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

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Loan T. T. Vo[1], Jordi Girones[1], Marie-Pierre Jacquemot[2], Frédéric Legée[2], Laurent Cézard[2], Catherine Lapierre[2], Fadi El Hage[2,3], Valérie Méchin[2], Matthieu Reymond[2] and Patrick Navard[1*]

[1]*MINES ParisTech, PSL Research University, CEMEF** - Centre de mise en forme des matériaux, CNRS UMR 7635, CS 10207 rue Claude Daunesse 06904 Sophia Antipolis Cedex, France*

[2] *Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, Versailles, France,*

[3] *Univ. Paris-Sud,Université Paris-Saclay, Orsay, France,Ecole Doctorale Sciences du Végétal, Bat. 360,Université Paris-Sud, 91405 Orsay, France ;*

* Contact person : Patrick Navard patrick.navard@mines-paristech.fr

[**]Member of the European Polysaccharide Network of Excellence (EPNOE), www.epnoe.eu

Abstract

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

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

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

1- Introduction

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

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

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

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

2- Materials and methods

2.1 Maize samples

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

From two blocs with four to six randomized rows per line, ten to twenty plants were harvested. In order to perform correlations between mechanical properties of the composites and biochemical composition of the plant stem or its histological structure, the stovers and the elongated internodes below the main cob from the same rows were harvested. The whole stem served as raw material to extract the stem fragments which were used to prepare composites. For preparing composites, leaves and cobs were removed from healthy harvested stovers (i.e. they were not rotted by any bacterial/fungal diseases) and 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^3	0.954 g/cm^3
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_2SO_4 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.

Figure 1.

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

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

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 µm and sieving to collect fraction between
199 the 200 µm and 300 µm 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 ± 2°C and 50 ± 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

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

3- Results and discussion

3.1 Characterization of the various maize stem fragments

Stem samples harvested of the six maize genotype sieved between 200 and 300 µm prior to the preparation of composites were biochemically characterized to determine their composition. Internodes above the main ears were also histologically characterized to depict the repartition of lignification in the main tissues of the cross section. We observed a wide range of variations for lignin, p-coumaric and ferulic acids contents and for all histological parameters (Table 2). The p-coumaric acid content was the most variable parameters which displayed values ranging from single to double. Not unexpectedly, the lowest LK lignin content and (Caest) value in the cell wall were observed for the F2bm3 mutant. The bm3 maize mutants have been widely used in genetic and cell wall biochemical approaches since the 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

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

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

	CWR	LK	p-coum. acids	est. Ferulic acids	Cellulosis	Hemicellulosis	StemArea	Lfract	Nlfract	Rfract	Bfract	Bnum	Bint
	(%DM)	(%CWR)	(‰CWR)	(‰CWR)	(%MIA)	(%MIA)	(cm²)	(%)	(%)	(%)	(%)		(1/cm²)
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

	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

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-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^2 for the
299 composites whilst this value for LDPE matrix is 44.7 ± 1.1 kJ/m^2. 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^2)
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^2)	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).

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

Figure 5.

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

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

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

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

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

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

3.4 Correlations between mechanical properties and the structure of stems

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

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

A major result is the total lack of correlation between the morphological descriptors and tensile properties. The majority of biomass of the maize internode is concentrated in the outer rind tissue, which is a mixture of densely packed vascular bundles embedded in a matrix of sclerenchymatous cells (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 plan-filled polyolefins, as seen by
493 their wide acceptance for being used as part of automobiles which a=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

Jordi Girones : preparation of composites, mechanical experiments, text writing

Marie-Pierre Jacquemot : samples harvesting and distribution to partners

Frédéric Legée : biochemical analyses

Laurent Cézard : biochemical analyses

Catherine Lapierre : biochemical analyses

Fadi El Hage : histological analyses

Valérie Méchin : sample harvesting, conceptualization of field trials, samples harvesting, data analysis, text writing

Matthieu Reymond : sample harvesting, conceptualization of field trials, samples harvesting, data analysis, text writing

Patrick Navard : overall supervision of the work, text writing

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Legends of figures

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 µm 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 ± 1.1 (kJ/m^2)). 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.

