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Exploring the potential of wild perennial grasses as a biomass source in semi-arid Mediterranean environments

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Abstract

In Mediterranean environments, few perennial grass species are available for cultivation in rainfed systems and marginal lands, where plants with excellent adaptation are required. The aim of the present work was to determine the potentiality of five native Mediterranean perennial grasses for lignocellulosic biomass production. Wild accessions of three hemicryptophytes (*Ampelodesmos mauritanicus*, *Hyparrhenia hirta*, and *Piptatherum miliaceum*) and two geophytes (*Saccharum spontaneum* ssp. *aegyptiacum* and *Sorghum halepense*) were collected at three Mediterranean sites (Sicily, Sardinia and Majorca), and their morphological, physiological, productivity and quality traits were evaluated in the field. The species differed in height, with *S. spontaneum* and *A. mauritanicus* being the tallest. The leaf mass ratio ranged from 0.23 to 1.0 g g^{-1} among species. Maximum net photosynthesis was measured in the C_4 species *S. spontaneum* and *S. halepense* (26.6 and 23.8 μmol CO_2 m^{-2} s^{-1}, respectively). *A. mauritanicus* showed the lowest transpiration rate and the highest instantaneous water use efficiency (2.7 mmol H_2O m^{-2} s^{-1} and 6.9 μmol CO_2 mmol H_2O^{-1}, respectively). *S. spontaneum* was the most productive species, yielding more than 18 Mg DM ha^{-1} as a three-year average. The highest content of acid detergent lignin was found in *P. miliaceum*, while *A. mauritanicus* was the species richest in hemicellulose and cellulose and poorest in ash. *S. spontaneum* showed the highest moisture content at harvest. Overall, the studied species showed interesting morphological, physiological, productive and qualitative traits. Nevertheless, additional research is necessary to investigate their long-term performance under different management strategies.

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Introduction

The interest in producing biomass to generate bioenergy has increased to a great extent during the last 20 years worldwide. Different crops, such as corn and rapeseed, have been used for this purpose (Firrisa *et al.*, 2013; Zentková and Cvengrošová, 2013; Karlen *et al*., 2014; Liu *et al*., 2014). However, the use of these crops as a biomass source has been criticised, since their use competes with food production in a direct or indirect way, increasing food prices and limiting the food supply in a food-limited world (Valentine *et al*., 2012; Hennecke and Rettenmaier, 2015). Due to this reason, second-generation biomass composed of inedible species has been suggested as an interesting alternative (Sanderson and Adler, 2008; Simmons *et al*., 2008; Naik *et al*., 2010). Several perennial grasses have been investigated as second-generation feedstock candidates both for generating bioenergy (*i.e*., heat and energy, bio-oil, syngas, *etc*.) and as a source of structural carbohydrates for biofuel production. In fact, perennial grasses have the advantage of producing relatively larger amounts of biomass than most annual species, even under low-fertility conditions. In addition, crop management costs for perennial grasses are much lower than those for annual species, since they are planted only at the beginning of the establishment of a long-lasting stand and require lower agronomic inputs to sustain yields (Zegada-Lizarazu *et al*., 2010). The benefit is reduced greenhouse gas emissions (Rettenmaier *et al*., 2010). Recently, it has been suggested that these advanced biomass crops should be grown in marginal lands to avoid competition with food crops in arable lands (Kang *et al*., 2013). Switchgrass (*Panicum virgatum* L.), miscanthus (*Miscanthus* spp.) and giant reed (*Arundo donax* L.) are some of the most investigated perennial grasses for producing second-generation bioethanol (Scordia *et al*., 2014), but in

Mediterranean environments, some constraints, especially water shortages during summer, often reduce the production of these species to various extents (Cosentino *et al*., 2007, 2014). Under a Mediterranean climate, characterised by 2-6 months of drought during summer and short dry periods occurring during the growth phase from autumn to spring, plant CO_2 assimilation is limited to a great extent (Gulías *et al*., 2009). Moreover, more limiting scenarios are forecasted due to climate change during the 21st century in the Mediterranean basin; even areas receiving more precipitation may get drier than today due to increased evaporation and changes in the seasonal distribution of rainfall and its intensity, higher air temperatures and increased occurrence of extreme weather events (Giannakopoulos *et al*., 2009; Cosentino *et al*., 2012). For these reasons, high yields of conventional energy crops can be achieved in southern Europe only in arable lands under irrigation (Cosentino *et al*., 2012).

Under rainfed conditions, plants with excellent adaptation are needed; hence, it would be worth focusing on perennial grass species well suited for specific sites and with low demand for inputs.

Currently, there is remarkable and still widely unexplored plant diversity in the Mediterranean basin. Tilman *et al*. (2006) highlighted that wild populations might represent a potential source of biomass plants in agriculturally degraded lands due mainly to traits of resistance and phenotypic plasticity. However, knowledge regarding their ecology, biology, physiology and agronomy must be prioritised before they can be recommended as candidate crops (Scordia *et al*., 2017).

The general objective of this work was to determine the potentiality of five native Mediterranean perennial grasses (*Piptatherum miliaceum* ssp. *miliaceum* and ssp. *thomasii, Ampelodesmos mauritanicus, Saccharum spontaneum* ssp. *aegyptiacum, Sorghum halepense* and *Hyparrenia hirta*) as biomass sources in the Mediterranean area under rainfed conditions. Our research focused on these species because they are widespread under a variety of conditions in the Mediterranean basin, including marginal lands, and are able to regrow under water shortages during summer. The specific objectives of our study were to: i) identify the key biological and physiological traits of five native Mediterranean perennial grasses; and ii) determine their potential aboveground biomass production and quality for energy uses.

