# 10.7251/AGSY1203209T UDK 633.15-152 LIGNOCELLULOSE FIBRES AND *IN VITRO* DIGESTIBILITY OF ZP MAIZE HYBRIDS AND THEIR PERENTAL INBRED LINES

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### Abstract

In the present study the dry matter yield, cell wall contents and *in vitro* digestibility of the whole plant of different ZP maize hybrids and their parental inbred lines were observed. Obtained results showed that the dry matter yield of the whole plant and yield of digestible dry matter of the whole plant of the observed inbred lines varied from 9.0 to 14.7tha<sup>-1</sup> and from 5.3 to 8.1tha<sup>-1</sup>, respectively. However, the dry matter yield of the whole plant and yield of digestible dry matter of the whole plant of the observed ZP hybrids varied from 22.1 to 26.2tha<sup>-1</sup> and from 14.1 to 15.5tha<sup>-1</sup>, respectively. The contents of lignocelulose fibres such as NDF, ADF, hemicelulose and cellulose in the whole maize plant of the observed inbred lines were higher in comparison to the tested ZP hybrids. Only ADL contents in the whole maize plant of the observed inbred lines and hybrids were in within the same range, i.e. 1.8-3.1%. The dry matter digestibility of the whole maize inbred lines and hybrids plants varied from 51.61% to 61.09% and from 57.22 to 63.81%, respectively. The difference in the digestibility of the dry matter of the whole plant amounted to 6.6%, i.e. 9.5% in hybrids, i.e. inbred lines, respectively. The differences in digestibility of the dry matter.

Key words: Maize inbred lines and hybrids, yield, lignocelullose fibres, digestibility

#### Introduction

Maize is one of the most important forage plants. In order to define the quality parameters of maize biomass it is necessary to study the structure of cell walls of the whole plant. It has been proven that there have been significant changes in the content of lignocellulose fibres in the whole maize hybrid plant, which considerably affected the digestibility of the dry matter and therefore the yield of digestible dry matter as well (Deinum et al., 1981; Coors, 1996; Kim et al., 1999; Frey et al., 2004). Research has revealed that plant genetics could affect the quality and digestibility of the whole plant maize silage (Jung, 1997). Previously published our results point out to a great importance and necessity of the characterisation of the released maize hybrids and their inbreds for both, the determination of purposes of certain hybrids and the development of new high yield potential hybrids for silage (Pejić, 1994, Terzić, 2006; Terzić et al., 2010 and Terzić et al., 2012). The objective of this study was to determine the dry matter yield, lignocellulose fibres contents and *in vitro* digestibility of the whole plant of new ZP maize hybrids and their parental inbred lines.

#### Material and methods

Seven ZP maize hybrids of the FAO maturity groups 600-800 (ZP623, ZP735, ZP749, ZP789, ZP 802, ZP 812 and ZP 873) and nine maize inbred lines were used in this study. Three tested inbred lines (L2, L3 and L4) originate from the USA. They are public inbred

lines from Iowa State University derived from the BSSS synthetic population. Reminding six local inbred lines (L1, L5, L6, L7, L8 and L9) were developed at the Maize Research Institute (MRI) by the pedigree selection method. All nine maize inbred lines are parental components of tested ZP hybrids. Good combining ability for the yield of both, grain and biomass (silage) was estimated in the studied inbred lines.

The two-replicate trail was set up according to the randomised complete-block design in the experimental field of the MRI. The experimental plot size amounted to  $21m^2$ , while the sowing density was 60,000 plants ha<sup>-1</sup>. Plants of each replicate were harvested at the full waxy maturity stage from the area of  $7m^2$  (two inner rows), and yields of fresh biomass of the whole plants, plants without ears and ears were estimated. Five average plants per replicate were selected for further tests. Samples of the whole plants were cut and dried at 60°C for 48h. In order to determine the content of the dry matter the whole plant samples were ground in the 1mm mesh mill. Then, the analysis of the absolute dry matter was done on the oven dry basis (105°C for 12 h) in order to estimate the total dry matter. Moreover, the analysis of the content of forage fibres (NDF - neutral detergent fibres, ADF - acid detergent fibres, ADL - acid detergent lignin, hemicelluloses and cellulose) was performed by the modified Van Soest detergent method (Van Soest, 1963). The method was modified by Mertens (1992). In vitro digestibility of the whole maize plant was done by the Aufréré method (Aufréré, 2006). This method is based on the hydrolysis of proteins of the whole plant in the pepsin acid solution (Merck 2000 FIP u/g Art 7190) at 40°C for 24 h, and then on the hydrolysis of carbohydrates in the cellulase solution (cellulase Onozuka R10) in duration of 24 h.

