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Miscanthus clones for cellulosic bioethanol production: Relationships between biomass production, biomass production components, and biomass chemical composition

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ABSTRACT

The perennial miscanthus crop is a promising feedstock for cellulosic ethanol production due to its high yield, low input and low environmental impacts. To be suitable for cellulosic ethanol production, cultivated *Miscanthus* clones need to present not only high aboveground biomass production, but also high cellulose and hemicellulose contents and low lignin, soluble, and ash contents. By testing *M.* × *giganteus*, *M. sacchariflorus*, and *M. sinensis* clones, we investigated the relationships between biomass production and biomass composition traits at two harvest dates over 3 years. High aboveground biomass production was associated with high canopy height, high stem diameter, late flowering, and high cellulose and lignin contents but low hemicellulose, soluble, and ash contents. The aboveground biomass production was positively correlated with the potential yields of cellulose, hemicelluloses, and lignins. These relationships were consistent throughout the years and the harvest dates. The most productive *Miscanthus* clones displayed high cellulose contents and low soluble and ash contents; however, they displayed low hemicellulose contents and high lignin contents. The total aboveground biomass composition was closer to the stem composition than to the leaf composition. Nevertheless, the leaves were interesting because of their high hemicellulose and low lignin contents. Lastly, all of the studied factors were significant, but the biomass production traits were mainly affected by the year of cultivation or clone, while the biomass composition traits were mainly affected by the harvest date. All of the traits showed low interaction effects. These results will guide the breeding of *Miscanthus* clones that are tailored for biofuel production.

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1. Introduction

Various crops are interesting candidates for the production of cellulosic ethanol. To be suitable for cellulosic ethanol production, such crops need to combine (i) a high aboveground biomass production with a composition maximizing the process conversion (Godin et al., 2010), (ii) low inputs, and (iii) a low environmental impact (Gabrielle et al., 2014).

The biomass composition and more particularly the cell wall composition are considered today as major challenges in enhancing the conversion efficiency of such processes. Several studies have reported that the cell wall architecture mainly influences the efficiency of the conversion process (Godin et al., 2010; Jakob et al., 2009; McCann and Carpita, 2008; Sticklen, 2006). The cell wall contains mainly cellulose, hemicelluloses, and lignins that are organized in a complex structure. Cellulose is a polymer of hexose sugars, and hemicelluloses are polymers of hexose and pentose sugars. These polymers are of particular interest for cellulosic ethanol production: they are hydrolyzed by enzymes in monomer of sugars, which are themselves fermented by microorganisms (DGEC, 2010). However, (i) lignins restrict the accessibility of enzymes to cellulose and hemicelluloses during enzymatic hydrolysis, and (ii) lignins are a source of inhibitory substances that can decrease the

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; DM, dry matter; NDF, neutral detergent fiber; PCA, principal component analysis; t, ton; VS, Van Soest.

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microorganism activity during fermentation (Godin et al., 2010; Klinke et al., 2004; McCann and Carpita, 2008; Tran and Chambers, 1985). This process requires, therefore, high cellulose and hemicellulose (holocellulose) contents (van der Weijde et al., 2013) but low lignin contents in the aboveground biomass (Chang and Holtzapple, 2000). In addition, low ash and soluble contents are preferable for such processes (Dumas and Navard, personal communication). From these considerations, the aboveground biomass production of the candidate crops is expected to be high in order to maximize the amounts in cellulose and hemicelluloses in the cell wall.

Among the candidate crops, the lignocellulosic C4 perennial miscanthus crop, and more specifically the species *Miscanthus* × *giganteus*, is of particular interest to target cellulosic ethanol production because it combines high biomass production with a low environmental impact (Cadoux et al., 2014; Clifton-Brown et al., 2004; Hastings et al., 2008; Lewandowski and Schmidt, 2006). Compared to other *Miscanthus* species, this species also contains a high cellulose content, which is expected for the cellulosic ethanol conversion process (Allison et al., 2011). However, this species shows a high lignin content, which is not suitable for this process (Allison et al., 2011).

