

A Review on Miscanthus Biomass Production and Composition for Bioenergy Use: Genotypic and Environmental Variability and Implications for Breeding

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Abstract The lignocellulosic C4 perennial crop miscanthus and, more particularly, one of its species, *Miscanthus × giganteus*, are especially interesting for bioenergy production because they combine high biomass production with a low environmental impact. However, few varieties are available, which is risky due to disease susceptibility. Gathering worldwide references, this review shows a high genotypic and environmental variability for traits of interest related to miscanthus biomass production and composition, which may be useful in breeding programs for enhancing the availability of suitable clones for bioenergy production. The *M. × giganteus* species and certain clones in the *Miscanthus sinensis* species seem particularly interesting due to high biomass production per hectare. Although the industrial requirements for biomass composition have not been fully defined for the different bioenergy conversion processes, the *M. × giganteus* and *Miscanthus sacchariflorus* species, which show high lignin contents, appear more suitable for thermochemical conversion processes. In contrast, the *M. sinensis* species and certain *M. × giganteus* clones with low lignin contents were interesting for biochemical conversion processes. The *M. sacchariflorus* species is also interesting as a progenitor for breeding programs, due to its low ash content, which is suitable for the different bioenergy conversion processes. Moreover,

mature miscanthus crops harvested in winter seem preferred by industry to enhance efficiency and reduce the expense of the processes. This investigation on miscanthus can be extrapolated to other monocotyledons and perennial crops, which may be proposed as feedstocks in addition to miscanthus.

Keywords Miscanthus · Phenotypic and genotypic variability · Biomass production · Biomass composition · Breeding · Bioenergy use

Abbreviations

DM	Dry matter
ha	Hectare
Gig	<i>Miscanthus × giganteus</i> species
Sacc	<i>Miscanthus sacchariflorus</i> species
Sin	<i>Miscanthus sinensis</i> species
t	Ton

Introduction

Currently, climate change and fossil fuel resource depletion are major global concerns. To limit climate change, reduce greenhouse gas emissions, and replace fossil fuel resources, renewable energy sources must be developed. Many reports have shown that biomass crops are significant contributors as bioenergy sources for heat, electricity, or biofuel production through thermochemical or biochemical processes [1–5].

Currently, a wide range of crops are biomass production candidates for bioenergy use: perennial C4 crops, such as miscanthus (*Miscanthus*), switchgrass (*Panicum virgatum*), or sugarcane (*Saccharum officinarum* L.); perennial C3 crops, such as reed canary grass (*Phalaris arundinacea* L.), giant reed (*Arundo donax* L.), short-rotation poplar coppices (*Populus*), or willow (*Salix*); annual C4 crops, such as fiber

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sorghum (*Sorghum bicolor* L.) or maize (*Zea mays*); or annual C3 crops, such as triticale (*Triticum sativum*) [6–15].

Among the few studies that have compared several such crops, the C4 perennial crops have been highlighted as advantageous for sustainable biomass feedstock [6].

For sustainable crops dedicated to producing bioenergy, the following must be combined: (i) high biomass production per hectare in various climates, (ii) suitable biomass composition for various bioenergy conversion processes, and (iii) positive environmental footprint (lowest water requirement, lowest N, P, and K fertilization, low greenhouse gas emissions, low invasiveness, etc.). For these requirements, C4 crops appear more promising as bioenergy crops than C3 crops because they are more productive and exhibit efficient water and nitrogen use [16] and efficient sunlight interception [17]. Among these C4 crops, C4 perennial crops such as miscanthus and switchgrass are particularly interesting because they yield a better environmental footprint than annual crops [6]. Moreover, perennial crops could be used to cover marginal lands for biomass production where annual crops are not suitable [18]. In comparison with switchgrass, miscanthus produces a higher biomass [16, 19] and also displays a larger solar energy conversion efficiency [20]. It is considered one of the most promising perennial bioenergy crops [15, 21–23]. More particularly, *Miscanthus × giganteus*, which is a triploid sterile hybrid descendent of a cross between *Miscanthus sacchariflorus* and *Miscanthus sinensis* [24], is particularly interesting because it combines high biomass production per hectare with a low environmental impact [6]. However, *M. × giganteus* also has certain disadvantages: (i) this species cannot produce high quantities of biomass under various climates because it is sensitive to heavy frost [25] and a lack of water [26], and further, (ii) *M × giganteus*, which has been the most commonly cultivated species until now, has only few clones [27, 28], which could be risky due to disease susceptibility [29].

Considering these disadvantages, developing miscanthus as suitable feedstock for bioenergy will enhance the miscanthus varieties available for cultivation. Therefore, miscanthus breeding programs must be developed to propose a range of miscanthus varieties that can produce high quantities of biomass per hectare under various climatic conditions. Other *Miscanthus* species could also be sought for breeding because the genus *Miscanthus* contains more than 20 species that originate from a broad geographical area [30]. Certain species, such as *M. sinensis*, show high genetic diversity compared with *M. × giganteus* [27, 28].

In addition to the above requirements for biomass production, using miscanthus as a feedstock for bioenergy requires that the biomass composition is adapted to various bioenergy conversion processes. This suitability remains a difficult challenge and constitutes a major bottleneck to proposing suitable lignocellulosic crops for bioenergy use.

First, the industrial requirements for lignocellulosic crop biomass composition have not been fully defined for the various bioenergy conversion processes. For instance, the most favorable balance between lignins, cellulose, and hemicelluloses in the cell wall for producing biofuels from biochemical conversion processes has not been defined [31].

In recent years, several global projects have been launched to precisely define these requirements and develop industrial-scale tests for several bioenergy conversion processes. The results from these projects should precisely define the industrial requirements for lignocellulosic crop biomass composition in bioenergy production.

Second, biomass composition can influence bioenergy conversion process efficiency. For instance, high lignin content is positive for thermochemical processes [32, 33], while it is negative for biochemical processes [34–36]. Regardless of the process, ash can be problematic because it deposits on the heat surfaces, which causes slagging and fouling [37, 38].

Third, the methods used to assess the biomass components and, more particularly, the cell wall components differ and are not reliable. Standardized methods have not been established for either miscanthus or other crops. This complicates the definition of the most suitable biomass composition for each bioenergy conversion process.

These observations demonstrate that biomass composition must be better understood and considered in miscanthus breeding programs to develop miscanthus as a suitable feedstock for bioenergy use.

Developing such breeding programs also requires consideration of interesting traits related to biomass production and composition as well as the factors of variation influencing such traits.

For the traits of interest, aboveground biomass production must be considered first. In addition, canopy height, stem number, or stem diameter, which are the main components that contribute to biomass production, must also be investigated [39–41]; cellulose, hemicellulose, lignin, and ash contents are the main traits considered in biomass composition.

For the factors of variation, studies on miscanthus have reported effects of species, clone, and ploidy level on biomass production and certain biomass production components [39–44] or biomass composition [33, 37, 43, 45–47]. Furthermore, these traits appear influenced by other factors, such as geographical area, climate conditions, or crop management practices, which can influence the conversion processes [33, 38, 44, 48–50].

Therefore, it is essential to consider the traits together with the factors of variation that can influence these traits in developing suitable miscanthus for bioenergy production.

Breeding program development requires knowing the genotypic variability related to the traits of interest [31]. As the

genotypic variability related to biomass production and biomass composition has not been well studied for miscanthus, it is crucial to explore the variability in this genus for biomass production and composition.

Therefore, the present paper reviews worldwide references about the phenotypic variability in the *Miscanthus* genus for the following traits related to biomass production and biomass composition: above-ground biomass production, canopy height, stem number per plant, stem diameter, cellulose content, hemicellulose content, lignin content, and ash content. The phenotypic variability was deconstructed into environmental variability and genotypic variability to highlight the genotypic variability contribution that may be used in breeding programs. This paper gathered the available data for the three most studied *Miscanthus* species: *M. × giganteus*, *M. sinensis*, and *M. sacchariflorus*.

This paper is presented in four sections. The first section describes the data set used in the present review and collected from scientific literature. A second section investigates the phenotypic variability of the traits of interest related to miscanthus biomass production and biomass composition. In this section, environmental and genotypic variability are investigated with a focus on the factors of variation that are most studied in the literature. Given the miscanthus genotypic variability for biomass production and composition, in the third section, we discuss the miscanthus breeding implications for bioenergy use considering the industrial requirements for bioenergy conversion processes. Finally, we provide certain proposals in the fourth section.

Database Description and Compilation

A literature search was conducted using the Thomson Reuters Web of Knowledge electronic database for several traits of interest related to miscanthus biomass production and biomass composition.

The first observation is that the studies reporting data on these traits are relatively recent, with publication dates mainly ranging from 1997 to 2014. Certain miscanthus traits have not been thoroughly studied. Traits related to biomass production studied in literature mainly concern aboveground biomass production, canopy height, stem number, and stem diameter. Among these traits, fewer studies have considered canopy height, stem number, and stem diameter than above-ground biomass production. Fewer studies have considered traits related to miscanthus biomass composition than miscanthus biomass production. For the present review, we will focus on traits related to cell wall components (cellulose content, hemicellulose content,

and lignin content) and ash content, which are interesting traits for industrial bioenergy production.

A second observation is that the studies differed.

First, the data from each publication were not comparable for a given trait; certain publications indicate individual values for each factor of variation (year, harvest date, nitrogen, location, genotype, etc.), and others reported only a mean value for a given trait. Therefore, to investigate the traits of interest related to miscanthus biomass production and biomass composition in this review, we selected publications that allowed us to collect individual data for each trait. Therefore, each individual data has the same weight and was comparable between publications.

Secondly, different factors of variation for such traits in the literature were discussed. In this review, we focus on the factors related either to crop age, climate conditions, geographical area, crop management practices (harvest date, nitrogen fertilization, irrigation, or plant density), species, clones, or ploidy level, which were the most commonly studied factors in the literature. For the crop age, most studies reported data for the first 3 years of cultivation. Therefore, we focus on these first 3 years to investigate the influence of crop age on these traits. In addition, only three *Miscanthus* species have been investigated in the literature for traits related to biomass production and composition: *M. × giganteus*, *M. sinensis*, and *M. sacchariflorus*. Moreover, the clone number and genetic background in the literature differed between the three species. Therefore, to compare the publications based on the *Miscanthus* species, we categorized the genotypes into three groups: (i) interspecific *M. × giganteus*-type hybrids that correspond to *M. × giganteus* clones or *M. sacchariflorus* × *M. sinensis* clones, (ii) *M. sinensis* species that correspond to *M. sinensis* clones or intra-specific hybrids between *M. sinensis* clones, and (iii) *M. sacchariflorus* species that correspond to *M. sacchariflorus* clones or intra-specific hybrids between *M. sacchariflorus* clones.

Third, the method used to determine the values for traits related to biomass composition differed between studies. The cellulose, hemicellulose, and lignin contents were determined using several chemical analysis methods, such as the Van Soest method, the KS M 7044 method to determine cellulose content, or the Klason method to determine lignin content [51, 52]. Based on the method used, the cellulose, hemicellulose, or lignin contents differed and may be underestimated or overestimated (Chabbert, personal communication). Therefore, to ensure reliable comparisons for such contents between publications, we focused only on the publications that used the Van Soest method as the method of reference [53] because it was most commonly used [33, 45, 54–56].

