

## **RAPESEED STUBBLE AS A RESOURCE FOR BIOENERGY AND BIREFINERIES. EFFECT OF GENOTYPE AND CULTIVATION CONDITIONS ON CHAFF AND STALK BIOMASS AND QUALITY**

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### **ABSTRACT**

The abundance and low price of the residual biomass of extensive crops makes them an attractive raw material for bioenergy and biorefineries. Residual biomass (stubble) is made up of biomass from different organs and may differ in its chemical composition. In rapeseed, the stubble is made up of the stalks and the pericarp of the siliques (chaff) and their characteristics have not been analyzed separately up to the present. Thus, it is impossible to determine the possible best uses for each fraction of the stubble based on its composition. This work aims to evaluate the quantity and composition of stalks and chaff biomass of 13 rapeseed genotypes in a variety of growing conditions, to test the following hypotheses: 1) the amount of stubble biomass per area is highly variable and cannot be estimated from grain yield, 2) the stubble-to-grain and the chaff-to-stalk ratios change with genotype and growth conditions, 3) the chemical composition of chaff and stalk is different, which justifies a separate use. The stubble biomass was between 2-6 t ha<sup>-1</sup> according to genotype and cultivation conditions. The chaff-stalk ratio was not stable and ranged from 0.8-2.2. The stalk biomass is better to produce energy, due to its high caloric power (17-18 MJ kg<sup>-1</sup>) and low ash content (6%), while chaff have less cellulose (<38%) and lignin (<13%) and have a higher ash content (5-14%), being more suitable for biorefinery use. We concluded that the rapeseed stubble biomass is high enough to consider its economic use, and it is recommended to consider the stalk and chaff separately. The differences found among genotypes provide elements to choose materials considering the use of the residual biomass for bioenergy or biorefinery.

**Key words:** Canola; ash content, lignocellulosic biomass, bioenergy, biorefinery.

## **EL RASTROJO DE COLZA COMO RECURSO PARA BIOENERGÍA Y BIORREFINERÍAS. EFECTO DEL GENOTIPO Y LAS CONDICIONES DE CULTIVO SOBRE LA BIOMASA Y CALIDAD DE VAINAS Y TALLOS**

### **RESUMEN**

La abundancia y bajo precio de la biomasa residual de cultivos extensivos (rastrojo) determina que sea una materia prima atractiva para bioenergía y biorrefinerías. Los rastrojos están conformados por biomasa proveniente de diferentes órganos y pueden diferir en su composición química. En colza, el rastrojo está conformado por tallos y el pericarpio de las silicuas (vainas), cuyas características no se han analizado de manera separada hasta el presente, lo que impide determinar las posibilidades de optimizar su aprovechamiento en función de su composición. Este trabajo tiene como objetivo evaluar la cantidad y la composición de la biomasa de tallos y vainas de 13 genotipos de colza en una variedad de condiciones de cultivo, para probar las siguientes hipótesis: 1) la cantidad de biomasa de rastrojo es altamente variable y no puede ser estimada a partir de los valores de

rendimiento de grano, 2) la proporción entre rastrojo y grano y entre vaina a tallo cambian con el genotipo y las condiciones de crecimiento del cultivo, 3) la composición química de la vaina y el tallo es diferente lo que justifica un aprovechamiento separado. La biomasa seca total de rastrojo fue de entre 2-6 t ha<sup>-1</sup> según genotipo y condiciones de cultivo. La relación vaina-tallo no estable y osciló entre 0.8-2.2. La biomasa del tallo es adecuada para producir energía, debido a su alto poder calórico (17-18 MJ kg<sup>-1</sup>) y bajo contenido de cenizas (6%). Las vainas tienen menos celulosa (<38%) y lignina (<13%) y un contenido mayor de cenizas (5-14%), siendo más adecuadas para aprovechamientos de biorrefinería. Se concluye que la cantidad de rastrojo de colza es elevada lo que permite considerar su aprovechamiento económico, pero se recomienda considerar los tallos y las vainas por separado. Las diferencias encontradas entre genotipos aportan elementos para elegir materiales considerando el uso de la biomasa de rastrojo para bioenergía o biorrefinería.

