agriculture

Article

Drought Impact on the Morpho-Physiological Parameters of Perennial Rhizomatous Grasses in the Mediterranean Environment

Claudia Arias [1,2,3], Gladys Lino [3,4], Elena Sánchez [3,5], Salvador Nogués [3] and Xavier Serrat [3,*]

[1] Department of Biological Foundation, Faculty of Agricultural Sciences, National University of Córdoba, Córdoba 5001, Argentina; claudia.arias@agro.unc.edu.ar
[2] Grupo de Investigación RIENU, Universidad Científica del Sur, Lima 15067, Peru
[3] Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, 08028 Barcelona, Spain; glino@cientifica.edu.pe (G.L.); elena.sanchez.sanchez@upc.edu (E.S.); salvador.nogues@ub.edu (S.N.)
[4] Facultad de Ciencias Ambientales, Universidad Científica del Sur, Lima 15067, Peru
[5] Department of Agri-Food Engineering and Biotechnology, Polytechnic University of Catalonia, 08860 Barcelona, Spain
* Correspondence: xserrat@ub.edu

Abstract: The selection of non-food crops for bioenergy production in limiting environments is a priority for energy security and climate change mitigation. Therefore, more studies are needed on the interactions between species and environmental factors in specific sites which allows their selection for biomass production. The objective of this work is to study the impact of drought on the morpho-physiological parameters of perennial rhizomatous grasses *Panicum virgatum* L., *Miscanthus* × *giganteus*, and *Arundo donax* L. in the Mediterranean environment. Plants were grown on field and trials were carried out under support-irrigation and rainfed conditions during two consecutive years. Morpho-physiological parameters were measured in May, June and August, and dry biomass at the end of the experiment. Under rainfed conditions, *A. donax* presented the highest photosynthesis rate (25, 15 and 10 CO_2 m^{-2} s^{-1}), relative water content (85–90%), and dry biomass (~4500 g $plant^{-1}$) compared with *P. virgatum* (20, 5 and 5 CO_2 m^{-2} s^{-1}, 65–85% RWC and ~1400 g $plant^{-1}$) and *Miscanthus* (18, 4 and 0 CO_2 m^{-2} s^{-1}, 80–10% RWC and ~260 g $plant^{-1}$). It is concluded that *A. donax* would be the best perennial rhizomatous grass to be used as bioenergy crop under Mediterranean conditions.

Keywords: drought; photosynthesis; biomass; *Panicum virgatum* L.; *Miscanthus* × *giganteus*; *Arundo donax* L.

Citation: Arias, C.; Lino, G.; Sánchez, E.; Nogués, S.; Serrat, X. Drought Impact on the Morpho-Physiological Parameters of Perennial Rhizomatous Grasses in the Mediterranean Environment. *Agriculture* **2023**, *13*, 1233. https://doi.org/10.3390/agriculture13061233

Academic Editor: Fadi Chen

Received: 5 May 2023
Revised: 24 May 2023
Accepted: 9 June 2023
Published: 12 June 2023

1. Introduction

The increased exploitation of renewable energy resources is expected to contribute to climate change mitigation and improve energy security [1]. It is expected that bioenergy will lead the growth of renewable energy in the coming decades [2]. In fact, bioenergy use has grown yearly by an average of 7% between 2010 and 2021 and its tendency is expected to increase and thus more efforts are needed to accelerate its use [3]. Much of the biomass currently used for bioenergy is still obtained from food crops, such as *Saccharum officinarum* L. or *Zea mays* L. However, non-food energy crops, such as *Phalaris* spp., *Salix* spp., *Populus*s spp. *Robinia* spp., *Eucalyptus* spp., and *Paulownia* spp., among others [4], are also expected to play an increasingly important role [5]. Therefore, the selection of bioenergy crops with no-food function, is an important priority for biomass production [6].

Three perennial rhizomatous grasses (PRGs), *Panicum virgatum* L., *Miscanthus* × *giganteus* (Greef et Deuter) and *Arundo donax* L., have been selected as candidates for bioenergy production out of almost 20 tested species [7,8]. The *P. virgatum* is a warm-season C4 photosynthetic metabolism species native to the North American prairies with a diverse geographical distribution and it is adapted to a wide range of climatic and edaphic conditions [9]. *Miscanthus* is also a typical C4 species, native to East Asia, found throughout a wide climatic range [10] and characterized by its rapid growth with low fertilizer requirements and high tolerance to drought, salinity and cold conditions [6]. *A. donax* has a C3 photosynthetic metabolism unlike the two previous species, but having as high photosynthetic rates as many C4 species [11]. This species is native to Asia and has been successfully established in the Mediterranean region and subtropical wetlands [12,13].

The main agronomic traits that define these three bioenergy crops are high biomass productivity, vigor, early growth and regrowth capacity [14]. All these species are characterized by a high content of lignin and cellulose that can be used for electricity, liquid fuel, biogas and hydrogen production, and to supply feedstocks for biorefineries [15]. Therefore, the cultivation of the PRGs, compared to food crop systems or typical annual crops, represent a viable alternative to minimize competition for land use for food production, and their adverse effects on food security, greenhouse gas emissions (GHG) and the loss of biodiversity [4,16].

Furthermore, these PRGs have a deep and extensive root system which, in the long term, benefits the soil in terms of structure, stability and soil quality by acting as carbon sinks [17]. In addition, PRGs have advantages over annual crops in terms of agricultural inputs (less fertilizers, pesticides and herbicides), production costs and harvest [18]. Another attribute that makes PGRs appealing for biomass production is their hardiness, which determines their survival during prolonged dry periods, and tolerance to salinity, waterlogging and pests [14]. Thus, these species can be grown on marginal lands where food production cannot take place because the land is not productive enough [19].

The most recent Intergovernmental Panel on Climate Change report [20] forecasts that climate change will expose plants to increasing occurrences of combined abiotic stresses, including drought, higher temperatures, and increased atmospheric CO_2 concentrations in the coming decades. This would be especially intense in the Mediterranean basin, which is characterized by 2–6 months of drought periods in summer and shorter drought winter periods from autumn to spring [21]. Thus, the crops grown in the semiarid Mediterranean area could be subjected to multiple stresses that are directly related to climate change. Due to increasing interest in the use of PRGs for bioenergy production, further studies on the interactions between species and environmental factors at specific sites, such as the Mediterranean region, can contribute to the understanding of the adaptive responses of these species to future climate change effects on biomass production [22].

Drought is the main environmental factor affecting plant physiological processes and negatively affecting crop production, from seed germination to adult plant growth and development [23]. Morpho-physiological traits play essential roles in growth and development of the plants. However, under limiting water conditions, some species can develop different morpho-physiological adaptation strategies, which give them increased tolerance to drought stress [24–26]. Thus, the identification of such drought-response traits in non-food biomass crops candidates is crucial. Leaf gas exchange and chlorophyll fluorescence parameters, relative water content, plant height and above-ground biomass production are among the most studied morpho-physiological traits in candidate biomass species [21,27]. We hypothesize that drought modulates the response of the morpho-physiological parameters of the studied species, which allows their selection for biomass production in a specific environment. Therefore, the objective of this work is to study the impact of drought on the morpho-physiological parameters of perennial rhizomatous grasses *Panicum virgatum* L., *Miscanthus* × *giganteus*, and *Arundo donax* L. in the Mediterranean environment.

2. Materials and Methods

2.1. Plant Material

Miscanthus × *giganteus* and *Arundo donax* L. seedlings were obtained from Piccoplant nursery (Pflanzenvertrieb und Verkauf GmbH, Oldenburg, Germany). *Panicum virgatum* L. (lowland ecotype, var. Alamo) seeds were donated by the INTA Anguil (La Pampa, Argentina). The seed germination of *P. virgatum* was performed according to the International Seed Testing Association [28] rules for this species. All the plants were transplanted to the field with four true leaves.

