Review

Arundo Donax L.: How High Photosynthetic Capacity is Maintained under Water Scarcity Conditions

Elena Sánchez [1,2], Pablo Rivera-Vargas [3], Xavier Serrat [2] and Salvador Nogués [2,*]

[1] Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya, 08860 Barcelona, Spain; elena.sanchez.sanchez@upc.edu

[2] Department of Evolutionary Biology, Ecology and Environmental Sciences, Plant Physiology Section, Universitat de Barcelona, 08028 Barcelona, Spain; xserrat@ub.edu

[3] Faculty of Education and Social Sciences, Universidad Andrés Bello, Santiago de Chile 7550196, Chile; pablorivera@ub.edu

[*] Correspondence: salvador.nogues@ub.edu; Tel.: +34-93-403-3717

Abstract: *Arundo donax* L. (giant reed) is a perennial rhizomatous grass and has been identified as an important non-food biomass crop with capacity for cultivation in marginal and degraded lands where water scarcity conditions frequently occur due to climate change. This review analyzes the effect of water stress on photosynthetic capacity and biomass production in multiple giant reed eco-types grown in different regions around the world. Furthermore, this review will attempt to explain the reason for the high photosynthetic capacity of giant reed even under changing environmental conditions as well as indicate other morphological reasons that could contribute to maintaining this high photosynthetic rate. Finally, future research in favor of selecting ecotypes with drought tolerance is proposed.

1. Introduction

Arundo donax L. (giant reed) is a species belonging to the Arundonoideae subfamily of the Gramineae family [1]. It is widely distributed throughout the world [2,3] since it can grow in a wide range of climatic conditions, from warm to cold [1] and highly humid to very dry areas, even those with long drought periods [4,5]. In addition, it maintains high biomass production under different nutrient availability conditions [6]. It has low input requirements while having high salinity and N tolerance [7], which increases its adaptability to marginal environments. It is well known that biomass crop production is competing with food production for arable land, hence the importance of using plant species with good adaptation to the climatic and unfavorable soil conditions of marginal lands. Thus, giant reed is considered one of the most interesting species for biomass production [4,8–14]. Regarding the use of giant reed as an energy crop, it has been characterized as a good material for combustion [15], although its biomass quality is under discussion [16]. Interestingly, it has been described as the best-suited species for biogas production due to its high C:N ratio [6], which is within the optimum (25–30) established by Maishanu and Hussani [17]. The ideal biomass crop needs to be not only adapted to marginal lands, but also have as high as possible biomass yield [18].

The photosynthetic rate of plants, and therefore their biomass production, depends to a large extent on the environmental conditions to which the plant is exposed and on the species' type of metabolism. Plant species are classified according to the photosynthetic mechanisms of carbon fixation as C3, C4 and CAM [19], with anatomical and biochemical differences between them. In C3 plants, whose name derives from the fact that the first carbon compound produced in the photosynthesis contains three carbon atoms,

carbon dioxide is fixed and transformed into sugar by the enzyme Rubisco through the Calvin–Benson cycle. However, the photosynthesis rate in C3 plants is slowed down by two key restrictions: (i) high photorespiration due to the fixation of oxygen molecules instead of carbon molecules in a high percentage of cases and (ii) high loss of water through the stoma during carbon uptake.

C4 plants are characterized by a Kranz anatomy, which includes two different photosynthetic tissues: an outer layer of mesophyll cells and an inner layer of bundle sheath cells surrounding the vascular bundles [19]. Carbon is converted into a four-carbon compound in the mesophyll cells by the enzyme phosphoenolpyruvate carboxylase (PEPc), which is characterized by a high affinity for CO_2. These C4 compounds are transported to the chloroplast of the bundle sheath cells, releasing CO_2 and increasing its concentration, which leads to a lower photorespiration due to the higher efficiency of Rubisco. Although C3 plants do not have PEP carboxylase to avoid photorespiration, giant reed has been described as a C3 species with a photosynthetic capacity similar to C4 species (like *Miscanthus* or *Panicum virgatum*), even on marginal lands [7,12,13,20].

2. Photosynthesis of Giant Reed in Optimal Growth Conditions

The values of some photosynthetic parameters that are important in the metabolism of *Arundo donax* L. as a biomass crop are presented and analyzed below.

The photosynthetic rate (Asat) is a gross measure of the rate at which a plant captures radiant energy and fixes it in organic carbon compounds. It is defined as a plant's carbon assimilation maximum per given leaf area unit and time exposed to saturating photosynthetically active radiation (μmol CO_2 m^{-2} s^{-1}) [21].

Although giant reed's Asat has been widely studied during the last two decades (Table 1) it is difficult to set in a range or compare, because the photosynthetic rate of giant reed in control conditions varies depending on several factors, such as light intensity during measurements, vapor pressure deficit (VPD), soil type, nutrient availability, the age of the canopy, and crop management [10,11]. However, high Asat values have been observed in plants regardless of age (plantlets or adult plants) or differences in growing conditions (in the field or in a greenhouse in pots).

Table 1. Photosynthetic assimilation rate (Asat; μmol CO_2 m^{-2} s^{-1}), stomatal conductance (Gs; mol m^{-2} s^{-1}), transpiration rate (E; mmol m^{-2} s^{-1}) and water use efficiency (WUE; μmol mmol^{-1}) of different *A. donax* L. ecotypes growth in different treatments (T): (i) control conditions (well-watered, WW) and (ii) under water stress (WS).

