

Late Emergence and Rapid Growth Maximize the Plant Development of *Miscanthus* Clones

H. W. Zub · C. Rambaud · L. Béthencourt ·
M. Brancourt-Hulmel

Published online: 29 April 2012

© The Author(s) 2012. This article is published with open access at Springerlink.com

Abstract To develop the perennial grass *Miscanthus x giganteus* as a highly productive crop for biomass production, new varieties need to be bred, and more knowledge about its growth behaviour has to be collected. Our aim was to identify an efficient function for assessing and comparing emergence date and canopy height growth (rate, duration, and final maximal height) of 21 clones of *Miscanthus* in Northern France. Flow cytometry made it possible to classify the clones into three clusters corresponding to 2x, 3x, and 4x ploidy levels. Three functions, 3- and 4-parameter logistic functions and Gompertz function, were tested to best describe the dynamics of crop emergence and of plant growth. The best functions were used to estimate emergence dynamics (Gompertz function), and growth dynamics (4-parameter logistic). All these traits showed a significant year, clone, and corresponding interaction effects (but not for harvest date). Species and ploidy level explained the clone and clone \times year interaction effects. *M. x giganteus* and *M. floridulus* clones were among the latest to emerge, and the tallest. *M. sinensis* clones showed the lowest height and growth rates. Higher final canopy height was correlated to late emergence and high growth rate. These findings could help early selection of interesting clones within *M. sinensis* populations, in order to breed new inter-species hybrids of *giganteus* type.

Keywords *Miscanthus* species · Growth function · Emergence date · Canopy height · Growth rate · Growth duration

Introduction

Species of the *Miscanthus* genus have advantages for biomass production. This perennial grass is a C₄ plant with a high productivity and low input requirements, particularly for pesticides and nitrogen fertiliser [1]. *Miscanthus* produces more biomass per hectare than other energy crops such as maize. Furthermore, this crop also fixes more atmospheric carbon in its biomass [2], and it sequesters the carbon present in the soil [3], which is beneficial for greenhouse gas reduction.

Research in Europe has mainly focused on a single species, *M. x giganteus*, which is a natural hybrid between *M. sacchariflorus* and *M. sinensis* [4]. This species presents little genetic variability [5, 6], and can be difficult to establish in a field. Despite its high biomass potential, *M. x giganteus* is sterile and propagated by rhizome, so that establishing this crop is expensive. It is also sensitive to lack of water [7, 8] and has a poor frost tolerance, especially during the first year of growth with a peak of sensitivity during the first winter [9, 10]. Potential improvement of the species could come from the *M. sinensis*, which has a diverse genetic pool for tolerance to water stress and frost [11], while still being high-yielding and adapted to biomass production.

In agronomy and plant breeding, there are models that can predict the distribution of crop yields from meteorological data, and models for assessing growth characteristics. Several models, such as MISCANFOR [12], MISCANMOD [13], or ecological modelling [14] have been in use to extrapolate *Miscanthus* yields, in particular *M. x giganteus*, to other environments. They use daily or monthly mean temperature, precipitation, photosynthetically active radiation, potential evapo-transpiration and irrigation time series for multiple or single years, and soil data for single or multiple locations [13].

However, no model has yet been proposed in *Miscanthus* to characterize the genetic variability of traits related to plant growth dynamics (e.g., growth rate, growth duration, maximal

H. W. Zub · C. Rambaud · L. Béthencourt ·
M. Brancourt-Hulmel (✉)
UMR 1281 Stress Abiotiques et Différenciation des Végétaux
cultivés, INRA,
BP 50136, 80203, Estrées-Mons, Péronne, France
e-mail: brancour@mons.inra.fr

growth). An attractive trait for the comparison of genotypes for their growth is the canopy height: this trait is simple to measure, and canopy height at maturity is strongly positively correlated with aboveground biomass (between 0.82 and 0.93 depending on year and harvest date) [15]. Monitoring plant height during the plant growth cycle could thus inform us about biomass production. As the potential biomass yield of a crop can be defined by the product of growth rate and growth duration [16], measuring the growth rate and duration of canopy height of *Miscanthus* clones may allow estimation of their potential biomass production. Emergence date is another trait of interest in *Miscanthus*, as this species is frost-sensitive. An early emergence exposes the crop to late frosts, in spring, which may kill the first shoots, and affect biomass production.

To characterise the variability in aboveground biomass growth of different *Miscanthus* clones and the effect of harvest date and year on this variability, we studied crop emergence and canopy height growth characteristics, particularly rates, durations, and maximum values. One question was how to best estimate these variables to allow comparison among clones. A function that takes time into account can estimate these variables. Various equations describe the non-linear sigmoid relationship between growth and time [17, 18]. Miguez et al. [19] used a logistic curve to describe biomass yield as a function of thermal time in *M. x giganteus*. However, it seems they chose this function arbitrarily. Very few studies on other plants have compared different functions to describe growth. Robert et al. [18] and Khamis et al. [20] suggest observing the scatter of the residuals to test how well non-linear models fit. The value of the mean square error (MSE) is useful to test the model performance [20]. However, this measure can only compare functions that have the same number of parameters to estimate. Robert et al. [18] recommend comparing models using the Akaike information criterion (AIC), which formalises the parsimony principle and is a compromise between likelihood and the number of parameters. This criterion has the advantage of allowing direct comparison between models and accounting for the difference in parameter number.

Our aims were therefore to identify the ‘best’ function for describing the dynamics of crop emergence and of height growth for different *Miscanthus* clones, in order to estimate the corresponding parameters and to compare the clones accordingly. We considered one main hypothesis. We predicted that in *Miscanthus*, the best function would be specific of the trait under consideration.

Materials and Methods

Field Experiment

We planted a randomized complete block trial at Estrées-Mons, in France (49°53 N, 3°00 E) in spring 2007. It consisted of six

blocks, each with 21 clones on plots of 16 m². Clones of four species were planted: three *M. x giganteus* clones, 15 *M. sinensis* clones, two *M. sacchariflorus* clones, and one *M. floridulus* clone. Table 1 gives the code, name, and species of each of them. Most of the species names corresponded to those given at the ornamental plant nurseries where the rhizomes came from, excepting for those we bought from U Jorgensen (University of Aarhus in Denmark), from Nordic Biomass (Denmark) and from ADAS (UK). We considered H8, a hybrid between *M. sacchariflorus* and *M. sinensis* [21], as a clone of *M. x giganteus*. The clone named “Flo” belonged to *M. floridulus* species, according to the ornamental plant nursery where we bought it (Table 1). Using the genetic similarity based on 328 AFLP markers, the 21 clones belonged to several distinct clusters [22]. All the clones of *M. sinensis* clustered together. The two clones of *M. sacchariflorus* were together. For the remaining clones, it appeared that the clone of *M. floridulus* was close from those of *M. x giganteus*. For more detailed descriptions of plant material characteristics, see Zub [22] and Zub et al. [15]. The “autumn harvest” always occurred on the three same blocks in autumn, at the end of the growing period. The winter harvest always took place on the three remaining blocks at the end of winter, at over-maturity. Each plot consisted of four rows of eight plants at a rhizome-planting density of two plants per m². There was a border row on either side of the plot using the same genotype, *M. sinensis* Malpartus, to edge all plots. A detailed description of the trial is given in Zub et al. [15].

In the second and third years after planting (spring of 2008 and 2009, March–April–May), we recorded the date of emergence in the field every 2–3 days. We then monitored growth height weekly during the vegetative growth period, on all plants in the plot (8 to 32 plants per micro-plot). We used canopy height as a predictor for aboveground biomass because it is a non-destructive, easy to measure trait and has a strong positive inter-genotypic correlation with aboveground yield [15]. For each plant, canopy height corresponded to the height from the ground to the ligula of the last ligulated leaf.