**Palabras clave:** Canola, cenizas, biomasa lignocelulósica, biorrefinería.

## INTRODUCTION

Over the last century, the world energy matrix has been based on fossil fuels, which have enormously contributed to the increase in the levels of CO<sub>2</sub> and thus, to global warming (Koçar and Civas, 2013). Many industrialized countries have committed themselves to reduce the use of petroleum derivatives and resort to renewable energy sources (Rutz and Janssen, 2007). Consequently, most long-term global energy scenarios rely on biomass as a promising source (Shyam, 2002), although biomass alone could hardly replace fossil fuels in most production, processing, and domestic activities. It is expected that global biofuel supply potential increases mainly from food and lignocellulosic crops (75%), whereas the remaining quarter would come from agricultural and forestry residues (Deng *et al.*, 2015). Therefore, it is crucial to improve the understanding of the sustainable and realistic potential of crop residual biomass as an energy source.

The abundance of crop residues makes them an attractive alternative for energy production and to avoid the controversy about using food that is necessary for an increasing world population. Biomass production for energy purposes has been mainly focused on short rotation forests, perennial grasses and stubble of summer crops, such as sugarcane, maize and sorghum (Ericsson *et al.*, 2009; Wright, 2006). Over the last decades, the global production of canola has steadily increased and its productivity can represent at

least 40-50% of wheat grain yield (Rondanini *et al.*, 2012). In addition, the feasibility of using rapeseed stubble for energy and second-generation biofuels has been evaluated (Bellarby *et al.*, 2010; Roberts *et al.*, 2015; Zabaniotou *et al.*, 2008). Sustainable agriculture and industrial processes move towards the use of lignocellulosic high-valued bio-wastes as bioethanol, biobased products, such as lactic acid, and nanocellulose within a biorefinery and green chemistry concept (Kim and Dale, 2004; Nechyporchuk *et al.*, 2016).

Harvest of grass and woody crops can be organized for recovery of the biomass only, while in grain annual crops, straw (stems and leaves of small cereals) and chaff (husks and glumes) are separated from fruits and seeds during threshing. Different harvesting systems are used for rapeseed around the world, including swathing, pushing and direct combining processes (Irvine and Lafond, 2010; Haile *et al.*, 2014). Also, whole crop harvest can be used, in this system the entire material is transferred to a central location where the harvested material is fractionated into grain and stubble. For example, some devices fractionate the harvested crop into stalk and chaff (a mixture of grain and chaff). The stalk is left on the field, whereas grain separation from chaff take place in a stationary system at the farmyard (McLeod Harvest Inc, Winnipeg, Canada). Thus, stalk and chaff biomass fractions can be handled separately for different end uses. Concomitantly, combine harvesters that collect sep-

arately the maize cob and the wheat chaff are being developed, allowing a selective harvesting of residual biomass from the crops (Bergonzoli *et al.*, 2020).

In several crops, stalk and chaff biomass fractions vary in quantity and quality, according to genotype, environment, and agricultural husbandry (Zabaniatou *et al.*, 2008; Roberts *et al.*, 2015). Chemical composition and heating value are relevant attributes for biomass end-use (Liang *et al.*, 2015; Windeatt *et al.*, 2014). Functional relationships among chemical compounds have been found in the biomass of some crops. For instance, for switchgrass, wheat and barley, biomass heating value decreases as ash content rises (Lehtikangas, 2001; Mani *et al.*, 2004). Chemical composition defines end-use of biomass: high concentrations of cellulose and hemicellulose with low concentration of lignin are desirable for second generation bioethanol production (Lemus *et al.*, 2002; Anwar *et al.*, 2014) while high lignin and cellulose contents are desirable for combustion (McKendry, 2002). Chemical characteristics of rapeseed residues have been focused on whole stubble or stalk biomass only (Monti *et al.*, 2008; Zabaniotou *et al.*, 2008; Mazhari Mousavi *et al.*, 2013; Roberts *et al.*, 2015). In contrast, the quantity and quality of chaff biomass have not been tested until now. Analyzing the chaff fraction in depth is relevant to determine its potential use in biorefinery and green chemistry, to identify promising genotypes, and to add value and economic sustainability to its use.

On the other hand, the effect of the cultivation conditions and stress on the quality of the stubble considering its use in biorefinery or bioenergy has been scarcely studied. In this sense, Franzaring *et al.* (2015) determined effects on the quantity and quality of biomass in two herbaceous crops for bioenergetic biomass (*Sida hermaphrodita* and *Silphium perfoliatum*) when they were exposed to different conditions of water availability,

temperature and concentration of carbon dioxide.

This work was aimed to evaluate the quantity and chemical composition of stalk and chaff biomasses from 13 spring rapeseed genotypes, some of which were exposed to stress conditions such as high temperatures or shading during grain filling or variable conditions of resource availability by manipulating the crop density. Changes in the quality of biomass associated with its lignin, cellulose and ash content will allow determining its aptitude as raw materials for bioenergy and biorefinery purposes. The hypotheses to be tested are: 1) the amount of stubble biomass per area is highly variable and cannot be estimated from grain yield, 2) the stubble-to-grain and the chaff-to-stalk ratios change with genotype and growth conditions, 3) the chemical composition of chaff and stalk are different enough to justify harvesting, handling, and end-use processing separately.