T	Asat	Gs	E	WUE	Experimental Conditions	Reference
WW	23.6	0.56–0.68			A two-year field trial of giant reed under different nitrogen fertilization and soil water availability in a semi-arid Mediterranean area.	[11]
WW	~33~38	0.548–0.770			Two well-established ecotypes (more than 15 years) from Sicily (Italy) in an experimental field.	[22]
WW	25–30	0.3–0.6			Rhizomes collected from Morocco, Sicily, and Florence and planted in plots in an experimental field.	[22]
WW	~38	0.750–0.800			Two well-established ecotypes (Central Italy and Morocco) with irrigation	[23]
WS	18–25	0.2–0.3			Two well-established ecotypes (Central Italy and Morocco) rain-fed for 6 weeks.	[23]
WW	11.91	–	3.36	3.54	Field capacity (20–35% moisture *v/v*; 0.0 MPa). Established cohort (16 weeks old; 8 weeks of treatment). Containers in greenhouse in California (USA).	[24]
WS	0.52		0.13	4.03	Mild drought (9% moisture). Established cohort (16 weeks old; 8 weeks of treatment). Containers in greenhouse in California (USA).	[24]
WS	0.12		0.16	0.75	Severe drought (5% moisture). Established cohort (16 weeks old; 8 weeks of treatment). Containers in greenhouse in California (USA).	[24]
WW	30.2				Natural stand located in Portugal.	[25]
WW	28.3	0.295		3.3	Plants collected in San Martí Sarroca (Spain).	[21]

WW	~20~28	0.170			Pot experiment with plantlets of a commercial clone.	[26]
WS	15.45	0.067			Progressive drought for 66 days (until 20%FC) in plantlets.	[26]
WW	~30	~1.1			Wild population from Florida (USA).	[27]
WW	27.13	0.644	6.31	4.2	Pot experiment. Rhizomes from California (USA).	[28]
WW	~23~27	0.250–0.450			Different clones in pots in greenhouse conditions.	[29]
WW	~27	~0.5	~9	~3	Plants in pots in greenhouse conditions.	[30]
WS	~18	~0.15	~3.7	~5.5	Mild stress in pots in greenhouse conditions.	[30]
WS	~9	~0.8	~1.8	~6.3	Several stresses in pots in greenhouse conditions.	[30]
WW	20–21	0.38–0.39	5.6		Two ecotypes (Central Italy and Morocco) planted in rhizotrons.	[31]
WS	14.1–14.4	0.153–0.157		3.2–2.9	Two ecotypes (Central Italy and Morocco) planted in rhizotrons.	[31]
WW	25–30	0.5-0.7			Rhizomes collected from Morocco, Sicily, and Florence and planted in plots in an experimental field.	[32]
WW	15.3–34.0				Pot experiment in experimental fields; *A. donax* L. from Greece.	[33]

~ Approximate values obtained from published figures.

The stomatal conductance (Gs) and transpiration (E) are also key parameters to assess plants' limitations in photosynthesis and growth potential, due to their strong effect on the CO_2 availability at photosynthetic cells. Gs conditions the net molar flux of CO_2 entering or water vapor exiting through the leaf stomata, for a given concentration difference of CO_2 or water vapor between the atmosphere and the sub-stomatal cavity. The molar fluxes conditioned by Gs are, for CO_2, the net CO_2 assimilation rate; and for water vapor, the transpiration rate [25].

Plants' ability to reduce transpiration while maintaining photosynthesis despite partial stomatal closure is called water use efficiency (WUE) and it is calculated according to the amount of carbon assimilated as biomass or grain produced per unit of water used by the crop. Giant reed, due to its C3 anatomy and metabolic pathway, has shown a higher transpiration rate (E) than C4 perennial grasses [11,24,27,28,34], reaching values of 9.5 mmol H_2O m^{-2} s^{-1} [20], and leading to lower water use efficiency [21,27,28]. Nevertheless, the substantial transpiration of giant reed does not affect the high photosynthetic rates, which in most cases are similar to a C4 species [25].

Comparing Asat data in control conditions (WW, Table 1), we note that they are not as high as those mentioned by Rossa et al. [20] (36.7 µmol m^{-2} s^{-1}), although they are within the range reported by Haworth et al. [22,23] (33–38 µmol m^{-2} s^{-1}). However, it is clearly observed that in most cases the Asat values are higher than the standard values in C3 plants (18–20 µmol m^{-2} s^{-1}) [35], and very similar to those of C4 species [21,24,25,36].

According to Rossa et al. [20], such a high photosynthetic rate in giant reed could be attributed to a higher level of irradiance for the saturation of electron transport through PSII and a lower photoinhibition compared to other grasses. However, instead of lower photorespiratory rates or high stomatal conductance, the high photosynthetic efficiency of giant reed might be related to the high capacity of RuBP-limited and RuBP-saturated photosynthesis (Vc,max 117 µmol CO_2 m^{-2} s^{-1} and Jmax 213 µmol CO_2 m^{-2} s^{-1}) [25], which is nearly double the average of other C3 species (Vc,max 64 µmol CO_2 m^{-2} s^{-1} and Jmax 134 µmol CO_2 m^{-2} s^{-1}) [37]. These high Vc,max and Jmax values in control conditions have been observed in other studies with giant reed [21,26].

