PRELIMINARY EVALUATION OF MACROPHYTE WETLAND BIOMASSES TO OBTAIN SECOND GENERATION ETHANOL

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ABSTRACT: The paper reports the preliminary analysis of a research in progress, which is studying different agronomic criteria and laboratory processing in order to find new macrophyte wetland plants able to give high biomass productivity and grow under irrigation with wastewater. The agronomic experiments took place in Italy and the following 19 species were considered: *Acorus calamus* L., *Arundo donax* L., *Canna indica* L., *Carex elata* All., *Cyperus longus* L., *Cyperus papyrus* L., *Glyceria maxima* (Hartm.) Holmb., *Iris pseudacorus* L., *Juncus effusus* L., *Lythrum salicaria* L., *Miscanthus x Giganteus* Greef et Deu., *Phalaris arundinacea* L., *Phragmites australis* (Trin.) Cav., *Scirpus sylvaticus* L., *Sorghum bicolor* (L.) Moench, *Symphytum officinale asperrimum* L., *Thalia dealbata* Fraser ex Roscoe, *Typha latifolia* L., *Vetiveria zizanoides* (L.) Nash. After the harvest each species was characterized in terms of fiber (hemicellulose, cellulose and lignin) and elements (C, N and S) in order to identify their potential ethanol productivity and select the ideal species for the field trials in northern and southern Italy.
Keywords: wastewater treatment, biomass, energy crops.

1 INTRODUCTION

Wastewater management is currently a central topic in agronomic and environmental systems. Several treatments have been introduced to control this problem and a wetland system is one of them.

In addition, growing herbaceous biomasses for energy production may represent an interesting prospect for the development of marginal areas and reuse of poor quality waters [1]. At the same time the gradual depletion of petroleum-derived transportation fuels has focused attention on both renewable and environmentally friendly resources [2], with ethanol from plants being a possible alternative [3]. At present corn and sugarcane are the major crops for world ethanol production [4-5]. But to combine all the above-mentioned positive aspects it is necessary to identify new species, i.e. plants able to grow with a huge quantity of sewage quality water and to produce high biomass. They should also be vigorous and perennial to minimize the yearly costs of sowing and soil tillage.

The aim of this research is to study potential new macrophyte wetland plants in order to increase the possibility of wastewater reuse and create an alternative renewable energy chain that is sustainable and economic.

2 METHODS

2.1 Project description

This experimentation started in November 2009 and is carried out by three Italian research groups:

- Department of Environmental Agronomy and Crop Production, University of Padua, with the field experiment in Veneto Region (northern Italy).
- Department of Agricultural Engineering, Hydraulics Division, University of Catania, with the field experiment in Sicily (southern Italy).
- The Biotechnical Laboratory Trisaia, Italian national agency for new technologies, energy and sustainable economic development, with the laboratory analyses.

Initially, the studies focused on the search for appropriate macrophytes, according to their potential productivity and adaptability to environmental conditions.

In both universities preliminary tests were done in 2009 on the following species: *A. calamus*, *A. donax*, *C. indica*, *C. elata*, *C. longus*, *C. papyrus*, *G. maxima*, *I. pseudacorus*, *J. effusus*, *L. salicaria*, *M. x Giganteus*, *Ph. arundinacea*, *Ph. australis*, *S. sylvaticus*, *S. bicolor*, *S. officinale asperrimum*, *T. dealbata*, *T. latifolia* and *V. zizanoides*.

After their analyses and the study of their potential ethanol productivity (cf. 3.1), all both experimental field research groups selected the final species for their own project and concentrated on organizing and managing the field experiments (cf. 2.1), which started in the summer of 2010. Plants and devices were monitored during the experiments and in November 2010 dried samples of all species from both sites were sent to the biotechnical laboratory for processing with pretreatment and fermentation to analyze their ethanol productivity.

2.2 Field experiments

2.2.1 The Veneto Case

The study was conducted at the experimental farm of Padua University, Faculty of Agricultural Science. In June 2010 the following species were planted: *A. donax*, *C. indica*, *Carex pseudocyperus* L., *Carex riparia* Curtis, *Cladium mariscus* (L.) Pohl., *I. pseudacorus*, *M. x Giganteus*, *S. sylvaticus*, *S. officinale asperrimum*. Some of these species were selected from preliminary tests on the 19 macrophytes, others were chosen on the basis of their potential productivity and adaptability to environmental conditions.

The plants were cultivated in growth boxes (2x2 m sided) installed with the top at 1.3 m above the field level and the open. In June 2010 they were also supplied with simulated slurry (being equivalent to 400 kg N/ha).

Controlled irrigations were applied from July to September (corresponding to 40 mm of water, supplied twice weekly) and samples of percolated water and humidity observations were taken regularly throughout the experiment. At harvest time (3rd November 2010), samples of each species were collected and dried in an oven at 65 °C in order to obtain the dry weight.

