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Field evaluation of *Arundo donax* clones for bioenergy production

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ABSTRACT

A collection of 24 clones of *Arundo donax*, from different geographical areas in Italy, Europe, and China, were evaluated during the first 3 years from transplant. A field trial with 3 replicates was set up in the Po valley (northern Italy) in a sandy loam soil. At harvest, at the end of the second and third year after plantation, biomass yield, average stem number, average stem diameter, and single plant area were determined for each clone. For a selection of clones, chemical analysis and biochemical methane potential (BMP) were also performed. A large variation among clones was found for all the biometric parameters considered and also for biomass yield. It was interesting to note that some clones, while achieving similar biomass yield, had contrasting growth patterns, with some clones producing just a few large stems and others producing many thin ones. As a consequence, a different number of stems per plant area was also found among clones. Chemical analysis highlighted a significant difference among clones for ash (from 5.3% to 8.1%), lignin (from 6.9% to 10.6%), and hemicellulose (from 25.1% to 29.2%) content, while cellulose content was on average 43.4%. BMP ranging from 147 ml g^{-1} VS to 243 ml g^{-1} VS and was partially affected by lignin and ash content.

1. Introduction

Various authors have indicated giant reed (*Arundo donax* L.) as a valuable source of biomass for its high yield, low input requirement, and adaptability to marginal environments (Angelini et al., 2005, 2009; Cosentino et al., 2006; Lewandowski et al., 2003; Mantineo et al., 2009; Nassi o Di Nasso et al., 2013). For its reputed drought tolerance, it is considered a suitable crop for Mediterranean environments even though it has large water requirements (Zegada-Lizarazu et al., 2010; Cosentino et al., 2014), as reported in recent studies (Nackley et al., 2014; Triana et al., 2014), that could, however, be satisfied by using low quality waters (Borin et al., 2013). Traditionally used as a source of musical reeds and industrial cellulose (Perdue, 1958), giant reed has been recently studied for bioethanol and thermochemical destinations (Jeguirim and Trouvé, 2009; Pilu et al., 2012; Scordia et al., 2013) and for anaerobic digestion (Schievano et al., 2012; Di Girolamo et al., 2013; Ragaglini et al., 2014).

Despite its positive features, there are still technical issues limiting a large scale commercial diffusion of giant reed as a dedicated biomass crop for bioenergy production. Moreover, concerns are raised over the weediness and invasiveness of giant reed, especially when cultivated in proximity of water courses (Barney and DiTomaso, 2008). Being sterile (Balogh et al., 2012) it cannot be planted by seed, but it must be propagated using portions of rhizomes, stem cuttings, or micro-propagated plantlets (Ceotto and Di Candilo, 2010; Mariani et al., 2010; Copani et al., 2013). The use of vegetative materials increases production cost and poses technical problems for the lack of suitable extraction and transplanting machines, even though prototypes are being designed and developed (Assirelli et al., 2013; Pari and Assirelli, 2011; Pari et al., 2011). Plantation costs could be reduced by using a low planting density, but very limited information is available on the effect of planting density on crop establishment and yield. Practically no information is available on the suitability of different genotypes to specific environments, cropping practices, and end use destination. Few studies have highlighted that due to the lack of sexual reproduction, clones of *A. donax* collected in contrasting environments have very limited genetic variability (Pilu et al., 2012; Mariani et al., 2010; Ahmad et al., 2008; Khudamrongsawat et al., 2004). Nevertheless, Cosentino et al. (2006), in a field trials set compare up to 39 clones collected across Sicily and Calabria, in the South of Italy, found that clones were significantly different in terms of biomass yield, which was positively correlated to stem weight, stem density, and stem height. In order to advance the knowledge on the effect of clone origin on biomass production, in this work 24 clones collected throughout Europe and in China were compared in the first three years after transplanting to evaluate yield potential, yield composition, and biomass quality for biogas production.

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2. Material and methods

A field trial was set up in Cremona (45.16°N, 9.96°E, 57 m a.s.l.) in the Po valley on a sandy loam soil to compare 24 clones of *A. donax* (23 from various parts of Europe and 1 from China, hereafter the clones will be coded with a suffix referring to the nation of origin, UO indicates unknown origin); minimum and maximum temperature and rainfall patterns are shown in Fig. 1 (Regional Meteorological Service – ARPA Lombardia). Cumulative rainfall during the growing season was 435 mm in 2012 and 688 in 2013, average temperature was 20 °C and 22 °C in 2012 and 2013, respectively. No sign of water stress were observed throughout the trial. Before planting, the solid fraction of digestate from a nearby biogas plant was distributed on the field at a rate of approximately 40 Mg ha^{-1}. The soil was then superficially ploughed (0.2 m depth) and harrowed. At least six rhizomes per clone were planted following a completely randomized block design, with two rhizomes per clone in each of the three blocks. Prior to transplanting, 0.25 m deep furrows were opened in the field, with 2 m distance between furrows. Rhizomes were cut into portions of approximately 350 g, paying attention that one or two large gems would be available per each rhizome portion. On 21st April 2011 the rhizomes were placed on the bottom of the furrow with a distance of 1.5 m between rhizomes (0.33 rhizomes m^{-2}), then the furrows were closed and the surface of the soil was flattened with a roller to facilitate soil adhesion to the rhizomes and prevent humidity loss. A supplementary row of rhizomes from a local genotype was planted all around the trial to avoid border effect.