Herein, Tables 1 and 2 gather the publications which reported data on the miscanthus phenotypic variability for the traits of interest. For each study, we provide information

Table 1 Description of the scientific references collected for the present review reporting data on aboveground miscanthus biomass production, canopy height, stem number per plant, and stem diameter

Trait	Reference	Year of publication	Factors of variation			Experimental design										Statistical data		
			Crop age	Country (number of sites)	Harvest date	Nitrogen fertilization (kg/ha)	Irrigation	Plant density (pl/m ²)	Number of <i>Miscanthus</i> species (name of species)	Number of clones	Number of replicated plots	Plot surface area (m ²)	Sampling area (m ²)	Std (min-max)	CV (%)	LSD (min-max) (0.05)	MSD (min-max)	SED (min-max)
Aboveground biomass production (t DM/ha)	Clifton-Brown et al. [44]	2001	1, 2, 3	Sweden, Denmark, England, Germany, Portugal	Autumn			2	3 (Gig, Sacc, Sin)	15	3, only 2 in Portugal	25	2.5				0.6–17.1	
	Lewandowski et al. [37]	2003	3	Sweden, Denmark, England, Germany, Portugal	Autumn and winter			2	3 (Gig, Sacc, Sin)	15	3, only 2 in Portugal	49	2				2.5–18.0	
	Christian et al. [57]	2008	1 to 14	UK	Winter	0, 160, 120 (three levels)		4	1 (Gig)	1	3	100	36					0.3–1.0
	Jezowski [40]	2008	1, 2, 3	Poland	Winter			1	2 (Gig, Sin)	6	3	20	10					
	Angelini et al. [58]	2009	1 to 12	Italy	Autumn			2	1 (Gig)	1	4	100	10					
	Manfinceo et al. [59]	2009	1 to 5	Italy	Winter	50, 100 (two levels)	75, 25 % Etm (two levels)	2	1 (Gig)	1		24						
	Jezowski et al. [60]	2011	1, 2, 3	Poland	Winter			1	2 (Gig, Sin)	6	3	20	10					
	Amougou et al. [51]	2011	2, 3	France	Autumn and winter	0, 120 (two levels)		1.5	1 (Gig)	1	3	360	4			4.9–9.6		
	Strulla et al. [61]	2011	2, 3	France	Autumn and winter	0, 120 (two levels)		1.5	1 (Gig)	1	3	360	4					
	Zub et al. [41]	2011	2, 3	France	Autumn and winter	0, 120 (two levels)		2	3 (Gig, Sacc, Sin)	21	3	16	16			1.2–4.5	1.8	
	Behnke et al. [62]	2012	2, 3	IL, USA	Winter	0, 60, 120 (three levels)		1	1 (Gig)	1	3	100			0.7–2.9			
	Dohleman et al. [63]	2012	5, 6, 7	IL, USA	Summer, autumn, and winter			1	1 (Gig)	1	4	100	0.38					
	Gauder et al. [39]	2012	1 to 14	Germany	Autumn			2	3 (Gig, Sacc, Sin)	15	3	25	1 to 4					
	Arundale et al. [19]	2014	3 to 10	IL, USA	Winter			1	1 (Gig)	1	4	25	0.38					
Canopy height (cm)	Arundale et al. [64]	2014	3 to 9	IL, USA	Winter	0, 67, 134, 202 (four levels)		1	1 (Gig)	1	4	25	0.19					
	Haines et al. [65]	2014	1, 2, 3	NC, USA	Winter	0, 45, 90, 135 (four levels)		1	1 (Gig)	1	4	16	4					
	Larsen et al. [66]	2014	1 to 20	Denmark	Autumn and winter	0, 75 (NPK and slurry), 150 (slurry) (four levels)	0, irrigated (two levels)	1.8, 1.1 (two levels)	2 (Gig, Sin)	2	3	160	22 and 37					
	Palmer et al. [67]	2014	1 to 4	NC, USA	Winter	0, 34, 67, 134 (four levels)		1	2 (Gig, Sin)	2	1	25	9					
	Jezowski [40]	2008	1, 2, 3	Poland	Winter			1	2 (Gig, Sin)	6	3	20				14–46		
Stem number per plant	Jezowski et al. [60]	2011	1, 2, 3	Poland	Winter			1	2 (Gig, Sin)	6	3	20	10					
	Zub et al. [41]	2011	2, 3	France	Winter			2	3 (Gig, Sin)	21	3	15	16			17–25	6	3
	Christian et al. [57]	2008	1 to 14	UK	Winter	0, 160, 120 (three levels)		4	1 (Gig)	1	3	100	1.25					2–4
	Jezowski [40]	2008	1, 2, 3	Poland	Winter			1	2 (Gig, Sin)	6	3	20			7–19			

Table 1 (continued)

Trait	Reference	Year of publication	Factors of variation			Experimental design					Statistical data							
			Crop age	Country (number of sites)	Harvest date	Nitrogen fertilization (kg/ha)	Irrigation	Plant density (pl/m ²)	Number of <i>Miscanthus</i> species (name of species)	Number of clones	Number of replicated plots	Plot surface (m ²)	Sampling area (m ²)	Std (min-max)	CV (%)	LSD (min-max) (0.05)	MSD (min-max)	SED (min-max)
Stem diameter (mm)	Jezowski et al. [60]	2011	1, 2, 3	Poland (two sites)					1	2 (Gig, Sin)	6	3	20	10				
	Zub et al. [41]	2011	2, 3	France (one site)					2	3 (Gig, Sacc, Sin)	21	3	16	16	15–49	17	3	
	Gauder et al. [39]	2012	1 to 14	Germany (one site)					2	3 (Gig, Sacc, Sin)	15	3	25	1 to 4				
	Jezowski [40]	2008	1, 2, 3	Poland (one site)					1	2 (Gig, Sin)	6	3	20					
	Jezowski et al. [60]	2011	1, 2, 3	Poland (two sites)					1	2 (Gig, Sin)	6	3	20					
	Zub et al. [41]	2011	2, 3	France (one site)					2	3 (Gig, Sacc, Sin)	21	3	16	16	1.9–2.4	6	1.2	
	Gauder et al. [39]	2012	1 to 14	Germany (one site)					2	3 (Gig, Sacc, Sin)	15	3	25	1 to 4				

In the study of Zub et al. [41], *M. floridulus* clone belongs to the *M. × giganteus* species according to the authors. Data written in italics were calculated in addition to the initial paper. The LSD values from Zub et al. [41] were calculated using Dagnelie [68]. In bold, all the references reported individual data which were used in the Figs. 1, 3, 4, 5, 6, and 7

Gig M. × giganteus species, *Sacc M. sacchariflorus* species, *Sin M. sinensis* species, *SED* standard error of difference, *LSD* least significant difference, *MSD* minimum significant difference

Table 2 Description of the scientific references collected for the present review reporting data on the miscanthus biomass composition for the aboveground biomass: cellulose, hemicellulose, lignin, and ash content

Trait	Reference	Year of publication	Factors of variation			Experimental design					Statistical data		
			Crop age	Country (number of sites)	Harvest date	Nitrogen fertilization (kg/ha)	Plant density (pl/m ²)	Number of <i>Miscanthus</i> species (name of species)	Number of clones	Number of replicated plots	Plot surface (m ²)	Sampling area (m ²)	Std (min-max)
Cellulose (% DM)	Hodgson et al. [33] ^a	2010	3	Sweden, Denmark, England, Germany, Portugal (five sites)	Autumn and winter		2	3 (Gig. Sacc, Sin)	15	3, only 2 in Portugal	49	2	1.2–6.8
Hemicellulose (% DM)	Hodgson et al. [33] ^a	2010	3	Sweden, Denmark, England, Germany, Portugal (five sites)	Autumn and winter		2	3 (Gig. Sacc, Sin)	15	3, only 2 in Portugal	49	2	1.0–5.1
Lignin (% DM)	Hodgson et al. [33] ^a	2010	3	Sweden, Denmark, England, Germany, Portugal (five sites)	Autumn and winter		2	3 (Gig. Sacc, Sin)	15	3, only 2 in Portugal	49	2	0.3–2.6
	Hayes [69] ^b	2013	1, 2, 3, 13	Ireland (six sites)	Autumn, winter, and spring			1 (Gig)	1				
Ash (% DM)	Clifton-Brown and Lewandowski [43]	2002	1, 2, 3	Germany (one site)	Autumn and winter		2	3 (Gig. Sacc, Sin)	15	3	25		0.8–4.2
	Lewandowski et al. [37]	2003	3	Sweden, Denmark, England, Germany, Portugal (five sites)	Autumn and winter		2	3 (Gig. Sacc, Sin)	15	3, only 2 in Portugal	49	2	0.5–3.2
	Baxter et al. [70]	2012	3, 4	UK (one site)	Autumn and winter			1 (Gig)	1	3			
	Hayes [69]	2013	1, 2, 3, 13	Ireland (six sites)	Autumn, winter, and spring	0, 100, 250 (three levels)		1 (Gig)	1				
	Meenan et al. [71]	2013	15, 16	Ireland (one site)	Autumn, winter, and spring			1 (Gig)	1	4			0.38

In bold, all the references reported individual data which were used in the Figs. 1, 3, 4, 5, 6, and 7

Gig M. × *giganteus* species, *Sacc M. sacchariflorus* species, *Sin M. sinensis* species, *SED* standard error of difference, *LSD* least significant difference, *MSD* minimum significant difference

^aThe cellulose, hemicellulose, and lignin contents were determined using the Van Soest method as the method of reference

^b Lignin content was determined using the Klason method

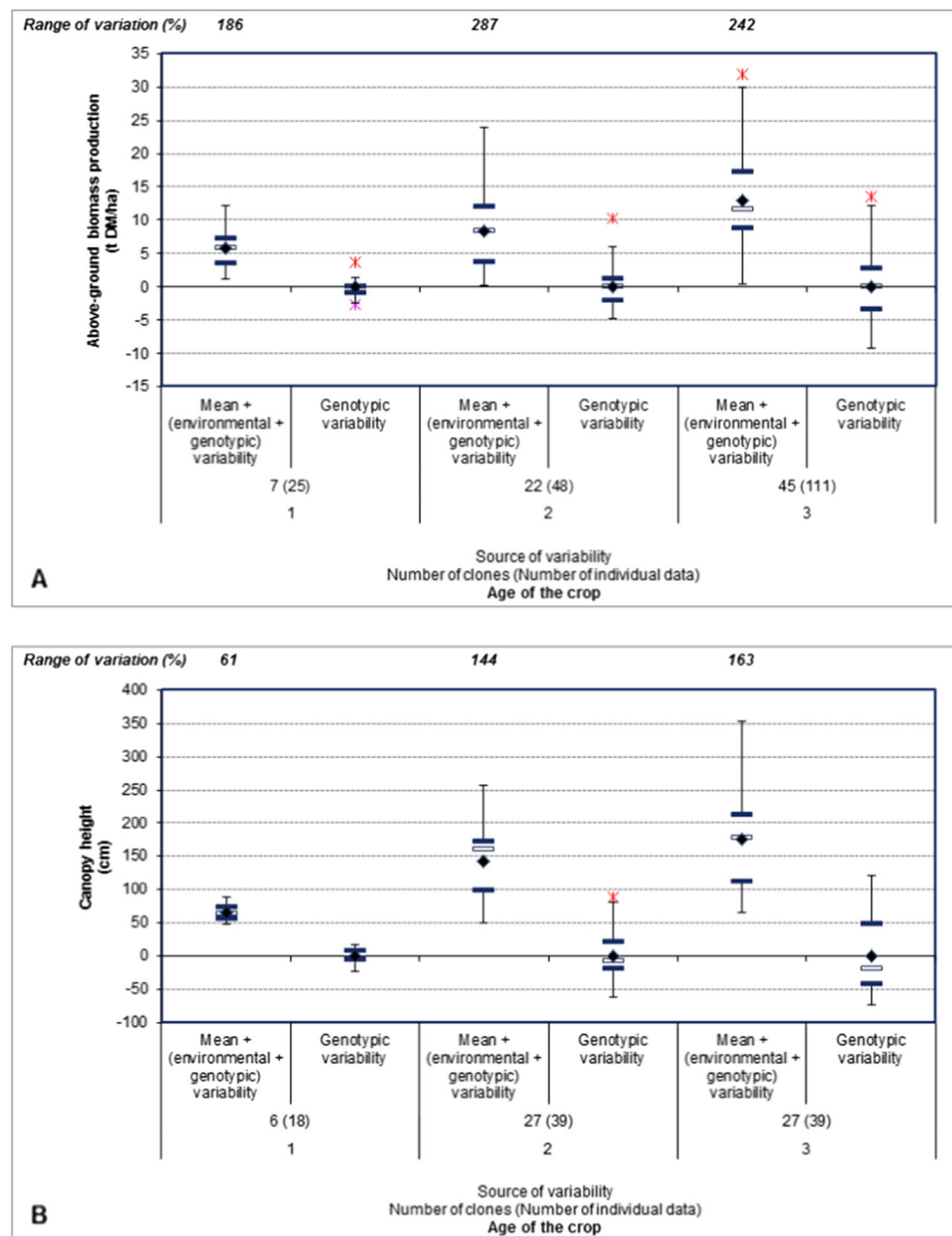


Fig. 1 Phenotypic variability and genotype contribution to the phenotypic variability in miscanthus for aboveground biomass production (**a**), canopy height (**b**), stem number per plant (**c**), and stem diameter (**d**) based on the crop age, from the first to third year of cultivation. Phenotypic variability corresponds to the mean + (environmental + genotypic variability); the genotype contribution to the phenotypic variability corresponds to genotypic variability. The aboveground biomass production was assessed for the winter harvest; the canopy height, stem number per plant, and stem diameter were obtained at the end of the growing season. The mean value (indicated by the *black diamond*) was calculated from the individual data collected in the literature for each year of cultivation. The *ends of the vertical lines* correspond to the minimum and maximum

values; the *black horizontal lines* correspond to the upper and lower quartiles; and the *white horizontal lines* correspond to the median for each year of cultivation. The *stars on both sides of the black horizontal lines* correspond to the outlier values. The *range of variation* corresponds to the difference between the maximum and minimum values and is expressed as a percentage of the mean. References used—**a** Christian et al. [57], Clifton-Brown et al. [44], Lewandowski et al. [37], Jezowski [40], Jezowski et al. [60], Mantineo et al. [59], Amougou et al. [51], Zub et al. [41]; **b** Jezowski [40], Jezowski et al. [60], Zub et al. [41]; **c** Jezowski [40], Christian et al. [57], Jezowski et al. [60], Zub et al. [41]; **d** Jezowski [40], Jezowski et al. [60], Zub et al. [41]

related to the factors of variation, experimental design construction, and statistical indices.