## MATERIAL AND METHODS

### Crop residues

Crop residues were obtained from four experiments carried out in 2012 and 2014 at the experimental field of the Faculty of Agronomy, University of Buenos Aires (34°35'S, 58°29'W). A total of 13 spring rapeseed genotypes were tested under different growing conditions (Table 1). All the experiments were sown on silty clay loam classified as Vertic Argiudoll according to the USDA taxonomy, in 2 x 1.5 m plots, 0.2 m row spaced. Plant density was 60 pl m<sup>-2</sup> (Piergentili, 2016) except for Exp. 1 and 3, which tested a 15-60 pl m<sup>-2</sup> range (Rondanini *et al.*, 2017). Shading treatments consisted of covering plots with dark plastic mesh (reducing incident solar radiation by 80%). Heat stress was applied by covering plots with transparent plastic film during 4 hours at midday (10:00-14:00 h) increasing air temperature by 10°C inside the plot, with respect to external air temperature, for 14 days from the beginning of full flowering

**Table 1.** Experimental details of spring rapeseed

Exp.	Rapeseed genotypes	Code	Sowing date	Growing conditions
1	Hyola 61	Hy61	May 8, 2012	Plant density (15, 30 and 60 pl m <sup>-2</sup> ) Rondanini <i>et al.</i> (2017)
2	Hyola 61	Hy61	May 8, 2012	Shade and heat stress at flowering. Plant density 60 pl m <sup>-2</sup> . Rondanini <i>et al.</i> (unpublished)
3	Hyola 61 SRM 2836	Hy61 SRM	June 30, 2014	Plant density (15 and 60 pl m <sup>-2</sup> ) Rondanini <i>et al.</i> (2017)
	Bioaureo 2386	Bio23		
	Bioaureo 2486	Bio24		
	Gladiator	Glad		
	Hyola 433	Hy433		
	Hyola 571 CL	Hy571		
4	Hyola 575	Hy575	June 30, 2014	Genotype screening. Plant density 60 pl m <sup>-2</sup> (Piergentili, 2016)
	Hyola 76	Hy76		
	Legacy	Leg		
	Rivette	Riv		
	Smilla	Smi		
	Solar CL	Sol		

(Rondanini *et al.*, unpublished). All the experiments were irrigated and fertilized to reach 60 kg ha<sup>-1</sup> of sulfur and 150 kg ha<sup>-1</sup> of nitrogen and were maintained free of weeds, pests and fungal diseases.

## MEASUREMENTS

At harvest, stalk, chaff and seeds were manually dissected, oven dried at 105°C and dry weight was measured with 0.1 g precision (dry matter, DM). The proportion of each fraction (biomass allocation) was expressed related to the total above-ground biomass. Stubble-to-grain and chaff-to-stalk ratios were also calculated.

In this article, stubble refers to both the canola above ground biomass left in the field after harvesting and the by-product after threshing, that are conformed by the stalks and pericarp of siliques (chaff).

Stalk and chaff fractions were milled to particle size of <2 mm in a laboratory mill. Chemical analyses were: ash content (muffle furnace at 550°C for 8 hours), heating value (automatic bomb calorimeter Shimatzu CA-

4P, according to JISM8814-1976), water soluble carbohydrates (Scott and Melvin, 1953), acid detergent fiber, neutral detergent fiber, acid detergent lignin (Van Soest *et al.*, 1991; Rocha *et al.*, 2017; van der Weijde *et al.*, 2017).

## STATISTICAL ANALYSIS

Experimental data were analyzed by box-plot and ANOVA and means were compared using Tukey's test to determine significant differences at 5%.

The experiments 1, 2 and 4, having a single factor (density, thermal stress, shading and genotypes), were analyzed using one-way ANOVA and Tukey's test to make multiple comparisons, while experiment 4, having two factors (genotype and density) was analyzed using two-way ANOVA and Sidak's Test to make multiple comparisons.

Linear regression analysis and Pearson's correlation test were used to relate chemical variables. Statistical analyses were performed using InfoStat (Di Rienzo *et al.*, 2013).

