Variability of stem solidness among miscanthus genotypes and its role on mechanical properties of polypropylene composites

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Abstract
Miscanthus (Miscanthus Andersson) is a perennial grass that is attracting growing interest from the biomaterial industry. Our aim was to compare miscanthus genotypes varying in stem solidness, a measure of degree to which pith fills cavity between the outer walls of the stem, and analyze whether this trait influences the mechanical properties of polypropylene composites reinforced with miscanthus particles. Six contrasting genotypes were chosen from a Miscanthus sinensis population to determine morphological variables, stem solidness, and mechanical properties of polypropylene composites including 30% of milled miscanthus particles of two sizes of 100 < x < 200 μm and 200 < x < 300 μm. Although aboveground biomass of miscanthus was closely related to the aboveground volume of the plant, namely stand volume, a few genotypes showed contrasting aboveground biomass production for similar stand volumes. This generated contrasting ratio between aboveground biomass and stand volume, namely plant-specific weights, for similar plant volumes. A principal component analysis showed that fully pith-filled stems, namely solid stems, were explained by a large stand volume and plant-specific weights as well as small stem cross-sections. Genotypes showing partially filled stems were taller with larger stem cross-sections but smaller plant-specific weights. They revealed high lignin and p-coumaric acid contents. Compared to neat-polypropylene, Young’s modulus increased significantly by 139% and 134% and tensile strength by 39% and 36% for genotypes with partially filled stems compared to genotypes with fully pith-filled stems, respectively. This difference in reinforcing capacity was similar to that of two particle sizes (139% and 134% for Young’s modulus, 41% and 34% for tensile strength, respectively). A good tensile strength was obtained with large cross-stem section, plant height and lignin and p-coumaric acid contents. It decreased with plant-specific weight, hemicellulose and ferulic acid contents. Wider morphological variations in other progenies or Miscanthus species should be explored further using the techniques reported here.

KEYWORDS
biomass, cell wall, composites, genotypes, Miscanthus sinensis, specific weight
**INTRODUCTION**

Miscanthus (*Miscanthus Andersson*) is a perennial grass that has attracted growing interest for the production of bioenergy, renewable fibers, and ecosystem services. This crop requires low nitrogen input due to its low nitrogen needs (Zapater et al., 2017) and to the capability of its rhizome to recycle nitrogen for the subsequent crop cycle (Strullu et al., 2011). The genus *sensu stricto* comprises about 12 species (Clifton-Brown et al., 2008), among which *Miscanthus × giganteus* and *Miscanthus sinensis* are the most cultivated in Europe. Morphological traits contribute to a high aboveground biomass production (Robson et al., 2013; Zub et al., 2011), and the aboveground biomass production itself is well predicted by a combination of morphological traits, such as aboveground volume or stand volume (Arnault et al., 2015; Zub et al., 2012). In a comparison of 21 miscanthus clones, the most productive clones displayed high cellulose and lignin contents but low hemicellulose contents (Arnault et al., 2015).

These different components of the miscanthus biomass constitute an abundant source of carbon, which leads miscanthus to be currently cultivated mainly for the production of bioenergy in Europe. Combustion was initially identified as an important potential use for miscanthus (Lewandowski et al., 1995) while the production of ethanol (Belmokhtar et al., 2017; van der Weijde et al., 2013) and anaerobic digestion were identified as interesting options later on (Kiesel et al., 2017; Thomas et al., 2019). Composites reinforced with miscanthus stems are being increasingly studied due to attractive mechanical properties associated with their lightweight compared to glass fibers (Girones et al., 2016). The first such study was conducted on *Miscanthus × giganteus* and centered on the preparation of composites by incorporating fibers that were pulped by the alkaline–methanol–anthraquinone process (Lundquist et al., 2004). They showed that cellulose fibers were thermostable at a temperature of 22.5 and 26.9 MPa with a flexural modulus of 3.2–3.8 GPa, although nothing is said about the size and origin of the fragments and the methodology used for their testing. The authors found that the incorporation of miscanthus fibers in starch-based polymer increases impact resistance, but they noted a huge scattering of impact data. In 2007, Kirwan et al. (2007) removed different types of extractives (tannins, gums, sugars and coloring matter, starches) from the stem fragments of *Miscanthus × giganteus* before their incorporation as a reinforcement in poly(vinyl alcohol). Both flexural modulus and, more significantly, strength increased with a combination of a hot washing regime and a 190°C processing temperature. The flexural strength reached up to 40–42 MPa while the mean flexural modulus decreased with the fragment sizes (5.7 and 6.8 GPa, for composites using 4 and 2 mm long stem fibers, respectively).

