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**Major agricultural factors influencing maize kernel  
contents as a raw material of bioethanol production**

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## INTRODUCTION

*“By the time a problem becomes obvious for everyone, it is often impossible to solve”*

*(Meadows)*

It is commonly known that the population of the Earth reached 6.2 billion people in 2000. If the demographic rate does not change, some estimations prognose 1 billion people more by 2015, resulting in 7.2 billion people total, while this number could even reach 8.7 billion by 2050, even though the growth on our planet is limited, such as the planet itself. This rather intensive growth will have further impact on the amount of extracted fossile energy resources which are already about to get exhausted. Nowadays, 80% of energy comes from burning the coal, oil and gas stocks. The EU forecast prepared in 2007 estimates that hard coal will be enough for 155 years, crude oil for 42 years and natural gas for 64 years based on similar usage tendencies. Therefore, this calculation does not take into account the fact that the extraction and usage rate of conventional energy resources will increase even faster due to the rapidly growing population, thereby causing unpredictable damages to the environment. Based on our current knowledge, it is impossible to tell how much and for how long the environment is able to withstand the increasing load as a result of growth. The extractable stocks can be temporarily increased by the improvement of extraction efficiency and the exploitation of newly discovered quarries, but it is for sure that significant changes need to take place in the field of energy production in our age, which means that we have to find some other, energy resource which does not harm the environment and is able to replenish the fossile energy resources which are harmful to the environment and are about to get exhausted.

It is generally known that bioethanol use contributes to the prevention of harmful Green House Gases (GHG) originating from the transport sector from getting emitted into the environment. This conclusion was also reinforced at the RIO+20 congress for sustainable development arranged in Brazil in June 2012, thereby encouraging the member states in this field. According to the research results of the University of Nebraska in the USA carried out in 2009 and acknowledged by the “Environmental Protection Agency” (EPA), maize-based bioethanol use reduces GHG production by 21-52% in comparison with petrol. Another problem also closely linked to the issue of energy supply and energy safety is the trap of energy dependence. The majority of EU member states (including Hungary) are in a similar situation as regards their level of energy supply. Since we do not have any significant energy stocks, we are forced to import energy resources. According to a survey, we imported 80% oil, 57% natural gas and 40% coal (in comparison with the amount we used from these

resources) in 2007. Regrettably, the survey represents the fact that the energy dependence of the EU is already extremely high, surrendering itself to the increasingly frequent and unfavourable price fluctuations. Based on the forecast of this report, these quotas will show a frightening energy dependence by 2030, as the predicted import values are 93% for oil, 84% for natural gas and 89% for coal. In 2009, Hungary and the neighbouring countries could experience what it is like to have restricted natural gas supply due to an unsettled account (the Russian – Ukrainian “gas debate”). In this particular case, the gas supply was temporarily cut, endangering the gas supply of several European countries in the middle of the rather tough Central European winter (- 20° C). Therefore, the oil price and the price of other fossile energy resources calculated from oil price significantly affect the change and development of the economic conditions of the world, more specifically of agriculture. Since the amount of fossile energy resource stocks is continuously decreasing and their extraction is becoming increasingly expensive, the increasing and steadily high-priced oil prices will have an impact on the costs of agricultural corp production and its economicalness, too. Nowadays, the prices of energy resources increase at a rate that we should take into account the alternative energy resources, especially biomass, in a wider scale. Based on this expected scenario, it is no wonder that increasing attention is paid to renewable energy resources, more specifically biomass and the energy gained from various algae strains, an innovative feedstock which is considered to be a way out from this unfavourable situation. The new, algae-based energy production systems – which we can call photobioreactors – will possibly spread on an industrial scale in the near future.

Hungary is seeking the solution in this respect, too. The strategic program of the Hungarian Academy of Science launched in 2008 focuses on seven increasingly important areas, one of which is the issue of long-term energy strategy and the environmental and food safety. During the last years, ensuring the usability of manifold energy resources became a national strategic objective. In terms of this strategy, the Hungarian Academy of Science published its book entitled “Overview of the Hungarian energy strategy” in it series called Public Body Strategic Programs. The authors of this book emphasise the importance of providing newer, manifold energy resources due to the increasingly rapid exhaustion of conventional energy resources. The forum of the discussion day of the EU project called Parliament and Civil Society in Technology Assessment (18<sup>th</sup> June 2012, the Danish Parliament) focused on the science which facilitates the decision-making in the process of political procedures, also involving energy supply. Maize utilisation has a great tradition in several countries (mainly the USA) concerning the manifold resources which can be used for

energy production purposes. In Hungary, the firstly established maize-based bioethanol production plant in Szabadegyháza increased its capacity in June 2012. This energy production alternative will have an increased attention in the future in the field of improving the opportunities of the Hungarian energy production.

## **Objectives**

The aim of this doctoral thesis was to select the most favourable maize varieties for bioethanol production based on the comparison of their kernels' content values – with special regard to starch content – by using a near infrared spectroscopic method, the so-called “NIT” technique. The other aim of the research was to detect the impacts of three important production factors: irrigation, fertilisation and cultivation methods on the starch, oil and protein contents of the kernels of various maize hybrids in three consecutive years from 2007 to 2009. Therefore, my objective was to compare more than 150 maize hybrids from different production sites during these three years.

### **Detailed objectives**

1. Examination of the maize kernels' contents with special regards to the starch content of fundamental importance from the aspect of bioethanol production in 2007 and 2008;
2. Comparative analysis of the maize hybrid samples collected from the same production sites in 2007 and 2008;
3. Evaluation of the impact of production factors: irrigation, fertilisation, cultivation on maize kernels' contents of three maize hybrids grown in the Látókép Experimental Site in 2007 and 2008;
4. Comparative analysis of the impact of production factors on the content values of the maize hybrid samples grown in the Látókép Experimental Site in 2007 and 2008;
5. Evaluation of the impact of production factors on the content values of the three maize hybrid samples grown in the Látókép Experimental Site in 2009.

## **MATERIALS AND METHODS**

During the work I spent with the experiment, I analysed the content values of 512 maize hybrids from 21 production sites using the Foss Infratech<sup>TM</sup> 1241 Grain Analyzer equipment, with special regard to the starch content which is of essential importance from the aspect of bioethanol production. As for the samples to be analysed which originated from the Látókép Experimental Site, I examined and evaluated the yield of all maize hybrids in three consecutive years (2007-2009), as well as the effect of weather, irrigation, nutrient replenishment and cultivation on the content values in each year in a complex, multifactoral long-term experiment. I analysed the effects of the meteorological differences of the given year and the various production technology alternatives (cultivation, fertilisation, irrigation) on the content values (oil, protein, moisture, starch) of the given maize hybrids. Furthermore, I also examined the yield of maize hybrids; therefore, I was able to determine the actual starch yield of various hybrids per hectare, which is one of the most important factor from the aspect of bioethanol production on an industrial scale. I performed a detailed analysis of the endowments of the Látókép Experimental Site.

### **Determination of the contents of maize hybrids with a Foss Infratech<sup>TM</sup> 1241 Grain Analyzer measurement device**

The maize samples necessary in order to perform the content value analyses were collected in the full ripening phase of maize during the field experiment. During the three years, I collected maize ear samples per hybrid from each replication at the Látókép Experimental Site in the experiment series which involved 9 maize hybrids. In the plots, I also determined the harvested yield. During the harvest of maize hybrids, I determined the yield per plot with a measurement vehicle. After determining the moisture content of the grain yield, I converted the moisture content of the harvested yield to 13% and determined maize yield. Before the laboratory analysis, I prepared the collected ear samples, since the Infratech Grain Analyzer can perform the measurement task only after preparation. I carried out the content value examinations of the maize hybrids with the Foss Infratec<sup>TM</sup> 1241 Grain Analyzer (FOSS Tecator AB, Hoeganaes, Sweden) yield analysing equipment in the Institute for Land Utilisation, Technology and Regional Development of the Centre for Agricultural and Applied Economic Sciences of the University of Debrecen.