To monitor temperatures around the rhizomes at 20 cm depth, four thermistances were set up at various points in the trial area. An air temperature sensor, installed at 10 cm, determined the difference in temperature experienced by shoots and rhizomes. Records took place every 15 minutes. These data were useful to determine cumulative degree days each day.

Determination of Ploidy Level

As the ploidy level was not available for most of the clones coming from the ornamental plant nurseries, it was measured by flow cytometry using nuclear suspensions of leaves from our field cultivated plants. To isolate and stain nuclei,

Table 1 The 21 *Miscanthus* clones used in the trial: code, species, clone name, ploidy level, and acquisition

Code	Species	Name	Ploidy level	Acquired from	Cited by
Flo	<i>M. floridulus</i>	Floridulus	3x	Chombart, France	
GigB	<i>M. x giganteus</i>	Britain	3x	ADAS	[24]
GigD	<i>M. x giganteus</i>	Denmark	4x	Aarhus University of Denmark	
H8	<i>M. x giganteus</i>	Hybrid 8	2x	Nordic Biomass	[21]
Aug	<i>M. sinensis</i>	August Feder	2x	Chombart, France	
Fla	<i>M. sinensis</i>	Flamingo	2x	Chombart, France	
Grz	<i>M. sinensis</i>	Graziella	2x	Chombart, France	
Mal	<i>M. sinensis</i>	Malepartus	2x	Chombart, France	
Rot	<i>M. sinensis</i>	Rotsilber	2x	Chombart, France	
Sil	<i>M. sinensis</i>	Silberspinne	2x	Chombart, France	
Str	<i>M. sinensis</i>	Strictus	2x	Chombart, France	
Fer	<i>M. sinensis</i>	Ferner Osten	2x	Bruckeveld, Belgium	
Her	<i>M. sinensis</i>	Herman Müssel	2x	Bruckeveld, Belgium	
Punk	<i>M. sinensis</i>	Punktchen	2x	Bruckeveld, Belgium	
Pur	<i>M. sinensis</i>	Purpurescence	2x	Bruckeveld, Belgium	
Yak	<i>M. sinensis</i>	Yaku Jima	2x	Bruckeveld, Belgium	
Gol	<i>M. sinensis</i>	Goliath	4x	Chombart, France	
GolD	<i>M. sinensis</i>	Goliath Danois	4x	Nordic Biomass	
H6	<i>M. sinensis</i>	Hybrid 6	3x	Aarhus University of Denmark	[21]
H5	<i>M. sacchariflorus</i>	Hybrid 5	4x	Aarhus University of Denmark	[21]
Sac	<i>M. sacchariflorus</i>	sacchariflorus	2x	Chombart, France	

we chopped about 40 mg of fresh leave tissue with a razor blade in a plastic Petri dish containing 2 ml of buffer [23] and 16 μ l of a filter sterilized solution of 4',6-Diamidino-2-Phenylindole (DAPI, Sigma D-9542) at a concentration of 250 μ g.ml⁻¹. Measurements were performed on a Partec PAS-II flow cytometer (Partec GmbH, 4400 Münster, Germany). Leaves of *Pisum sativum* cv Bilboquet corresponded to the internal standard. To estimate the ploidy level, we compared the position of peak of a given sample on a histogram with the reference of plants with known ploidy level. The clone H5 was tetraploid [21] and the clone of *M. x giganteus* acquired from ADAS (GigB) was triploid [24]. No reference was available for the diploid clones, but the results showed differences clear enough among the peaks to separate the diploids from the triploids.

Statistical Methods

Estimation of Parameters of Emergence and Growth Dynamics

Based on the literature, we compared three functions for describing the dynamics of emergence and growth in *Miscanthus*:

- Logistic function with three parameters (a, b and c) assuming a lower asymptote of 0 (L3)

- Logistic function with four parameters (a, b, c and d) assuming a lower asymptote other than 0 (L4)
- Gompertz function

Based on the work of Robert et al. [18], who modelled the grain-filling period of winter wheat, we characterised the different emergence and growth dynamics obtained with the following three relative measurements: (i) upper asymptote corresponding to maximum emergence percentage and maximum height at maturity (respectively Emax and Hmax), (ii) maximum emergence rate (i.e., speed) and maximum growth rate (RateE and RateG), and (iii) time required to reach 0.95 of the upper asymptote (DurE and DurG).

For emergence, the Emax parameter was more representative of establishment success in the first year than of differences between clones. The parameters for emergence rate and time required to reach upper asymptote were strongly correlated with the environment of the rhizome. We therefore added a variable: time required to reach 50 % of final emergence. We call it date of emergence (DatE) and it reflected the relative earliness of each clone at emergence. In the context of looking for the variables that contribute to biomass yield, we used the DatE rather than Emax, RateE and DurE.

The equations for the functions concerning maximum height, growth rate, growth duration, and emergence date are presented for each function in Table 2.

For crop emergence, the thermal time was expressed as cumulative degree days since the last negative soil temperatures

Table 2 Equations of the three functions used to estimate emergence and plant height growth of *Miscanthus* and references of authors who applied these functions on other plants

	Function	Growth rate RateG (cm.dd ⁻¹)	Growth duration DurG (dd)	Max value Hmax (cm)	Emergence date DatE (dd)
Gompertz (G) [18]	$f(t) = a + be^{-e^{(d-t)}}$	$\frac{bc}{e}$	$\frac{d - \ln\left(\frac{b}{0.95b - 0.05a}\right)}{c}$	$a + b$	$e^{(\ln d - \ln c)}$
3-parameters logistic (L3) : a, b and c [18]	$f(t) = \frac{a}{1 + e^{(b-t)}}$	$\frac{ac}{4}$	$\frac{1}{c} \left(b - \ln \frac{1}{19} \right)$	a	$e^{(\ln b - \ln c)}$
4-parameters logistic (L4) : a, b, c and d [17]	$f(t) = \frac{a}{1 + e^{(b-t)}}$	$\frac{ac}{4}$	$\frac{1}{c} \left(b - \ln \left(\frac{0.05(a+d)}{0.95a - 0.05d} \right) \right)$	$a + d$	$e^{(\ln b - \ln c)}$

dd, degree days

(*Miscanthus* rhizomes cannot emerge when the temperature is below 0 °C). For canopy height growth, thermal time was expressed as cumulative degree days since emergence. We calculated the sum of daily temperatures with the following formula, in particular already used for maize, sorghum, peas, and wheat [25]. In our study, we used the optimum temperature (Topt) of 32 °C and the maximum temperature (Tmax) of 45 °C obtained with sugar cane [26], and the corresponding maximum growth rate (Rmax) of 0.04691 dd⁻¹:

$$TT = \frac{1}{R_{max}} * \frac{T_{max} - T}{T_{max} - T_{opt}} * \left(\frac{T}{T_{opt}} \right)^{\frac{T_{opt}}{T_{max} - T_{opt}}} \quad (1)$$

where TT is the daily thermal time experienced by the plants, T is the mean daily temperature, Tmax is the maximum temperature for sugar cane and Topt the optimum temperature for sugar cane development. The base temperature defined from this equation is 6.8 °C.

For each non-linear model considered, the following equation described the curve of emergence and canopy height growth of each genotype in each plot:

$$Y_i = f(TT_i, \theta) + \varepsilon_i \quad (2)$$

where Y_i is either the % emergence on the plot i or the mean height of the plants in the plot i ; TT is an independent variable representing the thermal time as previously defined; and θ is a vector of parameters to be estimated (3 or 4); ε_i is the error term.