2.2.2 The Sicily Case

The research was conducted in a full-scale constructed wetland treatment plant and in an open field of *A. donax*, *M. x Giganteus*, and *V. zizanoides* located in San Michele di Ganzaria, a rural community of about 5,000 inhabitants in Sicily. The constructed wetland consists of two Horizontal SubSurface Flow beds (H-SSF1 and H-SSF2) that receive part of secondary effluent (4 L/s) of the conventional wastewater treatment plant of the village [6]. H-SSF1 and H-SSF2 have an almost equal surface area (about 2,000 m^2) but with different operational life: 10 and 4 years. *Ph. australis* was used as vegetation in both beds. Wastewater treated by constructed wetlands has been used for irrigation of crops. For this purpose, an experimental irrigation field of *A. donax*, *M. x Giganteus*, and *V. zizanoides* was established.

A randomized block design was adopted, with three repetitions for each plot of 9 m_2. Plants of *V. zizanoides* (4 plants/m_2) and *M. x Giganteus* (4 plants/ m_2) were planted in July 2008 and May 2009, respectively. In adjacent two blocks of about 500 m_2 each, *A. donax* (4 plants/m2) was planted in July 2008. The wastewaters were supplied by in-line labyrinth drippers system. A meteorological station was installed for the continuous measurement of: rainfall, temperature, air moisture content, wind speed, solar radiation and evaporation. The water volumes distributed were equal to 33%, 66% and 100% of the evapotranspiration losses calculated with the Penman-Monteith formula [7], implemented with the data taken from the meteorological station. More specifically, three irrigation levels (33%, 66% and 100% ET) were applied in vetiveria and myscantus plots and two irrigation levels (66% and 100% ET) in arundo plots. The irrigations were applied from May to October 2010.

Physical, chemical and microbiological analyses were conducted on wastewater samples collected before and after both CW. Percentage removal efficiencies of CW were computed for each parameter evaluated. Bio-agronomic analyses on tested species were done to evaluate the main parameters such as dimension, growth dynamics and productivity. In particular, plant samples for the evaluation of productivity were taken in November 2010. Biomass dry weight was determined by drying plant tissue samples in a thermo-ventilated oven at 65 °C until constant weight was reached.

2.3 Selected species

This section describes all the species cultivated in both field experiments in 2010.

2.3.1 *Arundo donax* L.

A. donax belongs to the subfamily Arundinoideae of the Gramineae family [10-11] and is the only wide-ranging species within the Arundo genus [12]. *A. donax* is thought to have originated from Asia and spread into the Mediterranean area without traces of hybridization with the other *Arundo* species [13]. Because of its widespread distribution and multiple uses, it has been given several common names, while the scientific community has adopted the common name of ‘giant reed’.

A. donax is a tall, perennial C3 grass and is one of the largest of the herbaceous grasses. The rhizomes form compact masses from which tough shoots arise that penetrate into the soil and usually lie close to the soil surface (5–15 cm deep, maximum 50 cm), while the roots are more than 100 cm long.

Giant reed grows in dense clumps; the stems can reach a height of up to 8–9 m, with growth rates of 0.3–0.7 m per week over a period of several months during the vegetative stage when conditions are favorable [15]. It tolerates a wide variety of ecological conditions and prefers well-drained soils with abundant soil moisture.

The potential productivity of giant reed can reach up to 100 t/ha/yr fresh matter in the second or third growing period under optimal conditions in a warm climate with sufficient irrigation [14-15]. Yields reported in Spain showed 45.9 t/ha DM on average, ranging from 29.6 to 63.1 t/ha [16].

2.3.2 *Canna indica* L.

C. indica is commonly called "Indian shot" and belongs to the Cannaceae family. *C. indica* is a native of tropical America and is a very popular ornamental and medicinal plant. Indian shot is a robust perennial herb, the ramifications that grow from a thick, branching, underground rhizome can be up to 1-3 m in height and form a compact mass, being enveloped by sheaths of leaves. The leaves are large, green or purplish green, with short petioles and elliptic plates, can measure from 30 to 60 cm long and 10 to 25 cm wide, with wide base and narrowing at the wedge and the apex is short sharp and acute. Inflorescence is in terminal clusters with 6-20 groups of 1-2 flowers. The fruits are ellipsoid capsules that are globose, warty, 1.5 to 3 cm in length, chestnut color, with large amounts of black and very hard seeds. Indian shot can be grown from sea level to 900 meters, but thrives in mountainous tropical or subtropical temperate climate, between 1,000 and 2,000 meters. It likes average temperatures from 14 to 27 °C and minimum annual rainfall of 500 mm to 1,200 mm. It grows very well in light soils.