Data reported for quality parameters of ZP hybrids and their parental inbred lines biomass were assessed by the analysis of variance (ANOVA) and the LSD multiple test was used for any significant differences at the P<0.05 level between the means. All the analyses were conducted using statistical software package STATISTICA 8.1. (StatSoft Inc. USA).

### **Results and discussion**

The data in Table 1 show that the dry matter yield of the whole plant and yield of digestible dry matter of the whole plant of the observed maize inbred lines varied from 9.0 (L9) to 14.7tha<sup>-1</sup> (L3) and from 5.3 (L9) to 8.1tha<sup>-1</sup> (L3), respectively. Differences in digestible dry matter yields were not statistically significant among the inbred lines L1, L2, L3, L4 and L7, and among the lines L5, L6, L8 and L9, as well. The inbred L3 had the highest yield of dry matter and the yield of digestible dry matter per hectare, while the inbred L9 had the lowest yield of dry matter and digestible dry matter per hectare. The whole plant dry matter content of tested inbred lines ranged from 28.09 (L8) to 36.71% (L2).

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Inbred line	Dry matter	Whole plant dry matter	Yield of digestible dry matter
mored mile	content (%)	yield (tha <sup>-1</sup> )	of whole plant (tha <sup>-1</sup> )
L1	32.44	12.1 <sup>c</sup>	7.4 <sup>a</sup>
L2	36.71	13.1 <sup>b</sup>	7.6 <sup>a</sup>
L3	28.80	14.7 <sup>a</sup>	8.1 <sup>a</sup>
L4	31.66	13.6 <sup>b</sup>	7.6 <sup>a</sup>
L5	31.26	10.1 <sup>de</sup>	5.7 <sup>b</sup>
L6	33.22	9.6 <sup>ef</sup>	5.8 <sup>b</sup>
L7	33.13	12.9 <sup>bc</sup>	7.7 <sup>a</sup>
L8	28.09	10.7 <sup>d</sup>	5.5 <sup>b</sup>
L9	31.06	$9.0^{\mathrm{f}}$	5.3 <sup>b</sup>
LSD 0.05		0.8	1.4

Table 1. Yield of whole plant dry matter and digestible dry matter of Maize Inbred Lines

Means in the same column with different superscripts differ (p < 0.05)

The dry matter yield of the whole plant and the yield of digestible dry matter of the whole plant of the observed ZP maize hybrids varied from 22.1 to 26.2tha<sup>-1</sup> and from 14.1 to 15.5tha<sup>-1</sup>, respectively (Tables 2). For all tested parameters of the yield structure there are no significant differences among the hybrids (p < 0.05). The hybrid ZP 802 had the highest dry matter yield of whole plant (26.2tha<sup>-1</sup>), while the hybrid ZP 812 had the highest yield of digestible dry matter (15.5tha<sup>-1</sup>). The whole plant dry matter content of tested hybrids varied from 32.69 (ZP 735) to 42.47% (ZP 749).

	Table 2. Yield Structure of ZP Maize Hybrids							
	Dry matter	Dry r	Yield of digestible					
Hybrid	Dry matter - content (%)	Whole plant	Whole plant	Ear	dry matter of			
	content (70)		without ear	Lai	whole plant (tha <sup>-1</sup> )			
ZP 623	36.82	25.2	11.5	13.7	14.4			
ZP 735	32.69	26.0	14.0	12.0	15.4			
ZP 749	42.47	23.2	11.2	12.0	14.8			
ZP 789	36.02	22.1	9.8	12.3	14.1			
ZP 802	37.10	26.2	13.0	13.2	15.0			
ZP 812	38.74	25.2	12.1	13.1	15.5			
ZP 873	37.53	23.0	10.9	12.1	14.1			
LSD 0.05		-	-	-	-			

Table 2. Yield Structure of ZP Maize Hybrids

Means in the same column with different superscripts differ (p < 0.05)