The comparison of different genotypes in their reinforcement capabilities occurred later, in particular with the development of an optimized small-scale protocol to prepare polypropylene composite reinforced with stem fragments (Girones et al., 2016). This protocol focused on the use of the whole crop that is usually comminuted into stem fragments during the winter harvest; after two milling rounds, stem size was reduced by sieves with varying open mesh pore sizes in their protocol. Their preparation contained a matrix of polypropylene reinforced with 30% w/w content of miscanthus fragments of varying sizes. The smallest fragment sizes yielded the best tensile strength while no significant effect was observed for the Young’s modulus and elongation at break, as smaller sieve sizes likely improved transfer between the matrix and the filler. In their study, the tensile strength varied from 32.5 to 38.2 MPa among genotypes while the standard without miscanthus fibers reached only 24 MPa. The Young’s modulus ranged from 2.77 to 3.25 GPa, in comparison to 1.15 GPa for the standard without fiber reinforcement. These results highlight the good reinforcing capacity of miscanthus fragments and shows a 17% variability among genotypes for both mechanical properties. Interestingly, the clone of the cultivated species in France, namely *Miscanthus × giganteus* (coded GIGB in the study of Girones et al., 2016), is among those showing the best performance (36.4 MPa and 3.250 GPa for tensile strength and Young’s modulus, respectively). A clone of the *M. sacchariflorus* species singularly outperforms this clone while all *Miscanthus sinensis* clones show lower performance with the exception of the Silberspinne clone.

Few studies related the initial miscanthus stem properties with the biochemical characteristics of the plant. Lundquist et al. (2004) used the smallest units and highest quality cellulose fibers from *Miscanthus × giganteus* stems thanks to an alkali–methanol–antraquinone pulping process (Lundquist et al., 2004). They showed that cellulose fibers were thermally stable up to 255°C and had an aspect ratio of 40, a tensile strength of 890 MPa and a Young’s modulus of 60 GPa. Other studies directly took the miscanthus stem fragments into account without extracting any component. In a comparison of several miscanthus species, Chapin et al. (2020) found evidence that elastic modulus was positively correlated with ferulic acid (0.58) and negatively correlated with p-coumaric acid content (−0.66), the latter being correlated with lignin (0.62) and the former with hemicelluloses (0.93).

Aside from the composition, the morphology and anatomy of the stem were partially taken into account. The stem of miscanthus is composed of successive nodes and internodes. While finding a genotype effect on fragments elastic moduli, Chapin et al. (2020) found evidence that the stem fragments elastic moduli were stable between composites based on stem...
internodes sampled at different positions along the stem. In addition, the miscanthus stem is made up of a dense outer tissue rind and a softer pith located in the center of the stem. In comparison with maize, Brancourt-Hulmel et al. (2021) highlight that the anatomy of the miscanthus stem is characterized by a thick rind and few but dense pith-bundles while its biochemistry shows high cell-wall, lignin, and cellulose concentrations. The latter were positively correlated with rind-fraction and pith-bundle-density, and these two groups of variables accounted for the good mechanical properties of stem-based composites in miscanthus in comparison to those of maize-based composites. Apart from stem-based composites, Kaack et al. (2003) also show that the modulus of elasticity measured on a stem significantly depends on the area of parenchyma vascular bundles and outer heavily lignified tissue (outer rind), and on the concentration of lignin and cellulose. This may contribute to a higher lodging resistance according to the authors.

Another interesting trait of miscanthus is the appearance of hollow stems at maturity, as for other grasses. This filling degree particularity of the stems is known in grasses as stem solidness (Kalous et al., 2015). Solid stems are completely filled with pith while hollow stems have no pith. Intermediate types are referred to as semi-solid stems (Sherman et al., 2015). Stem solidness can be an important architectural trait to support the erectness of the plant. It can also be used for the development of high yielding potential genotypes such as those found in maize stems contribute to the increased lodging resistance according to the authors.

In February 2018, each 4-year-old plant was measured for its height at total maturity and its circumference, taken at 50-cm plant height after maximally compressing the tillers together with a zip tie according to the protocol of Gifford et al. (2015). The corresponding stand volume was calculated by multiplying these two variables. Each plant was cut 5 cm above the ground. The corresponding aboveground total fresh matter was weighed, and a representative sample of approximately 500 g of fresh matter was collected for dry-matter content estimation and near-infrared (NIRS) analyses as described below. All samples were dried at 55°C for 4 days in a well-ventilated oven and ground with a hammer mill crusher to pass through a 1-mm grid.