Thus, to describe the relationships between emergence percentage and thermal time and between mean height in cm of a plot and its age in thermal time, we adjusted the data by three non-linear functions using the using the PROC NLIN non-linear regression procedure of the SAS system [27]. We obtained 63 parameter vectors per harvest date and per year.

Determination of the Best Function for Describing the Emergence and Plant Growth Dynamics

We used several statistical procedures to test the fit of the different non-linear models: observation of residual curves, comparison between the model mean square errors (MSE),

and comparison between the model performance indices according to the Akaike criterion [28].

Determination of Relationship Between Parameters Estimated for Emergence, Plant Growth, and Height at Maturity

For each harvest date and each year of growth, we determined the intergenotypic coefficients of correlation between mean values for each clone of final canopy height at maturity (Hmax), mean emergence date (DatE), mean growth rate (RateG), and mean growth duration (DurG). We also estimated the intergenotypic coefficients of correlation between emergence and each of the growth parameters (growth rate, growth duration, and final canopy height).

Estimation of the Effects of Clone, Harvest Date, and Year of Growth on Parameters of Emergence and Growth Dynamics

We performed an analysis of variance using a fixed-effects model to study the effects of clone, harvest date, and year of growth on the parameters derived from the emergence and growth dynamics using the SAS GLM procedure [27]. The ANOVA model was as follows:

$$Y_{ijkl} = \mu + g_i + a_l + h_j + ga_{il} + gh_{ij} + ah_{jl} + b(h)_{kj} + gah_{ilj} + gb(h)_{ikj} + ab(h)_{lkj} + \varepsilon_{ijkl}$$

where μ is the general mean of a given Y parameter studied for the clone i grown in block k for harvest date j during the year l ; g_i is the clone main effect; a_l is the year main effect (corresponding to the crop age and environmental effects such as climate, soil...); h_j is the harvest date main effect; $b(h)_{kj}$ is the effect of block k for the harvest date j ; ga_{ilj} is the interaction between clone and year; gh_{ij} is the interaction between clone and harvest date; ah_{jl} is the interaction between year and harvest date; gah_{ilj} is the interaction between clone, year, and harvest date; $gb(h)_{ikj}$ is the interaction between clone and block for harvest date; and $ab(h)_{lkj}$ is the interaction between year and block for harvest date. ε_{ijkl}

corresponds to the model residual error. We considered all terms as fixed effects.

Species and ploidy-level effects then partitioned the clone effect and its various interactions with the effects of harvest date and year as follows:

$$g_i = e_m + p_n + g'_i$$

where e_m is the species effect and p_n the ploidy level effect within the species. g'_i is the remaining clone effect (not explained by species or ploidy level).

The percentage partitioning the sum of squares of the clone effect by each of these newly introduced effects then estimated the efficiency of this partitioning by the successive effects of species and ploidy level.

To determine which factors have the most determinant effects, we took into account the highest F values for the effects that showed the greatest variability.

Results

Ploidy level is a very important factor to consider when analysing productivity and related traits. Hence, results on the determination of ploidy level by flow cytometry will be presented first. Secondly, we determined which of the non-linear models tested best suited for emergence date and growth dynamics. We then analysed the effects of year, species and ploidy level on observed differences among clones for emergence date on the one hand, and for growth parameters on the other. Lastly, we analysed the relationships between emergence date and growth parameters.

Determination of Ploidy Levels by Flow Cytometry

To estimate the ploidy level of the 21 studied *Miscanthus* clones, we compared the peak position for each clone sample on the histogram with the peak of a reference clone with known ploidy level. Clones could thus be classified in three clusters corresponding to 2x, 3x, and 4x ploidy levels: (i) clones with peaks overlaying the peak of the tetraploid H5 clone were declared tetraploid (Fig. 1c), (ii) clones overlaying the peak of the clone of *M. x giganteus* acquired from ADAS (GigB), were considered as triploid (Fig. 1b), and (iii) clones with peaks corresponding to less fluorescence intensity and that clearly separated from the triploid and tetraploid peaks (Fig. 1d shows a mixture of diploid, tetraploid and internal standard) were considered as diploid (Fig. 1a). In total, 14 were found to be diploid (Table 1), which encompassed 12 *M. sinensis* (Aug, Fla, Grz, Mal, Rot, Sil, Str, Fer, Her, Punk, Pur and Yak), one *M. sacchariflorus* (Sac), and one *M. x giganteus* (H8). Three clones were triploid (*M. x giganteus* (GigB), *M. sinensis* (H6) and *M. floridulus* (Flo)), and four clones were

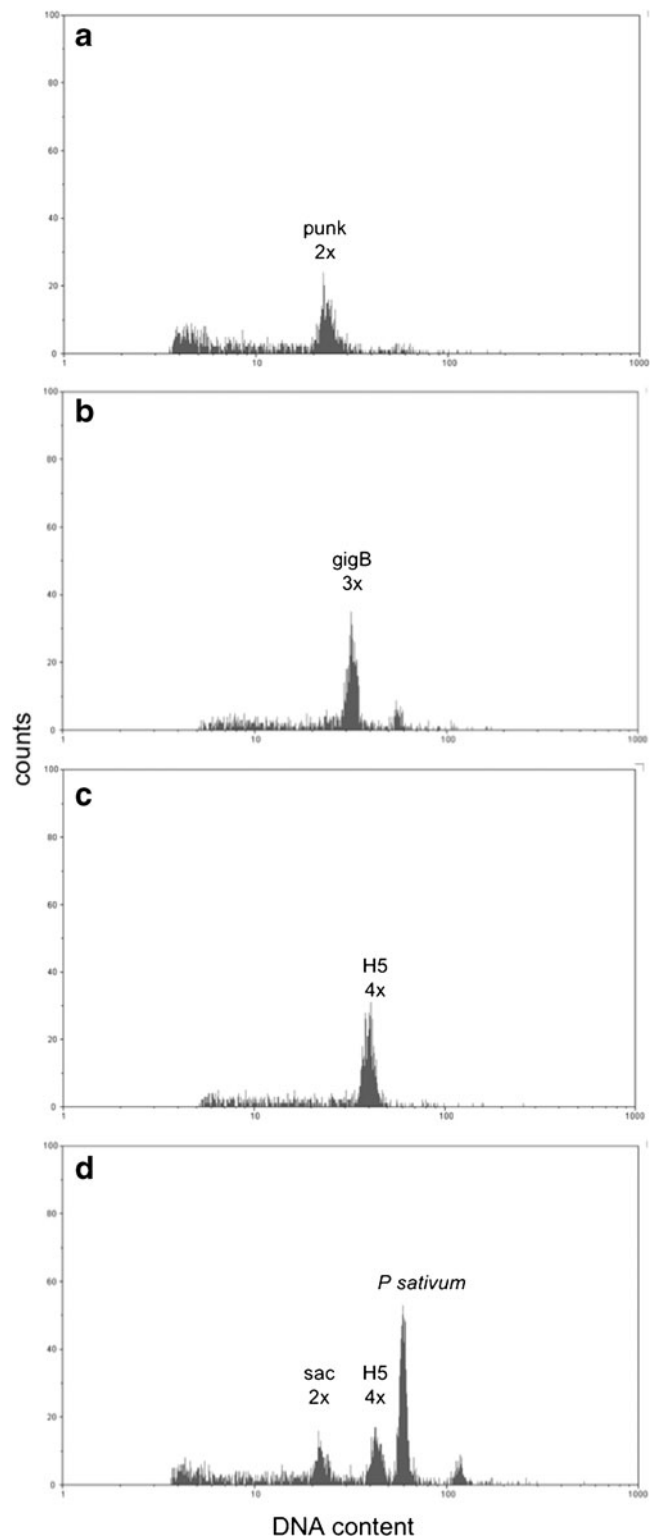


Fig. 1 Histograms of flow cytometric analysis that were used to determine nuclear DNA content in leaves of different *Miscanthus* clones. **a** A diploid plant of *M. sinensis* var Punktchen (Punk). **b** A triploid hybrid *M. x giganteus* (GigB). **c** A tetraploid hybrid of *M. sacchariflorus* (H5). **d** A mixture of a diploid plant *Miscanthus sacchariflorus* (Sac), a tetraploid *M. sacchariflorus* (H5), and the internal standard *Pisum sativum*

tetraploid clones (*M. sacchariflorus* (H5), *M. sinensis* (Gol and GolD) and *M. x giganteus* (GigD)) (Table 1). We then used this information about the ploidy level to perform further statistical analyses by species and ploidy level.