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-
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732
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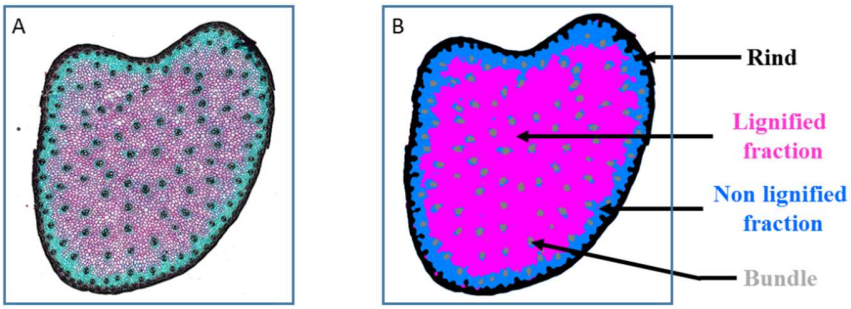

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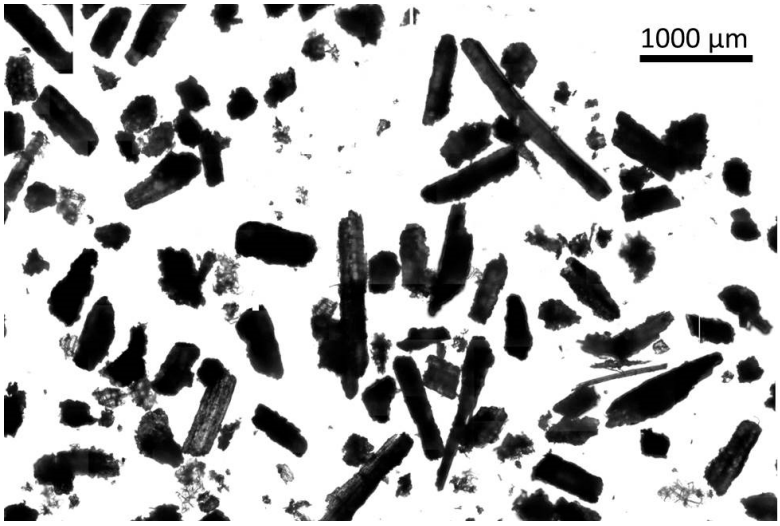

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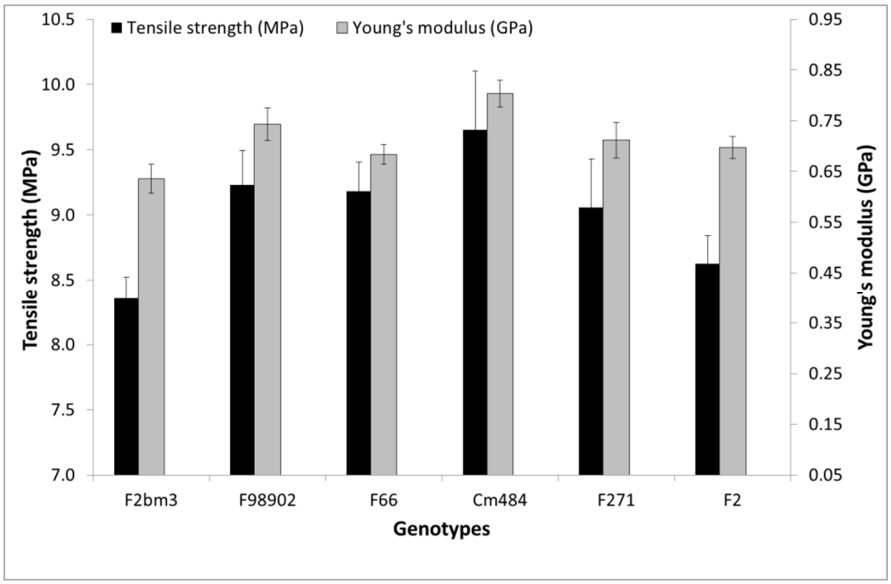

