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Lignocellulosic biomass production of Mediterranean wild accessions (*Oryzopsis miliacea*, *Cymbopogon hirtus*, *Sorghum halepense* and *Saccharum spontaneum*) in a semi-arid environment

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ABSTRACT

Sustainable biomass production mostly relies on cultivation practices employing low external input supply. Wild germplasm might be suited for low-input techniques while providing enough output. The present study investigated four native Mediterranean perennial grasses (*Oryzopsis miliacea*, *Cymbopogon hirtus*, *Sorghum halepense*, and *Saccharum spontaneum* L. ssp. *aegyptiacum*), with an autumn and winter harvest regime in a 4-year field trial in Sicily (south of Italy). Species, cultivation year and harvest time had significant effects on whole season crop water use efficiency, energy efficiency and biomass quality. Species and cultivation year also significantly affected aboveground biomass yield and net energy yield. The total accumulated harvested yield over the 4-year experiment was the highest in *Saccharum* in both autumn and winter harvests (79.5 and 79.2 Mg DM ha^{-1}), and the lowest in *Cymbopogon* (13.7 and 14.9 Mg DM ha^{-1}). In both harvests, less than 20% of this total biomass was collected for all species at the first year (9.5–11.3% in *Sorghum* and 15.7–18.1% in *Oryzopsis*). Peak biomass was reached at the third (*Saccharum* and *Sorghum*) or at the fourth year (*Orzyzopsis* and *Cymbopogon*) in autumn, and at the third year in winter harvest (all species). Water use efficiency was higher in the autumn than in the winter harvest (2.06 and 1.75 g L^{-1}, respectively), whilst the opposite was observed for energy efficiency (35.7 and 38.6 GJ ha^{-1}, respectively). Biomass structural compounds (hemicellulose, cellulose and acid detergent lignin) were higher in winter than autumn, while protein, lipid and ash contents were higher in autumn than winter.

1. Introduction

Research on perennial grasses for biomass production relies on a limited number of dedicated species suited to the different climatic conditions of Europe. The C_4 *Miscanthus* x *giganteus* and *Panicum virgatum* are high-yielding in temperate environments of northern and central Europe (Lewandowski et al., 2003; Zegada-Lizarazu et al., 2010), while the C_3 *Phalaris arundinacea* and *Arundo donax* are most suited further north and south, respectively (Lewandowski et al., 2003; Mantineo et al., 2009; Cosentino et al., 2014, 2016). Outstanding biomass yields have been obtained with these species when grown in non-limiting conditions; however, when managed in low-input systems (i.e., rainfed, unfertilized, etc.) yields were variable (Alexopoulou et al., 2015). These species are largely undomesticated and research in breeding and agronomic practices optimization is still needed (Zegada-Lizarazu et al., 2010). Nowadays, considerable attention has been paid to the effects of regional climate on plant development in order to identify the optimal genotype for a particular location (Nunn et al., 2017). However, there are considerable numbers of plants still largely unexplored, highly-resource-use-efficient and well-performing in locations with specific constraints.

In the Mediterranean area, for instance, water and heat stress usually limit plant production to different extents (Sánchez et al., 2015). In these conditions, species abundantly widespread in hot, drought- and harsh-prone environments should be investigated. However, the native germplasm of Mediterranean perennial grasses is nearly unexplored for bioenergy production to date (Sulas et al., 2015).

In this sense, Cosentino et al. (2015) proved that *Saccharum spontaneum* spp. *aegyptiacum,* native to northern Africa, is well adapted to the drought conditions of Southern Europe, showing biomass yields even higher than *Miscanthus* x *giganteus* and *Arundo donax* grown in the same experimental area under well-watered and rainfed conditions. In addition, Scordia et al. (2015a) indicated this species as a valuable candidate in environments characterized by drought stress, high

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temperatures and high vapor pressure deficits due mainly to the long green LAI maintenance and CO_2 assimilation, high net increase of biomass production per unit of intercepted light and per unit of transpired water. It would be worth investigating whether other Mediterranean perennial grasses have the typical biomass crop traits. For instance, *Cymbopogon hirtus* and *Oryzopsis miliacea,* which naturally occur in hash environments, are used as forage resources on the south side of the Mediterranean Basin and in South Africa (Foury, 1956; Lloyd and Moore, 2002). *Sorghum halepense* has recently been used to develop perennial sorghum (*S. bicolor*) as a bioenergy crop, although it is considered one of the worst noxious weeds in the USA (Price et al., 2006).

The native germplasm of Mediterranean perennial grasses could therefore represent a potential source of bioenergy crops or a resource for plant breeding and genetics due mainly to traits of resistance and phenotypic plasticity to several biophysical constraints (Tilman et al., 2006). On the other hand, the knowledge of their ecology, biology, physiology and agronomy must be improved before they can be recommended as candidate bioenergy crops.

In addition to the yield, biomass quality is of paramount importance to optimize bioconversion processes. One of the major determinants of biomass productivity (Beale and Long, 1997; Heaton et al., 2009; Hoagland et al., 2013), stand longevity (Heaton et al., 2009; O'Flynn et al., 2014) and quality of perennial grasses (Baxter et al., 2014; Kludze et al., 2013) is harvest time (Monti et al., 2015). Within the same species or genotype, harvest time affects cell wall composition, ash, stem to leaf ratio and biomass water content, in turn conditioning post-harvest logistics and bioconversion pathways (Monti et al., 2008, 2015).