Another important point to study regarding the giant reed's photosynthetic activity is how it behaves in light-limiting situations such as near dawn or dusk, during very cloudy days, or in the lower part of the canopy. The carbon uptake during low light flux periods may be aided by relatively high maximum quantum yields of CO_2 assimilation and high leaf absorptances. In this sense, the maximum absolute quantum yield of CO_2 assimilation (ΦCO$_2$,max; 0.056 mol·mol^{-1}) and the leaf absorptance (α, 0.80) observed by Webster et al. (2016) [25] in giant reed was 8% higher than the ΦCO$_2$,max values observed by Osborne and Garrett (1983) [38] and 14% higher than those α values observed by Long et al. (1993) [39] in other C3 grasses. Giant reed would therefore have an increase of 24%

in CO_2 uptake per unit incident light, which would be another reason for its high photosynthetic rates under optimal growing conditions [25].

The high photosynthetic rates mentioned above go together with the high biomass production reported under optimal cultivation conditions. A wide range of giant reed yield data has been published in recent years, accounting for variability in the ecotypes used in the experiment, the crop establishment age, and the production area. Yields reported in Spain, Italy, and Turkey have shown ranges of 29.6–63.1 t dry matter ha^{-1}, 20.4–51.5 Mg dry matter ha^{-1}, and 12.86–36.78 t dry matter ha^{-1}, respectively [3,40,41]. These are of the same magnitude as those obtained in other studies [7–9], and are even higher than the production reported in some C4 species [41].

It is worth highlighting here that despite the low genetic diversity found in clonal populations collected in different parts of the world due to lack of sexual reproduction [2,16,42–44], phenotypic differences have been described in other studies in terms of biomass parameters (LAI, SLA, etc.), and biomass yield and flowering time [3,9,44-46]. These phenotypic differences have also been observed between ecotypes in terms of Asat [29], Gs, and carbon isotope discrimination [46], whereas other studies have not found significant differences in Asat between ecotypes collected in different locations [22,23]. Accordingly, it is also of interest to focus on future multiyear and multisite investigations to search for cultivars that not only have a high photosynthetic rate but also a substantive biomass yield and which are of high quality for energy production.

3. Photosynthesis of Giant Reed under Drought Conditions

Many studies have shown the adaptability of giant reed to a number of unfavorable growth conditions, such as salinity [21,29] and submergence stress [24]. In this review, we will focus only on water stress (WS, Table 1) because it is well known that giant reed is a highly drought-tolerant species [1,4,5,21,24,29]. It cannot be ignored that the water stress tolerance of giant reed in adult stages is partially provided by the development of rhizomes, which improve the ability to accumulate water, nutrients, and carbohydrate reserves [13,24], and by deeply penetrating roots that can pursue water sources [4]. For this reason, we consider that a complete and thorough study of the rhizome's role in giant reed under drought conditions should be carried out, and we propose the use of stable isotope techniques to understand the carbon fluxes and allocation in this organ.

Despite this characterization as a drought-tolerant species, soil-drying-induced reductions in Asat and Gs depend on the level of water stress and its duration [10,11,21-24,26, 28-31]. Moreover, reductions in the use of light energy for photochemistry as well as damage to PSII function and increased heat dissipation (NPQ) have been observed in relation to control conditions [22,23]. This heat dissipation was also observed by Sánchez et al. [30], although no damage was shown in the PSII reaction centers due to similar Fv/Fm values during water stress, as shown by Zegada-Lizarazu et al. [31].

Haworth et al. [22] found a reduction of 66.7% in Asat and 91.2% in Gs 40 to 60 days after the cessation of irrigation (i.e., 35% of available soil water content in the first 0–30 cm soil depth and 20% of available soil water content at a soil depth of 60–90 cm). Similar values were observed by Sánchez et al. [29] after 60 days of water stress, with a 70.3% decrease in the Asat rate and a 91.3% decrease in Gs relative to the beginning of the experiment. The effect on Asat is not only noticed with prolonged water stress periods, as Sánchez et al. [21] have reported a decrease of more than 50% in the first 15 days of water deficit. Likewise, a greater reduction in Gs was observed for the same period. Moreover, a high and positive correlation of both parameters was observed in several studies related to drought or other abiotic stresses [21,22,30,32]. This would suggest that the temporal sequence in the reduction of Gs and Asat reported in other species [47], reflecting that stomatal closure is the first event restricting photosynthesis under water stress conditions, would also be observed in giant reed studies. Indeed, Romero-Munar et al. [26] observed a reduction in Gs ten days before the Asat reduction in plantlets grown under water deficit

conditions. The highly functional and effective control of stomata that is found in giant reed [32,48] is induced by an increase in foliar ABA content in response to drought [48].

As is well known, the stoma plays a critical role in the regulation of gas exchange between the interior of the leaf and the exterior environment, and its behavior is affected by environmental changes such as an increase in water deficit.

According to Flexas et al. [47], the biochemical capacity to assimilate CO_2 in C3 plants would not be affected by mild or severe stress levels, but rather the diffusion of CO_2 would be affected by a decrease in stomatal and mesophyll conductances. Under more severe stress, a general failure of metabolism would occur due to secondary oxidative stress, particularly under high light requirements [47], which are commonly observed in giant reed growth conditions [20]. Therefore, the photosynthetic capacity of giant reed under drought conditions would be preserved but limited by diffusive resistance. Accordingly, Romero-Munar et al. [26] observed no effect of water stress on the photosynthetic apparatus due to $V_{c,max}$ and J_{max} values, which were similar to control conditions, showing that photosynthesis is more limited by stomatal (93.3%) than by biochemical factors (4.7%). Even Haworth et al. [32] observed no strong influence on $V_{c,max}$ up to the point of severe water stress.