Materials and methods

Propagation material for five native perennial grasses was collected from three islands of the west Mediterranean basin: Sicily, Sardinia and Majorca. The five species, from the Poaceae family and having a perennial habit, belong to two groups (Table 1): i) hemicryptophytes [(smilo grass (*Piptatherum miliaceum* (L.) Coss.), diss grass (*Ampelodesmos mauritanicus* (Poir.) Dur. & Schinz), and coolatai grass (*Hyparrenia hirta* (L.)]; and ii) geophyte rhizomatous [(African fodder cane (*Saccharum spontaneum* L. ssp. *aegyptiacum* Willd. Hackel) and Johnsongrass (*Sorghum halepense* (L.) Pers.)].

P. miliaceum was grown at the three sites (ssp. *miliaceum* in Sicily, Sardinia and Majorca and ssp. *thomasii* also in Majorca), *A. mauritanicus* was grown in Sardinia and Majorca, and *H. hirta*, *S. halepense* and *S. spontaneum* were grown in Sicily.

All experimental sites are characterised by a typical Mediterranean climate with mild and wet winters and hot and dry summers. The total annual rainfall, based on long-term observations (30 years), is 447, 550 and 450 mm in Catania (Sicily, Italy), Sassari (Sardinia, Italy) and Palma (Palma de Majorca, Spain), respectively. The mean maximum temperature of the warmest month and the mean minimum temperature of the coldest month are 32.0ºC and 5.0ºC in Catania, 29.5ºC and 6.2ºC in Sassari, and 30.2ºC and 7.4ºC in Palma, respectively. Over the experimental period, main meteorological parameters, such as air temperature and precipitation, were continuously measured near the experimental fields at the three sites.

Plants were grown in experimental field plots under rainfed conditions with the aim to evaluate the aboveground biomass yield, physiological traits and biomass quality.

At all sites, gas exchange parameters, plant height, leaf mass ratio (LMR, leaf biomass/shoot biomass), aboveground biomass production and biomass quality were determined.

Field trial management

Site 1: Sicily

Propagation material of *H. hirta, P. miliaceum* ssp. *miliaceum, S. spontaneum* and *S. halepense* was collected from wild populations and established in an experimental field at the University of Catania (10 m a.s.l., 37°27’ N, 15°03’ E) in a typical Xerofluvent soil in spring 2010. Plantlets were obtained by the division of clumps and rhizomes and were directly transplanted into the field.

A randomised block design with three replicates was used. A single plot measured 100 m^2 (10×10 m), with a density of 1 plant m^{-2} (1×1 m). The agronomical practices comprised soil tillage in autumn and bed preparation just before transplanting by disk harrowing at 20 cm depth. At the same time, a basic fertiliser (50 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1} as ammonium sulfate and superphosphate, respectively) was applied, and otherwise all plots were unfertilised. Irrigation was supplied only from planting up to complete establishment (total amount: 150 mm). Weed control was performed when necessary by means of a grass trimmer. Following

Table 1. Botanical, ecological and physiological traits of *Ampelodesmos mauritanicus* (Ama), *Hyparrhenia hirta* (Hhi), *Piptatherum miliaceum ssp. miliaceum* (Pmm), *Saccharum spontaneum* ssp. *aegyptiacum* (Ssa) and *Sorghum halepense* (Sha). Annual precipitation and altitudinal ranges refer to our own observations from wild populations in Sicily, Sardinia and Majorca.

Species	Life form (Raunkiaer)	Habitat	Photosynthetic type	Annual precipitation	Altitudinal range
Ama	Hemicryptophyte	Mediterranean shrubland and open forests	C3	400-1000	10-750
Hhi	Hemicryptophyte	Dry meadows and disturbed areas	C4	430-850	10-125
Pmm	Hemicryptophyte	Disturbed areas and road margins	C3	300-880	10-600
Ssa	Geophyte	River bottoms and humid areas	C4	350-550	0-20
Sha	Geophyte	River bottoms and open fields	C4	450-650	0-600

the post-establishment year, crops were managed without any agronomical input supply; therefore, no irrigation, fertilisation or weed control was conducted. Harvest was carried out in September 2010, 2011 and 2012, corresponding to the first, second and third growing season, respectively. Biomass was measured in the centre of each plot after removing the edge plants from all sides to obtain a sampling area of 12 m^2 (4×3 m). Fresh biomass samples were oven dried at 65°C until a constant weight was reached to calculate the dry matter yield. The plant height and LMR were determined for ten random tillers per species in the year post-establishment.

Site 2: Majorca

Seeds of *A. mauritanicus*, *P. miliaceum* ssp. *miliaceum* and *P. miliaceum* ssp. *thomasii* were collected from wild populations in Majorca. Seeds were germinated in seed benches in December 2012. Plantlets of both subspecies of *P. miliaceum* were transplanted into fields at the University of the Balearic Islands (39º38' N, 2º38' E, 80 m a.s.l.) in February 2013, and those of *A. mauritanicus* were transplanted in November 2013. The experiment was carried out under rainfed conditions in 2 m^2 plots (plants were spaced 0.25 m apart within and between rows for both subspecies of *P. miliaceum* and 0.50 m for *A. mauritanicus*), with 4 plots per species in a completely randomised design. No fertiliser was applied during the experimental period, and the plants were only irrigated during the two weeks after transplantation into the field to ensure plant establishment. Weed control was performed by means of a grass trimmer only during the first growing season.

