PhD thesis

INVESTIGATION OF THE VALUABLE PARAMETERS OF SILAGE MAIZE AND SWEET SORGHUM GENOTYPES PRODUCED BY MUTATION AND HETEROSIS BREEDING, IN TERMS OF SILAGE AND BIOETHANOL PRODUCTION

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1. Introduction and aim of the research

1.1. Importance of the topic

Silage produced of silage maize is easily digestible due to its high fermentable carbohydrate content. Due to its high metabolizable energy content and easy digestibility, it is an ideal preservatized forage for intensive dairy cow stocks too.

The quality requirements for silage maize were different in different periods. In Hungary, crop production has been characterized by the quantitative approach for a long time. During these times, the primary aim of breeding was to make the maize lines to produce the highest amount of dry material possible. To fulfil this aim, grain and of double utilized maize hybrids were needed, which possessed wide genetic background and high productivity.

In the meantime, the breeding anticipation of producing silage and doubly utilized maize hybrids separately changed. In the case of maize hybrids, another view-point prospered, the one preferring better digestibility and high stalk dry material yield. To fulfil this aim, the back-cross method is used, with the help of which bm-genes coding low lignin content are integrated into isogenic maize hybrids. The brown midrib maize lines represent a new trend of breeding. The digestibility traits of silage made of brown midrib maize were better than those of their isogenes by 14%. Due to their lower fibre contents, better biogas yield can be achieved with them. These bm-genotypes are energy saving hybrids. The digestibility of the total silage is determined by the neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents of plant cells.

Due to the increasing energy demand of the population, the alternative utilization of maize (bioethanol, biogas) came into the limelight. In terms of the utilization aiming bioethanol production, the ethanol yield of the grain is important. The hybrids of high starch and low protein contents bred for bioethanol production can be produced in low input system too.

Among the alternative crops, silage sorghums are of special importance due to their high starch and sugar contents. As a consequence of the discovery of cytoplasmic male sterility, as a result of hybridization, the number of producible sorghum varieties and hybrids is increasing in Hungary too.

1.2.Aims

My breeding work between 2007 and 2010 are summarized as follows:

- Breeding, phenometric characterization of brown midrib maize hybrids, their isogenes and parental lines, study of the fibre fraction of plant parts and the connection between nutritional factors,
- Comparison of the lignin content in the vegetative plant parts of brown midrib and isogenes maize genotypes and gene effect studies,
- Investigation of the heterosis effect on the quantitative traits of the maize hybrids,
- Detailed analysis of the digestibility features of silages made of the bm_3 maize hybrids, sorghum and their mixed combinations;
- Investigation of amino acid and crude protein contents of traditional maize, sorghum and mixed (maize+sorghum) silages and the correlations of the amino acids;

❖ Breeding, morphological and phenometric characterization of sorghum lines and hybrids, description of the nutritional traits of the stalk juice,

❖ Description of the nutritional traits of the stalk juice in every phenophase and internode. Description of the average and median refractometric dry matter contents of the internodes, analysis of the alcohol yield,

❖ Cultivation of single cross sorghum and maize hybrids selected for bioethanol production with low input technology. Determination of heterosis and heterobeltiosis of starch, protein, and thousand kernel weight. Analysis of the correlation between the studied nutritional traits,

❖ Presentation of the mutation resulted in different generations of the grain sorghum experiment.

2. MATERIALS AND METHODS

2.1. Characteristics of the maize experimental system

The experimental work was carried out at the University of Debrecen Centre for Agricultural Sciences, Institute of Horticulture, Department of Genetics and the town of Kaba (Hungary) between 2008 and 2010. During the experiments, we conducted the studies listed in ***Table 1.***

Table 1. Experimental system of maize

Duration of experiment	Name of experiment	Place of experiment	Aim of experiment
15 Apr 2008−15 Oct 2008	Self-selected single-cross maize hybrids under low input circumstances	University of Debrecen Centre for Agricultural and Technical Sciences, Institute of Horticulture and Plant Biotechnology	Analysis of nutritional values essential in terms of bioethanol production in the grain
23 Apr 2008-11 Aug 2008	Field experiment of traditional maize, sorghum and mixed crop	Kaba (closed experimental area)	Comparison of silage samples on the basis of their amino acid contents
10 Oct 2009-20 Aug 2009	Maintaining and propagation experiment of brown midrib maize lines and their isogenes	University of Debrecen Centre for Agricultural and Technical Sciences, Institute of Horticulture	Comparison of plant parts of brown midrib and isogenic lines based on their detergent fibre contents Hybrid production
10 Apr 2010-15 Aug 2010	Field experiment on brown midrib and isogenic maize hybrids, traditional sorghum and their mixed crop	Kaba (closed experimental area)	Comparison of silage samples made of different genotypes based on their digestibility traits

2.2. Characteristics of the sorghum experimental system

The experimental work was carried out at the University of Debrecen Centre for Agricultural Sciences, Institute of Horticulture, Department of Genetics between 2005 and 2010. During the experiments, we conducted the studies listed in ***Table 2.***

2.3. Generation of the hybrids

The sorghum hybrids were produced according to the Comstock-Robinson (1952) II. factorial mating design. The male sterile female lines (A) were crossed with the restorer male lines (B) and we obtained male sterile female lines (A x B) which were crossing partners of the restorer male lines (R). The brown midrib single-cross maize hybrids and their inbred analogues were generated by crossing.

Table 2. Experimental system of sorghum

Duration of experiment	Name of experiment	Place of experiment	Aim of experiment
18 May 2005-15 Nov 2005 4 May 2006-20 Nov 2006 28 May 2007-25 Sept 2007	Three generation (M_0, M_1, M_2) mutation sorghum experiment (5.0; 7.5; 10.0; 12.5 Gy)	Centre of Agricultural Sciences, Department of Genetics and Plant Breeding	Detection of agronomically useful mutants (7.5-10.0 Gy) on the basis of the morpho-phenometric traits of the stock
18 Ápr 2007-10 Aug 2007	Descriptive and maintaining experiment of sorghum lines (preliminary experiment)	Centre of Agricultural Sciences, Department of Genetics and Plant Breeding	Selection of lines: for sweet sorghum hybrid production. Determination of breeding aims
2 May 2008-31 Aug 2008	Selection experiment of restorer sorghum lines	University of Debrecen Centre for Agricultural and Technical Sciences, Institute of Horticulture and Plant Biotechnology	The selection of lines with high degrees Brix for further studies out of the sweet sorghum lines
20 Sept 2008	Production of single-cross sorghum hybrids under low input circumstances	University of Debrecen Centre for Agricultural and Technical Sciences, Institute of Horticulture	Nutritional value analysis of the grains of sweet sorghum hybrids bred for bioethanol production. Determination of hybrid vigour regarding the qualitative traits.
14 Apr 2009-20 Sept 2009	Sampling experiment of restorer sorghum lines I.	University of Debrecen Centre for Agricultural and Technical Sciences, Institute of Horticulture	Refraction percentage studies of the stalk juices of the genotypes in every phenophase and internode. Comprehensive qualitative analysis of the stalk juice, Investigation of the alcohol content (FIRST YEAR)
27 Apr 2009-14 Sept 2009 .	Sampling experiment of single-cross sweet sorghum hybrids II.	University of Debrecen Centre for Agricultural and Technical Sciences, Institute of Horticulture	Determination of hybrid superiority based on the qualitative traits of the stalk juice and alcohol content (FIRST YEAR).
1 Apr 2010-24 Oct 2010	Sampling experiment of restorer sorghum lines I.	University of Debrecen Centre for Agricultural and Technical Sciences, Institute of Horticulture	Refraction percentage studies of the stalk juices of the genotypes in every phenophase and internode, Investigation of the alcohol content (SECOND YEAR)
1 Apr 2010-24 Oct 2010	Sampling experiment of single-cross sweet sorghum hybrids II.	University of Debrecen Centre for Agricultural and Technical Sciences, Institute of Horticulture	Determination of hybrid superiority based on the qualitative traits of the stalk juice and alcohol content (FIRST YEAR)