The effect of water scarcity is clearly observed in a reduction in biomass yield, not only in giant reed [14,21,22,26,29,31], but also in other plant species used as energy crops, such as *Dactylis glomerata* or *Miscanthus x giganteus* [49]. However, the reduction in biomass is lower than giant reed's decline in photosynthetic capacity [22], and this is due to the increase in water use efficiency (WUE) observed in plants subjected to water stress [21,26,30,49]. This means that the reduction in water consumption is greater than the reduction in biomass accumulation.

In addition to a reduction in shoot biomass rather than root biomass under water stress conditions [29], morphological changes have also been observed in giant reed leaves such as reductions in leaf area, the leaf area index (LAI), specific leaf area (SLA), and the leaf area ratio (LAR) [21,26,29–31]. These changes reflect anatomical acclimation and are a key component in the plant's ability to cope with water scarcity by controlling water loss through increases in WUE at the plant level [26,30,31]. Consequently, a more sustainable amount of water would be used, which is important at present in agriculture.

Another morphological response observed in giant reed as an adaptation to the environment is the variation in the xylem vessel diameter [50], which may regulate resistance to embolism. According to these authors, enlarged xylem vessel size initially allows ecotypes from arid regions to provide additional water to growing parts under drought conditions, but under more prolonged drought this causes vessel embolism and damage to the photosynthetic tissues, which then die off. The death of a plant's photosynthetic parts preserves rhizome viability, thus enabling regrowth when water is available again. In contrast, a reduction in the xylem vessel size in other ecotypes increases resistance to xylem embolism and allows plants to continue growing under milder drought conditions, and may be preferable for cropping systems, particularly where irrigation can be provided. Therefore, it would be interesting to carry out a larger study on the behavior of xylem size in other giant reed ecotypes subjected to water stress.

Phenotypic differences have been observed in different ecotypes subjected to water stress in relation to physiological and biomass parameters [23,29,50]. Therefore, it is imperative that future cultivar selection studies also include water stress tolerance to support growth in constrained marginal lands. Fabbrini et al. [46] suggested testing the drought tolerance of ecotypes in this way against differences in the Gs August/Gs July relationship. This means that the ideal ecotype would show i) low Gs July, keeping a high photosynthetic rate when some water is available in the soil, and ii) a large increase in Gs August, which would mean that the plant saves water when much less is available, or a low Gs August, meaning that water uptake still occurs due to an efficient and deep root system. One of the advantages of using Gs as a predictor of water stress is that it can be estimated indirectly through thermal infrared imaging, which provides useful information for

breeding ecotypes with improved drought tolerance [51]. In addition, the high and positive correlation between Gs and dry matter found by Kørup et al. [49] in other perennial grasses highlights its potential as a measure of water stress.

Moreover, Fabbrini et al. [46] suggested that giant reed's WUE should be more dependent on variability in photosynthetic capacity than on stomatal physiology, since carbon isotope discrimination is more related to stomatal resistance. Thus, he emphasized the need to study the variability and heritability of photosynthetic capacity for selection purposes.

In addition to an extensive root system, the existence of rhizomes and the physiological behavior of stomata allows giant reed to optimize the WUE. On one hand, some authors [23,31,52] have speculated that its ability to maintain high production under water stress is also due to modifications to the emission of isoprene and dimethylsulfoniopropionate (DMSP), which is part of the methionine (MET) pathway and plays a protective role in moderate drought stress tolerance. On the other hand, Lu et al. (2020) [53] observed that although photosynthetic pigment synthesis under drought stress was inhibited, the increase in superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and the decrease of malondialdehyde (MDA) indicate a response to oxidative damage by coordinating antioxidant enzymes to scavenge free radicals and accumulation soluble proteins. Therefore, studying the MET pathway and the response to oxidative damage would be another interesting line of exploration in future experiments to optimize the physiological response to drought stress and maximize growth.

When investigating giant reed as a promising grass to be grown in marginal lands, it is also important to consider its recovery capacity after a drought period. In this regard, the rapid restoration of key physiological functions that has been observed after rewatering and after O_2 deprivation [53–57] would confirm the environmental plasticity of giant reed in the face of different abiotic stresses [33].

4. Conclusions

Although we observe the effect of water scarcity on the decrease in Asat of giant reed (Table 1), it seems that the photosynthetic apparatus is not thereby damaged, and photosynthesis is more limited by stomatal limitations than by biochemical limitations.