Aboveground biomass production was evaluated for 5 plants placed at the centre of each plot at the beginning of summer in 2013 and 2014. Fresh biomass samples were oven dried at 65°C until a constant weight was reached to calculate the dry matter yield. Morphological traits (plant height and LMR) were determined on 5 random plants in the year post-establishment.

Site 3: Sardinia

Propagation material from 26 populations of *A. mauritanicus* and *P. miliaceum* ssp. *miliaceum* were collected across Sardinia from different microhabitats. Seeds of *P. miliaceum* were sown in pots, and the seedlings of the 8 best-germinating accessions were randomly transplanted into the field at the end of winter (February 2012). Clonal plantlets of *A. mauritanicus* were collected in October-November 2012. The plantlets, obtained through the division of clumps, were grown in pots until they were transplanted into the field in autumn 2013. The field experiment was carried out at the experimental station of CNR (40°45'12'' N, 8°25'17'' E, 27 m a.s.l.) in Sardinia (Italy). Three plots per species were established in a completely randomised design. Each plot contained 8 plants belonging to the same accession with a planting distance of 0.5×0.5 m. No fertiliser was applied during the experimental period, and emergency irrigation (50 mm) was provided just after transplanting to facilitate plant establishment. Afterwards, neither fertilisers nor irrigation were applied, and weed control was performed by means of a grass trimmer. For *P. miliaceum*, the aboveground plant biomass was estimated once a year in July 2012 (1st growing season), 2013 (2nd growing season) and 2014 (3rd growing season). Plant biomass was determined on an individual plant basis as the average dry weight (oven dried samples at 65°C) of 18 plants per population (6 plants per plot). For *A. mauritanicus*, the aboveground biomass was measured for the first time in July 2014, when a sufficient plant size was attained. Morphological traits (plant height and LMR) were determined for 16 random tillers per plot (48 tillers per species) in the year post-establishment for both species.

Biomass quality

Sub-samples of fresh biomass were placed in a ventilated oven to dry at 65°C until a constant weight was reached to determine the dry biomass and moisture content. The dry biomass was ground and stored for biomass quality analysis. Neutral and acid detergent fibres (NDF and ADF, respectively) and acid detergent lignin (ADL) were determined according to the method in Van Soest *et al*. (1991). The hemicellulose and cellulose content were calculated as NDF-ADF and ADF-ADL, respectively. The ash content was determined by placing 1 g samples in a muffle furnace at 550±50°C. All analytical determinations were replicated three times.

Gas exchange measurements

Gas exchange, as net photosynthesis (Pn, µmol CO_2 m^{-2} s^{-1}), and the leaf transpiration rate (E, mmol H_2O m^{-2} s^{-1}) were determined in fully developed and healthy leaves at mid-morning on sunny days using a portable photosynthesis system (Li6400, Li-Cor Inc., Lincoln, NE, USA). Four replicate plants from the centre of each plot were analysed for each species. Cuvette settings of 400 ppm [CO_2] and 1500 µmol m^{-2} s^{-1} of photosynthetically active radiation (10% blue and 90% red light) were used. Instantaneous water use efficiency (WUEi, µmol CO_2 mmol H_2O^{-1}) was calculated as Pn/E.

Statistical analysis

Statistical analysis was carried out separately for each site. For Catania, the biomass dry matter yield was analysed using one-way analysis of variance (ANOVA) with repeated measures on time. Measurements were performed across years within subjects, while species was a between-subjects effect. The tests of between-subjects effects were based on the average of the within-subjects effects. For Palma and Sassari, the biomass yield was analysed using one-way ANOVA, with species as a fixed effect.

For each location, plant height, LMR, Pn, E and WUEi were analysed using one-way ANOVA, with species as a fixed effect. The analyses were performed based on the randomised block design (CoStat version 6.003, CoHort software, Monterey, CA, USA). Percentage data were arcsine √% transformed before the analysis. The least significant difference was used for mean separation at the 95% confidence level.

Results

Meteorological conditions

Important differences among sites in the pattern of annual precipitation and maximum and minimum air temperatures were observed (Figure 1). Palma was the driest site, with a mean annual precipitation of 452 mm, and 588 and 639 mm yr^{-1} were registered for Catania and Sassari, respectively. Catania was the warmest site, with a mean annual temperature of 17.7ºC, while 16.9ºC and 15.5ºC, respectively, were registered in Palma and Sassari. The greatest difference between the average monthly maximum and minimum air temperatures was observed in Sassari (12.3ºC), while 11.3ºC and 10.8ºC were registered for Catania and Palma, respectively.

Morphological and physiological characteristics

The morphological and physiological characteristics of the plants reflected differences in growth form, leaf habit and photosynthetic type among the studied species (Table 2).