2.4. Maize breeding base materials

In the experimental field ('Bemutatókert') of the University of Debrecen Centre for Agricultural and Technical Sciences, we produced single-cross hybrids by crossings, using self-selected maize lines. The female and male lines of the maize hybrids containing the *bm_3* gene were provided by Oszkár Gyulavári maize breeder (***Table 3***).

Table 3. Maize hybrids and their parents

Maize hybrids	Female lines	Male lines
S_1 x S_4	S_1	S_4
S_1 x S_6	S_1	S_6
S_4 x S_6	S_4	S_6
S_5 x S_1	S_5	S_1
126 x S_1	126	S_1
GK49 izogén x GK42 izogén	GK49 izogén	GK42 izogén
GK49 bm_3 x GK42 bm_3	GK49 bm_3	GK42 bm_3
GK42 bm_3 x GK43 bm_3	GK42 bm_3	GK43 bm_3
GK42 izogén x GK43 izogén	GK42 izogén	GK43 izogén
SzeTC 465	(GK49 x GK59)	GK 57
SzeTC 465 bm_3	(GK49 bm_3 x GK59 bm_3)	GK 57 bm_3

2.5. Sorghum breeding base materials

The control sorghum hybrids were provided by the Cereal Research Non-Profit Ltd. (Szeged, Hungary) and Agroszemek Kft. The control sorghum hybrids were as follows: *Róna-4, Monori édes, Cellu édes, G1990.*

The silage maize and sorghum genotypes were co-cultivated in a 2:2 row order and the following combinations were generated: *SzeTC 465+Róna-4, SzeTC 465 bm_3+Róna-4, SzeTC 465+Monori édes, SzeTC 465 bm_3+Monori édes, 126 x S_1+Monori édes, Sze 521+Cellu édes.*

The restorer male lines (R) of our self-selected sorghum hybrids were ordered from Tápiószele, the Institute for Agrobotany (new name: Plant Diversity Center) and from the IPK (Institut für Pflanzengenetik und Kulturpflanzenforschung) Genebank of Gatersleben. The selected restorer male lines became as breeding base materials of the sweet sorghum hybrids suitable for bioethanol production (***Table 4***). The cytoplasmic male sterile female lines (A) and the maintainer male lines (B) were provided by János Berényi sorghum breeder from the Gardening and Agricultural Institute, Serbia.

The *Ria* and *Rib* grain sorghum lines, which were as breeding base materials in the mutation experiment and the *Zádor* grain sorghum hybrid were from the Research Institute of Karcag of the University of Debrecen Centre for Agricultural and Technical Sciences.

Table 4. The male sterile female lines, the restorer male lines and the sorghum hybrids

Genotype code	Sorghum genotype
Male sterile female lines (A)	
SL_1	Ria
SL_2	T x 3042 A= A_{17}
SL_3	T x 3118 A= A_{24}
SL_4	Wheatland A= A_{103}
SL_5	T x 398 A= A_{133}
Restorer male lines (B)	
CL_1	Rib
CL_2	Tx3042 B= B_{17}
CL_3	Tx3118 B= B_{24}
CL_4	Wheatland B= B_{103}
CL_5	Tx398 B= B_{133}
Three-cross sorghum hybrids (A x B) x R	
SL_1 x RL_3	Ria x Lady red
SL_1 x RL_4	Ria x Sorgho sücré
SL_1 x RL_7	Ria x Minnesota amber
SL_1 x RL_{10}	Ria x Odeskoee Rannee
SL_1 x RL_{12}	Ria x Early sumac
SL_1 x RL_{15}	Ria x 'Silosnoje 3'HUN
SL_2 x RL_3	A_{17}x Lady Red
SL_2 x RL_4	A_{17}x Sorgho sücré
SL_2 x RL_7	A_{17}x Minnesota amber
SL_2 x RL_9	A_{17}x Bulgarien
SL_2 x RL_{10}	A_{17}x Odeskoee Rannee
SL_2 x RL_{11}	A_{17} x Kubanskij Jantar
SL_2 x RL_{12}	A_{17} x Early sumac
SL_2 x RL_{15}	A_{17} x 'Silosnoje 3'HUN
SL_3 x RL_3	A_{24} x Lady Red
SL_3 x RL_4	A_{24} x Sorgho sücré
SL_3 x RL_7	A_{24} x Minnesota amber
SL_3 x RL_9	A_{24} x Bulgarien
SL_3 x RL_{10}	A_{24} x Odeskoee Rannee
SL_3 x RL_{11}	A_{24} x Kubanskij Jantar
SL_3 x RL_{12}	A_{24} x Early sumac
SL_3 x RL_{15}	A_{24} x 'Silosnoje 3'HUN
SL_3 x RL_{16}	A_{24} x 'Silosnoje 3'Sowj.
SL_3 x RL_{18}	A_{24} x Honey
SL_4 x RL_3	A_{103} x Lady red
SL_4 x RL_4	A_{103} x Sorgho sücré
SL_4 x RL_7	A_{103} x Minnesota amber
SL_4 x RL_{10}	A_{103} x Odeskoee Rannee
SL_4 x RL_{11}	A_{103} x Kubanskij Jantar
SL_4 x RL_{12}	A_{103} x Early sumac
SL_4 x RL_{15}	A_{103} x 'Silosnoje 3'HUN
SL_4 x RL_{16}	A_{103} x'Silosnoje 3'Sowj.
SL_4 x RL_{18}	A_{103} x Honey
SL_5 x RL_3	A_{133} x Lady Red
SL_5 x RL_4	A_{103} x Sorgho sücré
SL_5 x RL_7	A_{103} x Minnesota amber
SL_5 x RL_9	A_{133} x Bulgarien
SL_5 x RL_{10}	A_{133} x Odeskoee Rannee
SL_5 x RL_{11}	A_{133} x Kubanskij Jantar
SL_5 x RL_{12}	A_{133} x Early sumac
SL_5 x RL_{15}	A_{133} x 'Silosnoje 3 HUN'
SL_5 x RL_{16}	A_{133} x 'Silosnoje 3 'Sowj.
SL_5 x RL_{18}	A_{133} x Honey

2.6. Soil of the experimental areas

The experiments were conducted at the 'Bemutatókert' of the University of Debrecen Centre for Agricultural and Applied Economic Sciences and a farm in the town of Kaba, both chernozem soil. The soil of the 'Bemutatókert' was leached chernozem with a limeless upper layer. The humus layer was 50-70 cm in depth, medium category. The humus content of the soil was 2.57%. ***Table 5*** summarizes the results of the soil studies. As a result of the lack of lime, the upper layer may become cracky during a droughty season **(BÓDI, 2007)**.

