Perennial grasses as lignocellulosic feedstock for second-generation bioethanol production in Mediterranean environment

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Abstract

In this paper the suitability of three perennial, herbaceous, lignocellulosic grasses (*Arundo donax, Saccharum spontaneous* spp. *aegyptiacum* and *Miscanthus* x *giganteus*) for the production of second-generation bioethanol in semi-arid Mediterranean environment was studied. Crops were established in spring 2002, supplying irrigation and nitrogen fertilization up to 2004/2005 growing season. Subsequently, crops were grown without any agronomic input and harvested annually. Data reported in this paper refers to 2008/2009 and 2009/2010 growing seasons. Aboveground dry matter (DM) yield was higher in *Arundo* (35.4±2.1 Mg ha^{-1} in 2009 and 32.2±1.9 Mg ha^{-1} in 2010 harvest) than in *Saccharum* (27.3±2.0 and 23.9±1.9 Mg ha^{-1}, respectively) and *Miscanthus* (19.6±2.8 and 17.2±1.6 Mg ha^{-1}, respectively). Structural polysaccharides of the raw material were higher in *Miscanthus* (63.4% w/w) followed by *Saccharum* (61.5% w/w) and *Arundo* (57.6% w/w). The same trend was identified for the cellulose content (41.0%, 36.8% and 34.6%, respectively). The highest values in the total hemicellulose complex were observed in *Saccharum* (24.7%), followed by *Arundo* (23.1%) and *Miscanthus* (22.4%). The composition of structural polysaccharides leads to a higher theoretical ethanol yield (TEY) from one dry ton of *Miscanthus* feedstock (kg DM Mg^{-1}), followed by *Saccharum* and *Arundo*. On the other hand, the TEY per unit surface (Mg ha^{-1}) was greater in *Arundo* than in *Saccharum* and *Miscanthus*. When compared to other lignocellulosic sources used in the second-generation bioethanol technology, such as agricultural residues, woody species and other herbaceous perennial crops, *Arundo, Saccharum* and *Miscanthus* showed a great potential in terms of TEY ha^{-1}.

Given the high levels of biomass yield and composition of structural polysaccharides, the three species might be introduced into the Mediterranean cropping systems to supply lignocellulosic biomass for second-generation industrial plants or bio-refineries.

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Introduction

Bioethanol, a biofuel that can be used to replace gasoline or blended at high rates, is currently produced from starch and sugar-based raw materials. A mandatory target of 10% biofuel (bioethanol and biodiesel) share in total transport fuel consumption has been officially set by the European Union (2009/28/EC), which might impose to import raw materials or biofuels (European Commission, 2009). The growth in the international biomass trade and imports from third countries may lead to an unsustainable utilisation of this renewable resource. Furthermore, the production of bioethanol from starch (corn) or sugar (sugarcane), which are basically human foodstuffs, might possibly contribute to a food crisis. However, bioethanol can be produced from lignocellulosic material, which is abundant, produced at lower costs and from non-food sources.

Several studies have demonstrated the possibility of producing bioethanol from cellulose and hemicellulose (Scordia *et al.*, 2010, 2011, 2012, 2013a, 2013b) and recently the world's first commercial-scale plant for the production of bioethanol from lignocellulosic sources, with an annual forecast output of about 40,000 tons of bioethanol, was officially inaugurated in Crescentino, Italy (BIOLYFE project, Newsletter 13).

Perennial, herbaceous, non-food crops, being lignocellulosic feedstock, are very appealing for second-generation bioethanol production; the major component of its raw material is in fact cellulose, followed by hemicelluloses and lignin. It has been reported that perennial herbaceous crops have the potential to reduce the disadvantage associated with the change in land use (*e.g.* due to their potential introduction in marginal lands), competition of food *vs* fuels and in general environmental threats as compared to annual crops (Fernando *et al.*, 2010; Rettenmaier *et al.*, 2010).

Most herbaceous perennial crops, however, are largely undomesticated, so their cropping practices, their potential and actual yields, compositions and bioconversion characteristics are not as well-known as those of traditional agricultural crops (Scordia *et al.*, 2010).

At present, research should focus on the identification of an ideotype crop for a given geographic location, which can use abiotic resources efficiently (radiation, water, nutrients), is resistant to biotic stresses (pests and diseases), can give high biomass yields with minimum input supply or can grow well in sub-optimal soil conditions and with specific traits according to the end-uses.

There are several potentially available species to supply lignocellulosic biomass, however, only a few are recommended for the semi-arid Mediterranean environment, which is characterised by mild winters and very warm summers, with precipitations mainly during autumn-winter and to a lesser extent in spring, and drought summers. Out of several perennial, herbaceous, non-food species, giant reed (*Arundo donax* L.) has been indicated as the most suitable energy crop for southern European environments (Lewandowski *et al.*, 2003). This rhizomatous plant is native from Asia and widespread in the countries surrounding the Mediterranean Sea (Boose and Holt, 1999; Rossa *et al.*, 1998). It has a C3 photosynthetic pathway, but has a photosynthesis rate and productivity that are similar to those of C4 species (Lewandowski *et al.*, 2003). Recently, Ceotto *et al.* (2013) indicated that daily crop growth rate and radiation use efficiency (RUE) of giant reed is even higher than in C4 crops. Moreover, Nassi o di Nasso *et al.* (2013) stated that giant reed grows well even in marginal soils.