In Catania, the main effect of species was significant for all studied morphological and physiological characteristics. *S. spontaneum* was the tallest (2.21 m), and *H. hirta* was the shortest among species (1.00 m). *S. halepense* showed the highest leaf mass ratio (LMR, 0.68 g g^{-1}), while no differences were observed in the other species (0.26 g g^{-1} on average). Net photosynthesis (Pn) was the highest in the C$_4$ species *S. spontaneum* and *S. halepense* (26.6 and 23.8 μmol CO$_2$ m^{-2} s^{-1}, respectively). *P. miliaceum* showed the lowest Pn, 14.1 μmol CO$_2$ m^{-2} s^{-1}. The latter species also showed the lowest transpiration rate (E, 3.8 mmol H$_2$O m^{-2} s^{-1}), while *S. halepense* exhibited the highest E (5.1 mmol H$_2$O m^{-2} s^{-1}). The highest instantaneous water use efficiency (WUEi) was achieved in *S. spontaneum* (6.2 μmol CO$_2$ mmol H$_2$O^{-1}), the lowest was in *P. miliaceum* (3.8 μmol CO$_2$ mmol H$_2$O^{-1}), while *S. halepense* and *H. hirta* were in the middle of the range (4.7 μmol CO$_2$ mmol H$_2$O^{-1}).

In Palma, significant effects of species were observed in LMR, Pn, and WUEi. Plant height and E did not significantly differ between species.

Plant height was 0.92 m in *A. mauritanicus* and 0.93 m *P. miliaceum*, while LMR was higher in *A. mauritanicus* than in *P. miliaceum* (1.0 and 0.24 g g^{-1}, respectively), and Pn showed the same pattern (18.7 and 12.6 μmol CO$_2$ m^{-2} s^{-1}, respectively). Despite the different Pn values between species, E was similar (2.7 and 2.3 mmol H$_2$O m^{-2} s^{-1} in *A. mauritanicus* and *P. miliaceum,* respectively), leading to higher WUEi in *A. mauritanicus* in comparison to *P. miliaceum* (7.0 and 6.2 μmol CO$_2$ mmol H$_2$O^{-1}*,* respectively).

In Sassari, a significant difference between species was observed for LMR and Pn, while plant height, E and WUEi were not significantly different. LMR was higher in *A. mauritanicus* than in *P. miliaceum* (0.71 and 0.23 g g^{-1}, respectively), while Pn was higher in *P. miliaceum* than in *A. mauritanicus* (12.6 and 6.7 μmol CO$_2$ m^{-2} s^{-1}, respectively).

Biomass production and quality

In Catania and Palma, aboveground dry matter yield was significantly different among species ($P \leq 0.001$), while no differences were found in Sassari.

Based on the average among the three cultivation years, *S. spontaneum* was the most productive species in Catania, producing more than 18 Mg ha^{-1}, followed by S. *halepense*, with 5.4 Mg ha^{-1}. *P. miliaceum* and *H. hirta* showed similar results, 3.2 and 3.0 Mg ha^{-1}, respectively. The third growing season was the most productive across species (10.2 Mg ha^{-1}), and these values differed from those obtained in the second (7.8 Mg ha^{-1}) and the first year (4.7 Mg ha^{-1}). The species showing the most stable yield among years were *P. miliaceum* and *H. hirta* [coefficient of variation (CV) 17.1 and 21.5%]*,* while *S. halepense* and *S. spontaneum* showed the highest CV (40.5 and 39.5%, respectively) (Figure 2A).

In Palma, *A. mauritanicus* was the most productive species during establishment (7.5 Mg ha^{-1}), while the subspecies of *P. miliaceum* produced 2.4 Mg ha^{-1} (ssp. *miliaceum*) and 1.5 Mg ha^{-1} (ssp. *thomasii*). In the second growing season, no differences were observed between *P. miliaceum* and *P. thomasii,* with values of 1.7 and 1.8 Mg ha^{-1}, respectively. Of the two subspecies*, P. thomasii* showed the lowest CV among years (9%), while *P. miliaceum* had a CV of 16% (Figure 2B).

In Sassari, *P. miliaceum* showed high inter-annual variation in biomass production, exhibiting productivity of 2.1 Mg ha^{-1} in the first year, a sharp increase in the second year (23.3 Mg ha^{-1}) and a decrease during the third year (10.6 Mg ha^{-1}). However, *P. miliaceum* maintained relatively high productivity when compared to the other two sites. Across the three cultivation years, *P. miliaceum* showed a CV in biomass production of 72% (Figure 2C). The dry matter yield of *A. mauritanicus* in 2014 was comparable to that of *P. miliaceum* in the 3rd year (11.1 Mg ha^{-1}).

Biomass quality in terms of moisture, ash, ADL, hemicellulose and cellulose content significantly differed among species within sites (Table 3).

