



**HAL**  
open science

## Correlations between genotype biochemical characteristics and mechanical properties of maize stem - polyethylene composites

Loan T.T. Vo, Jordi Girones, Marie-Pierre Jacquemot, Frédéric F. Legée, Laurent L. Cezard, Catherine Lapierre, Fadi El Hage, Valérie Méchin, Matthieu Reymond, Patrick Navard

### ► To cite this version:

Loan T.T. Vo, Jordi Girones, Marie-Pierre Jacquemot, Frédéric F. Legée, Laurent L. Cezard, et al.. Correlations between genotype biochemical characteristics and mechanical properties of maize stem - polyethylene composites. *Industrial Crops and Products*, 2020, 143, pp.111925. 10.1016/j.indcrop.2019.111925 . hal-02424196

HAL Id: hal-02424196

<https://minesparis-psl.hal.science/hal-02424196>

Submitted on 21 Jul 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Correlations between genotype biochemical characteristics and mechanical**  
2 **properties of maize stem - polyethylene composites.**

3  
4 Loan T. T. Vo<sup>1</sup>, Jordi Girones<sup>1</sup>, Marie-Pierre Jacquemot<sup>2</sup>, Frédéric Legée<sup>2</sup>, Laurent Cézard<sup>2</sup>, Catherine  
5 Lapiere<sup>2</sup>, Fadi El Hage<sup>2,3</sup>, Valérie Méchin<sup>2</sup>, Matthieu Reymond<sup>2</sup> and Patrick Navard<sup>1\*</sup>

6  
7 <sup>1</sup>*MINES ParisTech, PSL Research University, CEMEF\*\* - Centre de mise en forme des matériaux,*  
8 *CNRS UMR 7635, CS 10207 rue Claude Daunesse 06904 Sophia Antipolis Cedex, France*

9 <sup>2</sup> *Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, Versailles,*  
10 *France,*

11 <sup>3</sup> *Univ. Paris-Sud, Université Paris-Saclay, Orsay, France, Ecole Doctorale Sciences du Végétal, Bat.*  
12 *360, Université Paris-Sud, 91405 Orsay, France ;*

13  
14 \* Contact person : Patrick Navard [patrick.navard@mines-paristech.fr](mailto:patrick.navard@mines-paristech.fr)

15  
16 \*\*Member of the European Polysaccharide Network of Excellence (EPNOE), [www.epnoe.eu](http://www.epnoe.eu)

17  
18 **Abstract**

19 This study was devoted to identifying specific biochemical traits that may be addressed in maize  
20 breeding programs for improving lignocellulosic composition for biomass utilization in the composites.  
21 To this aim, six maize contrasted genotypes were cultivated, harvested and compared in term of their  
22 capability to reinforce a low-density polyethylene (LDPE) matrix. Stem biochemical composition and  
23 histological pattern of a given internode were evaluated to determine the traits that impacted the

24 mechanical properties of the maize-LDPE composites. Across genotypes, maize stems with higher  
25 concentrations of total cell wall residue, lignin, p-coumaric acids and cellulose in conjunction with lower  
26 concentrations of ferulic acids and hemicellulose yielded better composite performances. **This strong**  
27 **influence of hydroxycinnamic acids is a new finding.** Cellulose is found to be the component dominating  
28 the mechanical properties of the fragments since the effects of cell wall residue and cellulose are  
29 following the same pattern towards composite properties. **Contrary to expectations,** the correlations  
30 between the histological structure of stems and the mechanical properties of the composites prepared  
31 with stem fragments is complex and cannot be interpreted in a simple manner. The two most contrasted  
32 genotypes in terms of mechanical performances (Cm484 and F2bm3) have the most contrasted  
33 biochemical and histological parameters.

34

35 **Keywords:** maize, genetic diversity, polymer composites, processing, mechanical properties

36

37

38

## 39 **1- Introduction**

40

41 The development of sustainable bio-based products from agricultural by-products has been attracting a  
42 lot of research interest in order to decrease the waste of precious resources and develop a specific  
43 economy from wastes (Väisänen et al., 2016; Ashori and Nourbakhsh, 2010; Lancaster et al., 2013).  
44 Maize accounts for approximately 40% of all of the cereal grains. Maize stover (i.e. the whole plant  
45 without cobs) is one of the most abundant agricultural residues, being an important renewable resource.  
46 With its three main components, cellulose, hemicelluloses and lignin, maize stovers have potential uses  
47 in many value-added applications. Recently, several researches have studied their prospective  
48 applications. Cellulose nanofibers and nanocrystals (Muniyasamy, 2016, Mtibe et al., 2015; Zheng et al.,  
49 2012), lignin and hemicelluloses (Sun et al., 2010; Sun et al., 2011; Jin et al., 2009; Xiao et al., 2001),  
50 ethanol (Schietzke et al. 2009), bio-energy (Sivasankar et al., 2012), cushioning packaging materials  
51 (Zhang et al., 2017), superabsorbent (Wan et al., 2014; Garvia-Rosales and Colin-Cruz, 2010), cement-  
52 bonded and low-density particleboards (Ajayi, 2006; Wang and Sun, 2002), and bio-composites (Chun  
53 et al., 2017; Ilie et al., 2015; Zhang et al., 2015) are among the lab-scale products obtained from maize  
54 stems, with the objective of reducing the environmental impact compared to oil-based plastics or with  
55 aim of saving non-renewable materials (Alavi, 2015). Such maize stem-based materials can be used to  
56 produce alternative products for conventional materials in a number of end-uses such as short life-span  
57 utensils, ceiling panels, bulletin boards and packaging, potentially reducing cost and providing a certain  
58 level of biodegradability (Yu and Xu, 2014). Various polymeric matrixes such as polypropylene (PP)  
59 (Ashori and Nourbakhsh, 2010; Ashori et al., 2014; Nourbakhsh and Ashori, 2010.; Nourbakhsh et al.,  
60 2011; Panthapulakkal and Sain, 2006), low and high density polyethylene (Shun, 2017; Trigui et al.  
61 2013; Thamae et al., 2008), poly  $\epsilon$ -caprolactone (Dauda et al., 2007) , unsaturated polyester resin (Ilie et

62 al., 2015; Ilie and Mosnegutu, 2016; Hassan et al., 2012a, 2012b; 2013), starch (Zhang, 2015), starch  
63 acetate (Ganjyal et al., 2004), and natural rubber (Chigondo et al., 2013) have been compounded with  
64 maize stems (either with or without extraction to obtain fibers) to prepare composites.

65 Maize stems as other plant stems have complex and highly variable mechanical properties because of its  
66 heterogeneous compositions and structure (Speck and Burgert, 2011; Dongdong and Jun, 2016; Barrios-  
67 Rios et al., 2012). The outer rind, which includes epidermis, parenchyma, sclerenchyma, and vascular  
68 bundles has high lignin and cellulose content and shows good mechanical strength. The inner pith is  
69 porous and highly deformable, comprising parenchyma cells and vascular bundles which have a lower  
70 density than those in the rind and are composed of several types of cells. Composition and structure of  
71 maize stems depend maize genotype showing large differences in the proportion of tissues and the cell  
72 wall composition of each tissues (El Hage et al., 2018). Understanding how these biochemical traits  
73 affect the mechanical properties of the obtained composites will provide valuable knowledge to improve  
74 the utilization of plant stems and to select genotypes with suitable overall cell wall composition and  
75 distributions of plant tissues in stem. There are a few published papers considering the impact of  
76 biochemical or histological stem variations within a species to the properties of polymer composites.  
77 Some papers studied the influence of the microfibrillar angle in wood and flax (Reiterer et al., 1999;  
78 Bourmaud et al., 2013) and the positions of extracted fibers along the stem (Charlet et al., 2009). Sano  
79 Neto et al. (2015) extracted fibers from the leaves of 12 different pineapple varieties to prepare  
80 composites. They observed direct correlations between the mechanical properties of the composite and  
81 the fiber diameter, the cellulose content and the thermal resistance. Stokke and Gardner (2003)  
82 investigated the structural and chemical characteristics of wood to provide better utilization of wood as  
83 filler for the polyvinyl chloride blends. The three main components of wood, cellulose, hemicelluloses  
84 and lignin, impart the anisotropy, hygroscopicity and thermoplastic softening behavior of wood.

85 Furthermore, the extractives affect the surface chemistry of wood and thus the chemical interaction with  
86 the thermoplastic matrix. Two papers from our teams used fragments from whole grass stems for  
87 reinforcing polymers and reported correlations between composite properties and genotypes. Girones et  
88 al. (2016) found that there is a clear influence of the genotype when preparing miscanthus-stem  
89 fragment-polypropylene composites with miscanthus and Vo et al. (2017) found similar results for  
90 sorghum, showing that different tissues are contributing to traction and impact resistances. There is  
91 currently no direct study relating composition and/or structure of the stem fragments used for preparing  
92 composites and the mechanical properties of the prepared composites, considering different genotypes  
93 from one given plant species. Understanding of these interactions could greatly expand the search of  
94 suitable genotypes or alleles at given genes to alter the stem compositions and structure for their  
95 application improvement.

96 Herein, apart from investigating the possibility to use maize stem fragments to reinforce polymer, the  
97 present paper will focus **for the first time** at the detailed correlations between biochemical compositions  
98 and histological structure of maize stem fragments with the mechanical performances of their  
99 composites. **In particular, a focus will be made towards hydroxycinnamic acids which were never looked**  
100 **at in this context.** The generated information could be used for the development and/or selection of  
101 varieties optimizing the distribution of lignified tissues in the stem and biomass composition for polymer  
102 composite applications. After removing cobs, leaf blades and leaf sheaths, dried maize stems from six  
103 different genotypes were milled in controlled and reproducible conditions to produce elongated stem  
104 fragments. In this work, the pith and rind parts were not separated since this is not relevant for future  
105 industrial applications and could save processing cost. Fragments with controlled size distribution were  
106 selected by sieving and used to prepare composites. The method for preparing composites and testing  
107 their mechanical properties was similar to what described in (Vo et al., 2017), in order to ensure that any

108 change in the mechanical performance of a composite prepared with a given composite was only due to  
109 the influence of the maize stem fragment variability.

110

## 111 **2- Materials and methods**

112

### 113 **2.1 Maize samples**

114 Six maize inbred lines were cultivated at Le Moulon (Gif sur Yvette, France): Cm484, F2, F271,  
115 F2bm3, F66, and F98902. The phenotypic variability of maize is broad for the parietal biochemical  
116 characters and for the tissue distribution within the stems (El Hage et al, 2018) and it is difficult to  
117 capture all of this variability in a small number of genotypes. However, the six selected lines have  
118 phenotypes that scan a wide range of phenotypic variation observed in maize lineages. Harvests were  
119 performed at silage stage for each line (~30 % of dry matter). All the lines except F98902 were  
120 harvested on 1<sup>st</sup> September 2014 whereas F98902 was on 22<sup>nd</sup> September 2014, due to the fact that this  
121 latter line was late flowering. These lines are maize public inbred lines and have been selected from  
122 previous studies (Barriere et al., 2009) to represent a wide range of cell wall degradability and lignin  
123 content in the cell wall (El Hage et al., 2018).