2.3.3 *Carex pseudocyperus* L. and *Carex riparia* Curtis

Carex is one of the largest plant genera, including more than 3000 species, a great many undoubtedly synonyms.

Besides the high number of species, the taxonomy of *Carex* is difficult due to the morphological reduction encountered in the shoot and inflorescence. In many sections, hybridization between species is a common phenomenon, and troublesome hybrid swarms exist.

Many species also have a very wide area of distribution (e.g. circumpolar *C. rostrata, C. aquatilis* and *C. nigra),* and within them many subspecies or varieties are recognized. These aspects make it difficult to evaluate the ecological and phytosociological data of the same taxa from various areas.

Although taxonomically cumbersome, the genus *Carex* is important for wetland scientists. For example, there are 90 Finnish species, 70 (78%) of which occur mainly in wetland sites. Of the 70, 31 are plants of mires (peatlands), 11 are typical of lake shores and alluvial sites, 7 of marine shores, 16 of wet meadows and heaths, and 5 are frequent in various kinds of wetlands. Similar figures could be given for many other areas, especially boreal regions. In more southern regions, the proportion of wetland species in the genus is still high, but Carices of deciduous forests or praries are also important in the flora. *Carex* flowers are unisexual and pollination is effected by wind.

2.3.4 *Claudium mariscus* (L.) Pohl.

Sawsedge, or sawgrass, *C. mariscus* is a robust perennial evergreen herbaceous plant belonging to the Cyperaceae family that can reach up to 2.5 m in height. It grows in wetland environments in shallow ponds, on the shores of lakes, lagoons and water channels, and in the surrounding wet grasslands. It is a cosmopolitan taxon with its main distribution area in Europe and the Mediterranean. The plant has a much branched rhizome, with runners that start from aerial organs formed by the stems, the long leaves are triangular and sharp edged. The hermaphrodite flowers are grouped in a panicle inflorescence, the flowering season is between May and September. The fruit is an acorn. The leaves and stem, even if dried, are very resistant.

2.3.5 *Iris pseudacorus* L.

I. pseudacorus is a species of Iris (Iridaceae family) that is native to Europe, western Asia and northwest Africa. Common names include “yellow iris” and “yellow flag”. It is a perennial herbaceous plant growing to 1–1.5 m tall, with robust rhizomes and erect leaves up to 90 cm long and 3 cm broad. The flowers are bright yellow, 7–10 cm across, with the typical iris form. The fruit is a dry capsule 4–7 cm long, containing numerous pale brown seeds. Plants can be grown in quite coarse grass, which can be cut annually in the autumn. *I. pseudacorus* grows best in very wet conditions and is often common in wetlands, where it tolerates submersion, low pH, and anoxic soils. Rhizomes have air spaces in the cellular tissues (lacunae) that facilitate survival in low oxygen conditions characteristic of flooding. The plant spreads quickly, by both rhizome and water dispersed seed. It fills a niche similar to that of *Typha* and often grows with it, though usually in less deep water. While it is primarily an aquatic plant, the rhizomes can survive prolonged dry conditions [17] *I. pseudacorus* has been used primarily as an ornamental plant in water gardens, but has also been widely planted for erosion control and in sewage treatment ponds [18] moreover yellow flag does not provide food for native animals and contains large amounts of glycosides that are poisonous to grazing animals [19]. The highest total both above-ground and below-ground biomass reached was 1.6841 kg/m^2, in planted microcosm units [20].

2.3.6 *Miscanthus x Giganteus* Greef et Deu

The genetic origin of Miscanthus is in East-Asia, where it is found throughout a wide climatic range from tropical, subtropical and warm temperate parts of Southeast Asia to the Pacific Islands [21]. The genotype widely used in Europe for productivity trials, *Miscanthus × giganteus*, was introduced from Japan to Denmark in 1930.

As a consequence of its triploidy, *M. × giganteus* is sterile and cannot form fertile seeds [22]. Miscanthus is self-incompatible. The two species that are of interest for breeding genotypes for bioenergy are *M. sacchariflorus* and *M. sinensis*. Miscanthus is a C_4 grass with high radiation and water use efficiencies [23].

The canopy of *Miscanthus × giganteus* can reach a height of 4 m. It is highly persistent; the estimated lifetime of a plantation is 20–25 years. It can be grown on a wide range of soils. The most important soil characteristic is the water holding capacity. Sites with stagnant water are unsuitable. The highest yields are produced on soils with a good water holding capacity. *M. × giganteus* begins growth from the dormant winter rhizome when soil temperatures reach 10–12 °C [24].

Most yields reported for miscanthus in Europe have been assessed using the ‘standard’ genotype *Miscanthus × giganteus*. The stands need 3–5 years to become fully established and reach the maximum yield. Yields above 30 t/ha DM are reported for locations in southern Europe with high annual incident global radiation and high average temperatures but only with irrigation. In central and northern Europe (from Austria to Denmark) where global radiation and average temperatures are lower, yields without irrigation are more typically 10–25 t/ha DM [25].