The sample handling unit of the Infratech 1241 spectrophotometer is the Grain Analyzer which is equipped with a “conveyor (6-33 mm) flour module” sample handling device. The infrared device works on a transmission basis and the wave-length of measurement was between 850 and 1048 nm with a 2 nm step in between. The number of spectrum data points of the device used in the examination was 100. I used five subsamples in the case of each sample to be able to obtain as high measurement accuracy as possible. The device separated the maize grains entering the sample holder into five subsamples automatically. The equipment was controlled by the ISW 3.10 (FOSS Tecator AB, Hoeganaes, Sweden, 2003) software. With this software, the numerical mean values were provided for all five subsamples’ total weight, moisture content in mass percentage and the starch, protein and oil content related to dry matter content, also expressed in mass percentage. The measurement results of the samples provided by the device were stored in an Excel file for all three years. I performed all further processing and evaluation of the obtained data in Microsoft Office Excel 2007.

### **Description of the Látókép Crop Production Experimental Site**

The Látókép Crop Production Experimental Site was established in 1983. The original size of the site was 70 hectares which was later extended to 192 hectares. The site is situated outside of Debrecen at the milestone 195 of the main road no. 33. On the site, production site-specific long-term field experiments are being carried out which are adopted to the special characteristics of the Hajdúság loess ridge. Based on the soil analysis results obtained in 2002, the average pH value of the soil is 6.6. The physical soil type is medium adobe. In the upper 20 cm layer of the soil, the Arany plasticity number is 37, the total salt content is 0.05 m/m%. The carbonic chalk content is 0 m/m% in the upper 80 cm of the soil, but it sharply increases by 11 m/m% between 100 and 160 cm. Compared to the soil analysis results in 1984, there is a continuous leaching of the carbonic chalk content and it appears in even deeper layers. During the last 26 years, the humus content of the soil decreased as a result of intensive cultivation. Currently, the humus content is 2.4 m/m% in the upper 20 cm of the soil and it does not exceed 1.00 m/m% at the 120 cm depth. The level of nitrogen and potassium supply is adequate, the phosphorus supply can be considered average. The groundwater is between 5-8 m in years with average weather. This groundwater level does not significantly decrease even during drier years due to the groundwater altering impact of the artificially developed Látókép Reservoir which was built in 1981.

## **Treatments of the multifactorial small plot field experiment**

I analysed the effect of a drought (2007), a favourable growing season (2008) and a growing season with average precipitation supply (2009) on the content values of 9 maize hybrids in a complex, multifactorial long-term cultivation experiment established at the Látókép Experimental Site of the University of Debrecen, Centre for Agricultural and Applied Economic Sciences. The multifactorial small plot long-term experiments covered the cultivation treatment, irrigation treatment and fertilisation.

The cultivation treatments were the following: A = autumn ploughing (at 27 cm depth), B = spring ploughing (at 23 cm depth), C = spring disk shallow cultivation (at 12 cm depth).

The irrigation treatment covered the irrigated ( $\ddot{O}_1$ ) and the non-irrigated ( $\ddot{O}_2$ ) categories. I separated the fertilisation treatments to three types. Type 1: N 0 kg/ha P<sub>2</sub>O<sub>5</sub> 0 kg/ha K<sub>2</sub>O 0 kg/ha, i.e., non-fertilised/control; type 2: N 120 kg/ha P<sub>2</sub>O<sub>5</sub> 90 kg/ha K<sub>2</sub>O 106 kg/ha; type 3: N 240 kg/ha P<sub>2</sub>O<sub>5</sub> 180 kg/ha, K<sub>2</sub>O 212 kg/ha. (The listed fertiliser amounts refer to active ingredient quantities).

The autumn NPK fertilisation was performed in the previously described concentration between 15<sup>th</sup>-20<sup>th</sup> October. The used fertiliser type was Kemira Power. The cultivation methods needed for successful maize production were the following in all three years (2007-2009): stubble-stripping, ploughing, finishing, seedbed preparation, crop care.

After sowing, the necessary crop protection procedures were performed in all three years. Of these, soil disinfection and weed control were of significant importance. Force 1.5 G was used in soil disinfection in a 14 kg/ha dose on the small plots before sowing. The following chemical was used in weed control: Guardian Tetra. The yearly dose applied in all three years was 4.5 litre per hectare and was applied few days after sowing. The sowing date was in the middle of April both in 2007, 2008 and 2009. As harvest dates were not identical in all three years, since the date of harvest was greatly dependent on the actual weather and soil conditions. The harvesting was performed on the following dates in each year: 12<sup>th</sup> October 2007, 13<sup>th</sup> October 2008 and 1<sup>st</sup>-2<sup>nd</sup> October 2009.

In all cases, I performed the NPK fertiliser dose experiments along with a non-fertilised control plot. Irrigation was carried out with a mobile linear irrigation equipment. This device was equipped with Wobler sprinkler heads, hydrant water feeding and a tracking tool and it

performed even water distribution. The experiment design was split-split-plot and the main plots featured the cultivation and irrigation treatments without replication. One cultivation block covered 8064 m<sup>2</sup> which is separated to an irrigated and a non-irrigated block. Maize hybrids used in the experiment are shown in Table 1.

**Table 1: Maize hybrids of the small plot experiment during the three years**

Year	Hybrid		
2007	MV Tarján	MV Koppány	DKC 4005
2008	DKC 4005	ED5110	Kamaria
2009	P9400	DKC5276	Kamaria

The size of the main plot with a given hybrid was 2688 m<sup>2</sup> and the plot size of the fertilisation treatment was 336 m<sup>2</sup>. The net basic area of a plot was equivalent to 15 m<sup>2</sup>.

**During the experimental year 2007**, I examined 106 hybrids produced in 6 production areas (*Hajdúdorog, Szentmártonkáta, Jászkisér, Hajdúböszörmény, Biharkeresztes, Zsámbok*). Altogether, I examined 106 maize samples collected from the fields which have different geographical locations. In 2007, the four maize samples I collected from six different production sites were used in field crop production in five, or even more locations, respectively. Therefore, I had the opportunity to carry out simultaneous evaluation and comparison of maize samples of the same genotype in the same year. After their harvesting, I measured the content values of the samples (oil (%), protein (%), starch (%), moisture (%), hectolitre (kg per 100 l), then processed the obtained results and compared identical hybrids which originated from different locations.

**During the experimental year 2008**, I collected 145 different hybrids – 403 unique samples altogether – from the fields in 12 different production areas (*Bakonszeg, Nádudvar, Földes, Hajdúböszörmény, Abony, Cegléd, Poroszló, Tiszaszőlős, Hort, Hosszúpályi, Biharkeresztes and Létavértes*). I collected the samples of the J1 maize hybrid from 10 different production sites during 2008. I measured the yield samples of the 10 J1 hybrid and compared them to each other. As a result of different production areas and the different weather and crop production circumstances, I obtained different content values. I followed the same method concerning the L1 maize hybrid which had the same genotype. In 2008, I collected, analysed and compared this hybrid from eight production areas with different geographical locations.

