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Key traits for biomass production identified in different *Miscanthus* species at two harvest dates

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ABSTRACT

To grow the bioenergy crop *Miscanthus x giganteus* on wider climate range requires enlarged genetic variability. Our objective was to identify key traits favouring the production of above-ground yield in a cropping environment of *Miscanthus* at two harvest dates. Canopy height, panicle height, shoot number, stem diameter and above-ground yield of twenty-one clones were studied and compared with emergence earliness and growth traits at autumn and winter harvests in Northern France, during the second and third crop years. Crop age, clone, harvest date, and corresponding double interactions were significant for all traits. Species and ploidy explained clone sum of square effects (92%, 78%, 80% and 89% for canopy height, panicle height, shoot diameter and yield, respectively) and clone × age and clone × harvest date interactions for yield (94% and 77%, respectively). Plant height and shoot number were higher in the third year than in the second, whatever the harvest date. The above-ground development between the two years was higher in winter harvest than in autumn, mostly for *M. sinensis*. Higher above-ground development and yields were observed for *M. x giganteus* and *M. floridulus* than *M. sinensis* and *M. sacchariflorus* as well as for triploid and tetraploid *M. sinensis* and *M. sacchariflorus* than diploids. Plant height, stem diameter, lateness at panicle emergence or flowering and growth rate were the main traits positively related to yield, in contrast to shoots number and growth duration. This would help to early identify high-yielding clones and breed new inter-specific hybrids.

1. Introduction

Miscanthus is a genus of C_4, perennial, rhizomateus grass, most species of which originate from East Asia. After establishment, the above-ground biomass can be harvested annually for 20–25 years [1]. Most European studies have focused on a single species, *M. x giganteus*, considered as a natural triploid hybrid between a diploid *M. sinensis* and a tetraploid *M. sacchariflorus* [2,3]. This clone has demonstrated its high biomass yield in Europe, which ranges from 15 to 25 t ha^{-1} without irrigation at the autumn harvest and from 7 to 19 t ha^{-1} at the late winter or winter harvest, depending on the climatic conditions [4,5]. In Northern Europe, it is necessary to delay harvest until the spring, and in Southern Europe until the end of winter. More detailed descriptions of this crop are given in the review by Zub and Brancourt-Hulmel [5].

Nevertheless, the development of *Miscanthus* production based on a single clone of *M. x giganteus* presents several limitations, such as its high establishment costs, its poor overwintering in some Northern parts of Europe and its poor adaptation to water stress in Southern Europe. Moreover, the propagation of a single clone in large areas increases the risk of attack by pests and diseases.

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Only one study has focused on assessing variations in the biomass yield of different *Miscanthus* species according to latitudinal gradient and climate in Europe [6,7,8] while several studies have compared different species in a single country (Table 1) [8,9,10,11].

Clifton-Brown et al. [6] and Lewandowski et al. [7] compared the biomass yield potential of four *M. x giganteus* clones, one *M. sacchariflorus* clone, five *M. sinensis* clones and five *M. sinensis* hybrids at five different sites in Europe (Sweden, Denmark, the UK, Germany and Portugal). During the third year of production (Table 1), the autumn and winter yields demonstrated the higher productivity of *M. x giganteus* in the UK, Germany and Portugal, whereas *M. x giganteus* clones did not survive the first winter in Sweden and Denmark. Mean biomass yields at autumn and winter harvests ranged from 15.9 to 11.6 t ha^{-1} in Northern Europe (UK) to 23.7 and 36.4 t ha^{-1} in Southern Europe (the highest yields being observed in Portugal but under irrigation, unlike the other countries). High yields were also reported for *M. sinensis* hybrids; the highest without irrigation being observed in Sweden with mean yields of 17.9 (autumn harvest) and 13 t ha^{-1} (winter harvest) for *M. sinensis* hybrids and 14.3 and 8.9 t ha^{-1} for *M. sinensis* at the autumn and winter harvests, respectively (Table 1). In another trial in Poland, Jezowski [10] concluded as to the superiority of various hybrids: 1. a hybrid between *M. sinensis* (2x) and *M. sacchariflorus* (4x), 2. a hybrid between *M. sinensis* (2x) and *M. sacchariflorus* (2x) or 3. a hybrid between two *M. sinensis* (2x). Their mean winter yields during the third year reached 20.5, 14.9 and 9.8 t ha^{-1}, respectively (Table 1).