To investigate each trait, we selected in Tables 1 and 2 the studies for which individual data were available (in bold in the

tables). We represented these individual data using box-plots (Figs. 1, 3, 4, 5, 6, and 7). Two types of box-plots were constructed for each illustration: a first type of box-plot shows the phenotypic variability that combines both environmental

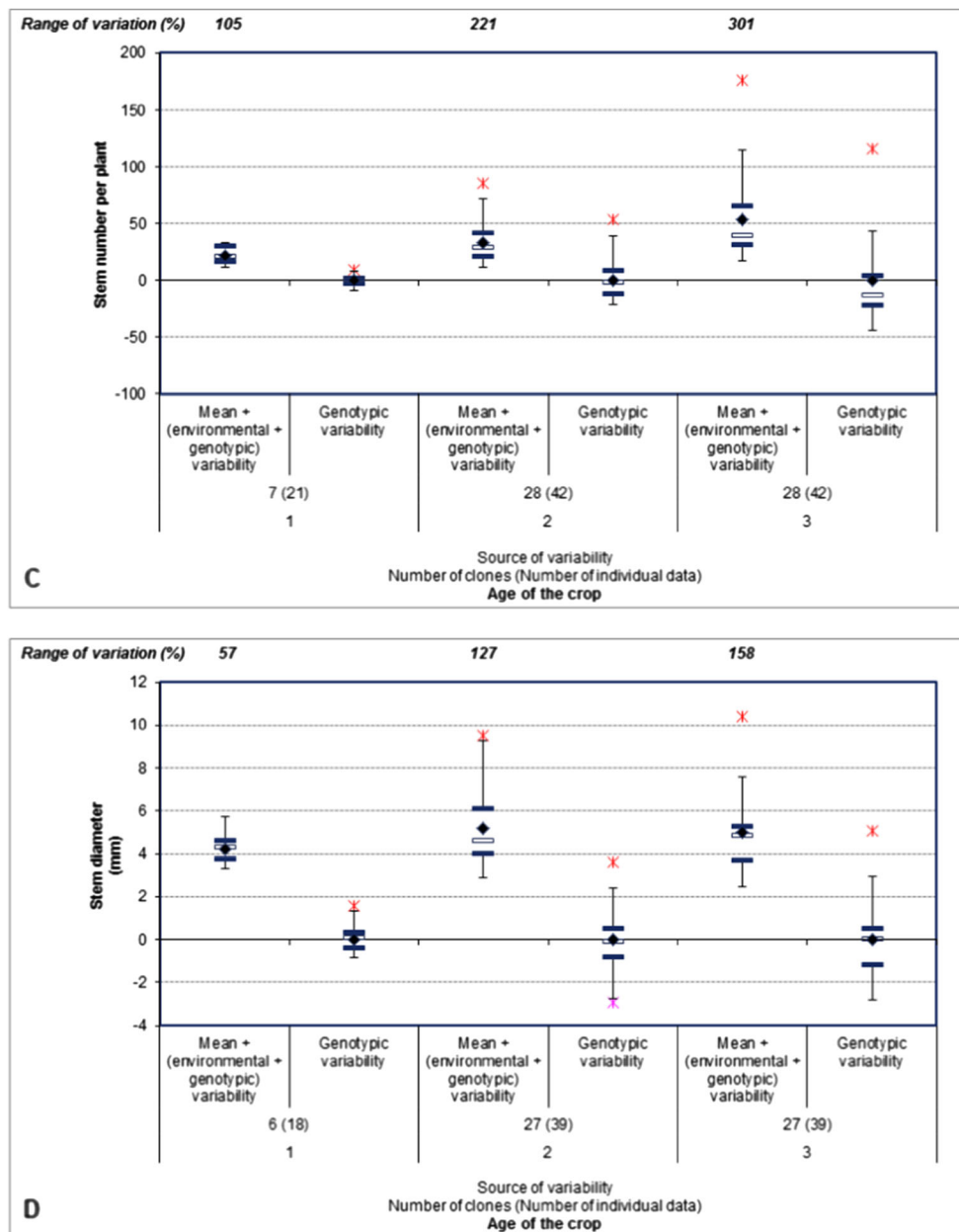


Fig. 1 (continued)

and genotypic variability, and a second type shows the part of the phenotypic variability due only to the genotype. This genotype effect (α_i) was calculated in each collected environment according to the following additive model including the studied factors:

$$E(Y_i) = \mu + \alpha_i + \text{other factor effects}$$

In addition, the range of variation was calculated for each trait.

Finally, the remaining references of the Tables 1 and 2 are cited in the text to support the discussion.

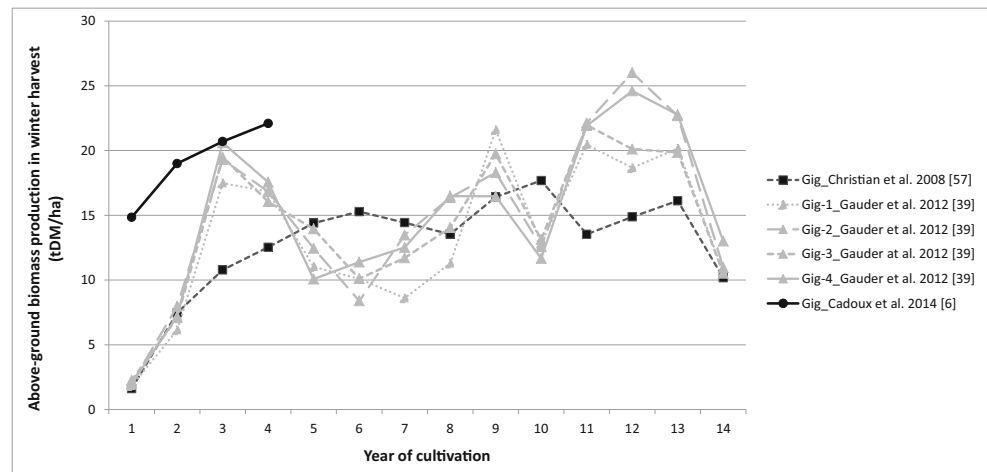
The Phenotypic Variability for the Traits of Interest

The Phenotypic Variability for Aboveground Biomass Production and Biomass Components

The Influence of Crop Age

Miscanthus is a perennial crop that can be cultivated for up to 25 years [72], during which miscanthus biomass is produced in two phases: a yield-building phase, where the biomass gradually increases, and an adult phase often described as a plateau phase, where the biomass production is maintained [29, 42, 73].

Fig. 2 *M. × giganteus* aboveground biomass production during the winter harvest for several successive years from Christian et al. [57], Gauder et al. [39], and Cadoux et al. [6]



Most studies in the literature have reported data on miscanthus aboveground biomass production and components for the first 3 years of cultivation, which correspond to the yield-building phase (Table 1). Data on these traits for subsequent years were less frequent in the literature and only concern the *M. × giganteus* species, which is the most commonly studied species [6, 38, 57, 59].

Therefore, in the following, we describe the influence of crop age on the aboveground biomass and its components based on the two biomass production phases. We better detail the first three cultivation years because more information is available in literature.

- The first years: the yield-building phase

The first year of cultivation corresponds to the year that the crop is established [29]. The biomass production is generally low during the first year with a 5.9 t dry matter/ha (DM/ha) average from experiments using irrigated or rainfall conditions (Fig. 1a).

During the 2 years that follow, the miscanthus biomass gradually increases: biomass production reaches 8.3 and 13.0 t DM/ha on average during the second and third years, respectively, under irrigated or rainfall conditions (Fig. 1a).

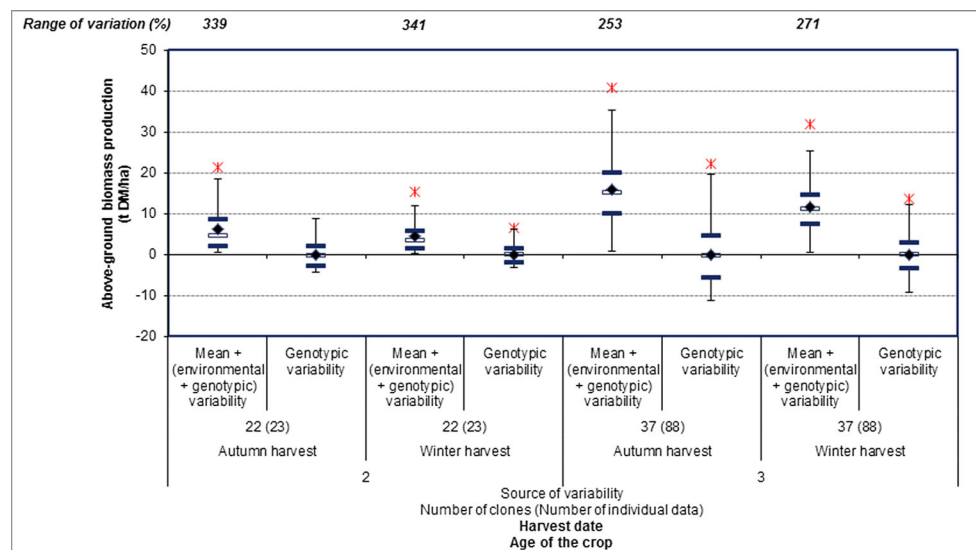


Fig. 3 The phenotypic variability and genotype contribution to the phenotypic variability for aboveground miscanthus biomass production based on the harvest date (autumn and winter harvests) during the second and third years of cultivation. The phenotypic variability corresponds to the mean + (environmental + genotypic variability); the genotype contribution to the phenotypic variability corresponds to the genotypic variability. The mean value (indicated by the black diamond) was calculated from the individual data collected in the literature for each year of cultivation and harvest date. The ends of the vertical lines correspond to

the minimum and maximum values; the black horizontal lines correspond to the upper and lower quartiles; and the white horizontal lines correspond to the median for each year of cultivation and harvest date. The stars on both sides of the black horizontal lines correspond to the outlier values. The range of variation corresponds to the difference between the maximum and minimum values and is expressed as a percentage of the mean. References used—Lewandowski et al. [37], Zub et al. [41], Amougou et al. [51]