In several experimental studies carried out in Southern Europe the aboveground biomass yield of giant reed was as high as 30 Mg ha^{-1} DM (Angelini *et al.*, 2005, 2009; Copani *et al.*, 2013; Cosentino *et al.*, 2005, 2006b; Nassi o di Nasso *et al.*, 2011). Due to its structural polysaccharide composition, giant reed has been extensively studied as feedstock for second-generation bioethanol production (Scordia *et al.*, 2011, 2012, 2013) or as biomass for combustion purposes (Nassi o di Nasso *et al.*, 2010).

Great attention has been paid worldwide to the *Miscanthus* genus as a potential dedicated biomass crop. *Miscanthus* is a C4, rhizomatous, perennial species native from East-Asia, where it can be found throughout a wide climatic range (Greef and Deuter, 1993). It was firstly introduced in Northern Europe as ornamental plant, while *Miscanthus* x *giganteus*, a sterile, triploid, interspecific hybrid, was selected for its high productivity, as biomass crop (Lewandowski *et al.*, 2000). In Central and Southern Europe *Miscanthus* x *giganteus* yielded up to 38 Mg ha^{-1} DM (Lewandowski and Heinz, 2003). However, in semi-arid Mediterranean areas, its productivity ranged between 12 and 27 Mg ha^{-1} DM, under rain-fed conditions and 100% maximum evapotranspiration restitution, respectively (Cosentino *et al.*, 2007). *Miscanthus* x *giganteus* has been recently studied for ethanol production from cellulose- and hemicellulose-derived sugars (Scordia *et al.*, 2013a).

In addition to giant reed, another wild species of the Mediterranean flora has been identified and assessed for the production of bioenergy due to its structural polysaccharide composition (Scordia *et al.*, 2010) and biomass yield (Cosentino *et al.*, 2012a). It is *Saccharum spontaneum* L. spp. *aegyptiacum* (Willd.) Hack., which proved to be well adapted to the semi-arid Mediterranean environment, yielding 9.6 Mg ha^{-1} in the first year and 17.9 Mg ha^{-1} DM in the second year after establishment under rain-fed conditions (Cosentino *et al.*, 2012a).

To this end, the present study aimed to ascertain the potential of *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* spp. *aegyptiacum* as lignocellulosic feedstock for second-generation bioethanol in semi-arid Mediterranean environment. The three species were compared in terms of aboveground biomass yield and biomass quality in order to accomplish the theoretical ethanol yield (TEY).

In addition, the TEY per dry matter ton (kg Mg^{-1}) and per unit surface (Mg ha^{-1}) of *Arundo*, *Miscanthus* and *Saccharum* has been compared with the most common feedstock used in the second-generation bioethanol process, such as agricultural residues, dedicated herbaceous species and woody crops.

Materials and methods

Site description and agronomic details

The field experiment was performed at the experimental fields of the University of Catania (10 m asl, 37°25' N lat., 15°03' E long.). Three species, belonging to *Poaceae* family, *Arundo donax* L., *Miscanthus* x *giganteus* Greef et Deu. and *Saccharum spontaneum* L. spp. *aegyptiacum* (Willd.) Hack., were studied. A randomised block experimental design with three replications was adopted.

Rhizomes of *Saccharum* and *Arundo* were collected by the coast and in riparian areas of Sicily (Cosentino *et al.*, 2006a), Italy, while rhizomes of *Miscanthus* were collected in an older plantation located at the same experimental fields (Cosentino *et al.*, 2007).

The previous crop was winter wheat. In autumn the soil has been ploughed (30-40 cm) and then harrowed at 20 cm before transplanting. Thus, fertilization with 80 kg N ha^{-1} as ammonium sulphate, and 100 kg P_2O_5 ha^{-1} as mineral superphosphate was applied. Potassium was not applied due to its high content in the soil. Rhizomes were cut into pieces and transplanted into small plots (16 m^2), with a density of 4 rhizomes m^{-2} in spring 2002.

The subsequent years (2003/2004 and 2004/2005 growing seasons, respectively), at the end of winter, 100 kg N ha^{-1} as ammonium nitrate were supplied.

Irrigation was applied in the summer period (between May and September), about every 20 days, for a total amount of 350 mm, according to the method of Cosentino *et al.* (2007). Briefly, the irrigation was determined on the basis of the maximum available soil water content in the first 60 cm of soil, where most of the root is expected to grow. Irrigation was applied when the sum of daily evapotranspiration (ETc) corresponded to 69.7 mm. The seasonal irrigation volume of the second and third year (2003/2004 and 2004/2005 growing seasons) was lower than the first year (about 150 mm), because of a prolonged lack of irrigation water.

Weeds have been controlled manually during the establishment year. Pesticides were not used.

Starting from the 2005/2006 growing season, plots were managed without any inputs supply and biomass harvested annually; weed control was no longer needed because of the well and uniform crop establishment. Harvest occurred every February when plants reach the minimum moisture content in these environments. In the present work, harvests of 2008/2009 and 2009/2010 growing seasons are reported.