Biomass and cell-wall components were estimated using the robust NIRS predictive equations developed for miscanthus (Table 1). In all, 244 samples of miscanthus harvested in February between 2009 and 2018 were biochemically analyzed to calibrate the predictive equations for cellulose, lignin, and hemicellulose. In all, 23 samples came from the Miscanthus sinensis population described above. The LANO laboratory (Saint-Lô, France) carried out the biochemical analyses to determine cellulose, hemicellulosic carbohydrates, and lignin contents, according to a protocol adapted from that of Van Soest and Wine (1967). More details are given by Belmokhtar et al. (2017). Spectra were obtained while scanning the miscanthus samples with an Antaris II NIRS analyser (ThermoScientific) providing spectra from 4000 to 10,000 cm⁻¹ with 1557 points (4 cm⁻¹ spacing). Equations were calibrated using the first derivative and a Norris filter smoothing over nine spectra points. The biochemical analyses of 40 samples were then used to independently validate the calibrations. In addition, the correlations between the NIRS predictions and the biochemical data were checked on the 23 samples belonging to the Miscanthus sinensis population. The composition values (cellulose, hemicellulose and lignin) were expressed as a percentage of the dry biomass (DM). In addition, 76 samples were used for the calibration of p-coumaric and ferulic acids. The corresponding biochemical analyses were carried out using to alkaline hydrolysis.

Due to the time-consuming nature of the determination of stem solidness, six genotypes were sampled (SiA225, SiA067, SiA191, SiA100, SiA095, and their best parent SIL). Using the data from 2017, the sampling was focused on the most contrasting 3-year-old genotypes for plant biomass for three given stand volumes (Figure 1).

The solidness of stems for each genotype was determined on two stems bearing panicles through the examination of the degree of stem filling of the 4th internode (from ground level

2 | MATERIALS AND METHODS

2.1 | Plant material and measurements

The plant material consisted of a Miscanthus sinensis progeny from a biparental cross between two parents of different stem number (Malepartus and Silberspinne). The offspring of 165 clones was established at INRA of Estrées-Mons (northern France) in 2014 according to a staggered-start design (Segura et al., 2008), with each year comprising five incomplete blocks. Each genotype was represented by four plants on average, that is, repeated four times, with a planting density of 1 plant m⁻².

Accordingly, the aim of the present study was to compare miscanthus genotypes for stem solidness and analyze the effect of genotype-specific stem solidness on mechanical properties of polypropylene composites reinforced with miscanthus.

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The solidness of stems for each genotype was determined on two stems bearing panicles through the examination of the degree of stem filling of the 4th internode (from ground level
upwards) after cutting them in half. This internode was chosen as it is known to be the most discriminating among the genotypes (M. Reymond, personal communication). Each half was then photographed, and the length and diameter of the hollow pith were measured and expressed as a percentage of the total internode volume (Figure 2). The solidness thus corresponds to the stem filling degree with a solid stem being completely filled.

2.2 | Composite preparation and measurement of mechanical properties

The miscanthus stems of the six previous genotypes were ground and milled according to previously established protocols (Girones et al., 2016) and subsequently sieved to select fragments with a narrow size distribution. As in the previous study of Girones et al. (2016), sieves with two open pore mesh sizes were used: 100 < x < 200 μm and 200 < x < 300 μm, respectively, coded particle size 1 and
2. For each size, composite blends comprising 30% w/w miscanthus stem fragments were prepared using polypropylene (Addilene from Arkema, melt volume index of 65 ml/10 min at 230°C/2.16 kg) as a polymeric matrix and a maleic anhydride-grafted polypropylene (Eastman G-3015) as a compatibilizer (5% ww on dry fragment basis). Test specimens were injection molded in a Haake Minijet II (Thermo Scientific) using a steel mold complying with ISO-527-2-1BA specifications, setting the chamber temperature at 180°C and rotor speed up to 60 rpm. At least six technical repetitions for each genotype and each particle size were tested. Each specimen was mechanically evaluated with a Zwick Z2.5 testing machine (Zwick-Roell) operating at 0.02 mm/s. Young’s modulus was calculated from the secant of the stress–strain curve at 0.05–0.25% deformation. The effect of the larger and smaller sieve sizes on particles incorporated in the plastic was then compared to the effect of the stem solidness effect among genotypes.