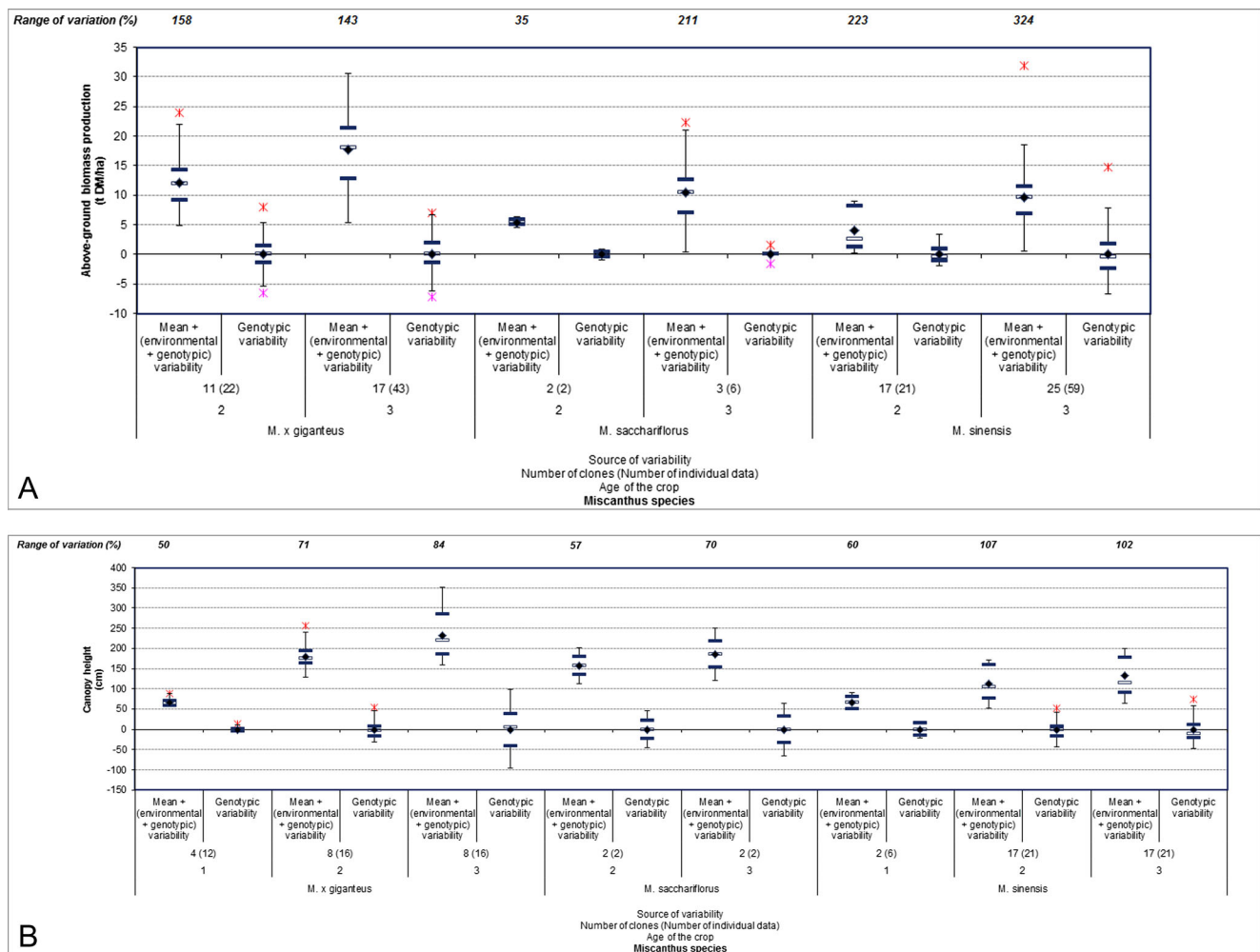


Fig. 4 The phenotypic variability and genotype contribution to the phenotypic variability for aboveground miscanthus biomass production (a), canopy height (b), stem number per plant (c), and stem diameter (d) among and within the three *Miscanthus* species *M. x giganteus*, *M. sacchariflorus*, and *M. sinensis* for the first 3 years of cultivation. The phenotypic variability corresponds to the mean + (environmental + genotypic variability); the genotype contribution to phenotypic variability corresponds to genotypic variability. The aboveground biomass production was assessed for the winter harvest; the canopy height, stem number per plant, and stem diameter were obtained at the end of the growing season. The mean value (indicated by the black diamond) was calculated from the individual data collected in the literature for each year of cultivation. The ends of the vertical lines correspond to the minimum and maximum values; the black horizontal lines correspond to the upper and lower quartiles; and the white horizontal lines correspond to the

median for each year of cultivation. The stars on both sides of the black horizontal lines correspond to the outlier values. The range of variation corresponds to the difference between the maximum and minimum values and was expressed as a percentage of the mean. For each trait, genotypic variability in the *M. sacchariflorus* species during the second year was the same as the phenotypic variability; the two *M. sacchariflorus* genotypes were observed in a single environment. No data were available for canopy height (b), stem number per plant (c), and stem diameter (d) on the first year of cultivation for the *M. sacchariflorus* species in the literature. References used—**a** Christian et al. [57], Lewandowski et al. [37], Jezowski [40], Jezowski et al. [60], Mantineo et al. [59], Amougou et al. [51], Zub et al. [41]; **b** Jezowski [40], Jezowski et al. [60], Zub et al. [41]; **c** Christian et al. [57], Jezowski [40], Jezowski et al. [60], Zub et al. [41]; **d** Jezowski [40], Jezowski et al. [60], Zub et al. [41]

Biomass components, such as canopy height and stem number per plant, also clearly increase from the first to third year; on average, the canopy height increases from 66 to 176 cm, and the stem number per plant increases from 21 to 53 (Fig. 1b, c). However, with mean values between 4.2 and 5.2 mm, the stem diameter was more consistent than the biomass production, canopy height, and stem number during the first 3 years (Fig. 1).

These observations confirmed that for miscanthus, the first 3 years correspond to a yield-building phase, where

the biomass production and its components, such as canopy height and stem number per plant, gradually increase.

The range of variation was large within each of the first 3 years of cultivation for each trait. This range of variation was generally smaller during the first year of cultivation compared with the second and third years (Fig. 1). In addition, the data scattering for biomass production, stem number, canopy height, and stem diameter, to a lesser extent, increased each year during the first 3 years; minimum values were consistent among the years, but the

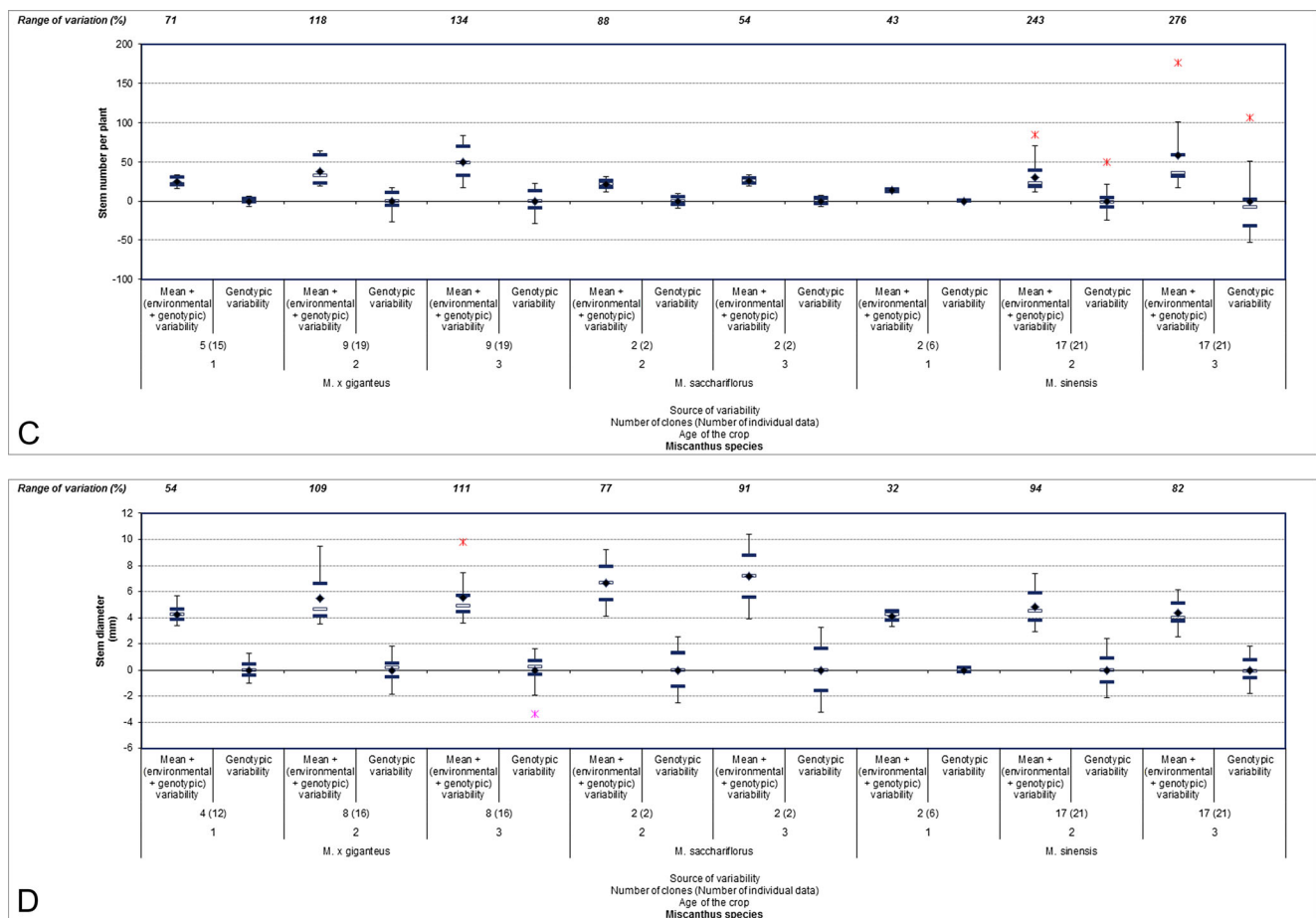


Fig. 4 (continued)

maximum values increased each year (Fig. 1). Similarly, genotypic variability was more visible in the second and third years than the first year for each trait studied; the higher variability in second and third years was certainly due to the most productive genotypes, which began to display their potential during the second year.

Finally, among the traits studied, stem number and aboveground biomass production appeared to be the most variable traits (301 and 242 %, respectively, in the third year) compared with canopy height and stem diameter, which seemed less variable (163 and 158 %, respectively, in the third year) (Fig. 1). This large observed range of variation for the traits related to biomass production was particularly interesting in breeding programs to select the best genotypes and achieve maximum biomass production.

- The subsequent years: the adult phase and decline

Several studies have reported data on miscanthus biomass production, canopy height, stem number, or stem diameter in long-term experiments. They mainly concerned long-term biomass production which was reported for the *M. x giganteus* species in Europe (France [6], UK

[57], Germany [39], Italy [58], Denmark [66]) and in the USA (Illinois [19, 63]). The winter harvest date corresponded to the harvest date commonly used by farmers. From the available individual data, we assume that the *M. x giganteus* biomass production crop reached a first peak at variable time: at 3 years in the study of Gauder et al. [39], at 6 years in the study of Christian et al. [57], and this first peak was not reached after 4 years of cultivation in the study of Cadoux et al. [6] (Fig. 2). Clifton-Brown et al. [42] in the UK and Miguez et al. [74] in the USA showed that biomass production plateaued after 2 to 5 years for *M. x giganteus* depending on the environmental conditions and crop management practices. Larsen et al. [66] reported in Denmark an increase of the *M. x giganteus* biomass production during the first years, optimum yields after 7 and 8 years, and a decrease to a lower level which remained relatively constant from year 11 to 20. In addition, Gauder et al. [39] found even more variable yields from the 3rd to the 14th years of cultivation.