The contents of NDF, ADF, ADL, hemicelluloses, cellulose and digestibility of the whole maize plant are presented in Tables 3 and 4. The results show that the NDF, ADF, ADL, hemicelluloses, cellulose contents and digestibility of dry matter in the whole maize plant of the observed different inbreds varied from 50.97 (L1) to 62.82% (L 8), 24.24 (L6) to 32.34% (L8), 1.84 (L7) to 3.07% (L8), 26.44 (L1) to 32.40% (L5), 21.94 (L6) to 29.27% (L8) and 51.61 (L8) to 61.09% (L1), respectively. Among the tested inbreds, the inbred L8 had the highest NDF (62.82%), ADF (32.34%), ADL (3.07%) and cellulose (29.27%) and the lowest dry matter digestibility (51.61%). However, the inbred L1, which had the highest dry matter digestibility (61.09%) had the lowest NDF (50.97%) and hemicellulose (26.44%) content of the whole plant. The differences in the dry matter digestibility among observed maize inbred lines were not significant between L4 and L5 and between L6 and L7 as well.

Table 3. Whole Plant Lignocellulose Fibres Content and Digestibility of Maize Inbred Lines

Inbred			%)		Dry matter	
line	NDF	ADF	ADL	Hemicellulose	Cellulose	digestibility (%)
L1	50.97 <sup>1</sup>	24.54 <sup>e</sup>	2.03 <sup>cd</sup>	26.44 <sup>f</sup>	22.51 <sup>ef</sup>	61.09 <sup>a</sup>
L2	55.98 <sup>f</sup>	26.03 <sup>d</sup>	1.91 <sup>cd</sup>	29.95 <sup>bc</sup>	24.12 <sup>d</sup>	57.86 <sup>d</sup>
L3	57.92 <sup>d</sup>	28.50 <sup>c</sup>	$2.76^{ab}$	29.42 <sup>cd</sup>	25.74 <sup>bc</sup>	54.78 <sup>f</sup>
L4	59.12°	28.44 <sup>c</sup>	3.06 <sup>abc</sup>	30.68 <sup>b</sup>	25.38 <sup>c</sup>	56.06 <sup>e</sup>
L5	61.72 <sup>b</sup>	29.32 <sup>b</sup>	2.85 <sup>ab</sup>	32.40 <sup>a</sup>	26.47 <sup>b</sup>	56.09 <sup>e</sup>
L6	52.54 <sup>h</sup>	24.24 <sup>e</sup>	$2.30^{bcd}$	28.30 <sup>e</sup>	21.94 <sup>f</sup>	60.27 <sup>b</sup>
L7	53.56 <sup>g</sup>	24.93 <sup>e</sup>	1.84 <sup>d</sup>	28.63 <sup>de</sup>	23.09 <sup>e</sup>	60.06 <sup>b</sup>
L8	62.82 <sup>a</sup>	32.34 <sup>a</sup>	3.07 <sup>a</sup>	30.48 <sup>b</sup>	29.27 <sup>a</sup>	51.61 <sup>g</sup>
L9	56.67 <sup>e</sup>	27.90 <sup>c</sup>	2.33 <sup>bcd</sup>	28.77 <sup>de</sup>	25.57 <sup>c</sup>	58.50 <sup>c</sup>
LSD 0.05	0.68	0.81	0.67	0.88	0.77	0.48
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Means in the same column with different superscripts differ (p < 0.05)

The results also show that the NDF, ADF, ADL, hemicelluloses, cellulose contents and digestibility of dry matter in the whole maize plant of the observed different ZP maize hybrids varied from 47.87 (ZP 749) to 52.71% (ZP 623), 23.53 (ZP 789) to 27.49% (ZP 735),

1.84 (ZP 873) to 2.99% (ZP 735), 22.14 (ZP 749) to 26.23% (ZP 873), 21.24 (ZP 789) to 24.50% (ZP 735) and 57.22 (ZP 623) to 63.81% (ZP 749), respectively. Among the tested hybrids, hybrid ZP 749 had the highest dry matter digestibility of the whole plant (63.81%) and the lowest NDF (47.87%) and hemicellulose (22.14%). The lowest digestibility (57.22%) and the highest contents of NDF (52.71%) were found for ZP 623 maize hybrid. The differences in the dry matter digestibility among observed ZP maize hybrids were not significant between the ZP 623 and ZP 802, ZP 749 and ZP 789 and also between ZP 812 and ZP 873.