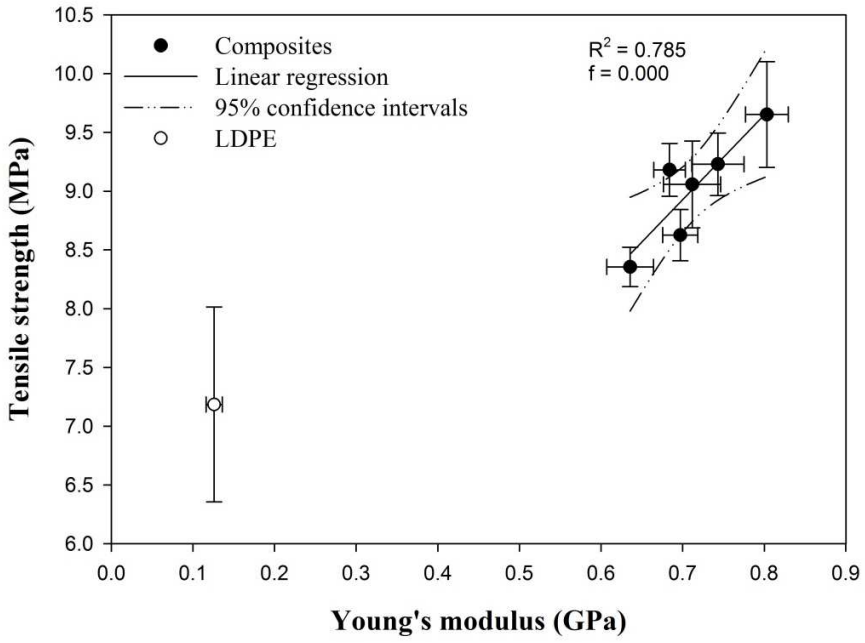

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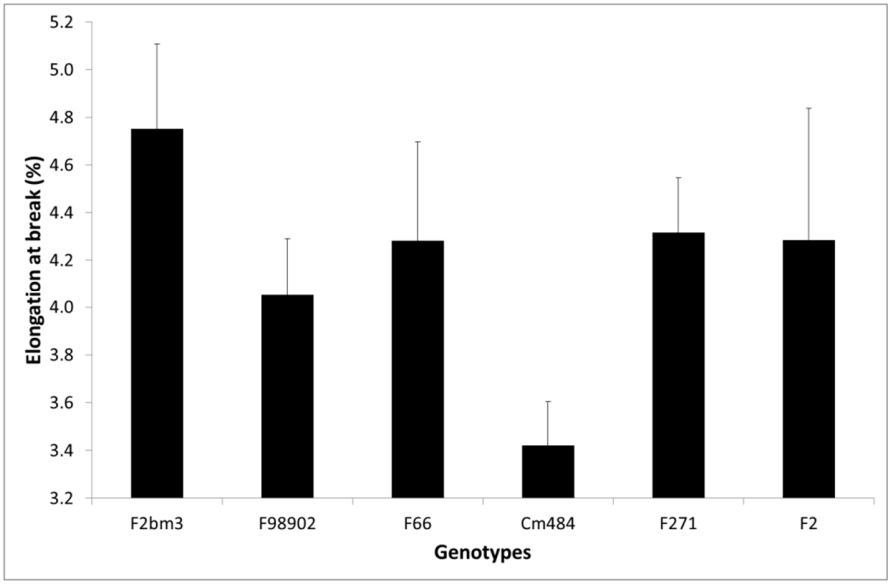
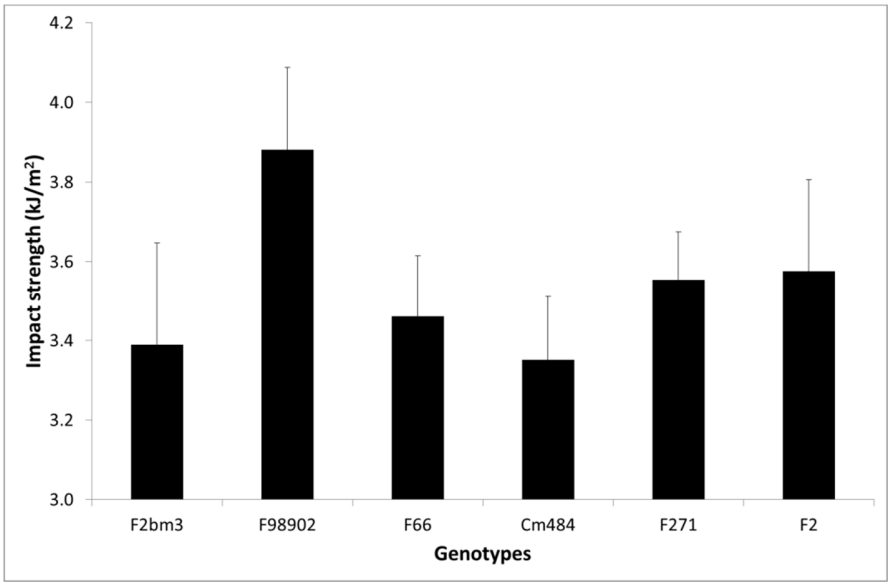