Best Models to Estimate Emergence Date and Growth Parameters

Three non-linear models were tested to describe the dynamics of the crop emergence and plant growth in each micro-plot for each year of growth and each harvest date. The scatter graphs of the residuals helped to detect possible deviations from the model. No heterogeneity of variance was observed for any model in any of the tested situations. The best models were those providing the lowest mean square error (MSE) and Akaike criterion (AIC) values (Table 3).

The Gompertz function best described emergence dynamics for the second and third years of development for both harvest dates, according to MSE and AIC (Table 3).

For growth dynamics, the L4 function was the only function that converged for all clones, at each harvest of each year. In contrast, the Gompertz function algorithm did not converge for data collected on 23 cases (a “case” correspond to one clone in one harvest date in one year) during the autumn harvest of the third year (Table 3). To a lesser extent, it also failed to converge with autumn harvest for both years of development. The function L3 failed to converge with autumn harvest in both years of development and winter harvest in the third year.

Emergence Date was Largely Influenced by the Crop Year

An analysis of variance was performed to analyse the effects of clone, harvest date, and year on emergence date (DatE) estimated with the Gompertz function (Table 4). The variance model explained 93 % of total variation in emergence date,

with a low residual coefficient of variation (CV) of 12 % (Table 4). The main effects of clone and year and the clone \times year and clone \times harvest date interactions were significant (at the 0.05 probability level). Grouping the clones by species and then by ploidy level resulted in partitioning 52 % of the total variation due to the clone effect. Similarly, partitioning the interaction between clone effect and year effect by species and then by ploidy level explained up to 70 % of the interaction.

Among the main effects, the year effect was more variable than the others (F value of 582 in Table 4). This was mainly due to an earlier emergence the third year compared to the second year (106 dd compared to 154 dd respectively).

Regarding the interactions, the clone \times year interaction was slightly higher than the clone main effect (Fig. 2a). Emergence date ranged between 138 dd for Flo and 169 dd for Punk in the second year, and from 79 dd for Punk to 146 dd for GigB in the third year. Grouping the clones by species explained a high part of this interaction (63 %). The interaction was clearly due to the earlier emergence dates the third year regardless of the harvest date (although the triple interaction clone \times year \times harvest date was significant): most *M. sinensis* clones, one *M. sacchariflorus* clone (Sac) and one *M. x giganteus* clone (H8), less distant to the bisecting line, contributed the most to this interaction (with circle on Fig. 2a), whereas the *M. floridulus* clone, two *M. x giganteus* clones (GigB and GigD), one *M. sacchariflorus* clone (H5) and two *M. sinensis* clones (Fer and Fla) (Fig. 2a), showed similar emergence dates in the second and third years for both autumn harvest and winter harvest.

Finally, there was a significant interaction between the effects of clone and harvest date, which was mainly due to the third year of growth (Fig. 2b). In the second year, the different clones broadly showed the same emergence date for both harvest dates (Fig. 2b). In contrast, five clones stood out in the third year: one *M. sinensis* clone (Fla) showed a much later emergence date with winter harvest than

Table 3 Comparison of three functions for estimating emergence and growth dynamics in *Miscanthus* in the second and the third year of growth at two harvest dates

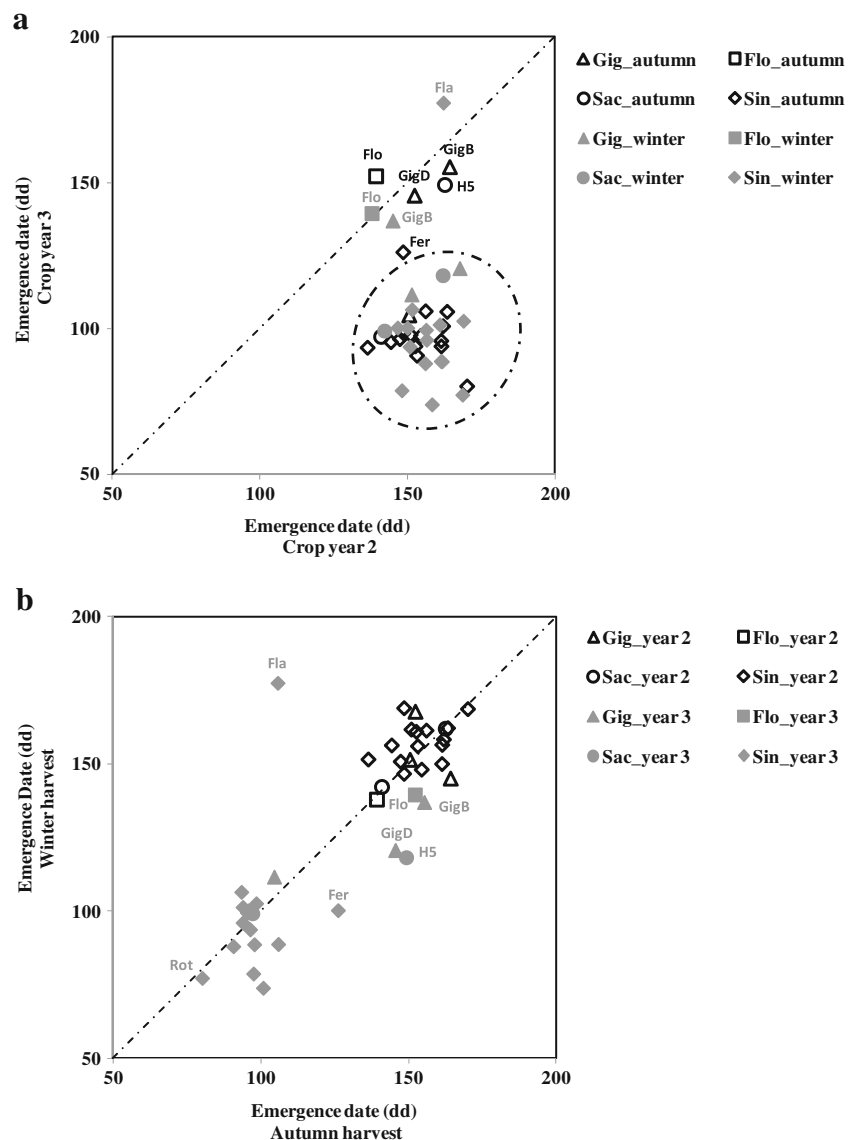
Year	Harvest date	Function	Emergence			Canopy height growth		
			MSE	AIC	NC	MSE	AIC	NC
A2	Autumn	Gompertz	0.002	−3,660	0	203	44,100	4
		Logistic 3 param	0.006	−2,962	0	219	44,789	1
		Logistic 4 param	0.016	−2,605	0	220	44,773	0
	Winter	Gomp	0.001	−3,678	1	230	53,152	0
		L3	0.006	−2,882	0	245	53,767	0
		L4	0.029	−2,191	0	245	53,755	0
A3	Autumn	Gomp	0.002	−2,919	0	453	80,574	23
		L3	0.010	−2,191	0	140	65,008	7
		L4	0.021	−2,034	0	140	64,991	0
	Winter	Gomp	0.003	−2,953	0	373	91,303	6
		L3	0.017	−2,096	0	155	77,753	0
		L4	0.026	−2,024	0	155	77,741	0