**In two consecutive years, 2007 and 2008**, I had the opportunity to collect samples from identical maize hybrids grown in the same area. Therefore, I could compare the content parameters of maize samples produced on totally identical soil but in two years with different weather. Compared to the long weather series, I measured and compared the relative starch content of identical hybrids produced in the plough-land in the periphery of Hajdúböszörmény (samples from three hybrids per two years) and Biharkeresztes (samples from 7 hybrids per two years) in 2007 which was a drought year and in 2008 that had average precipitation distribution which was favourable from the aspect of maize production.

**During 2009, which had average, slightly dry weather**, I analysed the three maize hybrids in the multifactorial long-term small plot experiment at the Látókép Experimental Site mainly from the aspect of the effect of the applied agrotechnical methods on the content values.

### **Statistical method of data evaluation**

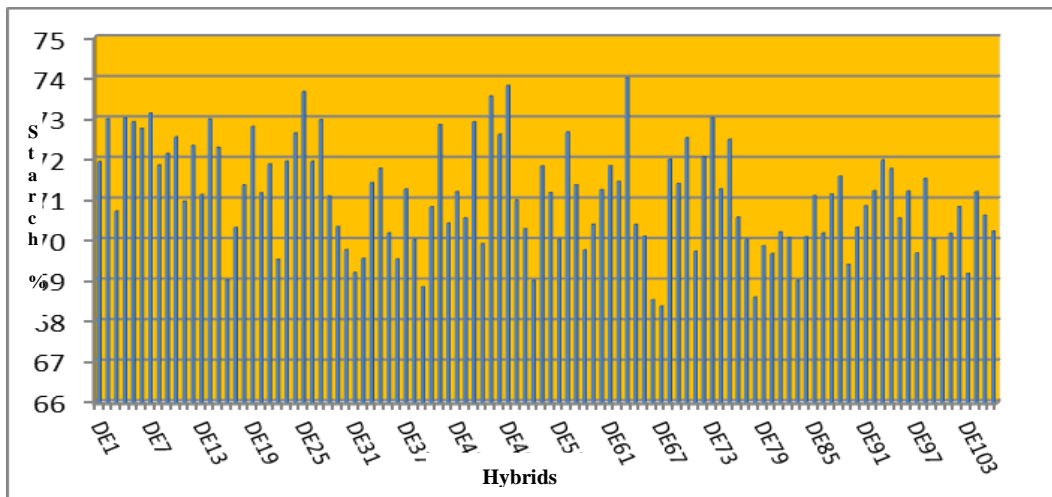
The evaluation of the obtained data was done with SPSS for Windows 17.0 (SPSS Inc., Chicago, Illinois, USA). I determined the distribution of the continuous data with a Kolmogorov-Smirnov test. I performed the comparison of my data which had Gaussian (normal) distribution with using an Independent-Samples T test, while I used the Mann-Whitney U test to compare the data showing non-Gaussian (non-normal) distribution. The comparison of category variables was performed with the “Analysis of Variance” (One-Way ANOVA) test and the “Least Significant Difference” (LSD) method. The effect of treatments (irrigation, fertilisation, cultivation) on the starch content (association) was evaluated with a general linear model (GLM). By using GLM, the statistical hypotheses related to significant differences between the groups of variables can be proven. The correlation analysis of the combinations of target variables with normal distribution and independent variables with continuous distribution was performed with GLM. I considered  $p < 0.05$  to be a statistically significant difference.

## RESULTS

### Analysis of maize kernel contents in 2007 with special regard to the starch content which is of fundamental significance from the aspect of bioethanol production

During the analysis of the 106 samples collected from the hybrids, I concluded that the average starch content of hybrids (at changing moisture content few days after harvesting, average value: 16.7%) was 59.2% which was determined with a NIT method.

Figure 1: Relative starch content of maize hybrids related to dry matter, 2007

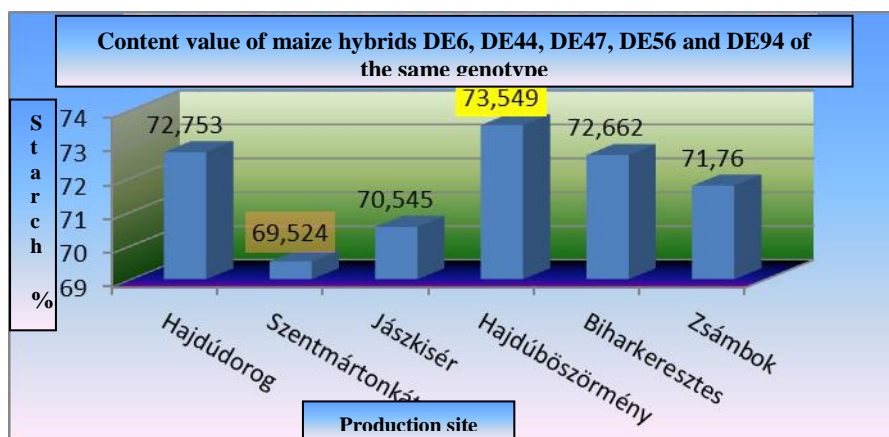


I measured 10.2% difference between the hybrids with the highest and lowest starch content which could result in significant yield differences during bioethanol production. In the case of approximately 13% moisture content, the starch content of the highest measured hybrid (13.7%) was 63.7% in 2007 and the lowest value was 53.5%. Of all collected samples, the maize hybrid with the highest relative starch content (74%) was the DE63 hybrid in 2007 which was produced on a Biharkeresztes field. The hybrid with lowest relative starch content was the DE67 hybrid with a 68.4% value. This maize hybrid was produced in Zsámbok. The difference between the minimum and maximum value is 5.6%. Figure 1 shows the analysis results of the starch content of all hybrids which were involved in the research.

## Comparison of the maize hybrid samples of the same genotype, collected from different fields in 2007

In 2007, I measured and compared the relative starch content of four maize hybrid samples of the same genotype, collected from different fields. I managed to collect and measure the samples of the identical hybrids with codes DE6, DE22, DE44, DE47, DE56, DE94 which were produced on all fields involved in the examination (Figure 2). The samples of the identical maize hybrids DE12, DE19, DE34, DE45, DE62, DE85 were also produced on all production areas in 2007. The samples of the DE13, DE24, DE35, DE48, DE61 hybrids were collected on five different fields. Samples from maize hybrids DE21, DE39, DE46 and DE52 of the same genotype were collected on four different locations in order to perform comparison.

Figure 2: Maize kernels' relative starch content grown in different locations, 2007



It can be seen in Figure 2 that maize hybrids which have totally identical genotype show different content values in different production sites, under different agrotechnical treatment and different meteorological conditions in 2007. During the measurement which I performed under the same conditions, I concluded that there is significant difference in the relative starch content related to dry matter and other content value parameters. Of the maize hybrids DE6, DE22, DE44, DE47, DE56, DE94 of the same genotype, the highest relative starch content (73.5%) was obtained in the case of the maize hybrid DE47 which was produced on one of the fields around Hajdúböszörmény. On the contrary, the DE22 maize hybrid of the same type produced in Szentmártonkő reached only 69.5%. The difference is 4%, which is considerable. The relative starch content of the hybrid produced in six

production sites related to average dry matter content is 71.8%. The ratio of oil and protein content was similarly various (non-demonstrated data).