## RESULTS

### Crop residues quantity and biomass allocation

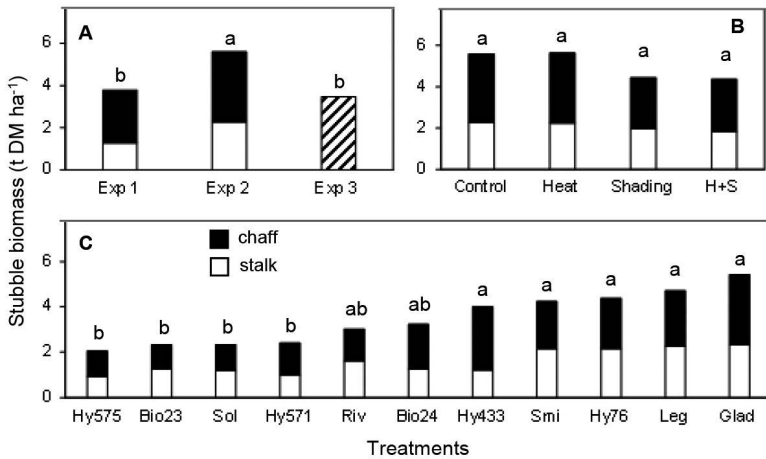
Total above-ground biomass varied markedly among experiments (Table 2). On average, the harvest index, defined as the proportion of grain over the total above-ground biomass, was 28%, which implies that 72% of the aerial biomass produced is considered stubble, thus, stubble-grain relationship varied between 1.3 to 6.1 (Table 2). Stubble biomass ranged from 2 to 6 t ha<sup>-1</sup> of dry matter (Figure 1) and was mainly located in the chaff fraction (55% of stubble biomass, on average), with chaff-to-stalk ratio ranging between 0.8-2.2 (Table 2).

Stalk and chaff biomasses were affected by genotype, year, and environmental stresses (Figure 1). Inter-annual variation in biomass allocation was observed for Hyola 61 grown at 60 pl m<sup>-2</sup> in experiments 1, 2 and 3 (Figure 1A). Chaff fraction was affected by heat and shading stresses at flowering stage in Exp. 4 (Figure 1B). In addition, significant genotypic effects were observed on total biomass produced by 11 spring genotypes in Exp. 4 (Figure 1C). Genotype choice was the main agronomic management decision generating variability in the stubble to grain ratio (6.14 to 1.38 range, Table 2) and the stubble biomass (2.33 to 0.94 t of stalk and 3.09 to 1.05 t of chaff, data calculated from Table 2). Geno-

**Table 2.** Total above-ground biomass at harvest (t of DM ha<sup>-1</sup>) and biomass allocation among stalk, seed and chaff (pericarp of siliques), stubble-to-grain ratio, and chaff-to-stalk ratio in spring rapeseed from Exp. 1-4. See experimental details in Table 1.

Exp.	Genotype	Treatment	Total above-ground biomass (t ha <sup>-1</sup> )	Above-ground biomass allocation			Stubble-to-grain ratio	Chaff-to-stalk ratio
				Stalk	Seed	Chaff		
1	Hy61	15 pl m <sup>-2</sup>	11.13 a	0.27 a	0.26 b	0.47 a	2.85 a	1.74 b
		30 pl m <sup>-2</sup>	9.98 ab	0.28 a	0.26 b	0.46 a	2.85 a	1.64 b
		60 pl m <sup>-2</sup>	5.40 b	0.24 a	0.31 a	0.46 a	2.26 a	1.92 a
		<i>p-value</i>	<i>0.0233</i>	<i>0.2333</i>	<i>0.0407</i>	<i>0.7563</i>	<i>0.4321</i>	<i>0.0391</i>
2	Hy61	Control	7.36 a	0.31 a	0.24 a	0.45 b	3.17 b	1.45 ab
		Heat	6.63 a	0.34 a	0.15 b	0.51 a	5.67 a	1.50 a
		Shading	5.63 a	0.35 a	0.21 ab	0.44 b	3.76 ab	1.26 b
		H+Sh	5.26 a	0.35 a	0.17 b	0.48 ab	4.88 ab	1.37 ab
		<i>p-value</i>	<i>0.2346</i>	<i>0.5891</i>	<i>0.0127</i>	<i>0.0369</i>	<i>0.0082</i>	<i>0.0409</i>
3	Hy61 SRM	15 pl m <sup>-2</sup>	5.10 a	---NA	0.32 ab	---	2.13	---
		60 pl m <sup>-2</sup>	5.42 a	---	0.36 a	---	1.78	---
		15 pl m <sup>-2</sup>	4.99 a	---	0.27 c	---	2.70	---
		60 pl m <sup>-2</sup>	5.00 a	---	0.31 b	---	2.23	---
		<i>p-value</i>	<i>0.6993</i>		<i>0.0008</i>		<i>0.0114</i>	
4	Bio23		3.10 b	0.41 a	0.25 ab	0.34 b	3.00 ab	0.83 b
	Bio24		5.48 ab	0.23 a	0.42 a	0.36 ab	1.38 b	1.57 ab
	Glad		6.31 a	0.37 a	0.14 b	0.49 a	6.14 a	1.32 ab
	Hy433		5.81 a	0.21 a	0.31 ab	0.48 a	2.23 ab	2.29 a
	Hy571		3.30 b	0.30 a	0.27 ab	0.43 ab	2.70 ab	1.43 ab
	Hy575		2.85 b	0.33 a	0.28 ab	0.39 ab	2.57 ab	1.18 ab
	Hy76		6.55 a	0.33 a	0.33 ab	0.34 ab	2.03 ab	1.03 ab
	Leg		6.83 a	0.33 a	0.32 ab	0.36 ab	2.13 ab	1.09 ab
	Riv		4.38 ab	0.37 a	0.31 ab	0.32 b	2.23 ab	0.86 b
	Smi		6.33 a	0.34 a	0.32 ab	0.33 b	2.13 ab	0.97 b
	Sol		3.34 b	0.36 a	0.31 ab	0.34 b	2.23 ab	0.94 b
<i>p-value</i>		<i>0.0419</i>	<i>0.6163</i>	<i>0.0256</i>	<i>0.0494</i>	<i>0.0125</i>	<i>0.0374</i>	