2.2. Experimental Design and Water Conditions

The field trial was carried out at the Experimental Fields of the Faculty of Biology, University of Barcelona, Spain (41°23′05.7″ N 2°07′12.9″ E) in a typical Calcic Luvisol soil. The physical and chemical characteristics of the experimental soil site have been described by Sauras-Yera et al. [29]. In brief, loamy texture classes are observed in this soil with mostly illitic clays. The organic carbon content was low as a result of rapid mineralization of organic matter under semiarid conditions, which decreases with depth. The soil has a basic pH and detectable quantities of calcium carbonate, and therefore are calcium saturated. This soil is derived from colluvial-alluvial sediments which were affected by old pedogenesis that produced the characteristic red-brown color of many Mediterranean and subtropical soils. To prepare the soil, a motor cultivator (Pasquali 956/603) with a tiller was used at a working depth of 0.50 m.

The trials were conducted during 2013 and 2014 in the dry seasons (May–August). The meteorological conditions, such as mean temperature (°C), precipitation (mm), and relative humidity (%), were registered by the meteorological station of the Department of Astronomy and Meteorology of the Faculty of Physics by the Experimental fields.

The experiment was arranged as a randomized complete block design, replicated three times for each species. The factors were watering levels (WA: watered) and rainfed (NW: non-watered) conditions. During the period May–August, the plants under the WA treatment were irrigated with 50 L m^{-2} $month^{-1}$ supplementary water using droppers in an automated watering system. Sixteen plants were planted in 3.24 m^2 plots (1.8 m × 1.8 m) and a 2.7 plants m^{-2} density (0.6 m between rows, and 0.6 m between plants) based on the procedure stated mentioned in Cosentino et al. [30]. At the beginning of spring, all plots were fertilized with 50 kg ha^{-1} N:P:K (15:15:15) fertilizer. During the two years of trial, no incidents of insects or diseases were observed, therefore, no pesticides or fungicides were applied. Different traits were measured in three times: May (T0), June (T1), and August (T2).

2.3. Physiological Measurements

2.3.1. Leaf Gas Exchange Parameters

Leaf-level gas exchange was measured using a Portable Photosynthesis System (Li6400, Li-Cor Inc., Lincoln, NE, USA) equipped with a 2 cm^2 Leaf Chamber Fluorometer (6400-40) and a 10% blue light source. The A/C_i curves with chlorophyll fluorescence determinations were conducted in fully expanded leaves from each species at 25 °C leaf temperature, 21% O_2 and a light rate saturated at 1200 μmol mol^{-1} of PPFD. The curves started at 400 μmol mol^{-1} CO_2, decreased stepwise until 250, 100, 50, and 0 μmol mol^{-1} CO_2 and restarted at 400 and increased stepwise until 750, 1000, and 1200 μmol mol^{-1} CO_2. Gas exchange measurements were performed in the middle area of the last unfolded leaf.

Net CO_2 assimilation rate (A_{sat}, μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) were measured using equations developed by von Caemmerer & Farquhar [31]. Intrinsic water use efficiency (WUE_i) was calculated from the gas exchange of CO_2 and H_2O as the ratio of CO_2 assimilation over stomatal conductance ($WUE_i = A_{sat}/g_s$ (μmol CO_2 mol H_2O^{-1}) under 400 μmol mol^{-1} CO_2, 21% O_2 and 1200 μmol m^{-2} s^{-1} of PPFD [32].

Estimations of the maximum Rubisco carboxylation rate (*Vc max* μmol CO_2 m^{-2} s^{-1}) and the rate of electron transport (*Jmax* μmol e- m^{-2} s^{-1}) were calculated using equations developed by McMurtrie and Wang [33].

In addition to the response to CO_2, photosynthetic light-response curves (A/PARi) were performed. The curves started at a maximum light level of 2000 μmol PAR m^{-2} s^{-1} and gradually decreased to 1200, 600, 300, 100, 50, and 30 down to 0 μmol PAR m^{-2} s^{-1}. Leaf temperatures of 25 °C, 21% O_2 and CO_2 at 400 μmol mol^{-1} were kept controlled.

2.3.2. Chlorophyll Fluorescence Analysis

Modulated chlorophyll fluorescence measurements were performed simultaneously using the Leaf Chamber Fluorometer coupled to Li6400 (Li-Cor Inc., Lincoln, NE, USA) to estimate the maximum quantum yield of PSII (F_v/F_m), the efficiency of excitation energy capture by opened PSII reaction centers (F_v'/F_m'), the relative quantum yield of photosystem II (Φ_{PSII}) and the photochemical quenching (*qp*), which were determined in a completely expanded leaf after 30 min of dark adaptation [34].

2.3.3. Relative Water Content and Leaf Greenness

Relative water content (RWC, %) of the leaves was determined as (FW-DW)/(TW-DW) × 100, where FW is the fresh weight, DW is the dry weight after drying in an oven at 60 °C until the weight was constant, and TW is the turgid weight of the leaves after equilibration in distilled water for 24 h at 4 °C [35]. Leaf greenness was estimated in the last fully expanded leaves using a portable meter (Minolta SPAD 502 Meter, Plainfield, IL, USA) [36]. Each measurement is the mean value of five measurements that were performed in the middle of each leaf.

2.4. Growth and Biomass Production

Plant height (H) was measured at T0, T1 and T2 from the soil surface to the end of the stem with a measuring tape. For the determination of dry weight (DW), above-ground biomass (leaves and stems) were harvested at the end of the growing period (T2), by cutting at 5 cm above-ground level. The samples were oven-dried at 60 °C until a constant weight expressed in g $plant^{-1}$ was reached.

2.5. Statistical Analysis

The drought impact on leaf gas exchange parameter was assessed using a factorial ANOVA analysis within three studied factors. The modulated fluorescence of chlorophylls, RWC, leaf greenness, H and DW parameters were compared using one-way ANOVA. The means ± standard errors (SE) were calculated for each parameter. When a particular F-test was significant, the means were compared using a Tukey multiple comparison test. The results were considered significant at $p < 0.05$. Statistical analysis was conducted using the SPSS software package (version 20.0; Inc., Chicago, IL, USA).

3. Results

3.1. Meteorological Conditions

Environmental temperature (°C), relative humidity (%), and precipitation (mm) data are shown in Figure 1. In the first year, the total rainfall was 555.6 mm y^{-1} and the annual distribution of precipitation was bimodal, which is characteristic of a Mediterranean environment. The highest amount of rainfall was distributed in two periods: (1) autumn (147.8 mm) and spring (246.3 mm), being the months of April and May where there was more precipitation which favored the implantation of crops; and (2) the driest periods corresponded to winter (95.0 mm) and summer (66.5 mm); in the latter, August was the month with the least rainfall and coincided with the period of greatest crop growth. In the second year, total rainfall was 606.9 mm y^{-1}; the highest amount of rainfall was again in autumn (284.5 mm) and spring (123.1 mm), but in this last period, rainfall was higher in

April and May. Winter was the driest period (84.3 mm), but summer was wetter (114.9 mm), with higher rainfall in July and August, compared to the first year. The total rainfall received by the crops between May (T0) and August (T2) was 121 mm in the first year and 165 mm in second year, which represents a 36% increase. Plots under WA, that had supplementary irrigation during May–August, received a total of 200 mm extra water (dosed at 50 l m^{-2} $month^{-1}$). Thus, WA plots received a total of 321 mm for the first year and 365 mm for the second year.