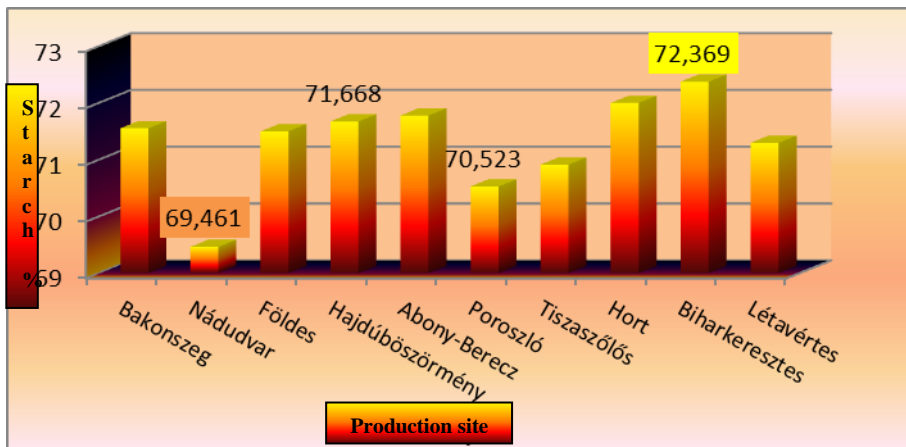
### **Analysis of maize kernel contents in 2008 with special regard to starch content which is of fundamental significance from the aspect of bioethanol production**

In 2008, the average starch content of the 403 samples (at changing moisture content few days after harvesting, average value: 16.9%) was 59.7% which was determined with a NIT method. There was a 12% difference between the highest and the shortest starch content (65.1% - 53.1%). After determining the starch content related to dry matter, the highest relative starch content was measured in the maize hybrid AGTC264 which was produced in 2008 on a field in the periphery of Hosszúpályi and its starch content related to dry matter was 75.4%. The lowest relative starch content (68.8%) was measured in the case of the hybrid AGTC361 whose sample was collected on a field around Biharkeresztes in 2008. In this case, the difference between the maximum and minimum value was 6.6%.

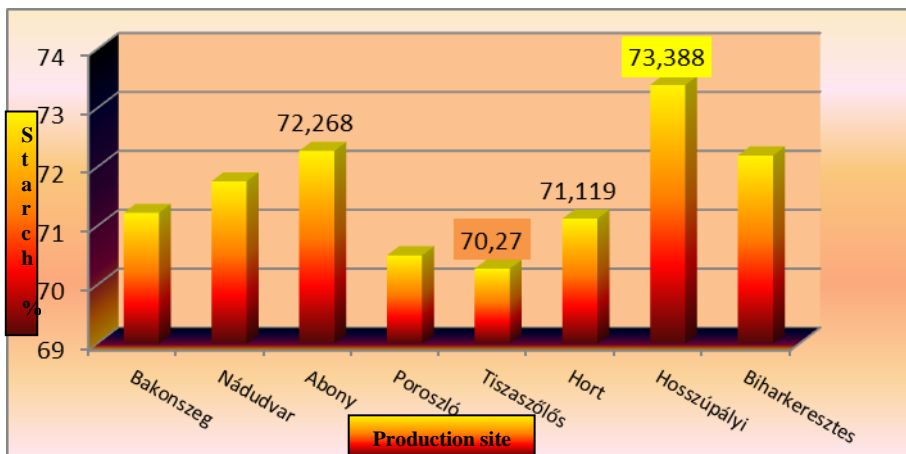
### **Comparison of the maize hybrid samples collected from different fields in 2008**

In 2008, the starch content related to dry matter of samples collected in 10 different geographical locations was between 69.5-72.4% (Figure 3). The average starch content of the 10 samples was 71.3%. The difference between the minimum obtained in the case of the hybrid J1 (AGTC86) produced in Nádudvar and the maximum of the hybrid J1 (AGTC362) produced in Biharkeresztes was 2.9 %.

**Figure 3: Starch content of the J1 maize hybrid in different production sites, 2008**



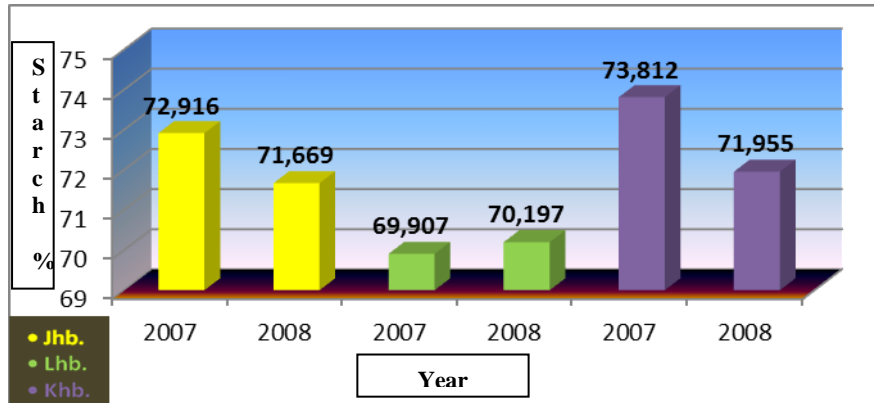
**Figure 4: Starch content of the L1 maize hybrid in different production sites, 2008**



I collected and measured the L1 maize hybrid samples of the same genotype in eight different locations in 2008 (Figure 4). The starch content of the samples related to dry matter was between 70.3 % - 73.4 %. The average starch content was 71.6 %. The difference between the starch content of the L1 (AGTC212) hybrid produced in Tiszaszőlős and the L1 (AGTC304) hybrid produced in Hosszúpályi was 3.1%.

## Comparative analysis of maize hybrid samples of same genotype, collected from the same production site in 2007 and 2008

Figure 5: Comparison of three vintages of identical hybrids, 2007-2008, Hajdúböszörmény



During the analysis of the 3-3 maize grains of the same genotype on the field outside of *Hajdúböszörmény*, the measured content values differed in the samples of the two years (Figure 5). The Jhb (DE45, AGTC112) hybrid contained 72.9% starch in the drought year of 2007, while this value was 71.7% in 2008. The difference was 1.2% in favour of the drought year. The Lhb (DE46, AGTC119) hybrid contained 69.9% starch in 2007 and 70.2% in 2008. The Khb (DE49, AGTC121) hybrid of the same genotype contained 73.8% starch in 2007 and 72% in 2008, the difference was 1.8%.

## Analysis of the effect of production factors: irrigation, fertilisation, cultivation on the content values of the three maize hybrids produced on the Látókép Experimental Site in 2007

The following hybrids were involved in the long-term small plot experiment carried out in the Látókép Experiment Site in 2007: *DKC 4005 (DE\_a)*; *MV Tarján (DE\_b)*; *MV Koppány (DE\_c)*. The following table (Table 2) presents the joint and individual evaluation of the effects of irrigation, fertilisation and cultivation on the measured starch content.

**Table 2: Starch content of hybrids at 14% moisture content. Látókép, 2007**

Name	Hybrid			Average
	DKC 4005(DE_a)	(DE_b) Koppány	(DE_c) Tarján	
	62,75	62,04	62,48	62,41

Of the three maize hybrids examined in 2007, DKC 4005 showed the highest starch content (62.7%). The lowest starch content was observed in the case of MV Koppány (62%). The difference between the two values was only 0.7%. There was no significant difference between the starch content measured in the case of the three different maize hybrid and the few decimal difference is negligible from the aspect of bioethanol production.