124 From two blocs with four to six randomized rows per line, ten to twenty plants were harvested. In order  
125 to perform correlations between mechanical properties of the composites and biochemical composition  
126 of the plant stem or its histological structure, the stovers and the elongated internodes below the main  
127 cob from the same rows were harvested. The whole stem served as raw material to extract the stem  
128 fragments which were used to prepare composites. For preparing composites, leaves and cobs were  
129 removed from healthy harvested stovers (i.e. they were not rotted by any bacterial/fungal diseases) and  
130 were dried in an air-circulating oven for at least one week at 55°C. The elongated internode below the

131 main cob was used to perform histological experiments (Legland et al., 2017; see below). This internode  
132 was selected because it is the most representative of stem internodes (El Hage, 2018).

133

## 134 **2.2 Polymer matrix and coupling agents**

135 A low-density polyethylene (LDPE) with low melting point produced by SABIC® (LDPE 1965T,  
136 SABIC Europe) was selected as the polymer matrix. Orevac® 18507 (Arkema, France), a high density  
137 polyethylene with a high content of maleic anhydride (MA-g-PE), was used as the coupling agent in a  
138 proportion of 5 wt% on dry stem fragment basis to improve the mechanical properties of the resulting  
139 composites and also increase any possible differences caused by the various genotypes. The main  
140 properties of the polymer matrix and coupling agent are shown in Table 1.

141

142 Table 1: Properties of the matrix and coupling agent used in this work

	LDPE	MA-g-PE
Melt index (190°C/2.16 kg)	65 g/10 min	5 g/10 min
Melting point	104°C	128°C
Density	0.919 g/cm <sup>3</sup>	0.954 g/cm <sup>3</sup>
Tensile strength at break	7 MPa	10 MPa

143

## 144 **2.3 Biochemical analysis**

145 Biochemical analyses were performed on the sieved maize stem fragments used for preparing  
146 composites. Cell wall residue (CWR) was obtained after a three-stage extraction of the dry matter by  
147 ethanol (twice) and water. Lignin content (LK) was estimated following the Klason procedure (Dence,  
148 1992). The concentration of esterified *p*-coumaric acids (Caest) and esterified ferulic acids (Faest)



149 corresponds to the amount of *p*-coumaric and ferulic acids released by mild alkaline hydrolysis. CWR  
150 fractions were treated with NaOH according to a previously described procedure (Méchin et al., 2000).  
151 Esterified *p*-coumaric and ferulic acids were released by subjecting CWR samples (50 mg) to a mild  
152 alkaline hydrolysis (2 N NaOH, 5 mL, 20 h at room temperature). The samples recovered from this mild  
153 alkaline hydrolysis were subjected to precipitation procedure prior to high-performance liquid  
154 chromatography–mass spectrometry (HPLC-MS) analysis.

155 The analyses of polysaccharides were performed on an alcohol insoluble material prepared as follows:  
156 100 mg dry weight of grounded samples were washed four times with 4 volumes of absolute ethanol for  
157 15 min at 80 °C, then rinsed twice in 4 volumes of acetone at room temperature for 10 min and left to  
158 dry under a fume hood overnight at room temperature. Neutral monosaccharide composition was  
159 determined from 10 mg of dried alcohol insoluble material after hydrolysis in 2.5 M trifluoroacetic acid  
160 for 2 h at 100 °C as described respectively in Harholt et al. (2006). To determine the cellulose content,  
161 the residual pellet obtained after the monosaccharide analysis was rinsed twice with 10 volumes of  
162 ethanol and once with 10 volumes of acetone and hydrolyzed with H<sub>2</sub>SO<sub>4</sub> as described in Updegraff  
163 (1969). The released monosaccharides of hemicellulose were diluted 500 times and the released glucose  
164 of cellulose was diluted 1000 times. Then the monosaccharides were quantified using an HPAEC-PAD  
165 chromatography as described in Harholt et al. (2006).

166

#### 167 **2.4 Histological quantifications**

168 Histological analyses were carried out on the internodes below the main cob, following a fully  
169 automated image processing workflow which was designed for identifying the different tissue regions  
170 that compose the internode sections, described in Legland et al., 2017). An example of a maize internode  
171 section prepared for the histological study is shown in Figure 1.

172

173

174 Figure 1.

175

176 Nineteen morphometric descriptors were considered. Seven of them corresponded to morphometric  
177 features (area, area fraction, or bundle number): "StemArea" is the area of the cross section of the  
178 internode ; "Lfract" stands for "Lignified fraction" (in %) which corresponds to the area stained in red  
179 with FASGA (from the initials of the Spanish names of its components : fucsina, alcian blue, safranina, glicerina  
180 and agua) staining ; "Nlfract" stands for "Non Lignified fraction" (in %) which corresponds to the area  
181 staining ; "Nlfract" stands for "Non Lignified fraction" (in %) which corresponds to the area stained in  
182 blue with FASGA staining ; "Rfract" stands for "Rind fraction" (in %) which corresponds to the external  
183 area of the cross section, encompassing the epidermis and a region with a high density of bundles ;  
184 "Bfract" stands for bundle fraction (in %) present in the pith ; "Bnum" stands for bundle number (in %)  
185 present in the pith and "Bint" stand for bundle intensity.

186 The remaining twelve parameters evaluated corresponded to the measure of colorimetry in a specific  
187 tissue regions: Lred , Lgreen and Lblue stands for the mean intensity of the three channels (RGB) of  
188 pixels present in the Lignified fraction; NLred , NLgreen and NLblue stands for the mean intensity of  
189 the three channels (RGB) of pixels present in the NonLignified fraction ; Rred , Rgreen and Rblue  
190 stands for the mean intensity of the 3 channels (RGB) of pixels present in the Rind fraction and Bred,  
191 Bgreen and Bblue stands for the mean intensity of the 3 channels (RGB) of pixels present in the Bundle  
192 fraction.

193

194 **2.5 Preparation of composites and mechanical testing**

195 The whole process from preparing the maize stem fragments to the composites with polyethylene matrix  
196 and the mechanical testing was following the protocols detailed in Vo et al. (2017) and briefly described  
197 here. In order to be used as reinforcement, stems were mechanically reduced by milling to elongated  
198 fragments with a mean long particle size in the order of 500  $\mu\text{m}$  and sieving to collect fraction between  
199 the 200  $\mu\text{m}$  and 300  $\mu\text{m}$  sieves. LDPE polyethylene composite blends comprising 30 wt% by mass of  
200 sieved maize stem fragments were prepared at 150°C to avoid thermal degradation. MA-g-PE (5 wt% on  
201 dry fragment basis or 1.5 wt% of the composite) was added to the mix once the maize stem fragments  
202 were well dispersed. The compounding of the three components (polymer, stem fragments and coupling  
203 agent) was performed in four steps to avoid clogging and to obtain the best homogeneous distribution of  
204 maize stem fragments inside the matrix:

- 205 • Step 1 (t = 0 min): Addition of 37.5 wt% of LDPE
- 206 • Step 2 (t = 1 min): Addition of 15 wt% of maize stem fragment and 12.5 wt% of LDPE
- 207 • Step 3 (t = 3 min): Addition of 15 wt% of maize stem fragment and 12.5 wt% of LDPE
- 208 • Step 4 (t = 5 min): Addition of 6 wt% of LDPE and 1.5 wt% of MA-g-PE
- 209 • Step 5 (t = 9 min): End of the mixing process

210 After compounding, the maize-LDPE blends were granulated. Tensile and impact bars were injection-  
211 molded, imposing 150 °C for the barrel and 40 °C for the molds. Test specimens were injection-molded  
212 in a Haake Minijet-II (Thermo Scientific) using a steel mold complying with the ISO specifications (ISO  
213 527-2-1BA for tensile and ISO 179 for impact). Test specimens were kept in a room under standard  
214 laboratory atmosphere (23  $\pm$  2°C and 50  $\pm$  5% relative humidity) for at least 5 days before performing  
215 the mechanical tests (ASTM D618 –Procedure A). Tensile tests were carried out in a Zwick Z2.5 tensile  
216 testing machine with a force cell of 2.5 kN, operating at 0.02mm/s (1.2mm/min) with 55 mm gap  
217 between grips and 2 kN pre-tension (ASTM D618 –Procedure A). Charpy V-notch impact tests were

218 conducted on a pendulum Ceast 9050 with a 1-J swinging arm. A 2-mm indent was conducted onto  
219 impact bars by a single tooth Ceast NotchVIS manual notching machine. These tests are following  
220 ASTM 638 and 790 standards. Each reported result is the mean value of ten tests. **The properties we**  
221 **selected to compare the different genotypes are the most relevant ones for the most probable**  
222 **applications of such composites, i.e. as engineering pieces in areas such as mechanical construction**  
223 **(automotive, small objects and tools, etc.). Other specific properties are of course important depending**  
224 **on the application such as electric properties, thermal resistance, resistance to solvents, to water intake,**  
225 **creep, and many others, but it was not the objective of this work to survey all these properties. As shown**  
226 **in Girones et al., 2016, processing is already taking care of since we have been using a robust procedure**  
227 **to ensure that it is not affecting the comparison of results between genotypes.**

228

### 229 **3- Results and discussion**

230

#### 231 **3.1 Characterization of the various maize stem fragments**

232 Stem samples harvested of the six maize genotype sieved between 200 and 300  $\mu\text{m}$  prior to the  
233 preparation of composites were biochemically characterized to determine their composition. Internodes  
234 above the main ears were also histologically characterized to depict the repartition of lignification in the  
235 main tissues of the cross section. We observed a wide range of variations for lignin, p-coumaric and  
236 ferulic acids contents and for all histological parameters (Table 2). The p-coumaric acid content was the  
237 most variable parameters which displayed values ranging from single to double. Not unexpectedly, the  
238 lowest LK lignin content and (Caest) value in the cell wall were observed for the F2bm3 mutant. The  
239 bm3 maize mutants have been widely used in genetic and cell wall biochemical approaches since the  
240 pioneering work of Kuc and Nelson (1964) on lignin composition in 1964. As reviewed in Barrière et al.

241 (2004), bm3 stems are poorly lignified, present low p-coumarate esters and are not heavily altered in  
242 their ferulate footprint even if we can notice a slighter release of ferulate esters. Not unexpectedly either,  
243 the highest values for lignin and p-coumaric acid contents were observed for F98902 samples as already  
244 described in El Hage et al. (2018). Histological traits quantified by automatic image analysis also  
245 consistently showed a wide range of variation.

246

247 Table 2. Variability of cell wall composition of the sieved maize stem fragments and of histological characteristics of the  
 248 corresponding internodes below the ear among the six genotypes.