During the course of the first year, emergence of the various clones was monitored so as to guarantee that at least two plants per clone and per replicate would be available for yield analysis. In the case of missing plants, additional rhizomes were planted when available. At the end of the first year the field was inhomogeneous with only a few stems per plant and no data were collected prior to stem cutting.

Data collection was carried out at the end of the second (8th February 2013) and third growing seasons (10th December 2013), when all the stems from each plant per clone and per block were harvested. Harvesting was carried out with a Stihl clearing saw mounted with a steel cutting blade. The fresh weight of each plant was measured on field with the use of a digital dynamometer. All stems for each plant were then counted and a subsample of 20 stems per plant was randomly collected to determine stem height and stem diameter at 0.1 m from the stem base. Immediately after harvesting, 3 stems per plant were weighed with a precision scale and subsequently brought to the lab for the determination of dry matter content at 105 °C. After biometrics determination, stems were air dried in a greenhouse and subsequently used for quality determinations.

The fiber fractions NDF, ADF, and ADL were determined using the AnkomII Fiber Analyzer (Ankom Technology Corporation, Fairport, NY, USA) and were corrected for the residual ash content, following the procedure described in Gallo et al. (2013).

The biochemical methane potential (BMP) was determined in duplicate in 250 ml batch digestion bottles, according to VDI 4630 (2006) guidelines. The inoculum was obtained from an anaerobic digester that was mainly fed with biomass crops (i.e., maize silage and triticale silage) at 43 °C. After collection, the inoculum was sieved (2 mm) and stored for one week at 43 °C. Each bottle was filled with 3 g dry weight of *A. donax* sample and 100 ml of inoculum, in order to achieve an inoculum to substrate ratio higher than 2:1 (on a DM basis), and 100 ml of medium (Goering and Van Soest, 1970) to ensure optimal nutrient conditions.

Bottles were incubated at 43 °C for 50 days and manually agitated every day. Gas production was recorded twice a day for the first two days, then once a day for three days, and then with a lower frequency, by a high precision digital pressure gauge (LabDMM, AEP, Cognento, IT).

Following the determination of biogas volume, the gas from each bottle was recovered into gas sampling bags (Standard FlexFoil, SKC Inc., Eighty Four, PA, USA) and CH_4 concentration was measured by a NIR analyzer (EC400, Eco-Control srl, Vimodrone, Italy).

The giant reed clone and the year effects on yield and quality related variables in a randomized complete-block design were tested by a two-way ANOVA using IBM – SPSS 21 (IBM Corporation, Armonk, New York, USA), given the normality of distributions (Shapiro and Wilks test; Shapiro and Wilk, 1965) and the homogeneity of variances (Levene's mean-based test; Levene, 1960). The two-way ANOVA was run on all the yield and quality related parameters, which were measured and estimated throughout the experiment. Year was taken as a fixed factor in the ANOVA model in order to test the significance of its interaction with clones and detect possible differences in clone growth rate and other plant related variables from the 2nd to the 3rd year after planting. Although Year is often considered a random factor, in a recent paper dealing with statistical analysis in agronomic research Year

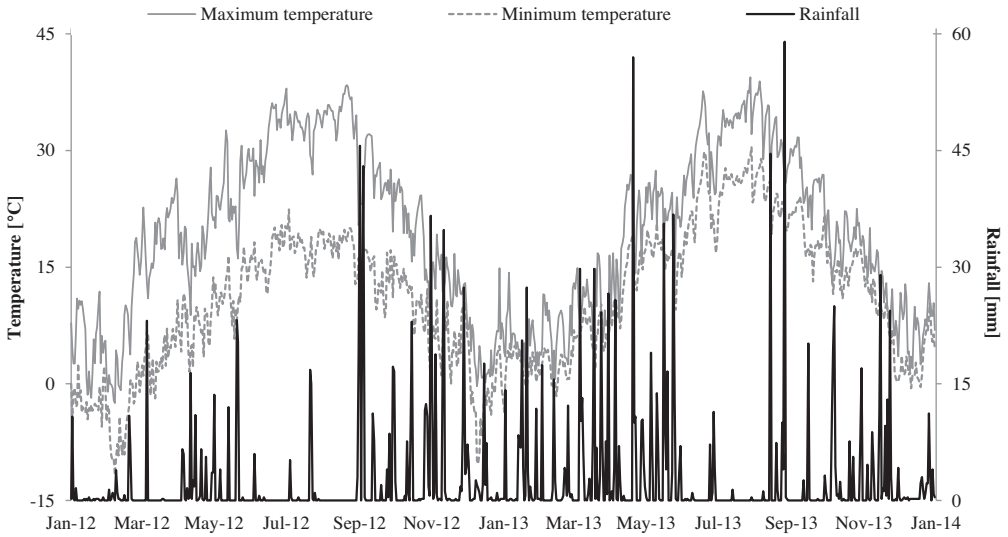

Fig. 1. Minimum and maximum temperatures and rainfall distribution in 2012 and 2013 at the experimental sites.