Different letters within each experiment indicate significant differences ( $p < 0.05$ ) according to Tukey's test. P-values are shown in italics. NA: data not available (because stalk and chaff were handled as a whole).



**Figure 1.** Stubble biomass (t of DM ha<sup>-1</sup>) assigned to stalk and chaff in (A) spring rapeseed Hyola 61 grown at 60 pl m<sup>-2</sup> from Exp. 1-3, (B) Hyola 61 under heat and shade stresses at flowering from Exp. 2, (C) eleven spring rapeseed genotypes from Exp. 4. Diagonal striped bar in (A) indicates stalk + chaff biomass. Different letters indicate significant differences (p < 0.05) in total biomass between treatments. See experimental details in Table 1.

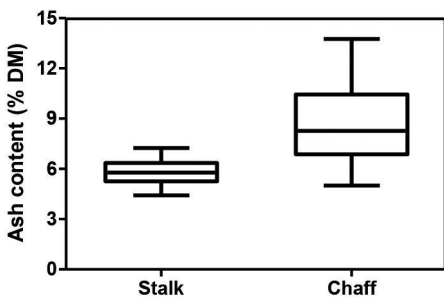
types such as Hyola 575, Bioaureo 2386, Solar and Hyola 571, produced less stubble biomass than Gladiator and Legacy (Figure 2).

Plant density had a significant effect on aerial biomass production and grain yield in a contrasting manner (Table 2). With high density (60 pl m<sup>-2</sup>), the highest grain yields and the lowest production of stubble biomass were observed. This generated significant (p < 0.05) changes in the chaff to stalk ratio, which was higher, while the stubble to grain ratio was not affected (Table 2).

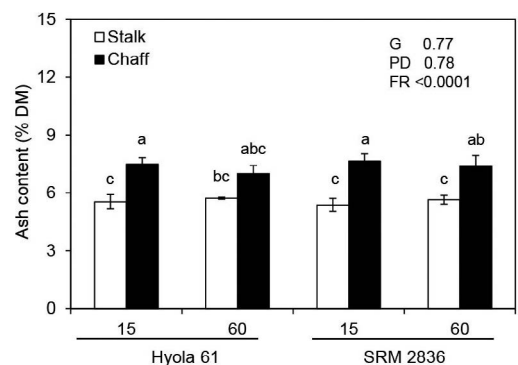
**Ash content from stalk and chaff biomass**

Ash content ranged from 4 to 15 % of DM, and was consistently higher in chaff than stalk (Figure 2). The ash concentration in chaff and stalk of two canola genotypes (Hyola 61 and

SRM 2836) growing at two different densities (15 vs 60 pl m<sup>-2</sup>) were not statistically different between treatments, but significant differences (p < 0.05) were found between chaff and stalk, with the exception of genotype Hyola 61 growing at density of 60 pl m<sup>-2</sup>, where no significant differences were found (Figure 3). Genotypic variation was also observed (Figure 4) with higher ash in chaff than stalk for all genotypes (note dots above the line 1:1). Legacy was the genotype with the highest ash content, whereas Hyola 575 and Hyola 433 were low-ash genotypes (Figure 4). Analyzing the data from the different treatments together,



**Figure 2.** Box-plot of ash content (% of DM) from stalk and chaff biomass of different rapeseed genotypes and growing conditions from Exp. 3 and 4. See experimental details in Table 1.