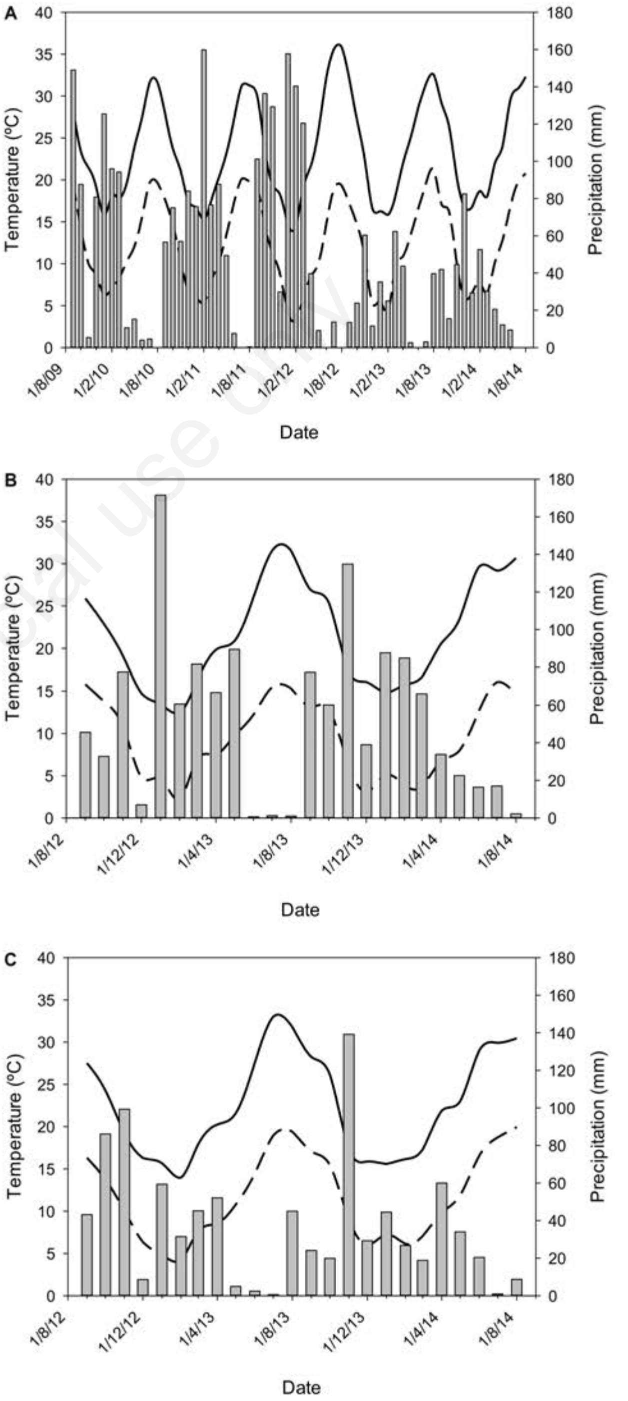

Figure 1. Monthly mean maximum (solid line) and minimum (dashed line) temperatures and monthly precipitation (filled bars) during the experimental period at the three experimental sites: Catania (A), Sassari (B) and Palma (C). Data were gathered by meteorological stations located near the experimental fields.

In Catania, the biomass moisture content was higher in *S. spontaneum* than in *S. halepense* (60.5 and 42.7%, respectively). *H. hirta* and *P. miliaceum* showed the lowest moisture (34.2 and 34.9%). The ash content was the highest in *P. miliaceum* (5.1%) and the lowest in *S. spontaneum* (3.7%). Both the ADL and cellulose content were greater in *S. spontaneum* (8.4 and 38.2%, respectively) than in the other species, while *S. halepense* showed the highest hemicellulose content (29.7%).

In Palma, *P. miliaceum* ssp. *thomasii, P. miliaceum* ssp. *miliaceum* and *A. mauritanicus* showed similar, although significantly different, moisture content (42.6-43.9%). The ash content was higher in *P. miliaceum* (7.5%) than in *P. thomasii* and *A. mauritanicus* (6.8 and 6.7%, respectively). The ADL content was the highest in *P. miliaceum* (6.8%) and the lowest in *A. mauritanicus* (5.3%). Hemicellulose was the highest in *P. thomasii* (29.3%) and the lowest in *P. miliaceum* (27.0%), while cellulose was the highest in *A. mauritanicus* (39.3%) and the lowest in *P. thomasii*.

In Sassari, moisture, ash, ADL, hemicellulose and cellulose significantly differed between species. The moisture content was higher in *A. mauritanicus* than in *P. miliaceum* (41.1 and 30.8%, respectively). The ash, ADL and cellulose content were higher in *P. miliaceum* (9.1, 12.6 and 36.7%, respectively) than in *A. mauritanicus* (2.8, 10.3 and 34.6%, respectively), while hemicellulose was higher in *A. mauritanicus* than in *P. miliaceum* (34.5 and 29.3%, respectively).

Discussion

Morphological and physiological traits

The five studied species are perennial grasses and have some common traits, such as the ability to regrow from buds at or near the soil surface after the season of stress. However, differences in photosynthetic type, environmental requirements (Table 1), plant height and LMR (Table 2) suggest differences in plant architecture

Table 2. Mean values ± standard errors of morphological and physiological traits of *Hyparrhenia hirta* (Hhi), *Piptatherum miliaceum* ssp. *miliaceum* (Pmm), *Saccharum spontaneum* ssp. *aegyptiacum* (Ssa), *Sorghum halepense* (Sha) and *Ampelodesmos mauritanicus* (Ama) at the three collection sites. Plant height (m), leaf mass ratio (LMR, leaf biomass/shoot biomass, g g^{-1}), maximum photosynthetic rate on an area basis (Pn, μmol CO$_2$ m^{-2} s^{-1}), leaf transpiration (E, mmol H$_2$O m^{-2} s^{-1}) and instantaneous water use efficiency (WUEi, Pn/E, μmol CO$_2$ mmol H$_2$O^{-1}). P-values for the main effect of species at each site according to one-way ANOVA.