2.3.7 *Scirpus sylvaticus* L.

The plant genus Scirpus consists of a large number of aquatic, grass-like species in the family Cyperaceae, many with the common names club-rush or bulrush or grassweed.

The genus has a cosmopolitan distribution, and grows in wetlands and moist soil. Some species specialize in saline, marshy, environments such as intertidal mud-flats; others prefer ponds or lake-sides and river-beds. They have grass-like leaves, and clusters of small spikelets, often brown in colour. Some species (e.g. *S. lacustris*) can reach a height of 3 m, while others (e.g. *S. supinus*) are much smaller, only reaching 20–30 cm tall.

2.3.8 *Symphytum officinale asperrimum* L.

S. officinale asperrimum (Boraginaceae) is a perennial herb known as comfrey, gum plant or boneset, and is employed topically as anti-inflammatory, emollient and mild anesthetic in phytotherapy, due to allantoin found in the underground organs and leaf. [26]. The plant is semi-sterile and is propagated vegetatively mainly through root cuttings and root offsets. The comfrey plant has a taproot up to 3m in length and the root system of a well-established comfrey plant is fleshy and extensive.

Once plants are well established, plenty of vegetative material can be harvested by cutting several times during the year; the plants regenerate quickly because of the large food reserves in the roots and can produce two to five crops per year [27], which are often utilized as a source of organic fertilizer, either as green manure or as an ingredient of compost [28]. In Uganda studies DM yields increased from about 2 t/ha at eight weeks of growth to 3 t/ha at 12 weeks of growth yields of 2-3 t DM/ha were sustained during four, five and six weeks of regrowth [29].

2.3.9 *Vetiveria zizanoides* (L.) Nash

V. zizanoides, or *Chrysopogon zizanioides* (L.) Roberty, is a C_4 perennial grass of the Poaceae family, is one of such species that could be grown all across the globe from tropical to Mediterranean climate. The vetiver grass has a gregarious habit and grows in clumps. Shoots growing from the underground crown make the plant frost and fire resistant and allow it to survive heavy grazing pressure. The leaves can become up to 120-150 cm long and 0.8 cm wide [30]. Seeds do not have endosperm, therefore there is no germination, and the two commonly used methods for large-scale propagation of vetiver are: separation or splitting and tissue culture. The grass fits well in an ecosystem service model contributing to regional and global economies with its multifarious environmental applications.

A native grass of India, initially valued for its aromatic oil, vetiver is now extensively used in soil conservation, land rehabilitation and pollution mitigation.

Its fast growing tufted roots that penetrate vertically deep into the soil reaching over 2 m in just six months to 6 m in three years. This species has annual biomass production potential of 100–120 t/ha which is distinctly higher compared to 30–40 t achievable for other biomass efficient plants e.g. Miscanthus grass [31].

2.4 Laboratory analyses

During the research dry biomass samples of each species have been sent to the Biotechnical Laboratory of Trisaia for evaluation in terms of fibers [32] and elements [33].

2.4.1 Analyses of fibers

The different constituents of fibers (hemicellulose, cellulose, lignin and ashes) were determined sequentially according to the Van Soest's scheme analysis [32].

The biomass (1 g), previously dried and sieved (1 mm), was treated with 100ml of a boiling neutral detergent solution for 1 hour. Soluble carbohydrates, pectins, most proteins, lipids and soluble mineral substances, constitute the soluble content, which is defined as neutral detergent solubles (NDS). The residue is composed by fibrous components (hemicellulose, cellulose, lignin and ashes) and defined as neutral detergent fibers (NDF). The NDF residue was then treated with a boiling acid solution of tensioactive agent for 1 hour to dissolve only the hemicellulose. The fibrous residue is composed of cellulose, lignin and ashes and is defined as ADF. The difference between NDF and ADF is given by hemicellulose. The ADF residue was treated with 25 ml of cold 72% sulfuric acid that dissolves only the cellulose. The residue is defined as ADL and is a "raw lignin" that can also contain cutin and ashes. The difference between ADF and ADL is given by cellulose. Finally the ADL residue was destroyed by ashing at 550 °C for 2 hours to determine the percentage of ashes.

All these analyses were done with the VELP FIWE6 instrument. This is an extraction apparatus purposely devised for this method that makes the standardization of analytical condition very easy.

2.4.2 Elemental analyses

For every plant species, 5 g of biomass was milled using an A10 IKA mill until a very thin and homogeneous powder was obtained.