**Figure 3.** Ash content (% of DM) in stalk and chaff biomass of two spring rapeseed genotypes (Hyola 61 and SRM 2836) grown at contrasting plant densities (15 and 60 plants m<sup>-2</sup>). Data are from Exp. 3. Different letters indicate significant differences (p < 0.05) according to Tukey's test. P-values for genotype (G), plant density (PD) and biomass fraction (FR) are also shown.



a significantly positive correlation between ash content in chaff and stalk was observed ( $p < 0.05$ ).

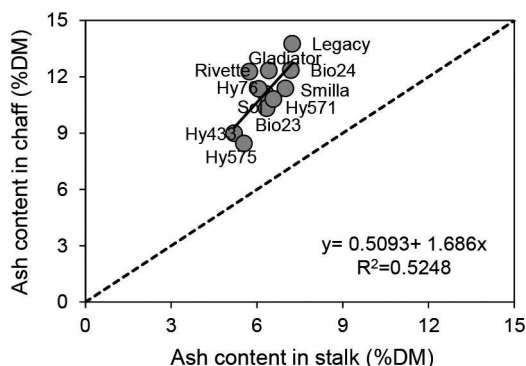


Figure 4. Linear regression between ash content (% of DM) in stalk and chaff biomass from eleven spring rapeseed genotypes. Data are from Exp. 4. Slope of lineal regression is significantly different from zero at  $p = 0.011$ . The dashed line indicates the relationship 1:1.

### Heating value

Heating values ranged between 15.5-18.4 MJ kg<sup>-1</sup> for stalk fraction, and between 16.7-18.4 MJ kg<sup>-1</sup> for chaff fraction. Heating value from stalk was slightly lower than chaff, although the difference was not statistically significant ( $p = 0.2187$ ). A negative relationship between ash content and heating value was observed for both stalk and chaff biomass fractions (Figure 5).

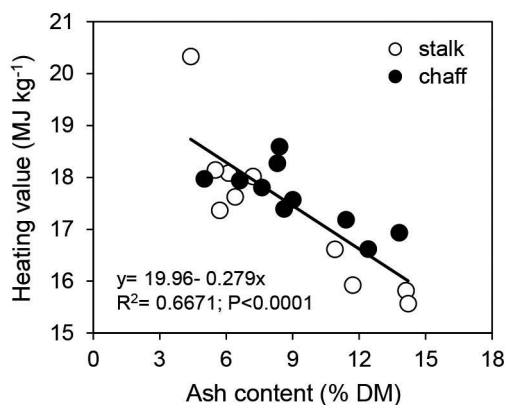


Figure 5. Relationship between ash content and heating value for stalk and chaff biomass of spring rapeseed. Data are from Exp. 1-4. Linear adjustment of the data is shown.

### Water soluble carbohydrates

Water soluble carbohydrates in stalks ranged from 1 to 3 mg per 100 mg of DM and were significantly ( $p < 0.05$ ) affected by genotype and environmental stress when shade and heat were applied together (Table 3). No effect of plant density was found on water soluble carbohydrates in stalk (Experiments 1 and 3, Table 3). For chaff, water soluble carbohydrates ranged between 1.8-3.0 mg per 100 mg of DM (Exp. 3 and 4, Table 3), and there were no significant differences between genotypes or plant density (Table 3).

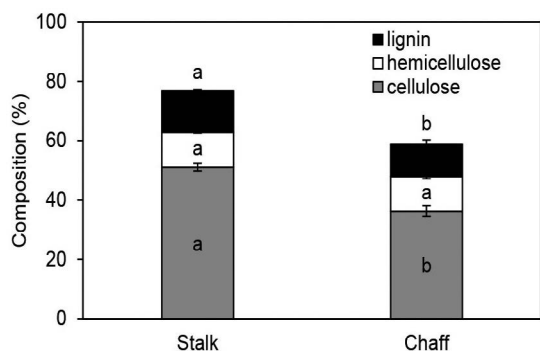
Table 3. Water soluble carbohydrates in stalk and chaff biomass fractions of spring rapeseed from Exp. 1-4. See experimental details in Table 1.