MSE, mean square error ; AIC, Akaike criterium ; NC, number of no convergence

Bold type: the most favorable statistical criteria for each year and each harvest date

Fig. 2 Emergence date (in degree days, dd) as a function of year (**a**) and harvest date (**b**). Root mean square error=15 dd.

a The clone \times year interaction was due to the fact that (i) two clones of *M. x giganteus*, one of *M. floridulus*, one of *M. sacchariflorus* and two of *M. sinensis* had similar emergence dates in their second and third years of growth, while (ii) the other clones emerged much earlier in the third year. **b** The interaction between clone effect and harvest date in the third year was due to the later emergence of *M. sinensis* Fla after winter harvest than after autumn harvest, and to the earlier emergence of one clone of *M. x giganteus*, one of *M. sacchariflorus* and two of *M. sinensis* after winter harvest than after autumn harvest



with autumn harvest and one clone of *M. sacchariflorus* (H5), two of *M. sinensis* (Fer and Rot) and one of *M. x giganteus* (GigD) showed emergence dates at least 26 dd earlier with winter harvest than with autumn harvest (Fig. 2b).

Note that the different species involved different numbers of clones, as did the different ploidy levels, which could introduce a bias into the results. However, the differences observed among species and ploidy levels were fairly marked, so we can assume that the broad trends among species and ploidy levels can be identified.

Crop Growth was Highly Influenced by the Year, Species, and Ploidy Level within the Clone Effect

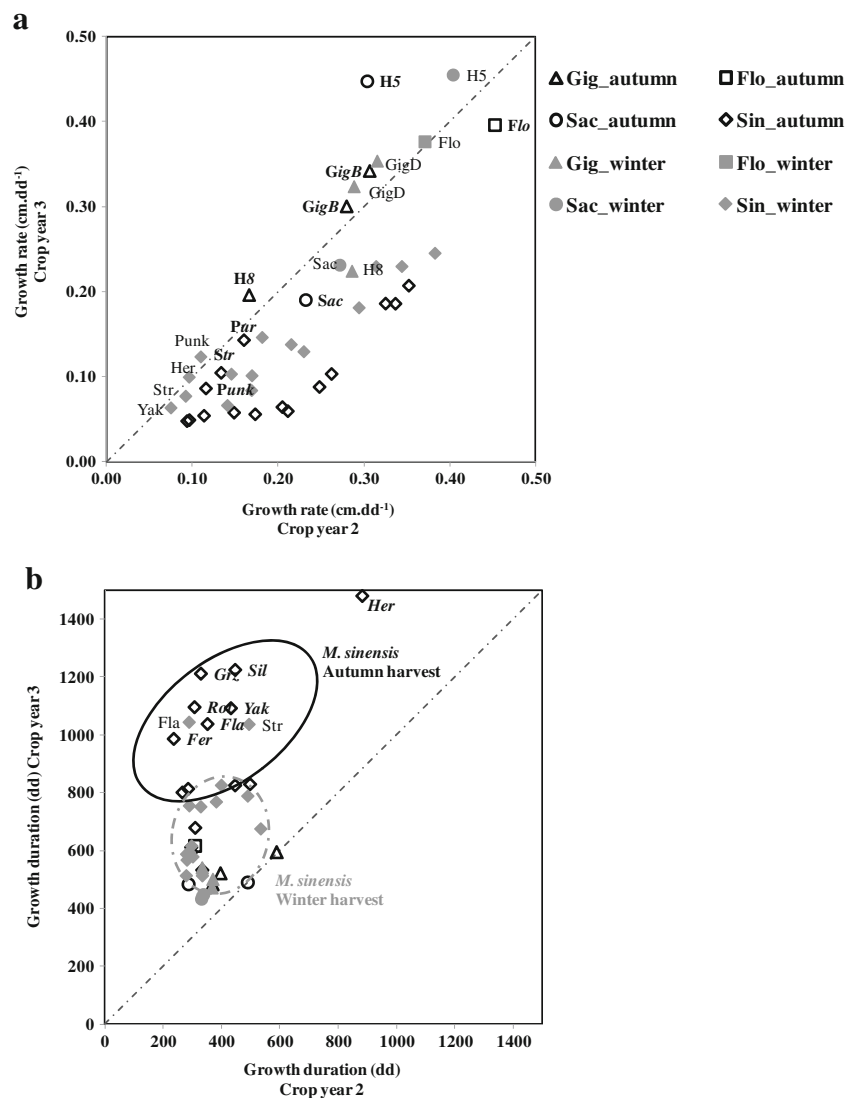
Table 4 shows the results of the analysis of variance of the height growth dynamics parameters (growth rate, growth duration, and final canopy height) of *Miscanthus* estimated

from function L4. Since most of the effects and the corresponding interactions were significant (at the 0.05 probability level), only the most important ones regarding the F values are pointed out here.

Firstly, the year had the greatest main effect (compared to harvest date and clone), whichever growth parameter was considered: the mean growth rate was slower in the third year than in the second year (0.18 cm.dd^{-1} compared to 0.23 cm.dd^{-1} on average), the growth duration in dd was almost twice as long in the third year than in the second year (mean value of 717 dd and 362 dd respectively), and finally the plants were 30 cm taller in the third year.

Secondly, the clone main effect also explained a lot of the variability in height growth rate and final canopy height and, to a lesser extent, the variability in duration of growth. There was an almost five-fold difference in growth rate between the extremes (Fig. 3a), which ranged from 0.07 cm.dd^{-1} for

Fig. 3 a Comparison of growth rates (in centimetres per degree days, $\text{cm}\cdot\text{dd}^{-1}$) for the two harvest years. Root mean square error= 0.03 dd^{-1} . The clone \times year interaction was due to mean growth rates per clone being generally slower in the third year of growth for clones of *M. sinensis*, and being similar in both years for clones of *M. x giganteus*, *M. floridulus* and *M. sacchariflorus*. **b** Growth (in degree days, dd) was longer in the third year of growth than in the second year. Root mean square error= 79 dd



clone Yak to $0.41\text{ cm}\cdot\text{dd}^{-1}$ for clone Flo. This variability among clones was largely due to species and ploidy level (91 %), which successively partitioned 54 % and 37 % of the variability in growth rate (Table 4). Duration of growth also varied among clones, mainly because of one *M. sinensis* clone (Her) which had a significantly ($P<0.05$) longer growth duration than the other clones (910 dd on average against 530 dd respectively) (Fig. 3b). Lastly, final canopy height varied threefold between the smallest clone, Yak (65 cm), and the tallest, H5 (220 cm). This variability among clones was largely due to species (56 %) and ploidy level (37 %). Taking into account the means assessed at the species level, the *M. floridulus* clone was the tallest, with a mean height of 204 cm, while the clones of *M. x giganteus* and *M. sacchariflorus* were significantly smaller, with a mean final canopy height of 175 and 172 cm respectively. The *M. sinensis* clones were significantly smaller, with a mean height of 105 cm. The *M. sinensis* 2x (*M. sin_2x*) clone was 45 % smaller than *M. sin_3x* and *M. sin_4x*, and

the *M. sacchariflorus* 2x (*M. sac_2x*) clone was 45 % smaller than *M. sac_4x*.