**Table 3: The effect of the examined cultivation methods on the starch content. Látókép, 2007**

Name	Cultivation type			Average
	Autumn ploughing	Disking	Spring ploughing	
	62,93	61,81	62,51	62,41

A change can be observed in the starch content depending on the applied cultivation method. The most successful cultivation method which resulted in higher starch content was autumn ploughing in 2007 when the average value of the three hybrids was 62.9%. The lowest starch content was observed in the case of spring ploughing (62.5%). The difference in starch content as a result of these two methods was 0.4% (Table 3). The most successful cultivation method which resulted in higher starch content was spring ploughing.

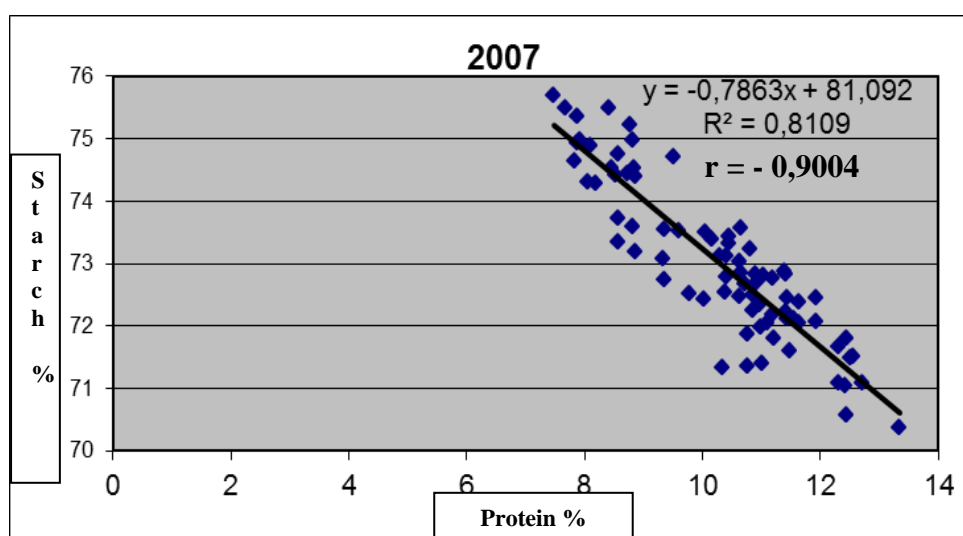
**Table 4: The effect of fertilisation on starch content. Látókép, 2007**

Name	Fertilisation			Average
	120 kg N/ha	240 kg N/ha	non-fertilised	
	62,18	61,68	63,28	62,41

The results obtained in 2007 reflect that there was higher starch content in non-fertilised hybrids (63,3 %) than in the case of applying 120 kg N/ha (62.2%) and 240 kg N/ha (61.7%). In the non-fertilised control treatments, the starch content of the maize hybrids was 1.1% higher than that of the same hybrids which received a 120 kg N/ha fertiliser treatment. This difference was 1.6% when compared to the 240 kg N/ha treatment on the same hybrids.

The difference in the starch content measured in the case of the 120 and 240 kg N/ha treatments is 0.5% in favour of the hybrids on which 120 kg N/ha fertiliser was applied (Table 4). Based on the measurements aiming at the effect of fertiliser treatments on starch content in 2007, it was concluded that while the yield was always higher as a result of higher fertiliser doses, the starch content of maize grains was always lower. When calculating these results, I did not take into account the actual starch yield produced per hectare which comes from the amount of yield and its starch content.

**Figure 6: Correlation between starch content and protein content. Látókép, 2007**



In 2007, the relative protein content of the three different maize hybrids produced under different agrotechnical circumstances on the Látókép Experimental Site showed a significant negative correlation with the relative starch content of the examined hybrids (Figure 6); therefore, the relative protein content of the maize grains increased at the expense of the relative starch content. The negative correlation was not changed by either the genetic characteristics of the hybrids, nor the impact of the various applied agrotechnical methods.

**Table 5. The effect of irrigation on the starch content. Látókép, 2007**

Name	Irrigation		
	Non-irrigated	Irrigated	Average
	62,36	62,46	62,41

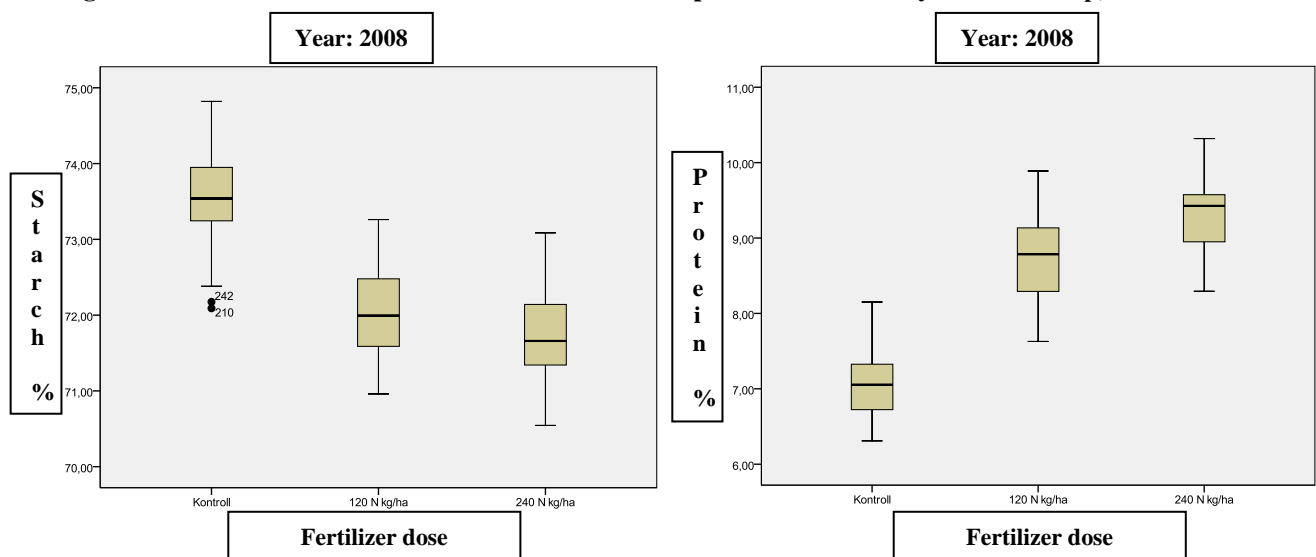
The 62.5% starch content measured in the irrigated maize population (14% moisture content) showed only a slight increase in comparison with the 62.4% measured in the case of

the non-irrigated small plot experimental treatment. The difference was 0.1% (Table 5). There is no significant difference between the two experimental treatments related to the starch content. There was only 0.1% difference between the starch content values of the irrigated and non-irrigated treatments of the three maize hybrids produced on the Látókép Experimental Site in 2007. Therefore, irrigation did not cause any significant difference on the starch content of grains, as opposed to yield and the starch content calculated from yield.