The milled biomass was then dried at 105 °C in a NSV9035-ISCO oven for 8 hours. With an AT21 Comparator METTLER TOLEDO balance 3 mg of biomass was weighed and then mixed with 0.6 g of V_2O_2 to catalyze the oxidation of SO_2 to SO_3. The samples were introduced, by means of ultra pure helium gas carrier, into a burner at a temperature of 1000 °C, and decomposed into N_2, CO_2, H_2O and SO_2 by the combined effect of the heat and catalysts like WO_3 and Cu.

The gas mix was then analyzed by a gas chromatograph with a thermo conductibility detector.

The results have been expressed as DM percentage and the O percentage calculated by difference of 100%. An AE 1110 CHNS-O (CE INSTRUMENTS) elemental analyzer was used for these analyses.

3 RESULTS AND DISCUSSION

3.1 Preliminary analyses

The cellulose enzymatic hydrolysis of lignocellulose biomass is a slow process that is influenced by its structural composition: high resistant crystalline structure and physical barrier of lignin surrounding the cellulose.

Every chemical or physical treatment, which decreases the crystalline structure of cellulose and increases the amount of the most reactive amorphous component of cellulose and disrupts the physical barrier of lignin, can optimize the accessibility of cellulose to enzymes increasing the hydrolysis rate [33].

Therefore, in this preliminary study (Table I), we can suppose that the selected species with the lowest lignin content and highest cellulose content are the most suitable candidates for the production of fermentable sugars and ethanol by means of the appropriate pretreatment, hydrolysis and fermentation [34].

The average composition of elements C, N, H and O is very similar and there are no differences among the selected species (Table II). The very similar chemical composition does not affect the whole process of ethanol production.

3.2 Mean productivity of the field experiment in Veneto Region

The highest value of dry biomass production is obtained by *A. donax* (32.7 t ha-1), followed by *M. x Giganteus* (16.3 t ha-1). All other species give lower and similar productions around 5 t/ha.

Comparing the elemental analyses in Table III, the highest carbon concentration values are in *M. x Giganteus, A. donax* and *C. mariscus* (45.10%, 44.52% and 44.61%) while *C. pseudocyperus* and *S. officinale asperrimum* have the lowest values (36.24% and 37.39%). The nitrogen concentration is very similar in all species (around 1.5%), with the exception of *S. officinale asperrimum* that reaches 2.3% and *M. x Giganteus* with only 0.70%. The same observation can be made on sulfur concentration with all species around 0.3%, apart from *M. x Giganteus* and *C. pseudocyperus* (0.15%) and *S. sylvaticus* that reaches 0.58%.

3.3 Mean productivity of the field experiment in Sicily

The rainfall recorded from January to November 2010 was about 500 mm. Irrigation water volumes applied in the open field experiment, during spring and summer 2010, were 200 mm, 400 mm and 600 mm, with 33% (S1), 66% (S2) and 100% (S3) restitution of crop evapotranspiration, respectively. The amounts of nitrogen and phosphorus released with irrigation water at S1, S2 and S3 plots were 75, 50 and 25 kg/ha and 22, 15 and 7 kg/ha, respectively.

The open field experiment was also fertilized with 140 kg/ha of N and 40 kg/ha of P.

As expected, irrigation was in general beneficial and full ET replenishment (100%) increased the biomass productivity as compared to the other two treatments (33 and 66% ET) (Table IV). However, while *V. zizanoides* and *M. x Giganteus* have substantially different agronomic values increasing the distributed water volume, *A. donax* has similar parameters. The same observations can be made for productivity: *A. donax* has less definite values (49.42 t/ha and 57.30 t/ha) while *V. zizanoides* and *M. x Giganteus* increase their dry biomass from 29.45 to 74.86 t/ha and from 13.49 to 33.97 t/ha, respectively. *V. zizanoides* has the highest production (74.86 t/ha), followed by *A. donax* with 57.30 t/ha. *M. x Giganteus* has the lowest value (33.97 t/ha, which corresponds to 60% less than vetiver plots.

Table I: Fiber analyses of the 19 preliminary species.