Exp.	Genotype	Treatment	Soluble carbohydrates mg (100 mg DM) <sup>-1</sup>	
			Stalk	Chaff
1	Hy61	15 pl m <sup>-2</sup>	1.20 a	---NA
		30 pl m <sup>-2</sup>	1.06 a	---
		60 pl m <sup>-2</sup>	1.35 a	---
		<i>p-value</i>	0.2653	
2	Hy61	Control	1.17 ab	---
		Heat	1.43 a	---
		Shading	1.37 a	---
		H+S	1.04 b	---
		<i>p-value</i>	<0.0001	
3	Hy61	15 pl m <sup>-2</sup>	1.96 ab	2.57 a
		60 pl m <sup>-2</sup>	1.56 b	1.79 a
		SRM	3.00 a	3.09 a
		60 pl m <sup>-2</sup>	2.40 ab	2.63 a
		<i>Genotype</i>	0.0036	0.078
		<i>Density</i>	<i>p-value</i>	0.0907
		<i>Interaction</i>	0.71	0.66
4	Bio23		3.37 a	2.69 a
	Bio24		1.61 b	---
	Glad		3.39 a	3.07 a
	Hy433		2.97 ab	---
	Hy571		1.98 ab	2.89 a
	Hy575		---	---
	Hy76		---	---
	Leg		---	---
	Riv		2.49 ab	2.73 a
	Smi		2.57 ab	2.56 a
	Sol		1.66 b	2.46 a
		<i>p-value</i>	0.0357	0.4662

Different letters within each experiment indicate significant differences ( $p < 0.05$ ) according to Tukey's test. P-values are shown in italics. NA: data not available.

## Cellulose, hemicellulose and lignin

Chemical composition in cellulose and lignin were different between stalk and chaff (Table 4, Figure 6). Cellulose was significantly higher in stalks than chaff ( $p=0.003$ ) for genotypes from Exp. 4 (Table 4). Hemicellulose showed no significant difference between biomass fractions ( $p=0.90$ ). Lignin did not differ between stalks and chaff, although there seemed to be a slight tendency to a higher level of lignin in stalks (Table 4). When the data from experiment 1 and 4 were analyzed together this trend was maintained and the lignin contents in stalks were statistically higher ( $p < 0.05$ ) than in chaff (Figure 6).



**Figure 6.** Cellulose, hemicellulose, and lignin content (% of DM) in stalk and chaff biomass of spring rapeseed. Data are from Exp. 1-4. Different letters indicate significant differences ( $p < 0.05$ ) between biomass fractions according to Tukey's test.

## DISCUSSION

Several models use grain yield as input to predict the amount of crop residual biomass, assuming a stubble-to-grain ratio of 1-2

(Roberts *et al.*, 2015; Vávrová *et al.*, 2014; McClellan *et al.*, 2012). In this work, that above-ground rapeseed biomass was highly variable ( $2-11 \text{ t DM ha}^{-1}$ ) as well as the stubble-to-grain ratio ranges between 1.3-6.1, suggesting that it was not a fixed parameter for estimating stubble biomass. The amount of stubble biomass (stalk+chaff) observed in this work ( $2-6 \text{ t DM ha}^{-1}$ ) exhibited greater variability than that reported in the literature for rapeseed ( $1-3 \text{ t DM ha}^{-1}$ ; Budzynski *et al.*, 2015; Roberts *et al.*, 2015; Vávrová *et al.*, 2014) indicating the high potential of rapeseed stubble biomass for energy and biorefinery purposes.

Available information about rapeseed biomass allocated on stalk and chaff is scarce for different genotypes and environmental conditions. In the present work, biomass allocation to stalk (0.32, mean of all treatments) tended to be lower and more stable than partition toward chaff, with a chaff-to-stalk ratio affected by genotype, heat stress and plant density. Knowledge about the genotypic and environmental effects on the chaff-to-stalk ratio is relevant to plan a sustainable energy use of stubble biomass, because it is necessary to leave a quantity of biomass in the field to maintain soil carbon stocks. The threshold for biomass removal depends on soil type, climate, and cropping system, but typically, 25-50% of the residual biomass can be removed without affecting soil carbon stocks (Blanco-Canqui, 2013; Vávrová *et al.*, 2014; Wienhold and Gilley, 2010). It is known that rapeseed stubble decomposes more quickly than

**Table 4.** Content of cellulose, hemicellulose, and lignin (% of DM) in stalk and chaff biomass from contrasting spring rapeseed genotypes. Data are from Exp. 4.