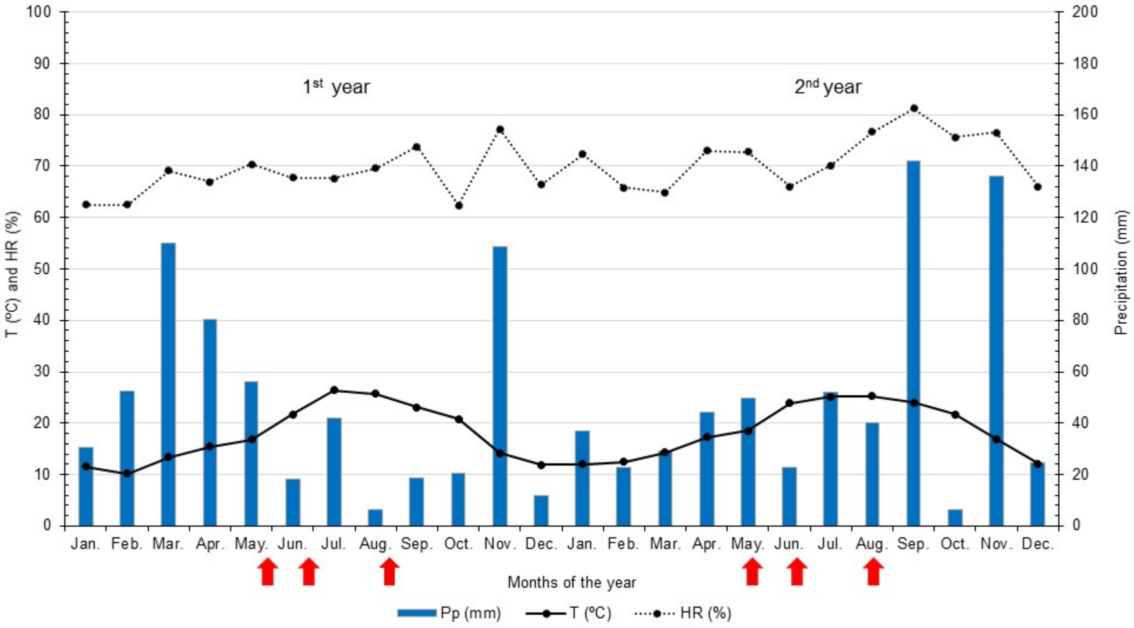

Figure 1. Monthly mean temperature (°C) (solid line), relative humidity (%) (dashed line), and precipitation (filled bars) per month during two consecutive years of the trial. Data were provided by the meteorological station of the Department of Astronomy and Meteorology of the Faculty of Physics at the University of Barcelona. Red arrows indicate the measurement moments (T0, T1 and T2).

3.2. Physiological Measurements

Significant differences (p = 0.001) were observed between species and treatments throughout the growing season for all physiological parameters and showed similar trends in the second year trial (Tables 1 and S1 and Figure S1). The highest Net CO_2 assimilation rate (*Asat*), stomatal conductance (g_s) (Table 1), maximum Rubisco carboxylation rate (*Vc max*) and the rate of electron transport (*Jmax*) (Table S1) values were measured at the beginning of the experiment (T0) and under WA conditions. The C4 species, *P. virgatum* and *Miscanthus*, did not present significantly different *Asat*, g_s, *Vcmax* and *Jmax* values (~18 µmol CO_2 m^{-2} s^{-1}, ~0.127 mol H_2O m^{-2} s^{-1}, ~50 µmol CO_2 m^{-2} s^{-1} and ~100 µmol e- m^{-2} s^{-1}, respectively). On the contrary, C3 species *A. donax*, showed the highest *Asat*, g_s, *Vcmax* and *Jmax* values (~25 µmol CO_2 m^{-2} s^{-1}, ~0.340 mol H_2O m^{-2} s^{-1}, ~100 µmol CO_2 m^{-2} s^{-1} and ~240 µmol e- m^{-2} s^{-1}, respectively) being even significantly higher than those form the C4 species. These values decreased in T1 and in T2, except for *A. donax*, which maintained their values similar to T0 under WA conditions in the first year. The three species were affected by drought under rainfed (NW) conditions, showing significantly decreased *Asat*, g_s, *Vcmax* and *Jmax* values when compared to WA. In NW *P. virgatum*, *Asat* and g_s values decreased around 80%, *Vcmax* decreased 55% in T1 and 85% in T2, *Jmax* decreased 66% in T1 and 77% in T2, in respect to WA and in both years.

In *Miscanthus*, *Asat* and g_s parameters have shown an 85% decrease during the first year in T1, and 60% during the second year. *Vcmax* also decreased 84% and *Jmax* decreased

64% in T1 both years. In contrast, the *Miscanthus* plants were senescent in T2. In *A. donax*, the *Asat* values decreased by 20 and 30% in T1, and 68 and 43% in T2, in the first and second year, respectively, while g_s values decreased by about 65% in both times and years (Table 1). *Vcmax* values in *A. donax* only showed a significant decrease (~50%) in T2 and in both years, while *Jmax* values decreased about 10–30% in T0 and T1, and 45% in T1 and T2, in both years.

In relation to intrinsic water use efficiency (*WUEi*) (Table S1) under WA conditions, significant differences were found between species, where *P. virgatum* and *Miscanthus*, showed a greater *WUEi* (~145 and ~140 µmol mol^{-1}, respectively) compared to *A. donax* (~72 µmol mol^{-1}) at T0. *WUEi* values were also affected by rainfed conditions and showed a significant increase under NW compared to WA, except for *Miscanthus* at T1 and *A. donax* at T2 during the 1st year.

Concerning the CO_2 assimilation rate in response to the absorbed light (*A*/*PARi*) (Figure S1), the highest assimilation rates were observed in WA conditions in the three species and in both years. In turn, it was observed that over time, the assimilation rates decreased within each treatment, being more marked in NW. If we compare the assimilation rate under NW, *P. virgatum* and *Miscanthus* presented values below 10 µmol CO_2 m^{-2}s^{-1} while *A. donax* showed rates above this value under high light between 600 and 2000 µmol PAR m^{-2} s^{-1}.

In relation to the chlorophyll fluorescence parameters, the maximum quantum yield values of photosystem II (*Fv*/*Fm*) were between 0.7 and 0.8 in all species. No significant differences were observed among the species, treatments or times for each species or year of measurement (Figure S2). In contrast, *ΦPSII* (Figure S3), *Fv'*/*Fm'* (Figure S4) and qP (Figure S5) parameters decreased exponentially when increasing the photosynthetically active radiation (PAR) in all species. However, this decrease was greater in *P. virgatum* and *Miscanthus* C4 species and under NW conditions. The *A. donax* values did not show differences between WA and NW conditions.

Under WA conditions, all three species presented a high relative water content (RWC) percentage (~75–95%) throughout the crop growing season in both years. *P. virgatum* and *Miscanthus* C4 species decreased the RWC significantly under NW conditions. *P. virgatum* decreased 22 and 10% in T2 and T0 in the first and second year, respectively (Figure 2A,B). *Miscanthus* under NW treatment was the most affected species, showing a decreased 20 and 40% RWC in T0 and T1 in the first and second year, respectively and becoming dry at the end of the trial (T2) in both years (Figure 2C,D). In contrast, *A. donax* maintained RWC values above 80% under NW conditions in both years (Figure 2E,F). At the same time, *A. donax* presented the highest values of leaf greenness (40–50% relative leaf greenness) under WA and NW when compared to the C4 species (25–35% relative leaf greenness), and similar values were observed in the second year. Again, *Miscanthus* was the most affected species under NW conditions and showed a decreased leaf greenness between T0 and T1 (20 and 42% in the first and second year, respectively), and plants were completely senescent at T2 (Figure S6).

Table 1. Net CO_2 assimilation rate at light saturation (*Asat,* μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) in *Panicum virgatum* L., *Miscanthus* × *giganteus* and *Arundo donax* L. plants grown under irrigated (WA) and non-water (NW) conditions during the first and second year at T0 (May), T1 (June) and T2 (August). *Miscanthus* plants exhibited foliar senescence under T2 and NW conditions; thus, these parameters could not be measured during either year of evaluation. Values represent the mean ± SE of nine replicates (n = 9). Different capital letters indicate significant differences ($p < 0.05$) between years for the same species, time and treatment. Different lowercase letters indicate significant differences ($p < 0.05$) between treatment for the same species, time and year.