Species	Site*	Plant height	LMR	Pn	E	WUEi
Hhi	1	1.00±0.18	0.24±0.03	19.7±0.92	4.2±0.27	4.7±0.09
Ssa	1	2.21±0.25	0.28±0.04	26.6±1.44	4.3±0.56	6.2±0.47
Sha	1	1.36±0.47	0.68±0.15	23.8±2.13	5.1±0.94	4.7±0.45
Pmm	1	1.06±0.10	0.25±0.06	14.1±0.38	3.8±0.21	3.7±0.10
Species effect	1	≤0.001	≤0.001	≤0.001	0.016	≤0.001
Ama	2	0.92±0.30	1.00±0.00°	18.7±1.37	2.7±0.20	7.0±0.52
Pmm	2	0.93±0.11	0.24±0.05	12.6±1.41	2.3±0.42	6.2±0.75
Species effect	2	0.504	0.002	≤0.001	0.084	0.026
Ama	3	1.98±34.5	0.71±0.14	6.7±0.95	2.3±0.25	2.9±0.45
Pmm	3	1.16±0.07	0.23±0.03	12.6±1.98	3.3±0.82	3.8±0.41
Species effect	3	0.054	0.017	0.009	0.095	0.067

*Site 1=Catania; site 2=Palma; site 3=Sassari; °young plants.

Table 3. Mean values (% w/w) ± standard error of biomass quality of *Hyparrhenia hirta* (Hhi), *Piptatherum miliaceum* ssp. *miliaceum* (Pmm), *Saccharum spontaneum* ssp. *aegyptiacum* (Ssa), *Sorghum halepense* (Sha), *Ampelodesmos mauritanicus* (Ama) and *Piptatherum miliaceum* ssp. *thomasii* (Pmt). P-values of the main effect of species at each site according to one-way ANOVA.

Species	Site*	Moisture	Ash	ADL	Hemicellulose	Cellulose
Hhi	1	34.2±1.7	4.7±0.7	6.3±0.6	25.9±1.4	33.6±1.1
Ssa	1	60.5±1.8	3.7±0.9	8.4±0.6	26.1±0.7	38.2±1.3
Sha	1	42.7±2.9	4.3±1.1	6.8±0.2	29.7±0.7	33.3±1.2
Pmm	1	34.9±2.5	5.1±0.8	6.2±0.2	27.3±1.3	35.5±0.9
Species effect	1	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Ama	2	43.4±0.7	6.7±0.1	5.3±0.3	28.3±1.1	39.3±0.4
Pmt	2	43.9±0.7	6.8±0.1	6.0±0.1	29.3±0.5	35.0±0.5
Pmm	2	42.6±0.7	7.5±0.1	6.8±0.2	27.0±0.4	36.5±0.3
Species effect	2	≤0.001	0.002	≤0.001	0.004	≤0.001
Pmm	3	30.8±1.2	9.1±0.4	12.6±0.5	29.3±1.1	36.7±0.7
Ama	3	41.1±2.8	2.8±0.4	10.3±0.3	34.5±0.5	34.6±0.4
Species effect	3	0.007	≤0.001	≤0.001	0.003	0.012

*Site 1=Catania; site 2=Palma; site 3=Sassari.

and biomass productivity. Biomass allocation to leaves was relatively low in *H. hirta*, *S. spontaneum* and *P. miliaceum* (from 0.28 to 0.23 g g^{-1}) in comparison to other grasses (Freschet, 2015). Nevertheless, it should be considered that the LMR values reported in this study correspond to adult flowering or leaf-senescent plants, in which the stem and panicle are important components of the total aerial biomass. By contrast, LMR values reported for *A. mauritanicus* correspond to young plants, in which neither stems nor panicles have been produced.

The maximum gas exchange rates were recorded in late spring for the C_4 species and early to mid-spring for the C_3 species, which is in accordance with the different temperature requirements for the two photosynthetic types (Berry and Björkman, 1980; Yamori *et al*., 2014). Moreover, maximum Pn values were recorded in *S. spontaneum* and *S. halepense*, both showing a C_4 photosynthetic pathway. However, it is noteworthy that relatively high Pn and low E values were recorded in *A. mauritanicus*, a C_3 species, leading to this species showing the highest WUEi. The high ability of *A. mauritanicus* to assimilate carbon with low water consumption is a highly interesting trait in terms of biomass production under marginal Mediterranean conditions, where water stress is the most important limiting factor, and this is likely related to some anatomical traits, such as the encrypted stomata of this species (Romero-Munar, unpublished results). In fact, encrypted stomata have been suggested to be an important contributor to reductions in leaf transpiration under well-watered conditions (Roth-Nebelsick *et al*., 2009), which would limit excessive plant water consumption during spring, leading to higher water availability in summer.