249 Cell wall residue CWR, lignin content LK, esterified *p*-coumaric acids Caest, esterified ferulic acids Faest, cellulose, hemicellulose  
 250 hemicellulose, stem area.

251

	CWR	LK	p-coum. acids	est. Ferulic acids	Cellulosis	Hemicellulosis	StemArea	Lfract	Nlfract	Rfract	Bfract	Bnum	Bint
	(%DM)	(%CWR)	(%CWR)	(%CWR)	(%MIA)	(%MIA)	(cm <sup>2</sup> )	(%)	(%)	(%)	(%)		(1/cm <sup>2</sup> )
Cm484	64(±1)	15.94(±0.02)	20.61(±0.06)	5.76(±0.03)	38.70(±0.81)	29.34(±0.49)	1.70(±0.35)	45.6(±7.5)	39.9(±7.3)	11.1(±0.5)	3.3(±0.4)	104(±13)	62(±5)
F2	59(±1)	17.78(±0.28)	20.58(±0.12)	6.29(±0.47)	35.33(±1.19)	27.61(±0.76)	1.739(±0.16)	64.7(±3.7)	19.1(±4.0)	10.8(±0.2)	5.3(±0.5)	115(±7)	66(±2)
F271	61(±0)	17.45(±0.38)	22.17(±0.16)	5.61(±0.14)	33.55(±1.43)	32.43(±0.64)	1.90(±0.35)	63.8(±3.5)	12.8(±1.5)	18.6(±1.3)	4.7(±0.7)	140(±1)	76(±13)
F2bm3	57(±1)	14.29(±0.18)	13.75(±0.16)	7.33(±0.06)	33.68(±0.23)	31.09(±0.31)	1.57(±0.18)	67.3(±1.2)	16.4(±0.8)	10.9(±0.1)	5.4(±0.6)	107(±10)	68(±1)
F66	63(±0)	14.83(±0.12)	19.01(±0.41)	6.37(±0.27)	34.03(±1.59)	31.54(±1.51)	1.59(NE)	53.6(NE)	27.4(NE)	13.9(NE)	4.9(±NE)	87(±NE)	55(NE)
F98902	63.5(±0.5)	19.34(±0.41)	28.25(±1.73)	5.98(±0.03)	35.07(±0.21)	25.99(±0.18)	4.04(±0.10)	64.8(±4.5)	13.6(±3.7)	15.4(±0.7)	6.1(±0)	170(±8)	42(±1)
min	56	14.11	13.59	5.47	32.1	25.8	1,35	38.1	9.9	10.6	2.9	87	41
max	65	19.75	29.99	7.39	39.5	33.1	4,14	69.2	47.2	19.9	6.1	178	90

252

253

	Lred	Lgreen	Lblue	NLred	NLgreen	NLblue	Rred	Rgreen	Rblue	Bred	Bgreen	Bblue
	(intensity)	(intensity)	(intensity)	(intensity)	(intensity)	(intensity)	(intensity)	(intensity)	(intensity)	(intensity)	(intensity)	(intensity)
Cm484	187(±1)	194(±3)	213(±0)	120(±8)	181(±5)	182(±4)	42(±3)	46(±1)	50(±2)	62(±12)	82(±9)	82(±9)
F2	177(±2)	174(±4)	196(±3)	95(±0)	151(±1)	150(±0)	43(±5)	41(±3)	43(±2)	46(±4)	62(±5)	63(±3)
F271	175(±5)	171(±3)	192(±4)	108(±9)	143(±4)	146(±3)	39(±1)	46(±3)	50(±2)	54(±2)	69(±1)	73(±2)
F2bm3	165(±1)	137(±2)	175(±1)	84(±4)	152(±1)	153(±1)	44(±4)	41(±0)	45(±0)	43(±5)	56(±7)	59(±7)
F66	175(NE)	176(NE)	201(NE)	93(NE)	157(NE)	167(NE)	41(NE)	45(NE)	53(NE)	54(NE)	65(NE)	67(NE)
F98902	174(±3)	167(±8)	196(±8)	85(±10)	147(±2)	158(±4)	37(±1)	28(±0)	37(±0)	50(±1)	62(±5)	68(±3)
min	164	135	174	75	139	143	37	28	37	37	49	52
max	188	198	213	128	186	186	48	49	53	74	91	92

254

255

### 256 **3.2 Mechanical properties of maize-LDPE composites**

257 Unlike processed fibers, like those produced by spinning solutions or melts which have a uniform cross-  
258 section, maize stem fragments have an irregular shape, as seen on Figure 2. In addition, after passing  
259 successive steps of milling and sieving, the aspect ratio of maize stem fragments is only 3 to 4,  
260 compared to an aspect ratio before processing of 350 or more for glass fibers.

261

262 Figure 2.

263

264 One of the potential problems in having such a short axial ratio is the fact that it could strongly decrease  
265 the stress transfer from matrix to filler, and thus decrease the overall mechanical properties. During the  
266 manufacturing of the composites, the processing temperature should be kept as low as possible since it  
267 affects the thermal degradation of lignocellulosic fillers. It was found that a nominal temperature of 150  
268 °C during blending was high enough to allow a correct mixing of the components. The temperature  
269 inside mixing chamber increased only to approximately 160 °C which ensured that no significant  
270 thermal degradation was expected to occur on the maize fillers. It can be considered that no loss of  
271 mechanical strength of the fillers which could affect the strength of the composite products occurred  
272 during blending.

273 The variations in the mechanical properties of the composites in term of tensile and impact strengths,  
274 Young's modulus and elongation at break are shown per genotype in Figures 3 and 4. The coefficient of  
275 variability (CV), which is defined as the ratio of the standard deviation to the mean value, for the  
276 different composite properties is given in Table 3 in order to depict the observed variability and to  
277 identify which mechanical properties exhibited the largest range of variability. The measurements of  
278 tensile and impact strengths showed similar coefficient of variation of 5.17 and 6.02 %, respectively;

279 while they were 8.79 and 11.69 % for Young's modulus and elongation at break respectively. Therefore,  
280 the elongation at break displayed the largest range of variability. The strength of the composites was  
281 positively related to their stiffness ( $R^2 = 0.785$  with  $f\text{-value} = 0.000$ ; Figure 3 bottom) and four  
282 genotypes lead to composites having an elongation at break above 4% (Figure 4) Genotype Cm484 had  
283 the highest reinforcing capacity, whereas F2bm3 had the lowest. Table 2 shows that these two genotypes  
284 are the most contrasted, having for a lot of parameters the lowest and the highest values, except for LK,  
285 Stem area, Rfract and Bnum, suggesting they have a similar global morphology, but very different  
286 values of parameters inside.

287 As can be seen in Figure 4 bottom, maize stem fragments have the ability to reinforce the LDPE matrix,  
288 despite having a low aspect ratio. The introduction of maize fragments into the LDPE matrix reduces the  
289 ductility of the matrix and enhances the tensile strength and stiffness of the composites, as compared  
290 with neat LDPE. These results are in line with our previous study with sorghum as a filler (Vo et al.,  
291 2017) where the reported values of mechanical properties obtained for sorghum-LDPE composites with  
292 the same PE matrix are similar to the ones found here (Table 4). The elastic modulus of the composites  
293 is more sensitive to the properties of the reinforcement than tensile strength. The capability to improve  
294 tensile strength and Young's modulus varies with the used genotypes. Compared with neat LDPE, an  
295 increase from 400 to 500 % of Young's modulus is observed when adding maize stem fragments  
296 (increase from 0.13 to about 0.70-0.80 GPa depending on genotypes). For tensile strength, the  
297 improvement is much lower, from 15 to 40 % (increase from 7.3 to 8.5-9.51 MPa).

298 The elongation at break ranges from 3.4 to 4.8 % and the impact strength from 3.2 to 3.9 kJ/m<sup>2</sup> for the  
299 composites whilst this value for LDPE matrix is  $44.7 \pm 1.1$  kJ/m<sup>2</sup>. This is a common observation with  
300 most of the filled composites. The decrease in elongation at break is due to the coupling of the filler to



301 the matrix, which hinders the motions of the local polymer chains, leading to a low elongation at break  
 302 and can decrease impact strength.

303 The effect of genotypes on the mechanical properties of the composites is contrasted. There is a very  
 304 little influence on Young's modulus and tensile strength. But the effect is much more pronounced on  
 305 Impact strength and even more on the elongation at break.

306

307 Table 3. Variability of Young's modulus, tensile strength, impact strength and elongation at break  
 308 among the six genotypes

	Young's modulus (GPa)	Tensile strength (MPa)	Elongation at break (%)	Impact strength (kJ/m <sup>2</sup> )
Mean value	0.72	9.10	4.16	3.53
Standard deviation	0.06	0.47	0.49	0.21
Coefficient of variation CV (%)	8.79	5.17	11.69	6.02

309

310 Figure 3.

311

312 Figure 4.

313

314 Table 4. Ratio of the Young's modulus, tensile strength over those values of the used PE with maize and  
 315 sorghum stem fragments and values of elongation at break and impact strength of the composites

316

Plant type (%) in composite)	Modulus comp/modulus PE	Strength comp/strength PE	Elongation at break (%)	Impact strength (kJ/m <sup>2</sup> )	Reference
---------------------------------	-------------------------------	---------------------------------	-------------------------------	--	-----------

---

Maize 30%	5.1-6.5	1.2-1.4	~4	3.5	This work
Sorghum 30 %	4.5-7.5	1-1.6	~4*	5-6	[44]

---

317

318 \* Note from the authors: The value of elongation at break which was reported in Tables 3 and 4 in  
 319 reference (Vo et al, 2017) was wrongly typed. The real value was ~4 %, instead of the reported one of  
 320 ~30 %, in agreement with the present article.

321

322 **3.3 Correlations between biochemical compositions of the maize stem fragments and the**  
 323 **mechanical properties of composites**

324 Many attempts have been carried out to correlate the properties of lignocellulosic fillers to the behaviors  
 325 of their reinforced composites. Maldas et al. (1989) reported that the differences in morphology, density  
 326 and aspect ratio of wood species accounted for the discrepancy of reinforcing behaviors in the  
 327 thermoplastic composites of polystyrene. Stark and Rowland (2003) found that the aspect ratio, not the  
 328 wood particle size, had the greatest influence on the strength and stiffness of the PP-composites.  
 329 However, the study of Ashori and Nourbakhsh (2010) showed that the fiber morphology (e.g. the aspect  
 330 ratio) does not primarily affect the mechanical properties of the composites but the chemical nature of  
 331 the fiber does. The latter comprehensively influences the interfacial interaction between two main  
 332 components of the composites.