			***Panicum virgatum* L.**		***Miscanthus* × *giganteus***		***Arundo donax* L.**	
Parameter	**Time**	**Treatment**	**1st Year**	**2nd Year**	**1st Year**	**2nd Year**	**1st Year**	**2nd Year**
Asat	T0	WA	19.33 ± 1.45 Aa	17.00 ± 1.00 Aa	17.67 ± 1.86 Aa	18.50 ± 1.50 Aa	25.00 ± 1.53 Aa	23.50 ± 2.50 Aa
	T0	NW	19.33 ± 1.45 Aa	17.00 ± 1.00 Aa	17.67 ± 1.86 Aa	18.50 ± 1.50 Aa	25.00 ± 1.53 Aa	23.50 ± 2.50 Aa
	T1	WA	21.00 ± 1.50 Aa	19.83 ± 3.35 Aa	16.17 ± 1.64 Aa	17.33 ± 0.44 Aa	18.67 ± 3.59 Ab	22.67 ± 0.67 Aa
	T1	NW	4.50 ± 0.50 Bb	5.07 ± 1.39 Bb	2.50 ± 0.76 Bb	7.00 ± 2.21 Cc	14.83 ± 1.59 Cc	15.67 ± 1.17 Cb
	T2	WA	15.50 ± 2.18 Cc	16.17 ± 1.17 Cc	9.83 ± 2.92 Cc	21.50 ± 0.50 Aa	22.50 ± 3.50 Aa	18.25 ± 3.75 Ac
	T2	NW	3.17 ± 0.93 Bb	5.33 ± 0.17 Bb	nd	nd	7.14 ± 1.30 Dd	10.33 ± 2.03 Ddc
g_s	T0	WA	0.130 ± 0.010 Aa	0.120 ± 0.010 Aa	0.124 ± 0.012 Aa	0.135 ± 0.005 Aa	0.320 ± 0.020 Aa	0.360 ± 0.050 Aa
	T0	NW	0.130 ± 0.010 Aa	0.120 ± 0.010 Aa	0.124 ± 0.012 Aa	0.135 ± 0.005 Aa	0.320 ± 0.020 Aa	0.360 ± 0.050 Aa
	T1	WA	0.120 ± 0.004 Aa	0.210 ± 0.005 Bb	0.120 ± 0.005 Aa	0.104 ± 0.004 Bb	0.280 ± 0.050 Aa	0.270 ± 0.050 Aa
	T1	NW	0.014 ± 0.010 Cc	0.030 ± 0.010 Cc	0.020 ± 0.003 Cb	0.035 ± 0.013 Cc	0.110 ± 0.020 Bb	0.109 ± 0.015 Bb
	T2	WA	0.090 ± 0.003 Dd	0.120 ± 0.003 Aa	0.060 ± 0.020 Dc	0.160 ± 0.028 Ed	0.300 ± 0.020 Aa	0.330 ± 0.080 Aa
	T2	NW	0.016 ± 0.004 Ce	0.030 ± 0.010 Cc	nd	nd	0.100 ± 0.040 Bb	0.082 ± 0.020 Cc

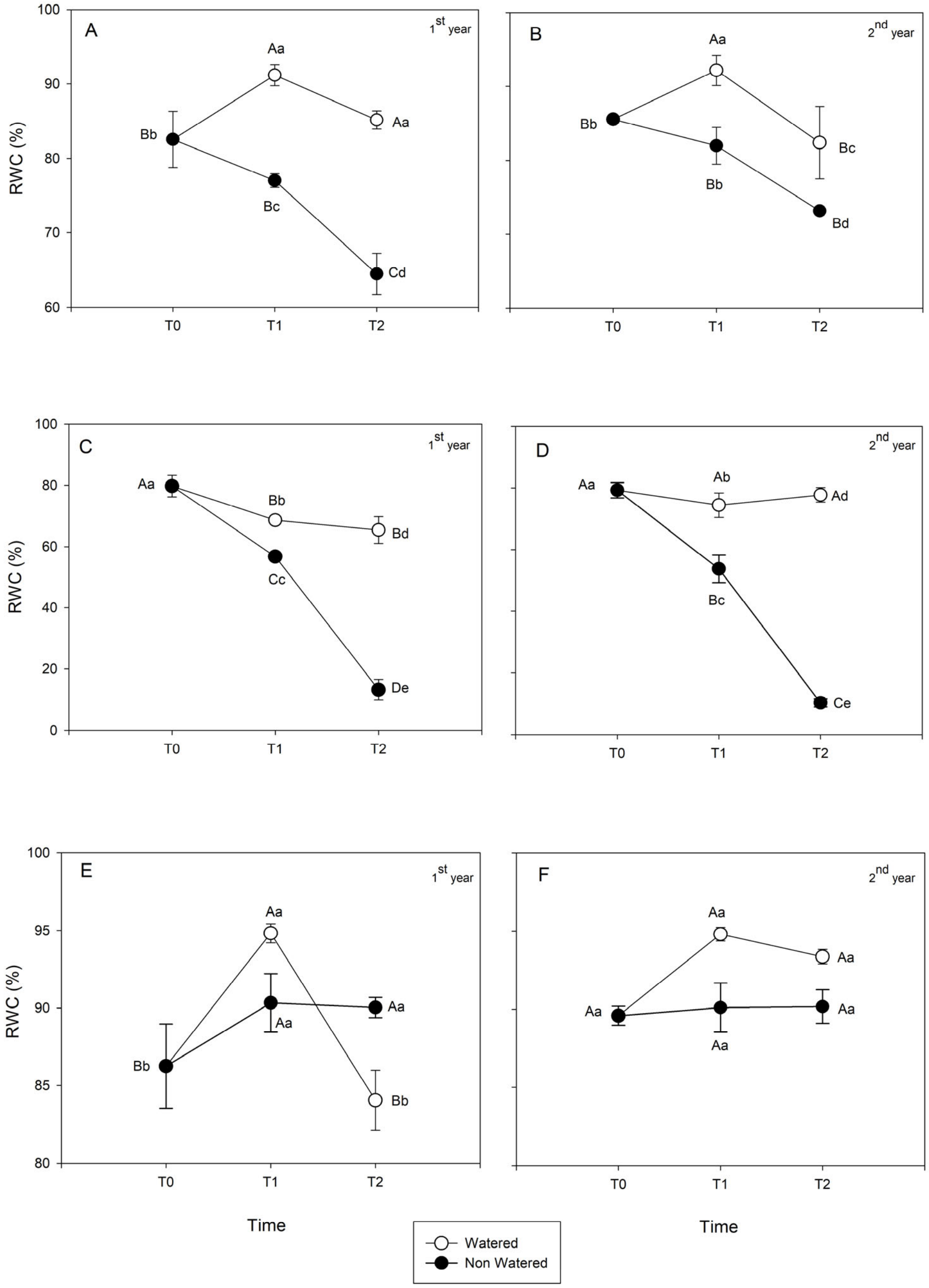

Figure 2. Relative water content (RWC) in leaves of *Panicum virgatum* L. (**A**,**B**), *Miscanthus × giganteus* (**C**,**D**) and *Arundo donax* L. (**E**,**F**) grown under irrigated (WA) and non-water (NW) conditions during the first and second year, at different times (T0: May, T1: June and T2: August). Values represent the mean ± SD of nine replicates (n = 9). Different capital letters indicate significant differences ($p < 0.05$) between time for the same treatment. Different lowercase letters indicate significant differences ($p < 0.05$) between treatment for the same time.

3.3. Agronomic Measurements

In relation to above-ground biomass, the highest DW was reached under WA conditions and for the three species studied. *A. donax* reached the greatest DW in both years (Figure 3). Under NW conditions, *P. virgatum* and *A. donax* presented a reduction in DW of around 30 and 25%, respectively, while *Miscanthus* was the species that showed the greatest decrease in DW, between 60 and 90% in the first and second year, respectively. The three species showed a significant increase in height under WA conditions throughout the whole growth period (T0, T1 and T2) (Figure S7). At T2, *P. virgatum* reached a height of approximately 2.00 m in both years (Figure S7A,B), *Miscanthus was* between 1.75 ± 0.10 and 2.47 ± 0.13 m high in the first and second year, respectively (Figure S7C,D) and *A. donax* was the tallest species reaching 3.00 ± 0.12 m height in the first year and 5.20 ± 0.25 m in the second year (Figure S7E,F). The C4 species growth was significantly lower in NW conditions. At the end of the trial (T2), the height of *P. virgatum* decreased between 40 and 30% and *Miscanthus* decreased between 50 and 80%, at first and second year, respectively. *A. donax* did not show significant differences between NW and WA treatments, except in T2 of the second year, where it grew more under WA conditions (Figure S7).