Furthermore, several mechanisms observed in giant reed, such as an extensive root system, the existence of rhizomes, the physiological behavior of stomata allowing WUE optimization, and the morphological responses in xylem vessels, underscore the maintenance of giant reed's high production under water stress (Figure 1).
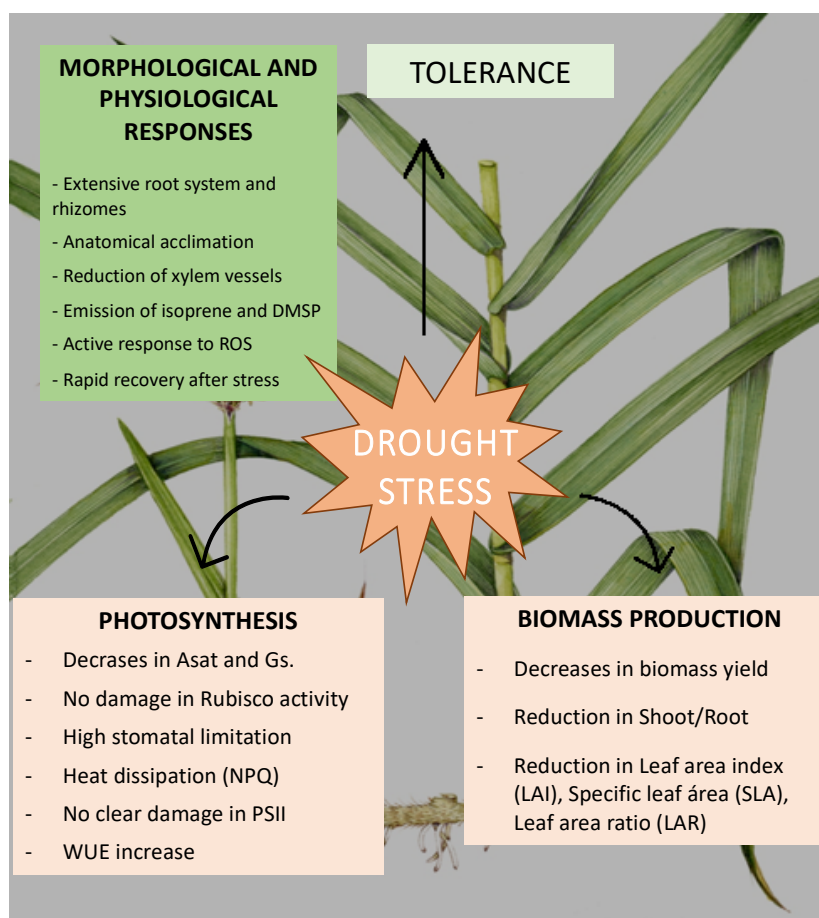
Figure 1. Summary figure describing the main findings of the study. Asat: photosynthetic assimilation rate; Gs: stomatal conductance; NPQ: non-photochemical quenching; PSII: photosystem II; WUE: water use efficiency; DMSP: dimethylsulfoniopropionate; ROS: reactive oxygen species.

Finally, new directions for research have been proposed in this paper that should help to reveal more information about giant reed's plasticity under water stress in order to improve the cultivation of giant reed on constrained marginal lands to produce biomass.

References

1. Perdue, R.E. *Arundo donax*—Source of musical reeds and industrial cellulose. *Econ. Bot.* **1958**, *12*, 368–404.
2. Mariani, C.; Cabrini, R.; Danin, A.; Piffanelli, P.; Fricano, A.; Gomarasca, S.; Dicandilo, M.; Grassi, F. Origin, diffusion and reproduction of the giant reed (*Arundo donax* L.): A promising weedy energy crop. *Ann. Appl. Biol.* **2010**, *157*, 191–202, doi:10.1111/j.1744-7348.2010.00419.x.
3. Amaducci, S.; Perego, A. Field evaluation of *Arundo donax* clones for bioenergy production. *Ind. Crop. Prod.* **2015**, *75*, 122–128, doi:10.1016/j.indcrop.2015.04.044.
4. Lewandowski, I.; Scurlock, J.M.O.; Lindvall, E.; Christou, M. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass. Bioener.* **2003**, *25*, 335–361, doi:10.1016/S0961-9534(03)00030-8.

5. Mack, R.N. Evaluating the credits and debits of a proposed biofuel species: Giant reed (*Arundo donax*). *Weed Sci.* **2008**, *56*, 883–888, doi:10.1614/WS-08-078.1.

6. Ren, L.; Eller, F.; Lambertini, C.; Guo, W.Y.; Brix, H.; Sorrel, B.K. Assessing nutrient responses and biomass quality for selection of appropriate paludiculture crops. *Sci. Total Environ.* **2019**, *664*, 1150–1161, doi:10.1016/j.scitotenv.2019.01.419.

7. Borin, M.; Barbera, A.C.; Milani, M.; Molari, G.; Zimbone, S.M.; Toscano, A. Biomass production and N balance of giant reed (*Arundo donax* L.) under high water and N input in Mediterranean environments. *Eur. J. Agron.* **2013**, *51*, 117–119, doi:10.1016/j.eja.2013.07.005.

8. Angelini, L.G.; Ceccarini, L.; Bonari, E. Biomass yield and energy balance of giant reed (*Arundo donax* L.) cropped in central Italy as related to different management practices. *Eur. J. Agron* **2005**, *22*, 375–389, doi:10.1016/j.eja.2004.05.004.

9. Cosentino, S.L.; Copani, V.; D'Agosta, G.M.; Sanzone, E.; Mantineo, M. First results on evaluation of *Arundo donax* L. clones collected in Southern Italy. *Ind. Crop. Prod.* **2006**, *23*, 212–222, doi:10.1016/j.indcrop.2005.06.004.

10. Cosentino, S.L.; Scordia, D.; Sanzone, E.; Testa, G.; Copani, V. Response of giant reed (*Arundo donax* L.) to nitrogen fertilization and soil water availability in semi-arid Mediterranean environment. *Eur. J. Agron.* **2014**, *60*, 22–32, doi:10.1016/j.eja.2014.07.003.