**Analysis of the effect of production factors: irrigation, fertilisation, cultivation on the content values of the three maize hybrids produced on the Látókép Experimental Site in 2008**

**The effect of fertilisation on the content values of hybrids in 2008**

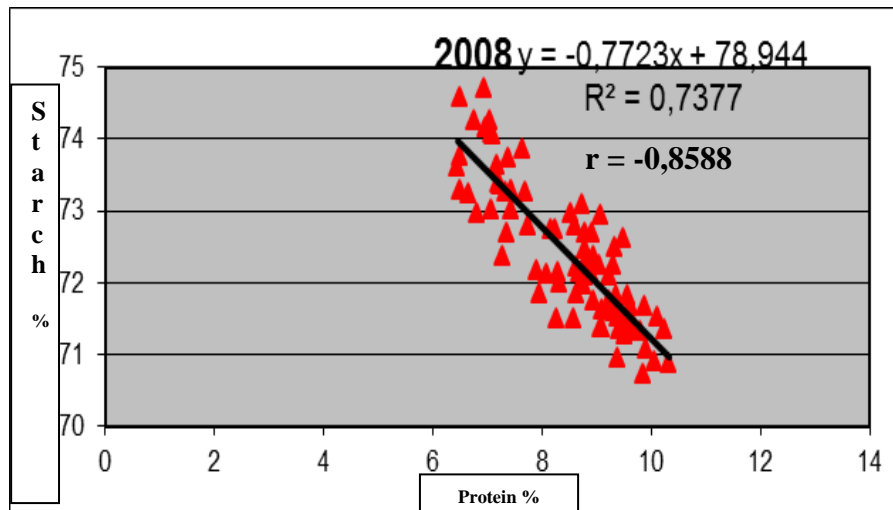
**Figure 7. The effect of fertiliser doses on the starch and protein content of hybrids. Látókép, 2008**



The relative starch and protein content of the three different maize hybrid samples (DKC4005, ED5110, Kamaria) collected in 2008 also show the tendency observed in 2007, that is the protein content of hybrids can be increased with fertiliser dosing, while it has an adverse effect on high starch content (Twin figure 7).

Irrigation did not have any significant effect on the relative starch content related to the dry matter of the three different maize hybrids in 2008.

Figure 8. Correlation between the starch and protein content. Látókép, 2008

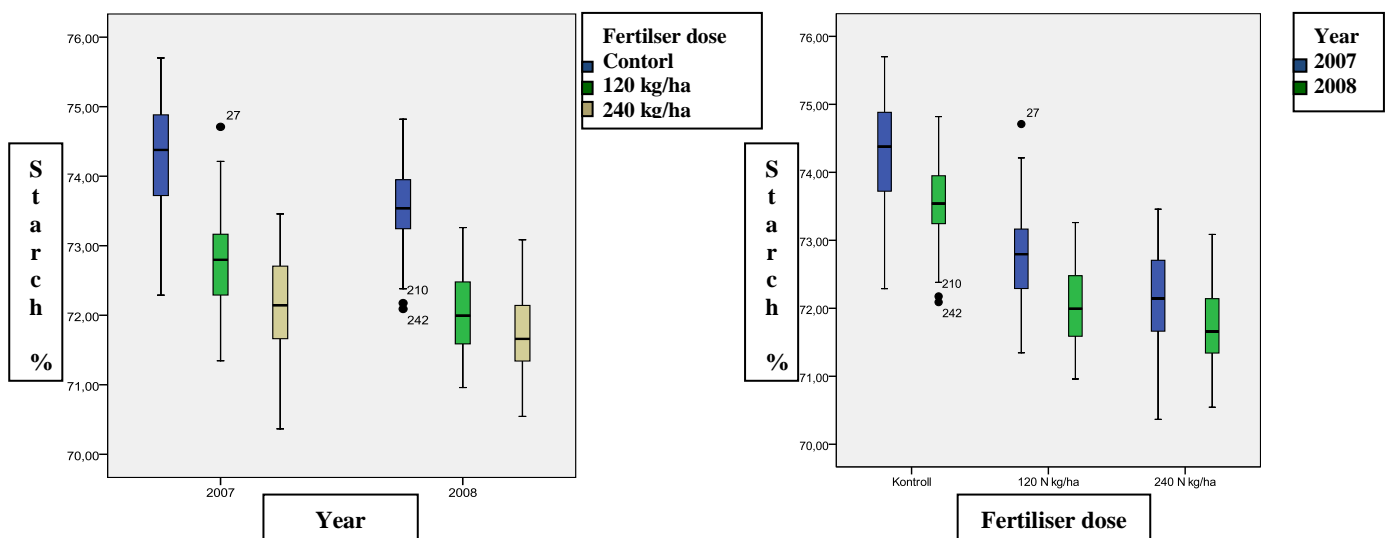


In 2008, the significant negative correlation of the relative protein and starch content could be observed, even though this year had significantly higher precipitation than 2007. The biological importance of this correlation is also shown by the fact that there was only one identical hybrid on the hybrids examined in 2007 and 2008, while the 2-2 hybrids in the two different years had different genotypes. The effect of the applied agrotechnical factors did not affect the negative correlation between the starch and protein content of the examined hybrids in 2008 (Figure 8.). The overwhelming majority of the examined hybrids contained 8-10 % protein at 71-73% starch content. Samples with higher (74-75%) starch content tended to contain less protein. The measurement device showed only 6-7% protein content in these samples.

**Analysis of the effect of production factors: irrigation, fertilisation, cultivation on the content values of the maize hybrids produced on the Látókép Experimental Site in 2007 and 2008**

**The effect of fertilisation on the content values of hybrids in 2007 and 2008**

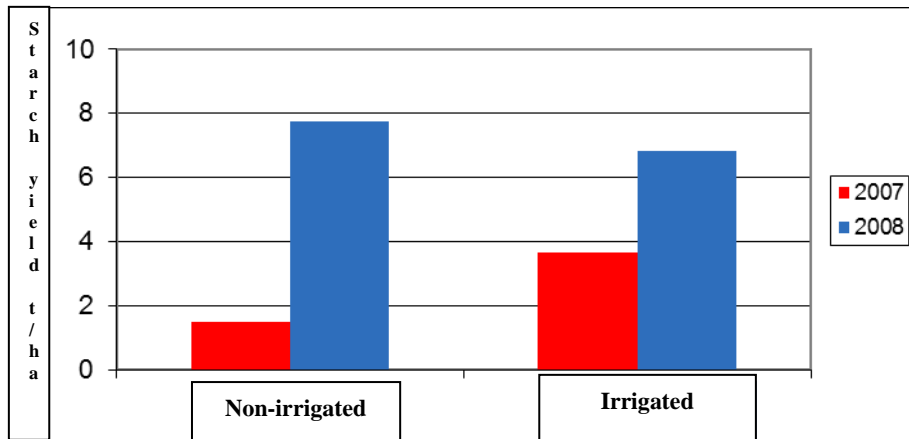
**Figure 9. The effect of the two years' fertiliser treatments on the starch content of hybrids. Látókép, 2007-2008**



Twin Figure 9 shows the starch content of the 5 hybrids of different genotypes related to dry matter against fertilisation, collected from the Látókép Experimental Site in 2007 and 2008. Between the 3-3 hybrids of the two years, there was a hybrid of completely identical genotype in the research. There were significant differences between the drought year of 2007 and 2008 when there was average weather as regards the starch contents of the hybrids which received the same fertiliser treatments. In the drought year of 2007 higher starch content was observed in the hybrids both altogether and individually under the same fertilisation circumstances than in 2008. Yield and the derived starch yield per hectare values were much lower than the values obtained in the case of the same hybrid in 2008, due to the low amount of precipitation.

In 2007, the relative starch content of the examined hybrids was higher than in 2008 when there was favourable precipitation supply both in the irrigated and the non-irrigated treatments. There have been the same tendency for years, that is, there was slightly more starch in the irrigated hybrids, but this difference was not significant in either year.