Table 5. Soil conditions of the experimental area in the 'Bemutatókert' in the DE ATC

pH		$CaCO_3$	Y_1	Y_2	Al soluble		Humus %	Soil plasticity index according to Arany
H_2O	KCL				P_2O_5 mg/kg	K_2O mg/kg		
7.0	6.5	Ny.	5.0	8.0	100	165	2.57	42

The soil of the experimental area in Kaba was lime-coated chernozem. The soil was rich in lime and organic materials, its heat and air balances were excellent. Its humus content was 3.54%, while the soil plasticity index according to Arany was 43 (***Table 6***).

Table 6. Characteristics of the experimental area in Kaba

pH		$CaCO_3$	KCL soluble		Al-oldható		Humus %	Soil plasticity index according to Arany
H_2O	KCL		Nitrate//nitrite (mg/kg)	Sulphite/sulphate (mg/kg)	P_2O_5 (mg/kg)	K_2O (mg/kg)		
6.77	5.63	0.624	13.4	9.09	152	213	3.54	43

2.7. Growing area and stock density of the maize experiments

We have sown the 5 self-selected maize lines, the 5 single-cross hybrids, the three brown midrib lines and the 3 isogenic lines in the experimental field ('Bemutatókert') of the University of Debrecen Centre for Agricultural and Technical Sciences. The experiment was set into plots of 5 m long rows, two parallels per lines and hybrids. The row distance between the plants was 75 cm, the tiller distance was 20 cm, thus 25 plants were placed in one row. In duplicates, 50 maize/genotype were sown. The experiment was located in one field (32 rows), therefore a total of 800 plants were sown.

A small plot field experiment was set in Kaba, consisting of the brown midrib maize, sorghum and their mixed crop combinations. The plant material of the experiment was as follows: 3 brown midrib hybrids and their isogenic varieties, 3 sorghum hybrids and 2 traditional maize hybrids. A total of 6 mixed crop combinations were formed.

The experimental field was a rectangle of 105 m in length and 8.5 m in width, its size was 892.5 m^2. 4-4 plots were set in the case of the maize and sorghum hybrids, while 2 in the case of the mixed crops. The size of a plot was 9 m^2 (3 m x 3 m). One plot was consisted of 4 rows, each of 3 m in length. The row distance between the maize plants was 70 cm, while the tiller distance was 20 cm. In the case of maize, 5 plants were placed within a running metre. Into the plot of 9 m^2, 60 maize plants were sown.

The row distance between the silage sorghum hybrids was 70 cm, while the tiller distances were 5-6 cm. Therefore, 17-20 germs were within a running metre. Thus, a total of 204-240 sorghum germs were sown into a 9 m^2 plot.

2.8. Growing area and stock density of the sorghum experiments

For the crosses, we used 5 male sterile female and 5 maintainer male lines. Every female and male line was set in a 5 metre long row, in duplicates. The applied row distance was 70 cm, the tiller distances varied between 5 and 10 cm. 15-25 seeds were placed in a running metre, thus 75-125 seeds were sown in a 5 m long row.

A total of 10 restorer male lines were used in the experiment, in 4 parallels/line. We have applied 75 cm as row, while 5-6 cm as tiller distance, 17-20 germs were placed within a running metre, therefore a total of 85-100 germs were sown in a 5 m long row.

During the experiment, 43 three-line sorghum hybrids were bred and studied. During the setting of the field experiment, we worked in duplicates per hybrid, a total of 3 fields were sown. The row distance was 75 cm, the tiller distance was 10-15 cm after manual tiller number setting. One running metre contained 10-15 germs, 50-75 germs were sown in a 5 m long row.

2.9. Applied agrotechniques during the research

Each year, the autumn ploughing was carried out until the middle of November, in the depth of 32 cm. In spring, the tillage and the preparation of the seed beds with early spring harrowing were conducted with combination before sowing. The maize seeds were sown with handy seeder, while the sorghum seeds with a seedling-machine for small seeds.

During the experiments we applied N, P_2O_5 and K_2O artificial fertilizers in the doses of 100, 90 and 90 kg/ha, respectively.

The whole amounts of P and K and the 30% of the N were applied before the autumn ploughing; while the remaining part of the N in spring before combination. To protect the plant stock from weeds, chemical and mechanical weed clearing were performed. RAMROD FLOW (5.5 ha/l) was employed for the basic treatment spread pre-emergently against the monocotyledonous weeds, while for the re-treatment BASAGRAN FORTE was used at the dosage of 1.5/l against the dicotyledonous ones.

2.10. The process of harvesting and the method of samplings

The harvesting and sampling of the sorghum experiments are illustrated by ***Table** 7*.

***Table** 7.* Demonstration of harvesting and sampling of sorghum experiments

Breeding aims of the experiment	Mutation sorghum experiment	Single-cross sorghum hybrids bred for bioethanol production	Qualitative analysis of restorer sorghum lines	Qualitative analysis of three-line sorghum hybrids
Genotypes	Rib, Zádor (5;7,5;10,0;12,5 Gy)	SL_1 x RL_4, SL_1 x RL_7, SL_1 x RL_{10}, SL_1 x RL_{12}	RL_3, RL_4, RL_7, RL_9, RL_{10}, RL_{11}, RL_{12}, RL_{15}, RL_{16}, RL_{18}	Table 4.
Time of sampling	7 Jun 2005 7 July 2005 7 Aug 2005		flow: 14 July 2009 20 July 2010	flow: 22 July 2009 20 July 2010
Time of harvesting	15 Nov 2005 20 Nov 2006 25 Sept 2007	full. mat. 20 Sept 2008	milky mat: 27 July 2009 20 July 2010	milky mat: 7 Aug 2009 18 Aug 2010
			waxy mat:7 Aug 2009 31 July 2010	waxy mat:20 Aug 2009 3 Sept 2009 pressing 20 Sept 2010
			full mat: 3 Sept 2009 29 Sept 2010	full mat:14 Sept 2009 8 Oct 2010
			over mat:20 Sept 2009 24 Oct 2010	over mat:14 Sept 2009 24 Oct 2010
Process of harvesting	Manual (snipping of panicle)		Cutting off the stalk by manual stalk-cutter	
Sampling, Sample preparation	Drying of the panicle at 40 °C, threshing of the grains with spike-thresher		Removal of leaves with cane-cutter 1. Chopping of the stalk with chaff-cutter 2. Pressing of the chaff (grape crusher, cylindrical stalk press) 3. Freezing of stalk juice 4. Fermentation 5. Determination of the alcohol content	
Names of studies	Leaf area index	Protein	Refractometric dry matter	
	Plant height measurement	Starch	Total sugar content	
	Measurement of panicle parameters	Kernel weight	Reducing sugar content	
Methods of studies	Morpho-phenometric description based on the descriptor list	NIR equipment (fast study)	Refractometric method Luff-Schrool-method Picnometric method	