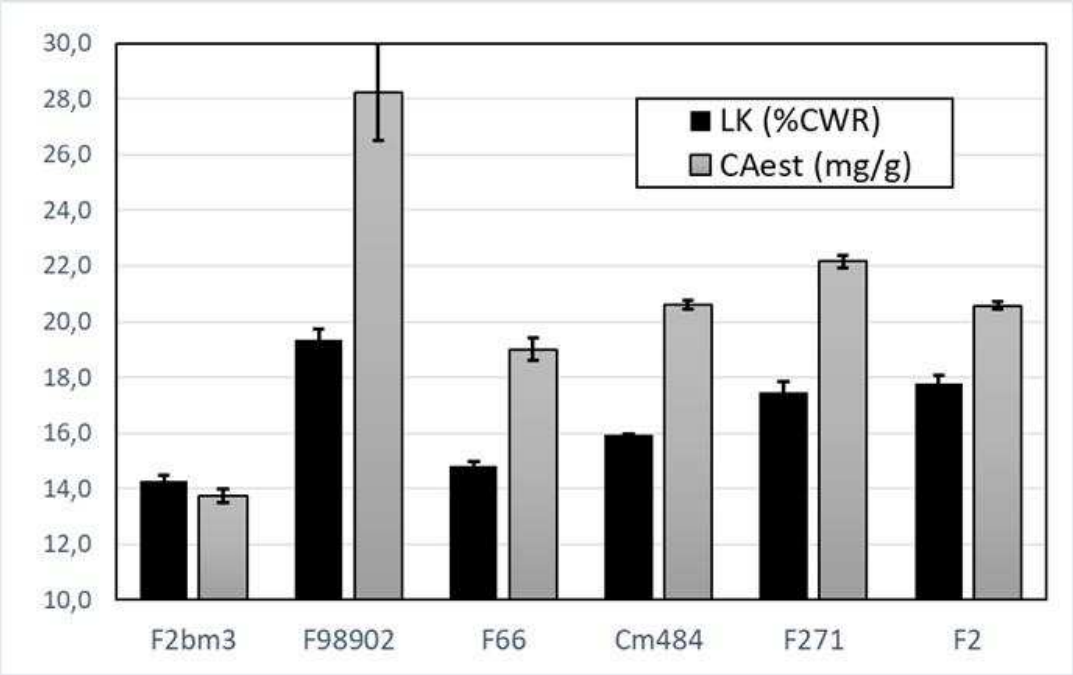

5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
Elongation at break (%)
F2bm3
F98902
F66
Cm484
F271
F2
Genotypes