Crop measurements

During the growing seasons, the main meteorological parameters (maximum, minimum temperature and rainfall) were measured by means of sensors connected to a data logger (CR 10 – Campbell Scient Inc., Logan, UT, USA) located close to the experimental field. At harvest, the following measurements were carried out on six random plants: height of the stem (about 4 cm aboveground to the last node except the inflorescence), number of nodes per stem (n.), basal stem diameter (cm), stem density (plants m^{-2}) and weight of one stem (g). The fresh biomass yield was determined in the centre of the plot (4 m^{-2}) after removing all plants from each plot edge. The moisture content (% w/w) was determined by placing sub-samples of stems and leaves in a ventilated oven dry at 65±5°C until constant weight was reached.

This made it possible to calculate aboveground dry biomass yield (Mg DM ha^{-1}).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using CoHort

Software (CoStat 6.003), according to the experimental layout. A one-way ANOVA for each experimental year was carried out considering the species as *fixed factors*. In case of significance of ANOVA, mean separation was calculated according to Student-Newman-Keuls test at 95% confidence level. Percentage values of moisture content at harvest (% w/w) and structural polysaccharides content (% w/w) were previously arcsin √% transformed.

Analytical methods

Structural carbohydrate content of the biomass harvested in 2009 was calculated in terms of percentage dry weight of the original sample (% w/w), using an improved high-performance anion exchange chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) with pulsed amperometric detection (HPAEC-PAD), according to the method of Davis (1998). Initially, samples were milled to pass a 1.0 mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) and vacuum dried at 45°C. Primary hydrolysis of 40-60 mg subsamples was performed with 1.0 mL 72% (w/w) H_2SO_4 for 1 h at 30°C. Hydrolysates were diluted to 4% (w/w) H_2SO_4 with distilled water, fucose added as an internal standard, and a secondary hydrolysis performed for 1 h at 120°C.

Following filtration through 0.45 μm Teflon syringe filters (National Scientific, Lawrenceville, GA, USA), 5 μL supernatant samples were injected directly onto the chromatographic system with no additional treatment.

Matrix hydrophobic components were removed by in-line solid-phase extraction. Sugar separation was achieved with Carbo-Pac PA1 guard and analytical columns (Dionex) connected in series. Eluent flow rate was 1.2 mL min^{-1} and the temperature was 22°C.

The solids after filtration were dried in an oven at 105°C until constant weight. After recording the dry weight the solid was transferred to a previously weighted crucible, which was allocated in a muffle furnace at 550±50°C for 8 h. The difference of weight was used to calculate the percentage of Klason lignin content. Ash content was measured before and after the two-step acid hydrolysis and referred to whole ash (before hydrolysis) and acid insoluble lignin ash (AL ash), namely the only ash left after the primary and secondary step acid hydrolysis, respectively.

Second-generation bioethanol production

Maximum theoretical ethanol yield (TEY) was calculated according to the following equation (Hettenhaus, 1998):

$$[(C6 \; x \; 1.111) + (C5 \; x \; 1.136)] \; x \; 0.511 \qquad (1)$$

Yields are expressed as weight base (kg ethanol DM Mg^{-1}). The weight yield of pentose from pentosan, as xylan and arabinan, is 1.136 g pentose per g pentosan. This number results from 150/132, the ratio of the molecular weight of pentose per molecular weight of anhydropentoses that make up pentosans. The yield of hexose from glucan, mannan and galactan, is 1.111 g hexose per g hexosan, the molecular weight ratio of 180/162 for hexose and anhydrohexoses, respectively.

The stoichiometric ethanol yield for fermenting microorganisms is 0.511 g ethanol per g of hexose or pentose. By multiplying TEY (kg Mg^{-1}) by the dry biomass yield per hectare (Mg DM ha^{-1}), the TEY (Mg ethanol ha^{-1}) with *Arundo*, *Miscanthus* and *Saccharum* is calculated. It is worth to note that the average biomass DM yield of the harvests 2008/2009 and 2009/2010 has been used for TEY (Mg ethanol ha^{-1}) calculation. TEY is then adjusted by taking into account the bioconversion efficiencies of C6 sugars to ethanol through simultaneous saccharification and fermentation (SSF), and C5 sugars (mainly xylose) to ethanol through fermentation by pentose fermenting yeast, as reported in previous studies with *Saccharum spontaneum* spp. *aegyptiacum* (Scordia *et al.*, 2010), *Arundo donax* (Scordia *et al.*, 2012, 2013b) and *Miscanthus* x *giganteus* (Scordia *et al.*, 2013a). Bioconversion efficiencies of C5 and C6 sugars to ethanol are listed in Table 1.

Results and discussion

Meteorological trend and biomass production

During the 2008/2009 growing season, the monthly minimum temperature increased linearly from 6°C in January to about 19-20°C in July-August to decrease at 4°C in February during the harvest of 2009. The monthly maximum temperature increased from 18°C in January to 34°C in July and August. A similar trend was recorded in the second growing season (2009/2010), however, minimum temperatures were higher than in the previous year during winter time. Slight differences between maximum and minimum temperatures were recorded, approaching 10-14°C during the harvest of 2010.

Rainfalls in 2008/2009 growing season were higher than in the subsequent one (779.8 and 638.6 mm, respectively), mostly in winter time (Figure 1). It is worth to note that rainfalls, in both growing seasons, were higher than in the past thirty-year period in the area (*i.e.*, 550-600 mm yr^{-1}). Furthermore, rainfall distribution was quite large during the vegetative growth of these species; indeed, after a dry period in summer time, rainfall at the end of August, September and October, coupled with favourable temperatures, still sustained the vegetative growth of these perennial grasses until November when flowering was observed and thus biomass accumulation levelled off.