333 Maize genotypes were selected to show an as-wide-as possible variation of biochemical composition. It  
 334 is known that many parameters are affecting the mechanical properties of composites, such as the  
 335 mechanical properties of the matrix, the quality of the interface between matrix and filler, the type of  
 336 manufacturing process, the processing parameters, the aspect ratio of the fillers, the filler loading level,  
 337 the distribution of filler inside the matrix, and the intrinsic mechanical resistance of the stem fragments.

338 In order to identify a possible effect of the composition of the genotypes, many of the above parameters  
339 influencing composite properties must be suppressed. However, during milling, different tissues with  
340 different mechanical characteristics would break and reduce their size in various modes because of the  
341 heterogeneity and the complexity of stems. Obviously, this fact would affect the aspect ratios of the stem  
342 fragments, which in turn would influence the fragment reinforcing capacity. Maize stems were easy to  
343 break and, after sieving, produced fragments with no significant differences in geometrical dimensions.  
344 So, to be able to compare genotypes, the concentration and size of the fillers were identical and fixed at  
345 the beginning of the work for all the studied maize genotypes. By using the same coupling agent, the  
346 interfacial properties between the matrix and the reinforcement were also fixed. In addition, the  
347 processing of the composites followed a well-established, robust protocol presented in materials and  
348 methods section, keeping constant temperatures and times of mixing or processing bars. As a result, any  
349 change which could occur on the mechanical properties of the composite products can then be safely  
350 attributed to histological structure and biochemical composition variations of the maize stem fragments  
351 used as reinforced elements. For practical reasons, it was not possible to deeply study the histological  
352 structure of the fragments. As said in the Materials and Methods section, histological investigations were  
353 performed on sections of the stem chosen at the first internode below the cob. The chemical composition  
354 was performed on the fragments used for processing composites.

355 The overall correlations, R values, between the mechanical properties and the biochemical compositions  
356 of maize fillers are given in Figure 5. The correlation is considered not significant if the F-value  
357 calculated is greater than 0.05. As seen in Figure 5, several strong and significant correlations were  
358 observed:

- 359 • Impact strength: positive correlations with the contents of lignin ( $R = 0.79$ ) and esterified *p*-  
360 coumaric acids Caest ( $R = 0.74$ ); negative correlation with xylose content ( $R = -0.75$ ).

- 361 • Tensile strength: positive correlation with the cell wall residue ( $R = 0.85$ ) and at the limit of  
362 significance, negative correlation with esterified ferulic acids Faest ( $R = -0.64$ ).
- 363 • Young's modulus: positive correlations with the cell wall residue ( $R = 0.77$ ) and glucose from  
364 cellulose ( $R = 0.75$ ); negative correlation with esterified ferulic acids Faest ( $R = -0.76$ ) and at the limit  
365 of significance, positive correlation with esterified *p*-coumaric acids Caest ( $R = 0.59$ ), .
- 366 • Elongation at break: positive correlations with esterified ferulic acids Faest ( $R = 0.74$ ); negative  
367 correlations with the cell wall residue ( $R = -0.75$ ) and glucose from cellulose ( $R = -0.79$ ).

368

369 Figure 5.

370

371 The overall comprehensive picture which can be drawn from these correlations is that the maize stem  
372 fragments with high contents of cell wall residue, lignin, *p*-coumaric acids, and cellulose along with the  
373 low content of ferulic acids and hemicellulose will have higher reinforced capacity leading to stronger  
374 composites in which they are incorporated.

375 Cellulose is a very strong component of plant stems. When extracted, cellulose nanofibrils have strength  
376 above 2 GPa due to its high linearity, high crystallinity and high molar mass (Saito et al., 2013). Rich in  
377 hydroxyl groups, it acts as backbone providing strength and stability to the plant cell wall. It was found  
378 that cellulose determined the properties in the longitudinal direction, while hemicellulose affected more  
379 the properties in the transverse direction of the wood cell wall (Bergander and Salmén, 2002). In  
380 agreement with this observation, our results evidenced a direct correlation between cellulose content and  
381 mechanical performance. Hence, the higher is the cellulose content, the larger is the strength of the  
382 elongated fragments, and consequently, the stronger are the mechanical properties of the composites.  
383 The low strength of neat LDPE and the low capacity of composites to sustain plastic deformation might

384 explain the correlation observed between the mechanical performance of composites and the cellulose  
385 content of the reinforcement. The low deformability of the fragments lead to a very large decrease of the  
386 elongation at break compared to the neat matrix.

387 Amorphous hemicellulose is the component cross-linking cellulose fibrils and also the filler between  
388 cellulose and lignin. It has a rather low molar mass and its lack of crystallinity is lowering its  
389 mechanical properties. It has also very low resistance to thermal decomposition. Its presence is  
390 detrimental to the mechanical properties of composites. Although it should glue tissues inside the  
391 fragment, its weak mechanical performance decreases the strength and modulus of these fragments,  
392 leading to an overall decrease of the mechanical properties of the composites.

393 Lignin did not show any correlation with Young's modulus, tensile strength and elongation at break of  
394 the composites. It acts as a structural scaffold, excluding water from the vicinity of cellulose, the  
395 strength of which depends on the moisture content. Contrary to what is found here, Neagu et al. (2006)  
396 reported that the stiffness of wood fiber was a function of lignin content, and that it thus played an  
397 important role in the reinforcement efficiency in the composites. Lignin with its distinctive network  
398 polymeric structure helps the bonding between fragment and matrix. Therefore, it promotes the ability of  
399 the materials to resist fracture under stress applied at high speed (i.e. impact), which is related to the  
400 overall toughness of the composites. Rozman et al. (1998, 2000) used lignin (added externally) as a  
401 compatibilizer in their composites and detected that lignin played a positive role in enhancing the tensile  
402 strength of rubberwood – and coconut fiber – PP composites. This is what is shown here with a clear  
403 correlation between impact strength and lignin content. This is a very important effect since impact  
404 strength is very low (see Figure 4).

405 The cell wall residue is the sum of three main components (cellulose, lignin and hemicelluloses). The  
406 fact that it shows a positive correlation with tensile strength and modulus (and a negative correlation

407 with elongation) and no correlation with impact is the clear sign that cellulose is the component  
408 dominating the mechanical properties of the fragments since the behavior of cell wall residue follows  
409 the trend of cellulose.

410 An interesting finding of this study is the very remarkable correlations found between *p*-hydrocinnamic  
411 acids (*p*-coumaric acid and ferulic acid) and the final properties of the composites. Looking at Figure 5,  
412 it can be noticed that the effect of *p*-coumaric acid is not closely following the one of lignin. This is  
413 unexpected because it is known to be bound mainly to lignin (Morrison et al., 1998). Grass lignins are *p*-  
414 coumaroylated up to 10-20% by weight of lignin. This *p*-coumaroylation is performed on monolignols  
415 involved in lignin formation (Lu and Ralph, 2008; Lapierre et al., 2018) and the *p*-coumaroylation level  
416 of lignins strongly affects their structure and properties (Zhang et al., 2011; Sibout et al., 2016). This is  
417 probably what explains the difference observed in Figure 5 between the correlations of lignin and *p*-  
418 coumaric acid. It is not only the fact that when there is more lignin there is also more *p*-coumaric acid. It  
419 is the fact that when there is more *p*-coumaric acid, the structure of lignin is affected in a positive way,  
420 i.e. having better mechanical performances and thus giving more strength to the maize stem fragments.  
421 In a previous study, Zhang et al (2011) demonstrated the impact of lignin *p*-coumaroylation on the  
422 limitation of cell wall degradability, highlighting the strengthening of tissues by this *p*-coumaroylation  
423 and its direct impact on cell wall recalcitrance. This is a very important result for the design of grass  
424 plants dedicated to be used as materials.

425 In contrast to *p*-coumaric acids, esterified ferulic acid has a clear negative effect on the mechanical  
426 properties of the composites. Ferulic esters were long presented as nucleation sites for lignification  
427 (Ralph et al., 1995; Grabber et al., 1998) demonstrated that ferulic esters were recruited before maize  
428 flowering to install lignification. Thus, ferulic esters detected at silage stage represented the unused  
429 lignification primers. An important quantity of unused ferulic primers could underline the establishment

430 of long lignin chains in a limited number of ferulic primers. The promotion of such a lignin network  
431 could explain a higher mechanical resistance.

432

### 433 **3.4 Correlations between mechanical properties and the structure of stems**

434 It has to be recalled that FASGA staining was performed on cross section of an internode (the one below  
435 the main cob) while the fragments used to prepare composites came from the whole stem. Histology can  
436 reflect the occurrence of tissues having biochemical composition favorable or not to the mechanical  
437 performances of the composites, as seen above. But it can also reflect the way stems are breaking, and  
438 the way sieving is selecting more or less favorable fragments. As shown by Vo et al. (2017) for  
439 sorghum, the different fractions obtained after sieving have different compositions, and such  
440 phenomenon is expected to occur also for maize stems since they possess a similar organization. This  
441 can not only affect the mechanical properties of fragments, but it may also change the type of tissues  
442 which are exposed at its surface, changing the interfacial adhesion with the matrix. Figure 5 gives the  
443 correlations between the mechanical properties of the maize-PE composites and the histological  
444 morphometric features traits of the internode below the main cob. Only a limited number of correlations  
445 seem significant.

446 Impact strength was shown to be strongly related to the lignin and *p*-coumaric acid contents (Figure 5).  
447 Figure 5 shows that impact strength is also correlated with the stem area , the fraction area and number  
448 of bundles. All other correlations regarding impact are non-significant.

449 A major result is the total lack of correlation between the morphological descriptors and tensile  
450 properties. The majority of biomass of the maize internode is concentrated in the outer rind tissue, which  
451 is a mixture of densely packed vascular bundles embedded in a matrix of sclerenchymatous cells  
452 (Sivasankar, 2012). The rind accounts for less than 20 % of the cross-sectional stem area, but more than

453 80 % dry mass. The remaining biomass (20 %) is attributed to the vascular bundles embedded in the  
454 parenchymatous cells in the pith. The differences between the genotypes will affect this ratio and the  
455 proportions of cell types in each tissue. Thus, after the sieving step, the selected fraction may include a  
456 high proportion of some types of tissues but low of the others. This could explain the poor overall  
457 correlations observed between the mechanical properties of the composites prepared from selected  
458 fragments and the morphology of the internode used for histology. This situation can also be observed  
459 when considering the correlation of tensile properties of the composites (Young's modulus, tensile  
460 strength and elongation at break) with the color descriptors, as shown on Figure 5.

461 These results suggest that it is not so easy to understand the correlations between the structure of stems  
462 and the mechanical properties of the composites prepared with stem fragments. The way stems are  
463 breaking needs the knowledge of crack propagation and thus of the location of the weakest tissues, the  
464 way sieving is selecting fragments, which may have a different composition depending on size, and the  
465 knowledge of the type of tissues or molecules exposed at the fragment surface are all influencing the  
466 final properties of composites.