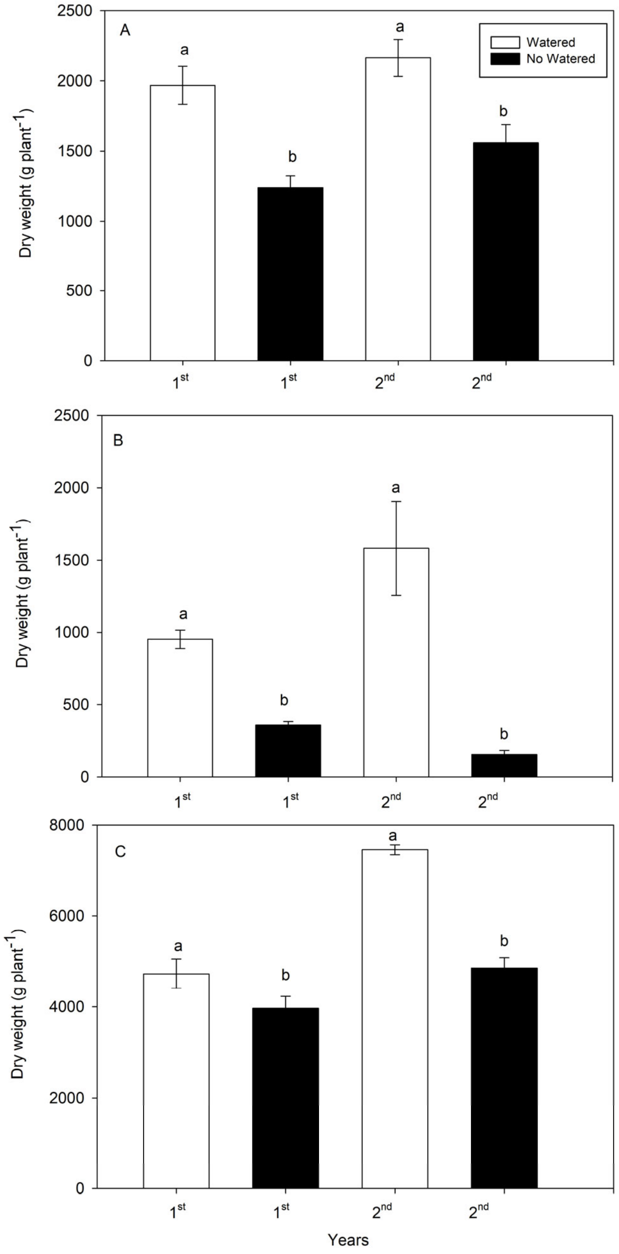

Figure 3. Above-ground dry weight (g plant^{-1}) from *Panicum virgatum* L. (**A**), *Miscanthus* × *giganteus* (**B**) and *Arundo donax* L. (**C**) plants grown under irrigated (WA) and non-water (NW) conditions

during the first and the second year at time T2 (August). Values represent the mean ± SD of nine replicates (n = 9). Different lowercase letters indicate significant differences ($p < 0.05$) between treatments for the same year.

4. Discussion

The variation in the annual distribution of precipitation and water availability (Figure 1) in the period of greatest growth of these species impacted the morpho-physiological parameters, which is in agreement with Gulías et al. [21], Cosentino et al. [37] and Scordia et al. [38]. The main differences at the morpho-physiological level between *A. donax* and the C4 species could be explained by their water requirements: 700–800 mm year^{-1} are required for *Miscanthus*, 450–750 mm year^{-1} for *P. virgatum* [39,40], and between 300 and 400 mm year^{-1} for *A. donax* [41]. Thus, only *A. donax* water requirements were satisfied by rainfall during both years (555.6 and 606.9 mm year^{-1}, respectively). Therefore, C4 species have severely limited production potential under suboptimal conditions [37]. Among the C4 species, *Miscanthus* was more affected by drought than *P. virgatum*, possibly due to its high water requirements. In the case of *P. virgatum*, although the annual precipitation would cover part of the range of water required, the lowland ecotype analyzed in this work is considered more susceptible to water stress than the upland ecotypes [40].

The growth and the biomass production are the result of the interactions between different factors, such as the physiology, water status, nutrient availability and plant morphology [30]. Under NW conditions, lower growth and biomass production were observed, similar to other crops in the Mediterranean environment [42]. At the same time, the decrease in water content causes the cells to lose turgor pressure [43], and the cells inhibit activities, such as cell division and cell expansion. It also inhibits the supply of organic and inorganic compounds necessary for the synthesis of protoplasm and cell walls, thereby directly affecting the growth of the entire plant [44].

Although *A. donax* is a C3 photosynthetic metabolism species, it showed the highest Net CO_2 assimilation rate, stomatal conductance, RWC, leaf greenness, height and biomass, even higher than those of C4 *P. virgatum* and *Miscanthus* species. The higher *Asat* and g_s values from *A. donax* agree with previous studies of Rossa et al. [12], Sánchez et al. [13], Ceotto et al. [14], Papazouglou et al. [45] and Nackley et al. [46]. Under NW conditions, the CO_2 assimilation rates decreased, mainly due to the decrease in g_s, but not because of the decrease in photosynthetic electron transport rate as reported in *P. virgatum* and *A. donax* by Sánchez et al. [24]. Furthermore, an increased leaf senescence was observed as a symptom of water deficit, mainly in *Miscanthus* at T2. This decreased photosynthesis response under water deficit could be a result of CO_2 diffusion limitation due to the decreased stomatal conductance, which would correspond to one of the immediate responses of plants under water deficit conditions in order to reduce water loss by evapotranspiration [43].

A. donax maintained a high RWC (>80%) during the growth period (Figure 2). On the contrary, RWC levels below 80% found in *P. virgatum* and *Miscanthus* under NW would imply a water potential of about ~1.5 MPa that causes changes in the metabolism and accumulation of proline and abscisic acid (ABA) [47]. As a consequence, ABA levels increase rapidly in leaves and induce stomatal closure and a decrease in stomatal conductance (g_s), thus limiting photosynthetic activity and carbon fixation [48]. *Asat* decreased under NW conditions, and the maximum velocity of RuBP carboxylation by Rubisco (Vc, max) and RuBP regeneration (*Jmax*) values also declined (Table S1). This response would be related to the loss or inactivation of Rubisco and with the reduction in sedoheptulose-1,7-bisphosphate, a key regulatory enzyme in the Calvin cycle, as reported by Nogués and Baker [49].

The leaf greenness is also directly related to the photosynthetic activity [50]. Our results showed a decreased leaf greenness in the C4 species when grown under NW conditions, as observed in other species under stress conditions [51]. Contrarily, *A. donax* showed the highest leaf greenness and the highest photosynthetic rate (Figure S6). Therefore, it is considered a good indicator of the plant's metabolic conditions.

In general, although C4 plant species showed a higher WUE than C3 plant species because of a lower stomatal conductance, C4 plant species are oversensitive to water stress since they quickly close their stomata and therefore decrease their stomatal conductance, causing a decrease in CO_2 assimilation [52]. Hence, in the face of a severe and prolonged water deficit, C4 plant species tend to be less tolerant than C3 plant species. Indeed, the C4 metabolism is an adaptation to increase the WUE under certain water limitations, but not against severe water stress [13,47]. Under NW conditions, all *Miscanthus* plants presented foliar senescence with RWC $<$20% at T2 because of the stomatal limitation caused by the strong water deficit they suffered. This difference between the NADP-ME (*Miscanthus*) and NAD-ME (*P. virgatum*) C4 photosynthetic metabolism subtypes in relation to their behavior towards the water deficit is due to a better adaptation to drought characterized by the NAD-ME C4 species. Thus, among the C4 species, the NAD-ME species are more tolerant to water deficit than NADP-ME and have a greater WUE [53], as the NADP-ME species are more frequently found in wetter environments [39,40]. This feature would indicate that the productivity of *Miscanthus* in a Mediterranean environment without supplementary irrigation would not be feasible since it has higher water requirements. In other words, the possibility of irrigation in the Mediterranean environment could be beneficial for the cultivation of this species since it responds positively to the increase in the irrigation dose [7], but this would suppose a greater economic cost in the production chain.