11. Cosentino, S.L.; Patanè; C; Sanzone, E.; Testa, G.; Scordia, D. Leaf gas exchange, water status and radiation use efficiency of giant reed (*Arundo donax* L.) in a changing soil nitrogen fertilization and soil water availability in a semi-arid Mediterranean area. *Eur. J. Agron.* **2016**, *72*, 56–69, doi:10.1016/j.eja.2015.09.011.

12. Mantineo, M.; D'Agosta, G.M.; Copani, V.; Patanè; C; Cosentino, S.L. Biomass yield and energy balance of three perennial crops for energy use in the semi-arid Mediterranean environment. *Field Crop Res.* **2009**, *114*, 204–213, doi:10.1016/j.fcr.2009.07.020.

13. Nassi o Di Nasso, N.; Roncucci, N.; Bonari, E. Seasonal dynamics of aboveground and belowground biomass and nutrient accumulation and remobilization in giant reed (*Arundo donax* L.): A three-year study on marginal land. *Bioenergy Res.* **2013**, *6*, 725–736, doi:10.1007/s12155-012-9289-9.

14. Impagliazzo, A.; Mori, M.; Fiorentino, N.; Di Mola, I.; Ottaiano, L.; De Gianni, D.; Nocerino, S.; Fagnano, M. Crop growth analysis and yield of a lignocellulosic biomass crop (*Arundo donax* L.) in three marginal areas of Campania region. *Ital. J. Agron.* **2017**, *12*, 7, doi:10.4081/ija.2016.755.

15. Dahl, J.; Obernberger, I. Evaluation of the combustion characteristics of four perennial energy crops (*Arundo donax*, *Cynara cardunculus*, *Miscanthus x Giganteus* and *Panicum virgatum*). In Proceedings of the 2nd World Conference on Biomass for Energy, Industry and Climate Protection, Rome, Italy, 10-14, May, 2004.

16. Pilu, R.; Bucci, A.; Badone, F.C.; Landoni, M. Giant reed (*Arundo donax* L.): A weed plant or a promising energy crop? *Afr. J. Biotechnol.* **2012**, *11*, 9163–9174, doi:10.5897/AJB11.4182.

17. Maishanu, S.M.; Hussani, H.B.N. Studies on factors affecting biogas generation from *Pistia stratiote*. In *Paper Presented at the 32nd Annual Conference of Nigeria Society for Microbiology*; University of Ilorin: Ilorin, Nigeria, 1991.

18. Jones, M.B.; Finnan, J.; Hodkinson, T.R. Morphological and physiological traits for higher biomass production in perennial rhizomatous grasses grown on marginal land. *GCB Bioenergy* **2015**, *7*, 375–385, doi:10.1111/gcbb.12203.

19. Sage, R.F. The evolution of C4 photosynthesis. *New Phytol.* **2004**, *161*, 341–370, doi:10.1111/j.1469-8137.2004.00974.x.

20. Rossa, B.; Rüffers, A.V.; Naidoo, G.; von Willert, D.J. *Arundo donax* L. (Poaceae)—A C3 species with unusually high photosynthetic capacity. *Plant Biol.* **1998**, *111*, 216–221, doi:10.1111/j.1438-8677.1998.tb00698.x.

21. Sánchez, E.; Gil, S.; Azcón-Bieto, J.; Nogués, S. The response of *Arundo donax* L. (C3) and *Panicum virgatum* (C4) to different stresses. *Biomass. Bioenergy* **2016**, *85*, 335–345, doi:10.1016/j.biombioe.2015.12.021.

22. Haworth, M.; Cosentino, S.L.; Marino, G.; Brunetti, C.; Scordia, D.; Testa, G.; Riggi, E.; Avola, G.; Loreto, F.; Centritto, M. Physiological responses of *Arundo donax* ecotypes to drought: A common garden study. *GCB Bioenergy* **2017**, *9*, 132–143, doi:10.1111/gcbb.12348.

23. Haworth, M.; Catola, S.; Marino, G.; Brunetti, C.; Michelozzi, M.; Riggi, E.; Avola, G.; Cosentino, S.L.; Loreto, F.; Centritto, M. Moderate drought stress induces increased foliar dimethylsulphoniopropionate (DMSP) concentration and isoprene emission in two contrasting ecotypes of *Arundo donax*. *Front. Plant Sci.* **2017**, *8*, 1016, doi:10.3389/fpls.2017.01016.

24. Mann, J.J.; Barney, J.N.; Kyser, G.B.; Di Tomaso, J.M. Miscanthus x giganteus and *Arundo donax* shoot and rhizome tolerance of extreme moisture stress. *GCB Bioenergy* **2013**, *5*, 693–700, doi:10.1111/gcbb.12039.

25. Webster, R.J.; Driever, S.M.; Kromdijk, J.; McGrath, J.; Leakey, A.D.B.; Siebke, K.; Demetriades-Shah, T.; Bonnage, S.; Peloe, T.; Lawson, T.; et al. High C3 photosynthetic capacity and high intrinsic water use efficiency underlies the high productivity of the bioenergy grass *Arundo Donax*. *Sci. Rep.* **2016**, *6*, 20694, doi:10.1038/srep20694.