467

#### 468 **4- Conclusions**

469 Contrary to most plant fillers where fibers with high cellulose content are extracted, the use of the whole  
470 stem fragments is opening new perspectives and raising new questions. This study demonstrated that  
471 preparation of composites from maize stems, an agro-waste product, is technically feasible.

472 Maize stems were found to differ in their reinforcing capacity across the range of genotypes evaluated.  
473 The relation between the composition and the mechanical properties show that cellulose content is the  
474 most important favorable parameter. *As shown here, this is true whatever is the influence of other  
475 parameters, whether they were of minor importance, of large importance such as hydroxycinnamic acids*



476 or of unknown importance as the effect of the structure of the stems. A very novel result regarding *p*-  
477 hydroxycinnamic acids was found. They are playing a significant, yet unclear role on the mechanical  
478 properties of composites due to their influence on the properties of stem fragments. *p*-coumaric acid has  
479 a favorable role, probably as a trigger for promoting stronger lignin fractions and the unfavorable effect  
480 of ferulic acid esters suggests the occurrence of a specific lignin network supported by long lignin chains  
481 anchored in a limited number of ferulic primers. Correlations between the histological traits and  
482 composite properties were difficult to interpret.

483 The practical implementation of this research is unknown. However, if the economic context is  
484 attractive, breeders will be able to identify lines that best meet the requirements for the use of biomass in  
485 the context of biomaterials. Lines in pre-breeding material may also meet these requirements. However,  
486 this paper is a part of a larger study aimed at comparing different plants of the same family (maize,  
487 sorghum and miscanthus) where stems can be used for preparing polymer composites. It is bringing  
488 valuable information regarding the role of different plant tissues on the manufacturing of polymer  
489 composites. These pieces of information will be compared with the ones obtained with miscanthus and  
490 sorghum (publications under preparation) in order to produce a general “composite” phenotyping data  
491 base picture enabling a potential genetic amelioration of these plants. These materials can bring several  
492 issues. Biodegradation is not seen as an issue when dealing with such plant-filled polyolefins, as seen by  
493 their wide acceptance for being used as part of automobiles which have to last more than 10 years in  
494 various harsh environment. However, recycling is known to be difficult, but its study is totally outside  
495 the scope of this research paper.

496

## 497 **5- Author Contributions**

498 Loan T. T. Vo : preparation of composites, mechanical experiments, text writing

499 Jordi Girones : preparation of composites, mechanical experiments, text writing  
500 Marie-Pierre Jacquemot : samples harvesting and distribution to partners  
501 Frédéric Legée : biochemical analyses  
502 Laurent Cézard : biochemical analyses  
503 Catherine Lapierre : biochemical analyses  
504 Fadi El Hage : histological analyses  
505 Valérie Méchin : sample harvesting, conceptualization of field trials, samples harvesting, data analysis,  
506 text writing  
507 Matthieu Reymond : sample harvesting, conceptualization of field trials, samples harvesting, data  
508 analysis, text writing  
509 Patrick Navard : overall supervision of the work, text writing

510

## 511 **6- Acknowledgement**

512 This work was supported by the French Government Grants (LabEx Saclay Plant Sciences-SPS, Grants  
513 ANR-10-LABX- 0040-SPS and ANR-11-BTBR-0006 BIOMASS FOR THE FUTURE), managed by  
514 the French National Research Agency under an Investments for the Future program (Grant ANR- 11-  
515 IDEX-0003-02). The authors benefited from the IJPB Plant Observatory facilities.

516

## 517 **7- References**

518 Ajayi, B., 2006. Properties of maize-stalk-based cement-bonded composites. Forest Prod. J. 56, 51-55.  
519 Alavi, S., Thomas, S., Sandeep, K.P., Kalarikkal, N., Vazrghese, J., Yarangalla, S., 2015. Polymers for  
520 packaging applications, Apple Academic Press, New Jersey (USA).

521 Ashori, A. Nourbakhsh, A., 2010. Bio-based composites from waste agricultural residues. *Waste Manag.*  
522 30, 680–684. doi:10.1016/j.wasman.2009.08.003.

523 Ashori, A. Nourbakhsh, A., Tabrizi, A.K., 2014. Thermoplastic Hybrid Composites using Bagasse, Corn  
524 Stalk and E-glass Fibers: Fabrication and Characterization. *Polym. Plast. Technol. Eng.* 53, 1-8.  
525 doi:10.1080/03602559.2013.832854.

526 Barrière, Y., Ralph, J., Méchin, V., Guillaumie, S., Grabber, J.H., Argillier, O., Chabbert, B., Lapierre,  
527 C., 2004. Genetic and molecular basis of grass cell wall biosynthesis and degradability. II. Lessons  
528 from brown-midrib mutants. *C. R. Biologies* 327, 847–860. doi:10.1016/j.crv.2004.05.010

529 Barrière, Y., Méchin, V., Lafarguette, F., Manicacci, D., Guillon, F., Wang, Lauressergues, D., Pichon,  
530 M., Bosio, M., Tatout, C., 2009. Toward the discovery of maize cell wall genes involved in silage  
531 quality and capacity to biofuel production. *Maydica*. 54 (2009) 161–198.  
532 <http://www.maydica.org/maydica2009.html> (accessed July 26, 2017).

533 Barros-Rios, J., Santiago, R., Malvar, R.A., Jung, H.J.G., 2012. Chemical composition and cell wall  
534 polysaccharide degradability of pith and rind tissues from mature maize internodes. *Anim. Feed Sci.*  
535 *Technol.* 172, 226–236. doi:10.1016/j.anifeeds.2012.01.005.

536 Bergander, A., Salmén, L., 2002. Cell wall properties and their effects on the mechanical properties of  
537 fibers. *J. Mater. Sci.* 37, 151-156. doi:10.1023/A:1013115925679.

538 Bourmaud, A., Morvan, C., Bouali, A., Placet, V., Perré, P., Baley, C., 2013. Relationships between  
539 micro-fibrillar angle, mechanical properties and biochemical composition of flax fibers. *Ind. Crops*  
540 *Prod.* 44, 343-351. doi:10.1016/j.indcrop.2012.11.031.

541 Charlet, K., Jernot, J.P., Gomina, M., Bréard, J., Morvan, C., Baley, C., 2009. Influence of an Agatha  
542 flax fibre location in a stem on its mechanical, chemical and morphological properties. *Compos. Sci.*  
543 *Technol.* 69. 1399-1403. doi:10.1016/j.compscitech.2008.09.002.

- 544 Chigondo, F., Shoko, P., Nyamunda, B.C., Moyo, M., 2013. Maize Stalk As Reinforcement In Natural  
545 Rubber Composites. *Int. J. Sci. Technol. Res.* 2, 263-271.
- 546 Chun, K.S., Yeng, C.M., Husseinsyah, S., Pang, M.M., Ismail, A., 2017. Effect of eco-degradant on  
547 properties of low density polyethylene/corn stalk eco-composites. *J. Eng. Sci. Technol.* 12-1165–  
548 1177.
- 549 Dauda, M. Yoshiba, M., Miura, K., Takahashi, S., 2007. Processing and mechanical property evaluation  
550 of maize fiber reinforced green composites. *Adv. Compos. Mater.* 16, 335-347.  
551 doi:10.1163/156855107782325168.
- 552 Dence, C.W., 1992. The Determination of Lignin, in: S.Y. Lin, C.W. Dence (Eds.), *Methods Lignin*  
553 *Chem.*, Springer Berlin Heidelberg, pp. 33–61. doi:10.1007/978-3-642-74065-7\_3.
- 554 Dongdong, D., Jun, W., 2016. Research on mechanics properties of crop stalks: A review. *Int J Agric*  
555 *Biol Eng.* 9, 10-19. doi:10.3965/j.ijabe.20160906.1499.
- 556 El Hage Fadi, 2018. Impact du déficit hydrique sur les relations entre la dégradabilité, la biochimie  
557 pariétale et la répartition des tissus lignifiés chez l'entre-noeud de maïs et déterminisme génétique de  
558 ces caractères. Ecole doctorale n°567. Université Paris Saclay, France.
- 559 El Hage, F., Legland, D., Borrega, N., Jacquemot, M.-P., Griveau, Y., Coursol, S., Méchin, V.,  
560 Reymond, M., 2018. Tissue Lignification, Cell Wall p-Coumaroylation and Degradability of Maize  
561 Stems Depend on Water Status. *J. Agric. Food Chem.* 2018, 66, 4800–4808. Doi:  
562 10.1021/acs.jafc.7b05755
- 563 Ganjyal, G.M., Reddy, N., Yang, Y.Q., Hanna, M.A., 2004. Biodegradable packaging foams of starch  
564 acetate blended with corn stalk fibers. *J. Appl. Polym. Sci.* 93, 2627-2633. doi:10.1002/app.20843.
- 565 García-Rosales, G., Colín-Cruz, A., 2010. Biosorption of lead by maize (*Zea mays*) stalk sponge. *J.*  
566 *Environ. Manage.* 91, 2079-2086. doi:10.1016/j.jenvman.2010.06.004.

567 Girones, J., Vo, L, Arnoult, S., Brancourt-Hulmel, M., 2016. Miscanthus stem fragment - reinforced  
568 polypropylene composites: development of an optimized preparation procedure at small scale and its  
569 validation for differentiating genotypes. *Polymer Testing*, 55, 166-172  
570 DOI: 10.1016/j.polymertesting.2016.08.023

571 J H Grabber, G.H., Hatfield, R.D., Ralph, J., 1998. Diferulate cross-links impede the enzymatic  
572 degradation of non-lignified maize walls. *J Sci Food Agric*, 77, 193-200  
573 [https://doi.org/10.1002/\(SICI\)1097-0010\(199806\)77:2<193::AID-JSFA25>3.0.CO;2-A](https://doi.org/10.1002/(SICI)1097-0010(199806)77:2<193::AID-JSFA25>3.0.CO;2-A)

574 Harholt, J., Jensen, J.K., Sørensen, S.O., Orfila, C., Pauly, M., Scheller, H.V., 2006. ARABINAN  
575 DEFICIENT 1 is a putative arabinosyltransferase involved in biosynthesis of pectic arabinan in  
576 *Arabidopsis*. *Plant Physiol.* 140, 49-58. doi:10.1104/pp.105.072744.

577 Hassan, S.B., Oghenevweta, J.E., Aigbodion, V.S, 2012a. Morphological and mechanical properties of  
578 carbonized waste maize stalk as reinforcement for eco-composites. *Compos. Part B Eng.* 43, 2230-  
579 2236. doi:10.1016/j.compositesb.2012.02.003.

580 Hassan, S.B., Oghenevweta, J.E., Aigbodion, V.S., 2012b. Potentials of Maize Stalk Ash as  
581 Reinforcement in Polyester Composites. *J. Miner. Mater. Charact. Eng.* 11, 445–459.  
582 doi:10.4236/jmmce.2012.114032.