Under NW conditions, all three species presented high F_v/F_m values and there were no significant differences between them under WA treatment (Figure S2). These results would indicate a good photosystem II efficiency or an absence of photoinhibition in the reaction centers of PSII, unlike other plant species that tend to decrease F_v/F_m as a response to biotic or abiotic stress, which would indicate the existence of photoinhibition damage [44,54,55]. The $\Phi PSII$, $F_{v'}/F_{m'}$, and *qp* parameters decreased with the increase in light intensity. However, differences were observed in the behavior of each species against NW conditions. In both studied C4 species, there was a difference between the results obtained under WA and in NW, the results being more pronounced in NW conditions. In *A. donax* there were no differences, which would indicate a greater sensitivity in the quantum efficiency of electron transport than in the C4 species under water deficit conditions [54]. Therefore, these results would suggest that these species would be avoiding photoinhibition processes due to excess excitation energy through thermal dissipation processes to maintain the integrity of the photosynthetic membranes [55].

In summary, although rainfed treatment decreased all the parameters, some morpho-physiological parameters, such as *Asat* and g_s, showed better performance in *A. donax* compared to C4 species. This ability to maintain higher rates of CO_2 assimilation under drought conditions from the stomatal regulation would be one of the adaptive strategies of this species, which is in agreement with Cosentino et al. [30].

5. Conclusions

In the selection of non-food crops for bioenergy production under environmental stresses, the perennial rhizomatous grass emerges as the main candidate. In this regard, choosing the best-adapted species was favored to study the impact of drought on the morpho-physiological parameters. *Arundo donax* L. showed the best response in all the studied morpho-physiological parameters compared to C4 species. In contrast, *Mischanthus x giganteus* was less tolerant to these environmental conditions and showed a significant decrease in all physiological parameters resulting in reduced growth, biomass production and an early senescence. Therefore, in the context of climate change, with increasing restriction in water availability, *A. donax* would be the best perennial rhizomatous grass candidate to be used as a bioenergy crop in the Mediterranean region.

Results achieved in this work are novel since the most of the literature address the study of these species separately and/or under controlled greenhouse conditions. At the same time, the research of these species in this region and at field conditions has been scarcely studied in comparison with other Mediterranean regions, such as central Spain, Greece, northern and southern Italy. On the other hand, since the perennial rhizomatous grasses have not been widely studied at the biochemical and molecular level, unlike other species, more studies are needed to increase productivity and biomass quality under Mediterranean environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13061233/s1, Table S1: Maximum photosynthetic carboxylation rate (*Vc max* μmol CO_2 m^{-2} s^{-1}), maximum rate of electron transport (*Jmax* μmol e- m^{-2} s^{-1}) and intrinsic water use efficiency (WUEi, μmol mol^{-1}) in *Panicum virgatum* L., *Miscanthus* × *giganteus* and *Arundo donax* L. plants grown under irrigated (WA) and non-water (NW) conditions during the first (1st) and second (2nd) year at T0 (May), T1 (June) and T2 (August). *Miscanthus* plants presented foliar senescence under T2 and NW conditions, thus, these parameters could not be measured during either year of evaluation. Values represent the mean ± SE of nine replicates (n=9). Different capital letters indicate significant differences (p < 0.05) between years for the same species, time and treatment. Different lowercase letters indicate significant differences (p < 0.05) between treatment for the same species, time and year; Figure S1: Photosynthetic light response curves (*A*/*PARi*) in *Panicum virgatum* L., *Miscanthus* × *giganteus* and *Arundo donax* L. plants grown under irrigated (WA) and non-water (NW) conditions, during the first (A, B, and C) and second year (D, E, and F), at different times (T0: May, T1: June and T2: August). *Miscanthus* plants presented foliar senescence under T2 and NW conditions, thus, this parameter could not be measured during both years of evaluation. Values represent the mean ± SD of nine replicates (n=9); Figure S2: Maximum quantum yield of photosystem II (*Fv/Fm*) in *Panicum virgatum* L. (A, B), *Miscanthus* × *giganteus* (C, D) and *Arundo donax* L. (E, F) plants grown under irrigated (WA) and non-water (NW) conditions during the first and second year, at different times (T0: May, T1: June and T2: August). *Miscanthus* plants presented foliar senescence under T2 and NW conditions, thus, this parameter could not be measured during either year of evaluation. Values represent the mean ± SD of nine replicates (n=9). Different lowercase letters indicate significant differences (p< 0.05) between treatment for the same time; Figure S3: Relative quantum efficiency of photosystem II electron transport (ΦPSII) in *Panicum virgatum* L., *Miscanthus* × *giganteus* and *Arundo donax* L. plants grown under irrigated (WA) and non-watered (NW) conditions, during the first (A, B, and C) and second year (D, E, and F), at different times (T0: May, T1: June and T2: August). *Miscanthus* plants presented foliar senescence under T2 and NW conditions; thus, this parameter could not be measured during both years of evaluation. Values represent the mean ± SD of nine replicates (n=9); Figure S4: Efficiency of excitation energy captured by the open reaction centers of photosystem II (*Fv´/Fm´*) in *Panicum virgatum* L., *Miscanthus* × *giganteus* and *Arundo donax* L. plants grown under irrigated (WA) and non-watered (NW) conditions, during the first (A, B, and C) and second year (D, E, and F), at different times (T0: May, T1: June and T2: August). *Miscanthus* plants presented foliar senescence under T2 and NW conditions, thus, this parameter could not be measured during both years of evaluation. Values represent the mean ± SD of nine replicates (n=9); Figure S5: Photochemical quenching (*qp*) in *Panicum virgatum* L., *Miscanthus* × *giganteus* and *Arundo donax* L. plants grown under irrigated (WA) and non-watered (NW) conditions, during the first (A, B, and C) and second year (D, E, and F) at different times (T0: May, T1: June and T2: August). *Miscanthus* plants presented foliar senescence under T2 and NW conditions, thus, this parameter could not be measured during both years of evaluation. Values represent the mean ± SD of nine replicates (n=9); Figure S6: Leaf greenness (SPAD units) in leaves of *Panicum virgatum* L. (A, B), *Miscanthus* × *giganteus* (C, D) and *Arundo donax* L. (E, F) grown under irrigated (WA) and non-water (NW) conditions, during the first and second year at different times (T0: May, T1: June and T2: August). *Miscanthus* plants presented foliar senescence under T2 and NW conditions; thus, this parameter could not be measured during both years of evaluation. Values represent the mean ± SD of nine replicates (n=9). Different capital letters indicate significant differences ($p < 0.05$) between time for the same treatment. Different lowercase letters indicate significant differences ($p < 0.05$) between treatment for the same time; Figure S7: Height (m) of *Panicum virgatum* L., *Miscanthus* × *giganteus* and *Arundo donax* L. plants grown under irrigated (WA) and non-water (NW) conditions during the first (A, C, and E) and second year (B, D, and F) at different times (T0: May, T1: June and T2: August). Values represent the mean ± SD of nine replicates (n=9). Different lowercase letters indicate differences (p<0.05) between treatments for the same time.