With regard to the interactions, there was a strong interaction between the effects of clone and year for all growth parameters, in contrast to the interaction of clone with harvest date (Table 4). For duration of growth, for instance, this interaction was mainly due to species, which accounted for 60 % of the corresponding variation. The *M. floridulus* clone and the *M. sinensis* clones showed growth duration almost twice as short in the second year (319 dd and 357 dd respectively) as in the third year (526 and 809 dd respectively). By contrast, the mean growth duration of the *M. sacchariflorus* and *M. x giganteus* clones was similar between the two years (Fig. 3b).

Finally, the interaction harvest date \times year affected mostly growth duration and final canopy height. The 9 % difference in duration of growth between autumn and winter harvests in the second year was not significant but duration of growth was significantly longer (22 %) for autumn

harvest than for winter harvest in the third year of growth (Fig. 3b). Final canopy height did not significantly differ between the two harvest dates in the second year (110 cm), but in the third year plants were taller in winter harvest than in autumn harvest (150 cm versus 137 cm respectively).

Inter-genotypic Correlations between Emergence Date and Growth Parameters

We identified the main traits contributing to the final canopy height by studying the inter-genotypic correlations with emergence date and growth parameters. We calculated these intergenotypic correlations from the means per year and per harvest date for each clone, on the one hand, and the means of the different *M. sinensis* clones on the other hand (Table 5).

Firstly, there was a significant positive correlation between final canopy height (Hmax) and emergence date irrespective of harvest date during the third year of growth (Table 5). Four clones (Flo, GigB, GigD and H5) strongly contributed to the correlation (Fig. 4): they stood out from the others by their late emergence dates and tall final canopy height (over 200 cm). But this correlation became non-significant (at the 5 % probability level) if we considered only the clones of *M. sinensis*.

Secondly, a significant positive correlation was found between final canopy height and growth rate irrespective of harvest date and growth year. Clones were taller when their growth rate was higher. This correlation was stronger in the third year than in the second year, irrespective of harvest date. This was the case for all the clones and also for the clones of *M. sinensis* (Table 5).

In addition, there was a significant negative correlation between final canopy height and duration of growth (Table 5). This was observed for winter harvest in the second year and above all for both autumn and winter harvests in the third year. This negative correlation was also found in the third year between species and ploidy level, and within species *M. sinensis*.

Lastly, we found a significant negative power relationship between growth rate and duration, irrespective of harvest date and year (Fig. 5). The faster the growth, the shorter its duration was.

Discussion

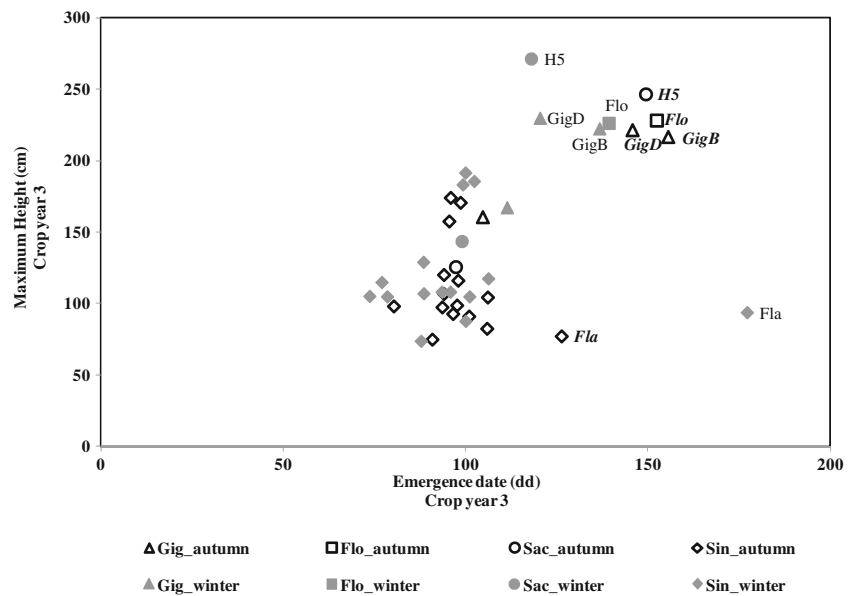
In *Miscanthus*, the use of the Akaike criterion, the mean square error and a graphical analysis of residuals led us to choose (i) the Gompertz function as the most efficient for describing the emergence data, and (ii) the 4-parameter logistic function as the best for describing canopy height growth. We have also shown the effects of clone and year on

Table 5 Comparison for Pearson correlation coefficients of all clones studied (A) with *M. sinensis* clones (B) for their emergence date (DatE), final canopy heights (Hmax), growth rate (RateG) and growth duration (DurG) at the autumn and winter harvests of the second and third years

		A All clones						B Only <i>M. sinensis</i> clones					
		DatE		Hmax		RateG		DurG		DatE		Hmax	
		Year 2	Year 3	Year 2	Year 3	Year 2	Year 3	Year 2	Year 3	Year 2	Year 3	Year 2	Year 3
Autumn harvest	DatE												
	Hmax	ns	0.68							ns	ns		
	RateG	ns	0.70	0.8	0.96					ns	ns	0.80	0.93
	DurG	ns	Ns	ns	-0.66	-0.5	-0.72			ns	ns	ns	-0.62
Winter harvest	DatE												
	Hmax	ns	0.34							ns	ns		
	RateG	ns	0.35	0.90	0.97					ns	ns	0.95	0.94
	DurG	ns	Ns	-0.27	-0.68	-0.61	-0.77			ns	ns	ns	-0.52

ns : non significant values

Fig. 4 In the third year of growth, the later a clone emerged, the greater the final canopy height was, irrespective of harvest date. Root mean square error (RMSE)=8 cm for height and RMSE=15 dd for emergence date



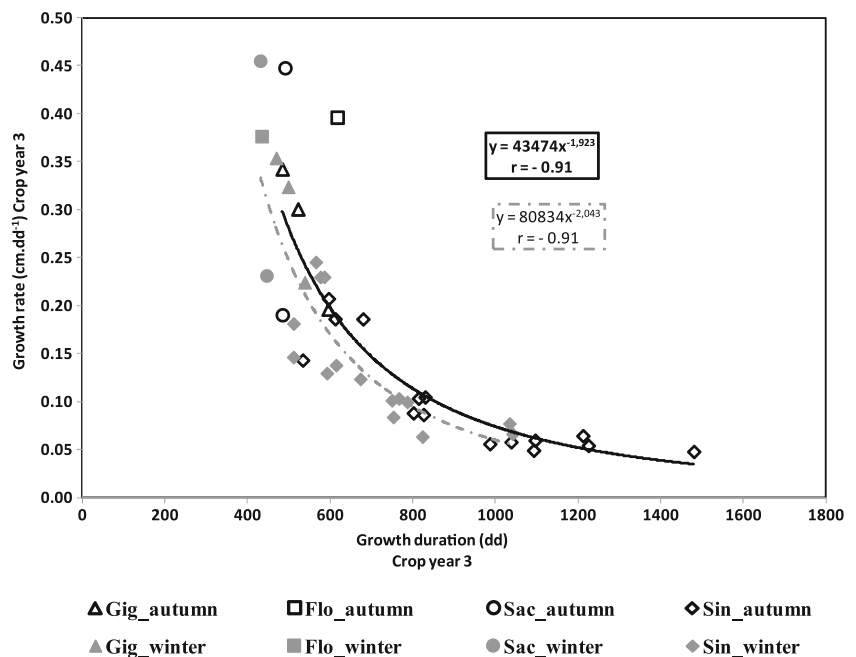
the different variables estimated by these functions, and also the interactions with the clone factor, the most important of these being clone \times year. The clone effect was quite strong due to species and then, to a lesser extent, to ploidy level; the species \times year effect strongly partitioned the clone \times year interaction.

Which Functions for which Trait?