2.11. Methods for the determination of digestibility characteristics

2.11.1. Determination of the raw protein content

For such purposes the macro-Kjeldahl method (MSZ EN ISO 5983:1:2005) is applied during which the N content is determined by a Kjel-Foss equipment. The received N content value is multiplied by 6.25 to get the raw protein content of the plant sample.

2.11.2. Determination of the raw fibre content

The raw fibre content of the samples was determined by FIBERTEC equipment according to the standard MSZ EN ISO 6865:2001.

2.12. Detection of the detergent fibre fractions

Neutral detergent fibre (NDF) is the material that cannot be dissolved in the presence of detergents under neutral conditions. The cell wall materials consist of this category. Hemicelluloses are removed by the next solution of more acidic pH; the remainder is the acid detergent fibre (ADF). Cellulose is dissolved in strong acid; the remainder part is the acid detergent lignin (ADL)**(KOTA, 2000)**.

2.13. Determination of the amino acid content

The amino acid content of the silages was determined at the Department of Animal Breeding of Kaposvár University, Hungary. The analysis of the forage samples were conducted according to the Hungarian Forage codex II./1. 1990.

2.14. Determination of the refractometric dry matter content

The dry matter content of the stalk juice was determined by a Falco-6 handy refractometer.

2.15. Determination of the sugar content

The sugar content was determined at the Equipment Centre and the Department of Animal Breeding of Kaposvár University according to the standard MSZ 6830-26 by the Luff-Schoorl method.

2.16. Determination of the alcohol content

The alcohol content of the bioethanol was measured by picnometer according to the MSZ 9589/2 standard.

2.17. The heterosis effect study

The levels of heterosis for starch content, protein content and thousand kernel weight were determined in the cases of the sorghum and maize hybrids. In the cases of the brown midrib maize hybrids the levels of heterosis were determined for plant height, lower ear attachment height, ear length and ear weight. Two heterosis types were calculated, average heterosis and heterobeltiosis with their absolute and percentage values.

Heterosis and heterobeltiosis can be calculated with the following two formulas:

$$\text{Heterosis} = \frac{\left[F1 - \frac{P1 + P2}{2}\right]}{\frac{P1 + P2}{2}} * 100$$

F1 = average of the studied trait of the hybrid,

P1 = average of the studied trait of one of the parents,

P2 = average of the studied trait of the other parent,

$$\text{Heterobeltiosis} = \frac{[F1 - HP]}{HP} * 100$$

HP = average of the highest parental performance

2.18. Evaluation of the results with mathematical-statistical methods

The results were evaluated using the SPSS 13.0 for Windows statistical software package. The effect of different treatments on the different nutritional traits was analyzed with monofactorial variance analysis. The connection between the quality factors were revealed by Pearson's correlation coefficient. In the case of strong correlation a regression line was drawn through the scattered points. For the comparison of the brown midrib maize hybrids and their isogenes t-probe was applied.

3. RESULTS

3.1. Morphological and phenometric characteristics of the brown midrib maize genotypes and their isogenes and the correlations between the factors

Out of the studied hybrids, the leaf-blades of the *GK42 isogenic x GK43 isogenic* and the GK42 *bm_3 x GK43 bm_3* hybrids enclosed low angle with the stalk. The angles enclosed by the leaf-blades and stalks of the SzeTC *465* and the *SzeTC 465 bm_3* maize hybrids were "high". Based on the position of their leaf-blades, the latter two hybrids were classified as "highly curved". The grains of the brown midrib maize hybrids were "flat", while those of their isogenic varieties were classified as "dent".

We have found very strong positive relationship between the internode weights and diameters of *GK49 bm_3 x GK42 bm_3* and *SzeTC 465 bm_3*: r=0.914** and r=0.916**, respectively. The correlation is also significant, **0.01.

3.2. Heterosis effect studies on the phenometric characteristics of the single-cross brown midrib maize genotypes and their isogenes

Among our self-selected single-cross maize hybrids, the highest average heterosis (67.25%) in plant height was achieved by *GK49 bm_3 x GK42 bm_3*, and also the superiority of the hybrid is clearly visible compared to the better inbred line (58.15%). In the case of ear height, the highest hybrid vigour (113.50%) was calculated in the case of the F_1 generation exceeded the average of the better parent by 93.06% (***Table 8***).

Table 8. Study of the heterosis effect of the plant heights and ear attachment heights of the brown midrib maize hybrids (Debrecen, 2010)

Hybrids	Heterosis value (plant height)				Heterosis value (ear attachment height)			
	Average heterosis		Heterobeltiosis		Average heterosis		Heterobeltiosis	
	%-os	absolute	%-os	absolute	%-os	absolute	%-os	absolute
GK49 izogén x GK42 izogén	**55.16**	**7.75**	**46.30**	**69.00**	**113.50**	**39.34**	**93.06**	**35.67**
GK49 bm_3 x GK42 bm_3	**67.25**	**89.67**	**58.15**	**82.00**	**76.60**	**20.17**	**43.82**	**14.17**
GK42 izogén x GK43 izogén	**29.38**	**49.66**	**15.69**	**29.66**	**39.24**	**15.5**	**14.58**	**7.00**
GK42 bm_3 x GK43 bm_3	**15.20**	**25.34**	-0.17	-0.33	**31.71**	**12.84**	**9.59**	**4.67**
SzeTC 465	**68.12**	**101.16**	**66.44**	**99.66**	**50.58**	**27.21**	**33.77**	**20.45**
SzeTC 465 bm_3	**38.87**	**58.51**	**30.29**	**48.60**	**29.39**	**15.22**	**20.50**	**11.40**

The highest heterosis values for ear length and ear weight were calculated in the case of the *GK49 isogenic x GK42 isogenic*. The average heterosis of ear length was 58.78%, while heterobeltiosis was 57.20%. The ear weight of the hybrid exceeded the average of the parents by 423.79%, while a 390.62% hybrid vigour was observed compared to the average of the better parent (***Table 9***).