Genotype	Cellulose (%)		Hemicellulose (%)		Lignin (%)	
	Stalk	Chaff	Stalk	Chaff	Stalk	Chaff
Bioaureo 2386	51.21	38.77	11.35	11.81	13.88	9.78
Gladiator	48.80	32.61	11.80	10.34	14.80	13.73
Rivette	53.15	37.37	12.21	12.88	13.77	9.49
<i>p-value</i>	<i>0.0028</i>		<i>0.8943</i>		<i>0.0886</i>	

P-values of mean test comparison between stalk and chaff are shown in italics.



wheat, with a mean net mineralization from stubble of 0.7 kg ha<sup>-1</sup> of nitrogen and 0.75 kg ha<sup>-1</sup> of phosphorus after 10-11 months (Soon and Arshad, 2002). This is relevant especially for soil carbon sequestration and nutrient cycling under reduced and no tillage (Alvarez, 2005). In this context, selective biomass removal of chaff or stalks can be planned according to the proper transformation process to be used (combustion, fermentation or thermo-chemical conversion) leaving the remaining biomass to be incorporated into the soil. Selective biomass removal is in line with novel biorefinery integration concept for lignocellulosic biomass, which proposes pre-treatment at the biomass sites, regional distributed conversion of biomass from various sectors (stubble, sawdust, black liquor), and centralized upgrading/separation of crude biofuels (Özdenkci *et al.*, 2017).

Chemical compositions of chaff and stalk differ strongly among rapeseed genotypes and environmental growing conditions. Observed ash values for stalk fraction fell within the range of 2-6% cited in literature for the whole rapeseed stubble (Kashaninejad and Tabil, 2011; Repić *et al.*, 2013; Svärd *et al.*, 2015; Zabaniotou *et al.*, 2008). By contrast, ash content in chaff fraction is higher and more variable than in stalk or stubble (5-14%), and there is no data available in the literature. Unlike other crops, rapeseed stubble does not have leaves, which is an advantage because they usually contain high ash and corrosive minerals affecting energy efficiency (Monti *et al.*, 2008; Zabaniotou *et al.*, 2008). The heating value is negatively associated with the ash content, as observed in other lignocellulosic materials (Lehtikangas, 2001; Mani *et al.*, 2004). Observed heating values in rapeseed are similar to those reported in stalks of wheat and lucerne (17-18 MJ kg<sup>-1</sup> DM) and slightly higher than rice hull and straw (15.8-15.0 MJ kg<sup>-1</sup> DM) being suitable to produce energy by direct combustion (Jenkins *et al.*, 1998; Friedl *et al.*, 2005).

Composition of rapeseed stalks and chaff justifies differential end uses for bioenergy and biorefinery, respectively. Stalk fraction is rich in cellulose, similar to bagasse (53%), corncob (55%), softwood and hardwood (Demirbas, 2004; Mazhari Mousavi *et al.*, 2013; Anwar *et al.*, 2014), and can be suitable to produce high value extraction compounds, as nanocelluloses, cellulose fibres, and soluble cellulosic macromolecules (Barana *et al.*, 2016; Frölander and Rødsrud, 2011). Interestingly, chaff fraction has low content of lignin and similar cellulose (<11 and 36 %, respectively) compared to corn stover, wheat straw, and rice hulls (15-22% of lignin and 30-45% cellulose; Barana *et al.*, 2016; Ho *et al.*, 2014). Also, rapeseed chaff fraction has lower lignin compared with other raw materials cited in the literature, as wood and sugar cane bagasse (18-35%, Anwar *et al.*, 2014). Low lignin content and 2-3% soluble sugars are suitable for enzymatic processing of chaff biomass, as lignin is a barrier to controlled breakdown of natural cellulose, and fermentable sugars can contribute to initiate processes of microbial degradation (Anwar *et al.*, 2014; Mithra *et al.*, 2018). Hemicellulose content is very stable between stalk and chaff in all rapeseed genotypes evaluated, being lower than corn stover and wheat straw (20-50% of hemicellulose, Ho *et al.*, 2014; Wu *et al.*, 2014).

## CONCLUSIONS

Stubble biomass of rapeseed is high enough (2-6 t DM ha<sup>-1</sup>) to withdraw some of the crop residues without affecting the sustainability of cropping systems, especially under no-tillage management. Chaff-to-stalk ratio is not a single value, on the contrary it ranges between 0.8-2.2 according to genotype and environment. Stalk biomass of rapeseed is suitable for producing energy by combustion, because of its high heating value (17-18 MJ kg<sup>-1</sup> DM) and low ash content (6 % of DM). Chaff has less cellulose (<38%) and slightly less lignin (<13%)

than stalks, being suitable for biorefinery purposes, as feedstock for high valued enzymes and bio-chemicals production. Differences among genotypes allow the identification of promising genotypes for specific end uses.

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