Species	Humidity (%)	Extract (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Ashes (%)
A. calamus	11.21±1.08	42.33±0.74	26.78±0.69	23.98±0.52	6.77±0.50	0.13±0.07
A. donax	6.29±0.12	14.13±0.13	33.57±0.57	43.69±0.30	8.68±0.25	0.56±0.04
C. indica	9.24±0.43	31.75±0.39	31.05±0.93	31.30±0.27	5.36±0.30	0.62±0.26
C. elata	9.61±0.38	19.32±0.01	34.60±0.38	33.30±0.59	11.09±0.70	0.14±0.04
C. longus	9.30±0.08	25.91±0.35	32.34±0.32	35.53±0.54	6.09±0.59	0.06±0.02
C. papyrus	7.39±0.02	32.38±0.08	27.76±0.73	33.72±1.20	5.83±0.31	1.10±0.14
G. maxima	8.79±0.06	27.98±0.42	37.01±0.36	31.32±0.22	2.49±0.14	0.56±0.08
I. pseudacorus	8.16±0.06	55.18±0.98	9.52±0.36	28.26±0.98	6.98±0.45	0.06±0.01
J. effusus	9.71±0.78	25.31±0.31	38.63±0.44	29.88±0.35	6.17±0.34	0.01±0.01
L. salicaria	8.57±0.56	19.65±0.15	18.49±0.11	45.65±0.31	14.36±0.21	0.01±0.01
M. x Giganteus	7.38±0.26	12.35±0.38	34.21±1.24	46.14±0.58	6.14±0.62	1.15±0.02
Ph. arundinacea	8.56±1.93	31.59±0.82	33.80±0.21	28.73±0.68	5.73±0.54	1.78±0.09
Ph. australis	8.02±1.10	31.40±1.72	33.33±0.03	26.22±0.31	7.30±1.03	1.18±0.16
S. sylvaticus	9.48±0.37	18.70±0.66	32.00±0.35	36.33±0.22	13.33±0.04	0.18±0.03
S. bicolor	8.43±0.34	25.03±0.57	34.14±0.27	35.52±0.23	4.69±0.51	0.69±0.09
S. officinale asperrimum	11.20±0.48	50.47±1.40	17.62±0.27	23.28±1.67	7.82±0.21	0.97±0.03
T. dealbata	9.49±0.51	29.98±1.37	29.82±0.34	35.19±0.69	4.37±0.16	1.15±0.15
T. latifola	8.14±0.07	31.47±0.49	30.74±0.28	32.34±0.22	5.44±0.01	0.01±0.01
V. zizanoides	7.19±0.31	18.98±0.33	39.77±0.45	34.88±0.46	5.39±0.04	0.80±0.11

Table II: Elemental analyses of the 19 preliminary species.

Species	C (%)	N (%)	H (%)	O (%)
A. calamus	42.75±0.32	2.02±0.07	4.87±0.15	50.52±0.18
A. donax	45.32±0.90	0.26±0.02	5.25±0.36	48.06±1.01
C. indica	40.58±0.27	1.32±0.09	5.01±0.19	52.49±0.48
C. elata	44.46±0.43	1.04±0.01	4.43±0.17	49.20±0.99
C. longus	43.96±0.03	0.34±0.04	4.41±0.38	51.25±0.50
C. papirus	40.86±0.23	0.43±0.01	5.25±0.05	52.35±0.16
G. maxima	45.74±0.15	0.93±0.03	4.66±0.06	49.52±0.30
I. pseudacorus	43.38±0.16	1.28±0.10	5.17±0.27	50.11±0.21
J. effusus	43.24±0.10	1.36±0.01	5.00±0.02	50.39±0.07
L. salicaria	46.20±0.08	0.35±0.01	4.86±0.24	48.85±0.40
M. x Giganteus	44.36±0.40	0.15±0.01	5.09±0.32	49.24±0.56
Ph. arundinacea	42.16±0.32	1.52±0.01	5.13±0.44	47.20±0.77
Ph. australis	44.34±0.27	1.34±0.02	4.45±0.11	48.89±0.26

S. sylvaticus	43.83±0.46	0.31±0.01	4.47±0.02	49.41±0.08
S. bicolor	42.38±0.84	0.70±0.01	5.24±0.36	49.54±0.90
S. officinale asperrimum	44.14±0.29	1.34±0.02	4.87±0.34	50.63±0.10
T. dealbata	41.12±0.29	0.72±0.02	4.80±0.07	52.19±0.47
T. latifola	45.60±1.42	1.45±0.04	5.75±0.22	47.20±1.96
V. zizanoides	43.83±0.46	0.43±0.01	5.05±0.36	49.93±0.07

Table III: Mean elemental characterization and productivity of the Veneto Case dry biomass.

Species	C (%)	N (%)	S (%)	Dry biomass (t/ha)
A. donax	44.52±0.14	1.17±0.08	0.31±0.03	32.73±10.71
C. indica	40.45±0.27	1.55±0.06	0.26±0.03	6.81±0.62
C. mariscus	44.61±0.13	1.73±0.10	0.33±0.06	5.98±1.13
C. pseudocyperus	36.24±3.87	1.48±0.07	0.32±0.06	4.79±0.21
C. riparia	42.95±0.33	2.09±0.26	0.35±0.06	4.85±0.50
I. pseudacorus	41.76±0.21	1.75±0.14	0.17±0.01	6.17±1.63
M. x Giganteus	45.10±0.08	0.70±0.06	0.15±0.03	16.33±0.97
S. officinale a.	37.39±0.54	2.31±0.14	0.29±0.04	3.82±0.30
S. sylvaticus	43.10±0.08	1.71±0.22	0.58±0.08	5.12±0.32

Table IV: Mean bio-agronomical characterization and productivity of the Sicily Case plants.