In summary, based on the long-term experimental data published, it is difficult to estimate the time of the first peak of production and the length of time that miscanthus maintains biomass production. It seems also that the yields

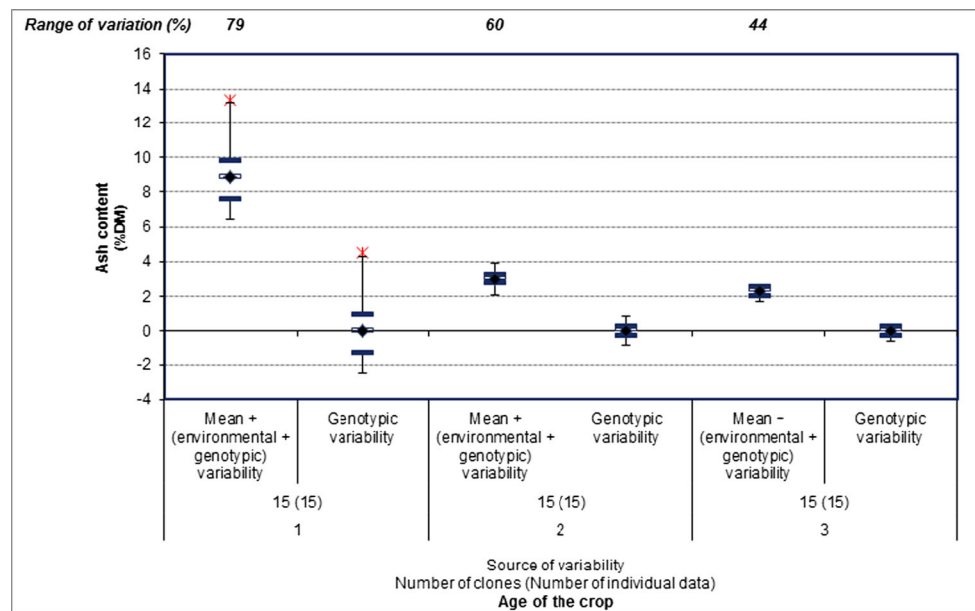


Fig. 5 The phenotypic variability and genotype contribution to the phenotypic variability for aboveground miscanthus ash content based on the crop age from the first to third year during the winter harvest based on Clifton-Brown and Lewandowski [43]. The phenotypic variability corresponds to the mean + (environmental + genotypic variability); the genotype contribution to the phenotypic variability corresponds to genotypic variability. The mean value (indicated by the black diamond) was calculated from the individual data collected in the literature for each year of cultivation. The ends of the vertical lines correspond to the minimum

and maximum values; the black horizontal lines correspond to the upper and lower quartiles; and the white horizontal lines correspond to the median for each year of cultivation. The stars on both sides of the black horizontal lines correspond to the outlier values. The range of variation corresponds to the difference between the maximum and minimum values and is as a percentage of the mean. For each of the 3 years, the genotypic variability equaled the phenotypic variability; the 15 genotypes were observed in a single environment

during the plateau phase are variable. Lastly, the time that biomass production begins to decline is also variable. This variability during the plateau phase and the variability of the time of the decline depend on possible causes such as the soil and climate conditions as well as crop management practices [74]. In addition, the biomass production decline depends itself on soil compaction and pest and disease pressure [19].

The Influence of Climate Conditions

Climate conditions, such as temperature, affect miscanthus biomass production more during the first year of crop establishment. For instance, a severe frost that occurs immediately after establishment can cause plant death in *M. × giganteus* and irreversibly damage biomass production [25]. In addition, low temperatures during the first winter after planting can damage miscanthus biomass production. For instance, *M. × giganteus* and *M. sacchariflorus* clones died the first winter after planting in Sweden and Denmark, whereas *M. sinensis* clones survived [44]. The authors explained the plant death by the winter soil temperatures, which fell below -4.5°C during the first year after planting in these countries.

During subsequent years of cultivation, Jezowski et al. [60] concluded that climate conditions significantly affected miscanthus biomass production based on the experiment location by comparing two sites in Poland during the first 3 years of cultivation. Clifton-Brown et al. [44] also concluded that biomass production varied based on the trial location; they showed that biomass production was generally higher in Central and Southern Europe than Northern Europe over the first 3 years of cultivation. Moreover, Clifton-Brown et al. [44] concluded that the clone and country significantly interacted; clones that produced the highest yields in Sweden and Denmark were among clones that produced the lowest yields in Portugal and Germany. Despite this interaction, the authors showed that certain *M. sinensis* hybrids can be found for a wide range of climate conditions in Europe. Gauder et al. [39] and Heaton et al. [75] also reported that climate parameters, such as rainfall and temperature, were important in miscanthus biomass accumulation. More particularly, in Germany, Gauder et al. [39] reported a strong correlation between the rainfall level during plant growth and miscanthus biomass production for a winter harvest.

Therefore, climate conditions can irreversibly damage biomass production during the first year of cultivation, which corresponds to the establishment year. This observation implies that climate conditions should be considered during the

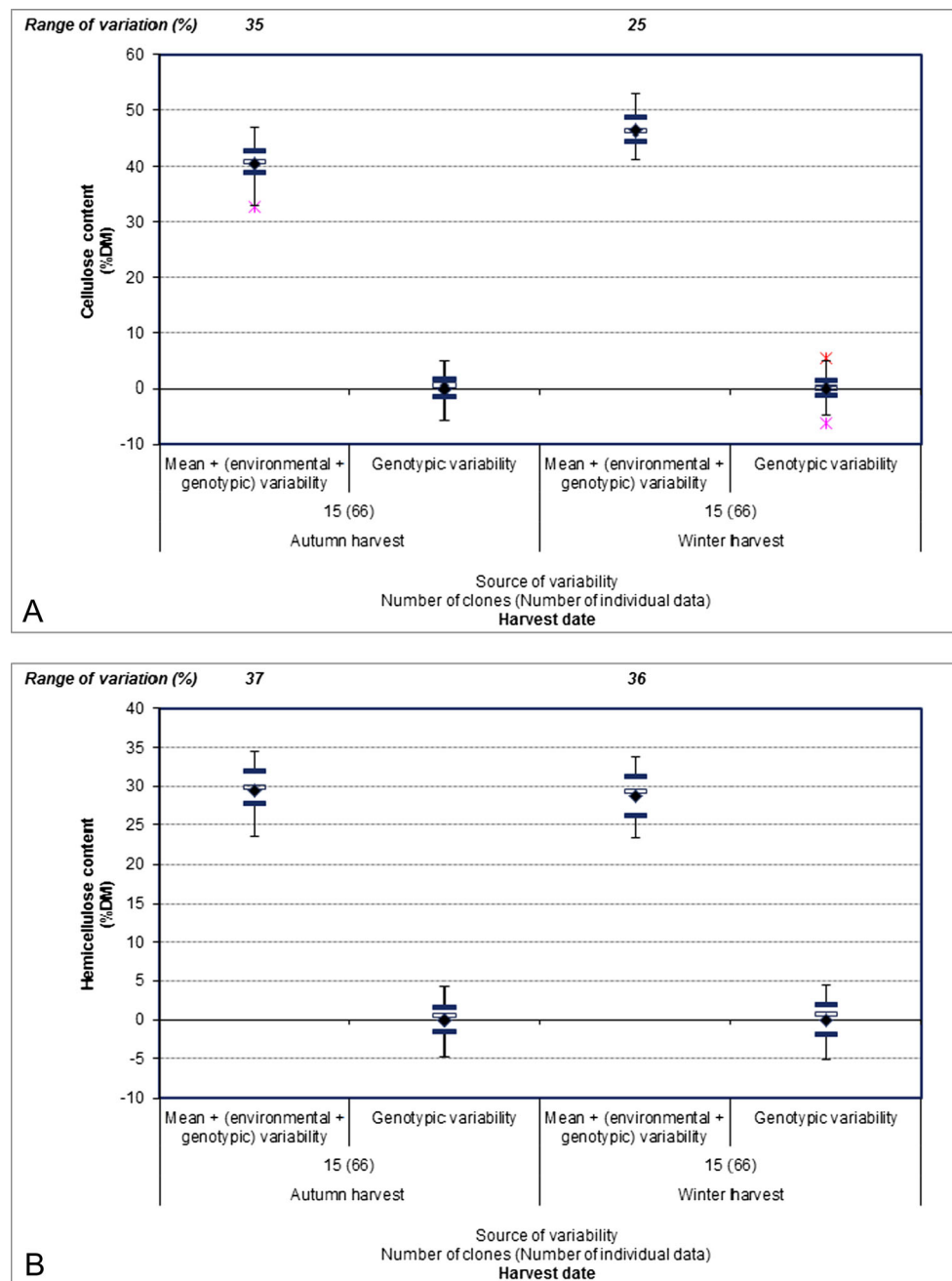


Fig. 6 The phenotypic variability and genotype contribution to the phenotypic variability for aboveground miscanthus cellulose content (**a**), hemicellulose content (**b**), lignin content (**c**), and ash content (**d**) between the autumn and winter harvests during the third year of cultivation. The phenotypic variability corresponds to the mean + (environmental + genotypic variability); the genotype contribution to phenotypic variability corresponds to the genotypic variability. Cellulose, hemicellulose, and lignin contents were determined using the Van Soest method as the method of reference. The mean value (indicated by the black diamond) was calculated from the individual data collected in the

literature for each year of cultivation. The ends of the vertical lines correspond to the minimum and maximum values; the black horizontal lines correspond to the upper and lower quartiles; and the white horizontal lines correspond to the median for each year of cultivation. The stars on both sides of the black horizontal lines correspond to the outlier values. The range of variation corresponds to the difference between the maximum and minimum values and is expressed as a percentage of the mean. References used—**a–c** Hodgson et al. [33]; **d** Clifton-Brown and Lewandowski [43], Lewandowski et al. [37]

first year, as it was the most critical phase for biomass production [76]. Variations in climate conditions, such as rainfall and temperature, can also influence biomass production during the subsequent years of cultivation. Therefore, to maximize

biomass production, *Miscanthus* genotypes have to be selected based on their climate condition requirements because an interaction between location and genotype was observed for biomass production [44].

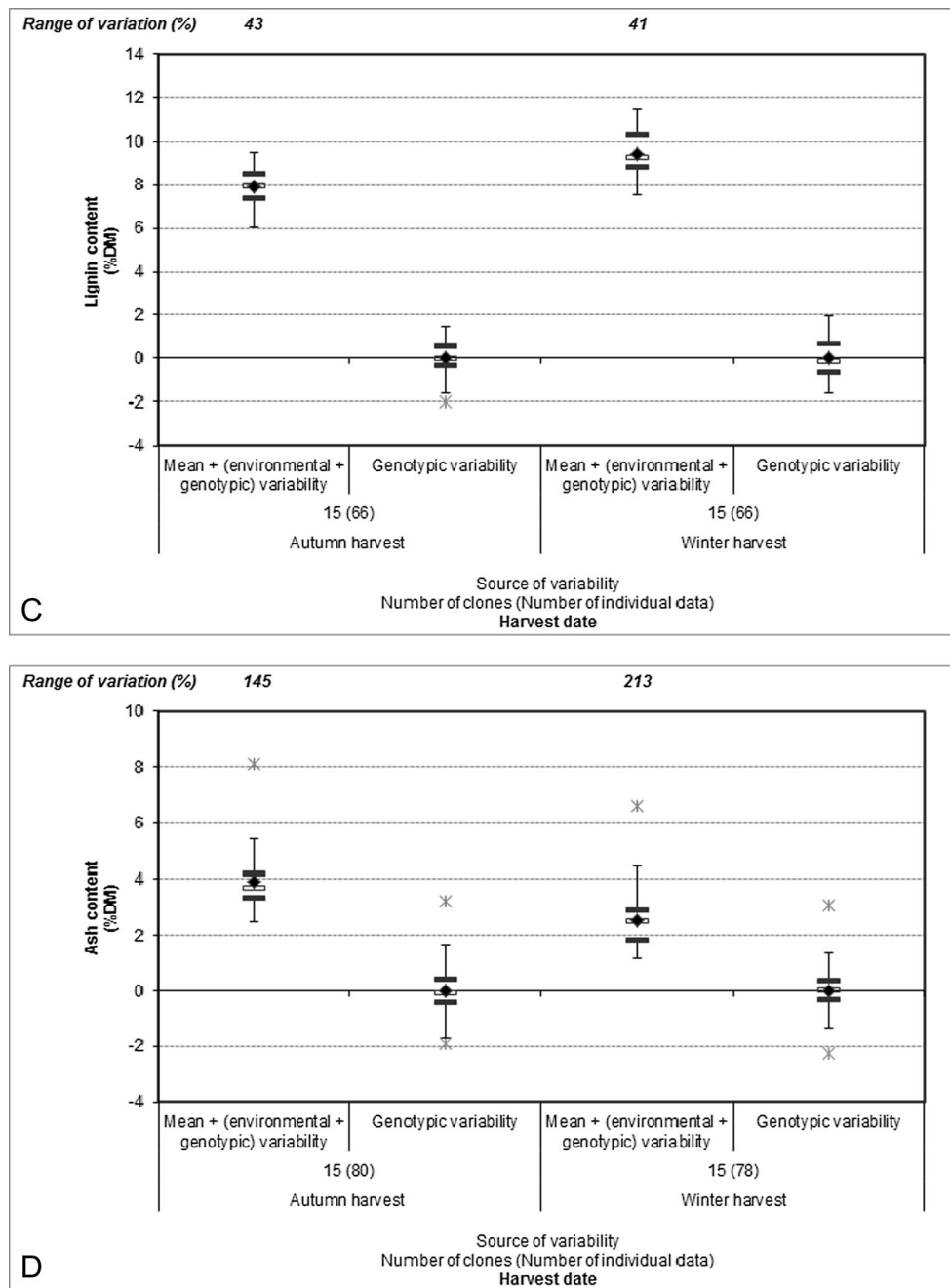


Fig. 6 (continued)

The Influence of Crop Management Practices

- The influence of harvest date
On average, 1.8 t DM/ha (29 %) and of 4.2 t DM/ha (26 %) decreases were observed between the autumn and winter harvests for the second and third years, respectively (Fig. 3). For each growing season, maximum biomass productions were obtained during autumn at flowering [26] then declined during winter mainly due to leaf loss, senescence, and assimilate translocation [41, 43, 77, 78].