583 Hassan, S.B., Oghenevweta, J.E., Nyior, G.B., Aigbodion, V.S., 2013. The Effect of Vacuum  
584 Carbonization of Waste Maize Stalk on the Thermal Resistance of Polyester / Maize Stalk Particulate  
585 Composites. *Plast. Polym. Technol.* 2 (2013) 68–76.

586 Ilie, R., Chiroiu, V., 2015. On the natural Fiber (Maize) Composite material. *PAMM Proc. Appl. Math.*  
587 *Mech.* 15, 303-304. doi:10.1002/pamm.201510142.

588 Ilie, R.D., Moşneguţu, V., 2016. A proposal for modeling the natural fibber composites . *ACTA*  
589 *Electrotech.* 57, 122-127.

590 Jin, A.X., Ren, J.L., Peng, F. Xu, F., Zhou, G.Y., Sun, R.C. Hu, J., 2009. Comparative characterization  
591 of degraded and non-degradative hemicelluloses from barley straw and maize stems: Composition,  
592 structure, and thermal properties. *Carbohydr. Polym.* 78, 609-619.  
593 doi:10.1016/j.carbpol.2009.05.024.

594 Kuc, J., Nelson, O.E., 1964. The abnormal lignins produced by the *brown-midrib* mutants of maize: I.  
595 The *brown-midrib-1* mutant. *Arch. Biochem. Biophys.* 105, 103-113. [https://doi.org/10.1016/0003-](https://doi.org/10.1016/0003-9861(64)90240-1)  
596 9861(64)90240-1

597 Lancaster, L., Lung, M.H., Sujana, D., 2013. Utilization of Agro-Industrial Waste in Metal Matrix  
598 Composites: Towards Sustainability. *World Acad. Sci. Eng. Technol.* 73, 1136-1144.  
599 <https://espace.curtin.edu.au/handle/20.500.11937/37923>.

600 Lapierre, C., Voxeur, A., Karlen, S.D., Helm, R.F., Ralph, J., 2018. Evaluation of feruloylated and  
601 p-coumaroylated arabinosyl units in grass arabinoxylans by acidolysis in dioxane/methanol. *J. Agric.*  
602 *Food Chem.* 66, 5418-5424.

603 Legrand, D., El Hage, F., Méchin, V., Reymond, M., 2017. Histological quantification of maize stem  
604 sections from FASGA-stained images. *Plant methods* 13, 84. [https://doi.org/10.1186/s13007-017-](https://doi.org/10.1186/s13007-017-0225-z)  
605 0225-z

606 Maldas, D., Kokta, B.V., Daneault, C., 1989. Thermoplastic composites of polystyrene: Effect of  
607 different wood species on mechanical properties. *J. Appl. Polym. Sci.* 38, 413-439.  
608 doi:10.1002/app.1989.070380303.

609 Méchin, V., Argillier, O., Menanteau, V., Barrière, Y., Mila, I., Pollet, B., Lapierre, C., 2000.  
610 Relationship of cell wall composition to in vitro cell wall digestibility of maize inbred line stems. *J.*  
611 *Sci. Food Agric.* 80, 574-580. doi:10.1002/(SICI)1097-0010(200004)80:5<574::AID-  
612 JSFA575>3.0.CO;2-R.

613 Morrison, T.A., Jung, H.G., Buxton, D.R., Hatfield, R.D., 1998. Cell-Wall Composition of Maize  
614 Internodes of Varying Maturity. *Crop Sci.* 38, 455-460.  
615 doi:10.2135/cropsci1998.0011183X003800020031x.

616 Mtibe, A., Liganiso, L.Z., Mathew, A.P., Oksman, K., John, M.J., Anandjiwala, R.D., 2015. A  
617 comparative study on properties of micro and nanopapers produced from cellulose and cellulose  
618 nanofibres, *Carbohydr. Polym.* 118, 1-8. doi:10.1016/j.carbpol.2014.10.007.

619 Muniyasamy, S., 2016. Development of Bio-based Composite Products from Agricultural Wastes/Crops  
620 Residues for Applications in Automotive sector, Green Packaging and Green Buildings in South  
621 Africa Utilization of agricultural biomass for sustainable bio-composites products : Op, in: *Green  
622 Econ. Postdr. Fellowships Induction Work.*, Cape Town, 2016.  
623 [http://ir.nrf.ac.za/bitstream/handle/10907/798/Muniyasamy\\_development.pdf?sequence=1](http://ir.nrf.ac.za/bitstream/handle/10907/798/Muniyasamy_development.pdf?sequence=1) (accessed  
624 June 21, 2017).

625 Neagu, R.C., Gamstedt, E.K., Berthold, F., 2006. Stiffness Contribution of Various Wood Fibers to  
626 Composite Materials. *J. Compos. Mater.* 40, 663-699. doi:10.1177/0021998305055276.

627 Nourbakhsh, A. Hosseinzadeh, A., Basiji, F., 2011. Effects of Filler Content and Compatibilizing  
628 Agents on Mechanical Behavior of the Particle-Reinforced Composites. *J. Polym. Environ.* 19, 908-  
629 911. doi:10.1007/s10924-011-0349-6.

630 Nourbakhsh, Ashori, A., 2010. Wood plastic composites from agro-waste materials: Analysis of  
631 mechanical properties. *Bioresour. Technol.* 101, 2525-2528. doi:10.1016/j.biortech.2009.11.040.

632 Panthapulakkal, S., Sain, M., 2006. Injection Molded Wheat Straw and Corn Stem Filled Polypropylene  
633 Composites. *J. Polym. Environ.* 14, 265-272. doi:10.1007/s10924-006-0021-8.

634 Ralph, J., Grabber, J.H., Hatfield, R.D., 1995. Lignin-Ferulate Crosslinks in Grasses: Active Incorporation of  
635 Ferulate Polysaccharide Esters into Ryegrass Lignins. *Carbohydrate Research*, 275, 167-178.  
636 [https://doi.org/10.1016/0008-6215\(95\)00237-N](https://doi.org/10.1016/0008-6215(95)00237-N)

637 Reiterer, A., Lichtenegger, H., Schegg, S.T., Fratzl, P., 1999. Experimental evidence for a mechanical  
638 function of the cellulose microfibril angle in wood cell walls. *Philos. Mag. A.* 79 (1999).

639 Rozman, H.D., Kumar, R.N., Adlli, M.R.M., Abusamah, A., Ishak, Z.A.M., 1998. The effect of lignin  
640 and surface activation on the mechanical properties of rubberwood-polypropylene composites, *J.*  
641 *Wood Chem. Technol.* 18, 471-490. doi:10.1080/02773819809349593.

642 Rozman, H.D., Tan, K.W., Kumar, R.N., Abubakar, A., Mohd. Ishak, Z.A., Ismail, H., 2000. The effect  
643 of lignin as a compatibilizer on the physical properties of coconut fiber/polypropylene composites.  
644 *Eur. Polym. J.* 36, 1483-1494. doi:10.1016/S0014-3057(99)00200-1.

645 Saito, T., Kuramae, R., Wohler, J., Berglund, L.A., Isogai, A., 2013. An ultrastrong nanofibrillar  
646 biomaterial: The strength of single cellulose nanofibrils revealed via sonication-induced  
647 fragmentation. *Biomacromolecules*, 14 (1), 248–253 DOI: 10.1021/bm301674e

648 Schwietzke, S., Kim, Y., Ximenes, E., Mosier, N., Ladisch, M., 2009. , Chapter 23: Ethanol Production  
649 from Maize, in: A.L. Kriz, B.A. Larkins (Eds.), *Mol. Genet. Approaches to Maize Improv.*  
650 *Biotechnol. Ion Agric. For.* Vol. 63, Springer Berlin Heidelberg, Berlin, Heidelberg, 2009: pp. 347–  
651 364. doi:10.1007/978-3-540-68922-5\_23.

652 Sena Neto, A.R., Araujo, M.A.M., Barboza, R.M.P., Fonseca, A.S., Tonoli, G.H.D., Souza, F.V.D.,  
653 Mattoso, L.H.C., Marconcin, J.M., 2015. Comparative study of 12 pineapple leaf fiber varieties for  
654 use as mechanical reinforcement in polymer composites. *Ind. Crops Prod.* 64, 68–78.  
655 doi:10.1016/j.indcrop.2014.10.042.

656 Sibout, R., Le Bris, P., Legée, F., Cézard, L., Renault, H., Lapierre, C., 2016. Structural redesigning  
657 arabidopsis lignins into alkali-soluble lignins through the expression of p-coumaroyl-coA:monolignol  
658 transferase PMT. *Plant Physiol*, 170(3):1358-1366.



659 Sivasankar, S. Collision, S., Gupta, R., Dhugga, S.K., 2012. , Chapter 16: Maize, in: C. Kole, C.P. Joshi,  
660 D. Shonnard (Eds.), Handbook Bioenergy Crop Plants, CRC Press, 2012: pp. 405 – 426.

661 Speck, T., Burgert, I., 2011. Plant stems: functional design and mechanics. *Ann. Rev. Mater. Res.* 41,  
662 169-193. doi:10.1146/annurev-matsci-062910-100425.

663 Stokke, D.D., Gardner, D.J., 2003. Fundamental aspects of wood as a component of thermoplastic  
664 composites. *J. Vinyl Addit. Technol.* 9 (2003) 96–104. doi:10.1002/vnl.10069.

665 Sun, X.F., Fowler, P., Rajaratnam, M., Zhang, G., 2010. Extraction and characterisation of  
666 hemicelluloses from maize stem. *Phytochem. Anal.* 21, 406-415. doi:10.1002/pca.1211.

667 Sun, X.F., Wang, H., Zhang, G., Fowler, P., Rajaratnam, M., 2011. Extraction and characterization of  
668 lignins from maize stem and sugarcane bagasse. *J. Appl. Polym. Sci.* 120, 3587-3595.  
669 doi:10.1002/app.33551.

670 Thamae, T., Marien, R., Chong, L., Wu, C. Baillie, C., 2008. Developing and characterizing new  
671 materials based on waste plastic and agro-fibre. *J. Mater. Sci.* 43, 4057-4068. doi:10.1007/s10853-  
672 008-2495-3.

673 Trigui, A., Karkri, M., Pena, L., Boudaya, C., Candau, Y., Bouffi, S., Vilaseca, F., Thermal and  
674 mechanical properties of maize fibres-high density polyethylene biocomposites. *J. Compos. Mater.*  
675 47, 1387-1397. doi:10.1177/0021998312447648.

676 Updegraff, D.M., 1969. Semimicro determination of cellulose in biological materials, *Anal. Biochem.* 32  
677 420–424. doi:10.1016/S0003-2697(69)80009-6.

678 Väisänen, T., Haapala, A., Lappalainen, R., Tomppo, L., 2016. Utilization of agricultural and forest  
679 industry waste and residues in natural fiber-polymer composites: A review. *Waste Manag.* 54, 62-73.  
680 doi:10.1016/j.wasman.2016.04.037.