Table 9. Study of the heterosis effect of the ear lengths and ear weights of the brown midrib maize hybrids (Debrecen, 2010)

Hybrids	Heterosis value (ear length)				Heterosis value (ear weight)			
	Average heterosis		Heterobeltiosis		Average heterosis		Heterobeltiosis	
	%-os	absolute	%-os	absolute	%-os	absolute	%-os	absolute
GK49 izogén x GK42 izogén	**58.78**	**5.82**	**57.20**	**5.72**	**423.79**	**109.72**	**390.62**	**107.97**
GK49 bm_3 x GK42 bm_3	**40.55**	**4.42**	**40.55**	**3.82**	**92.56**	**34.74**	**71.58**	**30.15**
GK42 izogén x GK43 izogén	**34.27**	**3.90**	**17.90**	**2.32**	**194.21**	**70.89**	**119.79**	**58.53**
GK42 bm_3 x GK43 bm_3	**28.50**	**3.13**	**22.69**	**2.61**	**126.75**	**54.44**	**83.89**	**44.43**
SzeTC 465	-24.76	-5.59	-4.76	-5.59	**187.52**	**85.40**	**106.36**	**67.49**
SzeTC 465 bm_3	**24.90**	**3.23**	**22.26**	**2.95**	**105.61**	**54.54**	**103.95**	**54.12**

3.3. Investigation of the detergent fibre content of the plant parts of brown midrib maize

The lignin content of the stalk of *GK49* bm_3 was lower (3.73%) than the ADL content of the *GK49 isogenic* variety (5.30%). Among all lines, the ADL content of the ear of the *GK43* bm_3 was the lowest (3.06%), while the ADL content of the *GK49 isogenic* variety was 3.94%.

The acid detergent fibre content of the ear of *GK43* bm_3 was 28.52%, which was lower than the ADF content of the *GK43 isogenic* (31.33%). The average ADF content of the *SzeTC 465* bm_3 maize hybrid was 29.64%, while that of the *SzeTC 465* hybrid was 32.99%.

Among the lines, the lowest average neutral detergent fibre content was measured in the case of *GK43* bm_3 (42.50%), while the isogenic variety of the line performed higher average NDF content (45.84%) than the brown midrib variety. The NDF content of the chaff of the *SzeTC 465* bm_3 hybrid was lower (49.96%) than that of the *SzeTC 465* hybrid (57.61%).

During the correlation analysis of the acid detergent and neutral detergent fibre contents of the chaffs, very strong positive significant relations were found in the following lines: *GK43 isogenic* (r=0.959**), *GK49 isogenic* (r=0.914*), and *GK49* bm_3 (r=0.979**).

3.4. Digestibility studies on the silage samples of brown midrib and isogenic maize hybrids, traditional sorghum hybrids and their mixed crops

We have measured 9.33% as the crude protein content of the *GK49 bm_3 x GK42 bm_3* maize hybrids, while the average crude protein content of the *GK49 isogenic x GK42 isogenic* hybrid was 8.14%.

The fibre content and the detergent fibre components of the *GK49 bm_3 x GK42 bm_3* maize hybrids performed lower percentage ratio than those of its *isogenic* variety. We have measured 19.71% as the crude fibre content of the *GK49 bm_3 x GK42 bm_3* hybrid, while the NDF of the hybrid was 46.39%, ADF content was 26.54%, and ADL content was 6.00%. The lowest crude fibre (17.63%) and detergent fibre components (ADF: 22.07%, ADL: 5.03%) were found in the case of the *SzeTC 465 bm_3* hybrid among the studied maize hybrids.

Among the mixed crops, favourable nutritional parameters in terms of digestibility were found in the *Róna+SzeTC 465 bm_3* combination: higher crude protein content (7.98%) and lower crude fibre (22.75%), NDF (48.41%), ADF (26.32%) and ADL contents (5.45%).

We have experienced very strong positive significant correlation between the neutral detergent and acid detergent fibre contents in the isogenic maize (r=0.934**) and brown midrib maize (r=0.986 *) hybrids.

3.5. Analysis of the amino acid contents of the silage samples

Regarding the essential amino acids, the *Sze 521* maize hybrid was the richest in methionine (2.37 g amino acid/100 g protein). The highest lysine contents were achieved by the *SzeTC 465* and the *126 x S_1* maize hybrids (4.84 g /100 g protein).

In the case of the sorghum silages, the lysine content of the *G1990* sample was the highest (4.93 g/100 g protein). *Monori édes* sorghum performed 2.18 g/100 g protein methionine content (***Figure 1***).

In the case of the silage samples of the mixed crops, among the essential amino acids, the lysine content of the *126 x S_1+Monori édes* mixed was 4.89 g/100 g protein. The methionine content of the *126 x S_1+Monori édes* mixed silage was 2.06 g/100 g protein.