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Hybrid -		Dry matter				
Hybrid -	NDF	ADF	ADL	Hemicellulose	Cellulose	digestibility (%)
ZP 623	52.71 <sup>a</sup>	26.60 <sup>bc</sup>	2.33	26.11 <sup>a</sup>	$24.27^{a}$	57.22 <sup>d</sup>
ZP 735	49.95 <sup>°</sup>	27.49 <sup>a</sup>	2.99	22.46 <sup>c</sup>	24.50 <sup>a</sup>	59.29 <sup>c</sup>
ZP 749	47.87 <sup>d</sup>	25.73 <sup>ab</sup>	2.49	22.14 <sup>c</sup>	23.24 <sup>b</sup>	63.81 <sup>a</sup>
ZP 789	48.29 <sup>d</sup>	23.53°	2.29	24.76 <sup>b</sup>	21.24 <sup>d</sup>	63.71 <sup>a</sup>
ZP 802	49.63 <sup>°</sup>	25.48 <sup>b</sup>	2.07	24.15 <sup>b</sup>	23.41 <sup>b</sup>	57.34 <sup>d</sup>
ZP 812	48.37 <sup>d</sup>	24.37 <sup>bc</sup>	2.16	$24.00^{b}$	22.21 <sup>c</sup>	61.39 <sup>b</sup>
ZP 873	50.99 <sup>b</sup>	24.76 <sup>bc</sup>	1.84	26.23 <sup>a</sup>	22.92 <sup>b</sup>	61.51 <sup>b</sup>
LSD 0.05	0.79	1.83	-	0.83	0.59	0.67

Table 4. Whole Plant Lignocellulose Fibres Content and Digestibility of ZP Maize Hybrids

Means in the same column with different superscripts differ (p < 0.05)

The correlation dependence between the whole plant digestibility and lignocellulose fibres of ZP maize hybrids and their parental inbred lines are presented in Tables 5 and 6.

A very significant negative correlation was determined between the digestibility the whole maize inbred plants and NDF, ADF, ADL, hemicelluloses and cellulose content (r=0.92, r=-0.94, r=-0.77, r=-0.68, r=-0.93). Furthermore, a highly significant correlation between NDF and ADF, ADL, hemicelluloses and cellulose (r=0.95, r=0.77, r=0.87, r=0.94) and between ADF and ADL, hemicelluloses and the cellulose content (r=0.80, r=0.68, r=0.99) of the whole maize inbred plants and a significant correlation between the content of ADL and the hemicellulose content (r=0.56) were established. A very significant correlation was determined between the contents of ADL and the cellulose (r=0.74) and between contents of hemicelluloses and cellulose (r=0.66).

Table 5. Correlation Dependence between Whole Plant Digestibility and Lignocellulose Fibres
of Maize Inbred Lines

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	NDF	ADF	ADL	Hemicellulose	Cellulose
Digestibility	-0.92**	-0.94**	-0.77**	-0.68**	-0.93**
NDF		0.95**	0.77**	0.87**	0.94**
ADF			0.80**	0.68**	0.99**
ADL				0.56*	0.74**
Hemicellulose					0.66**

\* and \*\* - significance at 0.05 and 0.01 probability levels, respectively.

A very significant negative correlation was determined between the digestibility and NDF and the cellulose content (r=-0.70, r=-0.67) and a significant negative correlation between the hemicelluloses content and ADF (r=-0.55). A highly significant correlation between NDF and hemicelluloses (r=0.66) and between ADF and the cellulose content (r=0.76) of the whole maize hybrid plants and a significant correlation between the content of NDF and the cellulose content (r=0.61) were established. The results gained in this study are in agreement with ones previously published (De Boever et al., 1997, Terzić, 2006, Terzić et al., 2010 and 2012).

Malze Hybrids					
	NDF	ADF	ADL	Hemicellulose	Cellulose
Digestibility	-0.70**	-0.35	-0.33	-0.27	-0.67**
NDF		0.17	0.47	0.66**	0.61*
ADF			0.29	-0.55*	0.76**
ADL				0.23	0.28
Hemicellulose					-0.16

Table 6. Correlation Dependence between Whole Plant Digestibility and Lignocellulose Fibres of ZP Maize Hybrids

\* and \*\* - significance at 0.05 and 0.01 probability levels, respectively.

# Conclusion

The inbred L3 had the highest yield of dry matter  $(14.7 \text{tha}^{-1})$  and the yield of digestible dry matter (8.1 tha^{-1}), while the inbred L9 had the lowest yield of dry matter (9.0 tha^{-1}) and digestible dry matter (5.3 tha^{-1}). The hybrid ZP 802 had the highest dry matter yield of the whole plant (26.2 tha^{-1}), while the hybrid ZP 812 had the highest yield of digestible dry matter (15.5 tha^{-1}).