Biomass production and quality

Most of the studied species showed relatively low biomass production, below 20 Mg ha^{-1}, when compared with other perennial grasses commonly used as biomass sources, such as giant reed, miscanthus and switchgrass, with yields ranging between 7 and 60 Mg ha^{-1} (Lewandowski *et al*., 2003; Zegada-Lizarazu *et al*., 2010). However, it is important to highlight that giant reed, miscanthus and switchgrass are commonly cultivated in temperate or Mesomediterranean areas, where the annual precipitation is usually greater than 700 mm and, most importantly, its distribution is much more regular during the year than in typical Mediterranean climate areas, where there is an uncoupling between the optimal temperature and water availability. In fact, few works have studied the production of those commonly used perennial grasses under semi-arid Mediterranean conditions. In this sense, Cosentino *et al*. (2007, 2014) showed that miscanthus and giant reed productivity used to be lower than 15 Mg ha^{-1} in southern Italy when grown under rainfed conditions, which is similar to the productivity of *P. miliaceum* reported in our study and well below the productivity of *S. spontaneum*. From a productivity point of view, *S. spontaneum* can be suggested to be a highly productive species in areas with a typical Mediterranean climate with annual precipitation between 400 and 500 mm, since its productivity was higher than 25 Mg ha^{-1} three years after it was established in spite of the low precipitation recorded. Its maximum reported productivity in this study (28 Mg ha^{-1}) was similar to that showed for energy cane under subtropical conditions (Lewandowski *et al*., 2003). Recently, Cosentino *et al*. (2015b) and Scordia *et al*. (2015) highlighted the agronomically desirable traits of *S. spontaneum*, such as its C_4 metabolism, high biomass yield, active CO_2 assimilation rates during drought-stress periods, ability to use water and radiation efficiently and satisfactory biomass quality.

P. miliaceum showed similar productivity to that of *S. spontaneum*, 23 Mg ha^{-1}, but this value was only attained under higher annual precipitation, since such high productivity was only recorded at the most humid site, Sassari, where the annual precipitation was greater than 600 mm during the experimental period. Moreover, this species showed high inter-annual variability in biomass production (Figure 2). *S. halepense* and *A. mauritanicus* showed intermediate biomass production, 8.1 and 7.5 Mg ha^{-1}, respectively, which are similar to the reported dry matter yield in humid semi-natural grasslands under temperate conditions (Tonn *et al*., 2010). However, *S. halepense* has been reported to produce between 14 and 17 Mg ha^{-1} under subtropical conditions (Lewandowski *et al*., 2003), and *A. mauritanicus* would likely increase its biomass production in subsequent years. By contrast,

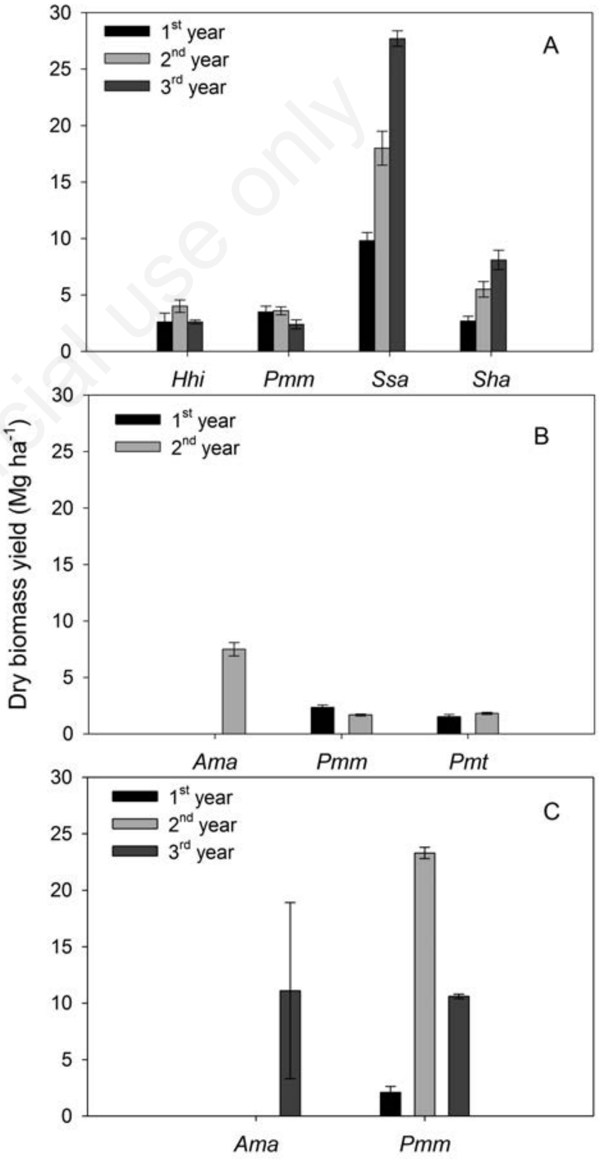

Figure 2. Dry biomass production of *Hyparrhenia hirta* (Hhi), *Piptatherum miliaceum* ssp. *miliaceum* (Pmm), *Saccharum spontaneum* ssp. *aegyptiacum* (Ssa), *Sorghum halepense* (Sha), *Ampelodesmos mauritanicus* (Ama) and *Piptatherum miliaceum* ssp. *thomasii* (Pmt) at the three experimental sites: Catania (A), Palma (B) and Sassari (C). Values are means ± standard error.

H. hirta, a C_4 species that is usually cultivated as a forage resource in semi-arid areas (Aydin *et al*., 1999), attained maximum biomass production of 4 Mg ha^{-1}, which is higher than the dry matter yield reported for dry semi-natural grasslands in temperate areas (Tonn *et al*., 2010). It is worth to note that in post-establishment seasons the two hemicryptophyte (*P. miliaceum* and *H. hirta*) were not able to cover the ground area like the geophyte rhizomatous (*S. halepense*, *S. spontaneum*) in Catania site, thus, the adopted plant density (1.0×1.0 m) was probably a limiting factor for biomass production. Further studies to optimise plant density could be envisaged.

