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Research paper

The response of *Arundo donax* L. (C_3) and *Panicum virgatum* (C_4) to different stresses

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ABSTRACT

In this work, two perennial rhizomatous grasses (*Arundo donax* L. (giant reed; C_3) and *Panicum virgatum* L. (switchgrass; C_4)) considered as promising energy crops have been subjected to four different types of stress in two experiments: (i) both species were subjected to four salinity and water stress treatments [well-watered with non-saline solution (WW S−), low-watered with non-saline solution (WS S-), well-watered with saline solution (WW S+) and low-watered with saline solution (WS S+)]; and (ii) both species were subjected to three temperature and light treatments [ambient temperature and light (C), ambient temperature and darkness (AD) and cold temperature and darkness (CD)]. Photosynthetic and physiological parameters as well as biomass production were measured in these plants. It can be hypothesized that a higher photosynthesis rate (A_{sat}) was be observed in switchgrass as a consequence of its C_4 metabolic pathway. However, our results indicated a similar A_{sat} at the beginning of the experiment for both species. This could be due to switchgrass being an NAD-ME C_4 type whereas giant reed has been reported as a C_3 species with a high photosynthetic rate. We showed that switchgrass seems to be more resistant to stresses such as water stress, salinity and cold than giant reed in our greenhouse conditions.

1. Introduction

Nowadays, climatic patterns in many regions of the world are being influenced by climate change [1], so many areas of agricultural production will be affected by abiotic stresses such as water stress (WS), salinity or flooding, creating the need to search resistant plants to these type of stresses. WS [2] and salinity [3] have been described as two of the most important environmental phenomena affecting plant growth, development and crop yield. Photosynthesis, together with cell growth, has been reported among the primary processes to be affected by WS [4]. Decreases in photosynthesis due to WS have been well studied and reviewed in C_3 plants [5–8] and may be directly associated with decreases in stomatal conductance in the early phase of stress [8,9], whereas the response of C_4 photosynthesis to WS has been less well studied. Ghannoum [10] suggested that although the C_4 CO_2-concentrating mechanism offers C_4 photosynthesis a greater buffering capacity against CO_2 shortages brought about by partial stomatal closure under WS, the biochemistry of C_4 photosynthesis is as—or even more - sensitive to WS than C_3 photosynthesis. According to Munns [11], early physiological plant responses to water and salt stress have much in common, and cell growth and photosynthesis would be affected by salinity in a similar way to WS [8,11,12]. Shoot growth is affected in the first phase of salinity stress, denominated "osmotic phase", and a reduction in leaf expansion, emergence of new leaves and lateral bud development is observed [3]. The second phase ("ion-specific phase") starts when salt accumulates to toxic concentrations in the old leaves, and a decrease in the photosynthetic capacity of the plant is noticed due to a greater rate of death of old leaves than production of new leaves.

Other factors limiting photosynthesis in C_3 and C_4 plants are temperature [13,14] and light [15]. Hurry et al. [16] have described

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Abbreviations: A_{sat}, light saturated net CO_2 assimilation rate; DLP, dry leaves percentage; FC, field capacity; F_v/F_m, maximum quantum efficiency of PSII; F'_v/F'_m, photochemical efficiency of PSII; GLP, green leaves percentage; g_s, stomatal conductance; H, height; J_{max}, maximum rate of electron transport contributing to RuBP regeneration; l, stomatal limitation to A_{sat}; LA, leaf area; LAI, leaf area index; LAR, leaf area ratio; LMA, leaf mass area; LWR, leaf weight ratio; NL, number of leaves; NS, number of stems; NPQ, non-photochemical quenching; PPFD, photosynthetic photon flux density; PSII, photosystem II; q_p, photochemical quenching; R, recovery; RQ, respiratory quotient; RWC, relative water content; SA, stem area; SLA, specific leaf area; S/R, shoot/root ratio; T, transpiration; TDW, total dry weight; $V_{c,mx}$, maximum velocity of Rubisco carboxylation; WS, water stress; WUE_{inst}, instantaneous water use efficiency; YLP, yellow leaves percentage; ϕ_{PSII}, relative quantum efficiency of PSII.

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how photosynthesis at low temperature is inhibited due to the low synthesis of sucrose, reaching the limits of inorganic phosphous (Pi) recycling. Tcherkez et al. [17] suggest that the plant reduces the rate of reserve consumption (primarily starch) under chilling conditions, but, on the other hand, an increase in temperature increases mitochondrial respiration with a faster degradation of reserves as a consequence. Light regulates the activity of the complex photosynthetic enzymes in both the Calvin cycle and electron transport. Therefore, lack of light causes the Calvin cycle to stop capturing atmospheric CO_2 because many enzymes, such as Rubisco and PEPC, are activated by red light via phytochrome. Some adaptation responses under low light or darkness are fast and reversible, such as chloroplast movements, but other functional and anatomical changes that occur in leaves are slow and irreversible [18]. The adaptation responses produce changes in leaf anatomy and enzyme activation cycles. Two important parameters for evaluating structural and biochemical acclimation are hydration and activity of Rubisco, respectively. In addition, the content of Rubisco decreases considerably after a period of darkness in C_3 plants [19,20], whereas the activity of Rubisco is not limited to the dark-acclimated C_4 leaves. In relation to continuous darkness, Nogués et al. [21] suggest that the respiratory substrate after a light period is a mixture in which the current photoassimilates are not the main component in physiological conditions. In addition, continuous darkness could be used as a tool to decrease the carbon pool of the plant.