To this end, the present study investigated autochthonous and undomesticated Mediterranean perennial grasses as novel candidate lignocellulosic bioenergy crops for the semi-arid Mediterranean area. *Oryzopsis miliacea*, *Cymbopogon hirtus*, *Sorghum halepense*, and *Saccharum spontaneum* ssp. *aegyptiacum* were compared in a four-year field trial under low-input agronomic practices with an autumn and winter harvest regime.

2. Materials and methods

2.1. Field trial set-up

The field trial was carried out from spring 2010 to winter 2014 at the Experimental farm of the University of Catania, Italy (37°25′ N., 15°03′ E., 10 m a.s.l.). In a randomized block, four species were compared in a split-plot design replicated three times: *Oryzopsis miliacea* (L.) Asch. & Schweinf. (sin. *Piptatherum miliaceum* L. Coss.), *Cymbopogon hirtus* (L.) Janchen (sin. *Hyparrhenia hirta* (L.) Stapf.), *Sorghum halepense* (L.) Pers., and *Saccharum spontaneum* L. ssp. *aegyptiacum* (Willd.) Hackel. The main plots were used for the species, while the sub-plots were used to analyse the harvest time, namely autumn and winter. Each main plot measured 100 m^2 (10 × 10 m), while each sub-plot 50 m^2 (10 × 5m).

Field and bed preparation followed an autumn ploughing and spring disk-harrowing before transplant. Local populations widespread near the experimental field provided enough material for clonal propagation either from rhizome (*Sorghum* and *Saccharum*) or clump division (*Oryzopsis* and *Cymbopogon*).

Clones were manually transplanted at a density of 1.0 plant m^{-2} (1.0 × 1.0 m). At transplant, basic fertilization with 50 kg N ha^{-1} as ammonium sulfate and 50 kg P_2O_5 ha^{-1} as superphosphate was applied. Irrigation was only applied in the first year to ensure plant establishment (150 mm), otherwise the plants were rainfed. Weeds were mechanically controlled during the first year. From the second year after planting, no fertilization, irrigation, weeding or other agronomic inputs were provided.

2.2. Measurements

The main meteorological parameters, such as maximum and minimum temperatures and rainfall, were measured by a weather station connected to a data logger (Delta-T, WS-GP1 Compact) located 150 m from the experimental field. The reference crop evapotranspiration (ET_0) was calculated from the evaporation pan (mm d^{-1}) by the pan coefficient of 0.80 (pan placed in dry fallow area, medium RH, light wind speed, windward side 1 m), as reported by Doorenbos and Pruitt (1977). Data were grouped on a ten-day basis, from sprouting to the end of each growing season.

The soil moisture (mm) was modelled to estimate the drought stress over the growing years. The available soil moisture was calculated as difference between the field capacity (27% of dry soil weight) and the wilting point (11% of dry soil weight) in a soil bulk density of 1.1 g cm^{-3} and a rooting depth of 0.9 m. This was decreased by an availability coefficient of 50% to obtain the maximum plant available water, which was increased by the rainfall and irrigation and decreased by the crop evapotranspiration by using the crop coefficients adopted for *Miscanthus* × *giganteus* and *Arundo donax* grown in the same area (Cosentino et al., 2007, 2014). On days when the soil moisture fell below 20% of the maximum plant available water, the plants were considered to be suffering from drought stress (Nunn et al., 2017).

Harvest took place at the beginning of autumn (end of September 2010, 2011, 2012 and 2013) and in winter-time (mid February 2011, 2012, 2013 and 2014).

At harvest, edge plants were removed in each plot to weight the biomass within 12 m^2. Dry biomass yield was calculated by weighing sub-samples of fresh biomass and after oven drying it at 65 °C until constant weight.

The whole season crop water use efficiency (g L^{-1}) was calculated as the ratio between dry biomass yield and crop water use (CWU) from spring-regrowth up to harvest, in both winter and autumn growing seasons. The CWU was calculated according to Cosentino et al. (2014) taking into account the irrigation at establishment (I), the rainfall (R) and the difference between soil water content at 0–90 cm soil depth between a first and a second measurement (ΔC). Soil water content was measured gravimetrically, collecting soil samples in three replicates per plot and oven drying at 105 °C until constant weight.

2.3. Biomass quality

Oven-dried samples (whole aboveground biomass) collected at the autumn or winter harvest were ground through a 1-mm screen in an IKA mill (IKA-WERFE, Gmbh & Co., KG, Staufenim Breisgau, Germany). Cellulose, hemicellulose, acid detergent lignin (ADL), proteins, lipids and ash were determined by a near-infrared spectrometer (NIR, SpectraStar™ 2500XL-R, Unity Scientific) provided with a tungsten halogen lamp as light source and a high performance ultra-cooled InGaAs extended range detector. Samples were placed in small powder cups and scanned in duplicate in diffuse reflection measurement mode, wavelength range of 680–2500 nm and accuracy < 0.1 nm. A previous calibration developed by the Ucal complete chemometric calibration software (InfoStar 3.11.0 version) was adopted. The calibration consisted of a regression that correlates spectra and analytic determinations of 240 different lignocellulosic raw materials of *Arundo donax* clones and *Miscanthus* species (stems, leaves or the whole biomass) grown under different agronomic practices and growing seasons. Following a first scan run, spectra of *Oryzopsis, Cymbopogon, Sorghum* and *Saccharum* were also used for further calibration development in the Ucal software. The same biomass samples were analytically determined in triplicate according to the Van Soest et al. (1991) method for structural carbohydrate and ADL by using a raw fiber extractor (FIWE 6, VELP Scientifica Srl, Usmate, Italy), the Kjeldahl method for proteins (Distillation unit B-324, Büchi Italia Srl), the Soxtec-Tecator extraction for lipids (FOSS Analytical, 15 Höganäs, Sweden) and the