26. Romero-Munar, A.; Baraza, E.; Cifre, J.; Achir, C.; Gulías, J. Leaf plasticity and stomatal regulation determines the ability of *Arundo donax* plantlets to cope with water stress. *Photosynthetica* **2018**, *56*, 698–706, doi:10.1007/s11099-017-0719-y.

27. Erickson, J.E.; Soikaew, A.; Sollenberger, L.E.; Bennet, J.M. Water use and water-use efficiency of three perennial bioenergy grass crops in Florida. *Agriculture* **2012**, *2*, 325–338, doi:10.3390/agriculture2040325.

28. Nackley, L.L.; Vogt, K.A.; Kim, S.H. *Arundo donax* water use and photosynthetic responses to drought and elevated CO_2. *Agr. Water Manag.* **2014**, *136*, 13–22, doi:10.1016/j.agwat.2014.01.004.

29. Sánchez, E.; Scordia, D.; Lino, G.; Arias, C.; Cosentino, S.L.; Nogués, S. Salinity and water stress effects on biomass production in different *Arundo donax* L. clones. *Bioenergy Res.* **2015**, *8*, 1461–1479, doi:10.1007/s12155-015-9652-8.

30. Sánchez, E.; Lino, G.; Arias, C.; Serrat, X.; Nogués, S. Photosynthesis, resource acquisition and growth responses of two biomass crops subjected to water stress. *J. Plant Sci.* **2018**, *6*, 68–86, doi:10.11648/j.jps.20180603.11.

31. Zegada-Lizarazu, W.; Della Rocca, G.; Centritto, M.; Parenti, A.; Monti, A. Giant reed genotypes from temperate and arid environments show different response mechanisms to drought. *Physiol. Plant.* **2018**, *163*, 490–501, doi:10.1111/ppl.12701.

32. Haworth, M.; Marino, G.; Riggi, E.; Avola, G.; Brunetti, C.; Scordia, D.; Testa, G.; Gomes, M.T.G.; Loreto, F.; Cosentino, S.L.; et al. The effect of summer drought on the yield of *Arundo donax* is reduced by the retention of photosynthetic capacity and leaf growth later in the growing season. *Ann. Bot.* **2019**, *124*, 567–579, doi:10.1093/aob/mcy223.

33. Papazoglou, E.G.; Karantounias, G.A.; Vemmos, S.N.; Bouranis, D.L. Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to heavy metals Cd and Ni. *Environ. Int.* **2005**, 31, 243–249, doi:10.1016/j.envint.2004.09.022.

34. Triana, F.; Nassi o Di Nasso, N.; Ragaglini, G.; Roncucci, N.; Bonari, E. Evapotranspiration, crop coefficient and water use efficiency of giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus x giganteus* Greed et Deu.) in a Mediterranean environment. *GCB Bioenergy* **2015**, *7*, 811–819, doi:10.1111/gcbb.12172.

35. Mohr, H.; Schopfer, P. *Plant Physiology*; Springer: Berlin, Germany, 1995; p. 629.

36. Dohleman, F.G.; Heaton, E.A.; Leakey, A.D.B.; Long, S.P. Does greater leaf-level photosynthesis explain the larger solar energy conversion efficiency of Miscanthus relative to switchgrass? *Plant. Cell Environ.* **2009**, *32*, 1525–1537, doi:10.1111/j.1365-3040.2009.02017.x.

37. Wullschleger, S.D. Biochemical limitations to carbon assimilation in C3 plants—A retrospective analysis of the A/Ci curves from 109 species. *J. Exp. Bot.* **1993**, *44*, 907–920, doi:10.1093/jxb/44.5.907.

38. Osborne, B.A.; Garrett, M.K. Quantum yields for CO2 uptake in some diploid and tetraploid plant species. *Plant. Cell Environ.* **1983**, *6*, 135–144.

39. Long, S.P.; Postl, W.F.; Bolhàr-Nordenkampf, H.R. Quantum yields for uptake of carbon-dioxide in C-3 vascular plants of contrasting habitats and taxonomic groupings. *Planta* **1993**, *189*, 226–234.

40. Hidalgo, M.; Fernandez, J. Biomass production of ten populations of giant reed (*Arundo donax* L.) under the environmental conditions of Madrid (Spain). In *Biomass for Energy and Industry: Proceeding of the First World Conference*; Kyritsis, S., Beenackers, A.A.C.M., Helm, P., Grassi A, Chiaramonti, D., Eds.; London: James & James (Science Publishers) Ltd.: Sevilla, Spain, 2000; pp. 1881–1884.

41. Nazli, R.I.; Tansi, V.; Öztürk, H.H.; Kusvuran, A. Miscanthus, switchgrass, giant reed and bulbous canary grass as potential bioenergy crops in a semi-arid Mediterranean environment. *Ind. Crop. Prod.* **2018**, *125*, 9–23, doi:10.1016/j.indcrop.2018.08.090.

42. Ahmad, R.; Liow, P.S.; Spencer, D.F.; Jasieniuk, M. Molecular evidence for a single genetic clone of invasive *Arundo donax* in the United States. *Aquat. Bot.* **2008**, *88*, 113–120, doi:10.1016/j.aquabot.2007.08.015.

43. Hardion, L.; Verlaque, R.; Saltonstall, K.; Leriche, A.; Vila, B. Origin of the invasive *Arundo donax* (Poaceae): A trans-Asian expedition in herbaria. *Ann. Bot.* **2014**, *114*, 455–462, doi:10.1093/aob/mcu143.