Species	Water distribution	Density (plants/m^2)	Height (cm)	Stems diameter (mm)	Total leaves (n°)	Dry leaves (n°)	Dry biomass (t/ha)
A. donax	66% ET	32.0	366	16.8	41	9	49.42
	100% ET	33.0	379	17.1	42	11	57.30
M. x Giganteus	33% ET	68.0	150	6.5	13	3	13.49
	66% ET	79.0	196	7.1	13	4	18.44
	100% ET	100.0	242	7.1	14	7	33.97
V. zizanoides	33% ET	3.7	142	-	-	-	29.45
	66% ET	3.6	188	-	-	-	56.05
	100% ET	4.0	209	-	-	-	74.86

4 CONCLUSIONS

The results in this paper belong to a preliminary study so they are indicative for the research in progress.

As concerns potential ethanol productivity the most interesting species are plants with the lowest lignin content and highest cellulose content, such as *M. x Giganteus*, *A. donax*, *C. longus*, *S. bicolor* and *T. dealbata.*

However the suitable candidates are also selected for their biomass production. In the Veneto trials *A. donax* has the highest value (32.7 t/ha), followed by *M. x Giganteus* (16.3 t/ha). In Sicily the productivity is strictly linked to irrigation level: in the plot with full ET replenishment the best biomass producer is *V. zizanoides* with 74.9 t/ha, followed by *A. donax* (57.3 t/ha), but decreasing the irrigation amount their productivity strongly diminishes (e.g. *M. x Giganteus* falls to 13.5 t/ha).

This is a long-term research, so more exhaustive conclusions should be reached during the upcoming experiments.

5 REFERENCES

[1] R. Venendaal, U. Jorgensen, C.A. Foster, European energy crops: a synthesis. Biomass and Bioenergy (1997), pp.147-185.

[2] X. Ge, D.M. Burner, J. Xu, G.C. Phillips, G. Sivakumar, Bioethanol production from dedicated energy crops and residues in Arkansas, USA. Biotechnology Journal (2011) 6, pp. 66-73.

[3] G. Sivakumar, D.R. Vail, J.F. Xu, D.M. Burner, J.O. Lay, X. Ge, P.J. Weathers, Bioethanol and biodiesel: Alternative liquid fuels for future generations. Engineering in Life Science (2010), 10, 1, pp. 8-18.

[4] C. Berg, Word Fuel Ethanol. Analysis and Outlook, Japan, (2004), available at: http://www.distill.com/World-Fuel-Ethanol-A&O-2004.html (accesed 15.05.2011).

[5] F.J. Davila-Gomez, C. Chuck-Hernandez, E. Perez-Carrillo, W.L. Rooney, S.O. Serna-Saldivar, Evaluation of bioethanol production from five different varieties of sweet and forage sorghums (Sorghum bicolor (L) Moench), Industrial Crops and Production (2011) 33, pp. 611-616.