Regarding the species used, giant reed and switchgrass are generating much interest in Europe as new renewable sources of biomass for energy production [22–24]. Several benefits are expected from the production and use of perennial grasses, as the important contribution in reducing of anthropogenic CO_2 emissions because the quantity of CO_2 released by combusting biomass does not exceed the amount that has been fixed previously by photosynthesis while the plants were growing [23]. Moreover, perennial grasses have other ecological advantages as a low requiring soil management, reducing the risk of soil erosion [25] and low demands for nutrient inputs due to the recycling of nutrients by their rhizome system [22]. On one hand, giant reed is a plant belonging to the *Poaceae* family. Recent studies [23,26] have proposed an Asiatic origin and a subsequent spread throughout the Middle East to southern Europe and Africa, being found widely in warm temperate regions all over the world. On the other hand, switchgrass is a native perennial warm-season grass from North America belonging to the *Gramineae* family [22], and is broadly adapted to the central and eastern United States [27]. Giant reed is not able to produce viable seeds due to failure of the megaspore mother cell to divide [26,28], whereas switchgrass can be established by seeding [22]. Therefore, several endemic species of switchgrass with wide ranges of adaptation can be found, but low genetic variability is found in giant reed [22]. However, spontaneous propagation can occur by rhizome fragmentation in both species [22,29]. High biomass productivity has been observed in giant reed [23] and switchgrass [22,30] even with low levels of crop inputs like irrigation, fertilization and plant density. The high yield of giant reed has been reported as being stable in long-term experiments [23,31].

Both species uses different photosynthetic pathway: Giant reed is a C_3 plant [22,32], whereas switchgrass is a NAD malic enzyme type C_4 grass [22,33]. The key feature of C_4 photosynthesis is the operation of a CO_2-concentrating mechanism in the bundle sheath that leads to the suppression of apparent photorespiration in air as well as the saturation of C_4 photosynthesis at a lower ambient $[CO_2]$ than for C_3 plants [10]. Therefore, a higher efficiency of radiation, nutrient and water use is expected in a C_4 species grown in an appropriate climate than a C_3 species [34]. Nevertheless, giant reed has been classified as a C_3 plant but it has been compared with C_4 plants due to its high photosynthetic potential [32], although the latest studies have suggested that giant reed has a relatively high transpiration rate and will therefore use more water than many C_3 and C_4 species used for biomass feedstock [35].

In response to the growing interest in these species as bioenergy crops, the aim of this paper is to study their physiological response to four stresses (water stress, salinity, cold and continuous darkness) to determine whether one of them is more or less tolerant to stress than the other.

2. Materials and methods

2.1. Plant material

Arundo donax L. plants collected in San Marti Sarroca (291m asl, 41°23′14″ N 1°36′43″ E, Catalonia, Spain), named *Arundo donax* clone Martinensis (giant reed), were obtained from multiplication of rhizomes on January 2013. Seeds of *Panicum virgatum* L. cv. Alamo (switchgrass) were donated by the Instituto Nacional de Tecnología Agropecuaria Anguil (INTA, Argentina) and germinated on moist filter paper in Petri dishes, placed in a long day chamber (16h of photoperiod) at a temperature of 22/18 °C day/night respectively and 70% HR.

Giant reed and switchgrass rhizomes, with an initial rhizome fresh weight of 33.2 g ± 4.4 and 16.3 g ± 2.8, were grown in a greenhouse at the Experimental Field Service of Barcelona University (Barcelona, Spain) in plastic pots containing 5 L of peat: perlite: vermiculite (3:1:1) and were irrigated with a complete Hoagland solution [36]. The average temperature and vapour pressure deficit (VPD) during growth was 25/15 °C day/night and 0.75 kPa, respectively. Relative humidity ranged from 40 to 65% and the maximum PPFD was ~1000 $\mu mol\ m^{-2}\ s^{-1}$.

In order to study the response of these two species to different stresses, plants were subjected to two different experiments.

2.2. Experiment 1: water stress and salinity

2.2.1. Plant material and experimental design

Three months after planting, plants were separated into four treatments. Half the plants were subjected to WS by withholding water until 25% of field capacity (FC). Then, well watered (maintained at FC) and WS plants were equally divided between salinity (e.g. 16 $mS\ cm^{-1}$ Hoagland solution) and non-salinity (e.g. 1 $mS\ cm^{-1}$ Hoagland solution). Saline solution was prepared by adding NaCl (PANREAC, 99% Sodium Chloride) to a complete Hoagland solution until the appropriate saline concentration was reached. Consequently, a total of three plants per ecotype were subjected to the following treatments: (i) well-watered with non-saline solution (WW S−), (ii) low-watered with non-saline solution (WS S-), (iii) well-watered with saline solution (WW S+) and, iv) low-watered with saline solution (WS S+).

Plants were subjected to these four treatments during two months. Measurements were carried out every fifteen days (i.e. T15, T30, T45) until the end of the experiment (i.e. T60).

2.2.2. Measurements

2.2.2.1. Gas exchange. Leaf-level gas exchange was measured using a portable photosynthesis system (Li6400, Li-Cor Inc., Lincoln, NE, USA) provided with a Leaf Chamber Fluorometer (6400-40) of 2 cm^2 and a 10% blue light source.

Firstly, before the experiment (T0), A/C_i curves with chlorophyll fluorescence determinations were conducted in fully expanded leaves from each species (n = 3) at 25 °C with a light rate saturated at 1200 $\mu mol\ m^{-2}\ s^{-1}$ of PPFD and an airflow rate of 8 $cm^3\ s^{-1}$. The response of A to the intercellular CO_2 concentration (C_i) was