With variations ranging from 253 to 341 %, biomass production was highly variable during each year and each harvest date (Fig. 3). Interestingly, the data appeared more scattered during the autumn harvests than winter harvests (Fig. 3).

Finally, the genotype contribution to the phenotypic variability was high for aboveground biomass production and more visible during the third than second year for both harvest dates (Fig. 3).

In addition, Strullu et al. [61] showed on *M. × giganteus* an interaction of the harvest date with the nitrogen

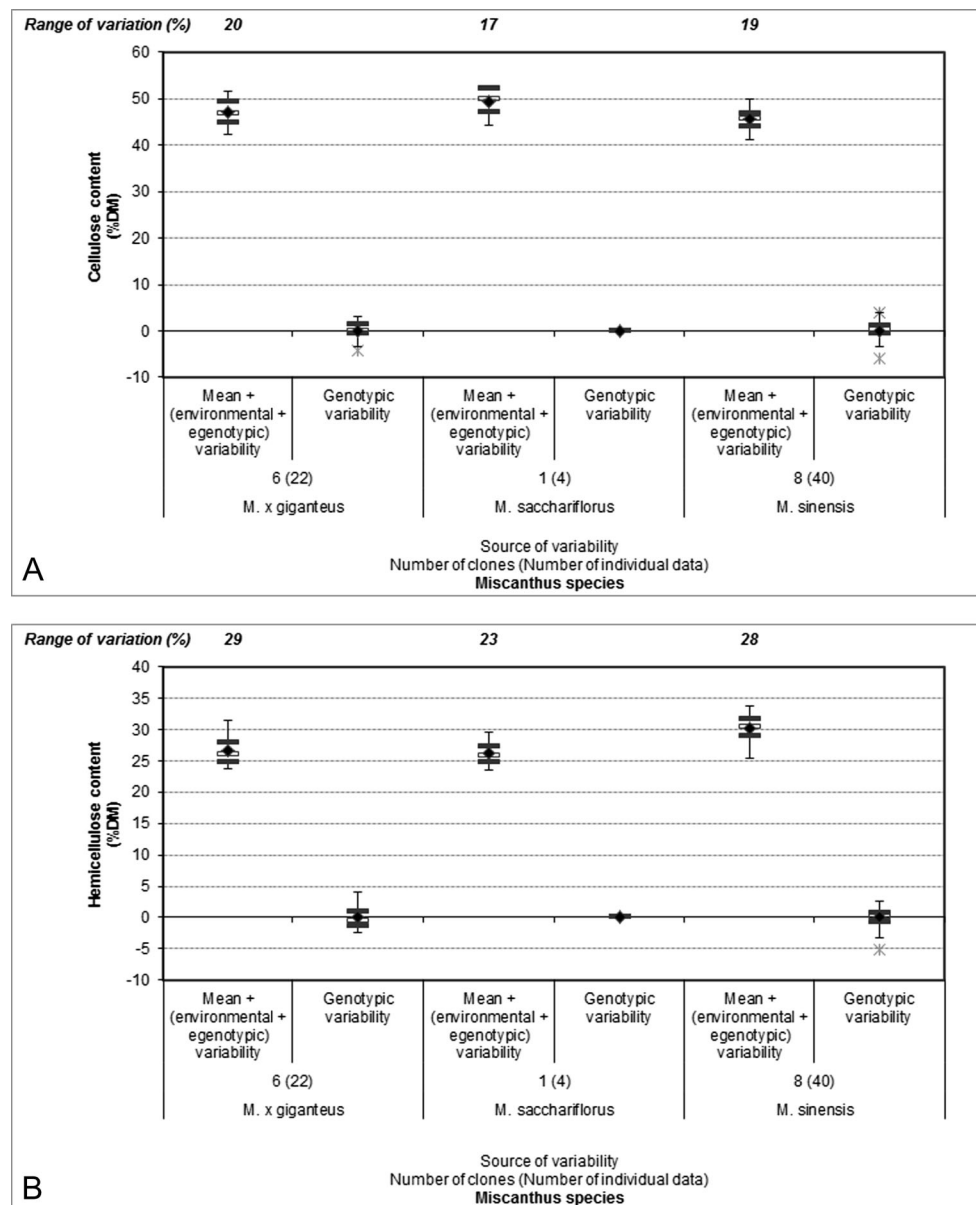


Fig. 7 The phenotypic variability and genotype contribution to the phenotypic variability in miscanthus among and within the three *Miscanthus* species *M. x giganteus*, *M. sacchariflorus*, and *M. sinensis* for cellulose content (a), hemicellulose content (b), lignin content (c), and ash content (d) in the aboveground biomass during winter harvest in the third year of cultivation. The phenotypic variability corresponds to mean + (environmental + genotypic variability); the genotype contribution to the phenotypic variability corresponds to genotypic variability. The cellulose, hemicellulose, and lignin contents were determined using the Van Soest method as the method of reference. The mean value (indicated by the black diamond) was calculated from the individual data collected in

the literature for each year of cultivation. The ends of the vertical lines correspond to the minimum and maximum values; the black horizontal lines correspond to the upper and lower quartiles; and the white horizontal lines correspond to the median for each year of cultivation. The stars on both sides of the black horizontal lines correspond to the outlier values. The range of variation corresponds to the difference between the maximum and minimum values and is expressed as a percentage of the mean. For each trait, the genotypic variability of the *M. sacchariflorus* species equaled zero; only one *M. sacchariflorus* genotype was observed. References used—**a–c** Hodgson et al. [33]; **d** Clifton-Brown and Lewandowski [43], Lewandowski et al. [37]

fertilization: in winter harvest, there was no effect of the nitrogen treatment whereas in autumn harvest, the biomass production increased with the nitrogen fertilization.

- The influences of nitrogen fertilization and irrigation
Most studies investigated the effects of nitrogen input

levels and irrigation on *M. x giganteus* biomass production until now, excepting the study of Palmer et al. [67].

In a review, Cadoux et al. [79] concluded that the effect of nitrogen fertilization on aboveground *M. x giganteus* biomass production varied. Certain studies reported no effect from nitrogen fertilization on aboveground *M. x giganteus* biomass production [51, 57, 59, 62, 80]. Other

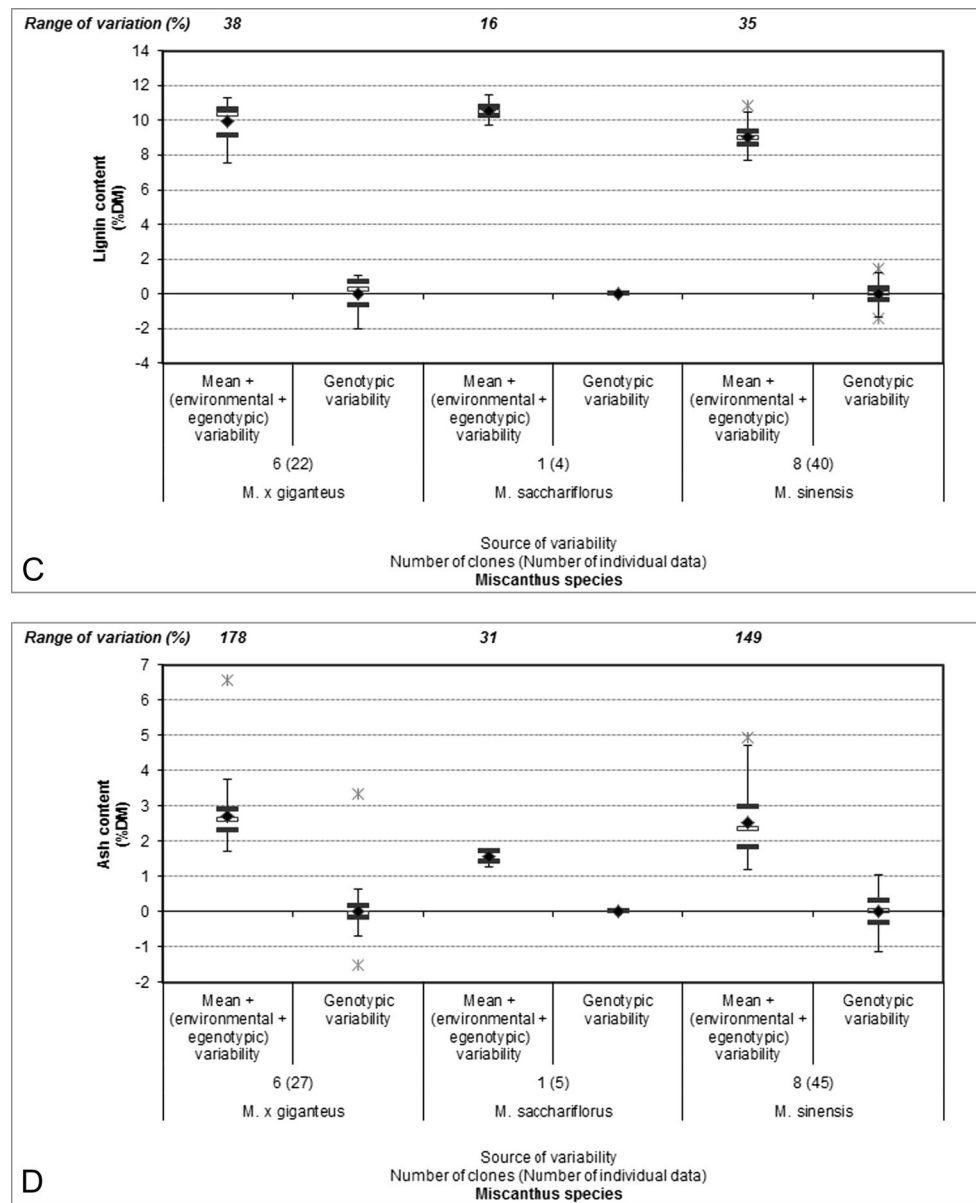


Fig. 7 (continued)

studies have reported a positive effect on the aboveground biomass for nitrogen input ranging from 0 to 200 kg N/ha [6, 26, 64, 81, 82]. More recently, variable effects for nitrogen fertilization were even observed on the biomass production between years [65].

As reported by Cadoux et al. [79], the effect from nitrogen fertilization on aboveground *M. x giganteus* biomass production was generally limited but the effect was amplified with irrigation. For instance, during the third year of cultivation, Mantineo et al. [59] observed maximum *M. x giganteus* biomass production with the highest nitrogen fertilization level (100 kg N/ha) and under the best soil water content conditions (75 % water restoration in the soil with maximum evapotranspiration). Moreover, Ercoli et al.

[82] concluded that the effect of irrigation on *M. x giganteus* biomass production increased with the nitrogen level, whereas irrigation did not affect biomass production without nitrogen fertilization. With 100 kg N/ha, irrigation increased the biomass production by 3.7 t DM/ha, and with 200 kg N/ha, irrigation increased the biomass production by 9.8 t DM/ha compared with the rainfed treatment over the first 4 years of cultivation.

These observations suggest that high *M. x giganteus* yields could be obtained with low nitrogen input levels because biomass production increases recorded with 0 and 200 kg N/ha were moderate or insignificant. However, because the irrigation and nitrogen level influenced *M. x giganteus* biomass production in the interaction,

it would be interesting to perform a more detailed investigation of the miscanthus capacity to produce high biomass under low nitrogen input and low irrigation levels with other *Miscanthus* species than *M. × giganteus*. Identifying clones that can produce a high biomass with the lowest environmental footprint (lowest water requirement and nitrogen fertilization levels) is necessary for improving miscanthus growth for bioenergy use.