3 | RESULTS AND DISCUSSION

3.1 | Large differences in plant weights were observed for similar stand volumes

Aboveground biomass ranged between 0.5 and nearly 24 Mg ha⁻¹ (in dry matter) while the stand volume varied from approximately 0–25 000 cm³ ha⁻¹ (Figure 1). A tight significant correlation was observed between aboveground biomass of the plant and its stand volume (r = 0.98).

Zub et al. (2012) compared the predictions of aboveground biomass production using four variables: the number of stems per plant, vegetative plant height, the ground occupied per plant, and the stand volume. The stand volume yielded the best predictions of the biomass produced in the second and third years. Arnoult et al. (2015) confirmed these findings over a longer time period. Here, we confirmed the good predictability of aboveground biomass by stand volume over a larger number of genotypes. However, in contrast to the studies of Zub et al. (2012) and Arnoult et al. (2015) and despite the high correlation between aboveground biomass and stand volume, wide ranges of plant weights were observed for similar stand volumes (Figure 1). This difference among genotypes may be due to the presence or absence of hollow pith at the stem level, leading to the following principal component analysis.

3.2 | Relationships of solidness with morphological and biomass composition variables

An initial principal component analysis was carried out on a correlation matrix of morphological variables and variables related to the composition of the biomass that were determined on the sample of Miscanthus genotypes (Figure 3). The first component captured 51.7% of the variation in the correlation matrix. In the plot of variables, this component indicated that the specific weight (SW) and contents in hemicellulose (HC), ferulic acid (FA) and ratio between ferulic and p-coumaric acid (FA:pCA) increased when the height (HT), lignin content (L) and p-coumaric acid (PCA) decreased. The component described the contrast between the specific weight and height (correlation of −0.97 in the correlation matrix, Figure 4). The plants that displayed the highest specific weights were among the smallest. To a lesser extent, the stem cross-sections (section) tended to be small (correlation of −0.54 in the correlation matrix, Figure 4). Regarding their biomass composition, they showed high hemicellulose and ferulic acid contents. The loadings of stand volume (SV) and plant circumference (C50) were near 0, which indicated that these variables were not related to this first component. They were indeed related to the second component, which revealed a contrast between these two variables and the cellulose content (correlation of −0.72 and −0.61 in the correlation matrix, respectively). Plants with large stand volumes displayed large plant circumference while their biomass showed low cellulose content. This second component accounted for 26%
of the variation in the correlation matrix. As a supplementary variable in this principal component analysis, totally filled stems were explained by a large stand volume and plant-specific weights and small stem cross-sections. In contrast, genotypes with partially filled stems displayed large plant heights and stem cross-sections, and accordingly small plant-specific weights. In terms of biomass composition, they revealed high lignin and \( p \)-coumaric acid contents.

In miscanthus, the study of the stem filling percentage is time-consuming, which may explain its lack of documentation. In addition, it also appears that stem density is rarely studied in herbaceous plants. This is in contrast to tree species, where the effects of tree size on stem wood density, moisture content, and proportion of branch biomass are well documented, in particular for the development of allometric equations to assess stem biomass, branch biomass, and aboveground woody biomass (Fortier et al., 2017). Our results show that miscanthus genotypes with fuller stems appeared to have larger plant-specific weight.

### 3.3 | Effect of particle size on mechanical properties

In comparison to neat-polypropylene, the composites prepared with the sample of Miscanthus sinensis offspring increased the mechanical properties (Figure 5), with a better increase for the Young’s modulus (137% on average) than for tensile strength (38% on average). This reinforcing capacity of miscanthus is in agreement with previous literature results (Girones et al. 2016; Johnson et al. 2005). Interestingly, the smallest particle sizes yielded the best tensile strength on average (33.8 MPa), as well as the best Young’s modulus (2.726 GPa), which corresponded to different increases between the two particle sizes: Young’s modulus recorded an increase of 140% and 133% for 100 < \( x \) < 200 \( \mu \)m and 200 < \( x \) < 300 \( \mu \)m particle sizes, respectively, and tensile strength increased by 41% and 34% for 100 < \( x \) < 200 \( \mu \)m and 200 < \( x \) < 300 \( \mu \)m particle sizes, respectively. Differences in stem fragment sizes with respect to reinforcement originate from at least two factors. The main one is linked to the aspect ratio which is directly related to the stress transfer between matrix and filler. The second is due to the fact that the composition, and thus intrinsic mechanical properties, may vary depending on the size (Vo et al., 2017). Despite the reinforcing capacity observed here, the performances of these clones from the Miscanthus sinensis species were below those of previously reported with the same polymer matrix on a clone of the interspecific hybrid Miscanthus × giganteus (Girones et al., 2016).