681 Vo, L.T.T., Girones, G., Beloli, C., Chupin, L., Di Giuseppe, E., Vidal, A., Soutiras, A., Pot, D.,  
682 Bastianelli, D., Bonal, L., Navard, P., 2017. Processing and properties of sorghum stem fragment-  
683 polyethylene composites. *Ind. Crops Prod.* 107, 386-398. doi:doi.org/10.1016/j.indcrop.2017.05.047.

684 Wan, T., Xiong, L., Huang, R., Zhao, Q., Tan, X., Qin, L., Hu, J., 2014. Structure and properties of corn  
685 stalk-composite superabsorbent. *Polym. Bull.* 71, 371-383. doi:10.1007/s00289-013-1066-1.

686 Wang, D., Sun, X.S., 2002. Low density particleboard from wheat straw and corn pith. *Ind. Crops Prod.*  
687 15, 43-50. doi:10.1016/S0926-6690(01)00094-2.

688 Xiao, B., Sun, X., Sun, R., 2001. Chemical, structural, and thermal characterizations of alkali-soluble  
689 lignins and hemicelluloses, and cellulose from maize stems, rye straw, and rice straw. *Polym.*  
690 *Degrad. Stab.* 74, 307-319. doi:10.1016/S0141-3910(01)00163-X.

691 Yu, X., Xu, H., 2014. Chapter 12: Lightweight composites reinforced by agricultural byproducts, in:  
692 *ACS Symp. Ser. Vol. 1175*, pp. 209–238. doi:10.1021/bk-2014-1175.ch012.

693 Zhang, B. C., Liu, X. L., Qian, Q. A., Liu, L. F., Dong, G. J., Xiong, G. Y., Zeng D, Zhou Y., 2011.  
694 Golgi nucleotide sugar transporter modulates cell wall biosynthesis and plant growth in rice. *Proc.*  
695 *Natl. Acad. Sci. U.S.A.* 108, 5110–5115. doi: 10.1073/pnas.1016144108

696 Zhang, F., Wang, W., Tong, J. Zhou, J., 2015. Moisture absorption properties of biomimetic laminated  
697 boards made from cross-linking starch/maize stalk fiber. *Int. J. Agric. Biol. Eng.* 8, 65-71.  
698 doi:10.3965/IJABE.V8I2.1495.

699 Zhang, L., Yang, Z., Qiang Zhang, E., Zhu, X., Hu, L., 2017. Mechanical behavior of corn stalk pith :  
700 an experimental and modeling study. *INMATEH - Agric. Eng.* 51, 39-48.  
701 [http://www.inmateh.eu/INMATEH\\_1\\_2017/51-04 Lixian Zhang.pdf](http://www.inmateh.eu/INMATEH_1_2017/51-04 Lixian Zhang.pdf).

702 Zheng, L., Zhu, C., Dang, Z., Zhang, H., Yi, X., Liu, C., 2012. Preparation of cellulose derived from  
703 corn stalk and its application for cadmium ion adsorption from aqueous solution. Carbohydr. Polym.  
704 90, 1008–1015. doi:10.1016/j.carbpol.2012.06.035.

705

## 706 **Legends of figures**

707 Figure 1. A typical example of maize internode section for histology with differentiated cells (genotype  
708 F2bm3).

709

710 Figure 2. Microscopy image of maize stem fragments collected between 200 and 300  $\mu\text{m}$  sieves  
711 showing their low axial ratio and irregular shapes.

712

713 Figure 3. (top) Tensile strength and Young's modulus of the LDPE-maize reinforced composites  
714 (standard errors correspond to ten test bar replicates per genotype) and (bottom) the relationship between  
715 the two parameters.

716

717 Figure 4. Top: elongation at break (elongation at break of LDPE is above several hundreds of %).  
718 Middle: notched impact strength of LDPE-maize reinforced composites (notched impact strength of  
719 LDPE =  $44.7 \pm 1.1$  (kJ/m<sup>2</sup>)). Standard errors correspond to ten test bar replicates per genotype. Bottom:  
720 lignin content (LK) and esterified *p*-coumaric acids (CAest) concentration for the same genotypes,  
721 showing visually the correlation between biochemical composition of stem fragments and impact  
722 strength of composites.

723

724 Figure 5. Correlations combined with a significant test between the mechanical properties of the  
725 composites (Young's modulus YM, Tensile strength TS, Elongation at break EB, Impact strength IS)  
726 and the biochemical compositions of the fillers (Cell wall residue CWR, lignin content LK, esterified *p*-  
727 coumaric acids Caest, esterified ferulic acids Faest, arabinose ARA, xylose XYL, glucose GLU,  
728 hemicellulose HCEL) and the measure of colorimetry of the internode. (Lfract = lignified fraction,  
729 Nlfract = non-lignified fraction, Rfract = rind fraction, Bfract = bundle fraction, Bnum = bundle number,  
730 Bint = bundle intensity, red-green-blue channels were computed for each tissue region – lignified, non-  
731 lignified, rind and bundle).

732

733

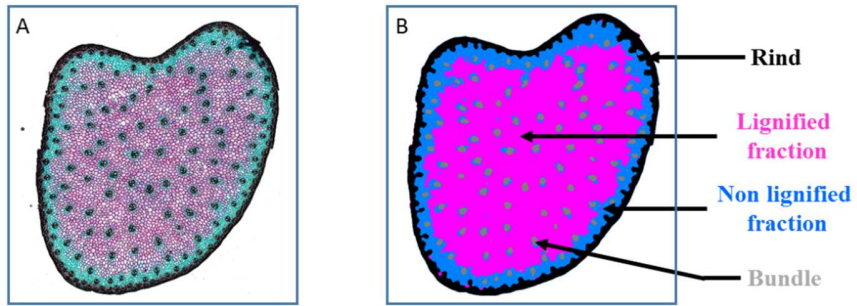


Figure 1. A typical example of maize internode section for histology with differentiated cells (genotype F2bm3).

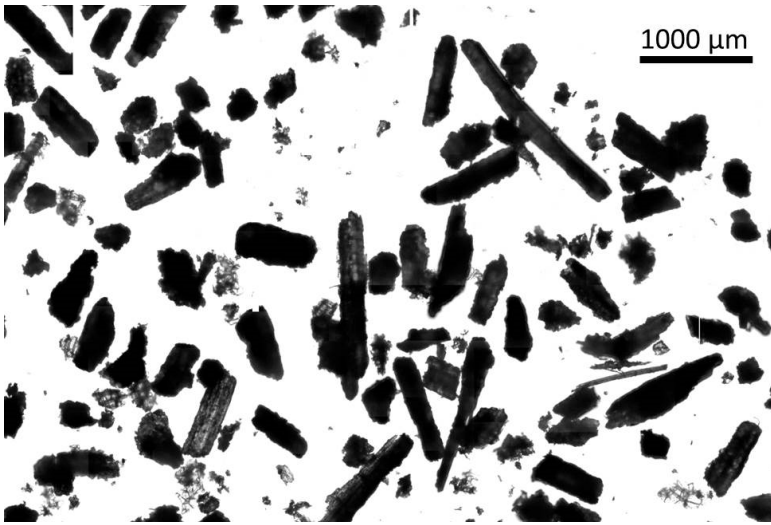


Figure 2. Microscopy image of maize stem fragments collected between 200 and 300  $\mu\text{m}$  sieves showing their low axial ratio and irregular shapes.

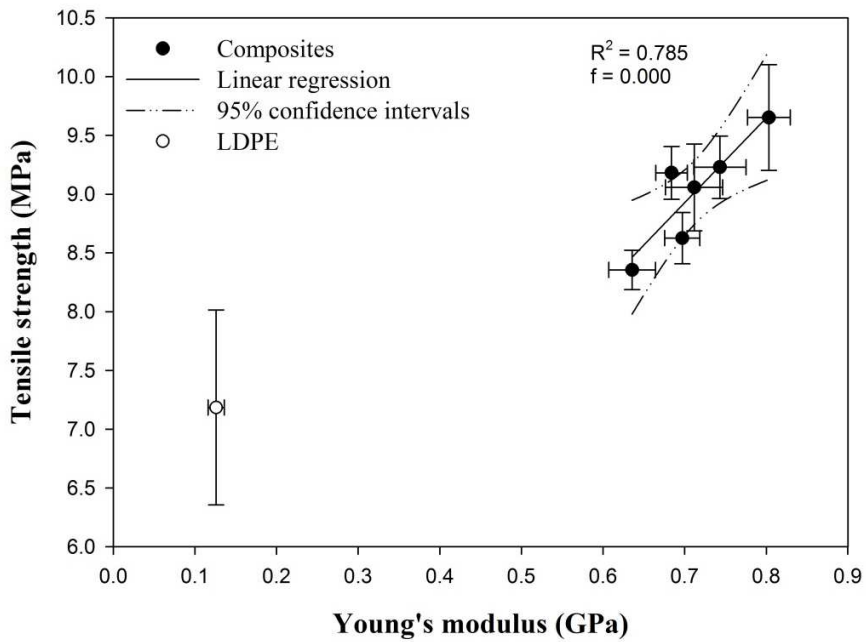
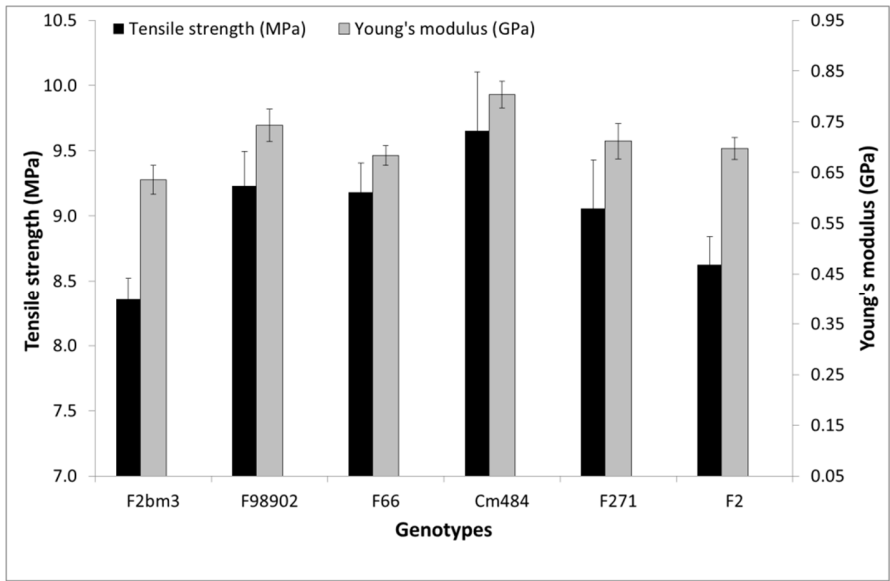
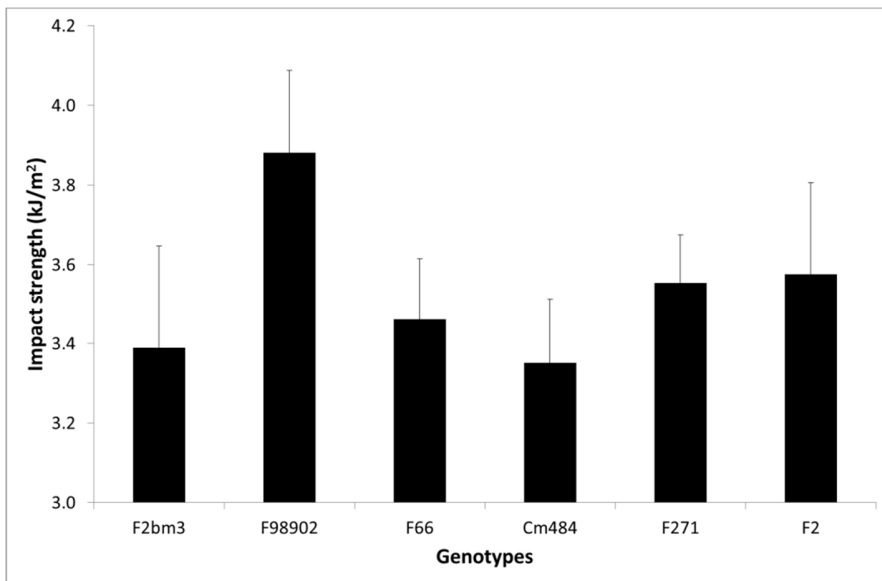
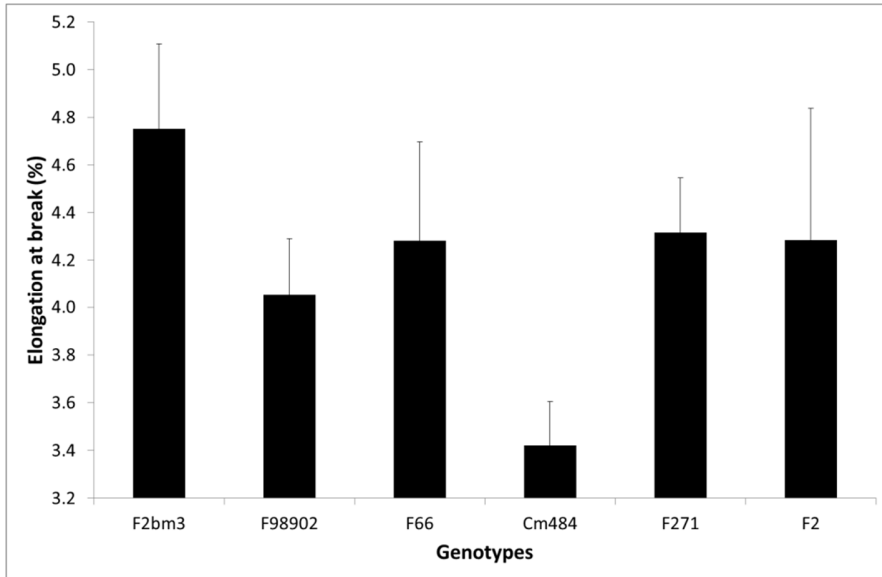