Author Contributions: Conceptualization, C.A. and S.N.; methodology, C.A.; validation, C.A.; formal analysis, C.A., G.L., E.S., X.S. and S.N.; writing—original draft preparation, C.A.; writing—review and editing, G.L., E.S., X.S. and S.N.; visualization, C.A., G.L., E.S., X.S. and S.N.; and project administration, S.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European project OPTIMA (Optimization of Perennial Grasses for Biomass Production, Grant Agreement n° 289642). Collaborative project, FP7-KBBE-2011.3.1-02.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to acknowledge the European Union, for financially supporting this work through the OPTIMA project and to Horacio Petruzzi from INTA Anguil (La Pampa, Argentina) for the *Panicum virgatum* L seeds.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Taylor, G.; Donnison, I.S.; Murphy-Bokern, D.; Morgante, M.; Bogeat-Triboulot, M.B.; Bhalerao, R.; Hertzberg, M.; Polle, A.; Harfouche, A.; Alasia, F.; et al. Sustainable Bioenergy for Climate Mitigation: Developing Drought-Tolerant Trees and Grasses. *Ann. Bot.* **2019**, *124*, 513–520. https://doi.org/10.1093/aob/mcz146.
2. Oliver, R.J.; Finch, J.W.; Taylor, G. Second Generation Bioenergy Crops and Climate Change: A Review of the Effects of Elevated Atmospheric CO_2 and Drought on Water Use and the Implications for Yield. *GCB Bioenergy* **2009**, *1*, 97–114. https://doi.org/10.1111/j.1757-1707.2009.01011.x.
3. International Energy Agency Renewables 2018. Available online: https://www.iea.org/reports/renewables-2018 (accessed on 20 January 2023).
4. Clifton-Brown, J.; Hastings, A.; von Cossel, M.; Murphy-Bokern, D.; McCalmont, J.; Whittaker, J.; Alexopoulou, E.; Amaducci, S.; Andronic, L.; Ashman, C.; et al. Perennial Biomass Cropping and Use: Shaping the Policy Ecosystem in European Countries. *GCB Bioenergy* **2023**, *15*, 538–558. https://doi.org/10.1111/gcbb.13038.
5. Blair, M.J.; Gagnon, B.; Klain, A.; Kulišić, B.K. Contribution of Biomass Supply Chains for Bioenergy to Sustainable Development Goals. *Land* **2021**, *10*, 181. https://doi.org/10.3390/land10020181.
6. Hu, S.; Wu, L.; Persson, S.; Peng, L.; Feng, S. Sweet Sorghum and Miscanthus: Two Potential Dedicated Bioenergy Crops in China. *J. Integr. Agric.* **2017**, *16*, 1236–1243. https://doi.org/10.1016/S2095-3119(15)61181-9.
7. 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 Bioenergy* **2003**, *25*, 335–361. https://doi.org/10.1016/S0961-9534(03)00030-8.
8. Scordia, D.; Testa, G.; Cosentino, S.L. Perennial Grasses as Lignocellulosic Feedstock for Second-Generation Bioethanol Production in Mediterranean Environment. *Ital. J. Agron.* **2014**, *9*, 84–92. https://doi.org/10.4081/ija.2014.581.
9. Hashemi, M.; Sadeghpour, A. Establishment and Production of Switchgrass Grown for Combustion: A Review. *Int. J. Plant Biol. Res.* **2013**, *1*, 1002.
10. Greef, J.M.; Deuter, M. Miscanthus Gigantheus, J.M. Greef & Deuter. *Angew. Bot.* **1993**, *67*, 87–90.
11. Faralli, M.; Williams, K.; Corke, F.; Li, M.; Doonan, J.H.; Varotto, C. Interspecific and Intraspecific Phenotypic Diversity for Drought Adaptation in Bioenergy Arundo Species. *GCB Bioenergy* **2021**, *13*, 753–769. https://doi.org/10.1111/gcbb.12810.
12. Rossa, B.; Tuffers, a v; Naidoo, G.; von Willert, D.J. *Arundo donax* L. (Poaceae)—A C-3 Species with Unusually High Photosynthetic Capacity. *Bot. Acta* **1998**, *111*, 216–221.
13. Sánchez, E.; Rivera-Vargas, P.; Serrat, X.; Nogués, S. *Arundo donax* L.: How High Photosynthetic Capacity Is Maintained under Water Scarcity Conditions. *Agronomy* **2021**, *11*, 1089. https://doi.org/10.3390/agronomy11061089.
14. Ceotto, E.; di Candilo, M.; Castelli, F.; Badeck, F.-W.; Rizza, F.; Soave, C.; Volta, A.; Villani, G.; Marletto, V. Comparing Solar Radiation Interception and Use Efficiency for the Energy Crops Giant Reed (*Arundo donax* L.) and Sweet Sorghum (*Sorghum bicolor* L. Moench). *Field Crops Res.* **2013**, *149*, 159–166. https://doi.org/10.1016/j.fcr.2013.05.002.
15. Taylor, G. Biofuels and the Biorefinery Concept. *Energy Policy* **2008**, *36*, 4406–4409. https://doi.org/10.1016/J.ENPOL.2008.09.069.
16. Pulighe, G.; Bonati, G.; Colangeli, M.; Morese, M.M.; Traverso, L.; Lupia, F.; Khawaja, C.; Janssen, R.; Fava, F. Ongoing and Emerging Issues for Sustainable Bioenergy Production on Marginal Lands in the Mediterranean Regions. *Renew. Sustain. Energy Rev.* **2019**, *103*, 58–70. https://doi.org/10.1016/j.rser.2018.12.043.
17. Angelini, L.G.; Ceccarini, L.; Nassi o Di Nasso, N.; Bonari, E. Comparison of *Arundo donax* L. and Miscanthus × Giganteus in a Long-Term Field Experiment in Central Italy: Analysis of Productive Characteristics and Energy Balance. *Biomass Bioenergy* **2009**, *33*, 635–643. https://doi.org/10.1016/J.BIOMBIOE.2008.10.005.
18. Heaton, E.A.; Clifton-Brown, J.; Voigt, T.B.; Jones, M.B.; Long, S.P. Miscanthus for Renewable Energy Generation: European Union Experience and Projections for Illinois. *Mitig. Adapt. Strateg. Glob. Change* **2004**, *9*, 433–451. https://doi.org/10.1023/B:MITI.0000038848.94134.be.