4.2
4.0
3.8
3.6
3.4
3.2
3.0
Impact strength (kJ/m²)
F2bm3
F98902
F66
Cm484
F271
F2
Genotypes



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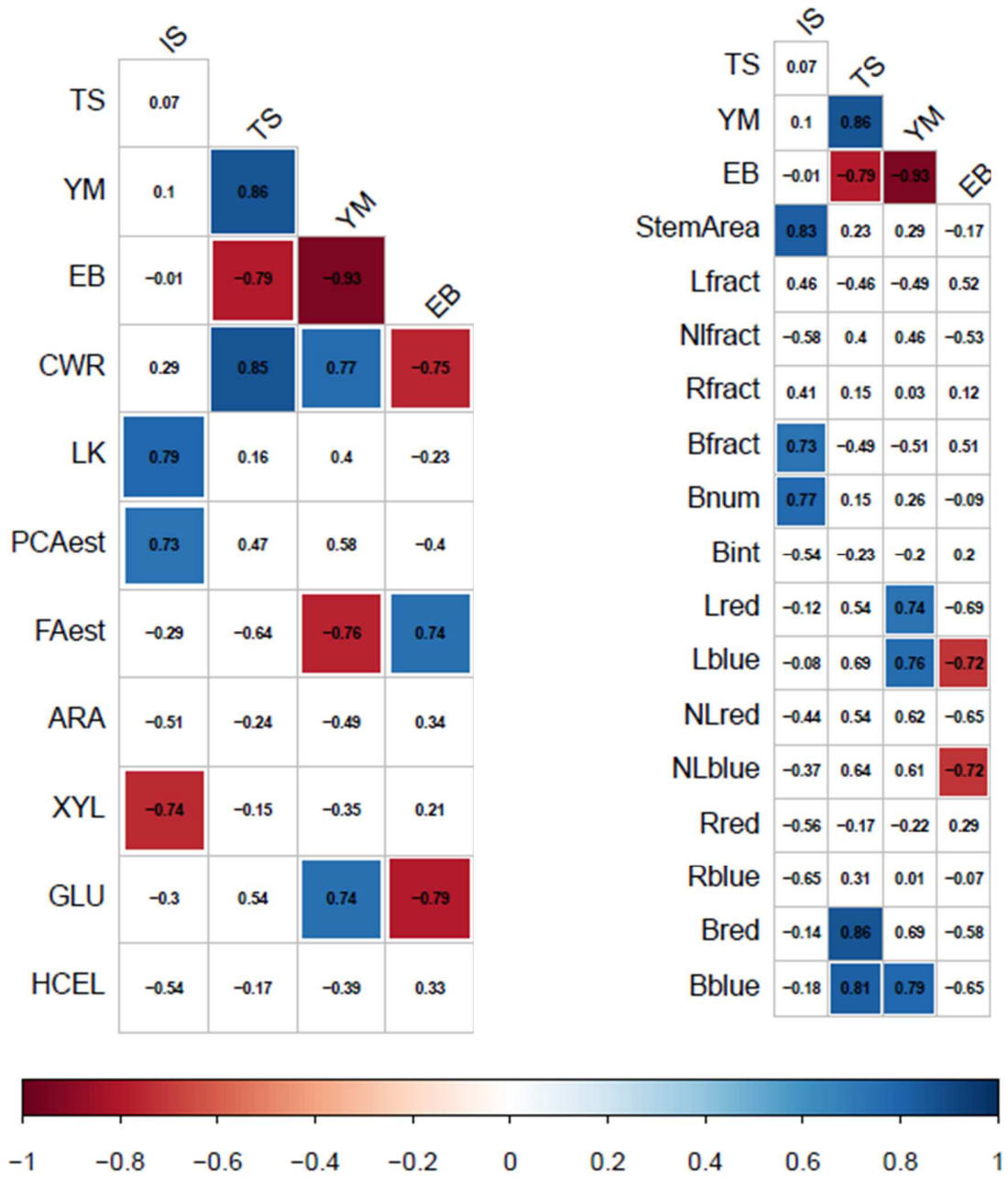

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