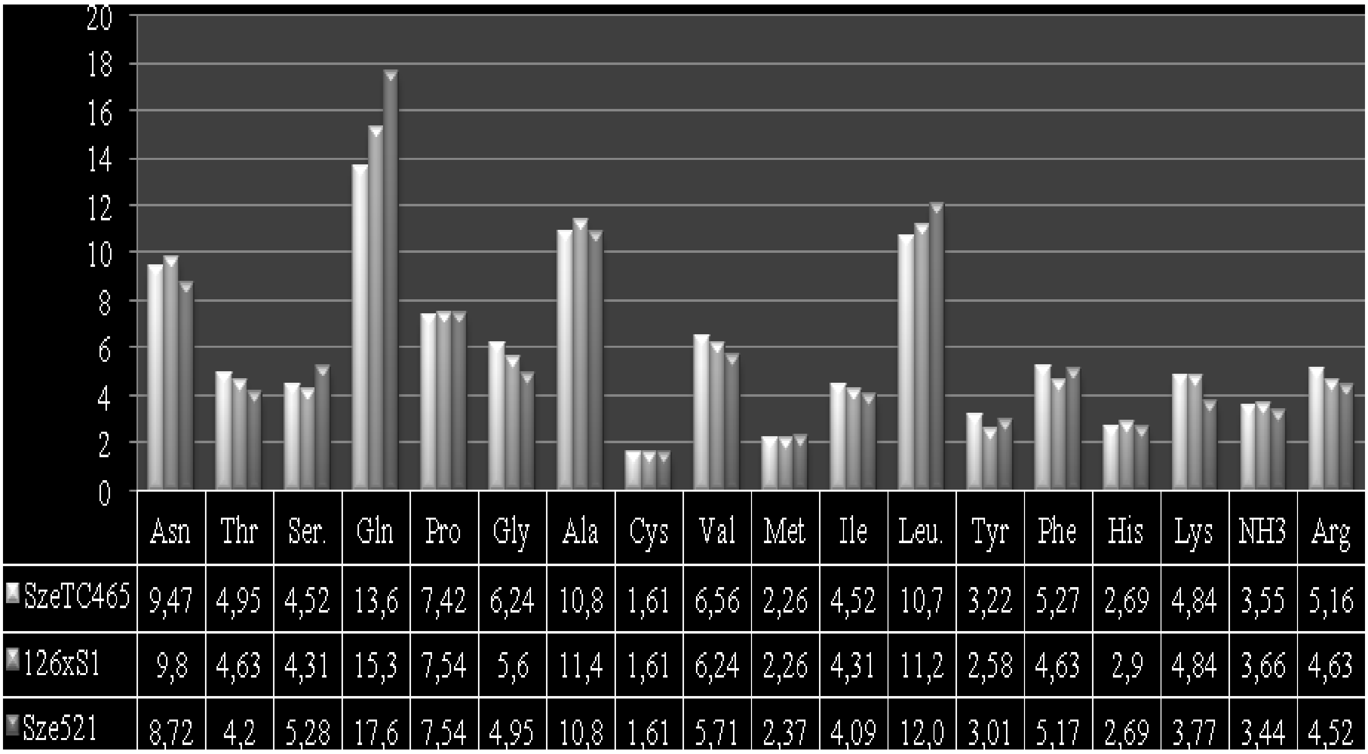

Figure 1. Amino acid content values of the maize silages (g AA/100 g protein)
(Kaba, 6 Aug 2008)

3.6. Heterosis values calculated for the nutritional traits of maize grains

Considering the starch contents of the hybrids, the relative values of the average heterosis varied between 0.89 and 6.46%. The average of the parents was exceeded by the S_4 x S_6 hybrid by 6.46%. The starch content of the hybrid was 71.33%, while those of the S_4 and S_6 lines were 69.63% and 64.37%, respectively. It exceeded the starch content of the S_4 line by 2.44%, which means 1.70%, expressed in absolute heterosis.

Regarding thousand kernel weight, the range of the average heterosis was between 23.8 The hybr respectively.

9.47	4.95	4.52	13.6	7.42	6.24	10.8	1.61	6.56	2.26	4.52	10.7	3.22	5.27	2.69	4.84	3.55	5.16
9.80	4.63	4.31	15.3	7.54	5.60	11.4	1.61	6.24	2.26	4.31	11.2	2.58	4.63	2.90	4.84	3.66	4.63
8.72	4.20	5.28	17.6	7.54	4.95	10.8	1.61	5.71	2.37	4.09	12.0	3.01	5.17	2.69	3.77	3.44	4.52

3.7. Description of the quantitative traits of the sorghum genotypes and correlation studies on the features of the internodes

The average stalk weight of the RL_{15} line (48.27 g) was higher than that of the RL_{16} line (44.08 g). In the case of both studied plants, the internode weights increased up to the fifth internode, as follows: RL_{16}: (23.60-53.53 g) and $RL_{15:}$ (22.10-63.66 g).

The average stalk weight (46.93 g), length (24.28 cm) and diameter (1.48 cm) of SL_2 x RL_{12} were higher than those of the SL_1 x RL_{12}. The internode weights of both hybrids increased up to the fourth internode.

We have found strong positive significant difference between the internode weight and diameter values in the cases of the RL_{16} line (r=0.836**) and the SL_2 x RL_{12} hybrid (r=0.768**).

3.8. The average values and medians of the refraction percentages in the different phenophases of the restorer sorghum lines

Among the studied lines, RL_9 and RL_{18} were of long vegetative periods. Their stalks were thick, and they produced higher amounts of juices and possessed lower refractometric dry material contents – RL_9: 15.88% in 2009, RL_{18}: 17.01% in 2010 – than the lines of shorter vegetation periods and higher sugar contents (8-internodal period), RL_{12}: (22.35% in 2009) in yellow ripeness. Lines of shorter vegetation times belonged to the groups of 7 and 8 internodes. The refraction percentage values of the RL_{16} line were higher during the vegetation period (7.01-18.76% in 2009 and 6.57-18.02% in 2010) than those of the RL_{10} line (6.90-16.77% in 2009 and 4.85-18.02% in 2010). RL_4 és az RL_{11} can be considered as lines of very short vegetation periods, applicable for syrup production.

With the help of the determination of the medians of the refraction percentage, we had the possibility to compare the sorghum lines possessing different internode numbers. We have determined that there were significant differences between the refractometric dry material contents of the studied sorghum lines in both years and in every phenophase. Among the lines of 8 internodes, the highest refractometric sugar content median was measured in the case of the RL_{12} line (10.32-21.11%) from flowering to yellow ripeness, both in 2009 and 2010 (10.61-22.50%). Among the lines of 11 internodes, the median of the refraction percentage of the RL_9 line increased from flowering to yellow ripeness (7.12 to 16.33%) and performed higher value in 2009 than in 2010.

3.9. The average values and medians of the refraction percentages in the different phenophases of the sweet sorghum hybrids

The *SL_1 x RL_{12}* hybrid of early ripening had a 12.36% average refraction value at milky ripeness, while the late ripening hybrid *SL_5 x RL_{10}* performed the highest degrees Brix (14.66%) during this period among the 8-internodal hybrids. The refraction percentage of the control 8-internodal hybrid *Róna-4* was exceeded by our self-selected genotypes in milky ripeness in 2010: *SL_1 x RL_{12}* (20.95%), *SL_5 x RL_{10}* (19.79%) and *SL_2 x RL_{11}* (19.41%).

Based on the medians of the refraction percentages of our self-selected hybrids, we have compared the plants belonging to different internodal groups. The *SL_5 x RL_{10}* hybrid was of thick stalk, late vegetation period, whose sugar contents – except of milky ripeness (14.30% in 2009 and 15.00% in 2010) – performed lower refactometric sugar contents in each phenophase.

The lower refractometric sugar content is related to higher juice yield. The *SL_1 x RL_{12}* hybrid performed linearly increasing sugar accumulation from flowering to yellow ripeness (10.77-19.00% in 2009; 5.21-21.33% in 2010). The Brix percentages regarding No. 5 gradually increased from the beginning of the vegetation period to yellow ripeness.

Among the hybrids the more favourable ones – in terms of refraction percentage – were *SL_2 x RL_7* and *SL_5 x RL_3*. The median of the degree Brix of the *SL_2 x RL_7* hybrid was higher in 2010 than in 2009. During flowering, we have measured 9.66% refractometric sugar in the fifth internode, which increased to 12.52 to milky ripeness in 2009.

3.10. The tendencies of the average refraction percentages of the sorghum genotypes in the case of different internodes

We have observed that the refraction percentages increased from the first to the fourth and fifth internodes in both years. A linear increase could also be observed from the first to the seventh internode (10.73-14.26%) during wax-ripening in 2010. The refractometric sugar content of the fourth, medium internode performed 7.83% during flowering. Approaching to the phenophase of wax-ripening, we have measured 16.83%, which increased to 20.00% to dead ripeness in 2009.