Table 1. Bioconversion efficiency of C5 and C6 sugars to ethanol following hemicellulose hydrolysate fermentation and simultaneous saccharification and fermentation of *Arundo donax* L. (Scordia *et al.*, 2012, 2013b), *Miscanthus x giganteus* Greef et Deu. (Scordia *et al.*, 2013a) and *Saccharum spontaneum* spp. *aegyptiacum* (Willd.) Hack (Scordia *et al.*, 2010).

	Bioconversion efficiency (%)	
	C5	C6
Arundo donax	64	51
Miscanthus x giganteus	75	73
Saccharum spontaneum	69	53

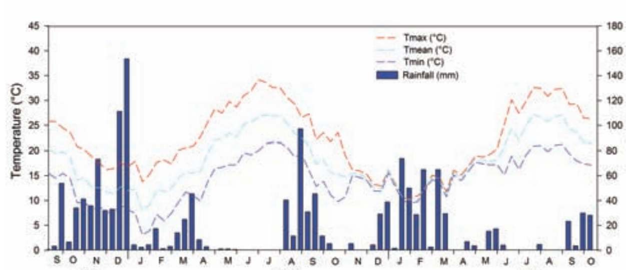

Figure 1. Maximum, minimum, mean temperatures and rainfall during the growing seasons 2008/2009 and 2009/2010 at the Experimental Farm of Catania University (10 m asl, 37°25' N lat., 15°03' E long.).

Biometric characters of the two harvests are shown in Table 2. *Arundo* showed the tallest stem height in both years, followed by *Saccharum* and *Miscanthus*, statistically different from each other. Consequently, the number of nodes per stem was significantly different between the species: higher in *Arundo* than *Saccharum* and *Miscanthus*. The same trend was recorded for the basal stem diameter, with *Arundo* thicker than *Saccharum* and *Miscanthus*.

An opposite trend was seen in the stem density per square meter. Indeed, this character was significantly higher in *Miscanthus* followed by *Saccharum*, while *Arundo* showed the lowest statistically significant value.

The weight of a single stem was higher in *Arundo* than in *Saccharum* and *Miscanthus*, which were statistically different from each other.

The moisture percentage at harvest was significantly higher in *Saccharum* followed by *Arundo*. *Miscanthus* showed the lowest statistically significant value, however, proved to have a higher quality in terms of thermochemical conversion (*e.g.*, combustion), since it is strictly related to logistics, affecting transportation, storage, handling and plant efficiency as well.

The higher moisture content detected in *Arundo* and *Saccharum* may be explained by the fact that they are naturalized and well adapted to Southern Mediterranean environments, can maintain gas exchange activities with the atmosphere even in early winter when the climatic conditions are favourable. Vice versa, *Miscanthus*, native from a tropical area and adapted to live in dry cold temperate environments, showed senesced stems and leaves in winter time. For this reason, stem water content is about 15% in *Miscanthus* and more than 35% in the other two species, as well as leaves at the top canopy are still green in *Saccharum* and *Arundo,* while completely dry in *Miscanthus.*

Indeed, due to leaf senescence and losses, *Miscanthus* showed the lowest amount of leaves at harvest (8.0%) and consequently the highest stem content (92.0%). *Arundo* and *Saccharum* showed no differences (84.0% stems and 16.0% leaves) (data not shown).

Fresh aboveground biomass yield resulted significantly higher in *Arundo* in both years (53.1±4.0 and 52.1±3.8 Mg ha^{-1} in 2009 and 2010 harvest, respectively) than in *Saccharum* (44.8±1.5 and 42.3±3.0 Mg ha^{-1}, respectively) and *Miscanthus* (19.4±4.0 and 22.3±5.1 Mg ha^{-1}, respectively), as shown in Figure 2. Accordingly, the aboveground dry matter (DM) yield was highest in *Arundo*, with 35.4±2.1 Mg ha^{-1} in 2009 and 32.2±1.9 Mg ha^{-1} in 2010. *Saccharum* yielded 27.3±2.0 and 23.9±1.9 Mg ha^{-1} in 2009 and 2010 harvest, respectively, while *Miscanthus* 19.6±2.8 and 17.2±1.6 Mg ha^{-1}, respectively.

Owing to the absence of agronomic input since the 2005/2006 growing season, biomass DM yield might be considered higher than what expected for these crops in this environment. The high rainfalls during the two growing seasons (779.8 and 638.6 mm, respectively), higher than what generally observed in the last decade in same area (~550 mm) and most importantly rainfall distribution (very large during vegetative growth), might have boosted biomass accumulation beyond actual yields in rain-fed conditions.

Our findings are in agreement with Mantineo *et al.* (2009), who reported similar values in a five-year study with *Arundo* and *Miscanthus* in a semi-arid Mediterranean area (from 22.2 to 43.0 Mg ha^{-1} with *Arundo* and from 11.0 to 30.6 Mg ha^{-1} with *Miscanthus*), however, nitrogen fertilisation (50 and 100 kg ha^{-1}, respectively) and maximum evapotranspiration restitution (25 and 75%, respectively) were supplied to the crop. In the fourth and fifth year of that study the crops did not receive any input, however, *Arundo* still maintained a high productivity level in both harvests (34.9 and 27.0 Mg ha^{-1}, respectively), while *Miscanthus* started to be more affected (27.0 Mg ha^{-1} at the fourth and 18.2 Mg ha^{-1} at the fifth year). Thus, our results on biomass DM yield are quite comparable to those reported by Mantineo *et al.* (2009) in a similar cultivation area.