The reported biomass quality parameters in this study suggest the interesting potential use of these species in low-temperature thermochemical conversions, since the ash content of all the species was similar to that reported for cardoon (Solano *et al*., 2010), giant reed (Scordia *et al*., 2016) and different semi-natural temperate grasslands (Tonn *et al*., 2010). In contrast, the ash content found in miscanthus was lower than that observed in our findings (Scordia *et al*., 2013, 2016). However, a more detailed analysis of biomass quality, such as the evaluation of lower heating values, ash melting behaviour, and bulk and energy density, taking also into account N, Cl, K, Si and other mineral contents, should be conducted in the future for an accurate assessment of the potentiality of these species in thermochemical conversions. In addition, the moisture content at harvest was high in all species, ranging from 30% in *P. miliaceum* to 60% in *S. spontaneum* (Table 3), which is well above the optimal values reported for switchgrass, miscanthus and other common herbaceous biomass crops (Mckendry, 2002; Karp and Shield, 2008). The moisture content at harvest is a key point in determining economic viability in lignocellulosic biomass production chains, since storage and transportation are highly limited by biomass humidity (Smeets *et al*., 2009). Moisture resulted highly variable from site to site and year to year. In this sense, environmental conditions and management practices largely determine the biomass humidity at harvest (Monti *et al*., 2015).

The three most important components of lignocellulosic feedstock, cellulose, hemicellulose and lignin, play a major role in determining the economic viability of the conversion of such feedstocks to ethanol (Menon and Rao, 2012). The results reported here suggest that the theoretical ethanol yield of most of the studied species might be quite comparable to that reported for other monocot species, such as miscanthus and giant reed, owing to the similar content of hemicellulose and cellulose (Scordia *et al*., 2014). *S. spontaneum* has been used in a dilute oxalic acid pretreatment, and both cellulose- and hemicellulose-derived sugars have been successfully fermented to ethanol (Scordia *et al*., 2010). Further studies might include the use of the other species for an accurate assessment of their conversion efficiency.

In a bioeconomy context, these wild species could be a resource for uses other than biomass. For instance, *A. mauritanicus* has traditionally been used in South Italy in the manufacture of artisanal baskets (Novellino, 2007; Restaino, 2008) and as traditional building material in Algeria (Merzoud and Habita, 2008; Merzoud *et al*., 2009). *S. spontaneum* has been suggested to be a species able to mitigate soil erosion in sloping areas (Cosentino *et al*., 2015a). *P. miliaceum* has been included in several seed mixtures for degraded land revegetation in xeric and arid Mediterranean areas (Oliveira *et al*., 2014) and in the remediation of heavy metal-contaminated areas (Kabas *et al*., 2012; Parraga-Aguado *et al*., 2014). Moreover, *P. miliaceum* and *H. hirta* can be potentially used in both extensive turf grass and green roofs in xeric and arid Mediterranean areas or as forage in early autumn and late spring for grazing animals (Melis *et al*., 2016).

H. hirta has also been considered to be an interesting species because of its content of flavonolignans, an interesting group of compounds with potential uses in the pharmaceutical industry (Bouaziz *et al*., 2002), as well as its usefulness in ameliorating many liver disorders (Bouaziz-Ketata *et al*., 2014).

However, *H. hirta* and *A. mauritanicus* are also a concern because of their invasion ability in several areas (Grigulis *et al*., 2005; Chejara *et al*., 2010), and the rhizomatous species are not an exception, which highlights the importance of improving the knowledge regarding the physiological, agronomical and ecological traits of these species in order to use them in economically viable and environmentally safe ways.

On the other hand, wild accessions might serve as a source of genes for biomass and grain improvements and to confer resistance to abiotic and biotic stresses of cultivated crops. Although breeding activities are still at the infancy on this front, perennial sorghum lines [from *Sorghum bicolor* (L.) Moench × *Sorghum halepense* (L.) Pers.] showed grain and biomass yields comparable with the relative *S. bicolor,* with perennial behaviour depending mostly upon increased dosage of *S. halepense* genome in *S. bicolor* background (Habyarimana *et al*., 2017). These models might be useful in defining suitable perennial ideotypes, and in the development of new biomass crops.

Conclusions

The ability of five native species to produce lignocellulosic biomass in terms of quantity and quality was assessed under different Mediterranean conditions. *S. spontaneum* appeared to be the most interesting species for biomass production, since it showed the highest productivity regardless of the annual precipitation. *P. miliaceum* showed similar productivity but only when the annual precipitation was greater than 600 mm. The studied native perennial grasses showed comparable biomass quality to that of other herbaceous bioenergy crops. In particular, *A. mauritanicus* showed the highest hemicellulose and cellulose and the lowest ash content.

All studied species appeared to be of interest under common Mediterranean conditions, *i.e.*, annual precipitation below 700 mm, taking into consideration their potentiality as second-generation biomass sources in marginal lands. However, as undomesticated species, additional research is necessary to explore other populations from native environments to increase the genetic diversity for important phenological, morphological and physiological traits, investigate key traits for non-invasiveness, optimise cultivation practices and long-term performances, and demonstrate environmental benefits under different management practices.

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