19. Fernando, A.L.; Costa, J.; Barbosa, B.; Monti, A.; Rettenmaier, N. Environmental Impact Assessment of Perennial Crops Cultivation on Marginal Soils in the Mediterranean Region. *Biomass Bioenergy* **2017**, *111*, 174–186. https://doi.org/10.1016/j.biombioe.2017.04.005.
20. Adler, C.; Wester, P.; Bhatt, I.; Huggel, C.; Insarov, G.E.; Morecroft, M.D.; Muccione, V.; Prakash, A. Climate Change 2022: Impacts, Adaptation and Vulnerability. In *Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Pörtner, H.O., Roberts, D.C., Tignor, M., Poloczanska, E.S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., et al., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2022; pp. 2273–2318. ISBN 9781009325844.
21. Gulías, J.; Melis, R.; Scordia, D.; Cifre, J.; Testa, G.; Cosentino, S.L.; Porqueddu, C. Exploring the Potential of Wild Perennial Grasses as a Biomass Source in Semi-Arid Mediterranean Environments. *Ital. J. Agron.* **2018**, *13*, 103–111. https://doi.org/10.4081/ija.2018.937.
22. Freitas, E.N.; Salgado, J.C.S.; Alnoch, R.C.; Contato, A.G.; Habermann, E.; Michelin, M.; Martínez, C.A.; Polizeli, M. de L.T.M. Challenges of Biomass Utilization for Bioenergy in a Climate Change Scenario. *Biology* **2021**, *10*, 1277. doi.org/10.3390/biology10121277.
23. Moustakas, M.; Sperdouli, I.; Moustaka, J.; Şaş, B.; İşgören, S.; Morales, F. Mechanistic Insights on Salicylic Acid Mediated Enhancement of Photosystem II Function in Oregano Seedlings Subjected to Moderate Drought Stress. *Plants* **2023**, *12*, 518. https://doi.org/10.3390/plants12030518.
24. Sanad, M.N.M.E.; Smertenko, A.; Garland-Campbell, K.A. Differential Dynamic Changes of Reduced Trait Model for Analyzing the Plastic Response to Drought Phases: A Case Study in Spring Wheat. *Front. Plant Sci.* **2019**, *10*, 504. https://doi.org/10.3389/fpls.2019.00504.
25. Kherif, O.; Haddad, B.; Bouras, F.-Z.; Seghouani, M.; Zemmouri, B.; Gamouh, R.; Hamzaoui, N.; Larbi, A.; Rebouh, N.-Y.; Latati, M. Simultaneous Assessment of Water and Nitrogen Use Efficiency in Rain-Fed Chickpea-Durum Wheat Intercropping Systems. *Agriculture* **2023**, *13*, 947. https://doi.org/10.3390/agriculture13050947.
26. Bouras, F.-Z.; Hadjout, S.; Haddad, B.; Malek, A.; Aitmoumene, S.; Gueboub, F.; Metrah, L.; Zemmouri, B.; Kherif, O.; Rebouh, N.-Y.; et al. The Effect of Nitrogen Supply on Water and Nitrogen Use Efficiency by Wheat–Chickpea Intercropping System under Rain-Fed Mediterranean Conditions. *Agriculture* **2023**, *13*, 338. https://doi.org/10.3390/agriculture13020338.
27. 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. https://doi.org/10.11648/j.jps.20180603.11.
28. International Seed Testing Association (ISTA). The Germination Test. In *International Rules for Seed Testing*; International Seed Testing Association: Zurich, Switzerland, 2015; pp. 5–56.
29. Sauras-Yera, T.; Vallejo, V.R.; Valcke, E.; Colle, C.; Förstel, H.; Millán, R.; Jouglet, H. 137Cs and 90Sr Root Uptake Prediction under Close-to-Real Controlled Conditions. *J. Environ. Radioact.* **1999**, *45*, 191–217. https://doi.org/10.1016/S0265-931X(98)00113-1.
30. Cosentino, S.; 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. https://doi.org/10.1016/j.eja.2015.09.011.
31. von Caemmerer, S.; Farquhar, G.D. Some Relationships between the Biochemistry of Photosynthesis and the Gas Exchange of Leaves. *Planta* **1981**, *153*, 376–387. https://doi.org/10.1007/BF00384257.
32. 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. https://doi.org/10.1038/srep20694.
33. McMurtrie, R.E.; Wang, Y.P. Mathematical Models of the Photosynthetic Response of Tree Stands to Rising CO2 Concentrations and Temperatures. *Plant Cell Environ.* **1993**, *16*, 1–13.
34. Nogues, S.; Allen, D.J.; Morison, J.I.; Baker, N.R. Ultraviolet-B Radiation Effects on Water Relations, Leaf Development, and Photosynthesis in Droughted Pea Plants. *Plant Physiol.* **1998**, *117*, 173–181.
35. González, L.; González-Vilar, M. Determination of Relative Water Content. In *Handbook of Plant Ecophysiology Techniques*; Reigosa Roger, M.J., Ed.; Springer Netherlands: Dordrecht, The Netherlands, 2001; pp. 207–212. ISBN 978-0-306-48057-7.
36. Uddling, J.; Gelang-Alfredsson, J.; Piikki, K.; Pleijel, H. Evaluating the Relationship between Leaf Chlorophyll Concentration and SPAD-502 Chlorophyll Meter Readings. *Photosynth. Res.* **2007**, *91*, 37–46. https://doi.org/10.1007/s11120-006-9077-5.
37. 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. https://doi.org/10.1016/J.EJA.2014.07.003.
38. Scordia, D.; Testa, G.; Cosentino, S.L.; Copani, V.; Patanè, C. Soil Water Effect on Crop Growth, Leaf Gas Exchange, Water and Radiation Use Efficiency of *Saccharum spontaneum* L. ssp. aegyptiacum (Willd.) Hackel in Semi-Arid Mediterranean Environment. *Ital. J. Agron.* **2015**, *10*, 185–191. https://doi.org/10.4081/ija.2015.672.
39. Zegada-Lizarazu, W.; Wolter Elbersen, H.; Cosentino, S.L.; Zatta, A.; Alexopoulou, E.; Monti, A. Agronomic Aspects of Future Energy Crops in Europe. *Biofuels Bioprod. Bioref.* **2010**, *4*, 674–691. https://doi.org/10.1002/bbb.242.
40. van der Weijde, T.; Alvim Kamei, C.L.; Torres, A.F.; Vermerris, W.; Dolstra, O.; Visser, R.G.; Trindade, L.M. The Potential of C4 Grasses for Cellulosic Biofuel Production. *Front. Plant Sci.* **2013**, *4*, 107. https://doi.org/10.3389/fpls.2013.00107.
41. DiTomaso, J.M. Biology and Ecology of Giant Reed. In *Proceedings of the Arundo and Saltcedar: The Deadly Duo, Toronto, ON, Canada, 17 June 1998*; Bell, C.E., Ed.; University of California Cooperative Extension: Napa, CA, USA, 1998; pp. 1–5.

42. Nogués, S.; Alegre, L. An Increase in Water Deficit Has No Impact on the Photosynthetic Capacity of Field-Grown Mediterranean Plants. *Funct. Ecol.* **2002**, *29*, 621–630. https://doi.org/10.1071/PP01117.
43. Lawlor, D.W.; Cornic, G. Photosynthetic Carbon Assimilation and Associated Metabolism in Relation to Water Deficits in Higher Plants. *Plant Cell Environ.* **2002**, *25*, 275–294. https://doi.org/10.1046/j.0016-8025.2001.00814.x.
44. Passioura, J.B. Drought and Drought Tolerance. *Plant Growth Regul.* **1996**, *20*, 79–83. https://doi.org/10.1007/BF00024003.
45. Papazoglou, E.G.; Karantounias, G.A.; Vemmos, S.N.; Bouranis, D.L. Photosynthesis and Growth Responses of Giant Reed (*Arundo donax* L.) to the Heavy Metals Cd and Ni. *Environ. Int.* **2005**, *31*, 243–249. https://doi.org/10.1016/j.envint.2004.09.022.
46. Nackley, L.L.; Vogt, K.A.; Kim, S.-H. Arundo donax Water Use and Photosynthetic Responses to Drought and Elevated CO_2. *Agric. Water Manag.* **2014**, *136*, 13–22. https://doi.org/10.1016/j.agwat.2014.01.004.
47. Xu, J.; Trainotti, L.; Li, M.; Varotto, C. Overexpression of Isoprene Synthase Affects ABA- and Drought-Related Gene Expression and Enhances Tolerance to Abiotic Stress. *Int. J. Mol. Sci.* **2020**, *21*, 4276. https://doi.org/10.3390/ijms21124276.
48. Flexas, J.; Diaz-Espejo, A.; Gago, J.; Gallé, A.; Galmés, J.; Gulías, J.; Medrano, H. Photosynthetic Limitations in Mediterranean Plants: A Review. *Environ. Exp. Bot.* **2014**, *103*, 12–23. https://doi.org/10.1016/J.ENVEXPBOT.2013.09.002.
49. Nogues, S.; Baker, N.R. Effects of Drought on Photosynthesis in Mediterranean Plants Grown under Enhanced UV-B Radiation. *J. Exp. Bot.* **2000**, *51*, 1309–1317. https://doi.org/10.1093/jexbot/51.348.1309.
50. Nasar, J.; Khan, W.; Khan, M.Z.; Gitari, H.I.; Gbolayori, J.F.; Moussa, A.A.; Mandozai, A.; Rizwan, N.; Anwari, G.; Maroof, S.M. Photosynthetic Activities and Photosynthetic Nitrogen Use Efficiency of Maize Crop Under Different Planting Patterns and Nitrogen Fertilization. *J. Soil Sci. Plant Nutr.* **2021**, *21*, 2274–2284. https://doi.org/10.1007/s42729-021-00520-1.
51. Santos, C.V. Regulation of Chlorophyll Biosynthesis and Degradation by Salt Stress in Sunflower Leaves. *Sci. Hortic.* **2004**, *103*, 93–99. https://doi.org/10.1016/j.scienta.2004.04.009.
52. Ghannoum, O. C_4 Photosynthesis and Water Stress. *Ann. Bot.* **2009**, *103*, 635–644. https://doi.org/10.1093/aob/mcn093.
53. Ghannoum, O.; von Caemmerer, S.; Conroy, J.P. The Effect of Drought on Plant Water Use Efficiency of Nine NAD-ME and Nine NADP-ME Australian C4 Grasses. *Funct. Plant Biol.* **2002**, *29*, 1337–1348. https://doi.org/10.1071/FP02056.
54. de Stefano, R.; Cappetta, E.; Guida, G.; Mistretta, C.; Caruso, G.; Giorio, P.; Albrizio, R.; Tucci, M. Screening of Giant Reed (*Arundo donax* L.) Ecotypes for Biomass Production under Salt Stress. *Plant Biosyst.* **2018**, *152*, 911–917. https://doi.org/10.1080/11263504.2017.1362059.
55. Maxwell, K.; Johnson, G.N. Chlorophyll Fluorescence—A Practical Guide. *J. Exp. Bot.* **2000**, *51*, 659–668. https://doi.org/10.1093/jxb/51.345.659.