In a more northern Mediterranean environment (North Italy), long- and mid-term studies reported DM yields with giant reed of 37 and 20 Mg ha^{-1} yr^{-1} in productive and marginal soil respectively (Angelini *et al.*, 2009; Nassi o Di Nasso *et al.*, 2013).

Angelini *et al.* (2009) suggested two yielding phases in giant reed: a maturity phase from the 3rd to the 8th year of growth, with a mean value around 45 Mg ha^{-1} yr^{-1}, and a decreasing phase from the 9th to the 12th year of growth, with a mean value about 25 Mg ha^{-1} yr^{-1}.

In addition, a growth analysis performed by Nassi o Di Nasso *et al.* (2011) on giant reed and *Miscanthus* crop at the 7th and 8th year of

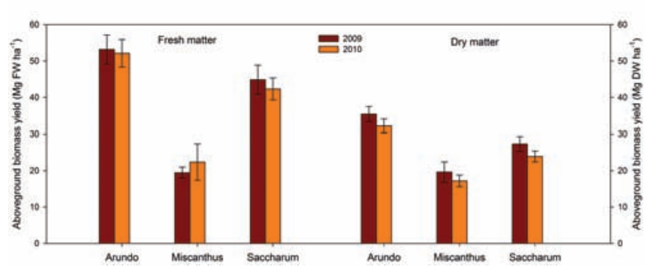

Figure 2. Aboveground fresh and dry matter yield (Mg ha^{-1}) of *Arundo donax* L., *Miscanthus x giganteus* Greef et Deu. and *Saccharum spontaneum* spp. *aegyptiacum* (Willd.) Hack. in two subsequent growing seasons ± standard deviation.

Table 2. Biometric parameters according to first (2009) and second (2010) year harvest of *Arundo donax* L., *Miscanthus x giganteus* Greef et Deu. and *Saccharum spontaneum* spp. *aegyptiacum* (Willd.) Hack.

Year		2009			2010	
Parameter/species	*Arundo*	*Miscanthus*	*Saccharum*	*Arundo*	*Miscanthus*	*Saccharum*
Stem height (cm)	317.2[a]	136.1[c]	242.5[b]	384.8[a]	132.8[c]	294.1[b]
Node number (n.)	45.0[a]	11.0[c]	13.3[b]	49.0[a]	11.4[c]	16.3[b]
Basal diameter (mm)	1.5[a]	0.8[c]	1.1[b]	1.9[a]	0.6[c]	1.3[b]
Stem density (n. m^{-2})	31.2[c]	161.2[a]	59.1[b]	50.0[c]	140.8[a]	79.9[b]
Weight one stem (g)	77.8[a]	13.4[c]	31.2[b]	89.4[a]	10.8[c]	42.6[b]
Moisture content (%)	33.4[b]	11.6[c]	39.1[a]	38.2[b]	12.3[c]	43.5[a]

[a,b,c]Within each experimental year (2009 and 2010), different letters in the same row indicate significance (P≤0.05). Percentage values were previously arcsin √% transformed.

growth showed stable yields, in an environment where water availability, temperature and solar radiation were not limiting factors, with maximum at 30 and 40 Mg ha^{-1}, respectively. On the other hand, Angelini *et al.* (2009) reported an average yield of 28.7 Mg ha^{-1} yr^{-1} and 37.7 Mg ha^{-1} yr^{-1} DM for *Miscanthus* and giant reed, respectively, in a 12-year field trial without irrigation.

Only few studies are available for *Saccharum spontaneum* L. spp. *aegyptiacum* (Willd.) Hack. Cosentino *et al.* (2012a), in semi-arid Mediterranean area, reported 9.6 Mg ha^{-1} at the first and 17.9 Mg ha^{-1} DM at the second year after the establishment under rain-fed conditions, while higher than 30.0 Mg ha^{-1} DM when 50% or 100% ETm restitution was applied in an older *Saccharum* stand (Cosentino *et al.*, 2012b). According to Angelini *et al.* (2009), aboveground biomass DM yield of the three species was positively correlated to some biometric characters, such as stem height, basal stem diameter and weight of one stem. Literature results, as well as results from the present study, allow to point out the potential of these species in the Mediterranean environment, where temperatures and solar radiation are optimum for growth development and yields, while water availability strictly affect biomass yields, allowing to achieve high levels when abundant and well distributed throughout the growing season.

Biomass quality

Structural polysaccharides (% w/w) of the raw material were higher in *Miscanthus* (63.4%) followed by *Saccharum* (61.5%) and *Arundo* (57.6%), as shown in Table 3. Cellulose, made up exclusively by glucans, had the greatest impact on the total dry weight. The content in glucans was significantly higher in *Miscanthus* than *Saccharum*, which in turn was significantly higher compared to *Arundo* (41.0%, 36.8% and 34.6%, respectively). Significantly highest values in the total hemicellulose complex were observed in *Saccharum* (24.7%), followed by *Arundo* (23.1%) and *Miscanthus* (22.4%). The greater proportion of hemicellulose is represented by xylans (20.4% in *Arundo*, 19.9% in *Miscanthus* and 21.5% in *Saccharum*), while arabinans exceed 2.0% only in *Saccharum*. Galactans, mannans and rhamnans were detected in small amounts in the three species (<1.0%).