[6] S. Barbagallo, G.L. Cirelli, A. Marzo, M. Milani, A. Toscano, Hydraulics and removal in two HSSF constructed wetlands for wastewater reuse with different operational life. In: Proceedings of 12th IWA International Conference on Wetland Systems for Water Pollution Control. Island of San Servolo, Venice, Italy, 4-8 October (2010).
[7] ASCE-EWRI. The ASCE Standardized Reference Evapotranspiration Equation. Technical Committee Report to the Environmental and Water Resources Institute of the American Society of Civil Engineers from the Task Committee on Standardization of Referente Evapotranspiration (2004).
[8] P.J.Van Soest, J.B. Robertson, System of analysis evaluating fibrous feeds. Cornell University Ithaca-N.Y. (1979).
[9] AOAC International. Methods of analysis of nutrition labeling. Arlington, Texas. (1993).
[10] R.E. Perdue, Arundo donax—Source of musical reeds and industrial cellulose. Economic Botany 1958;12 pp. 368–404.
[11] G.C. Tucker, The genera of Arundinoideae (Gramineae) in the southeastern United States. Journal of the Arnold Arboretum (1990);71(2) pp. 145–77.
[12] A.B. Boose, J.S. Holt, Environmental effects on asexual reproduction in Arundo donax. Weed Research (1999), 39 pp. 117–27.
[13] Mariani C. et al. Origin, diffusion and reproduction of the giant reed (Arundo donax L.): a promising weedy energy crop - Ann Appl Biol 157 (2010), pp. 191–202
[14] K.P. Sharma, S.P.S. Kushwaha, B. Gopal, A comparative study of stand structure and standing crops of two wetland species, Arundo donax and Phragmites karka, and primary production in Arundo donax with observations on the effect of clipping. Tropical Ecology (1998);39(1) p. 39.
[15] A.A. Shatalov, H. Pereira. Arundo donax L. (giant reed) as a source of 3bres for paper industry: perspectives for modern ecologically friendly pulping technologies. In: S. Kyritsis, A.A.C.M. Beenackers, P. Helm, A. Grassi, D. Chiaramonti, editors. Biomass for Energy and Industry: Proceeding of the First World Conference, Sevilla, Spain, 5–9 June 2000. London: James & James (Science Publishers) Ltd., (2001). pp. 1183–1186.
[16] M. Hidalgo, J. Fernandez, Biomass production of ten populations of giant reed (Arundo donax L.) under the environmental conditions of Madrid (Spain). In: S. Kyritsis, A.A.C.M. Beenackers, P. Helm, A. Grassi, D. Chiaramonti, editors. Biomass for Energy and Industry: Proceeding of the First World Conference, Sevilla, Spain, 5–9 June 2000. London: James & James (Science Publishers) Ltd., (2001). p. 1881–1884.
[17] Z. Yousefi, A. Mohseni-Bandpei, Nitrogen and phosphorus removal from wastewater by subsurface wetlands planted with Iris pseudacorus Ecological Engineering 36 (2010) 777–782).
[18] W.J. Sutherland,. Biological Flora of the British Isles: Iris pseudacorus L. No. 169. Journal of Ecology (1990) 78, pp. 833-848.
[19] IPANE. 2001. University of Connecticut, Invasive Plant Atlas of New England (IPANE: Iris pseudacorus. http://webapps.lib.uconn.edu/ipane/browsing.cfm?descriptionid=59. Accessed January 16, 2003.)
[20] W. Haiming, Z. Jian, L. Peizhi, Z. Jinyong, X. Huijun, Z. Bo, Nutrient removal in constructed microcosm wetlands for treating polluted river water in northern China. Ecological Engineering 37 (2011) pp. 560–568.
[21] J.M. Greef, M. Deuter, Syntaxonomy of Miscanthus × giganteus GREEF et DEU. Angewandte Botanik (1993)67 pp. 87–90.
[22] I.B. Linde-Laursen, Cytogenetic analysis of Miscanthus 'Giganteus', an interspeci3c hybrid. Hereditas (1993)119:297–300.
[23] C.V. Beale, S.P. Long. Can perennial C4 grasses attain high efficiencies of radiant energy conversion in cool climates? Plant, Cell and Environment (1995);18:641–50.
[24] J.C. Clifton-Brown, The importance of temperature in controlling leaf growth of Miscanthus in temperate climates. Dissertation. University of Dublin, Trinity College, Dublin, (1997).
[25] I. Lewandowski, J.C. Clifton-Brown, J.M.O. Scurlock, W. Huisman, Miscanthus: European experience with a novel energy crop. Biomass and Bioenergy (2000);19:209–27.
[26] A. Toledo, Caracterização morfoanatômica de raiz e rizoma de Symphytum officinale L. (Boraginaceae) / Morpho-anatomical characterization of the root and rhizome of Symphytum officinale L. (Boraginaceae) - Rev. bras. farmacogn;16(2):185-191, abr.-jun. 2006.
[27] Bremness, The Complete Book of Herbs. Dorling Kindersley, London, UK. (1988).
[28] A. Wilkinson, A laboratory evaluation of comfrey (Symphytum officinale L.) as a forage crop for ensilage. Animal Feed Science and Technology 104 (2003) pp. 227–233.
[29] F. B. Bareeba, W. O. Odwongo , J. S. Mugerwa, The potential of Russian comfrey (Symphytum officinale) as an animal feedstuff in Uganda - - Department of Animal Science - Faculty of Agriculture and Forestry - Makerere University.
[30] J. A. Duke, J. L. duCellier CRC Handbook of Alternative Cash Crops January (1993), CRC Press ISBN:0849336201.
[31] J. C. Clifton-Brown, P.F. Stampfl, M.B. Jones, Global Change Biol., 2004, 10, pp. 509–518.
[32] L.T. Fan, M.M Gharpuray, Y.H. Lee, Cellulose Hydrolysis, Springer Publisher (1987).
[33] N. Curreli, M.B. Fadda, A. Rescigno, A.C. Rinaldi, G. Soddu, F. Sollai, S. Vaccargiu, E. Sanjust, A. Rinaldi, Mild alkaline/oxidative pretreatment of wheat straw. Process Biochemistry (1997) 32, pp. 665-670.
[34] N. Curreli, M. Agelli, A. Rescigno, E. Sanjust, A. Rinaldi, Complete and efficient enzymic hydrolysis of pretreated wheat straw. Process Biochemistry, (2002) 37, pp. 937-941.

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