### 3.4 | Effect of solidness on mechanical properties

Regarding stem solidness, a better increase was observed for both mechanical properties for the genotypes showing partially filled stems compared to totally filled (Figure 5): Young’s modulus increased by 139% and 134% and tensile strength by 39% and 36%, for the former and latter genotypes, respectively. Albeit small, the difference in reinforcing capacity between the two groups of the Miscanthus sinensis offspring was similar to the difference between the two particle sizes. An improved performance for hollow stems in winter wheat was similarly described by Solle et al. (2019), where in particular the performances of composites reinforced with hollow stems were better than those with filled stems. These authors also observed that the hollow stem of wheat was most comparable to hemp.

### 3.5 | Relationships with mechanical properties

A second principal component analysis was carried out on the correlation matrix of morphological variables including solidness and variables related to the composition of the biomass that were determined on the offspring sample (Figure 6). The first principal component gathered 50.9% of the variation in the correlation matrix and the second reached 28.4%. Solidness contributed equally to both components. For both particle sizes (TS1 and TS2), tensile strength was best explained in comparison to Young’s modulus (YM1 and YM2). Young’s modulus was found to be better explained for the smallest particle size (YM1). There was a clear contrast between solidness and tensile strength, particularly

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**FIGURE 4**  Correlation matrix between morphological and biochemical variables. The variables are coded as in Figure 3.
for the smallest size particle (TS1) as shown in the correlation matrix (Figure 7). Interestingly, the tensile strength increased with the cross-stem section, plant height, and lignin and p-coumaric acid contents. Moreover, we noted that the mechanical properties decreased with plant-specific weight, hemicellulose, and ferulic acid contents.

That is, hollower stems tended to contain more lignin and less hemicellulose. This relationship can be explained by the variability of the “pith to rind ratio” between hollow and filled stems, as well as by the differences in chemical composition observed between these two parts of the stem. In *M. × giganteus*, Ji et al. (2016), indeed, found that the pith contained 38.2% cellulose, 25.8% hemicelluloses, and 16.6% lignin in percent of biomass, whereas the rind had corresponding values of 46.9%, 22.4%, and 21.7%. Regarding hemicellulose content, similar results were found by Hosseinaei et al. (2012), who showed the positive effect of hemicellulose extraction from Southern Yellow Pine on tensile strength. In this study, the tensile strength seemed to be independent of the cell-wall content due to a small difference among the genotypes for this trait (78.1%–81.7% of the dry biomass) in contrast to the study of Brancourt-Hulmel et al. (56.0%–96.8% of the dry biomass). But in both studies, other common variables such as the lignin content and conversely hemicellulose content were highlighted. The composition of stem fragments changes the intrinsic mechanical properties of composites and plays a role in their mechanical performances, which are a function of the mechanical properties of both the matrix and reinforcing elements. This is what drives the different mechanical properties of the composites for different genotypes. In addition, roughness of the surface of stem fragments is also important. It is known that when treating plant fibers with alkali, roughness is increased, which may play a role in the anchoring of fibers with the polymer matrix (Le Moigne et al. 2018). However, the variability observed for stem solidness among genotypes contributes at least in part to the differences observed in the mechanical properties of polymer composites based on the corresponding stems.

4 CONCLUSION AND PROSPECTS

Aboveground biomass of miscanthus was observed to be closely related to stand volume. Nevertheless, a few contrasted
genotypes showed large differences in aboveground biomass production for similar stand volumes, generating different plant-specific weights. In addition, larger plant-specific weights in these contrasted genotypes were found to correspond to fuller stems. Using these contrasted genotypes for the preparation of composites, we found that hollow stems provided better mechanical properties than solid or filled stems. Albeit small, this difference between partially filled and totally filled stems was similar to the difference between the two particle sizes studied. Wider morphological variations in other progenies or other Miscanthus species and their hybrids should be explored further using the techniques reported in this paper. This may help to improve the adaptation of miscanthus in the future, in particular to breed Miscanthus sinensis to be more suited to composite end-use by combining stem solidness with other stem anatomy traits, in particular size of the stem cross-section and thickness of the outer rind, and stem composition at optimal levels.

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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FIGURE 7 Correlation matrix between morphological, biochemical variables, and mechanical properties (tensile strength and Young’s modulus at the two particle sizes). The morphological and biochemical variables are coded as in Figure 3. The mechanical properties are coded as in Figure 6.
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