Hemicellulose composition confirmed the intrinsic chemical composition of these monocot species, since arabinoxylans have been identified as the main hemicelluloses in other monocots residues as corn stover, wheat, barley, oat, rice and sorghum (Ebringerova and Heinze, 2000). Acid insoluble lignin, for the three crops, is within the range reported for other herbaceous species. *Miscanthus* showed the highest statistically significant value (22.4%), while in *Saccharum* and *Arundo* no significant differences were observed (20.0 and 20.4%, respectively). The ash content of both whole raw material and acid insoluble lignin (AL ash) were significantly higher in *Arundo* (7.20 and 1.7%, respectively), followed by *Saccharum* (5.4 and 1.2%, respectively) and *Miscanthus* (4.8 and 0.8%, respectively). The polysaccharide content can be used to indicate initially the potential of these grasses, whether they are suitable for the application as energy crops for second-generation bioethanol production. Hence, the determination of polysaccharides can be applied to quantify the theoretical production of ethanol from *Arundo*, *Saccharum* and *Miscanthus* species.

Second-generation ethanol production

The theoretical ethanol yield (TEY) from one DM ton (kg ethanol DM Mg^{-1}) of the three perennial grasses is shown in Figure 3.

Arundo TEY was 196.0 kg of ethanol from glucose, 3.7 kg from galactose and 0.7 kg from mannose, corresponding to 200.5 kg on the whole C6 sugars. TEY from xylose amounted to 118.2 kg and 10.5 kg from arabinose, for an overall production of 128.7 kg from C5 sugars. Summing up the ethanol from C6 and C5, 329.2 kg of ethanol can be obtained from one DM ton of *Arundo donax*.

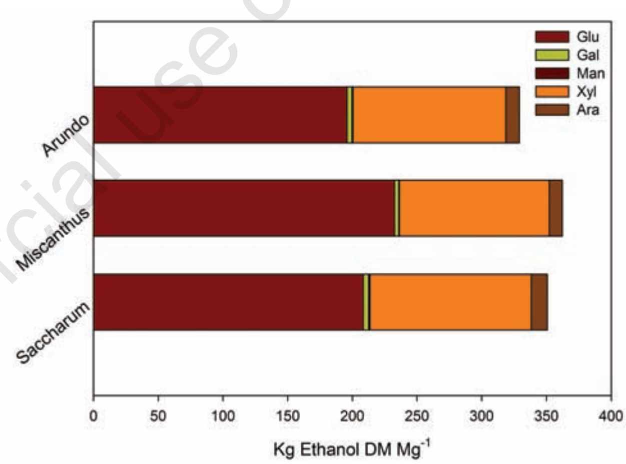

Figure 3. Theoretical ethanol yield (kg Mg^{-1}) from one dry ton of *Arundo donax* L., *Miscanthus* x *giganteus* Greef et Deu. and *Saccharum spontaneum* spp. *aegyptiacum* (Willd.) Hack.

Table 3. Structural polysaccharides content (% w/w) of the raw material of *Arundo donax* L., *Miscanthus x giganteus* Greef et Deu. and *Saccharum spontaneum* ssp. *aegyptiacum* (Willd.) Hack.

Composition	*Arundo* (% w/w)	*Miscanthus* (% w/w)	*Saccharum* (% w/w)
Glucan	34.60^{c}	40.99^{a}	36.81^{b}
Xylan	20.41^{b}	19.98^{c}	21.53^{a}
Galactan	0.66^{a}	0.57^{a}	0.72^{a}
Arabinan	1.81^{b}	1.74^{b}	2.16^{a}
Mannan	0.12^{a}	0.09^{a}	0.16^{a}
Rhamnan	0.06^{b}	0.02^{c}	0.14^{a}
Total polysaccharides	57.66^{c}	63.39^{a}	61.52^{b}
K. Lignin	20.44^{b}	22.40^{a}	20.03^{b}
Whole Ash	7.20^{a}	4.80^{c}	5.40^{b}
AL Ash	1.67^{a}	0.84^{c}	1.21^{b}

[a,b,c]Different letters in the same row indicate significance ($P \leq 0.05$). Percentage values were previously arcsin $\sqrt{\%}$ transformed.

The TEY from hexoses and pentoses of *Miscanthus* was equal to 236.0 and 125.8 kg respectively, for a total amount of 361.8 kg DM Mg^{-1}. The total TEY from one DM ton of *Saccharum* amounted to 350.8 kg, partitioned as 213.6 kg from C6 and 137.2 kg from C5 sugars.

These results indicate that *Saccharum*, *Arundo* and *Miscanthus* biomass is comparable, in its carbohydrates composition of the raw material and consequently to the TEY, to other substrates used in the lignocellulosic-to-ethanol technology, such as wood (eucalyptus, poplar and willow), herbaceous agricultural residues (corn stover, corn cobs, wheat straw, rice straw and sugarcane bagasse) and herbaceous perennial species (switchgrass), making these perennial grasses suitable feedstock for second-generation bioethanol production (Table 4).

Although some agricultural residues theoretically overyield *Arundo*, *Miscanthus* and *Saccharum*, the yield potential of a species or residue should be referred to a unit land, namely the hectare.