No previous study has been published that compares functions to describe aboveground growth in *Miscanthus*. On one hand, Miguez et al. [19] used the logistic curve to describe *Miscanthus* yield trends over the course of its

growth cycle, but did not justify their choice. Robert et al. [29] compared the Gompertz, Weibull, and 3- and 4-parameter logistic functions for studying varieties of winter wheat for grain-filling dry matter as a function of time. They concluded that the 3-parameter logistic curve was best, based on the AIC criterion and analysis of residuals as an indicator of the quality and performance of the models. Khamis et al. [20] reached the same conclusion when comparing 12 models on palm-oil yield dynamics over the years following planting. On the other hand, Mohanty et al. [30] considered that rice emergence was best represented by a Gompertz function, based on an assessment of the fit with the root mean square error (RMSE). In accordance with all

Fig. 5 Negative power relation observed between duration of growth (in degree days, dd) and growth rate (in centimeters per dd), for the third year of growth. Root mean square error (RMSE)=79 dd for growth duration and RMSE=0.03 dd⁻¹ for growth rate. It was identical for the two harvest dates



these studies, our results confirm the superiority of the logistic function for studying *Miscanthus* plant growth over time, and the superiority of the Gompertz function to analyse crop-emergence dynamics.

The convergence problems that arose particularly with the Gompertz function and the 3-parameter logistic function for growth dynamics may be due to the shape of the curves. The 4-parameter logistic function considers a period during which plant height varies little, followed by an acceleration of growth. The Gompertz and 3-parameter logistic functions, which have no lower asymptote, do not represent this phenomenon very well [31].

Species Comparisons for their Growth Dynamics

While the literature describes relatively well biomass production during the growth cycle of *M. x giganteus* [19, 21, 32], little is known about its genotypic variability. Riche et al. [33] compared the biomass output of four *M. x giganteus* clones, one *M. sacchariflorus* clone, five *M. sinensis* clones and five hybrids of *M. sinensis* over 11 consecutive growth cycles in England, but they did not compare the growth dynamics during the plants' annual cycle. Fifteen clones have also shown variability in plant height at maturity during the third year of the crop in five European sites [21] but there were no dynamic measurements taken during the growth period. Jorgensen et al. [34] showed the height growth dynamics of the same clones as above, in Denmark, but the comparisons of the clones corresponded only to the end of the growth period.

By studying the growth during two annual growth cycles, our study revealed that genotypic differences observed in plant height at maturity are due, at least partly, to difference in emergence date and growth rate (Fig. 4).

Effects of Clone, Year, and Harvest Date

Clone differences in emergence date, growth rate, and growth durations could be due to differences in the base temperatures for each clone. Farrell et al. [10] revealed differences in base temperatures between four *Miscanthus* clones of three species. *M. x giganteus* and *M. sacchariflorus* (Gig2 and H5) had a base temperature of 8.5 °C, which is equivalent to an hybrid between two *M. sinensis* (H9) but which is significantly higher than the 6 °C base temperature of a hybrid selected from a population of *M. sinensis* (H6). In our study, we assigned an identical base temperature for all the clones, of around 6.8 °C during the active growth phase (close to the base temperature observed in H6 [10]). These differences in base temperature could lead to a 1.7 °C difference in daily cumulative temperature, which would add up to a 150 dd overestimation in *M. x giganteus* and *M. sacchariflorus* for the emergence period of

February–March–April, and a 300 dd overestimation for the active growth period from April to September. However, this should be nuanced, as final-height differences between clones were strongly correlated with yield differences (data published in [15]).

M. sinensis clones had a slower growth rate during the third year of growth than during the second year, which might be due the higher sensitivity to water stress of this species compared to *M. x giganteus* and *M. sacchariflorus*. In the third year of growth, the plants had to cope with water stress from late July onwards, whereas no water stress occurred during the second year. *M. sinensis* is known to close its stomata more quickly when it lacks water in order to limit evapotranspiration [35], which increases its tolerance to water stress. In counterpart, this response could also slow its growth by reducing photosynthesis.

Further, the behaviour differences between clones varied between the second and third years after an autumn harvest, but not after a winter harvest: we found no significant differences in growth duration in the second year, but in the third year it was significantly longer with autumn harvest (22 %) than with winter harvest. Yet emergence dates were virtually identical for the two harvests, in both years (Fig. 2). It might be due to the fact that the winter harvest micro-plots flowered (or their panicles emerged) earlier than with an autumn harvest, particularly with *M. sinensis* [15].

Options for Genetic Improvement

The inter-species hybrid between *M. sinensis* and *M. sacchariflorus* (clone H8), which is of medium height, suggests an ideotype for genetic improvement. A high final plant height (Hmax) combined with a rapid growth rate and late emergence date could be the criteria for selecting most biomass productive plants at an early stage. The clones which associated late emergence dates, fastest growth, and tallest plants were mainly clones of *M. floridulus*, and of the *M. x giganteus* inter-species hybrid. This hybrid is actually the main source currently used for biomass production.

Nevertheless, the canopy height variable might be preferable to early emergence and growth rate, because it is by far the easiest to estimate. However, Hmax varied widely within the *M. sinensis* species, but not so much within *M. x giganteus*. These results are in accordance with Clifton-Brown et al. [21], who demonstrated the low variability in aboveground development (number of stems and plant height at end of growth period) among four clones of *M. x giganteus*. Vonwuhlich et al. [5] and Hodgkinson et al. [6], using isozymes, showed that *M. x giganteus* has a low genetic variability, suggesting that there is little room for genetic improvement in this species. Creating new hybrids would therefore be a significant breeding avenue to explore. It would increase genetic variability for

traits of interest such as final plant or canopy height, while making use of the variability found within the species *M. sinensis*, as demonstrated from the small sample of 15 clones tested in our study.

In the *M. floridulus* species, some native clones appeared as highly productive in Chinese conditions [36]. Nevertheless, using 328 AFLP markers to measure the genetic similarity between our 21 clones, we found that the clone of *M. floridulus* that we studied was genetically close to *M. x giganteus* [22]. Therefore, this clone might be a hidden cv Floridulus from the *M. x giganteus* species. It appears that *M. floridulus* needs more investigation to be considered as a genetic improvement option for European conditions.

Conclusion

Our study has shown that *Miscanthus* vary in their emergence dates and growth dynamics as estimated by the Gompertz and 4-parameter logistic functions, respectively. We have also found that to obtain greater aboveground development, it would be useful to combine a late emergence date, a short duration of growth and a high growth rate. It seems that genetic improvement to increase aboveground biomass yield in *Miscanthus* should therefore focus on increasing the growth rate rather than increasing the duration of the growth cycle. This study also arose some perspectives to improve seasonal management of *Miscanthus* crops, as winter harvests cause growth to stop quicker than autumn harvests, especially in *M. sinensis*.

Earlier studies had shown a strong correlation between plant height and aboveground biomass [15, 21]. To find new selection criteria for the genetic improvement of *Miscanthus*, it might be useful to study the relationship between aboveground biomass and the emergence and growth parameters estimated in our study [15]. Some of those traits might also be useful when studying the effect of stress, such as water deficit and nitrogen limitation. Other criteria might also be worth considering such as stem numbers and earliness of flowering (or earliness of panicle emergence). After that, a focus on the heritability of the most relevant traits would provide insights on existing possibilities for genetic improvement at the intra-species level.

Acknowledgments The authors would like to acknowledge the financial help they received from the Picardie region (PEL project), and the help they received from P. Devaux and the Florimond-Desprez French Company for the ploidy level determination. They thank N. Robert, S. Arnoult, J. Le Gouis, and K. Chenu for their relevant comments on the manuscript. They also thank M.C. Mansard, R. Mendez-Guizoni, M. Leleu, and all the staff of the French experimental unit at INRA's Estrées-Mons for their technical work and their valuable assistance with crop management and measurements. They also thank U. Jorgensen, who provided three clones of *Miscanthus*.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution and reproduction in any medium, provided the original author(s) and source are credited.