- The influence of plant density

Different plant densities were used in miscanthus experiments (0.6 to 4 plants/m², see Tables 1 and 2), but only a few studies reported the effect of plant density on miscanthus biomass production. Danalatos et al. [80] observed that a 1- and 2-plants/m² density improved miscanthus biomass production compared with 0.67 plants/m² in central Greece. Based on this result, we suggest that the higher plant density increased biomass production. Based on this observation, two hypotheses can be formulated for the evolution of miscanthus biomass production over several years: (i) at higher plant density, biomass production will plateau more rapidly, and (ii) a higher plant density will yield higher biomass production levels during the plateau phase. More investigation is necessary to confirm these hypotheses and select an optimal plant density to maximize aboveground miscanthus biomass production. In addition, this choice should also depend on the cost of the miscanthus rhizomes because the expense of establishing the rhizome remains high.

Producing plantlets through vitro culture or producing seed-propagated varieties may be good alternatives for combining high plant densities, high biomass production, and lower establishment costs. Nevertheless, the environmental footprint, such as non-invasiveness, must also be preserved. For example, sterility must be obtained for seed-propagated varieties.

The Influence of Species and Ploidy Level

First, the *Miscanthus* species differed in biomass production and biomass components. With the average values 12 and 18 t DM/ha for the winter harvest during the second and third years, respectively, *M. × giganteus* seemed the best biomass producer compared with the *M. sacchariflorus* and *M. sinensis* species (Fig. 4a). The *M. × giganteus* species also seemed better than the *M. sacchariflorus* and *M. sinensis* species. Moreover, the *M. sinensis* species seemed smaller than the *M. × giganteus* and *M. sacchariflorus* species (Fig. 4b). During the third year, the *M. × giganteus* species reached 231 cm on average, whereas the *M. sacchariflorus* and *M. sinensis* species reached 185 and 132 cm, respectively (Fig. 4b). For stem number per plant, the *M. sinensis* and *M. × giganteus* species appeared to produce

more stems than the *M. sacchariflorus* species. For example, the *M. sinensis* and *M. × giganteus* species produced 49 and 58 stems per plant on average, respectively, during the third year compared with the *M. sacchariflorus* species, which produced 26 stems (Fig. 4c). Finally, for stem diameter, the *M. sacchariflorus* and *M. × giganteus* species showed the highest values compared with the *M. sinensis* species for the second and third years of cultivation (Fig. 4d). For example, during the third year, the *M. sacchariflorus* and *M. × giganteus* species showed the mean stem diameters 7.2 and 5.6 mm on average, respectively, compared with *M. sacchariflorus* species, which exhibited the stem diameter 4.4 mm (Fig. 4d).

The range of variation within species was higher for *M. sinensis* than both the *M. × giganteus* and *M. sacchariflorus* species, particularly for biomass production and stem number per plant (Fig. 4a, c). For example, during the third year, the range of variation for aboveground biomass production and stem number per plant in the *M. sinensis* species reached 324 and 276 % on average, respectively (Fig. 4a, c). In contrast, the range of variation for canopy height and stem diameter appeared similar among the three *Miscanthus* species (Fig. 4b, d).

For each trait, the genotype contribution to the phenotypic variability was high. This genotypic variability was more visible in the second and third years than the first year (Fig. 4). Moreover, this genotypic variability was generally more visible for the *M. sinensis* species than the *M. × giganteus* and *M. sacchariflorus* species (Fig. 4). This trend confirmed that the genotypic diversity among miscanthus is relatively high, particularly among the *M. sinensis* species [28].

Second, certain *Miscanthus* clones exhibited high biomass production and biomass component values and, thus, appeared suitable for biomass production in contrast to other clones that exhibited low values.

Interestingly, certain *M. sinensis* clones produced more biomass than certain *M. × giganteus* clones. Based on the studies shown in Fig. 4a, the maximum biomass production 31.9 t DM/ha was recorded for a hybrid composed of two *M. sinensis* clones (EMI no. 7) in Portugal with irrigation compared with a 30.6 t DM/ha maximum for a *M. × giganteus* clone (Greef et Deu.) in Italy with irrigation, as well as 100 kg N/ha from a winter harvest during the third year of cultivation. Although a higher aboveground biomass production of 49 t DM/ha was recorded for a *M. × giganteus* clone in France under irrigated conditions [83], based on these observations, we suggest that specific *M. sinensis* clones can produce more biomass than certain *M. × giganteus* clones under specific climate conditions and locations. This suggestion was confirmed by the results from Clifton-Brown et al. [44]. In addition, certain studies have reported the superiority of certain *Miscanthus* hybrids for biomass production compared with non-hybrids. For example, Clifton-Brown et al. [44]

observed that *M. sinensis* hybrids (EMI nos. 6–10) produced 31 % more biomass on average than non-hybrid *M. sinensis* clones (EMI nos. 11–15) during a winter harvest in the third year of cultivation.

Considering the biomass components, two *M. sinensis* clones tested in Northern France [41] showed a high stem number per plant: *M. sinensis* Herman Müssel reached 85 stems during the second year of cultivation, and *M. sinensis* Silberspinne reached 176 stems during the third year of cultivation (Fig. 4c). One *M. × giganteus* clone (Flo) that was also tested in Northern France [41] showed a high stem diameter, reaching 9.8 mm in the third year (Fig. 4d).

Finally, ploidy level influenced the biomass production and its components. Glowaka et al. [84] observed significant but contrasting effects on biomass production and its components from increasing the ploidy level, depending on the genotype. Interestingly, the authors generated polyploid forms for certain genotypes, which showed more dry matter, higher plants, or a greater stem diameter compared with the corresponding controls. The results from Zub et al. [41] suggest that the miscanthus tetraploid or triploid forms were more productive than the diploid forms, and although the miscanthus forms studied were from distinct backgrounds, they reinforced the results from Glowaka et al. [84]. These results show that a greater ploidy level can improve biomass production.

Despite these encouraging results, this investigation must be considered in its context for several reasons. First, the species compared from various environments did not have the same genetic background. Second, the number of clones used differed between the three *Miscanthus* species studied: fewer clones were observed for the *M. × giganteus* and *M. sacchariflorus* species than the *M. sinensis* species; this could partially explain the lower variability in the *M. × giganteus* and *M. sacchariflorus* species. Finally, the effect of ploidy level on biomass production and its components seemed to differ based on the genotype.

The Phenotypic Variability for the Traits Related to Biomass Composition

The Influence of Crop Age

Allison et al. [45] studied the effect of year of cultivation on cellulose, hemicellulose, and lignin contents in miscanthus. Based on their experiments, the authors suggested that miscanthus biomass composition remained relatively consistent over the successive second, third, and fourth years.

Clifton-Brown and Lewandowski [43] compared several miscanthus clones for ash content in aboveground biomass during the first 3 years of cultivation. They observed that this content decreased from 8.9 to 2.3 % DM on average between the first and third years during the winter harvest (Fig. 5). Moreover, with a 79 % range of variation during the first year,

the ash content appeared more variable during the first year compared with the second and third years (Fig. 5).

The Influence of Geographical Area

By studying the biomass compositions of 15 *Miscanthus* genotypes in five European locations during the third year of cultivation, Lewandowski et al. [37] showed that the ash content in the aboveground biomass varied based on the experiment location. Hodgson et al. [33] also highlighted a significant effect from location on cellulose and hemicellulose contents in the aboveground biomass. In contrast, these authors showed that this effect was not significant for lignin content in the aboveground biomass.

The influence of location on biomass composition could be partly explained by climatic conditions. The rainfall differences between locations could cause mineral leaching. Wind force differences between locations could affect the composition due to leaf loss [38].

These observations demonstrated that miscanthus biomass composition differed based on location. However, in contrast to biomass production, for which clear trends between Northern Europe countries and Central/Southern Europe countries have been highlighted [37], no clear trends identified an area that is most suitable for miscanthus biomass composition.

The Influence of Crop Management Practices

• The influence of harvest date

The cellulose and lignin contents in the aboveground biomass increased from 40.6 to 46.4 % DM and 8.0 to 9.4 % DM on average between the autumn and winter harvests, respectively (Fig. 6a, c). In contrast, the hemicellulose content tended decreased between the two harvest dates with the averages 29.4 and 28.8 % DM during the autumn and winter harvests, respectively (Fig. 6b). These cellulose, hemicellulose, and lignin content differences between the autumn and winter harvests could be due to leaf loss between the two harvest dates [69].

Delaying the harvest time also significantly decreased the ash content [37, 43, 70] as well as cutting the biomass and leaving it in the field [71]. For example, the ash content decreased from 3.9 to 2.5 % DM on average between the autumn and winter harvests during the third year of cultivation (Fig. 6d).

In addition, the genotype contribution to phenotypic variability was high for cellulose, hemicellulose, lignin, and ash contents in the aboveground biomass (Fig. 6).

Finally, with variations ranging from 25 to 213 % during the third year of cultivation, the cellulose, hemicellulose, lignin, and ash contents were highly variable traits (Fig. 6). Interestingly, the highest ranges of variation for

ash content (145 and 213 %) showed that this trait was more variable than cellulose, hemicellulose, and lignin contents regardless of the harvest date (Fig. 6).

- The influence of nitrogen fertilization

Hodgson et al. [32] showed that the nitrogen input negatively impacted the *M. × giganteus* biomass quality; when nitrogen input increased, the cellulose, hemicellulose, and lignin contents in the aboveground biomass decreased, while ash increased. The authors concluded that low nitrogen fertilization yielded better quality biomass for thermochemical processes. These observations were encouraging as environmentally and economically beneficial and as contributor to cropping system sustainability.

The Influence of Species

Differences among *Miscanthus* species were highlighted for cellulose, hemicellulose, lignin, and ash contents during the winter harvest of the third year of cultivation (Fig. 7). With the average values of 47.3 and 49.4 % DM, respectively, the *M. × giganteus* and *M. sacchariflorus* species appeared to contain more cellulose than the *M. sinensis* species (Fig. 7a). Moreover, with the average values of 10.0 and 10.6 % DM, respectively, the *M. × giganteus* and *M. sacchariflorus* species also seemed to exhibit higher lignin content than the *M. sinensis* species (Fig. 7c). In contrast, with the average value 30.2 % DM, the *M. sinensis* species exhibited higher hemicellulose content than the *M. × giganteus* and *M. sacchariflorus* species (Fig. 7b). Finally, with the average value of 1.6 % DM, the *M. sacchariflorus* species exhibited lower ash content in the aboveground biomass than the *M. × giganteus* and *M. sinensis* species (Fig. 7d).

In addition, range of variation seemed variable based on the *Miscanthus* species and its traits. The range of variation for the cellulose and hemicellulose contents seemed to be the same as for the *M. × giganteus*, *M. sinensis*, and *M. sacchariflorus* species. In contrast, the range of variation for the lignin and ash contents appeared significantly lower for the *M. sacchariflorus* species than for the *M. × giganteus* and *M. sinensis* species. Among the traits studied, the ash content seemed the most variable trait with a range of variation of up to 178 % compared to the cellulose, hemicellulose, and lignin contents in the aboveground biomass (Fig. 7).

Finally, for each trait, the portion of phenotypic variability due to the genotype was high, except for the *M. sacchariflorus* species, for which a single clone has been studied (Fig. 7).

The biomass composition observations among the *Miscanthus* species can only be considered trends because only few clones were used in the studies reported in Fig. 7, wherein only a single *M. sacchariflorus* clone, six *M. × giganteus* clones, and eight *M. sinensis* clones were used.

Interestingly, certain *Miscanthus* clones showed high cellulose and hemicellulose contents, and low lignin and ash contents. Certain *M. sinensis* clones presented lower minimal ash content than *M. sacchariflorus* clones (Fig. 7d): Clifton-Brown and Lewandowski [43] and Lewandowski et al. [37] identified two *M. sinensis* clones (Sin-11 and Sin-15) with the lowest ash content. Moreover, one *M. × giganteus* clone (EMI08) studied in England by Hodgson et al. [33] showed lower minimum lignin content than *M. sinensis* clones (Fig. 7c).

These observations suggest that certain *Miscanthus* species or clones are useful for breeding programs considering biomass composition. In addition, two papers reinforced the suggestions based on these data: the study by Allison et al. [45] which did not indicate individual values, and the study by Hodgson et al. [46] which concerned older crops that were 9 years old.