44. Pilu, R.; Cassani, E.; Landoni, M.; Badone, F.C.; Passera, A.; Cantaluppi, E.; Corno, L.; Adani, F. Genetic characterization of an Italian Giant Reed (*Arundo donax* L.) clones collection: Exploiting clonal selection. *Euphytica* **2014**, *162*, doi:10.1007/s10681-013-1022-z.

45. Touchell, D.H.; Ranney, T.G.; Panthee, D.R. Genetic diversity, cytogenetics and biomass yields among taxa of giant reed (*Arundo* species). *J. Am. Soc. Hort. Sci.* **2016**, *141*, 256–263, doi:10.21273/JASHS.141.3.256.

46. Fabbrini, F.; Ludovisi, R.; Alasia, O.; Flexas, J.; Douthe, C.; Ribas-Carbó; M; Robson, P.; Taylor, G.; Scarascia-Mugnozza, G.; Keurentjes, J.J.B.; et al. Characterization of phenology, physiology, morphology and biomass traits across a broad Euro-Mediterranean ecotypic panel of the lignocellulosic feedstock *Arundo donax*. *GCB Bioenergy* **2019**, *11*, 152–170, doi:10.1111/gcbb.12555.

47. Flexas, J.; Bota, J.; Loreto, F.; Cornic, G.; Sharkey, T.D. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biol.* **2004**, *6*, 269–279, doi:10.1055/s-2004-820867.

48. Haworth, M.; Marino, G.; Cosentino, S.L.; Brunetti, C.; De Carlo, A.; Avola, G.; Riggi, E.; Loreto, F.; Centritto, M. Increased free abscisic acid during drought enhances stomatal sensitivity and modifies stomatal behaviour in fast growing giant reed (*Arundo donax* L.). *Environ. Exp. Bot.* **2018**, *147*, 116–124, doi:10.1016/j.envexpbot.2017.11.002.

49. Kørup, K.; Laerke, P.E.; Baadsgaard, H.; Andersen, M.N.; Kristensen, K.; Munnich, C.; Didion, T.; Jensen, E.S.; Martensson, L.M.; Jørgensen, U. Biomass production and water use efficiency in perennial grasses during and after drought stress. *GCB Bioenergy* **2018**, *10*, 12–27, doi:10.111/gcbb.12464.

50. Haworth, M.; Centritto, M.; Giovannelli, A.; Marino, G.; Proietti, N.; Capitani, D.; De Carlo, A.; Loreto, F. Xylem morphology determines the drought response of two *Arundo donax* ecotypes from contrasting habitats. *GCB Bioenergy* **2017**, *9*, 119–131, doi:10.1111/gcbb.12322.

51. Pipatsitee, P.; Eiumnoh, A.; Praseartkul, P.; Taota, K.; Kongpugdee, S.; Sakulleerungroj, K.; Cha-um, S. Application of infrared thermography to assess cassava physiology under water deficit condition. *Plant Prod. Sci.* **2018**, *21*, 398–406, doi:10.1080/1343943X.2018.1530943.

52. Velikova, V.; Brunetti, C.; Tattini, M.; Doneva, D.; Ahrar, M.; Tsonev, T.; Stefanova, M.; Ganeva, T.; Gori, A.; Ferrini, F.; et al. Physiological significance of isoprenoids and phenylpropanoids in drought response of Arundinoideae species with contrasting habitats and metabolism. *Plant Cell Environ.* **2016**, *39*, 2185–2197, doi:10.111/pce.12785.

53. Xu, L.; Cao, Y.; Tang, S.; Lu, Y.; Luo, S.; Ma, Y. Effects of drought stress and rewatering on physiological characteristics of *Arundo donax* var. *Versicolor*. *Sci. Soil Water Conserv.* **2020**, *18*, 59–66, doi:10.16843/j.sswc.2020.03.007.

54. Pompeiano, A.; Huarancca Reyes, T.; Moles, T.M.; Villani, M.; Volterrani, M.; Guglielminetti, L.; et al. Inter- and intraspecific variability in physiological traits and post-anoxia recovery of photosynthetic efficiency in grasses under oxygen deprivation. *Physiol. Plant.* **2017**, *161*, 385–399, doi:10.1111/ppl.12608.

55. Pompeiano, A.; Landi, M.; Meloni, G.; Vita, F.; Guglielminetti, L.; Guidi, L. Allocation pattern, ion partitioning, and chlorophyll a fluorescence in *Arundo donax* L. in responses to salinity stress. *Plant Biosyst.* **2017**, *151*, 613–622, doi:10.1080/11263504.2016.1187680.

56. Pompeiano, A.; Remorini, D.; Vita, F.; Guglielminetti, L.; Miele, S.; Morini, S. Growth and physiological response of *Arundo donax* L. to controlled drought stress and recovery. *Plant Biosyst.* **2017**, *151*, 906–914, doi:10.1080/11263504.2016.1249427.

57. Pompeiano, A.; Huarancca Reyes, T.; Moles, T.M.; Guglielminetti, L.; Scartazza, A. Photosynthetic and growth responses or *Arundo donax* L. plantlets under different oxygen deficiency stresses and reoxygenation. *Front. Plant Sci.* **2019**, *10*, 408, doi:10.3389/fpls.2019.00408.