We have measured the highest sugar content in the fourth internode of *SL_2 x RL_{12}* during milky ripeness (11.76%), which increased to 19.00% to the yellow ripeness in the first experimental year.

In milky ripeness, the highest sugar content was measured in the stalk juice of the fifth internode (12.66%) in 2010. During wax-ripening, also the sugar content of the fifth internode performed the highest value (15.00%) in 2010 (***Table 10***).

Table 10. The values of the averages of the refractometric dry material contents in the internodes of the ***SL_2 x RL_{12}*** 9-internodal sorghum hybrid (Debrecen, 2009-2010)

Hybrid	I.n.	Flowering		Milky mature		Waxy mature		Full mature	
	s.sz.	2009	2010	2009	2010	2009	2010	2009	2010
SL_2 x RL_{12}	1.	3.33	5.66	9.60	11.10	13.27	11.33	16.83	21.50
	2.	3.40	5.66	9.43	12.23	13.50	12.60	17.83	20.83
	3.	3.57	6.33	11.33	12.00	13.44	13.60	18.83	**21.66**
	4.	3.99	6.00	**11.76**	12.33	13.97	**15.53**	**19.00**	21.50
	5.	3.99	6.50	10.93	**12.66**	**15.00**	15.40	18.41	21.22
	6.	4.33	**6.66**	11.33	12.43	14.33	14.33	18.57	20.83
	7.	4.66	6.33	11.00	11.33	14.05	13.66	18.40	20.66
	8.	5.16	6.16	10.66	11.93	14.00	14.33	17.00	20.16
	9.	**5.66**	6.00	11.00	11.66	14.26	13.00	15.50	18.00
average		**4.23**	**6.14**	**10.78**	**11.96**	**13.96**	**13.75**	**17.82**	**20.70**
$SD_{5\%}$		**1.18**	0.75 (ns)	1.72 (ns)	1.70 (ns)	1.93 (ns)	**1.91**	3.01 (ns)	**2.11**

Abbreviations: In. No.: number of internodes

3.11. The main components of the stalk juice contents of the restorer sorghum lines and the tendencies in the nutritional values

In the case of lines of early ripening, increasing refractometric sugar contents could be detected from milky ripeness to yellow ripeness, e.g. in line RL_{11}. The refraction percentage of the line RL_{11} gradually increased from 8.68% to 18.33%. The restorer lines of late vegetation period reached their highest degrees Brix during wax-ripening, which were the following: 15.00% in RL_9 and 16.66% in RL_{18}. These values decreased to 14.66 % in RL_9 and to 14.17% in RL_{18} to milky ripeness.

In the case of RL_{11}, the increase of the total sugar content was observed from milky ripeness to yellow ripeness (6.60-14.26%). The other lines reached their maximum values during wax-ripening, the range of which was the following: 10.07-17.50%.

The highest reducing sugar contents were detected during wax-ripening, whose range was the following: 0.55-4.11%, the values decreased to yellow ripeness to 0.76-2.71%. The highest reducing sugar content in the case of RL_{18} was 4.11% during wax-ripening, this value decreased to 2.23% to yellow ripeness.

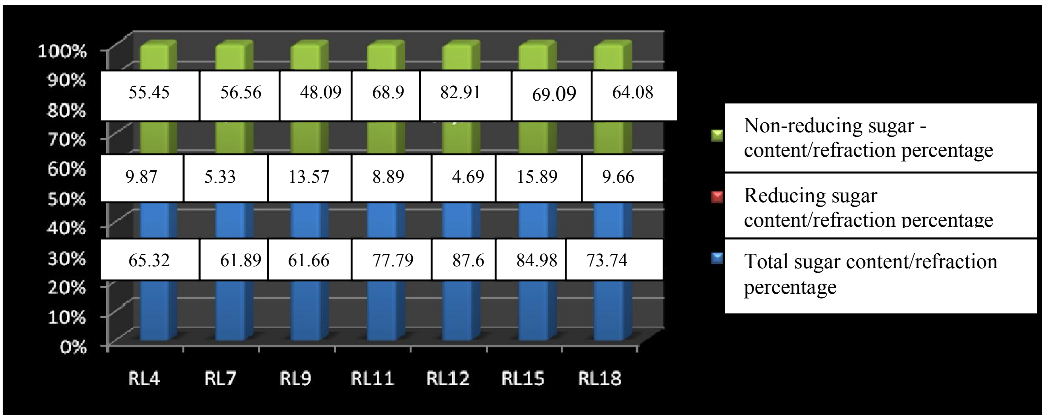

Figure 2. The values of the nutritional parameters in the case of the examined restorer sorghum lines during yellow maturation

We have determined that among the dry materials, the range of the total sugar content was 61.66-87.60% in refraction percentage during yellow ripeness, within which the reducing sugar varied between 4.68 and 15.89%. The highest ratio was experienced in the line RL_{15}. The reducing sugar contents of the late ripening lines performed 9.66% (RL_{18}) and 13.57%-ot (RL_9) within the dry material content of the stalk juice (***Figure 2***).

3.12. The main components of the stalk juice contents of the sorghum hybrids and the nutritional values

On the basis of the refraction percentage, the values of the hybrids qualified as "good" varied between 17.05 and 18.33%. The lowest Brix values were performed by SL_5 x RL_9. The highest values were experienced in the case of the SL_2 x RL_{11} hybrid. Based on the degrees Brix, the SL_5 x RL_{10} (14.16%), the SL_3 x RL_7 (15.83%) and the SL_2 x RL_{15} (16.00%) hybrids were classified as "medium".

The end values of the total sugar contents of the examined genotypes varied between 3.93 and 11.81%. The lowest total sugar content was measured in the parenchyma cells of the SL_3 x RL_4 hybrid. The highest total sugar was experienced in the stalk juice of SL_2 x RL_{12}. We have experienced hybrid vigour in the case of the reducing sugar contents of the stalk juices of the SL_3 x RL_7 (2.10%), the SL_5 x RL_9 (3.25%), the SL_3 x RL_{15} (4.05%), and the SL_3 x RL_4 (1.87%) hybrids. Their reducing sugar contents were higher than that of the control. The total sugar content is the 43.37-87.70% of the refraction percentages of the hybrids. The lowest relative ratio was found in the SL_2 x RL_{11} hybrid, while the highest one in the case of the SL_4 x RL_{10} hybrid.

The relative ratios of the reducing sugar content and the refraction percentage varied between 2.39 and 32.03%. The highest reducing sugar/refraction percentage ratio was performed by the SL_4 x RL_{10} hybrid.

3.13. Analysis of the alcohol yield of the sorghum genotypes in the waxy mature

The alcohol contents of the SL_1-SL_4 hybrids varied between 2.93 and 6.23 V/V. The highest alcohol yields were achieved by the SL_4 hybrid group. The average alcohol content of the hybrid groups was 4.8 V/V in 2009. During the second experimental year, we compared the alcohol yields of the hybrid and line groups. The hybrid groups had identical alcohol contents (2.45 V/V). The restorer lines performed lower alcohol contents (2.38 V/V) than the hybrids.