Key Points and Conclusions on Phenotypic Variability for *Miscanthus* Biomass Production and Biomass Composition

The present review shows that the traits of interest related to biomass production and biomass composition were generally highly variable for *Miscanthus*. Among these traits, aboveground biomass production and stem number per plant appeared more variable than canopy height and stem diameter with values up to 300 % during the third year, the range of variation for the aboveground biomass production and stem number per plant were approximately 2-fold greater than the range of variation for the canopy height and stem diameter. Interestingly, for the biomass composition, the ash content in the aboveground biomass appeared to be the most variable trait; with values that could reach over 200 %, the range of variation for ash content reached levels 5-fold greater than the range of variation for cellulose, hemicellulose, and lignin contents. Moreover, the genotype contribution to the phenotypic variability was high for each trait studied.

Therefore, the high phenotypic variability for the traits studied and high genotype contribution to the variability were important for enhancing *Miscanthus* breeding for bioenergy.

In addition, most of the factors of variation investigated in this review influenced the traits related to biomass production and biomass composition and, therefore, must be considered in breeding programs.

Miscanthus biomass production and canopy height appeared to mainly be influenced by crop age. With the maximum mean up to 13.0 t DM/ha and 176 cm during the third year of cultivation, the biomass production and canopy height may be approximately 2.2- and 2.7-fold greater for a 3-year-old crop than a 1-year-old crop, respectively.

Similarly, the stem number per plant seemed mainly affected by the crop age and species: (i) With the maximum mean at 53 stems per plant in a 3-year-old crop, the stem number per plant increased 2.5-fold from the first to third year, and (ii) in comparison, with the maximum mean 58 stems in the *M. sinensis* species, the stem number per plant was 2.2-fold greater in the *M. sinensis* species than in the *M. sacchariflorus* species, which showed the lowest mean in the third year of cultivation.

The stem diameter appeared to mainly be influenced by species; with the maximum mean 7.2 mm for the *M. sacchariflorus* species, the stem diameter was 1.6-fold greater than for the *M. sinensis* species, which showed the lowest mean during the third year of cultivation.

For biomass composition, the cellulose, hemicellulose, and lignin contents in the aboveground biomass appeared to be, at a same level, most affected by the harvest date and species, while it was not affected by crop age.

With a 46.4 % DM average during the winter harvest, the cellulose content was 1.1-fold greater during the winter harvest than the autumn harvest during the third year of cultivation. Similarly, with a 9.4 % DM average during the winter harvest, the lignin content was 1.2-fold higher during the winter harvest than the autumn harvest during the third year of cultivation. In contrast, with the mean 28.8 % DM during the winter harvest, the hemicellulose content was similar during the winter and autumn harvests in the third year of cultivation. In comparison, with means up to 49.4 and 10.6 % DM in *M. sacchariflorus* species, the cellulose and lignin contents were, respectively, 1.1- and 1.2-fold greater in this species than in the *M. sinensis* species, which exhibited the lowest average values. With the maximum mean 30.2 % DM in the *M. sinensis* species, the hemicellulose content was 1.2-fold greater in this species than in the *M. sacchariflorus* species, which exhibited the lowest average value.

The ash content in the aboveground miscanthus biomass appeared mainly affected by the crop age. With the average 2.3 % DM in a 3-year-old crop, the ash content can decrease by 3.9 from the first to the third year.

Therefore, developing miscanthus as sustainable feedstock for bioenergy required tackling such factors that influence traits related to biomass production and composition.

With bioenergy use as the breeding objective, the phenotypic variability, the genotype contribution to this variability, and the factors of variation investigated in the present review yielded guidelines (i) to select the traits for consideration in breeding programs, (ii) to propose the miscanthus species or clones that appear interesting as progenitors for breeding, and (iii) to select the optimal crop age, crop management practices, or geographical areas.

Implications for Miscanthus Breeding Considering the Industrial Requirements for Bioenergy Use

Few studies have investigated the ability to transform lignocellulosic crops through the different bioenergy conversion processes on an industrial scale. However, the researchers and industrial companies involved in recent bioenergy projects have begun to generate more precise requirements for optimizing lignocellulosic crop biomass production and composition.

First, for biomass production, the industry supports the notion that biomass production per hectare must be maximized under various climates with a lower environmental footprint for both biomass conversion processes: biochemical and thermochemical processes (Table 3(A)).

Second, although the lignocellulosic crop biomass composition requirements have not been fully defined by the industry, data are available from several private partners or publications. These data concern cellulose, hemicellulose, lignin, and ash contents in aboveground biomass, which are considered the main traits that impact biomass conversion efficiency.

Lignin and ash content appear to be the main traits that affect thermochemical process efficiency. High lignin content is preferable for this process because high lignin content aids in improving calorific value [32, 33, 85] (Table 3(A)). In contrast, low ash content is preferred, especially for combustion, as it implies a more efficient combustion processes, higher heating values, lower NO_x emissions and, thus, lower combustion costs [37, 38] (Table 3(A)).

Cell wall composition and, more particularly, cellulose, hemicelluloses, and lignins appear to be the main traits that affect biochemical process efficiency. The cell wall composition and its digestibility must be optimized to ensure high sugar extraction efficiency in biochemical processes [31, 86]. Among cellulose, hemicelluloses, and lignins, lignins seem the main restrictive factors because they apparently limit microbial accessibility of fermentescible sugars stored in the cell wall during fermentation [34–36]. Cellulose and hemicellulose contents also affect the efficiency of such processes during hydrolysis or fermentation [31, 87]. Based on these observations and the initial results from recent projects, the industry expects high cellulose and hemicellulose contents but low lignin and ash contents to enhance the efficiency of biochemical processes [Colonna, Dumas, personal communication, 88] (Table 3(A)). However, a favorable balance between lignin, cellulose, and hemicellulose for this process has not been fully defined because (i) data for the influence of biomass composition on this process' efficiency have not been published, and (ii) the methods used to assess biomass cell wall components differ, are not standardized, and yield variable results [31].

All these industrial requirements regarding biomass production and composition as well as the high genotypic

Table 3 (A) Summary of the requirements expected in the industry for lignocellulosic crop biomass production and composition for each biomass conversion process to produce bioenergy and (B) *Miscanthus* species characteristics relevant to biomass production and composition based on the observations in this review

Biomass conversion process	Trait					
	Type of conversion	Bioenergy end-use	Aboveground biomass production per hectare	Cellulose content	Hemicellulose content	Lignin content
(A)	Thermochemical	Heat, electricity, cogeneration, or biofuels	+	NA	NA	+
	Biochemical	Heat, electricity, cogeneration, or biofuels	+	+	+	–
(B)	Thermochemical	Heat, electricity, cogeneration, or biofuels	+, Gig, Sin (some clones); –, Sacc, Sin	+	+, Gig, Sacc; –, Sin, Gig (some clones)	+, Gig, Sin; –, Sacc, Sin (some clones)
	Biochemical	Heat, electricity, cogeneration, or biofuels	+, Gig, Sin (some clones); –, Sacc, Sin	+, Gig, Sacc; –, Sin	+, Sin; –, Gig, Sacc	+, Gig, Sin; –, Sacc, Sin (some clones)

“+” corresponded to high value, “–” corresponded to low value, NA data not available

variability described for these miscanthus traits herein aid in proposing guidelines for developing miscanthus breeding programs to produce bioenergy.

Firstly, the industry must maximize lignocellulosic crop biomass production for each bioenergy conversion process (Table 3(A)). Based on the current investigation, breeding programs must include developing varieties that (i) reach their maximum production potential per hectare in a specific environment and (ii) are adapted to a wide range of climates. Among the three *Miscanthus* species studied in this review, the *M. × giganteus* species and certain *M. sinensis* species clones appear particularly interesting because they produce a high quantity of biomass per hectare (Table 3(B)). Although the *M. sacchariflorus* species and *M. sinensis* species produce lower biomass per hectare on average than the *M. × giganteus* species (Table 3(B)), these species can offer progenitors for breeding programs because they are adapted to a wider range of climates than *M. × giganteus*. Moreover, breeding programs must consider nitrogen and water use efficiencies for a low environmental impact. In addition, traits such as canopy height, stem number per plant, or stem diameter must be considered in miscanthus breeding programs because these traits correlate with biomass production [40, 41].

Secondly, although the industrial requirements for lignocellulosic crop biomass composition have not been fully defined, these requirements appear to contrast based on the conversion processes (Table 3(A)). Therefore, currently, improving biomass composition is the most important challenge for bioenergy breeding programs to improve the efficiency and economic expense of conversion processes [31]. Based on the present review, we conclude that the species to consider may differ based on the conversion process. A given *Miscanthus* species may be suitable for one conversion process but not another. For thermochemical processes, such as combustion, pyrolysis, or gasification, the *M. × giganteus* and *M. sacchariflorus* species seemed better adapted than the *M. sinensis* species due to their high lignin content (Table 3(B)). For biochemical processes, such as hydrolysis, fermentation, or methanation, the *M. × giganteus* and *M. sacchariflorus* species were also interesting because they had high cellulose content. However, these species also had high lignin content, which can reduce efficiency for these processes (Table 3(B)); therefore, they were not entirely suitable. One *M. × giganteus* clone (EMI08) and the *M. sinensis* species showed lower lignin content and, therefore, may be particularly interesting for improving lignin content from biochemical processes in breeding programs (Table 3(B)). As low ash content is more suitable for all of these processes (Table 3(A)), it may also be interesting to consider the *M. sacchariflorus* species for improving this trait because this species contained less ash than the *M. × giganteus* and *M. sinensis* species (Table 3(B)). The two *M. sinensis* clones

(Sin-11 and Sin-15) also appeared interesting because they had the lowest ash content.

In addition to these guidelines, other factors that influence miscanthus biomass production and composition must be considered. The variability described in this reviews allow us to suggest that mature miscanthus crops are more suitable than younger crops for these processes due to (i) the maximum canopy height, stem number, and biomass production, as well as (ii) the lower ash content. Moreover, for thermochemical processes and, more particularly, combustion, delaying the miscanthus harvest date from autumn to winter yielded a better biomass composition, which leads to better combustion efficiency [38]. Hodgson et al. [33] also predicted that the winter harvest is more suitable for thermochemical processes because the calorific value of the winter-harvested biomass should increase. Finally, delaying the harvest date decreased the drying and transport expenses because the moisture content was lower for the winter harvest. Therefore, the winter miscanthus harvest appeared more economical than the autumn harvest for such conversion processes [89, 90]. Interestingly, the crop age and harvest date recommendations are consistent with recommendations from agronomical and environmental perspectives [91].

Prospects

The present review provides relevant information on genotypic and environmental variability for the biomass production and composition of the three *Miscanthus* species which gained most of the attention today. The broad genotypic variability in these traits shows that the potential for improving miscanthus as a bioenergy feedstock is great. However, miscanthus is a recent undomesticated crop; this review shows that little information on miscanthus biomass production and composition has been reported compared with major crops, and it must be genetically improved. Therefore, more information is necessary.

The relationships between the traits related to biomass production and composition that have not been studied for miscanthus until now must be investigated. It is important to improve the biomass composition while at least maintaining the biomass production. Moreover, little information about the heritability has been reported up to now. Heritability for these traits must be thoroughly investigated in the future. The present review showed extreme clones for many traits of interest, which can guide the breeder to create the relevant populations for assessing such estimates of heritability. In addition, miscanthus germplasm collections must be developed because the current collections do not cover the broad genetic diversity in the *Miscanthus* genus. Exploring miscanthus species other than the three *Miscanthus* species discussed herein may be promising for assessing new and interesting variables for miscanthus breeding. In addition, the breeding considerations

for biomass composition should be accompanied by a complete definition of the industrial requirements for the different bioenergy conversion processes.

Finally, the results from the present investigation on miscanthus can be extrapolated to other monocotyledons or perennial crops that are close to miscanthus from a taxonomic perspective; sugarcane, sorghum, or maize are also promising feedstock candidates for bioenergy production. Enhancing other lignocellulosic crops is particularly interesting for the industry. The most sustainable scenario currently proposed includes developing crop areas close to local facilities. The most suitable feedstock for each facility will have to fulfill a continuous supply of the facility and will depend on the area's climatic and soil conditions. Therefore, this feedstock will be composed not only of a single crop but a set of several crops specifically adapted to the production area and harvested at different times throughout the year to continuously supply the facilities.

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