References

- Lewandowski I, Schmidt U (2006) Nitrogen, energy and land use efficiencies of miscanthus, reed canary grass and triticale as determined by the boundary line approach. *Agr Ecosyst Environ* 112 (4):335–346
- Ceotto E (2008) Grasslands for bioenergy production. A review. *Agron Sustain Dev* 28:47–55
- Benbi DK, Brar JS (2009) A 25-year record of carbon sequestration and soil properties in intensive agriculture. *Agron Sustain Dev* 29:257–265
- Greef JM, Deuter M (1993) Syntaxonomy of *Miscanthus x giganteus* GREEF et DEU. *Angew Bot* 67:87–90
- Vonwuhlich G, Deuter M, Muhs HJ (1994) Identification of *Miscanthus* varieties by their isozymes. *J Agron Crop Sci* 172 (4):247–254
- Hodkinson TR, Chase MW, Renvoize SA (2002) Characterization of a genetic resource collection for *Miscanthus* (Saccharinae, Andropogoneae, Poaceae) using AFLP and ISSR PCR. *Ann Bot* 89(5):627–636
- Christian DG, Haase E (2001) Agronomy of *Miscanthus*. In: Jones M, Walsh M (eds) *Miscanthus for energy and fibre*. James and James, London, pp 21–45
- Heaton EA, Clifton-Brown J, Voigt TB, Jones MB, Long SP (2004) *Miscanthus* for renewable energy generation: European Union experience and projections for Illinois. *Mitig Adapt Strateg Glob Chang* 9(4):21–30
- Clifton-Brown JC, Lewandowski I (2000) Overwintering problems of newly established *Miscanthus* plantations can be overcome by identifying genotypes with improved rhizome cold tolerance. *New Phytol* 148(2):287–294
- Farrell AD, Clifton-Brown JC, Lewandowski I, Jones MB (2006) Genotypic variation in cold tolerance influences the yield of *Miscanthus*. *Ann Appl Biol* 149(3):337–345
- Zub HW, Brancourt-Hulmel M (2010) Agronomic and physiological performances of different species of *Miscanthus*, an important energy crop. A review. *Agron Sustain Dev* 30(2):201–214
- Clifton-Brown JC, Neilson B, Lewandowski I, Jones MB (2000) The modelled productivity of *Miscanthus giganteus* (Greef et Deu.) in Ireland. *Ind Crop Prod* 12:97–109
- Hastings A, Clifton-Brown J, Wattenbach M, Mitchell CP, Smith P (2009) The development of MISCANFOR, a new *Miscanthus* crop growth model: towards more robust yield predictions under different climatic and soil conditions. *GCB Bioenergy* 1:154–170
- Pogson M (2011) Modelling *Miscanthus* yields with low resolution input data. *Ecol Model* 222(23–24):3849–3853
- Zub HW, Arnoult A, Brancourt-Hulmel M (2011) Key traits for biomass production identified in different *Miscanthus* species at two harvest dates. *Biomass Bioenergy* 35(1):637–651
- Ritchie JT, Singh U, Godwin DC (1998) Cereal growth, development and yield. In: Tsuji GY et al (eds) *Understanding options for agricultural production*. Kluwer Academic, Dordrecht, pp 79–98
- Murray WA, Nunn PA (1987) A non-linear function to describe the response of %N in grain to applied fertilizer. *Asp Appl Biol* 15:219–225
- Robert N, Huet S, Hennequet C, Bouvier A (1999) Methodology for choosing a model for wheat kernel growth. *Agronomie* 19:405–417
- Miguez FE, Villamil MB, Long SP, Bollero GA (2008) Meta-analysis of the effects of management factors on *Miscanthus x*

- giganteus* growth and biomass production. *Agric For Meteorol* 148 (8):1280–1292
20. Khamis A, Ismail Z, Haron K, Mohammed AT (2005) Nonlinear growth models for modelling oil palm yield growth. *J Math Stat* 1 (3):225–233
 21. Clifton-Brown JC, Lewandowski I, Andersson B, Basch G, Christian DG, Kjeldsen JB, Jorgensen U, Mortensen JV, Riche AB, Schwarz KU, Tayebi K, Teixeira F (2001) Performance of 15 *Miscanthus* genotypes at five sites in Europe. *Agron J* 93(5):1013–1019
 22. Zub HW (2010) The ability of different *Miscanthus* species to produce biomass in a cropping environment in Northern France under two harvest dates. Thèse Université de Picardie Jules Verne, Amiens, p 189
 23. Bergounioux C, Perennes C, Miège C, Gadal P (1986) Male sterility effect upon protoplast division in *Petunia hybrida*. *Protoplasma* 130:138–144
 24. Hodkinson TR, Chase MW, Takahashi C, Leitch IJ, Bennett MD, Renvoize SA (2002) The use of DNA sequencing (ITS and trnL-F), AFLP, and fluorescent in situ hybridization to study allopolyploid *Miscanthus* (Poaceae). *Am J Bot* 89:279–286
 25. Yan W, Hunt LA (1999) An equation for modelling the temperature response of plants using only the cardinal temperatures. *Ann Bot* 84(5):207–214
 26. Keating BA, Robertson MJ, Muchow RC, Huth NI (1999) Modelling sugarcane production systems I. Development and performance of the sugarcane module. *Field Crop Res* 61(3):253–271
 27. SAS Institute (1990) SAS/STAT user's guide. Version 6, fourth ed. SAS Institute Inc., Cary NC, pp 893–997
 28. Akaike H (1974) A new look at the statistical identification model. *IEEE Trans Auto Control* 19:716–723
 29. Robert N, Berard P, Hennequet C (2001) Dry matter and nitrogen accumulation in wheat kernel. Genetic variation in rate and duration of grain filling. *J Genet Breed* 55:297–306
 30. Mohanty M, Painuli DK (2004) Modeling rice seedling emergence and growth under tillage and residue management in a rice–wheat system on a Vertisol in Central India. *Soil Tillage Res* 76(2):167–174
 31. Debouche C (1979) Présentation coordonnée de différents modèles de croissance. *Rev Stat Appl* 27(4):5–22
 32. Cosentino SL, Patane C, Sanzone E, Copani V, Foti S (2007) Effects of soil water content and nitrogen supply on the productivity of *Miscanthus x giganteus* Greef et Deu. in a Mediterranean environment. *Ind Crop Prod* 25(1):75–88
 33. Riche AB, Yates NE, Christian DG (2008) Performance of 15 different *Miscanthus* species and genotypes over 11 years. *Asp Appl Biol* 90:207–212
 34. Jorgensen U, Mortensen J, Kjeldsen JB, Schwarz KU (2003) Establishment, Development and yield quality of fifteen *Miscanthus* genotypes over three years in Denmark. *Acta Agric Scand B-S P* 53(4):190–199
 35. Clifton-Brown JC, Lewandowski I, Bangerth F, Jones MB (2002) Comparative responses to water stress in stay-green, rapid- and slow-senescing genotypes of the biomass crop, *Miscanthus*. *New Phytol* 154(2):335–345
 36. Zheng B, Feng XP, Ren J, Chen M, He Y, Huang Y, Wu J, Xia G, Huang J, Jiang D, Brancourt-Hulmel M (2010) The primary analysis of genetic diversity in *Miscanthus*. BIT's 3rd Annual World Congress of Industrial Biotechnology, Dalian, China, July 25–27, pp 357