Among the tested inbreds, the inbred L8 had the highest NDF (62.82%), ADF (32.34%), ADL (3.07%) and cellulose (29.27%) and the lowest dry matter digestibility (51.61%). The inbred L1, which had the highest dry matter digestibility (61.09%) had the lowest NDF (50.97%) and hemicellulose (26.44%) content of the whole plant. The differences in the dry matter digestibility among observed maize inbred lines were not significant between L4 and L5 and between L6 and L7 as well. The hybrid ZP 749 had the highest dry matter digestibility of the whole plant (63.81%) and the lowest NDF (47.87%) and hemicellulose (22.14%). The lowest digestibility (57.22%) and the highest contents of NDF (52.71%) were found for ZP 623 maize hybrid. The differences in the dry matter digestibility among observed ZP maize hybrids were not significant between the ZP 623 and ZP 802, ZP 749 and ZP 789 and, also, between ZP 812 and ZP 873.

A very significant negative correlation was determined between the digestibility of the whole maize inbred plants and NDF, ADF, ADL, hemicelluloses and cellulose content (r=0.92, r=-0.94, r=-0.77, r=-0.68, r=-0.93). However, a very significant negative correlation for tested whole maize hybrid plants was determined only between the digestibility and NDF and the cellulose content (r=-0.70, r=-0.67).

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### References

- Aufréré J. (2006): Prevision de la digestibilite des fourages et aliments concentres et composes des herbivores par une methode enzymatique pepsine-cellulase. AQ 353, 1-6.
- Coors J.G. (1996): Findings of the Wisconsin corn silage consortium. In Conference Proceedings, Seeds of Animal Nutrition Symposium. October 22, 1996. Rochester, New York, NY.
- Deinum, B., Bakker, J. J. (1981): Genetic differences in digestibility of forage hybrids. Neth. J. Agric. Sci., 29, 93-98.
- De Bover J.L., Cottyn B.G., De Brabander D.L., Vanacker J.M., Boucque CH.V. (1997): Prediction of the feeding value of maize silages by chemical parameters, in vitro

digestibility and NIRS. Animal Feed Science Technology, 66, 211-222.

- Frey T.J., J.G. Coors, R.D. Shaver, J.G. Lauer, D.T. Eilert, P.J. Flannery (2004): Selection for silage quality in the Wisconsin quality synthetic and related maize populations. Crop Science, 44(4), 1200-1208.
- Jovanović, R., Koljajić, V., Magoč, M. (1993): Najnovija dostignuća u ishrani krava visoke mlečnosti. Zbornik radova 23-24, 9-13.
- Jung H-J. G. (1997): Analysis of forage and cell walls in ruminant nutrition. Conference: New Developments in Forage Science Contributing to Enhanced Fiber Utilization by Ruminants. The Journal of Nutrition (supplement), 810S-813S.
- Kim J.D., C.H. Kwon, D.A. Kim (2001): Yield and Quality of Silage Corn as Affected by Hybrid Maturity, Planting Date and Harvest Stage. Asian-Aust. J. Anim. Sci., 14(12), 1705-1711.
- Mertens D.R. (1992): Critical conditions in determining detergent fibers. In: Forage Analysis Workshop, Proceedings, NFTA Forage Testing Assoc. Denver, CO. Natl. Forage Testing Assoc. Omaha, NE., pp. C1-C8.
- Pejić, Đ. (1994): Silažni kukuruz. Institut za kukuruz "Zemun Polje", Beograd-Zemun.
- Terzić D. (2006): Parametri kvaliteta kukuruzne biomase za silažu. XI Savetovanje o biotehnologiji. Zbornik radova, , 247-253.
- Terzić D., M. Milašinović-Šeremešić M. Radosavljević, M. Filipović (2012): Fibers and cell wall content and in vitro digestibility of different maize hybrids. 6<sup>th</sup> Central European Congress on Food, Novi Sad 23-26.05.2012. Proceedings, 1630-1634.
- Terzić D., Radosavljević M., Žilić S., Milašinović M., Semenčenko V. (2010): Quality parameters of ZP hybrids biomass. XII International Symposium on Forage Crops of Republic of Serbia "Forage Crops basis of the Sustainable Animal Husbandry Development", Kruševac, 26-28.05.2010. Biotechnology in Animal Husbandry, (spec. issue), 491-496.
- Van Soest P.J. (1963): Uses of detergents in the analysis of fibrous feeds. A rapid method for the determination of fibre and lignin. Journal of Association of Agricultural Chemistry, 46, 829-835.