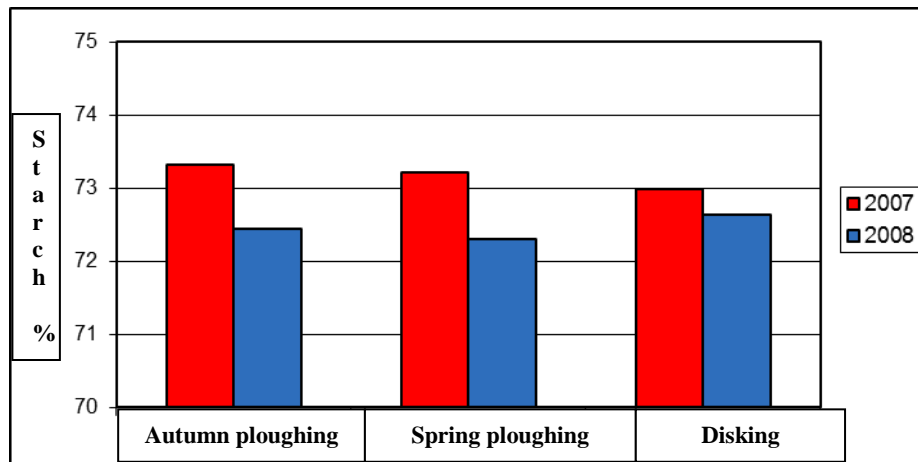
**Figure 10. The effect of irrigation on the starch yield of the hybrids. Látókép, 2007 and 2008**



As regards the starch yield (t/ha) of the hybrids (Figure 10.), there was a significant difference between the total starch yield (2.2 t/ha) of the irrigated and non-irrigated hybrids as a result of irrigation in the drought year of 2007. On the contrary, there was a nearly 1 t/ha decrease in the starch yield in the irrigated hybrids in 2008 when the precipitation supply was favourable. The biological cause of the observed reduction might have been related to the dilution of the nitrogen source in the soil caused by the water abundance in the soil.

## The effect of cultivation on the Látókép hybrids in 2007 and 2008

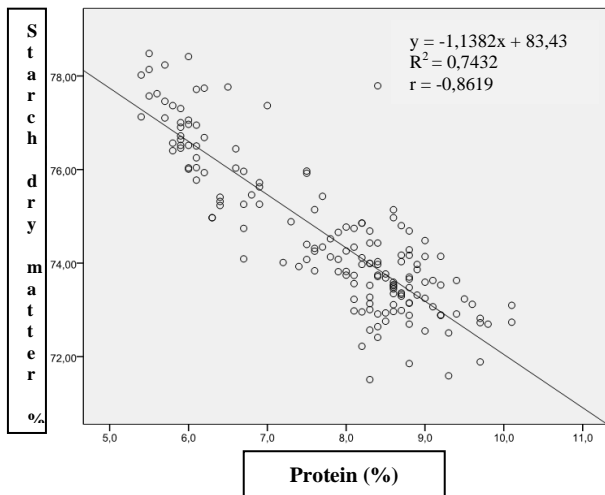
Figure 11. The effect of cultivation on the kernels' starch content. Látókép, 2007 and 2008



The cultivation treatments compared in course of the research in both years (Figure 11.) did not cause any significant difference in the relative starch content of the hybrid samples.

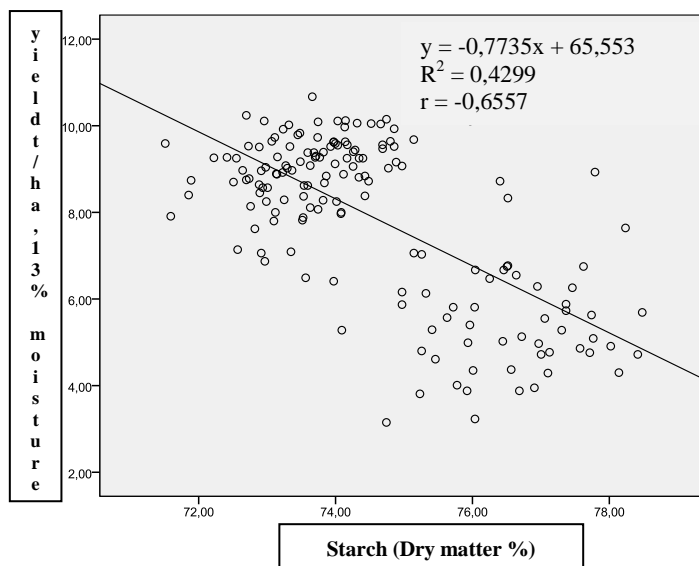
**Analysis of the effect of production factors: irrigation, fertilisation, cultivation on the content values of the maize hybrid samples produced in the Látókép Experimental Site in 2009**

**Figure 12. Spearman's correlation analysis of the association between starch content and protein content. Látókép, 2009**



The correlation between the relative starch content and protein content of the three maize hybrid samples obtained from the Látókép Experimental Site in 2009 is shown in figure 12. In addition to the visible negative correlation, it can also be concluded based on the high  $R^2$  value (0.7432) that the regression line could have been narrowly fitted to the data points in the experimental year of 2009 (Figure 12). The overwhelming majority of the examined samples contained 72-73.5% starch at 8-9% protein content.

**Figure 13. Starch content and yield of the Látókép hybrid samples in 2009**



Based on the relationship between yield and starch content as demonstrated on Figure 13, there was a negative correlation, i.e. higher yields (9-10 t/ha) were associated with lower relative starch contents (72-74%). The fitting of the regression line was weak ( $R^2 = 0.43$ ). The yields of the high starch content (76-78%) samples were significantly lower than the average ( $9.2 \text{ t ha}^{-1}$ ).

## UTILIZATION POSSIBILITIES OF RESULTS

Between 2007 and 2009, I was among the first ones internationally to analyse 176 maize hybrids from 10 different production sites, that is 512 samples altogether with using a *Foss Infratech Grain Analyzer* device that is based on the NIT technology. As expected, the near-infrared method made it possible to accurately determine the content values of a significant number of maize samples in a relatively short period of time, which may provide a helps in the complex and quick evaluation of the effects of several agricultural and agrotechnical factors. The NIT-based measurement device was capable of simultaneously determining all content value parameters of special importance from the aspect of the multiple use/processing of maize. Therefore, it took only a few minutes to obtain data about the dry matter, protein, starch and oil content of the sample in only one measurement event. Also, the device performed the determination of the hectolitre parameter of the given sample. Chinese researchers found the reliability of the evaluation performed with NIR spectroscopy in relation to the content of maize grains to be of similar accuracy as the analysis performed with chemical methods. There were relatively few other researchers who used the NIR spectroscopy method to analyse the starch content of maize grains. The findings of my dissertation also demonstrate that it is possible to perform the high accuracy evaluation of a significant amount of samples from year to year with *Foss Infratech Grain Analyzer*. This method provides an opportunity to evaluate and to compare the newly developed Hungarian hybrids to older and foreign competitors, an aspect which is particularly important for the Hungarian maize production sector. Accordingly, I was the first to be able to determine the content of 10 new Hungarian maize hybrids in my study. It can be considered a further significant advantage that this method helps in analysing the effect of different production sites and climatic conditions by accurately evaluating several hundreds or even thousands of

samples. Last but not least, this method also makes it possible to evaluate the cost-efficiency of the various agrotechnical procedures in case of hybrids of the same genotype that are produced on the same production site. The quick implementation of the measurement provides an opportunity to determine the best harvest date by collecting test yield results.

The measurements based on the NIR/NIT method getting increasingly widespread in analytical technics, started to become more acknowledged in the agriculture, more specifically in the characterisation of maize yield only in the recent past. In Hungary, it was our working group that first used the NIR/NIT technology to determine the content values of numerous hybrid samples in the laboratory of the Centre for Agricultural and Applied Economic Sciences of the University of Debrecen. In this doctoral dissertation I am describing some of the results of my experimental work. Our working group is still using this technology to carry out similar evaluations with a different focus. The first results of this research have recently been published in a thesis.