Irrespective of the environment and management cultivation practices used, results from literature indicate that biomass yields of agricultural residues, such as corn cobs and corn stover, range from 0.45 to 1.75 Mg DM ha^{-1} and 5.2 to 13.2 Mg DM ha^{-1}, respectively (Kim and Dale, 2004; Lorenz *et al.*, 2009; Dobermann *et al.*, 2002), or 1.9 to 7.0 Mg DM ha^{-1} and 3.5 to 6.0 Mg DM ha^{-1} of wheat straw and rice straw, respectively (Mckendry, 2002; Kim and Dale, 2004; Nemeikšien *et al.*, 2011; Naresh, 2013) and from 11.0 to 22.9 Mg DM ha^{-1} of sugarcane bagasse (Kim and Dale, 2004; van der Weijde *et al.*, 2013). Higher yields are reported for dedicated species for biomass production, such as the woody willow (8.2-15.0 Mg DM ha^{-1}), poplar (10.7-15.0 Mg DM ha^{-1}), eucalyptus (15.0-20.0 Mg DM ha^{-1}) (Venendaal *et al.*, 1997; Kauter *et al.*, 2003; Rettenmaier *et al.*, 2010; Cosentino *et al.*, 2012c), or the herbaceous perennial switchgrass (5.0-20.0 Mg DM ha^{-1}) (Elbersen *et al.*, 2013; Lewandowski *et al.*, 2003).

Hence, according to the biomass DM yield achieved in this study, *Arundo*, *Miscanthus* and *Saccharum* showed a great TEY potentiality as compared to the other lignocellulosic feedstock analysed (Figure 4).

Looking at the literature data, *Arundo* and *Miscanthus* (11.21±5.46 Mg ha^{-1} and 8.86±6.01 Mg ha^{-1}) performed better than switchgrass (4.58±3.06 Mg ha^{-1}) as far as herbaceous perennials are concerned. *Eucalyptus* was the best feedstock within the woody species analysed (6.29±0.90 Mg ha^{-1}), while among the agricultural residues the lowest TEY was observed in the corncob (0.52±0.48 Mg ha^{-1}) and the highest in sugarcane bagasse (6.87±2.41 Mg ha^{-1}).

Our data on TEY with *Miscanthus* and *Arundo* (6.66±0.54 Mg ha^{-1} and 11.13±0.86 Mg ha^{-1}) are within the range reported by the literature data however the great variability observed in the literature suggests that very high or very low yields have been measured worldwide. Further studies are needed to assess the real potential of these grasses for biomass production in different cultivation areas.

Saccharum TEY was 8.98±0.63 Mg ha^{-1}, showing an intermediate value between *Miscanthus* and *Arundo*.

It is worth nothing that values reported in Figure 4 are purely theoretical and do not take into account the efficiency of the bioconversion process.

Second-generation process comprises several steps including bottlenecks and therefore loss of efficiency. It consists of a i) pre-treatment step to remove hemicelluloses, disrupt or rearrange lignin structure

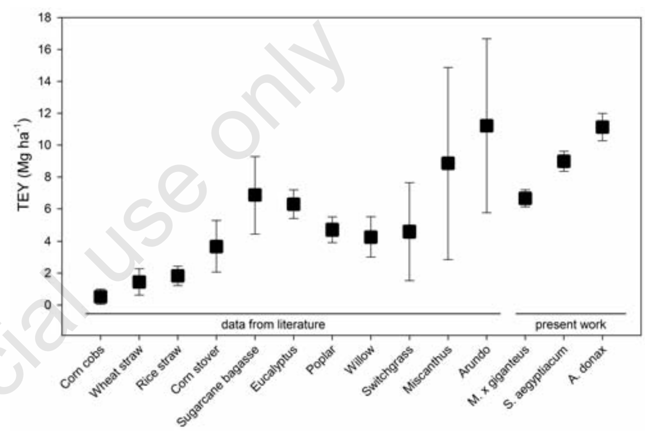

Figure 4. Theoretical ethanol yield (TEY, Mg ha^{-1}) from one hectare with *Arundo donax* L., *Miscanthus* x *giganteus* Greef et Deu. and *Saccharum spontaneum* spp. aegyptiacum (Willd.) Hack., agricultural residues, woody species and perennial herbaceous grasses (including *Arundo donax* and *Miscanthus x giganteus* from literature). Square symbol represents mean value, while vertical error bars the lowest and highest value, respectively.

Table 4. Structural carbohydrate composition and theoretical ethanol yield of the most studied lignocellulosic feedstock for second generation bioethanol production.

Feedstock	Composition (% w/w)								
	Glucan	Xylan	Arabinan	Mannan	Galactan	Lignin	Ash	TEY (kg DM Mg^{-1})	Reference
Corn stover	40.0	21.0	5.0	NR	2.0	23.0	3.5	398.8	Sassner *et al*., 2008
Wheat straw	32.6	20.1	3.3	NR	0.8	26.5	4.6	324.8	Linde *et al*., 2008
Rice straw	41.7	20.7	3.3	0.5	1.2	15.3	11.0	384.9	Wi *et al*., 2013
Sugarcane bagasse	43.0	26.0	1.5	NR	0.4	24.6	NR	405.2	Rudolf *et al*., 2008
Corn cobs	37.0	27.8	2.19	NR	NR	13.9	NR	383.4	Lee *et al*., 2011
Eucalyptus	44.4	17.5	1.1	NR	NR	27.7	0.2	359.2	Romaní *et al*., 2010
Poplar	43.8	14.9	0.6	3.9	1.0	29.1	1.1	365.7	Wyman *et al*., 2009
Willow	42.5	15.0	1.5	3.0	2.5	26.0	2.0	367.6	Sassner *et al*., 2008
Swithgrass	32.0	17.9	1.9	NR	1.7	21.4	3.8	305.7	Xu *et al*., 2010
A. donax	34.60	20.41	1.81	0.12	0.66	20.44	7.20	329.2	Present study
M. x giganteus	40.99	19.98	1.74	0.09	0.57	22.40	4.80	361.8	Present study
S. spontaneum	36.81	21.53	2.16	0.16	0.72	20.03	5.40	350.8	Present study

