energy crops is that they must be both efficient and sustainable. In this regard, *A. donax* is a species requiring low or no fertilization and it is also able to grow on marginal lands (Pilu *et al.*, 2012; Sanchez *et al.*, 2015), thereby ensuring cost optimization and the ability to use areas not destined for food cultivation.

### Acknowledgements

This paper is published with the assistance of Dr. Gladys Lino in this work.

### Sources of financing

This study was funded by the research department of Universidad Científica del Sur.

### Authorial contribution

MS was responsible for the conception, data gathering and writing of this article.

### Potential conflicts of interest

The author has no potential conflicts of interest to declare.

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