Among the 20 species within the *Miscanthus* genus (Hodkinson et al., 2002), many studies have reported various potentials in biomass production and various cellulose, hemicellulose, lignin, and ash contents in the aboveground biomass (Allison et al., 2011; Baxter et al., 2012; Clifton-Brown et al., 2001, 2008; Clifton-Brown and Lewandowski, 2002; Gauder et al., 2012; Hodgson et al., 2010; Jezowski, 2008; Qin et al., 2012; Zub et al., 2011). These results are encouraging for the selection of other *Miscanthus* clones than *M.* × *giganteus* clones, which will combine favorable biomass production and composition traits for cellulosic ethanol production. However, (i) the miscanthus aboveground biomass production is a complex trait that is determined by earliness and morphological traits, such as the canopy height, stem number per plant, and stem diameter (Robson et al., 2013; Zub et al., 2011). Regarding the miscanthus biomass composition, (ii) Allison et al. (2011) highlighted variable correlations between cellulose, lignin, and hemicellulose contents. In addition, miscanthus biomass composition differs between the stems and leaves, and the ratio between the stems and leaves varies according to the *Miscanthus* clones (Kalembasa et al., 2005). Lastly, (iii) the traits that are related to biomass production and composition vary according to the *Miscanthus* clone, the year of cultivation, and the harvest date (Allison et al., 2011; Clifton-Brown and Lewandowski, 2002; Gauder et al., 2012; Godin et al., 2013a,b; Kaack et al., 2003; Larsen et al., 2014; Lewandowski and Kicherer, 1997; Qin et al., 2012; Zub et al., 2011).

Previous studies have been conducted on miscanthus biomass production and composition but mainly separately. Little attention has been paid to the relationships between both types of traits that are related to biomass production and biomass composition in miscanthus. However, based on previous observations, we first hypothesized that there are synergistic and antagonistic relationships between the traits of interest that are related to biomass production and composition, resulting in most productive *Miscanthus* clones not always being the most suitable clones for the biomass composition. Second, we hypothesized that the relationships between the biomass production and the biomass composition traits were consistent in the function of the year of cultivation and the harvest date in miscanthus.

The aim of this study was therefore to characterize the relationships between the traits that are related to biomass production and biomass composition in miscanthus. For this objective, we first generated preliminary results on the variability of the traits that are related to biomass production and biomass composition in 21 *Miscanthus* clones. Here, we focused on the main effects and interactions of the clone, the harvest date, and the year of cultivation. The biomass composition was investigated in different parts of the plant: the total aboveground part, the stems, and the leaves. Second, we explored the relationships between these traits to identify the synergistic and antagonistic traits between the biomass production and biomass composition traits. Here, we considered separately the composition of the total aboveground biomass, the stems, and the leaves. Third, we explored the stability of the relationships between the aboveground biomass production and each of the cellulose, hemicellulose, lignin, soluble, and ash contents in the total aboveground biomass. We focused on the stability of these relationships over years of cultivation and harvest dates, which had a significant effect on the traits of interest. Fourth, we explained the variations in the composition of the total aboveground biomass by the contribution of the leaves and the stems. We lastly investigated the characteristics of the studied clones to determine whether the most productive *Miscanthus* clones are also the most suitable clones in terms of biomass composition for cellulosic ethanol production.

This study will contribute to new knowledge regarding the relationships between the miscanthus biomass production and composition traits, which will be particularly useful in breeding suitable *Miscanthus* clones for cellulosic ethanol production.

2. Materials and methods

2.1. Experimental site and climatic conditions

The experimental site is in the Picardie region of Northern France (49°53 N, 3°00 E) at the INRA experimental unit in Estrées-Mons. The soil is a deep loam soil (Orthic Luvisol, FAO classification). The climate is oceanic. The rainfall and temperature data (see Table 1) were collected throughout the experiment by a local meteorological station 1 km away from the trial.

2.2. Experimental design

The experimental design of the trial was a split plot design: the harvest date factor was the whole-plot factor (main plot) and the clone factor was the subplot factor. The levels of the harvest date factor were randomly assigned to whole-plots. The levels of the clone factor were randomly assigned to split plots (subplots) within each whole-plot. Two levels of the harvest date factor were tested: an autumn harvest was carried out in autumn at the end of the growing period, and a winter harvest was carried out at the end of winter at over-maturity. Twenty one clones of *Miscanthus* were studied: 4 clones were identified as *M.* × *giganteus* clones, 15 clones as *M. sinensis* clones, and 2 clones as *M. sacchariflorus* clones (Table 2). Among the 4 clones of *M.* × *giganteus*, H8 was considered as a *M.* × *giganteus* clone as it was a hybrid between *M. sacchariflorus* and *M. sinensis*. Using AFLP markers, the clone named "Flo" belonged to the *M.* × *giganteus* species (Rambaud, personal communication). The design contained three blocks.

Each subplot of 16 m^2 consisted of four rows of eight plants that were planted at a density of 2 plants per m^2. A border row was planted on each side of the plot for edging using the same clone, *M. sinensis* Malepartus.

2.3. Management of the trial

The trial was planted by hand in spring 2007 at a rhizome planting density of 2 plants per m^2. The plants were watered immediately after planting to ensure good root contact with the soil. No irrigation was applied during the following years of cultivation. No fertilizer was applied during the entire experiment. The weeds were controlled each year by hand and machine hoeing.

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