It is a known fact that the kernels' content values of a given maize variety are significantly affected by external factors in addition to its basic genetic endowments. These external factors can be divided into two main groups: ecological factors during the growing season and the applied production factors. I approached the justification of the hypothesis in two ways during my analyses. In three consecutive years, I analysed the content of 512 maize hybrids from 22 production sites. Furthermore, I examined the change of the content values of three maize hybrid samples produced in the Látókép Experiment Site as related to different production methods between 2007 and 2009. In my publications and doctoral dissertation, I mainly focused on starch content which is the most significant ingredient from the aspect of bioethanol production, in addition to obtaining other important measurement data.

## **SUMMARY**

The rapidly growing population of the Globe results in a surging energy consumption which will lead to the depletion of energy resources in the short term. Several countries understanding these phenomena have started studies and produce alternative sources of energy based on research. The Hungarian energy supply has faced many problems in the past years. Not having significant energy sources added up with the European financial situation that resulted in an unstable situation characterized by growing energy prices. This shows in the dramatically rising fuel prices. Using cost efficient alternative energy production technologies to substitute partially a share of currently used fuel types might have a great importance. The

outstanding agricultural environment present in Hungary may provide an excellent opportunity to achieve this goal by establishing cost efficient methods for bioethanol production based on appropriate corn yields and starting mass production in as many places as possible. Aiming at this objective requires a careful analysis on pedological capabilities and the selection of the optimal corn hybrids considering the influence of irrigation, application of fertilizers, and tillage methods on the given production sites. This way it is possible to choose the best corn hybrids to be grown in any given area for purposes of bioethanol production.

In the course of my research carried out during the three years of PhD studies I demonstrated that measurements utilizing the NIT/NIR method yielded in data retrieval facing all expectations. Using among the first ones in Hungary the *Infratech Grain Analyzer* that operates with NIT technology I was able to provide an accurate high-throughput analysis of the inner contents of different corn type kernels. Altogether 512 samples of 176 different corn hybrids including 10 new Hungarian strains were analyzed between 2007-2009. The examined corn kernels' inner contents, including their starch content, varied in a wide range. I have investigated the effects of genetical and external factors by two approaches. I studied the variations of inner content parameters of corn seeds collected from 22 different production sites particularly considering the dissimilarities of precipitation conditions of the three years. Moreover, I analyzed the variance of three-three maize hybrids' inner contents, respectively regarding the effects of irrigation, nutrient supply and different tillage methods grown in small-plot field experiments at the Experimental Station of Látókép.

The research results showed that the corn yields of 2008, characterized by optimal amount of precipitation in the different production sites and the Experimental Station of Látókép notably exceeded the yields of the same corn hybrids grown in same circumstances in the draught-stricken year of 2007. The starch content of the maize kernels was decreased by the effect of fertilization and irrigation. However, both of the two factors had a positive influence on the starch yield due to increased crop yields collected from irrigated and fertilized areas. The planting rate had no significant effect on the starch content. The used tillage methods did not have a major influence on the starch content of the corn seeds however, the crop and, in parallel, the starch yields grew significantly when using the autumn ploughing method compared to the disking and spring ploughing cultivation types. Nitrogen nutrient supply had the largest effect among the external factors, the 120 N kg/ha dose compared to the unfertilized control statistically significantly enhanced the starch yield and crop yield in all of the three examined years. At the same time the double dose of (240 N kg/ha) fertilizer did not result any further large scale increase in the crop yield or starch yield. A significant

negative correlation between the kernels' starch content and protein content was established year by year.

In conclusion, I have characterized inner contents of kernels of ten new Hungarian maize hybrid in course of my Ph.D. research. One of these was a genetically identical DKC corn hybrid grown at the Látókép Experimental Station. I compared the changes in the inner content parameters of the seeds elicited by the effects of different doses of N-fertilizer, irrigated and non-irrigated treatments and cultivation types. My results may contribute to the optimization of Hungarian corn based energy production conditions and in the selection of maize hybrids with optimal characteristics. These results also help in choosing the most cost efficient production factors among different weather conditions and at different production sites.

## **NEW RESULTS AND OBSERVATIONS**

1. Using among the first ones in Hungary the *Infratech Grain Analyzer* that operates with NIT technology I was able to provide an accurate high-throughput analysis of the inner contents of different corn type kernels. Altogether 512 samples of 176 different corn hybrids including 10 new Hungarian strains were analyzed between 2007-2009.
2. The use of NIR/NIT analytical method gives an outstanding possibility to detect the influence of tillage methods, irrigation, application of fertilizers and planting rate on the maize kernels' inner contents. The near-infrared method made it possible to determine even more accurately the content values of maize samples starch content in a relatively short period of time compared with other analytical methods.
3. The application of this analysis makes it possible to select the most appropriate corn hybrids suitable for bioethanol production in dry- and wet-milling ethanol plants alike.
4. I have established that the positive influence of the utilization of fertilizers on the starch yield was due to the increased crop yields collected from small plot field experiments.

5. I was the first to compare the impact of production factors: irrigation, fertilisation, cultivation on three, genetically identical DKC maize hybrids inner contents grown at the Látókép Experimental Site in 2007, 2008 and 2009.

#### **Utilization of new results in practice:**

1. I selected the 10 most favorable maize hybrids for bioethanol production purpose from the 176 Hungarian grown hybrids analyzed in the three years. I took the relative starch content and the starch yield as well into concern of the selection.  
The 10 most favorable hybrid with code: DE63, DE25, DE49, DE63, DE73, AGTC264, AGTC133, AGTC168, AGTC206, AGTC237
2. I determined through multifactorial small field plot experiments the best agricultural growing methods (irrigation, application of fertilizers, tillage methods, growing rates) for the chosen corn hybrids to be grown in any given area for purposes of bioethanol production.

## **PUBLICATIONS IN THE SUBJECT OF THE DISSERTATION**

### Scientific publication in foreign language in Hungarian lectured review:

1. **Kiss, Cs.,** Andorkó I. (2008): Small-plot field experiments with maize hybrids, aiming to study starch content. Cereal Research Communications. 36:0133-3720. (IF: 1,19)
2. Rátonyi T., Harsányi E., **Kiss Cs.,** Megyes A. (2009): Effects of precipitation on the starch yield of maize grown for bioethanol production. Cereal Research Communications. 37:89-92. (IF: 1,037)

### Foreign language non lectured conference publication:

3. Rátonyi T., Harsányi E., Megyes A., **Kiss Cs.** (2009): Evaluation of quality parameters of maize grown for bio-ethanol production in Hungary in relation to crop production factors. International Soil Tillage Research Organisation 18th Triennial Conference. pp. T7 – 006 – 1 – T7 - 006 – 5

Hungarian lectured conference publication:

4. **Kiss Cs., Harsányi E., Rátonyi T. (2007):** Maize as an alternative feedstock in Hungary. Kiss T., Somogyvári M. (Eds.). *Via Futuri 2007*. Biomass-based energy production. BOKOM Kft. Pécs. ISBN: 978-963-06-5993-2. 150-156.
5. **Kiss Cs. (2012a):** The analysis of corn hybrids starch content using NIT spectroscopy method. *Acta Agraria Debreceniensis. (in press)*.
6. **Kiss Cs. (2012b):** The detailed assay of the main steps of dry-milling bioethanol production. *Acta Agraria Debreceniensis. (in press)*.

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