Figure 3. (top) Tensile strength and Young's modulus of the LDPE-maize reinforced composites (standard errors correspond to ten test bar replicates per genotype) and (bottom) the relationship between the two parameters.





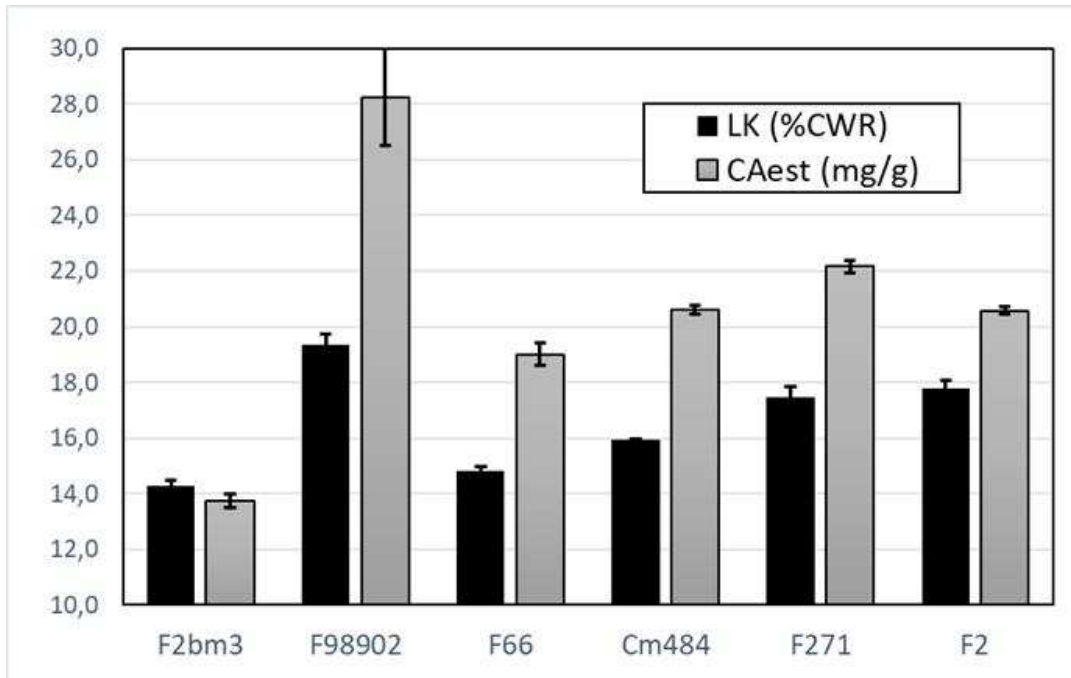


Figure 4. Top: elongation at break (elongation at break of LDPE is above several hundreds of %). Middle: notched impact strength of LDPE-maize reinforced composites (notched impact strength of LDPE =  $44.7 \pm 1.1$  (kJ/m<sup>2</sup>)). Standard errors correspond to ten test bar replicates per genotype. Bottom: lignin content (LK) and esterified *p*-coumaric acids (CAest) concentration for the same genotypes, showing visually the correlation between biochemical composition of stem fragments and impact strength of composites.

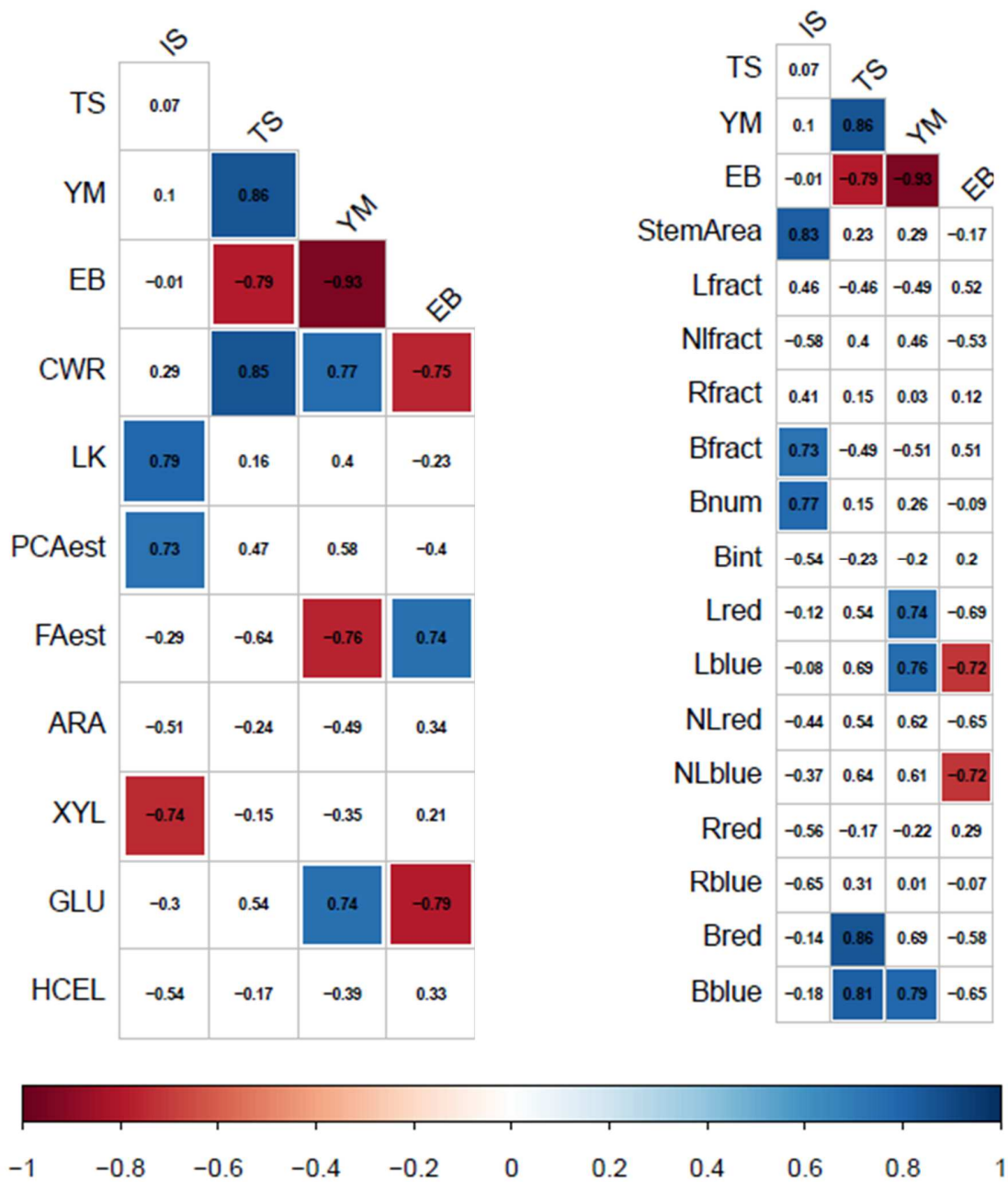


Figure 5. Correlations combined with a significant test between the mechanical properties of the composites (Young's modulus YM, Tensile strength TS, Elongation at break EB, Impact strength IS)

and the biochemical compositions of the fillers (Cell wall residue CWR, lignin content LK, esterified *p*-coumaric acids Caest, esterified ferulic acids Faest, arabinose ARA, xylose XYL, glucose GLU, hemicellulose HCEL) and the measure of colorimetry of the internode. (Lfract = lignified fraction, Nlfract = non-lignified fraction, Rfract = rind fraction, Bfract = bundle fraction, Bnum = bundle number, Bint = bundle intensity, red-green-blue channels were computed for each tissue region – lignified, non-lignified, rind and bundle).