During the same comparison of fifteen clones, Clifton-Brown et al. [6] also observed a strong effect of the clone × country interaction on the autumn yield during the third year. For instance, hybrid H8 displayed the highest yield of 24.7 and 19.2 t ha^{-1} among the hybrids used in Sweden and Germany, whereas it showed the lowest yield of 6.5 and 21 t ha^{-1} in the UK and Portugal, respectively. By contrast, hybrid H7 displayed the lowest yield of 11 t ha^{-1} among the hybrids studied in Sweden and the highest yield of 40.9 t ha^{-1} in Portugal (Table 1). However, France did not participate in this study, therefore no reference is available at present on the potential yields of different *Miscanthus* species in this country.

During the same experiment, Clifton-Brown and Lewandowski [12], Lewandowski et al. [6] observed the effect of delaying the harvest date until winter on mean biomass yields at five sites in Europe. The mean losses of yield between the autumn and winter harvests ranged from 23% in Germany to 40% in Denmark. The authors observed that yield losses decreased with the time elapsing between the autumn and winter harvests. The longer this period, the greater were the yield losses. These losses thus corresponded to the positioning of harvest during the year. Because these trial crops received nitrogen fertilizer each year, the authors did not observe this harvest date effect on the yield of the subsequent year or the response of several *Miscanthus* clones to such an autumn harvest, which indirectly involves a reduction in nitrogen availability.

Because the inter-genotype variability of *Miscanthus* yield has not yet been documented in France, although *Miscanthus* is being grown on a thousand hectares using a single clone in France (E de Maupeou, personal communication), the aim of this paper was to identify the key traits that promoted the production of its above-ground biomass so as to broaden genotype variability in all these countries. In each clone, particular attention was paid to the corresponding species and ploidy level, and to the relationships between subsequent crop years (2 and 3 year-old crops). Twenty-one clones were compared at two harvest dates (autumn and winter) in two successive crop years during a field replicated trial in Northern France. The study focused on four of the *Miscanthus* species studied in Europe for biomass production: *M. x giganteus*, *M. floridulus*, *M. sinensis* and *M. sacchariflorus*. The effects of clones, age (year) of the crop and harvest date on their above-ground biomass yield and plant traits were studied. We firstly compared the clones with respect to above-ground development and biomass yield, and studied the partitioning of clone variability that could be explained by species and ploidy level. We then defined the relationship between above-ground development, growth traits and biomass yield, and the relationship between yield in subsequent years as a function of harvest date.

2. Materials and methods

2.1. Soil and climatic conditions

The experiment was carried out at the INRA experimental unit in Mons (49°53N, 3°00E), Northern France during 2007–2010. The experimental field is characterized as a deep loam soil (Ortic luvisol, FAO, classification). Rainfall and radiation were recorded by a meteorological station at a distance of 1 km from the field trial. Rhizome temperature (at −20 cm under the soil surface) and air temperature (at +10 cm above the soil surface) were recorded by respectively four and one thermistances within the trial plot.

2.2. Description of the trial

The trial concerned 21 clones: three *M. x giganteus* (*M. x gig*) clones that were inter-specific hybrids between *M. sinensis* and *M. sacchariflorus*, fifteen *M. sinensis* (*M. sin*)clones, two *M. sacchariflorus* (*M. sacc*) clones and one *M. floridulus* (*M. flo*) clone. Ploidy level of each clone was presented in Table 2. As for *M. sinensis* and *M. sacchariflorus* which could have different ploidy level, and because the *M. x giganteus* is defined as an inter-specific hybrid between these two species, it can be expected different ploidy levels within the *M. x giganteus* species. Three clones, one of *M. sac* (H5), one of a hybrid between *M. sin* and *M. sac* (H8) and one of *M. sin* (H6), had previously been studied by Clifton-Brown et al. [6] and Lewandowski et al. [7] with respect to biomass yield and quality (Table 1). The clones were planted by hand at a density of 2 plants m^{-2} in a randomized complete block design with six replicates in the spring of 2007, following a winter wheat crop. Plot size was 3.2 m × 4.8 m. During the first year, the plots were irrigated one month after planting. No fertilization was applied during the three years of the experiment, and residual nutrients into the soil were estimated each year by soil sampling to verify that the crop did