3.14. Nutritional values of the sorghum grains

In the case of every hybrid, we have experienced positive average and absolute heterosis for the starch content. The relative heterosis values varied between 0.49 and 21.7%. The hybrid that performed the highest starch content (62.63%) was SL_1xRL_{12}, the relative heterosis was calculated as 1.9%, while the absolute heterosis as 1.17%.

Calculated for thousand kernel weight, the average heterosis and heterobeltiosis were positive in the case of two hybrids. The thousand kernel weight of the SL_1xRL_{12} hybrid was 32.7 g, the relative average heterosis was 29.09%, while the absolute 7.37%. It exceeded the average of the better parent by 6.65%, and with 2.04% calculated for absolute heterobeltiosis.

3.15. Results of the mutation breeding experiments

Agronomically beneficial mutants were found at 7.5 and 10 Gy irradiation doses; because of the rudimentary shoots developed highly productive panicles, the leaf area indices as well as the Harvest index and the grains increased. The most pronounced change was observed for 12.5 Gy *Zádor* with the appearance of twin panicles. The callus induced from the albino seedlings with chlorophyll deficiency experienced in the M_2 generation began to grow. In the M_1 generation irradiated with 12.5 Gy agronomically unfavourable mutants were obtained. As a consequence of the sterile panicles - unproductive panicle development -, the Harvest index was low.

4. NEW AND NOVEL SCIENTIFIC RESULTS

The following results are considered as new scientific results of our work:

1. We have found relationship between the following qualitative traits:

- internode weight and diameter: *GK 49* bm_3 *x GK42* bm_3 (r=0.914**); SL_1 *x* RL_{12} (r=0.801**), RL_{16} (r=0.836**);
- brown midrib maize silage: dry material content and NDF (r=-0.926**),
- silage (maize, sorghum, mixed) amino acids: LYS-ILE (r=0.78*); MET-ALA (r=0.80*); GLN-LEU (r=0.90**).
- Chaff of the line *GK 43* bm_3: ADL-NDF (r=0.957*),
- Ear of the line *GK 49* bm_3: ADL-ADF (r=0.943*) and chaff: NDF-ADF (r=0.979*);
- total and non-reducing sugar content: sorghum lines (r=0.954**), sorghum hybrids (r=0.856**).

   Such correlations have never been published in domestic or foreign literature. We have found strong positive significant correlation between the factors influencing the digestibility of the silage. These are as follows: crude fibre and ADL (r=0.923**); NDF and ADF (r=0.986*).

2. For the traits essential in terms of bioethanol production, starch content and thousand kernel weight, positive heterosis and heterobeltiosis were experienced in the case of every single-cross maize hybrids. In the case of the sorghum hybrids, positive heterosis was observed for the starch content and the bioalcohol yield.
3. We have compared the studied sorghum genotypes in terms of refractometric dry material during the whole vegetation period. The genotypes best adequate for the breeding aims are the following:

   by internodal groups:

   8-internodal: RL_{12} (r.f.%$_{2009}$=10.33-22.35%, r.f.%$_{2010}$=10.69-20.95%),

   SL_5 *x* RL_{10} (r.f.%$_{2009}$=10.17-17.27%,r.f.%$_{2010}$=12.15-9.79%);

4. We have determined the percent ratios of the individual qualitative traits of the stalk juice. Based on the obtained data, we have evaluated the studied sorghum genotypes. The RL_{12} (*Early sumac*) sorghum restorer line and the $SL_2 \, x \, RL_{12}$ hybrid proved to be the most favourable among the studied genotypes in yellow ripeness.

   Value of RL_{12} Brix scale: 20.66%, total sugar content: 18.10%, reducing sugar content: 0.97%, total sugar content/refraction percentage: 87.6%, reducing sugar content/refraction percentage: 4.69%, reducing sugar content/total sugar content: 5.36%,

   Value of $SL_2 \, x \, RL_{12}$ Brix scale: (17.41%), total sugar content: (11.81%), reducing sugar content: (0.65%), total sugar content/refraction percentage: (67.83%), reducing sugar content/refraction percentage: (3.73%), reducing sugar content/total sugar content: (94.5%).
5. Among the *Zádor* grain sorghum population, agronomically useful mutants were found in the stock treated with 7.5 and 10 Gy irradiation doses (productive tillering, increasing leaf area index and thousand kernel weight). The thousand kernel weight in the stock of *Zádor* treated with 12.5 Gy (32.98 g) was higher than that of the stock treated with the irradiaton dose of 5 Gy (27.97 g).

5. RESULTS UTILIZABLE IN PRACTICE

1. During our experiments, we have conducted detailed analyses on silage maize hybrids, among them two, self-selected brown midrib ones (*GK49 bm_3 x GK42 bm_3, GK42 bm_3 x GK43 bm_3*), which were considered as energy saving. The determination of the morpho-phenometric traits were performed in favour of the prognosis of the hybrid effect and the selection of the crossing partner.
2. We have determined the detergent fibre contents (among the dry materials) of the different plant parts (leaf, ear, stalk, chaff) and compared the brown midrib and isogenic maize genotypes based on the fibre fractions. The ADL contents of the A *GK49 bm_3* ear, stalk and chaff were considerably lower than those of the *GK49 isogenic* lines. The ADF and NDF contents of the *SzeTC 465 bm_3* chaff were also significantly lower than those of the *SzeTC 465*. The bm_3 gene made significant effect on the genotype.
3. We have selected the features determining the quality of the silage (maize, sorghum, mixed). The optimal dry material content at harvesting, the high crude protein content (7.58-9.33%), the low crude fibre content (17.63-27.43%), the low detergent fibre fractions as NDF (40.05-53,01%), ADF (22.07-30.12%), and ADL (5.03-6.72%) are favourable.
4. On the basis of our results, we have characterized the studied hybrids. The lowest fibre fractions were experienced in the case of the *SzeTC 465 bm_3* hybrid, which were the following: crude fibre (17.63%) and detergent fibre components (ADF: 22.07%, ADL: 5.03%). Among the mixed crops, the most favourable digestibility traits were found in the case of the *Róna+SzeTC 465 bm_3* combination, which were as follows: higher crude protein content (7.98%), lower crude fibre (22.75%) and NDF (48.41%).
5. Among the studied maize hybrids, we have detected the highest amino acid contents in the silage samples of *Sze 521* and *126 x S_1* (lysine: 4.84 g/100 g protein; methionine 2.26 g/100 g protein; cysteine: 1.61 g/100 g protein). The highest methionine content was found in the case of the hybrid *Monori édes* (2.18 g/100 g protein).
6. We have selected the parameters determining the quality of the sorghum stalk juice. In terms of ethanol yield, the high refraction percentage, the high total sugar content and the low reducing sugar content are important.

6. PUBLICATIONS RELATED TO THE DISSERTATION

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