TEY, theoretical ethanol yield; NR, not reported.

Table 5. Ethanol production from one hectare of *Arundo donax* L., *Miscanthus* x *giganteus* Greef et Deu. and *Saccharum spontaneum* ssp. aegyptiacum (Willd.) Hackel.

	Biomass yield* ($Mg\ ha^{-1}$)	TEY C5 ($Mg\ ha^{-1}$)	TEY C6 ($Mg\ ha^{-1}$)	Bioconversion C5 (%)	Bioconversion C6 (%)	EY C5 ($Mg\ ha^{-1}$)	EY C6 ($Mg\ ha^{-1}$)	Total EY ($Mg\ ha^{-1}$)	Total EY ($L\ ha^{-1}$)
A. donax	33.8	4.35	6.78	64	51	2.78	3.46	6.24	7908
M. x giganteus	18.4	2.31	4.34	75	73	1.73	3.17	4.90	6210
S. spontaneum	25.6	3.51	5.74	69	53	2.42	3.05	5.47	6933

*Biomass yield as average of two year harvests (2009 and 2010). TEY, theoretical ethanol yield; EY, ethanol yield.

and make cellulose more available for ii) enzymatic hydrolysis by cellulase/β-glucosidase to free sugars and iii) ferment the free sugars to ethanol. All those steps need to be optimized to achieve maximum yields and/or lower energy consumption. Various methods of pre-treatment can be used, including mechanical, steam explosion, ammonia fibre explosion, alkali, sulphite and dilute acid, either inorganic or organic (Mosier *et al.*, 2005; Zhu *et al.*, 2010) with different degree of strength and weakness (Chandel and Singh, 2011). Enzymatic hydrolysis carried out by enzyme complexes known as cellulases are involved in cellulose digestibility after the pre-treatment enhancing glucose yield, even though end products as cellobiose and glucose at high concentrations act as inhibitors (Philippidis *et al.*, 1993). One of the most successful methods to improve enzymatic hydrolysis was the SSF. In this process, glucose produced by the hydrolysing enzymes is consumed immediately by fermenting microorganisms present in the media, minimizing the inhibitory effect of cellobiose and glucose and increasing ethanol yields (Eklund *et al.*, 1995).

Recent bioconversion studies carried out with *Saccharum spontaneum* spp. *aegyptiacum* (Scordia *et al.*, 2010), *Arundo donax* (Scordia *et al.*, 2012, 2013b) and *Miscanthus* x *giganteus* (Scordia *et al.*, 2013a), using a pre-treatment with oxalic acid, the SSF of cellulose and the fermentation of hemicellulose hydrolysate by C5 and C6 fermenting yeasts (*Scheffersomyces stipitis* CBS 6054), have highlighted that bioconversion yields obtained, with respect to the maximum theoretical, are from 51% to 75%, as shown in Table 1.

Thereby, TEY reduced in all species and amounted to 6.24 Mg ethanol ha^{-1} with *Arundo*, 4.90 Mg ha^{-1} with *Miscanthus* and 5.46 Mg ha^{-1} with *Saccharum* (Table 5). By taking into account the ethanol density (0.789 g cm^{-3}), 7908 L ethanol ha^{-1} are achieved with *Arundo*, 6210 L ha^{-1} with *Miscanthus* and 6933 L ha^{-1} with *Saccharum*.

Conclusions

The choice of a species for a particular location depends on factors such as geographical and climate conditions, amount of rainfall and distribution, annual temperature profile, soil conditions and bioconversion technology adopted.

A sustainable cultivation system with perennial species requires a high level of annual biomass yield with minimum energy input supply. Hence, the three crops seemed particularly suited to the semi-arid Mediterranean area, giving high yield with minimum or no energy input. In particular, *Arundo* and *Saccharum* performed better than *Miscanthus* in these conditions, since they are naturalized and well adapted to the climatic conditions of this environmental zone.

Present results indicate that *Arundo, Saccharum* and *Miscanthus* biomass is comparable, in its carbohydrate composition of the raw material, and consequently to the TEY, to other lignocellulosic sources used in the second-generation bioethanol technology.

The overall TEY (Mg ethanol ha^{-1}) strengthened the hypothesis of the great potential of *Arundo, Saccharum* and *Miscanthus* over agricultural residues, woody species and herbaceous perennial crops employed worldwide.

Owing to the productive traits with minimum energy input, we could also speculate on the environmental benefits of their cultivation management; thus, the three species might be easily introduced into the Mediterranean cropping systems in order to supply lignocellulosic biomass for second-generation industrial plants or biorefineries.

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